Piperazine squaric acid diamides, a novel class of allosteric P2X7 receptor antagonists

Patberg, M.; Isaak, A.; Füsser, F.; Ortiz Zacarías, N.V.; Vinnenberg, L.; Schulte, J.; ... ; Junker, A.

European Journal of Medicinal Chemistry 226 (2021) 113838

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Piperazine squaric acid diamides, a novel class of allosteric P2X7 receptor antagonists

Marius Patberg [a, 1], Andreas Isaak [a, 1], Friederike Füsser [b], Natalia V. Ortiz Zacarías [c, f], Laura Vinnenberg [d], Janine Schulte [a], Lucia Michetti [a], Lucie Grey [b], Cas van der Horst [c], Petra Hundehege [d], Oliver Koch [b], Laura H. Heitman [c, f], Thomas Budde [e], Anna Junker [a, *]

[a] European Institute for Molecular Imaging (EIMI), Waldeyerstr. 15, 48149, Münster, Germany
[b] Institut für Pharmazeutische und Medizinische Chemie der Universität Münster, Corrensstr. 48, 48149, Münster, Germany
[c] Leiden Academic Centre for Drug Research (LACDR), Leiden University, Division of Drug Discovery and Safety, Einsteinweg 55, 2333, CC Leiden, the Netherlands
[d] Klinik für Neurologie Mit Institut für Translationale Neurologie, ICB, Mendelstr. 7, 48149, Münster, Germany
[e] Institut für Physiologie I, Robert-Koch-Str. 27a, 48149, Münster, Germany
[f] Oncode Institute, Leiden, the Netherlands

ARTICLE INFO

Article history:
Received 21 June 2021
Received in revised form
6 September 2021
Accepted 7 September 2021
Available online 16 September 2021

Keywords:
P2X
P2X7 receptor
Antagonists
Squaric acid
Allosteric

ABSTRACT

The P2X7 receptor (P2X7R) stands out among the purinergic receptors due to its strong involvement in the regulation of tumor growth and metastasis formation as well as in innate immune responses and afferent signal transmission. Numerous studies have pointed out the beneficial effects of P2X7R antagonism for the treatment of a variety of cancer types, inflammatory diseases, and chronic pain. Herein we describe the development of novel P2X7R antagonists, incorporating piperazine squaric diamides as a central element. Besides improving the antagonists' potency from pIC_{50} values of 5.7–7.6, ADME properties ($logD_{7.4}$ value, plasma protein binding, *in vitro* metabolic stability) of the generated compounds were investigated and optimized to provide novel P2X7R antagonists with drug-like properties. Furthermore, docking studies revealed the antagonists binding to the allosteric binding pocket in two distinct binding poses, depending on the substitution of the central piperazine moiety.

1. Introduction

Seven different P2X subunits, termed P2X1 to P2X7, have been identified. The P2X subunits can assemble predominantly as trimeric homo- or heteromers, but multimers of higher-order were also reported [1]. Each subunit consists of two transmembrane domains, a larger extracellular loop and intracellular N- and C-termini. The chalice-formed, assembled trimers bear three ATP binding sites in the upper region of the extracellular part [2,3]. The location of the ATP binding pocket was further confirmed by the X-ray crystal structures of the closely related zfP2X4R [4] and the hP2X3R [5] in the closed-form and the X-ray crystal structures of pdP2X7R, bound to structurally distinct antagonists, that revealed three binding sites for allosteric inhibitors juxtaposed to the ATP binding pockets [6]. Most recently, Cryo-EM structures of apo and ATP-bound state rP2X7R were reported [1].

The homotrimeric P2X7 receptor (P2X7R) is an ATP-gated, unselective ion channel that has been found to be involved in various pathological conditions and has been increasingly gaining attention as a promising target for the treatment of inflammation, neuropathic pain, and cancer [7–10].

The P2X7R stands out in its physiological and pharmacological profile, as its activation requires 10-fold higher concentrations of ATP than other P2X receptor subtypes and is dependent on the level of bivalent cations (Mg^{2+}/Ca^{2+}) [2]. The intracellular C-terminal domain of the P2X7 subunit is significantly longer compared to other P2X subtypes and its extended length was found crucial for channel gating [11,12]. An important functional property of the P2X7R is the increase of membrane permeability of cells expressing this receptor upon prolonged or repeated activation with ATP, caused by the formation of large, unselective macropores, leading to its cytotoxic activity [2,13,14]. The P2X7 receptor is expressed on

* Corresponding author.
E-mail address: anna.junker@wwu.de (A. Junker).
[1] the authors contributed equally.

https://doi.org/10.1016/j.ejmech.2021.113838
0223-5234/

virtually all immune cells [7], where it triggers the secretion of IL-1β and IL-18 by activation of caspase-1, which promotes the maturation of interleukins and their subsequent release [15]. Hereby the P2X7R antagonism was shown to be beneficial as the potential treatment for diabetic retinopathy [16–19]. The P2X7R is further expressed on neuronal cells like microglia [20] and Schwann cells [21]. Upregulated expression of this receptor was demonstrated in injured nerves of dorsal root ganglia of patients suffering from chronic pain. P2X7R knockout displayed protection from neuropathic pain and lowered hypersensitivity [22].

Many different kinds of tumors exhibit overexpression of P2X7Rs [23–25]. The role of the P2X7R in tumor progression is diverse. Tumor microenvironments (TME), as sites of inflammation, usually show increased concentrations of ATP, thus enhancing cancer cell migration and invasion via P2X7R as demonstrated e.g. in breast cancer models [25,27]. P2X7R antagonists have been shown to inhibit cell proliferation of pancreatic duct adenocarcinoma cells (PDAC) [24] and to suppress the metastasis formation in breast cancer [2,26]. It should be mentioned that P2X7R activation can also lead to apoptosis in acute myeloid leukemia cells [28] and high levels of ATP (>20 μM) were shown to inhibit migration of human breast cancer cells [25]. However, local concentrations of ATP in these experiments were above the physiologically relevant range and, therefore, beyond the scope of possible therapeutic activation of P2X7Rs. It is now clear that P2X7R antagonists bear promising potential as therapeutic agents for the treatment of neuropathic pain, (neuro-)inflammatory diseases and cancer [29,30].

Herein we report the design, synthesis and pharmacological evaluation of piperazine-based squaric acid diamide-based P2X7 antagonists. Dihydroxy-3-cyclobutene-1,2-dione (a.k.a. squaric acid) is a structural motif that has been shown to work as a suitable bioisoster of polar substituents like phosphate groups and carboxylic acids and has been successfully implemented in biologically active compounds [31]. Furthermore, ester derivatives of squaric acid are easily accessible and allow the simple and selective formation of squaric acid amides and diamides with drug-like properties. In 2008, Betschmann et al. published a series of active N-cyanoguanidine-piperazine P2X7R antagonists, the most active containing 2-methylphenyl and 3,4-dimethoxybenzoyl moieties (**1**) [32]. We were interested in replacing the cyanoguanidine linker in **1** squaric acid diamide moiety, creating more potent P2X7R antagonists, and optimizing the physicochemical properties as well as metabolic stability of the novel compounds to generate drug-like lead structures suitable for further preclinical evaluation. In the course of these studies, a series of simplified piperazine amine derivatives was synthesized to determine crucial moieties for ligand-binding pocket interactions. Consecutive structural modifications generated compounds with improved physicochemical properties and increased potency (e.g. compounds **8a** and **8p**).

1.1. Chemistry

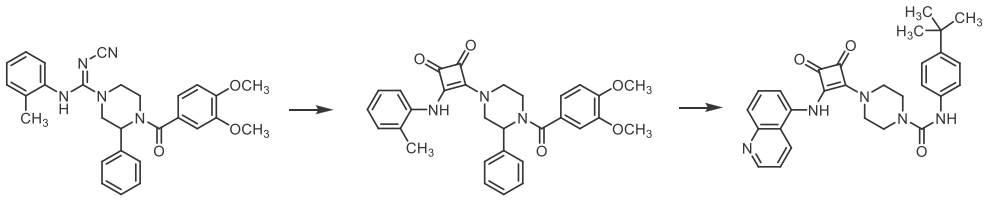
Piperazine amines (Scheme 1, A) were prepared by alkylation of the free nitrogen of Boc-protected piperazines **2a** or **2b**, respectively, achieved either by nucleophilic substitution using bromoalkanes or by reductive amination with the respective aldehyde to give intermediates **3a**-**3h**, latter method giving significantly better yields. Deprotection of the piperazine was performed by using hydrochloric acid in ether, followed by the formation of the amide with the respective acid using COMU™ coupling reagent, giving piperazine amides **4d**-**4m**.

For the preparation of 2-phenylpiperazine amides, the order of synthetic steps was interchanged. Benzoylation of the more hindered nitrogen atom and subsequent deprotection gave amide **3i**, followed by alkylation of the nitrogen in 4-position in the second step (compounds **4a**-**c**). Compound **4n** was prepared by reacting 2-phenylpiperazine with an excess of 2-chlorophenyl isocyanate. Compound **4o** was prepared through benzoylation of commercially available 1-(benzo[*b*]thiophen-4-yl)piperazine.

For the synthesis of the squaric acid diamide series (Scheme 1, B), the piperazine building blocks **2a** and **2c** were synthesized by mono-Boc protection of the less hindered nitrogen. The protected piperazines **2a** or **2c** were reacted with benzoic acid derivatives to the respective amides using COMU™ coupling reagent or reacted with phenyl isocyanates to afford the respective urea derivatives. Subsequent deprotection using hydrochloric acid in diethyl ether provided the respective amide (**6a**) and urea (**6b**-**6g**) intermediates. Squaric acid monoamides **5a** and **5b** were prepared by addition of toluidine or 5-aminoquinoline, respectively, at room temperature. Conversion of piperazines **6a**-**6g** with squaric acid amide **5a** or **5b** yielded the desired squaric acid diamides. For the synthesis of squaric acid diamides containing unsubstituted piperazine, protected piperazine **2c** was reacted with squaric acid amide **5a** and subsequently deprotected to give building block **7**, which allows quick and easy implementation of different amide and urea linked aryl substituents. The latter route is more convenient in terms of late-stage modification, although the purification of building block **7** is lavish. Both routes gave the desired final compounds **8a**-**8p** in satisfying yields.

2. Results and discussion

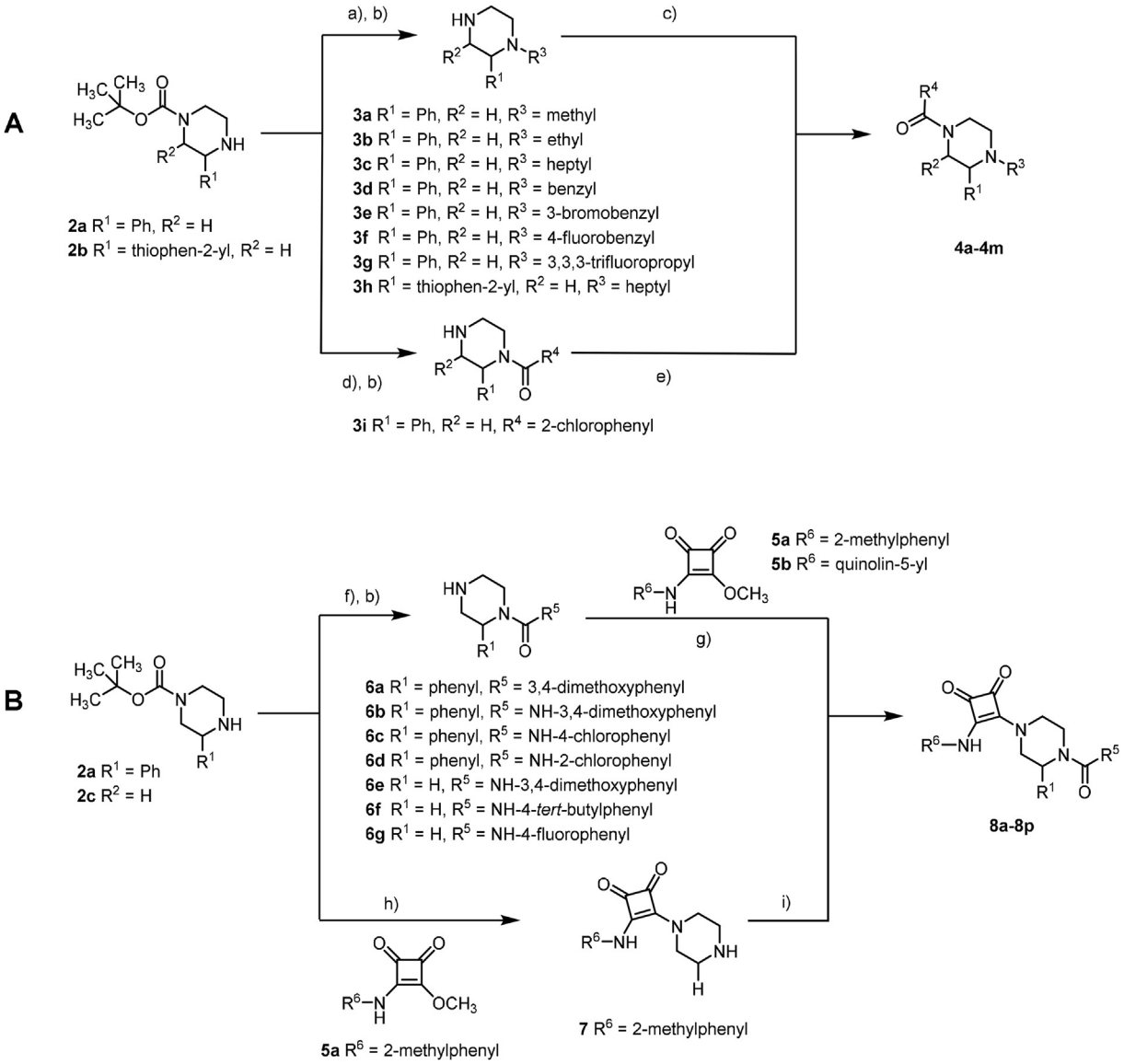
Compounds were tested for their antagonistic activity in a hP2X7R YO-PRO-1 dye uptake assay. First, the cyanoguanidine linker was removed from the molecule and a propyl (**4a**), heptyl (**4b**) or benzyl (**4c**) substituent was implemented, which led to a complete loss of activity. Moving the 2-phenyl substituent at the piperazine to the 3-position in combination with methyl (**4d**), ethyl (**4e**) and heptyl (**4f**) substituents did not restore potency. Similar



1
pIC_{50} (hP2X7R) = 5.7

8a
pIC_{50} (hP2X7R) = 6.3

8p
pIC_{50} (hP2X7R) = 7.6



Scheme 1. Reactions and conditions: a) R [3]-Br, DMF, K_2CO_3 (2 eq), rt to 80 °C, 6 d; or R^3 = O, $NaBH(OAc)_3$, THF, rt, 1 d; b) Et_2O x HCl, MeOH, 2 d, rt; c) R^4-COOH, COMU, CH_3CN, DIPEA, rt, overnight; d) R [3]-COOH, COMU, CH_3CN, DIPEA, rt, overnight; e) R [3]-Br, DMF, K_2CO_3 (2 eq), rt to 80 °C, 6 d; or R^3 = O, $NaBH(OAc)_3$; f) R [5]-NCO, toluene, rt to 50 °C, overnight; or R [5]-COOH, COMU, CH_3CN, DIPEA, rt, overnight; g) toluene, rt to 50 °C, overnight; h) toluene, rt, overnight; i) R [5]-NCO, toluene, rt to 50 °C, overnight.

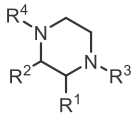
observations were made for the benzyl (**4g**), 3-bromobenzyl (**4h**) and 4-fluorobenzyl (**4i**) derivatives. Bioisosteric exchange of the 3-phenyl substituent with thiophen-2-yl in **4j** showed also no improvement in antagonistic potency (see Table 1). The 4-heptyl substituted 3-phenylpiperazine in combination with 2-chloro (**4f**), 2-chloro-3-trifluoromethyl (**4k**) and 2,3-dichloro (**4l**) substitution pattern in the benzoyl moiety were also inactive. All tested compounds mentioned so far exhibited inhibition of the P2X7 receptor lower than 50% at a concentration of 10 μM. However, exchange of the heptyl chain by a trifluoropropyl substituent in the latter compound gave derivative **4m**, which exhibited moderate potency (pIC_{50} (hP2X7(YOPRO) = 6.0). A comparable activity was determined for piperazine biscarboxamide **4n** (pIC_{50} (hP2X7(YOPRO) = 6.3, see Table 1).

The loss of P2X7R activity in the first series of piperazine-based compounds clearly demonstrated the requirement for the introduction of additional H-bond donors and acceptors. Herefore the 3,4-diaminocyclobut-3-ene-1,2-dione (squaric acid diamide) linker appeared highly attractive to us as squaric acid diamides are inexpensive, easy to synthesize and provide multiple opportunities for the formation of H-bond interactions.

Consequently, in the second approach, bioisosteric exchange of the cyanoguanidine linker in the lead structure **1** by a squaric acid diamide moiety led to compound **8a**. Thereby, the potency of the antagonist was significantly increased from pIC_{50} (hP2X7(YOPRO) = 5.71 to 6.26. In analogy to the investigations of Betschmann in 2008 [31], a 5-aminoquinoline squaric acid amide instead of the toluidine substituent was implemented in **8b** (pIC_{50}(hP2X7(YOPRO) = 6.65)) and the aryl amide was exchanged for a urea moiety in **8c** (pIC_{50}(hP2X7(YOPRO) = 7.19, see Table 2)) respectively. Both compounds showed increased activity, although aminoquinolinone **8b** exhibited poor overall solubility.

Exchanging the 3,4-dimethoxyphenyl (**8c**, pIC_{50}(hP2X7(YOPRO) = 6.65)) by a 4-chlorophenyl substituent (**8d**, pIC_{50}(hP2X7(YOPRO) = 6.87)) or by 2-chlorophenyl substituent (**8e**, pIC_{50}(hP2X7(YOPRO) = 7.06)) is well tolerated in terms of P2X7R

Table 1
Antagonistic activities of the piperazine amines in hP2X7R YO-PRO-1 assay, (n = 3), *% inhibition of test compounds' concentration of 10 μM.



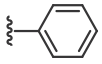
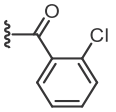
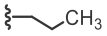
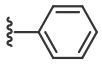
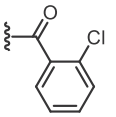
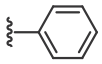
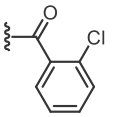
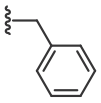
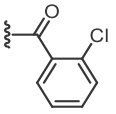
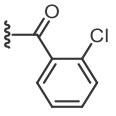
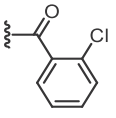
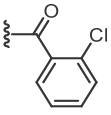
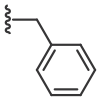
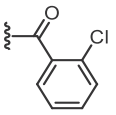
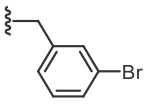
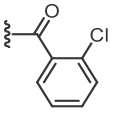
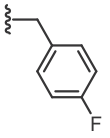
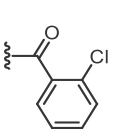
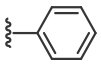
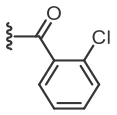
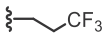
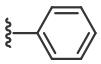
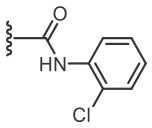
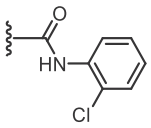
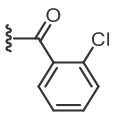
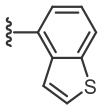
Cmpd.	R^1	R^2	R^3	R^4	pIC_{50} ± SEM
4a					4.08 ± 0.40
4b					−11 ± 14*
4c					14 ± 10*
4d					4.31 ± 0.28
4e					4.31 ± 0.30
4f					4 ± 5*
4g					21 ± 12*
4h					29 ± 11*
4i					35 ± 18*
4j					31 ± 24*
4k					29 ± 11*
4l					19 ± 10*

Table 1 (*continued*)

Cmpd.	R^1	R^2	R^3	R^4	pIC_{50} ± SEM
4m					6.00 ± 0.04
4n					6.31 ± 0.18
4o					34 ± 10*

activity. With the emphasis on water solubility, the 2-phenyl substituent at the piperazine ring was discarded in compound **8f** (pIC_{50}(hP2X7(YOPRO) = 6.52)). This modification caused a minor drop in the potency, but a significant increase of solubility in MeOH and DMSO was observed. In addition, NMR data indicated higher structural flexibility; therefore, we decided to proceed with our investigation of squaric acid diamides with unsubstituted piperazine.

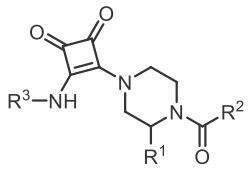
A broad selection of different substituents in 4-position of the carboxamide-linked phenyl ring was prepared and tested. Implementation of a primary amine in para-position **8g** (pIC_{50}(hP2X7(YOPRO) = 6.42)) showed no effect on potency, presence of fluorine (**8h**, pIC_{50}(hP2X7(YOPRO) = 6.12)) in this position lowered the activity slightly. Less polarizing groups like chlorine (**8i**, pIC_{50}(hP2X7(YOPRO) = 6.80)) and methyl (**8j**, pIC_{50}(hP2X7(YOPRO) = 6.51)) as well as the unsubstituted phenyl derivative **8k** (pIC_{50}(hP2X7(YOPRO) = 6.82)) exhibited a small increase in potency. Focusing on addressing lipophilic interactions in this region of the binding pocket with the introduction of the 4-*tert*-butyl group in **8l** (pIC_{50}(hP2X7(YOPRO) = 6.96)) was even more beneficial for antagonistic activity; a similar increase was observed through the introduction of electron-withdrawing nitro (**8m**, pIC_{50}(hP2X7(YOPRO) = 7.13)) and ester (**8n**, pIC_{50}(hP2X7(YOPRO) = 6.99)) functionalities. These findings also suggest that bulkier substituents in this position are preferred. A significant drop in activity was found with cyclohexyl derivative **8o** (pIC_{50}(hP2X7(YOPRO) = 6.13)), therefore suggesting the necessity of aromatic moieties in this position. In an attempt to combine motifs from the most active compounds in this series, compound **8p**, bearing an aminoquinoline squaric acid amide and a *tert*-butyl group in 4-position of the carboxamide-linked phenyl substituent was synthesized, in fact, leading to the most potent compound of this series with a pIC_{50} value of 7.62 (see Table 2). In Fig. 1A the representative dose-response curves (pooled data) of antagonists **8a**, **8c**, **8f** and **8p** and the reference antagonist JNJ47965567 (**9**) are shown.

For a selection of the prepared squaric acid diamides **8a**, **8c**, **8f** and **8p**, representing the major modifications in molecular structure conducted in this study, we recorded ionic currents in whole-cell patch-clamp experiments. In the first set of recordings, we tested whether to test whether the P2X7Rs receptors were functionally active in stably transfected HEK293 cells. After establishing the whole-cell configuration, cells revealed resting potentials and membrane capacitances of −40.6 ± 1.3 mV and 32.2 ± 2.2 pF (n = 102), respectively. In order to establish the ATP response profile, cells were challenged with different concentrations of ATP (1 μM–5 mM). For all applications with concentrations ≥10 μM measurable currents were reliably evoked. Since current responses to the same ATP concentration varied considerably between cells and revealed a clear correlation to the cell size, we determined current densities in the following. Plotting of mean current densities in response to different ATP concentrations and fitting to Hill equation yielded an EC_{50} value of 327 ± 37.5 μM. In order to evoke inward currents of adequate amplitude, we decided to use 400 μM ATP applications to assess the potent inhibition of agonist-induced currents by the P2X7R selective antagonists. All recordings were performed on at least 3 different days at least 7 different cells per compounds concentration (summary of the data and the dose-response curves are provided in Supporting information). In Fig. 1, B the dose-response curves (pooled data) for the antagonists **8a**, **8c**, **8f** and **8p** are provided. All antagonists revealed a concentration-dependent complete block of ATP-induced currents, with pIC_{50} values of **8a** (pIC_{50} = 7.00 ± 0.08, IC_{50} = 100 ± 8.6 nM), **8c** (pIC_{50} = 8.21 ± 0.05, IC_{50} = 6.1 ± 0.3 nM), **8f** (pIC_{50} = 7.73 ± 0.08, IC_{50} = 18.5 ± 1.5 nM) and **8p** (pIC_{50} = 8.69 ± 0.08, IC_{50} = 2.0 ± 0.16 nM) (Fig. 1 B). The whole-cell patch-clamp recordings followed the same trend as the results of the YO-PRO-1 assay, with compound **8a** being the weakest and **8p** being the most potent antagonist of this series.

2.1. Docking studies at P2X7R

In order to check if our compounds bind to the allosteric binding pocket docking experiments were performed. The compounds **8a**-**8p** were docked into the P2X7 binding site of an allosteric antagonist (pdb 5U1X) [6]. Although the docking poses are quite similar, two distinct groups of nearly identical poses can be identified based on an existing phenyl-moiety in R^1 (see Fig. 2c and e). Due to the steric hindrance of the phenyl moiety, compounds **8a** to **8e** are moved more deeply into the protein structure in comparison to compounds **8f** to **8p**. This can be seen in Fig. 2d where compounds **8a** and **8p** (the representatives of both groups) are overlayed. Interestingly, an induced fit of Phe65 helps to stabilize both groups of binding poses. For the group of poses around compound **8p**, Phe65 is moved down into the binding pocket (Fig. 2b) in comparison to the group containing an additional phenyl moiety (Fig. 2a). These highly conserved groups of docking poses clearly indicate the binding of our compounds into the allosteric binding pockets.

Table 2
Antagonistic activities of the squaric acid diamides in hP2X7R YO-PRO-1 assay, (n = 3).



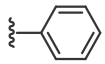
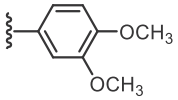
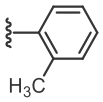
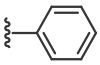
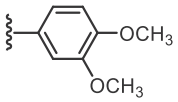
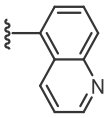
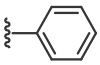
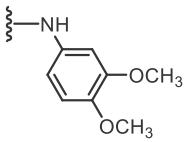
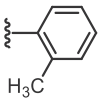
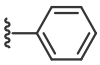
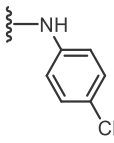
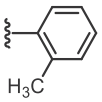
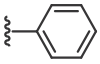
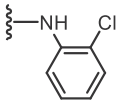
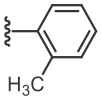
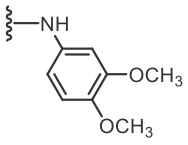
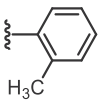
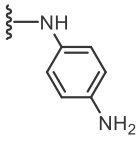
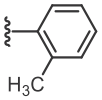
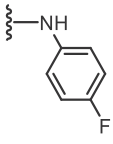
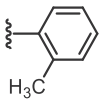
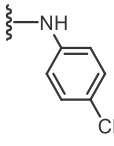
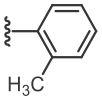
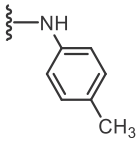
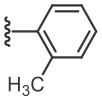
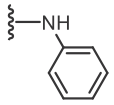
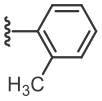
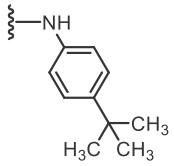
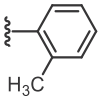
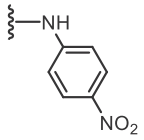
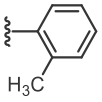
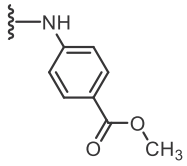
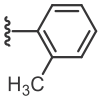
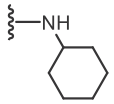
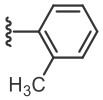
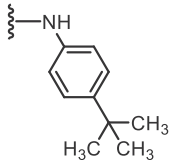
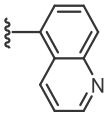
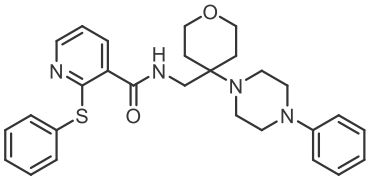
Cmpd.	R^1	R^2	R^3	pIC_{50} ± SEM
8a				6.26 ± 0.12
8b				6.87 ± 0.07
8c				6.65 ± 0.14
8d				6.80 ± 0.16
8e				6.96 ± 011
8f				6.51 ± 0.19
8g				6.12 ± 0.09
8h				7.06 ± 0.02
8i				7.19 ± 0.16
8j				6.52 ± 0.18

Table 2 (*continued*)

Cmpd.	R^1	R^2	R^3	pIC_{50} ± SEM
8k				6.82 ± 0.10
8l				6.13 ± 0.15
8m				6.99 ± 0.31
8n				6.42 ± 0.15
8o				7.13 ± 0.11
8p				7.62 ± 0.15
9	 JNJ47965567			7.80 ± 0.09

2.2. Off-target studies

The most potent P2X7R antagonists **8a**, **8c**, **8f** and **8p** were tested for their functional activity at the human P2X1R, P2X2R, P2X3R and P2X4Rs in Ca^{2+}-flux assay (Fluo-4 NW assay kit, Thermo Fisher Scientific), demonstrating no antagonistic activity (<30% at 10 μM compounds concentration) at these homotrimeric P2X receptor subtypes.

Due to structural similarities in the general structure of the squaric acid diamides with AZD2423 (Fig. 3), a highly potent CCR2 receptor antagonist [33], a selection of squaric acid diamides showing the closest structural similarity to AZD2423 were tested for their affinity and inhibitory potency towards the CCR2 receptor. All tested compounds (**8a**, **8c**, **8f** and **8p**) were not able to displace [^{3}H]CCR2-RA-[*R*] at a concentration of 1 μM (data not shown). Furthermore, at the same concentration, none of the compounds led to >25% inhibition of CCL2-induced β-arrestin recruitment in CCR2 (data not shown).

2.3. Physico-chemical properties and metabolic stability

The ADME-Tox properties such as logS at pH 7.4, logD at pH 7.4, logP, CYP 2C9 pK_i value, hERG pIC_{50}, BBB log([brain]:[blood]), BBB category, human intestinal absorption (HIA) category, P-gp category, CYP 2D6 affinity category, PPB90 category (high at > 90% bound to plasma), CYP 3A4 composite site lability (CSL) were predicted for all compounds (see Supporting Information) using Stardrop 7.0. All compounds were predicted to be orally bioavailable with human intestinal absorption (HIA) > 30%. Compounds **4a**-**4m** and **4o** were predicted to penetrate the blood-brain barrier (BBB ratio ≥ -0.5), whereas compounds **1**, **4n**, **8a**-**8p** were predicted to have no or very low CNS availability (BBB ratio ≤ -0.5).

The lead compound **1** as well as a selection of the novel squaric acid diamides **8a**, **8c**, **8f**, **8l** and **8p**, representing the major modifications in molecular structure conducted in this study, were experimentally evalauted for their water solubility ($logD_{7.4}$ values), plasma protein binding (PPB) and their metabolic stability in mouse liver microsomes. The $logD_{7.4}$ values were determined by the

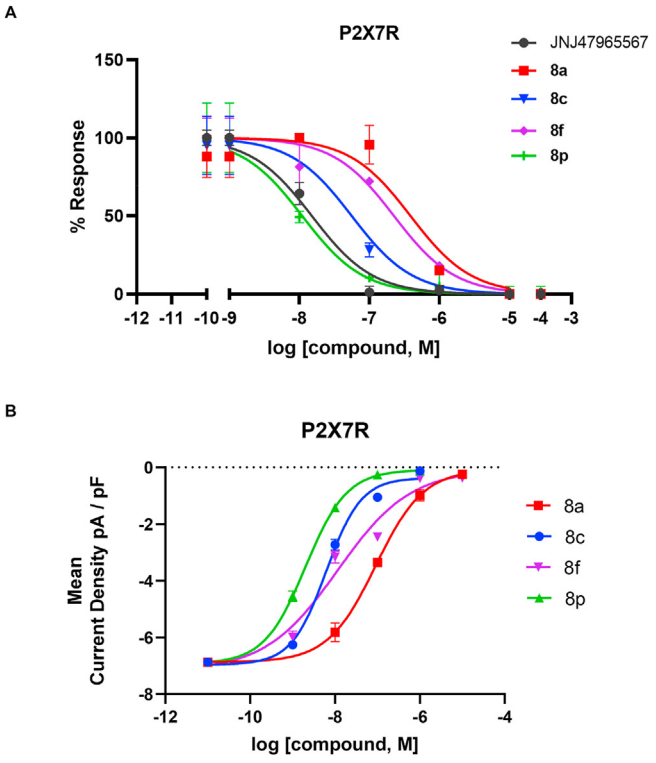


Fig. 1. A. Dose-response curves of antagonists **8a**, **8c**, **8f** and **8p** reference antagonist JNJ47965567 (**9**) (n = 3) in YO PRO-1 dye uptake assay and **B** dose-response curves of the P2X7R inhibition of ATP-currents through **8a**, **8c**, **8f** and **8p** in patch-clamp cell recordings (n = 3, pooled data).

shake-flask method in a 3-(N-morpholino)propanesulfonic acid buffered water/DMSO system at pH = 7.4. Plasma protein binding was investigated by high-performance affinity chromatography (HPAC) using human serum albumin (HAS) coated column. For the evaluation of metabolic stability, the respective compound was

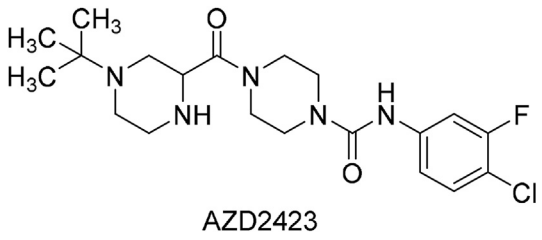


Fig. 3. AZD2423.

incubated with mouse liver microsomes (MLM) for 90 min and the digest was quantified via HPLC-MS analysis.

In Table 3 the determined values and their predicted counterparts for compounds **1**, **8a**, **8c**, **8f**, **8l** and **8p** are summarized. The calculated $logD_{7.4}$ values ($clogD_{7.4}$) did not match the experimentall determined $logD_{7.4}$ values. Whereas the predicted plasma protein binding (PPB90 category) matched well the determined values. The solubility was unaffected by the exchange of the cyanoguanidine in compound **1** with the squaric acid diamide in compound **8a** ($logD_{7.4}$ = 2.8 and 2.6), similarly to the plasma protein binding (92–97%). Compounds **8a** and **8c** were prepared and tested as racemic mixtures. However, the enantiomers have been separated on the chiral HSA column, therefore providing two slightly different values. Extension of the amide linker to a urea linker in **8c** improved the solubility in water significantly ($logD_{7.4}$ of 1.2), but did not affect the plasma protein binding (95% or 97%, respectively). Removal of the phenyl substituent in **8f** reduced plasma protein binding to 69% and unexpectedly caused a slight drop in water solubility ($logD_{7.4}$ = 1.6). The metabolic landscape (CSL) of compounds **1**, **8a**, **8c**, **8f**, **8l** and **8p** is shown in Supporting information. The most potent compound **8p** was predicted to be metabolically equally stable to the lead compound **1**. The experimentally determined stability in mouse liver microsomes was comparable for compounds **1**, **8a** and **8c**, ranging between 40% and 65% over 90 min. However, compound **8p** was found to be metabolically more stable compared to lead compound **1**. Implementation of the

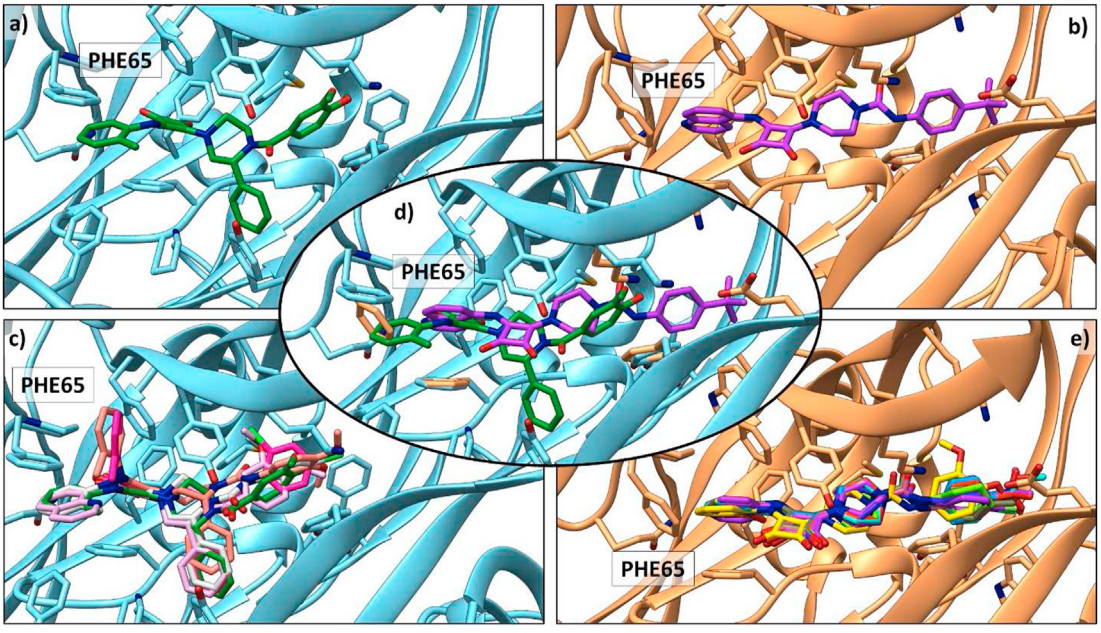


Fig. 2. Docking poses of compounds **8a**-**8p** in the allosteric binding pocket of P2X7R (pdb5U1X): a) **8a** (green), b) **8p** (purple), c) **8a**-**8e**, d) **8a** and **8p**, e) **8f**-**8p**.

Table 3
Determined versus predicted physico-chemical properties of selected squaric acid diamides and their metabolic stability in mouse liver microsomes.

Cmpd.	$logD_{7.4}$	$clogD_{7.4}$	Plasma protein binding (HSA)	PPB90 category	Metabolic stablity (MLM, 5 μM, 90 min)
1	2.84 ± 0.11	2.467	92%	high	54 ± 4.3%
8a	2.63 ± 0.01	3.492	96%/98%	high	40 ± 2.7%
8c	1.23 ± 0.02	3.857	95%/97%	high	64 ± 2.0%
8f	1.55 ± 0.04	2.727	69%	medium	21 ± 6.2%
8l	4.23 ± 0.25	3.315	>98%	high	39 ± 1.1%
8p	3.36 ± 0.04	3.450	>98%	high	22 ± 4.2%

tert-butyl substituent in compound **8l** instead of dimethoxy phenyl (**8f**) expectedly causes a significant increase in $logD_{7.4}$ values, and a decrease in metabolic stability while plasma protein binding remains unaffected. However, the introduction of the quinoline moiety (**8p**) instead of the 2-methyl phenyl moiety (**8l**) on the other side of the molecule restores the metabolic stability without affecting any other values (see Table 3).

3. Conclusion

A novel series of piperazine squaric acid diamides was synthesized and tested for their antagonistic activity at the hP2X7 receptor in YO-PRO-1 dye uptake assay and an electrophysiological patch-clamp assay. An inactive series of piperazine amides with unpolar aliphatic and aromatic substitution proved the necessity of polar linkers in 4-position of the piperazine. Replacement of the cyanoguanidine moiety in lead compound **1** by squaric acid increased the antagonistic activity $pIC_{50} = 5.7$ to 6.3 (**8a**). SAR studies pointed out that a urea linker in 1-position of the piperazine (**8c**) instead of an amide is well tolerated and improves its solubility in water ($logD_{7.4} = 2.6$ to 1.2). Additionally, to the prediction of ADME-Tox properties of all compounds, the physicochemical parameters $logD_{7.4}$, PPB and the metabolic stability were determined for a selected series of antagonists, representing the major modifications of the molecular scaffold, **1**, **8a**, **8c**, **8f**, **8l** and **8p**. The 2-phenyl substitution of the piperazine was found not to be essential for antagonistic activity but had an effect on metabolic stability as well as the plasma protein binding (**8c** vs. **8f**). The carbamoyl-linked phenyl substituent, while definitely contributing to the interaction with the receptor, tolerates modifications in the steric demand and the electronic density to a certain extend. Modification of the scaffold according to these latter findings allowed the synthesis of compound **8p** (pIC_{50}(hP2X7(YOPRO) = 7.62) with the highest antagonistic potency in this series. Off-target studies found no activities of the investigated scaffold at the P2X1-4 and CCR2 receptors.

4. Experimental section

4.1. Docking studies

The crystal structure of P2X7 in complex with an allosteric antagonist JNJ47965567 (pdb 5U1X) [6] was used to dock the compounds **8a**-**8p**. The protein structure and ligand database were prepared using the Molecular Operating Environment program version 2019 (MOE) [34]. Structural preparation of the protein included 3D protonation (at pH 7.4), deleting water molecules and the automatic correction step. The Ligand database was prepared by a washing step with a dominant protonation at pH 7.4 followed by an energy minimization with the forcefield MMFF94x.

Docking was performed using Gold version 5.8.1. The binding pocket was defined by the extracted Ligand JNJ47965567 and a radius of 6 Å, the side chains Phe95A, Phe95B, Phe95C, Lys297B and Tyr298C were defined flexible by using the rotamer library included in Gold. Ligands were docked using the scoring function ChemPLP and creating 100 diverse solutions, while the early termination was switched off. The option “flip ring corners” for ligand flexibility was switched on and the automatic search efficiency was set to 200%.

4.2. ADME-tox prediction

Drug metabolism and pharmacokinetic data were calculated using StarDrop 7.0 (Optibrium Ltd., United Kingdom) with the modules ADME QSAR and P450. The predicted data included the partition coefficient logD (octanol/buffer at pH 7.4), partition coefficient logP (octanol/water), solubility in water logS, solubility in PBS logS(pH7.4), half-maximum inhibitory concentration of the hERG channel hERG IC50, Blood-Brain-Barrier penetration log([-Brain]:[Blood]), Human intestinal absorption HIA (+indicates absorption ≥30%; - < 30%), indication of P-glycoprotein substrate P-gp, Plasma protein binding PPB90 (High ≥90%; Low <90% bound to plasma) and Cytochrome P450 affinity (2D6-affinity) (Low pKi <5; Medium pKi 5–6; High pKi 6–7; Very High pKi >7) and composite site lability (P450-3A4_CSL).

4.3. Chemistry general

Unless otherwise noted, moisture-sensitive reactions were conducted under dry nitrogen. Flash column chromatography (fc): Silica gel 60, 40–64 μm; parentheses include: diameter of the column, length of the column, fraction size, eluent, R_f value. Melting point: melting point apparatus Stuart Scientific® SMP 3, uncorrected. IR: IR spectrophotometer FT-ATR-IR (Jasco®). ^{1}H NMR (400 MHz): Unity Mercury Plus 400 spectrometer (Varian®), AV400 (Bruker®), JEOL JNM-ECA-400. ^{13}C NMR (100 MHz): Unity Mercury plus 400 spectrometer (Varian®) JEOL JNM-ECA-400; δ in ppm relative to tetramethylsilane; coupling constants are given with 0.5 Hz resolution, the assignments of ^{13}C and ^{1}H NMR signals were supported by 2D NMR techniques; MS: APCI = atmospheric pressure chemical ionization, EI = electron impact, ESI = electro-spray ionization: MicroTof (Bruker Daltronics, Bremen), calibration with sodium formate clusters before measurement. All solvents were of analytical grade quality and demineralized water was used. HPLC solvents were of gradient grade quality, and ultrapure water was used. All HPLC eluents were degassed by sonication prior to use. Thin-layer chromatography was conducted with silica gel F_{254} on aluminium plates in a saturated chamber at room temperature. The spots were visualized using UV light (254 nm) or reagents such as cerium molybdate dipping bath with additional heating using a standard heat gun. Hence the retention factor values strongly depend on the temperature, the chamber saturation and exact ratio of components of the eluent (highly volatile); the given retention factor values represent just approximate values. Flash column chromatography was conducted with silica gel 600 (40–63 μm, Macherey-Nagel).

4.4. HPLC purity measurements

Equipment: UV-detector: UltiMate 3000 variable Wavelength Detector; autosampler: UltiMate 3000; pump: Ultimate 3000; degasser: Ultimate 3000: data acquisition: Chromeleon Client 8.0.0 (Dionex Corpor.). Method: column: guard column: Zorbax SB-Aq 12.5 × 4.6 mm catridge, column: Zorbax SB-Aq StableBond analytical 150 × 4.6 mm, flow rate: 1.00 mL/min; injection volume: 5.0 μL; detection at λ = 210 nm;

Method A: solvents: A: Tetrabutylammonium phosphate buffer (5 mM) in H_2O, B: CH_3CN, gradient elution: (A %): 0–20 100 to 90%, 20–30 min: gradient from 90% to 100%.

Method B: solvents: A: Tetrabutylammonium phosphate buffer (5 mM) in H_2O, B: CH_3CN, gradient elution: (A %): 0–20 min 80 to 20%, 20–30 min: gradient from 20% to 80%.

Method C: solvents: A: Tetrabutylammonium phosphate buffer (5 mM) in H_2O, B: CH_3CN, gradient elution: (A %): 40–100%, 20–30 min: gradient from 100% to 40%.

Method D:HPLC method for determination of the product purity: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; Method: column: LiChrospher® 60 RP-select B (5 μm), 250 × 4 mm^2 column; flow rate: 1.00 mL/min; injection volume: 5.0 μL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A %): 0–4 min: 90%, 4–29 min: gradient from 90% to 0%, 29–31 min: 0%, 31–31.5 min: gradient from 0% to 90%, 31.5–40 min: 90%.

4.5. Determination of the partition coefficient (log D (exp.)

Aqueous 3-(N-morpholino)propanesulfonic acid buffer (20 mM) and *n*-octanol were mixed and stirred overnight at room temperature and 500 rpm to ensure saturation. 75 μL of a 100 μM solution of the respective compound in DMSO was added to 2 mL tube containing 1.5 mL of buffer/octanol mixture in the ratio 1:1, 2:1 and 1:2. Prepared mixtures have been vortexed for 2 min and subsequently centrifuged at 4 °C and 16000 rpm. The amount of compound in the buffer layer was quantified by ion count in single ion mode via HPLC-MS (ACN/water). Injection volume was 1 μL or 10 μL in case of lower ion count yield. Every experiment was triplicated.

4.6. Stability in mouse liver microsomes

An aqueous buffer of 75 mM PBS, 12.5 mM $MgCl_2$, 0.6 mM NADPH, 1 mg/mL mouse liver microsomes and 5 μM of the respective compound was prepared. 200 μL of these samples were incubated for 90 min at 37 °C and 900 rpm. Afterwards, acetonitrile/methanol (400 μL, 1:1) was added and the samples were cooled to 0 °C for 10 min. 600 μL buffer was added and the solution was centrifuged for 15 min at 16000 rpm. The supernatant was centrifuged again for 15 min at 16000 rpm and 4 °C. The obtained solution was diluted with H_2O/ACN/MeOH (1:1:1) to a suitable concentration for quantification. Shortly before injection into HPLC, the solution was centrifuged again for 2 min at 16000 rpm and 4 °C. The amount of remaining compound in the solution was quantified by ion count in SIM mode via HPLC-MS (ACN/water). Injection volume was 1 μL. Every experiment was triplicated.

4.7. HSA binding

HSA binding was determined by retention time on a HSA HPLC column. A 2 mM solution of the respective compound in DMSO was diluted with eluent (ammonium acetate (50 mM, pH = 7.4):isopropanol = 96:4 (v/v)) resulting in a compound concentration of 20 μM. Reference substances were D-Glucose, Metronidazole, Paracetamol, Salbutamol, Sulfamethoxazole, Ramipril, Propranolol, Phenytoin, Haloperidol, Imipramin and Chlorpromazine. Every test compound and reference substance was measured threefold. Injection volume was 1 μL.

4.8. Data analysis

NMR spectra were processed with MestReNova 12.0 (MestreLab Research).

4.9. Synthesis procedures

Due to the hindered rotation around the amide bond and the limited movement of the piperazine ring of the 2-phenyl substituted piperazines provide 4 sets of NMR signals at rt at an approximate ratio 1:1:1:1. Since the exact assignment of each of the conformers was not performed, the NMR signals are reported as signal sets. Temperature experiments have confirmed rotational isomerism as a cause for the splitting of NMR signals.

4.9.1. tert-Butyl 3-phenylpiperazine-1-carboxylate (2a)

2-Phenylpiperazine (2.82 g, 17.4 mmol, 1.0 eq) was dissolved in dry CH_2Cl_2 (20 mL), then di-*tert*-butyl dicarbonate (3.79 g, 17.4 mmol, 1.0 eq) and triethylamine (2.6 mL, 18.9 mmol, 1.09 eq) were added. The mixture was stirred at room temperature until the starting material was consumed (1 d). The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography (cyclohexane:ethyl acetate = 1:2 + 1% Et_3N, Ø = 3 cm, h = 30 cm, V = 20 mL) to provide **2a** as colorless solid, 3.60 g (13.7 mmol, 79%), $C_{15}H_{22}N_2O_2$ (262.4 g/mol). TLC (Silica): R_f = 0.41 (cyclohexane:ethyl acetate = 1:1 + 1% triethylamine), mp: 105.4 °C**.** Exact mass (APCI): *m/z* = calcd. for $C_{15}H_{23}N_2O_2$ [M + H^+] 263.1754, found 263.1747. Purity (HPLC, method D): 99%, R_t = 15.0 min.^{1}H NMR (600 MHz, MeOH-d_4): δ = 7.42–7.38 (m, 2H, 2-C*H*$_{phenyl}$, 6-C*H*$_{phenyl}$), 7.35 (t, *J* = 7.6 Hz, 2H, 3-C*H*$_{phenyl}$, 5-C*H*$_{phenyl}$), 7.31–7.27 (m, 1H, 4-C*H*$_{phenyl}$), 4.01 (ddt, *J* = 13.1, 3.5, 1.8 Hz, 2H, 2-C*H*H$_{piperazine}$, 6-C*H*H$_{piperazine}$), 3.66 (dd, *J* = 10.8, 3.2 Hz, 1H, 3-C*H*H$_{piperazine}$), 3.04 (d, *J* = 11.6 Hz, 1H, 5-C*H*H$_{piperazine}$), 3.00–2.64 (m, 3H, 2-C*H*H$_{piperazine}$, 5-C*H*H$_{piperazine}$, 6-C*H*H$_{piperazine}$), 1.47 (s, 9H, C*H*$_3$). ^{13}C NMR (151 MHz, MeOH-d_4): δ = 156.4 (1C, C=O), 142.1 (1C, *C*-1$_{phenyl}$), 129.7 (1C, *C*-3$_{phenyl}$, *C*-5$_{phenyl}$), 128.9 (1C, *C*-4$_{phenyl}$), 128.0 (2C, *C*-2$_{phenyl}$, *C*-6$_{phenyl}$), 81.4 (1C, *C*(CH$_3$)$_3$), 61.3 (1C, *C*-3$_{piperazine}$), 52.2 and 51.1 (1C, *C*-2$_{piperazine}$), 46.6 (1C, *C*-5$_{piperazine}$), 45.3 and 44.7 (1C, *C*-6$_{piperazine}$), 28.7 (9C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3329 (N–H), 2958, 2924, 2889 (C–H), 1681 (C=O), 1400 (C-C$_{arom}$).

4.9.2. tert-Butyl (3-thiophen-2-yl)-piperazine-1-carboxylate (2b)

2-Thiophenyl-piperazine (1.0 eq, 1.48 mmol, 250 mg) was dissolved in dry dichloromethane (8 mL) under N_2 atmosphere. The solution was cooled to 0 °C and di-*tert*-butyl dicarbonate (1 eq, 1.48 mmol, 323 mg) was added. The mixture was stirred for 1 h at 0 °C. Silica was added and the mixture was filtered. The silica plug was washed with dichloromethane (40 mL). The mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 14 cm, v = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 3% methanol) to provide **2b** as a yellow solid, 141 mg (0.52 mmol, 36%), $C_{13}H_{20}N_2O_2S$ (268.38 g/mol). mp: 84 °C.

TLC (Silica): 0.34 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 3% methanol). Exact mass (APCI): *m/z* = calcd. for $C_{13}H_{21}N_2O_2S$ [M + H^+] 269.1318, found 269.1276. Purity (HPLC, method D): 95%, R_t = 14.35 min ^{1}H NMR (600 MHz, MeOH-d_4): δ (ppm) = 7.32 (dd, *J* = 5.1/2.1 Hz, 1H, 3-C*H*$_{thiophenyl}$), 7.07 (dt, *J* = 3.5/1.0 Hz, 1H, 5-C*H*$_{thiophenyl}$), 7.00 (dd, *J* = 5.1/3.5 Hz, 1H, 4-

$CH_{thiophenyl}$), 4.06 (br, 1H, 2-CH$H_{piperazine}$), 4.00 (ddd, J = 10.1/3.3/0.8 Hz, 1H, 3-$CH_{eq.,\ piperazine}$), 3.93 (d, J = 12.1 Hz, 1H, 6-CH$H_{eq.,\ piperazine}$), 3.00 (br, 3H, 2-CH$H_{piperazine}$, 5-CH$H_{eq.,\ piperazine}$, 6-CH$H_{ax.,\ piperazine}$), 2.79 (ddd, J = 12.4/11.4/3.4 Hz, 1H, 5-CH$H_{ax.,\ piperazine}$), 1.47 (s, 9H, CH_3). ^{13}C NMR (151 MHz, MeOH-d_4): δ (ppm) = 156.3 (1C, C=O), 145.1 (1C, C-$2_{thiophenyl}$), 127.3 (1C, C-$3_{thiophenyl}$), 125.6 and 125.5 (2C, C-$4_{thiophenyl}$, C-$5_{thiophenyl}$), 81.5 (1C, $C(CH_3)_3$), 56.3 (1C, C-$3_{piperazine}$), 52.5 and 51.4 (1C, C-$2_{piperazine}$), 46.2 (1C, C-$5_{piperazine}$), 45.2 and 44.0 (1C, C-$6_{piperazine}$), 28.6 (3C, CH_3) FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3317 (N–H), 2974, 2928, 2855 (C–H), 1686 (C=O), 1412 (C-C_{arom}).

4.9.3. tert-Butyl piperazine-1-carboxylate (2c)

Piperazine (4.00 g, 46.0 mmol, 2 eq) was dissolved in CH_2Cl_2 (150 mL) and the mixture was cooled to 0 °C. Di-*tert*-butyldicarbonate (5.10 g, 23.0 mmol, 1 eq) was dissolved in CH_2Cl_2 (25 mL) and the resulting solution was added dropwise and the mixture was stirred over night while allowed to warm up to room temperature. Formed white precipitate was filtered off and the residue was washed with cold CH_2Cl_2. The solvent was removed *in vacuo* and the resulting residue was dissolved in water (20 mL) and saturated, aqueous solution of K_2CO_3 (10 mL) was added. The mixture was extracted with Et_2O (3 × 40 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo* to provide **2c** as a colorless solid, 2.60 g (14.0 mmol, 61%), $C_9H_{18}N_2O_2$ (186.26 g/mol). mp: 50 °C. TLC (Silica): 0.15 (CH_2Cl_2 + 5% MeOH + 1% Et_3N). Exact mass (ESI): m/z = calcd. for $C_9H_{19}N_2O_2$ [M + H^+] 187.1441, found 187.1454. ^{1}H NMR (400 MHz, $CHCl_3$-d, 25 °C): δ = 3.42–3.31 (m, 4H, 2-, 6-$CH_{2,piperazine}$), 2.83–2.72 (m, 4H, 3-, 5-$CH_{2,piperazine}$), 1.72 (s, 1H, NH), 1.44 (s, 9H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6, 25 °C): δ = 155.0 (1C, C=O), 79.7 (1C, $C(CH_3)_3$), 46.0 (2C, C-3, -$5_{piperazine}$), 44.9 (2C, C-2, -$6_{piperazine}$), 28.6 (1C, CH_3). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3321(N–H), 2974, 2943, 2866, 2819 (C-H_{aliph}), 1674 (C=O).

4.9.4. 1-Methyl-2-phenylpiperazine dihydrochloride (3a)

tert-Butyl 3-phenylpiperazine-1-carboxylate **2a** (1.11 g, 4.23 mmol, 1.0 eq) was dissolved in dry dimethylformamide (17 mL) und nitrogen atmosphere and K_2CO_3 (877 mg, 6.35 mmol, 1.5 eq) and iodomethane (0.29 mL, 4.65 mmol, 1.1 eq) were added. The mixture was stirred at room temperature for 5 d. The solvent was removed under reduced pressure, the residue was taken up in ethyl acetate, filtered and the filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography. (ethyl acetate:cyclohexane = 4:6, Ø = 3 cm, h = 30 cm, V = 20 mL). 340 mg of the obtained colorless oil was dissolved in methanol (25 mL), diethyl ether x HCl (2 M, 6 mL) was added and the mixture was stirred for 7 h. The mixture was filtered and the residue was washed with Et_2O (25 mL). The residue was dissolved in methanol (25 mL) and concentrated *in vacuo*, to provide **3a** a colorless solid, 215 mg (0.86 mmol, 52%), $C_{11}H_{18}Cl_2N_2$ (249.2 g/mol). TLC (Silica): R_f = 0.47 (dichloromethane + 10% methanol + 4% triethylamine). Exact mass (APCI): m/z = calcd. for $C_{11}H_{17}N_2$ [M + H^+] 177.1386, found 177. 1383. Purity (HPLC, method D): 72%, R_t = 4.6 min ^{1}H NMR (600 MHz, MeOH-d_4): δ (ppm) = 7.78–7.73 (m, 2H, 2-CH_{phenyl} and 6-CH_{phenyl}), 7.59–7.54 (m, 3H, 3-CH_{phenyl}, 4-CH_{phenyl} and 5-CH_{phenyl}), 4.82–4.76 (m, 1H, 2-$CH_{piperazine}$), 3.94 (ddt, J = 15.4/11.9/2.6 Hz, 2H, 5-CHH$_{piperazine}$ and 6-CHH$_{piperazine}$), 3.88 (d, J = 12.9 Hz, 1H, 3-CHH$_{piperazine}$), 3.86–3.70 (m, 3H, 5-CH$H_{piperazine}$, 3-CH$H_{piperazine}$ and 6-CH$H_{piperazine}$), 2.72 (s, 3H, CH_3). ^{13}C NMR (151 MHz, MeOH-d_4): δ (ppm) = 132.3 (1C, C-4_{phenyl}), 131.4 (1C, C-1_{phenyl}), 131.1 (2C, C-3_{phenyl} and C-5_{phenyl}), 129.9 (2C, C-2_{phenyl} and C-6_{phenyl}), 66.5 (1C, C-$2_{piperazine}$), 52.6 (1C, C-$6_{piperazine}$), 47.1 (1C, C-$3_{piperazine}$), 42.1 (1C, C-$5_{piperazine}$), 41.4 (1C, CH_3). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3375 (N–H), 2951 and 2928 (C-$H_{aliphatic}$), 1454 (C-C_{arom}).

4.9.5. 1-Ethyl-2-phenylpiperazine (3b)

tert-Butyl 3-phenylpiperazine-1-carboxylate **2a** (350 mg, 1.33 mmol, 1.0 eq) was dissolved in dry dimethylformamide (17 mL) und nitrogen atmosphere and K_2CO_3 (277 mg, 2.00 mmol, 1.5 eq) and iodomethane (0.15 mL, 1.47 mmol, 1.1 eq) were added. The mixture was stirred at room temperature for 5 d. The solvent was removed under reduced pressure, the residue was taken up in ethyl acetate, filtered and the filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography. (ethyl acetate:cyclohexane = 1:9, Ø = 3 cm, h = 20 cm, V = 10 mL). 304 mg of the obtained yellow oil was dissolved in methanol (14 mL), diethyl ether x HCl (2 M, 23 mL) was added and the mixture was stirred for 3 d. The mixture was filtered and the residue was washed with Et_2O (25 mL). The residue was dissolved in methanol (25 mL) and concentrated *in vacuo* to provide **3b** as a red solid, 226 mg (0.86 mmol, 62%), $C_{11}H_{18}Cl_2N_2$ (249.2 g/mol). TLC (Silica): R_f = 0.55 (dichloromethane + 10% methanol + 4% triethylamine). Exact mass (APCI): m/z = calcd. for $C_{11}H_{17}N_2$ [M + H^+] 191.1543, found 191.1553. Purity (HPLC, method D): 92%, R_t = 5.2 min ^{1}H NMR (400 MHz, MeOH-d_4): δ (ppm) = 7.85–7.77 (m, 2H, 2-CH_{phenyl} and 6-CH_{phenyl}), 7.60–7.53 (m, 3H, 3-CH_{phenyl}, 4-CH_{phenyl} and 5-CH_{phenyl}), 4.88–4.85 (m, 1H, 2-$CH_{piperazine}$), 4.08–3.96 (m, 2H, 6-CHH$_{piperazine}$ and 5-CHH$_{piperazine}$), 3.93 (d, J = 13.9 Hz, 1H, 3-CH$H_{piperazine}$), 3.89–3.80 (m, 1H, 5-CH$H_{piperazine}$), 3.76 (ddd, J = 14.1/3.4/1.9 Hz, 1H, 3-CHH$_{piperazine}$), 3.67 (td, J = 13.9/13.4/3.5 Hz, 1H, 6-CH$H_{piperazine}$), 3.20–2.98 (m, 2H, $CH_{2,ethyl}$), 1.28 (t, J = 7.4 Hz, 3H, $CH_{3,ethyl}$). ^{13}C NMR (101 MHz, MeOH-d_4): δ (ppm) = 132.2 (1C, C-4_{phenyl}), 131.5 (1C, C-1_{phenyl}), 131.1 (2C, C-3_{phenyl} and C-5_{phenyl}), 130.0 (2C, C-2_{phenyl} and C-6_{phenyl}), 75.2 (1C, $CH_{2,ethyl}$), 65.6 (1C, C-$2_{piperazine}$), 50.6 (1C, C-1_{ethyl}), 48.9 (1C, C-$6_{piperazine}$), 47.2 (1C, C-$3_{piperazine}$), 42.1 (1C, C-$5_{piperazine}$), 8.9 (1C, $CH_{3,ethyl}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3375 (N–H), 2630, 2600 and 2558 (C-$H_{aliphatic}$), 1431 (N–H).

4.9.6. 1-Heptyl-2-phenylpiperazine (3c)

tert-Butyl 3-phenylpiperazine-1-carboxylate **2a** (1.0 eq, 1.90 mmol, 500 mg) was dissolved in dry DMF (8 mL) under N_2 atmosphere. Heptyl bromide (1.4 eq, 2.67 mmol, 419 μL) and K_2CO_3 (2 eq, 3.80 mmol, 525 mg) were added and the mixture was stirred for 6 d at room temperature. The reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and saturated aqueous solution of $NaHCO_3$ (20 mL), then was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was dissolved in methanol (8 mL), then diethyl ether x HCl (2 M, 6 mL) was added and the mixture was stirred for 3 d at room temperature. The reaction mixture was diluted with aqueous solution of NaOH (1 M, 12 mL) and was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 3 cm, l = 12 cm, V = 10 mL, cyclohexane:ethyl acetate = 4:1 + 1% Et_3N + 2% methanol) to give **3c** as a yellow oil, 329 mg (1.26 mmol, 67%), $C_{17}H_{28}N_2$ (260.43 g/mol). TLC (Silica): 0.30 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 10% methanol). Exact mass (APCI): m/z = calcd. for $C_{17}H_{29}N_2$ [M + H^+] 261.2325, found 261.2329. Purity (HPLC, method D): 89%, R_t = 15.31 min ^{1}H NMR (600 MHz, $CHCl_3$-d): δ (ppm) = 7.40–7.28 (m, 4H, 2-, 3-, 5-, 6-CH_{phenyl}), 7.27–7.23 (m, 1H, 4-CH_{phenyl}), 3.16 (dd, J = 10.6/3.2 Hz, 1H, 2-$CH_{ax.,piperazine}$), 3.08 (dt, J = 11.7, 2.6 Hz, 1H, 6-$CH_{eq.,piperazine}$), 2.99 (ddt, J = 12.8/3.5/1.8 Hz, 1H, 5-$CH_{eq.,piperazine}$), 2.91 (ddd, J = 12.7/11.8/3.0 Hz, 1H, 5-$CH_{ax.,piperazine}$), 2.82 (ddd, J = 12.6/3.2/1.6 Hz, 1H, 3-$CH_{eq.,piperazine}$), 2.68 (dd, J = 12.7/10.6 Hz, 1H, 3-$CH_{ax.,piperazine}$), 2.43 (ddd, J = 12.7/9.5/6.8 Hz, 1H, 1-CHH_{heptyl}), 2.22 (td, J = 11.8/3.2 Hz, 1H, 6-$CH_{ax.,piperazine}$), 1.94 (ddd, J = 12.7/9.5/4.8 Hz, 1H, 1-

CHH_{heptyl}), 1.46–1.31 (m, 2H, 2-C$H_{2heptyl}$), 1.29–1.02 (m, 8H, 3-, 4-, 5-, 6-C$H_{2heptyl}$), 0.85 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CHCl_3$-d): δ (ppm) = 142.7 (1C, C-1_{phenyl}), 129.7 (2C, C-3_{phenyl}, C-5_{phenyl}), 129.0 (2C, C-2_{phenyl}, C-6_{phenyl}), 128.6 (1C, C-4_{phenyl}), 69.8 (1C, C-$2_{piperazine}$), 56.2 (1C, C-1_{heptyl}), 54.8 (1C, C-$3_{piperazine}$), 53.6 (1C, C-$6_{piperazine}$), 46.5 (1C, C-$5_{piperazine}$), 32.9, 30.1, 28.3 and 23.6 (4C, C-3_{heptyl}, C-4_{heptyl}, C-5_{heptyl}, C-6_{heptyl}), 26.6 (1C, C-2_{heptyl}), 14.4 (1C, CH_3). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3271 (N–H, val), 2924 (C–H, val).

4.9.7. 1-Benzyl-2-phenylpiperazine (3d)

tert-Butyl 3-phenylpiperazine-1-carboxylate **2a** (1.0 eq, 0.76 mmol, 200 mg) was dissolved in dry THF (3 mL) under N_2 atmosphere. Freshly destilled benzaldehyde (1.2 eq, 0.92 mmol, 93 μL) was added and the mixture was stirred for 6 h at room temperature. Sodium triacetoxyborohydride (1.4 eq, 1.07 mmol, 227 mg) was added and the mixture was stirred for 15 h at room temperature. The reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was dissolved in methanol (8 mL), then diethyl ether x HCl (2 M, 6 mL) was added. The mixture was stirred for 16 h at room temperature, was then diluted with aqueous solution of NaOH (1 M, 10 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 13 cm, v = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 5% methanol) to provide **3d** as a yellow oil, (166 mg, 0.66 mmol, 87%), $C_{17}H_{20}N_2$ (331.26 g/mol). TLC (Silica): R_f = 0.21 (cyclohexane:ethyl acetate = 2:1 + 1% Et_3N + 5% methanol). Exact mass (APCI): m/z = calcd. for $C_{17}H_{21}N_2$ [M + H^+] 253.1699, found 253.1692. Purity (HPLC, mehod D): 99%, R_t = 14.68 min ^{1}H NMR (600 MHz, MeOH-d_4): δ (ppm) = 7.49 (d, J = 7.5 Hz, 2H, 2-CH_{phenyl}, 6-CH_{phenyl}), 7.39–7.34 (m, 2H, 3-CH_{phenyl}, 5-CH_{phenyl}), 7.30–7.21 (m, 5H, 4-CH_{phenyl}, 2-CH_{benzyl}, 3-CH_{benzyl}, 5-CH_{benzyl}, 6-CH_{benzyl}), 7.21–7.17 (m, 1H, 4-CH_{benzyl}), 3.73 (dd, J = 13.2/0.9 Hz, 1H, PhCHHN), 3.26 (dd, J = 10.6/3.2 Hz, 1H, 2-C$H_{ax.,piperazine}$), 2.92–2.85 (m, 3H, PhCHHN, 3-C$H_{eq.,piperazine}$, 5-C$H_{piperazine}$), 2.85–2.79 (m, 2H, 5-C$H_{piperazine}$, 6-C$H_{piperazine}$), 2.72 (dd, J = 12.8/10.5 Hz, 1H, 3-C$H_{ax.,piperazine}$), 2.13 (m, 1H, 6-C$H_{piperazine}$). ^{13}C NMR (151 MHz, MeOH-d_4): δ (ppm) = 143.1 (1C, C-1_{phenyl}), 139.6 (1C, C-1_{benzyl}), 130.1 (2C, C-2_{benzyl}, C-6_{benzyl}), 129.6 (2C, C-3_{phenyl}, C-5_{phenyl}), 129.2 (2C, C-3_{benzyl}, C-5_{benzyl}), 129.0 (2C, C-2_{phenyl}, C-6_{phenyl}), 128.7 (1C, C-4_{phenyl}), 128.0 (1C, C-4_{benzyl}), 69.6 (1C, C-$2_{piperazine}$), 60.4 (1C, PhCH_2N), 55.2 (1C, C-$3_{piperazine}$), 53.5 (1C, C-$6_{piperazine}$), 46.5 (1C, C-$5_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3275 (N–H), 3028, 2947, 2792 (C–H), 1600 (C-C_{arom}).

4.9.8. 1-(3-Bromobenzyl)-2-phenylpiperazine (3e)

tert-Butyl 3-phenylpiperazine-1-carboxylate **2a** (1.0 eq, 0.38 mmol, 100 mg) was dissolved in dry THF (5 mL) under N_2 atmosphere. Bromobenzaldehyde (1.2 eq, 0.46 mmol, 54 μL) was added and the mixture was stirred for 4 h at room temperature. Sodium triacetoxyborohydride (1.4 eq, 0.53 mmol, 112 mg) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with saturated aqueous solution of $NaHCO_3$ (10 mL) and was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was dissolved in diethyl ether (5 mL) and methanol (2 mL), then diethyl ether x HCl (2 M, 5 mL) was added. The mixture was stirred for 72 h at room temperature. The solvent was removed under reduced pressure und the residue was washed with diethyl ether (30 mL). The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 12 cm, V = 10 mL, cyclohexane/ethyl acetate = 2:1 + 1% Et_3N + 5% methanol) to provide **3e** as a yellow oil, 87 mg (0.26 mmol, 69%), $C_{17}H_{19}BrN_2$ (331.26 g/mol). TLC (Silica): R_f = 0.31 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 10% methanol). Exact mass (APCI): m/z = calcd. for $C_{17}H_{20}^{79}BrN_2$ [M + H^+] 331.0804, found 331.0795. Purity (HPLC, method D): 97%, R_t = 18.26 min ^{1}H NMR (600 MHz, MeOH-d_4): δ (ppm) = 7.46 (d, J = 7.5 Hz, 2H, 3-CH_{phenyl}, 5-CH_{phenyl}), 7.41 (s, 1H, 2-C$H_{Br\text{-}phenyl}$), 7.36 (m, 3H, 2-CH_{phenyl}, 6-CH_{phenyl}, 5-C$H_{Br\text{-}phenyl}$), 7.28 (t, J = 7.4 Hz, 1H, 4-CH_{phenyl}), 7.24–7.16 (m, 2H, 4-C$H_{Br\text{-}phenyl}$, 6-C$H_{Br\text{-}phenyl}$), 3.69 (dd, J = 13.7/1.0 Hz, 1H, NCHHPh), 3.27 (dd, J = 10.5/3.2 Hz, 1H, 2-C$H_{ax.,piperazine}$), 2.90 (m, 2H, 3-C$H_{eq.,piperazine}$, 5-C$H_{piperazine}$), 2.88 (m, 1H, NCHHPh), 2.84 (m, 1H, 5-C$H_{piperazine}$), 2.80 (m, 1H, 6-C$H_{eq.,piperazine}$), 2.73 (dd, J = 12.7/10.6 Hz, 1H, 3-C$H_{ax.,piperazine}$), 2.16 (td, J = 11.7/3.2 Hz, 1H, 6-C$H_{ax.,piperazine}$). ^{13}C NMR (151 MHz, MeOH-d_4): δ (ppm) = 142.9 (1C, C–$3_{Br\text{-}phenyl}$), 142.6 (1C, C-1_{phenyl}), 132.7 (1C, C–$2_{Br\text{-}phenyl}$), 131.1 (1C, C–$5_{Br\text{-}phenyl}$), 131.0 (1C, C–$6_{Br\text{-}phenyl}$), 129.8 (2C, C-3_{phenyl}, C-5_{phenyl}), 128.9 (2C, C-2_{phenyl}, C-6_{phenyl}), 128.8 (1C, C-4_{phenyl}), 128.7 (1C, C–$4_{Br\text{-}phenyl}$), 123.3 (1C, C–$1_{Br\text{-}phenyl}$), 69.5 (1C, C-$2_{piperazine}$), 59.7 (1C, NCH_2Ph), 55.1 (1C, C-$3_{piperazine}$), 53.6 (1C, C-$6_{piperazine}$), 46.5 (1C, C-$5_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3271 (N–H), 2939, 2792 (C–H), 1593 (C-C_{arom}).

4.9.9. 1-(4-Fluorobenzyl)-2-phenylpiperazine (3f)

tert-Butyl 3-phenylpiperazine-1-carboxylate **2a** (1.0 eq, 0.38 mmol, 100 mg) was dissolved in dry THF (10 mL) under N_2 atmosphere. 4-Fluorobenzaldehyde (1.2 eq, 0.45 mmol, 48 μL) and sodium triacetoxyborohydride (1.4 eq, 0.53 mmol, 112 mg) were added and the mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the residue was concentrated *in vacuo*. The residue was dissolved in methanol (5 mL), then diethyl ether x HCl (2 M, 5 mL) was added. The mixture was stirred over night at room temperature, was afterwards diluted with aqueous solution of NaOH (1 M, 10 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 15 cm, V = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 3% methanol) to provide **3f** as s brown solid, 40 mg (0.15 mmol, 39%), $C_{17}H_{19}FN_2$ (270.35 g/mol). TLC (Silica): R_f = 0.05 (cyclohexane:ethyl acetate = 2:1 + 1% Et_3N). mp: 78.2 °C. Exact mass (APCI): m/z = calcd. for $C_{17}H_{20}FN_2$ [M + H^+] 271.1605, found 271.1577. Purity (HPLC, method D): 97%, R_t = 15.96 min ^{1}H NMR (600 MHz, MeOH-d_4): δ (ppm) = 7.48 (d, J = 7.5 Hz, 2H, 2-CH_{phenyl}, 6-CH_{phenyl}), 7.37 (td, J = 7.3/1.2 Hz, 2H, 3-CH_{phenyl}, 5-CH_{phenyl}), 7.30–7.26 (m, 1H, 4-CH_{phenyl}), 7.26–7.23 (m, 2H, 2-C$H_{fluorobenzyl}$, 6-C$H_{fluorobenzyl}$), 6.99 (t, J = 8.8 Hz, 2H, 3-C$H_{fluorobenzyl}$, 5-C$H_{fluorobenzyl}$), 3.68 (dd, J = 13.4/1.3 Hz, 1H, PhCHHN), 3.27 (dd, J = 10.6/3.2 Hz, 1H, 2-C$H_{piperazine}$), 2.94–2.79 (m, 5H, PhCHHN, 3-CH$H_{piperazine}$, 5-C$H_{2piperazine}$, 6-CH$H_{piperazine}$), 2.73 (dd, J = 12.8/10.6 Hz, 1H, 3-CH$H_{piperazine}$), 2.14 (td, J = 11.9/3.1 Hz, 1H, 6-CH$H_{piperazine}$). ^{13}C NMR (151 MHz, MeOH-d_4): δ (ppm) = 163.4 (d, J = 243.3 Hz, 1C, C-$4_{fluorobenzyl}$), 142.9 (1C, C-1_{phenyl}), 135.6 (d, J = 3.1 Hz, 1C, C-$1_{fluorobenzyl}$), 131.6 (d, J = 8.1 Hz, 2C, C-$2_{fluorobenzyl}$, C-$6_{fluorobenzyl}$), 129.8 (1C, C-3_{phenyl}, C-5_{phenyl}), 128.9 (2C, C-2_{phenyl}, C-6_{phenyl}), 128.8 (1C, C-4_{phenyl}), 115.8 (d, J = 21.4 Hz, 2C, C-$3_{fluorobenzyl}$, C-$5_{fluorobenzyl}$), 69.4 (1C, C-$2_{piperazine}$), 59.5 (1C, PhCH_2N), 55.0 (1C, C-$3_{piperazine}$), 53.3 (1C, C-$6_{piperazine}$), 46.5 (1C, C-$5_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3271 (N–H), 2939, 2808 (C–H), 1600 (C-C_{arom}).

4.9.10. 2-Phenyl-1-(3,3,3-trifluoropropyl)piperazine (3g)

Under N_2 atmosphere, trifluoropropanal (47 mg, 0.42 mmol, 1.1 eq) was dissolved in dry THF, then *tert*-Butyl 3-phenylpiperazine-1-carboxylate **2a** (100 mg, 0.38 mmol, 1 eq) was added and the mixture was stirred at room temperature for 6 h. Then sodium triacetoxyborohydride (97 mg, 0.46 mmol, 1.2 eq) was added and the mixture was stirred at room temperature overnight. Reaction control by TLC showed remaining starting material. Therefore, more sodium triacetoxyborohydride (75 mg, 0.35 mmol, 0.9 eq) was added. After stirring for 5 h at room temperature, an aqueous solution of NaOH (saturated, 10 mL) was added. The mixture was extracted with ethyl acetate (3 × 20 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The residue was dissolved in MeOH (4 mL), HCl (concentrated, 1 mL) was added and the solution was stirred overnight. Aqueous solution of NaOH (1 M, 10 mL) added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (cyclohexane:ethyl acetate = 2:1 + 4% MeOH + 1% triethylamine, Ø = 2 cm, h = 8 cm, V = 7 mL) to provide **3g** as a colorless oil, 43 mg (0.17 mmol, 44%), $C_{13}H_{17}F_3N_2$ (258.29 g/mol). TLC (Silica): R_f = 0.2 (ethyl acetate + 5% MeOH, 1% NEt_3). Exact mass (APCI): *m/z* = calcd. for $C_{13}H_{18}F_3N_2$ [M + H^+] 259.1417, found 259.1423. Purity (HPLC, method D): 96%, R_t = 15.4 min ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.44–7.21 (m, 5H, CH_{phenyl}), 4.85 (H_2O), 3.21 (dd, *J* = 10.5, 3.2 Hz, 1H, 2-C$H_{piperazine}$), 3.07–2.97 (m, 2H, 5-CH$H_{piperazine}$, 6-CH$H_{piperazine}$), 2.95–2.81 (m, 2H, 3-CH$H_{piperazine}$, 5-CH$H_{piperazine}$), 2.77–2.62 (m, 2H, 3-C$H_{piperazine}$, 1-CHH_{propyl}), 2.25 (m, 4H, 6-CH$H_{piperazine}$, 1-CHH_{propyl}, 2-C$H_{2,propyl}$). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 142.2 (1C, *C*-1_{phenyl}), 129.73 (2C, *C*-3, -5_{phenyl}), 128.9 (3C, *C*-2, -4, -6_{phenyl}), 128.2 (q, *J* = 276 Hz, 1C, *C*F_3), 69.2 (1C, *C*-$2_{piperazine}$), 55.0 (1C, *C*-$3_{piperazine}$), 53.4 (1C, *C*-$6_{piperazine}$), 48.6 (q, *J* = 2.3 Hz, 1C, *C*-1_{propyl}), 46.6 (1C, *C*-$5_{piperazine}$), 31.5 (q, *J* = 27.5 Hz, 1C, *C*-2_{propyl}). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3282 (N–H), 2947, 2816 (C–H), 1492 (C-C_{arom}).

4.9.11. 1-Heptyl-2-(thiophen-2-yl)-piperazine (3h)

tert-Butyl (3-thiophen-2-yl)-piperazine-1-carboxylate **2b** (1.0 eq, 0.37 mmol, 100 mg) was dissolved in dry DMF (5 mL) under N_2 atmosphere. Heptyl bromide (1.2 eq, 0.45 mmol, 71 μL) and K_2CO_3 (1.5 eq, 0.56 mmol, 77 mg) were added and the mixture was stirred for 7 d at room temperature. The reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was dissolved in methanol (4 mL), then diethyl ether x HCl (2 M, 2 mL) was added and the mixture was stirred for 3 d at room temperature. The reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 12 cm, V = 10 mL, cyclohexane:ethyl acetate = 4:1 + 1% Et_3N + 2% methanol) to provide **3h** as s yellow oil, 20 mg (0.08 mmol, 20%), $C_{15}H_{26}N_2S$ (266.45 g/mol).TLC (Silica): R_f = 0.19 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 10% methanol). Exact mass (APCI): *m/z* = calcd. for $C_{15}H_{27}N_2S$ [M + H^+] 267.1889, found 267.1896. Purity (HPLC, method D): 86%, R_t = 9.62 min ^{1}H NMR (600 MHz, MeOH-d_4): δ (ppm) = 7.32 (ddd, *J* = 5.1/1.3/0.6 Hz, 1H, 5-C$H_{thiophenyl}$), 7.01 (dd, *J* = 3.4/1.2 Hz, 1H, 3-C$H_{thiophenyl}$), 6.95 (dd, *J* = 5.1/3.5 Hz, 1H, 4-C$H_{thiophenyl}$), 3.53 (dd, *J* = 10.4/3.2 Hz, 1H, 2-C$H_{ax.,piperazine}$), 3.04 (dt, *J* = 11.8/2.7 Hz, 1H, 6-CH$H_{eq.,piperazine}$), 2.98 (ddt, *J* = 12.7/3.4/1.9 Hz, 1H, 5-CH$H_{eq.,piperazine}$), 2.94 (ddd, *J* = 12.6/3.3/1.6 Hz, 1H, 3-C$H_{eq.,piperazine}$), 2.89 (ddd, *J* = 12.6/11.6/3.0 Hz, 1H, 5-C$H_{ax.,piperazine}$), 2.77 (dd, *J* = 12.7/10.3 Hz, 1H, 3-C$H_{ax.,piperazine}$), 2.56 (ddd, *J* = 12.6/10.2/6.4 Hz, 1H, 1-CHH_{heptyl}), 2.22 (ddd, *J* = 11.7/3.2 Hz, 1H, 6-C$H_{ax.,piperazine}$), 2.01 (ddd, *J* = 12.6/9.8/4.5 Hz, 1H, 1-CHH_{heptyl}), 1.51–1.34 (m, 2H, 2-C$H_{2heptyl}$), 1.33–1.07 (m, 8H, 3-C$H_{2heptyl}$, 4-C$H_{2heptyl}$, 5-C$H_{2heptyl}$, 6-C$H_{2heptyl}$), 0.87 (t, *J* = 7.2 Hz, 3H, C$H_{3heptyl}$). ^{13}C NMR (151 MHz, MeOH-d_4): δ (ppm) = 145.9 (1C, *C*-$1_{thiophenyl}$), 127.3 (1C, *C*-$4_{thiophenyl}$), 126.7 (1C, *C*-$3_{thiophenyl}$), 125.8 (1C, *C*-$5_{thiophenyl}$), 64.3 (1C, *C*-$2_{piperazine}$), 56.2 (1C, *C*-1_{heptyl}), 55.6 (1C, *C*-$3_{piperazine}$), 53.5 (1C, *C*-$6_{piperazine}$), 46.4 (1C, *C*-$5_{piperazine}$), 32.9 (1C, C_{heptyl}), 30.2 (1C, C_{heptyl}), 28.3 (1C, C_{heptyl}), 26.6 (1C, *C*-2_{heptyl}), 23.7 (1C, C_{heptyl}), 14.4 (1C, *C*-7_{heptyl}). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3271 (N–H), 2924 (C–H).

4.9.12. 1-(2-Chlorobenzoyl)-2-phenylpiperazine (3i)

To a solution of 2-chlorobenzoic acid (209 mg, 1.33 mmol, 1.0 eq) in dry tetrahydrofuran (6 mL) under nitrogen atmosphere, *N,N*-diisopropylethylamine (0.45 mL) and COMU (628 mg, 1.47 mmol, 1.1 eq) were added. The mixture was stirred for 10 min, *tert*-Butyl 3-phenylpiperazine-1-carboxylate **2a** was added and the mixture was stirred at room temperature for 3 d. The solvent was removed under reduced pressure and the residue was taken up in ethyl acetate, filtered over silica and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (ethyl acetate:cyclohexane = 1:3, Ø = 3 cm, h = 20 cm, V = 20 mL). 328 mg of the obtained white foam was dissolved in methanol (10 mL) and diethyl ether x HCl (2 M, 15 mL) was added. The mixture was stirred at room temperature for 8 d, then filtered and the residue was washed with diethyl ether (25 mL). The residue was dissolved in methanol (25 mL) and concentrated *in vacuo* to provide **3i** as a colorless foam, 226 mg (0.86 mmol, 62%), $C_{17}H_{17}ClN_2O$ (337.2 g/mol). TLC (Silica): R_f = 0.62 (dichloromethane + 10% methanol + 4% triethylamine). Exact mass (APCI): *m/z* = calcd. for $C_{17}H_{18}ClN_2O$ [M + H^+] 301.1102, found 301.1087. Purity (HPLC, Purity D): 98%, R_t = 13.9 min ^{1}H NMR (600 MHz, MeOH-d_4): δ (ppm) = 7.73–7.16 (m, 9H, CH_{phenyl} and C$H_{Cl\text{-}phenyl}$), 6.23 (s, 1H, 2-C$H_{piperazine}$), 4.35–4.03 (m, 1H, 3-CHH$_{piperazine}$), 3.77–3.36 (m, 3H, 3-CHH_{piper}azine, 6-C$H_{2,piperazine}$), 3.29–3.10 (m, 2H, 5-C$H_{2,piperazine}$). ^{13}C NMR (151 MHz, MeOH-d_4): δ (ppm) = 170.0 (1C, *C*=O), 140.6 (1C, *C*–$1_{Cl\text{-}phenyl}$), 135.5 (1C, *C*-1_{phenyl}), 132.5 (2C, *C*–$3_{Cl\text{-}phenyl}$ and *C*–$4_{Cl\text{-}phenyl}$), 131.2 (1C, *C*–$5_{Cl\text{-}phenyl}$), 130.8 (1C, *C*–Cl), 130.5 (2C, *C*-3_{phenyl} and *C*-5_{phenyl}), 129.4 (1C, *C*–$6_{Cl\text{-}phenyl}$), 129.0 (1C, *C*-4_{phenyl}), 127.7 (2C, *C*-2_{phenyl} and *C*-6_{phenyl}), 50.8 (1C, *C*-$2_{piperazine}$), 45.4 (1C, *C*-$3_{piperazine}$), 44.2 (1C, *C*-$5_{piperazine}$), 40.9 (1C, *C*-$6_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3271 (N–H), 2936, 2916 (C–H), 1636 (C=O).

4.9.13. (2-Chlorophenyl) (3-phenyl-4-propylpiperazin-1-yl) methanone (4a)

3-Phenyl-1-propylpiperazine x HCl **3i** (50 mg, 0.15 mmol, 1 eq) was dissolved in dry dimethylformamide (4 mL) and K_2CO_3 (31 mg, 0.22 mmol, 1.5 eq) and 1-iodopropane (53 mg, 0.31 mmol, 2.1 eq) were added. The mixture was stirred for 3 d at room temperature. The mixture was concentrated *in vacuo* and the residue was taken up in ethyl acetate, filtered and the filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography (ethyl acetate:cyclohexane = 1:3, Ø = 2 cm, h = 30 cm, V = 7 mL). Afterwards, the obtained substance was purified by preparative HPLC (C18): 5 mL/min, gradient (ACN/H_2O) to provide **4a** as a colorless oil, 14 mg (0.04 mmol, 26%), $C_{20}H_{23}ClN_2O$ (342.9 g/mol). TLC (Silica): R_f = 0.43 (ethyl acetate:cyclohexane = 1:3). Purity (HPLC, method D): 99%, R_t = 15.7 min ^{1}H NMR (400 MHz, MeOH-d_4): δ (ppm) = Each H provides two signals: 7.72–7.58 (m, 3H, 2-CH_{phenyl} and 6-CH_{phenyl}), 7.57–7.18 (m, 15H, 3-CH_{phenyl}, 4-CH_{phenyl}, 5-

CH_{phenyl}, 3-C $H_{Cl\text{-}phenyl}$, 4-$CH_{Cl\text{-}phenyl}$, 5-$CH_{Cl\text{-}phenyl}$ and 6-$CH_{Cl\text{-}phenyl}$), 4.67–4.50 (m, 1H, 6-$CH_{piperazine}$), 3.66–3.60 (m, 1H, 3-$CH_{piperazine}$), 3.54–3.43 (m, 1H, 6-$CH_{piperazine}$), 3.29–3.23 (m, 1H, 3-C $H_{piperazine}$), 3.23–3.14 (m, 2H, 3-$CH_{piperazine}$, 6-$CH_{piperazine}$), 2.81–2.71 (m, 1H, 5-C $H_{piperazine}$), 2.60 (dd, J = 12.1/4.1 Hz, 1H, 6-$CH_{piperazine}$), 2.53–2.43 (m, 1H, 3-CH $_{piperazine}$), 2.39–2.30 (m, 4H, 1-$CH_{2propyl}$), 2.29–2.20 (m, 2H, 5-$CH_{piperazine}$), 2.07 (td, J = 11.7/3.4, 1H, 5-$CH_{piperazine}$), 1.66–1.39 (m, 4H, 2-$CH_{2propyl}$), 0.97–0.83 (m, 6H, $CH_{3propyl}$). ^{13}C NMR (101 MHz, MeOH-d_4): δ (ppm) = 172.8 and 169.64 (1C, C=O), 140.4 and 140.1 (1C, C-1_{phenyl}), 136.9 and 136.7 (1C, C–$1_{Cl\text{-}phenyl}$), 133.1 (1C, C–$6_{Cl\text{-}phenyl}$), 131.93 and 131.86 (1C, C-4_{phenyl}), 130.82 (1C, C–$3_{Cl\text{-}phenyl}$), 129.4 (3C, C-3_{phenyl}, C-5_{phenyl} and C–$4_{Cl\text{-}phenyl}$), 129.25, 129.23, 128.8 and 128.7 (4C, C-2_{phenyl} and C-6_{phenyl}), 61.4 and 61.3 (1C, C-1_{propyl}), 55.9 and 55.8 (1C, C-$3_{piperazine}$), 54.5 (1C, C-$5_{piperazine}$), 52.9 and 52.7 (1C, C-$2_{piperazine}$), 44.3 (1C, C-$6_{piperazine}$), 20.8 and 20.7 (1C, C-2_{propyl}), 12.2 and 12.1 (1C, $CH_{3propyl}$). The signal for C–$6_{Cl\text{-}phenyl}$ was determined using HSQC. FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = N. D.

4.9.14. 1-(2-Chlorobenzoyl)-4-heptyl-2-phenylpiperazine (4b)

1-(2-Chlorobenzoyl)-2-phenylpiperazine **3i** (1.0 eq, 0.23 mmol, 70 mg) was dissolved in dry DMF (2.2 mL) under N_2 atmosphere. K_2CO_3 (1.5 eq, 0.35 mmol, 47 mg) and heptyl bromide (1.2 eq, 0.28 mmol, 43 μL) were added and the mixture was stirred for 3 d at room temperature. The reaction mixture was diluted with ethyl acetate (20 mL), the organic layer was washed with H_2O (2 × 10 mL) and brine (10 mL). The combined aqueous layers were extracted with ethyl acetate (2 × 10 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 15 cm, V = 10 mL, cyclohexane:ethyl acetate = 4:1) to provide **4b** as a colorless oil, 61 mg (0.15 mmol, 67%), $C_{24}H_{31}ClN_2O$ (398.98 g/mol). TLC (Silica): R_f = 0.46 (cyclohexane:ethyl acetate = 3:1 + 5% methanol). Exact mass (APCI): m/z = calcd. for $C_{24}H^{35}_{32}ClN_2O$ [M + H^+] 399.2198, found 399.2207. Purity (HPLC, method D): 98%, R_t = 20.51 min ^{1}H NMR (400 MHz, MeOH-d_4): δ (ppm) = 7.68 (d, J = 8.2 Hz, 0.8H, 2-CH_{phenyl}, 6-CH_{phenyl}), 7.60 (d, J = 7.2 Hz, 0.7H, 2-CH_{phenyl}, 6-CH_{phenyl}), 7.54–7.17 (m, 7.5H, 2-CH_{phenyl}, 3-CH_{phenyl}, 4-CH_{phenyl}, 5-CH_{phenyl}, 6-CH_{phenyl}, 3-$CH_{benzoyl}$, 4-$CH_{benzoyl}$, 5-$CH_{benzoyl}$, 6-$CH_{benzoyl}$), 5.91 (s, 0.7H, 2-$CH_{piperazine}$), 4.78 (s, 0.1H, 2-$CH_{piperazine}$), 4.62 (m, 0.2H, 6-CH$H_{piperazine}$), 4.58 (s, 0.2H, 2-$CH_{piperazine}$), 3.63 (d, J = 12.2 Hz, 0.7H, 3-CH$H_{piperazine}$), 3.47 (m, 0.3H, 3-CH$H_{piperazine}$, 5-CH$H_{piperazine}$), 3.27 (m, 0.4H, 6-CH$H_{piperazine}$), 3.17 (m, 1.2H, 6-$CH_{2piperazine}$), 2.94 (t, J = 10.7 Hz, 0.3H, 1-CHH_{heptyl}), 2.75 (t, J = 10.7 Hz, 0.7H, 5-CH$H_{piperazine}$), 2.59 (dd, J = 12.1/4.0 Hz, 0.2H, 3-CH$H_{piperazine}$, 5-CH$H_{piperazine}$), 2.47 (m, 0.8H, 3-CH$H_{piperazine}$), 2.41–2.31 (m, 1.8H, 1-$CH_{2heptyl}$), 2.24 (td, J = 10.8/4.1 Hz, 0.6H, 5-CH$H_{piperazine}$), 2.06 (td, J = 11.7/3.4 Hz, 0.4H, 5-CH$H_{piperazine}$), 1.53 (m, 2H, 2-$CH_{2heptyl}$), 1.40–1.19 (m, 8H, 3-$CH_{2heptyl}$, 4-$CH_{2heptyl}$, 5-$CH_{2heptyl}$, 6-$CH_{2heptyl}$), 0.90 (m, 3H, CH_3). ^{13}C NMR (101 MHz, MeOH-d_4): δ (ppm) = 168.5, 168.2 and 168.0 (1C, C=O), 139.0 and 138.7 (1C, C-1_{phenyl}), 135.5 and 135.3 (1C, C-$2_{benzoyl}$), 130.5 and 130.5 (1C, $C_{benzoyl}$), 130.0 and 129.7 (1C, C-$1_{benzoyl}$), 129.5, 129.4 and 129.3 (1C, $C_{benzoyl}$), 128.3 and 128.1 (1C, $C_{benzoyl}$), 127.8, 127.8 and 127.7 (1C, C-2_{phenyl}, C-6_{phenyl}), 127.4, 127.3, 127.3 and 127.2 (1C, C-2_{phenyl}, C-6_{phenyl}), 127.0 (1C, C-4_{phenyl}), 126.9, 126.9 (2C, C-3_{phenyl}, C-5_{phenyl}), 126.3 (1C, $C_{benzoyl}$), 58.0, 57.9 and 52.8 (1C, C-1_{heptyl}), 57.8, 51.6 and 51.3 (1C, C-$2_{piperazine}$), 56.1, 54.5 and 54.5 (1C, C-$3_{piperazine}$), 53.4 and 53.2 (1C, C-$5_{piperazine}$), 43.9, 42.9 and 38.4 (1C, C-$6_{piperazine}$), 31.7 and 31.6, 28.9 and 28.8, 27.1 and 27.0, 22.3 (4C, C-3,-4,-5,-6_{heptyl}), 26.2 and 26.1 (1C, C-2_{heptyl}) 13.0 (1C, C-7_{heptyl}). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2924, 2854, 2808 (C–H), 1635 (C=O), 1593 (C-C_{arom}).

4.9.15. 4-Benzyl-1-(2-chlorobenzoyl)-2-phenylpiperazine (4c)

1-(2-Chlorobenzoyl)-2-phenylpiperazine **3i** (1.0 eq, 0.23 mmol, 70 mg) was dissolved in dry DMF (2.2 mL) under N_2 atmosphere. K_2CO_3 (1.5 eq, 0.35 mmol, 47 mg) and benzyl bromide (1.2 eq, 0.28 mmol, 33 μL) were added and the mixture was stirred for 2 d at room temperature. The reaction mixture was diluted with ethyl acetate (20 mL), the organic layer was washed with H_2O (2 × 10 mL) and brine (10 mL). The combined aqueous layers were extracted with ethyl acetate (2 × 10 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 15 cm, v = 10 mL, cyclohexane/ethyl acetate = 4:1 + 1% methanol). The obtained product was dissolved in diethyl ether (5 mL) and diethyl ether x HCl (5 mL) was added. The resulting precipitate was filtered, washed with diethyl ether (10 mL), dissolved in methanol (5 mL) and NaOH (2 M, 4 mL) was added. The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure to provide **4c** as a colorless oil, 67 mg (0.17 mmol, 75%), $C_{24}H_{23}ClN_2O$ (390.91 g/mol). TLC (Silica): R_f = 0.48 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 5% methanol). Exact mass (APCI): m/z = calcd. for $C_{24}H^{35}_{24}ClN_2O$ [M + H^+] 391.1572, found 391.1552. Purity (HPLC, method D): 97%, R_t = 17.92 min ^{1}H NMR (400 MHz, MeOH-d_4): δ (ppm) = 7.58 (d, J = 8.1 Hz, 1H, 2-CH_{phenyl}, 6-CH_{phenyl}), 7.53–7.15 (m, 13H, 2-, 3-, 4-, 5-, 6-CH_{benzyl}, 3-, 4-, 5-, 6-$CH_{benzoyl}$, 2-, 3-, 4-, 5-, 6-CH_{phenyl}), 5.90 (t, J = 8.1 Hz, 0.7H, 2-$CH_{piperazine}$), 4.73 (s, 0.1H, 2-$CH_{piperazine}$), 4.60 (d, J = 13.8 Hz, 0.2H, 6-$CH_{piperazine}$), 4.53 (s, 0.2H, 2-$CH_{piperazine}$), 3.58 (m, 1H, NCHHPh), 3.50 (m, 1.7H, 3-$CH_{piperazine}$, NCHHPh), 3.35 (s, 0.2H, 3-$CH_{piperazine}$), 3.20 (m, 1.7H, 6-$CH_{piperazine}$), 2.95 (m, 0.3H, 5-$CH_{piperazine}$), 2.74 (m, 0.7H, 5-$CH_{piperazine}$), 2.63 (dd, J = 12.0/4.0 Hz, 0.2H, 3-$CH_{piperazine}$), 2.51 (td, J = 12.0/4.2 Hz, 0.8H, 3-$CH_{piperazine}$), 2.33 (td, J = 11.4/3.90 Hz, 0.6H, 5-$CH_{piperazine}$), 2.14 (td, J = 11.64/3.34 Hz, 0.41 H, 5-$CH_{piperazine}$). ^{13}C NMR (101 MHz, MeOH-d_4): δ (ppm) = 169.7 and 169.4 (1C, C=O), 140.3 and 140.0 (1C, C-1_{phenyl}), 138.9 (1C, C-1_{benzyl}), 136.9, 136.7 and 136.6 (1C, C-$2_{benzoyl}$), 131.4 (1C, C-$1_{benzoyl}$), 131.9, 131.9, 130.9, 130.8, 130.8, 130.4, 130.4, 130.2, 129.6, 129.5, 129.3, 129.3, 129.3, 129.3, 129.2, 129.1, 128.8, 128.7, 128.7, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.0 and 127.9 (14C, C-2, -3, -4, -5, -6_{benzyl}, C-3, -4, -5, -$6_{benzoyl}$, C-2, -3, -4, -5, -6_{phenyl}), 63.8, 63.8 and 63.6 (1C, NCH_2Ph), 59.3, 52.9 and 52.7 (1C, C-$2_{piperazine}$), 56.9, 55.2 and 55.2 (1C, C-$3_{piperazine}$), 54.7, 54.5 and 54.0 (1C, C-$5_{piperazine}$), 45.3, 44.3 and 39.9 (1C, C-$6_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2924, 2854, 2808 (C–H), 1635 (C=O), 1593 (C-C_{arom}).

4.9.16. (2-Chlorophenyl) (4-methyl-3-phenylpiperazin-1-yl) methanone (4d)

1-Methyl-2-phenylpiperazine x 2 HCl (**3a**, 125 mg, 0.5 mmol, 1 eq) was dissolved in dry CH_2Cl_2 (10 mL) and Et_3N (0.7 mL, 5.0 mmol, 10 eq) was added. An excess of 2-chlorobenzoyl chloride dissolved in dry CH_2Cl_2 (1 mL) was added and the mixture was stirred for 1 h at room temperature. Solvent was removed *in vacuo* and the crude product was purified by flash chromatography (ethyl acetate:cyclohexane = 4:6) to provide **4d** as a colorless solid, 81 mg (0.26 mmol, 51%), $C_{18}H_{19}ClN_2O$ (314.8 g/mol). TLC (Silica): R_f = 0.23 (ethyl acetate:cyclohexane = 4:6). mp: 154 °C. Exact mass (APCI): m/z = calcd. for $C_{18}H_{20}ClN_2O$ [M + H^+] 315.1259, found 315.1238. Purity (HPLC, method A): 99%, R_t = 14.6 min ^{1}H NMR (600 MHz, MeOH-d_4): δ = 7.58–7.16 (m, 9H, 2-, 3-, 4-, 5-, 6-CH_{phenyl}, 3-, 4-, 5-, 6-$CH_{Cl\text{-}phenyl}$), 4.75–4.64 (m, 0.5H, 2-CHH$_{piperazine}$), 4.52 (tdd, J = 13.3, 3.3, 2.1 Hz, 0.5H, 2-CHH$_{piperazine}$), 3.48 (ddd, J = 13.4, 12.2, 3.1 Hz, 0.25H, 6-CHH$_{piperazine}$), 3.44–3.36 (m, 1H, 0.75H, 6-CHH$_{piperazine}$), 3.27–3.03 (m, 2.75H, 2-CHH$_{piperazine}$, 3-$CH_{piperazine}$, 5-CHH$_{piperazine}$, 6-CHH$_{piperazine}$), 2.99–2.84 (m, 1H, 1.25, 2-

C*H*H$_{piperazine}$, 3-C*H*$_{piperazine}$, 5-C*H*H$_{piperazine}$), 2.45–2.33 (m, 0.75H, 5-C*H*H$_{piperazine}$), 2.24 (td, *J* = 12.1, 3.4 Hz, 0.25H, 5-C*H*H$_{piperazine}$), 2.13–1.99 (m, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, MeOH-d$_4$): δ = 168.82, 168.75, 169.57, 168.45 (1C, *C*=O), 140.99, 140.96, 140.54, 140.51 (1C, *C*-1$_{phenyl}$), 136.56, 136.47, 136.38, 136.31 (1C, *C*–1$_{Cl\text{-}phenyl}$), 132.07, 132.06, 131.97, 131.96, 131.33, 131.30, 131.22, 131.06, 130.91, 130.78, 130.77, 130.76, 129.91, 129.89, 129.88, 129.35, 129.32, 129.29, 129.28, 129.09, 128.98, 128.96, 128.88, 128.73, 128.70, 128.68, 128.66, 128.64 (10C, *C*-2, -3, -4, -5, -6$_{phenyl}$, *C*-2, -3, -4, -5, -6$_{Cl\text{-}phenyl}$), 70.8, 70.4, 70.0, 69.9 (1C, *C*-3$_{piperazine}$), 56.7, 56.3, 55.94, 55.92 (1C, *C*-5$_{piperazine}$), 55.0, 54.2, 48.4, 47.6 (1C, *C*-6$_{piperazine}$), 49.3, 49.2, 42.84, 42.75 (1C, *C*-2$_{piperazine}$), 48.4, 47.6 (1C, *C*-6$_{piperazine}$), 43.67, 43.63, 43.58, 43.57 (1C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2986, 2936, 2843 (C-H$_{aliphatic}$), 1628 (C=O), 1593.

4.9.17. (2-Chlorophenyl) (4-ethyl-3-phenylpiperazin-1-yl) methanone (4e)

1-Ethyl-2-phenylpiperazine x 2 HCl (**3b**, 132 mg, 0.5 mmol, 1 eq) was dissolved in dry CH_2Cl_2 (10 mL) and Et_3N (0.7 mL, 5.0 mmol, 10 eq) was added. An excess of 2-chlorobenzoyl chloride dissolved in dry CH_2Cl_2 (1 mL) was added and the mixture was stirred for 1 h at room temperature. Solvent was removed *in vacuo* and the crude product was purified by flash chromatography (ethyl acetate:cyclohexane = 2:3) to provide **4e** as a colorless solid, 20 mg (0.06 mmol, 12%), $C_{19}H_{21}ClN_2O$ (328.8 g/mol). TLC (Silica): R_f = 0.23 (ethyl acetate:cyclohexane = 4:6). mp: 121 °C. Exact mass (APCI): *m*/*z* = calcd. for $C_{19}H_{21}ClN_2O$ [M + H^+] 329.1415, found 329.1424. Purity (HPLC, method D): 99%, R_t = 15.3 min ^{1}H NMR (600 MHz, $CHCl_3$-d): δ (ppm) = 8.06–7.58 (m, 4H, 2x 3-C*H*$_{Cl\text{-}phenyl}$, 2x 4-C*H*$_{phenyl}$), 7.56–7.28 (m, 14H, each H provides two signal sets: 2-, 3-, 5-, 6-C*H*$_{phenyl}$, 4-, 5-C*H*$_{Cl\text{-}phenyl}$ and 6-C*H*$_{Cl\text{-}phenyl}$), 5.00 (d, *J* = 14.4 Hz, 1H, 2-C*H*$_{2,piperazine}$), 4.94 (d, *J* = 14.0 Hz, 1H, 2-C*H*$_{2,piperazine}$), 4.60–4.49 (m, 1H, 6-C*H*$_{2,piperazine}$), 4.33–4.22 (m, 1H, 2-C*H*$_{2,piperazine}$), 4.20–4.13 (m, 1H,6-C*H*$_{2,piperazine}$), 4.12–4.06 (m, 1H, 3-C*H*$_{piperazine}$), 3.99–3.92 (m, 1H, 3-C*H*$_{piperazine}$), 3.88 (t, *J* = 12.7 Hz, 1H, 2-C*H*$_{2,piperazine}$), 3.80 (d, *J* = 11.9 Hz, 1H, 5-C H$_{2,piperazine}$), 3.66–3.58 (m, 1H, 5-C*H*$_{2,piperazine}$), 3.58–3.52 (m, 1H, 6-C*H*$_{2,piperazine}$), 3.47 (d, *J* = 14.3 Hz, 1H, 6-C*H*$_{2,piperazine}$), 3.21–3.11 (m, 2H, 1-CH*H*$_{ethyl}$), 3.10–2.96 (m, 2H, 2x 5-C*H*$_{2,piperazine}$), 2.81–2.68 (m, 2H, 1-C*H*H$_{ethyl}$), 1.37–1.27 (m, 6H, 2x C*H*$_{3,ethyl}$). ^{13}C NMR (151 MHz, MeOH-d$_4$): δ = 166.7 and 166.6 (1C, *C*=O), 134.3 (1C, *C*-1$_{phenyl}$), 132.2 (1C, *C*–1$_{Cl\text{-}phenyl}$), 131.23 and 131.20 (1C, *C*–2$_{Cl\text{-}phenyl}$), 130.7 and 130.6 (1C, *C*–6$_{Cl\text{-}phenyl}$), 130.1 (1C, *C*–5$_{Cl\text{-}phenyl}$), 129.9 (1C, *C*-4$_{phenyl}$), 129.8 (1C, *C*–3$_{Cl\text{-}phenyl}$), 128.9 and 128.8 (2C, *C*-3$_{phenyl}$ and *C*-5$_{phenyl}$), 128.4 and 128.3 (2C, *C*-2$_{phenyl}$ and *C*-6$_{phenyl}$), 128.2 and 128.0 (1C, *C*–4$_{Cl\text{-}phenyl}$), 69.1 and 68.5 (1C, *C*-3$_{piperazine}$), 51.2 and 50.9 (1C, *C*-5$_{piperazine}$), 50.6 (1C, *C*-6$_{piperazine}$), 49.0 (1C, *C*-1$_{ethyl}$), 45.1 (1C, *C*-2$_{piperazine}$), 43.8 (1C, *C*-6$_{piperazine}$), 38.5 (1C, *C*-2$_{piperazine}$), 8.14 (1C, *C*H$_{3ethyl}$).FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2936, 2789 (C-H$_{aliphatic}$), 1628 (C=O).

4.9.18. 1-(2-chlorobenzoyl)-4-heptyl-3-phenylpiperazine (4f)

1-Heptyl-2-phenylpiperazine (**3c**, 1.0 eq, 0.36 mmol, 120 mg) was dissolved in dry dichloromethane (5 mL) under N_2 atmosphere. Et_3N (10 eq, 3.6 mmol, 0.5 mL) and three drops of pyridine were added. 2-chlorobenzoic acid chloride (1.2 eq, 0.43 mmol, 55 μL) was added and the mixture was stirred over night at room temperature. The reaction mixture was diluted with ethyl acetate (40 mL), the organic layer was washed with H_2O (3 × 20 mL) and saturated solution of $NaHCO_3$ (2 × 20 mL). The combined aqueous layers were extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 12 cm, v = 10 mL, cyclohexane/ethyl acetate = 3:1 + 1% Et_3N + 4% methanol) to provide **4f** as a yellow oil, 134 mg (0.34 mmol, 93%), $C_{24}H_{31}ClN_2O$ (398.98 g/mol). TLC (Silica): R_f = 0.55 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 5% methanol). Exact mass (APCI): *m*/*z* = calcd. for $C_{24}H_{32}^{35}ClN_2O$ [M + H^+] 399.2198, found 399.2197. Purity (HPLC, method D): 97%, R_t = 21.00 min ^{1}H NMR (400 MHz, MeOH-d$_4$): δ (ppm) = 7.55–7.20 (m, 9H, 3-, 4-, 5-, 6-C*H*$_{benzoyl}$, 2-, 3-, 4-, 5-, 6-C*H*$_{phenyl}$), 4.69 (d, *J* = 13.0 Hz, 0.5H, 2-CH*H*$_{piperazine}$), 4.48 (t, *J* = 10.3 Hz, 0.5H, 2-CH*H*$_{piperazine}$), 3.50–3.32 (m, 1.2H, 2-CH*H*$_{piperazine}$, 3-CH*H*$_{piperazine}$, 5-CH*H*$_{piperazine}$), 3.25 (m, 1H, 3-CH*H*$_{piperazine}$, 6-CH*H*$_{piperazine}$), 3.22–3.00 (m, 2.3H, 3-CH*H*$_{piperazine}$, 5-CH*H*$_{piperazine}$, 6-CH*H*$_{piperazine}$), 2.92 (m, 0.5H, 2-CH*H*$_{piperazine}$), 2.46 (m, 1H, 1-CH*H*$_{heptyl}$), 2.30 (m, 0.8H, 5-CH*H*$_{piperazine}$, 6-CH*H*$_{piperazine}$), 2.15 (m, 0.2H, 5-CH*H*$_{piperazine}$), 1.97 (m, 1H, 1-CH*H*$_{heptyl}$), 1.41 (m, 2H, 2-C*H*$_{2heptyl}$), 1.30–1.00 (m, 8H, 3-C*H*$_{2heptyl}$, 4-C*H*$_{2heptyl}$, 5-C*H*$_{2heptyl}$, 6-C*H*$_{2heptyl}$), 0.85 (td, *J* = 7.1/3.4 Hz, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, MeOH-d$_4$): δ (ppm) = 168.5 and 168.4 (1C, *C*=O), 141.6, 141.5, 141.14 and 141.07 (1C, *C*-1$_{phenyl}$), 136.6, 136.53, 136.45 and 136.4 (1C, *C*-2$_{benzoyl}$), 132.0, 131.9, 131.3, 131.1, 130.9, 130.8, 129.8, 129.3, 129.1, 128.93, 128.86, 128.7 and 128.6 (10C, *C*-1, -3, -4, -5, -6$_{benzoyl}$, *C*-2, -3, -4, -5, -6$_{phenyl}$), 69.2, 68.9 and 68.4 (1C, *C*-3$_{piperazine}$), 55.45, 54.7, 52.3 and 52.2 (1C, *C*-6$_{piperazine}$), 55.35, 55.30, 55.28 and 55.26, (1C, *C*-1$_{heptyl}$), 53.1, 52.7, 48.7 and 47.9 (1C, *C*-5$_{piperazine}$), 49.8, 49.7, 43.1 and 43.0 (1C, *C*-2$_{piperazine}$), 32.9, 30.1, 28.2 and 23.6 (4C, *C*-3,-4,-5,-6$_{heptyl}$), 26.9 (1C, *C*-2$_{heptyl}$), 14.4 (1C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2924, 2854, 2800 (C–H), 1643 (C=O), 1593 (C-C$_{arom}$).

4.9.19. 1-(2-Chlorobenzoyl)-4-benzyl-3-phenylpiperazine (4g)

2-Chlorobenzoic acid (1.1 eq, 0.26 mmol, 41 mg) was dissolved in dry acetonitrile (2 mL) under N_2 atmosphere. COMU® (1.1 eq, 0.26 mmol, 112 mg) and *N*,*N*-diisopropylethylamine (2.0 eq, 0.48 mmol, 81 μL) were added and the mixture was stirred for 15 min at room temperature. Then 1-benzyl-2-phenylpiperazine **3d** (1.0 eq, 0.24 mmol, 60 mg) was dissolved in dry acetonitrile (1 mL) and the solution was added to the mixture. After stirring for 1 d, the reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and brine (5 mL) and was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 3 cm, l = 14.5 cm, V = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et_3N) to provide **4g** as a yellow oil, 59 mg (0.21 mmol, 58%), $C_{24}H_{23}ClN_2O$ (390.91 g/mol).TLC (Silica): R_f = 0.68 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 8% methanol). Exact mass (APCI): *m*/*z* = calcd. for $C_{24}H_{24}ClN_2O$ [M + H^+] 391.1572, found 391.1554. Purity (HPLC, method D): 99%, R_t = 18.22 min.^{1}H NMR (600 MHz, MeOH-d$_4$): δ (ppm) = 7.59 (d, *J* = 8.1, 1H) and 7.53–7.15 (m, 13H, 2-, 3-, 4-, 5-, 6-C*H*$_{benzyl}$, 3-, 4-, 5-, 6-C*H*$_{benzoyl}$, 2-,3-,4-,5-,6-C*H*$_{phenyl}$), 4.63–4.53 (m, 1H, 2-C$_{piperazine}$, 6-C$_{piperazine}$), 3.76 (d, *J* = 13.4 Hz, 0.5H, PhCH*H*N), 3.73 (dd, *J* = 13.4/5.9 Hz, 0.5H, PhCH*H*N), 3.46 (dd, *J* = 9.4/4.6 Hz, 0.3H, 3-CH$_{piperazine}$), 3.41–3.32 (m, 0.8H, 3-CH$_{piperazine}$, 6-CH$_{piperazine}$), 3.30–3.22 (m, 1.2H, 2-CH$_{piperazine}$, 3-CH$_{piperazine}$, 6-CH$_{piperazine}$), 3.22–3.15 (m, 0.7H, 2-CH$_{piperazine}$), 3.06 (dtd, *J* = 12.9/11.9/3.3 Hz, 0.5H, 6-CH$_{piperazine}$), 3.02–2.95 (m, 1H, 2-CH$_{piperazine}$, 5-CH$_{piperazine}$), 2.93–2.86 (m, 1H, PhC*H*HN), 2.79 (ddt, *J* = 12.6/9.9/2.8 Hz, 0.5H, 5-CH$_{piperazine}$), 2.31–2.17 (m, 0.7H, 5-CH$_{piperazine}$), 2.08 (td, *J* = 12.0/3.5 Hz, 0.3H, 5-CH$_{piperazine}$). ^{13}C NMR (151 MHz, MeOH-d$_4$): δ (ppm) = 168.74, 168.65, 168.5 and 168.4 (1C, *C*=O), 141.73, 141.67, 141.3 and 141.2 (1C, *C*-1$_{phenyl}$), 139.47, 139.44 and 139.40 (1C, *C*-1$_{benzyl}$), 136.6, 136.5, 136.39 and 136.36 (1C, *C*-2$_{benzoyl}$), 132.04, 132.01, 131.95, 131.9, 131.33, 131.28, 131.2, 131.10, 130.9, 130.77, 130.76, 130.02, 130.00, 129.94, 129.92, 129.88, 129.87, 129.35, 129.33, 129.31, 129.29, 129.28, 129.26, 129.12, 129.11, 128.9, 128.8, 128.68, 128.66, 128.6, 128.2 and 128.1 (15C, *C*-2,-3,-4,-5,-6$_{benzyl}$, 69.1, 68.8 and 68.2 (1C, *C*-3$_{piperazine}$), 59.64, 59.60, 59.56 and 59.5

(1C, PhCH$_2$N), 55.7, 54.9, 50.0 and 49.9 (1C, *C*-2$_{piperazine}$), 53.0, 52.6, 52.2 and 52.1 (1C, *C*-5$_{piperazine}$), 48.6, 47.8, 43.1 and 43.0 (1C, *C*-6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2901, 2801, (C—H), 1639 (C=O), 1593 (C-C$_{arom}$).

4.9.20. 1-(2-Chlorobenzoyl)-4-(3-bromobenzyl)-3-phenylpiperazine (4h)

2-Chlorobenzoic acid (1.1 eq, 0.23 mmol, 37 mg) was dissolved in dry acetonitrile (2 mL) under N_2 atmosphere. COMU® (1.2 eq, 0.25 mmol, 109 mg) and *N*,*N*-diisopropylethylamine (2.2 eq, 0.42 mmol, 72 μL) were added and the mixture was stirred for 15 min at room temperature. Then 1-(3-bromobenzyl)-2-phenylpiperazine (**3e**, 1.0 eq, 0.21 mmol, 70 mg) was dissolved in dry acetonitrile (1 mL) and the solution was added to the mixture. After stirring for 1 d, the reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 3 cm, l = 10 cm, V = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et$_3$N) to provide **4h** as a colorless solid, 89 mg (0.23 mmol, 99%), $C_{24}H_{22}BrClN_2O$ (469.81 g/mol). TLC (Silica): R_f = 0.68 (cyclohexane:ethyl acetate = 3:1 + 1% Et$_3$N + 8% methanol). Exact mass (APCI): *m*/*z* = calcd. for $C_{24}H_{23}^{79}BrClN_2O$ [M + H$^+$] 469.0677, found 469.0654. Purity (HPLC, method D): 99%, R_t = 20.44 min ^{1}H NMR (600 MHz, MeOH-d$_4$): δ (ppm) = 7.60—7.01 (m, 13H, 2-, 4-, 5-, 6-C*H*$_{benzyl}$, 3-, 4-, 5-, 6-C*H*$_{benzoyl}$, 2-, 3-, 4-, 5-, 6-C*H*$_{phenyl}$), 4.63—4.50 (m, 1H, 2-C*H*$_{piperazine}$, 6-C*H*$_{piperazine}$), 3.66 (t, *J* = 14.2 Hz, 1H, PhC*H*HN), 3.44 (dd, *J* = 8.7/5.3 Hz, 0.3H, 3-C*H*$_{piperazine}$), 3.34 (td, *J* = 11.4/3.2 Hz, 0.5H, 3-C*H*$_{piperazine}$), 3.29—3.13 (m, 2.2H, 2-C*H*$_{piperazine}$, 3-C*H*$_{piperazine}$, 6-C*H*$_{piperazine}$), 3.03 (dd, *J* = 12.6/3.2 Hz, 0.5H, 6-C*H*$_{piperazine}$), 2.99—2.77 (m, 2H, PhC*H*HN, 2-C*H*$_{piperazine}$, 5-C*H*$_{piperazine}$), 2.65 (ddd, *J* = 14.6/11.7/3.0 Hz, 0.5H, 5-C*H*$_{piperazine}$), 2.28—2.14 (m, 0.7H, 5-C*H*$_{piperazine}$), 2.07—1.99 (m, 0.3H, 5-C*H*$_{piperazine}$).^{13}C NMR (151 MHz, MeOH-d$_4$): δ (ppm) = 168.6, 168.5, 168.4 and 168.2 (1C, *C*=O) 142.40, 142.36 and 142.3 (1C, *C*-1$_{benzyl}$) 141.4, 141.3, 141.0 and 140.9 (1C, *C*-1$_{phenyl}$), 136.5, 136.4, 136.32, 136.27, 132.52, 132.48, 132.45, 131.99, 131.98, 131.90, 131.87, 131.3, 131.24, 131.23, 131.20, 131.18, 131.15, 131.13, 131.11, 131.0, 130.9, 130.74, 130.72, 130.0, 129.41, 129.38, 129.35, 129.34, 129.2, 129.1, 129.0, 128.9, 128.8, 128.8, 128.7, 128.62, 128.61, 128.59, 128.57, 128.50, 123.4 and 123.3 (16C, *C*-2, -3, -4, -5, -6$_{benzyl}$, *C*-1, -2, -3, -4, -5, -6$_{benzoyl}$, *C*-2, -3, -4, -5, -6$_{phenyl}$), 68.9, 68.6, 68.1 (1C, *C*-3$_{piperazine}$), 58.92, 58.89, 58.87 and 58.8 (1C, PhCH$_2$N), 55.5, 54.8, 49.9 and 49.8 (1C, *C*-2$_{piperazine}$), 53.0, 52.7, 52.3 and 52.2 (1C, *C*-5$_{piperazine}$), 48.5, 47.7, 43.0 and 42.9 (1C, *C*-6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2901, 2801 (C—H), 1643 (C=O), 1593, 1566 (C-C$_{arom}$).

4.9.21. 1-(2-Chlorobenzoyl)-4-(4-fluorobenzyl)-3-phenylpiperazine (4i)

2-Chlorobenzoic acid (1.1 eq, 0.14 mmol, 22 mg) was dissolved in dry acetonitrile (2 mL) under N_2 atmosphere. COMU® (1.1 eq, 0.14 mmol, 60 mg) and *N*,*N*-diisopropylethylamine (2.0 eq, 0.28 mmol, 48 μL) were added and the mixture was stirred for 15 min at room temperature. Then 1-(4-fluorobenzyl)-2-phenylpiperazine (**3f**, 1.0 eq, 0.13 mmol, 35 mg) was dissolved in dry acetonitrile (1 mL) and the solution was added to the mixture. After stirring for 1 d, the reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and brine (5 mL), then was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 3.5 cm, l = 15 cm, V = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et$_3$N). After removing the solvent *in vacuo*, the residue was dissolved in acetonitrile and H_2O (1:3, 5 mL) and the mixture was lyophilisated for 2 d, compound **4i** was obtained as a colorless solid, 89 mg (0.23 mmol, 99%), $C_{24}H_{22}ClFN_2O$ (408.90 g/mol). TLC (Silica): R_f = 0.60 (cyclohexane:ethyl acetate = 3:1 + 1% Et$_3$N + 8% methanol). mp: 67 °C. Exact mass (APCI): *m*/*z* = calcd. for $C_{24}H_{23}ClFN_2O$ [M + H$^+$] 409.1478, found 409.1459. Purity (HPLC, method D): 99%, R_t = 18.38 min ^{1}H NMR (600 MHz, MeOH-d$_4$): δ (ppm) = 7.63—7.20 (m, 11H, 2-,6-C*H*$_{F\text{-}benzyl}$, 3-, 4-, 5-, 6-C*H*$_{benzoyl}$, 2-, 3-, 4-, 5-, 6-C*H*$_{phenyl}$), 7.06—6.93 (m, 2H, 3-C*H*$_{F\text{-}benzyl}$, 5-C*H*$_{F\text{-}benzyl}$), 4.66—4.51 (m, 1H, 2-C*H*H$_{piperazine}$, 6-C*H*H$_{piperazine}$), 3.70 (t, *J* = 12.6 Hz, 1H, PhC*H*HN), 3.46 (dd, *J* = 8.8/5.2 Hz, 0.3H, 3-C*H*$_{piperazine}$), 3.39 (m, 0.8H, 6-C*H*H$_{piperazine}$), 3.34—3.24 (m, 1.2H, 2-C*H*H$_{piperazine}$, 3-C*H*$_{piperazine}$, 6-C*H*H$_{piperazine}$), 3.23—3.13 (m, 0.7H, 2-C*H*H$_{piperazine}$), 3.13—3.03 (m, 0.5H, 6-C*H*H$_{piperazine}$), 3.03—2.94 (m, 1H, 2-C*H*H$_{piperazine}$, 5-C*H*H$_{piperazine}$), 2.94—2.86 (m, 1H, PhC*H*HN), 2.73 (m, 0.5H, 5-C*H*H$_{piperazine}$), 2.33—2.16 (m, 0.5H, 5-C*H*H$_{piperazine}$), 2.06 (td, *J* = 11.8/3.7 Hz, 0.5H, 5-C*H*H$_{piperazine}$). ^{13}C NMR (151 MHz, MeOH-d$_4$): δ (ppm) = 168.8, 168.7, 168.5 and 168.4 (1C, *C*=O), 164.7, 164.6, 162.3 and 162.2 (d, *J* = 243.8 Hz, *C*—4$_{F\text{-}benzyl}$), 141.7, 141.6, 141.2 and 141.1 (1C, *C*-1$_{phenyl}$), 136.6, 136.5, 136.39 and 136.35 (1C, *C*-2$_{benzoyl}$), 135.47, 135.45, 135.42 and 135.39 (1C, *C*-1$_{benzoyl}$), 132.1, 132.02, 131.96, 131.9, 131.62, 131.60, 131.56, 131.55, 131.52, 131.49, 131.47, 131.34, 131.29, 131.2, 131.1, 130.9, 130.8, 130.04, 130.02, 129.4, 129.4, 129.34, 129.31, 129.27, 129.12, 129.09, 128.9, 128.84, 128.81, 128.68, 128.66 and 128.6 (12C, *C*-2, -6$_{F\text{-}benzyl}$, *C*-1, -3, -4, -5, -6$_{benzoyl}$, *C*-2, -3, -4, -5, -6$_{phenyl}$), 116.05, 116.03, 116.01, 116.00, 115.83, 115.81, 115.80 and 115.78 (d, *J* = 21.4, 1C, *C*—3$_{F\text{-}benzyl}$, *C*—5$_{F\text{-}benzyl}$), 69.00, 68.7 and 68.2 (1C, *C*-3$_{piperazine}$), 58.8, 58.73, 58.68 and 58.66 (1C, PhCH$_2$N), 55.6, 54.9, 50.0 and 49.9 (1C, *C*-2$_{piperazine}$), 52.9, 52.6, 52.2 and 52.1 (1C, *C*-5$_{piperazine}$), 48.6, 47.8, 43.1 and 43.0 (1C, *C*-6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2901, 2808, (C—H), 1643 (C=O), 1601 (C-C$_{arom}$).

4.9.22. 1-(2-Chlorobenzoyl)-4-heptyl-3-(thiophen-2-yl)-piperazine (4j)

2-Chlorobenzoic acid (1.1 eq, 0.07 mmol, 12 mg) was dissolved in dry acetonitrile (2 mL) under N_2 atmosphere. COMU® (1.1 eq, 0.07 mmol, 32 mg) and *N*,*N*-diisopropylethylamine (2.0 eq, 0.14 mmol, 23 μL) were added and the mixture was stirred for 15 min at room temperature. Then 1-heptyl-2-(thiophen-2-yl)-piperazine (**3h**, 1.0 eq, 0.06 mmol, 18 mg) was dissolved in dry acetonitrile (1 mL) and the solution was added to the mixture. After stirring for 1 d, the reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and brine (5 mL), then was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 3 cm, l = 8 cm, V = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et$_3$N) to provide **4j** as a yellow oil, 20 mg (0.05 mmol, 71%), $C_{22}H_{29}ClN_2OS$ (405.00 g/mol). TLC (Silica): R_f = 0.63 (cyclohexane/ethyl acetate = 3:1 + 1% Et$_3$N + 8% methanol). Exact mass (APCI): *m*/*z* = calcd. for $C_{22}H_{29}ClN_2OS$ [M + H$^+$] 405.1762, found 405.1721. Purity (HPLC, method D): 95%, R_t = 20.26 min ^{1}H NMR (400 MHz, MeOH-d$_4$): δ (ppm) = 7.55—7.27 (m, 4.5H, 3-, 4-, 5-, 6-C*H*$_{benzoyl}$), 7.22 (dd, *J* = 7.5/1.7 Hz, 0.3H, C*H*$_{benzoyl}$), 7.12 (td, *J* = 3.6/1.2 Hz, 0.5H, 3-C*H*$_{thiophenyl}$), 7.02 (ddd, *J* = 5.6/3.5/2.3 Hz, 0.5H, 5-C*H*$_{thiophenyl}$), 6.96—6.85 (m, 1H, 3-C*H*$_{thiophenyl}$, 5-C*H*$_{thiophenyl}$), 4.65 (dd, *J* = 13.0/2.7 Hz, 0.2H, 6-CH*H*$_{piperazine}$), 4.52 (ddd, *J* = 13.1/3.5/1.7 Hz, 0.3H, 2-CH*H*$_{piperazine}$), 4.43 (dddd, *J* = 13.1/11.0/3.6/1.7 Hz, 0.2H, 2-CH*H*$_{piperazine}$), 3.78 (dt, *J* = 9.5/3.9 Hz, 0.5H, 3-C*H*$_{piperazine}$), 3.68 (dd, *J* = 10.3/3.4 Hz, 0.3H, 3-C*H*$_{piperazine}$), 3.51 (dd, *J* = 10.2/3.7 Hz, 0.2H, 3-C*H*$_{piperazine}$), 3.44—3.04 (m, 3.5H, 2-C*H*$_{2piperazine}$, 5-CH*H*$_{piperazine}$, 6-CH*H*$_{piperazine}$), 3.00 (ddd, *J* = 12.4/6.9/3.3 Hz, 0.5H, 5-CH*H*$_{piperazine}$), 2.65—2.46 (m, 1H, 1-CH*H*$_{heptyl}$), 2.43—2.33 (m, 0.5H, 5-CH*H*$_{piperazine}$), 2.28 (td,

J = 12.0/3.3 Hz, 0.2H, 5-CH$H_{piperazine}$), 2.23–2.13 (m, 0.3H, 5-CH$H_{piperazine}$), 2.12–1.99 (m, 1H, 1-CHH_{heptyl}), 1.54–1.33 (m, 2H, 2-C$H_{2heptyl}$), 1.32–1.08 (m, 8H, 3-, 4-, 5-, 6-C$H_{2heptyl}$), 0.92–0.81 (m, 3H, CH_3). ^{13}C NMR (101 MHz, MeOH-d_4): δ (ppm) = 168.8, 168.61 and 168.56 (1C, *C*=O), 144.5, 144.0, 143.8 and 143.4 (1C, *C*-2$_{thiophenyl}$), 136.5, 136.4, 136.2 (1C, *C*-2$_{benzoyl}$), 132.1, 132.04, 131.95, 131.4, 131.33, 131.27, 131.1, 130.9, 130.8, 130.7, 129.3, 129.2, 129.1, 128.9, 128.7, 128.64 and 128.61 (5C, *C*-1,-3,-4,-5,-6$_{benzoyl}$), 127.54, 127.53, 127.48, 127.43, 127.35, 127.3, 127.2, 126.47, 126.45, 126.4 (3C, *C*-3,-4,-5$_{thiophenyl}$), 63.5, 63.1, 62.9 and 62.4 (1C, *C*-3$_{piperazine}$), 55.6, 50.2 and 50.0 (1C, *C*-2$_{piperazine}$), 55.4, 55.33, 55.29 and 55.25 (1C, *C*-1$_{heptyl}$), 52.3, 52.2, 52.1 and 51.4, (1C, *C*-5$_{piperazine}$), 48.3, 47.7, 42.91 and 42.87 (1C, *C*-6$_{piperazine}$), 32.9, 30.2, 30.1, 28.21, 28.19, 27.20, 27.17, 27.1, 27.0 and 23.7 (4C, *C*-3$_{heptyl}$, *C*-4$_{heptyl}$, *C*-5$_{heptyl}$, *C*-6$_{heptyl}$), 14.4 (1C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2954, 2924, 2854 (C–H), 1643 (C=O), 1593 (C-C$_{arom}$).

4.9.23. 1-(2-Chloro-3-trifluoromethyl-benzoyl)-4-heptyl-3-phenylpiperazine (4k)

2-Chloro-3-trifluoromethylbenzoic acid (1.1 eq, 0.25 mmol, 51 mg) was dissolved in dry acetonitrile (2 mL) under N_2 atmosphere. COMU® (1.2 eq, 0.28 mmol, 120 mg) and *N*,*N*-diisopropylethylamine (2.2 eq, 0.51 mmol, 86 μL) were added and the mixture was stirred for 15 min at room temperature. Then 1-heptyl-2-phenylpiperazine (**3c**, 1.0 eq, 0.23 mmol, 60 mg) was dissolved in dry acetonitrile (1 mL) and the solution was added to the mixture. After stirring for 3 d at room temperature, the reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 12 cm, v = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et_3N) to provide **4k** as a yellow oil, 90 mg (0.19 mmol, 84%), $C_{25}H_{30}ClF_3N_2O$ (466.97 g/mol). TLC (Silica): R_f = 0.72 (cyclohexane:ethyl acetate = 2:1 + 1% Et_3N + 5% methanol). Exact mass (APCI): *m*/*z* = calcd. for $C_{25}H_{31}^{35}ClF_3N_2O$ [M + H$^+$] 467.2072, found 467.2071. Purity (HPLC, method D): 98%, R_t = 22.42 min ^{1}H NMR (600 MHz, MeOH-d_4): δ (ppm) = 7.90 (m, 0.5H, 4-C$H_{benzoyl}$), 7.83 (m, 0.5H, 4-C$H_{benzoyl}$), 7.73 (ddd, *J* = 21.0/7.7/1.6 Hz, 0.5H, 6-C$H_{benzoyl}$), 7.61 (m, 1H, 5-C$H_{benzoyl}$, 6-C$H_{benzoyl}$), 7.51 (m, 0.5H, 5-C$H_{benzoyl}$), 7.45 (ddd, *J* = 8.1/2.7/1.3 Hz, 1H, 2-, 6-CH_{phenyl}), 7.39 (m, 1H, 3-, 5-CH_{phenyl}), 7.36–7.21 (m, 3H, 2-, 6-CH_{phenyl}, 3-, 5-CH_{phenyl}, 4-CH_{phenyl}), 4.70 (m, 0.5H, 6-CHH$_{piperazine}$), 4.50 (m, 0.5H, 2-CH$H_{piperazine}$), 3.50–3.32 (m, 1.3 H, 3-C$H_{piperazine}$, 6-CHH$_{piperazine}$), 3.28 (m, 1H, 3-C$H_{piperazine}$, 5-CHH$_{piperazine}$), 3.26–3.12 (m, 1.3H, 3-C$H_{piperazine}$, 6-CHH$_{piperazine}$), 3.12–3.00 (m, 1H, 2-CH$H_{piperazine}$, 5-CHH$_{piperazine}$), 2.95 (ddd, *J* = 13.2/10.9/8.1 Hz, 0.5H, 2-CH$H_{piperazine}$), 2.46 (m, 1H, 1-CHH$_{heptyl}$), 2.31 (m, 0.8H, 5-CHH$_{piperazine}$), 2.18 (td, *J* = 11.9/3.3 Hz, 0.2H, 5-CHH$_{piperazine}$), 2.01–1.93 (m, 1H, 1-CHH$_{heptyl}$), 1.47–1.35 (m, 2H, 2-C$H_{2heptyl}$), 1.30–1.02 (m, 8H, 3-C$H_{2heptyl}$, 4-C$H_{2heptyl}$, 5-C$H_{2heptyl}$, 6-C$H_{2heptyl}$), 0.85 (td, *J* = 7.1/5.6 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, MeOH-d_4): δ (ppm) = 167.4, 167.3, 167.1 and 166.9 (1C, *C*=O), 141.5, 141.5, 141.1 and 140.9 (1C, *C*-1$_{phenyl}$), 139.5, 139.5 and 139.2 (1C, *C*-1$_{benzoyl}$), 133.0 and 132.8 (1C, *C*-6$_{benzoyl}$), 132.7 and 132.6 (1C, *C*-5$_{benzoyl}$), 130.1 (d, *J* = 31.2 Hz, 1C, *C*-2$_{benzoyl}$), 129.8 (1C, *C*-3$_{benzoyl}$), 129.6 (m, 1C, *C*-4$_{benzoyl}$), 129.3, 129.3, 129.20, 129.17 and 129.1 (m, 4C, *C*-2, -3, -5, -6$_{phenyl}$), 128.9 and 128.8 (1C, *C*-4$_{phenyl}$), 124.1 (d, *J* = 272.4 Hz, 1C, *C*F$_3$), 69.2, 68.8, 68.3 and 68.3 (1C, *C*-3$_{piperazine}$), 55.5, 54.6, 49.8 and 49.7 (1C, *C*-2$_{piperazine}$), 55.31, 55.25 and 55.2 (1C, *C*-1$_{heptyl}$), 53.1, 52.6, 52.2 and 52.1 (1C, *C*-5$_{piperazine}$), 48.7, 47.8, 43.2 and 43.1 (1C, *C*-6$_{piperazine}$), 32.9, 30.1, 28.1 and 26.9 (4C, *C*-3, -4, -5, -6$_{heptyl}$), 23.6 (1C, *C*-2$_{heptyl}$), 14.4 (1C, *C*H$_{3heptyl}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2955, 2924, 2855 (C–H), 1643 (C=O), 1593 (C-C$_{arom}$).

4.9.24. 1-(2-,3-Dichlorobenzoyl)-4-heptyl-3-phenylpiperazine (4l)

2-,3-Dichlorobenzoic acid (1.1 eq, 0.25 mmol, 48 mg) was dissolved in dry acetonitrile (2 mL) under N_2 atmosphere. COMU® (1.2 eq, 0.28 mmol, 120 mg) and *N*,*N*-diisopropylethylamine (2.2 eq, 0.51 mmol, 86 μL) were added and the mixture was stirred for 15 min at room temperature. Then 1-heptyl-2-phenylpiperazine (**3c**, 1.0 eq, 0.23 mmol, 60 mg) was dissolved in dry acetonitrile (1 mL) and the solution was added to the mixture. After stirring for 1 d, the reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 2 cm, l = 12 cm, V = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et_3N) to provide **4l** as a yellow oil, 98 mg (0.23 mmol, 99%), $C_{24}H_{30}Cl_2N_2O$ (433.42 g/mol). TLC (Silica): R_f = 0.69 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N + 10% methanol). Exact mass (APCI): *m*/*z* = calcd. for $C_{24}H_{31}^{35}Cl_2N_2O$ [M + H$^+$] 433.1808, found 433.1845. Purity (HPLC, method D): 98%, R_t = 21.67 min ^{1}H NMR (400 MHz, MeOH-d_4): δ (ppm) = 7.64 (ddd, *J* = 8.1/2.7/1.6 Hz, 0.5H, 6-C$H_{benzoyl}$), 7.61–7.54 (m, 0.5H, 6-C$H_{benzoyl}$), 7.48–7.15 (m, 7H, 4-C$H_{benzoyl}$, 5-C$H_{benzoyl}$, 2-CH_{phenyl}, 3-CH_{phenyl}, 4-CH_{phenyl}, 5-CH_{phenyl}, 6-CH_{phenyl}), 4.67 (m, 0.5H, 6-CHH$_{piperazine}$), 4.48 (dddd, 13.0/11.2/3.7/1.6 Hz, 0.5H, 2-CHH$_{piperazine}$), 3.51–3.33 (m, 1.2H, 3-C$H_{piperazine}$, 6-CHH$_{piperazine}$), 3.30–3.23 (m, 1H, 3-C$H_{piperazine}$, 5-CHH$_{piperazine}$), 3.23–3.02 (m, 2.2H, 2-C$H_{piperazine}$, 3-CHH$_{piperazine}$, 5-CHH$_{piperazine}$, 6-CHH$_{piperazine}$), 2.92 (ddd, *J* = 13.1/10.9/5.4 Hz, 2-CHH$_{piperazine}$), 2.53–2.38 (m, 1H, 1-CHH$_{heptyl}$), 2.36–2.24 (m, 0.7H, 5-CHH$_{piperazine}$), 2.16 (td, *J* = 11.8/3.6 Hz, 0.3H, 5-CHH$_{piperazine}$), 2.01–1.89 (m, 1H, 1-CHH_{heptyl}), 1.49–1.32 (m, 2H, 2-C$H_{2heptyl}$), 1.31–1.04 (m, 8H, 3-CHH_{heptyl}, 4-CHH_{heptyl}, 5-CHH_{heptyl}, 6-CHH_{heptyl}), 0.85 (td, *J* = 7.0/3.3 Hz, CH_3). ^{13}C NMR (101 MHz, MeOH-d_4): δ (ppm) = 167.74, 167.65, 167.5 and 167.4 (1C, *C*=O), 141.52, 141.48, 141.1 and 141.0 (1C, *C*-1$_{phenyl}$), 138.8, 138.68, 138.66 and 138.6 (1C, *C*-1$_{benzoyl}$), 134.7, 134.58, 134.56 and 134.54 (1C, *C*-3$_{benzoyl}$), 132.48, 132.46 and 132.4 (1C, *C*-2$_{benzoyl}$), 129.9, 129.2, 129.14, 129.12, 129.24, 129.20, 129.14, 129.12, 128.86, 127.60, 127.41, 127.26 and 127.20 (8C, *C*-4$_{benzoyl}$, *C*-5$_{benzoyl}$, *C*-6$_{benzoyl}$, *C*-2$_{phenyl}$, *C*-3$_{phenyl}$, *C*-4 $_{phenyl}$, *C*-5$_{phenyl}$, *C*-6$_{phenyl}$), 69.2, 68.8, 68.32 and 68.30 (1C, *C*-3$_{piperazine}$), 55.4, 54.6, 49.8 and 49.7 (1C, *C*-2$_{piperazine}$), 55.31, 55.27, 55.24 and 55.23 (1C, *C*-1$_{heptyl}$), 53.1, 52.6, 52.2 and 52.1 (1C, *C*-5$_{piperazine}$), 48.7, 47.8, 43.2 and 43.1 (1C, *C*-6$_{piperazine}$), 32.9, 30.1, 28.2 and 23.6 (4C, *C*-3$_{heptyl}$, *C*-4$_{heptyl}$, *C*-5$_{heptyl}$, *C*-6$_{heptyl}$), 27.0 (1C, *C*-2$_{heptyl}$), 14.4 (1C, CH_3). FTIR (neat): (cm^{-1}) = 2951, 2924, 2855 (C–H), 1643 (C=O), 1585 (C-C$_{arom}$).

4.9.25. 1-(2,3-Dichlorophenyl)-1-[3-phenyl-4-(3,3,3-trifluoropropyl)piperazine-1-yl]methanone (4 m)

2,3-Dichlorobenzoic acid (31 mg, 18 mmol, 1.1 eq) was dissolved in acetonitrile (2 mL), then COMU (77 mg, 0.18 mmol, 1.1 eq) and DIPEA (55 μL, 0.32 mmol, 2 eq) were added. The mixture was stirred for 3 h at room temperature, then 2-phenyl-1-(3,3,3-trifluoropropyl)piperazine (3g, 35 mg, 0.16 mmol, 1 eq) was added. After stirring over night at room temperature, an aqueous solution of $NaHCO_3$ (saturated, 10 mL) was added and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over $NaSO_4$, filtered and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N, Ø = 2 cm, h = 8 cm, V = 7 mL) to provide **4m** as a red oil, 60 mg (0.15 mmol, 96%), $C_{20}H_{19}Cl_2F_3N_2O$ (431.28 g/mol). TLC (Silica): R_f = 0.83 (cyclohexane:ethyl acetate = 1:1 + 5% MeOH). Exact mass (APCI): *m*/*z* = calcd. for $C_{20}H_{20}Cl_2F_3N_2O$ [M + H$^+$] 431.0899, found 431.0928. Purity (HPLC, method D): 99%, R_t = 20.6 min ^{1}H NMR (400 MHz, MeOH-d_4): δ = 7.67–7.16 (m, 8H, CH_{phenyl} and CH_{Cl}-

$_{phenyl}$), 4.84 (H_2O_{water}), 4.75–4.64 (m, 0.5H, 6-CH$H_{piperazine}$), 4.55–4.44 (m, 0.5H, 2-CH$H_{piperazine}$), 3.49–3.36 (m, 1.75H, 3-C$H_{piperazine}$, 6-CH$H_{piperazine}$), 3.35 ($CH_3OH_{methanol}$), 3.28–2.99 (m, 2.75H, 2-, 3-, 5-, 6-CH$H_{piperazine}$), 2.94 (ddd, J = 13.1, 10.8, 5.5 Hz, 0.5H, 2-CH$H_{piperazine}$), 2.82–2.69 (m, 1H, 1-CHH_{propyl}), 2.46–2.14 (m, 4H, 2-$CH_{2,propyl}$, 5-CH$H_{piperazine}$). ^{13}C NMR (101 MHz, MeOH-d_4): δ = 167.8, 167.5 and 167.4 (1C, C=O), 140.9, 140.8, 140.4, 140.3 (1C, C-1_{phenyl}), 138.7, 138.6, 138.5, 134.60, 134.57, 134.55, 132.52, 132.50, 132.45, 132.44, 129.99, 129.98, 129.9, 129.8, 129.53, 129.49, 129.44, 129.1, 128.8, 127.6, 127.4, 127.3, 127.2 (11C, C-1, -2, -3, -4, -5, -6$_{dichlorophenyl}$, C-2, -3, -4, -5, -6$_{phenyl}$), 68.7, 68.2, 67.8, 67.7 (1C, C-$3_{piperazine}$), 55.4, 54.6, 49.7 (1C, C-$2_{piperazine}$), 52.9, 52.4, 52.1, 52.0 (1C, C-$5_{piperazine}$), 49.8 ($CH_3OH_{methanol}$), 47.88, 47.85, 47.81 (1C, C-1_{propyl}), 47.7, 43.1, 43.0 (1C, C-$6_{piperazine}$), 32.0, 31.9, 31.8, 31.7, 31.6 (1C, C-2_{propyl}). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 1736 (C=O), 2959, 2924, 2855, 2816 (C–H), 1431, 1411 (C-C_{arom}).

4.9.26. N^1,N^2-bis(2-chlorophenyl)-2-phenylpiperazine-1,4-dicarboxamide (4n)

tert-Butyl 3-phenylpiperazine-1-carboxylate (**2a**, 300 mg, 1.14 mmol, 1 eq) was dissolved under N_2 atmosphere in dry toluene (3 mL) and 2-chlorophenyl isocyanate (138 μL, 1.14 mmol, 1 eq) was added. The mixture was stirred for 16 h at room temperature. Then, Et_2O x HCl (2 mL) was added and the mixture was stirred overnight. Due to incomplete conversion, more Et_2O x HCl (2 mL) was added and the mixture was stirred for 3 d at room temperature. Then, an aqueous, saturated solution of $NaHCO_3$ (20 mL) was added and the mixture was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by automated flash chromatography (25 g silica column, ethyl acetate: cyclohexane = 1:1 + 1% NEt_3, gradient: 1 cV 0% MeOH, 10 cV 0%–12% MeOH, 5 cV 12% MeOH) to provide 4n as a colorless solid, 76 mg (0.16 mmol, 14%), $C_{24}H_{22}Cl_2N_4O_2$ (469.37 g/mol). mp: 145 °C. TLC (Silica): R_f = 0.79 (ethyl acetate:cyclohexane = 1:1 + 5% MeOH + 1% Et_3N). Exact mass (APCI): m/z = calcd. for $C_{24}H_{23}Cl_2N_4O_2$ [M + H$^+$] 469.1193, found 469.1182. Purity (HPLC, method D): 99%, R_t = 21.4 min ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.14 (s, 1H, NH_B), 8.10 (s, 1H, NH_A), 7.60 (dd, J = 8.1, 1.6 Hz, 1H, 6-CH_A), 7.48–7.40 (m, 5H, 3-C$H_{Cl\text{-}Ph}$, 3-, 6-CH, 2-, 6-CH_{phenyl}), 7.38 (t, J = 7.7 Hz, 2H, 3-, 5-CH_{phenyl}), 7.32–7.24 (m, 3H, 5-C$H_{Cl\text{-}Ph}$, 6-C$H_{Cl\text{-}Ph}$, 4-CH_{phenyl}), 7.13 (qd, J = 7.8, 1.6 Hz, 2H, 4-C$H_{Cl\text{-}Ph}$, 4-C$H_{Cl\text{-}Ph}$), 5.41 (t, J = 4.3 Hz, 1H, 2-C$H_{piperazine}$), 4.38 (dd, J = 13.8, 4.0 Hz, 1H, 3-CHH$_{piperazine}$), 4.15–4.06 (m, 1H, 6-CHH$_{piperazine}$), 3.91–3.85 (m, 1H, 5-CHH$_{piperazine}$), 3.65 (dd, J = 13.8, 4.4 Hz, 1H, 3-C$H_{piperazine}$), 3.41–3.33 (m, 2H, 5-, 6-CHH$_{piperazine}$). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 154.9 (1C, C=O), 154.8 (1C, C=O), 139.4 (1C, C-1_{phenyl}), 136.40 and 136.39 (2C, C–$1_{Cl\text{-}Ph}$, C–$1_{Cl\text{-}Ph}$), 129.2 (2C, C–$3_{Cl\text{-}Ph}$, C–$3_{Cl\text{-}Ph}$), 128.6 (2C, C-3, -5$_{phenyl}$), 128.2 (1C, C–$2_{Cl\text{-}Ph}$), 127.3 (1C, C–$5_{Cl\text{-}Ph}$), 127.21 and 127.19 (2C, C–$5_{Cl\text{-}Ph}$, C–$5_{Cl\text{-}Ph}$), 127.0 (1C, C–$6_{Cl\text{-}Ph}$), 126.7 (2C, C-2, -6$_{phenyl}$), 126.1 (1C, C–$6_{Cl\text{-}Ph}$), 125.7 (1C, C–$4_{Cl\text{-}Ph}$), 125.4 (1C, C–$4_{Cl\text{-}Ph}$), 54.6 (1C, C-$2_{piperazine}$), 45.3 (1C, C-$3_{piperazine}$), 43.5 (1C, C-$5_{piperazine}$), 39.3 (1C, C-6). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3426 (C-H_{arom}), 2953, 2916 (C-H_{aliph}), 1680, 1665 (C=O), 1595, 1517 (C=C_{arom}).

4.9.27. 1-(2-Chlorobenzoyl)-4-(benzo[b]thiophen-4-yl)-piperazine (4°)

2-Chlorobenzoic acid (1.1 eq, 0.43 mmol, 67 mg) was dissolved in dry acetonitrile (3 mL) under N_2 atmosphere. COMU® (1.2 eq, 0.47 mmol, 201 mg) and *N*,*N*-diisopropylethylamine (2.0 eq, 0.98 mmol, 164 μL) were added and the mixture was stirred for 15 min at room temperature. Then 1-(benzo[*b*]thiophen-4-yl)-piperazine (1.0 eq, 0.39 mmol, 100 mg) was dissolved in dry acetonitrile (1 mL) and the solution was added to the mixture. After stirring for 1 d, the reaction mixture was diluted with aqueous solution of NaOH (1 M, 10 mL) and brine (5 mL), then was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, Ø = 3 cm, l = 15 cm, V = 10 mL, cyclohexane:ethyl acetate = 3:1 + 1% Et_3N) to provide **4o** as a yellow oil, 97 mg (0.27 mmol, 70%), $C_{19}H_{17}ClN_2OS$ (356.87 g/mol). TLC (Silica): R_f = 0.26 (cyclohexane:ethyl acetate = 3:1 + 1% Et_3N). Exact mass (APCI): m/z = calcd. for $C_{19}H_{18}ClN_2OS$ [M + H$^+$] 357.0823, found 357.0787. Purity (HPLC, method D): 97%, R_t = 22.51 min ^{1}H NMR (400 MHz, MeOH-d_4): δ (ppm) = 7.58 (d, J = 8.1 Hz, 1H, 7-C$H_{benzothiophenyl}$), 7.52 (m, 2H, 2-C$H_{benzothiophenyl}$, 6-C$H_{benzoyl}$), 7.49–7.38 (m, 4H, 3-C$H_{benzothiophenyl}$, 3-, 4-, 5-C$H_{benzoyl}$), 7.27 (t, J = 7.9 Hz, 1H, 6-C$H_{benzothiophenyl}$), 6.95 (d, J = 7.6 Hz, 1H, 5-C$H_{benzothiophenyl}$), 4.04 (t, J = 4.6 Hz, 2H, 2-, 6-C$H_{2piperazine}$), 3.51 (m, 2H, 2-, 6-C$H_{2piperazine}$), 3.28–3.17 (m, 2H, 3-, 5-C$H_{2piperazine}$), 3.18–3.01 (m, 2H, 3-, 5-C$H_{2piperazine}$). ^{13}C NMR (101 MHz, MeOH-d_4): δ (ppm) = 169.1 (1C, C=O), 149.1 (1C, C-$1_{benzothiophenyl}$), 142.6 (1C, C-$7a_{benzothiophenyl}$), 136.7 (1C, C-$2_{benzoyl}$), 135.6 (1C, C-$3a_{benzothiophenyl}$), 132.0, 130.8, 129.1 and 128.7 (4C, C-3, -4, -5, -6$_{benzoyl}$), 131.3 (1C, C-$1_{benzoyl}$), 126.5 (1C, C-$2_{benzothiophenyl}$), 126.1 (1C, C-$6_{benzothiophenyl}$), 122.7 (1C, C-$3_{benzothiophenyl}$), 118.6 (1C, C-$7_{benzothiophenyl}$), 113.7 (1C, C-$5_{benzothiophenyl}$), 53.5 and 53.1 (2C, C-3, -5$_{piperazine}$), 48.6 and 43.4 (2C, C-2, -6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2978, 2900, 2816 (C–H), 1732 (C-S_{arom}), 1635 (C=O), 1593 (C-C_{arom}).

4.9.28. 3-Methoxy-4-[(2-methylphenyl)amino]cyclobut-3-ene-1,2-dione (5a)

3,4-Dimethoxycyclobut-3-ene-1,2-dione (1 g, 7.04 mmol, 1 eq) was dissolved in dry methanol (8 mL) under nitrogen atmosphere. 2-Methylphenylamin (toluidine, 189 μL, 1.76 mmol, 0.25 eq) was added and the mixture was stirred for 30 min at room temperature. Next more toluidine (189 μL, 1.76 mmol, 0.25 eq) was added and the mixture was stirred overnight at room temperature, followed by the final addition of toluidine (377 μL, 3.52 mmol, 0.5 eq) and stirring for additional 4 h. Then 17 mL methanol was added and the mixture was heated to 70 °C. The mixture was filtered while still hot and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2 + 2% MeOH, Ø = 2 cm, h = 18 cm, V = 20 mL) to provide 5a as a yellow solid, 0.977 g (4.50 mmol, 64%), $C_{12}H_{11}NO_3$ (217.22 g/mol). TLC (Silica): R_f = 0.33 (CH_2Cl_2 + 2% MeOH). Exact mass (APCI): m/z = calcd. for $C_{12}H_{12}NO_3$ [M + H$^+$] 218.0812, found 218.0801. Purity (HPLC, method D): 95%, R_t = 15.4 min ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.31 (s, 1H, NH), 7.21 (m, 2H, 3-C$H_{aminophenyl}$, 6-C$H_{aminophenyl}$), 7.13 (m, 2H, 4-C$H_{aminophenyl}$, 5-C$H_{aminophenyl}$), 4.28 (s, 3H, CH_3O), 2.27 (s, 3H, CH_3Ph). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.6 (2C, C=O), 178.7 (1C, C-$3_{cyclobutene}$), 170.9 (1C, C-$4_{cyclobutene}$), 131.9 (1C, C-$1_{aminophenyl}$), 136.0 (1C, C-$2_{aminophenyl}$), 130.9 (1C, C-$3_{aminophenyl}$), 126.7 (1C, C-$6_{aminophenyl}$), 126.5 (1C, C-$4_{aminophenyl}$), 124.8 (1C, C-$5_{aminophenyl}$), 60.7 (1C, CH_3O), 18.0 (1C, CH_3Ph). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3263 (N–H), 2978 (C-H_{aliph}), 1801, 1693 (C=O), 1616 (C–N), 1593 (C-C_{arom}).

4.9.29. 3-Methoxy-4-(quinolin-5-ylamino)cyclobut-3-ene-1,2-dione (5b)

3,4-Dimethoxycyclobut-3-ene-1,2-dione (296 mg, 2.08 mmol, 1 eq) was dissolved in MeOH (14 mL) and 5-aminoquinoline (300 mg, 2.08 mmol, 1 eq) was added. The mixture was stirred at room temperature for 3 d. The mixture was cooled to 0 °C and filtered through paper filter. The residue was washed with cooled MeOH (0 °C, 10 mL) and the solvent was removed *in vacuo*. The obtained solid was dried at 75 °C at atmospheric pressure to provide **5b** as a

red solid, 404 mg, 1.59 mmol, 76%, $C_{14}H_{10}N_2O_3$ (254.25 g/mol). TLC (Silica): R_f = 0.51 (CH_2Cl_2 + 8% MeOH). mp: 150 °C (dec.). Exact mass (APCI): *m/z* = calcd. for $C_{14}H_{11}N_2O_3$ [M + H^+] 255.0764, found 255.0774. Purity (HPLC, method D): 89%, R_t = 8.9 min ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.07 (s, 1H, N*H*), 8.94 (dd, *J* = 4.1, 1.6 Hz, 1H, 2-C*H*$_{quinolinyl}$), 8.54 (ddd, *J* = 8.6, 1.7, 0.9 Hz, 1H, 4-C*H*$_{quinolinyl}$), 7.91 (dt, *J* = 8.3, 1.0 Hz, 1H, 8-C*H*$_{quinolinyl}$), 7.76 (dd, *J* = 8.5, 7.5 Hz, 1H, 7-C*H*$_{quinolinyl}$), 7.60 (dd, *J* = 8.6, 4.1 Hz, 1H, 3-C*H*$_{quinolinyl}$), 7.43 (dd, *J* = 7.5, 1.1 Hz, 1H, 6-C*H*$_{quinolinyl}$), 4.31 (s, 3H, C*H*$_3$), 3.17 (s, C*H*$_{3,\ methanol}$). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 188.4 (1C, *C*-1$_{cyclobutenyl}$), 184.8 (1C, *C*-2$_{cyclobutenyl}$), 178.9 (1C, *C*-3$_{cyclobutenyl}$), 170.8 (1C, *C*-4$_{cyclobutenyl}$), 150.9 (1C, *C*-2$_{quinolinyl}$), 147.9 (1C, *C*-8a$_{quinolinyl}$), 133.1 (1C, *C*-5$_{quinolinyl}$), 131.6 (1C, *C*-4$_{quinolinyl}$), 129.0 (1C, *C*-7$_{quinolinyl}$), 126.8 (1C, *C*-8$_{quinolinyl}$), 122.5 (1C, *C*-4a$_{quinolinyl}$), 121.4 (1C, *C*-3$_{quinolinyl}$), 121.0 (1C, *C*-6$_{quinolinyl}$), 60.4 (1C, *C*H$_3$), 48.6 (*C*H$_{3,\ methanol}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2978 (N—H), 2345, 2326, 2307 (C—H), 1798, 1712 (C═O), 1627, 1608, 1589, 1558 (C-C$_{arom}$).

4.9.30. (3,4-Dimethoxyphenyl)(2-phenylpiperazin-1-yl)methanone (6a)

3,4-Dimethoxybenzoic acid (191 mg, 1.04 mmol, 1.1 eq) was dissolved in acetonitrile (4 mL). COMU (445 mg, 1.04 mmol, 1.1 eq) was added, followed by DIPEA (322 μL, 1.90 mmol, 2.0 eq). The mixture was stirred at room temperature for 15 min, then *tert*-butyl 3-phenylpiperazine-1-carboxylate (**2a**, 250 mg, 0.95 mmol, 1 eq) was added. The mixture was stirred at room temperature overnight, then the solvent was removed *in vacuo*. The crude product was purified by automated flash chromatography (10 g silica column, ethyl acetate:cyclohexane = 1:1 + 1% Et_3N, gradient: 2 cV 0% MeOH, 8 cV 0—10% MeOH, 5 cV 10% MeOH) to provide **6a** as a colorless solid, 240 mg (0.73 mmol, 77%), $C_{19}H_{22}N_2O_3$ (326.40 g/mol). TLC (Silica): R_f = 0.24 (cyclohexane:ethyl acetate = 2:1 + 1% Et_3N + 8% MeOH). mp: 94 °C. Exact mass (APCI): *m/z* = calcd. for $C_{19}H_{23}N_2O_3$ [M + H^+] 327.1703, found 327.1690. Purity (HPLC, method D): 93%, R_t = 12.9 min ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.43 (d, *J* = 7.3 Hz, 2H, 2-, 6-C*H*$_{phenyl}$), 7.37 (t, *J* = 7.7 Hz, 2H, 3-, 5-C*H*$_{phenyl}$), 7.25 (t, *J* = 7.2 Hz, 1H, 4-C*H*$_{phenyl}$), 7.02—6.93 (m, 3H, 2-, 5-, 6-C*H*$_{dimethoxyphenyl}$), 5.29 (s, 1H, 2-C*H*$_{piperazine}$), 3.85 (d, *J* = 13.6 Hz, 1H, 6-CH*H*$_{piperazine}$), 3.80 (s, 3H, C*H*$_3$O(4-C$_{dimethoxyphenyl}$)), 3.73 (s, 3H, C*H*$_3$O(3-C$_{dimethoxyphenyl}$)), 3.50 (d, *J* = 12.9 Hz, 1H, 3-CH*H*$_{piperazine}$), 3.12—3.00 (m, 2H, 3-, 6-CH*H*$_{piperazine}$), 2.94 (*H*$_2$O), 2.86 (dd, *J* = 12.3, 3.4 Hz, 1H, 5-CH*H*$_{piperazine}$), 2.73 (td, *J* = 12.0, 3.6 Hz, 1H, 5-CH*H*$_{piperazine}$), 2.12 (s, 1H, N*H*). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.3 (1C, *C*═O), 149.8 (1C, *C*-4$_{dimethoxyphenyl}$), 148.6 (1C, *C*-3$_{dimethoxyphenyl}$), 139.5 (1C, *C*-1$_{phenyl}$), 128.5 (1C, *C*-1$_{dimethoxyphenyl}$), 127.7 (2C, *C*-3, -5$_{phenyl}$), 126.6 (1C, *C*-2, -6$_{phenyl}$), 125.9 (1C, *C*-4$_{phenyl}$), 119.3, 112.0, 111.4 (3C, *C*-2, -5, -6$_{dimethoxyphenyl}$), 55.5 (2C, *C*H$_3$), 53.7 (1C, *C*-2$_{piperazine}$), 48.0 (1C, *C*-3$_{piperazine}$), 45.1 (1C, *C*-5$_{piperazine}$), 41.8 (1C, *C*-6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3263 (N—H), 2978 (C—H), 1689, 1593 (C═O), 1519, 1489 (C-C$_{arom}$).

4.9.31. N-(3,4-dimethoxyphenyl)-2-phenylpiperazine-1-carboxamide (6b)

tert-Butyl 3-phenylpiperazine-1-carboxylate (**2a**, 300 mg, 1.14 mmol, 1 eq) was dissolved in dry toluene (3 mL) under nitrogen atmosphere. 3,4-Dimethoxyphenyl isocyanate (205 mg, 1.14 mmol, 1 eq) was added and the mixture was stirred over night at room temperature. The solvent was removed under reduced pressure and the colorless residue was dissolved in MeOH (8 mL). Et_2O x HCl (1 M, 2 mL) was added 4 times over 2 days. Then, an aqueous, saturated solution of $NaHCO_3$ (15 mL) was added and the mixture was extracted with ethyl acetate (3 × 25 mL, 2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by automated flash chromatography (25 g silica column, ethyl acetate: cyclohexane = 1:1 + 1% NEt_3, gradient: 2 cV 2% MeOH, 10 cV 2%—10% MeOH, 15 cV 10% MeOH) to provide 6b as a colorless solid, 168 mg (0.63 mmol, 72%), $C_{19}H_{23}N_3O_3$ (341.41 g/mol). mp: 71 °C. TLC (Silica): 0.2 (ethyl acetate + 5% MeOH). Exact mass (ESI): *m/z* = calcd. for $C_{19}H_{24}N_3O_3$ [M + H^+] 342.1812, found 342.1800. Purity (HPLC, method D): 98%, R_t = 12.8 min ^{1}H NMR (600 MHz, MeOH-d_4): δ = 7.43—7.37 (m, 2H, 3-, 5-C*H*$_{phenyl}$), 7.34 (d, *J* = 8.4 Hz, 2H, 2-, 6-C*H*$_{phenyl}$), 7.29—7.25 (m, 1H, 5-C*H*$_{phenyl}$), 7.06 (t, *J* = 1.4 Hz, 1H, 2-C*H*$_{dimethoxyphenyl}$), 6.85 (d, *J* = 1.4 Hz, 2H, 5-, 6-C*H*$_{dimethoxyphenyl}$), 5.39—5.31 (m, 1H, 2-C*H*$_{piperazine}$), 3.96 (dt, *J* = 13.5, 1.5 Hz, 1H, 6-C*H*H$_{piperazine}$), 3.80 (s, 3H, 3-OC*H*$_{3,dimethoxyphenyl}$), 3.79 (s, 3H, 4-OC*H*$_{3,dimethoxyphenyl}$), 3.63 (ddd, *J* = 13.4, 2.3, 1.2 Hz, 1H, 3-CH*H*$_{piperazine}$), 3.23—3.14 (m, 2H, 3-, 6-CH*H*$_{piperazine}$), 2.94 (ddt, *J* = 12.6, 3.4, 1.5 Hz, 1H, 5-CH*H*$_{piperazine}$), 2.83 (td, *J* = 12.3, 3.7 Hz, 1H, 5-CH*H*$_{piperazine}$). ^{13}C NMR (151 MHz, MeOH-d_4): δ = 158.7 (1C, C═O), 150.4 (1C, *C*-3$_{dimethoxyphenyl}$), 146.9 (1C, *C*-4$_{dimethoxyphenyl}$), 140.2 (1C, *C*-1$_{phenyl}$), 134.4 (1C, *C*-3$_{dimethoxyphenyl}$), 129.9 (2C, *C*-3, -5$_{phenyl}$), 128.0 (1C, *C*-4$_{phenyl}$), 127.9 (1C, *C*-2, -6$_{phenyl}$), 115.1 (1C, *C*-6$_{dimethoxyphenyl}$), 113.3 (1C, *C*-5$_{dimethoxyphenyl}$), 108.3 (1C, *C*-2$_{dimethoxyphenyl}$), 56.8 (1C, OCH$_3$-3$_{dimethoxyphenyl}$), 56.4 (1C, OCH$_3$-4$_{dimethoxyphenyl}$), 54.7 (1C, *C*-2$_{piperazine}$), 49.4 (1C, *C*-3$_{piperazine}$), 46.2 (1C, *C*-5$_{piperazine}$), 42.0 (1C, *C*-6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3319 (N—H) 2933, 2833 (C-H$_{aliph}$), 1502 (C═O), 1462, 1447 (C-C$_{arom}$).

4.9.32. N-(4-chlorophenyl)-2-phenylpiperazine-1-carboxamide (6c)

tert-Butyl 3-phenylpiperazine-1-carboxylate (**2a**, 300 mg, 1.14 mmol, 1 eq) was dissolved in dry toluene under N_2, then 4-chlorophenyl isocyanate (175 mg, 1.14 mmol, 1eq) was added. The mixture was stirred at room temperature for 30 h. The solvent was partially removed *in vacuo* and MeOH (3 mL) was added, followed by addition of Et_2O x HCl (1 M, 2 mL). After 3 h, another portion of Et_2O x HCl was added and the mixture was stirred at room temperature overnight. An aqueous solution of $NaHCO_3$ (saturated, 20 mL) was added and this mixture was extracted with ethyl acetate (3 × 30 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude product was purified by automated flash chromatography (25 g silica column, ethyl acetate:cyclohexane = 1:1 + 1% Et_3N, gradient: 1 cV 0% MeOH, 10 cV 0—12% MeOH, 5 cV 12% MeOH). The product **6c** was obtained as a colorless solid, 228 mg (0.72 mmol, 65%), $C_{17}H_{18}ClN_3O$ (315.80 g/mol). TLC (Silica): R_f = 0.2 (ethyl acetate:cyclohexane = 3:1 + 1% Et_3N + 10% MeOH). mp: 128 °C. Exact mass (APCI): *m/z* = calcd. for $C_{17}H_{19}ClN_3O$ [M + H^+] 316.1211, found 316.1204. Purity (HPLC, method A): 95%, R_t = 4.32 min ^{1}H NMR (400 MHz, MeOH-d_4): δ = 7.42—7.31 (m, 6H, 2-, 6-C*H*$_{Cl\text{-}phenyl}$, 2-, 3-, 4-, 5-, 6-C*H*$_{phenyl}$), 7.30—7.21 (m, 3H, 3-, 5-C*H*$_{Cl\text{-}phenyl}$, 4-C*H*$_{phenyl}$), 5.37 (s, 1H, 2-CH*H*$_{piperazine}$), 4.84 (H$_2$O), 4.10 (q, *CH*$_{2,\ ethyl\ acetate}$), 3.97 (ddd, 1H, *J* = 13.5, 2.0, 1.1 Hz, 6-CH*H*$_{piperazine}$), 3.63 (ddd, 1H, *J* = 13.5, 2.3, 1.2 Hz, 3-CH*H*$_{piperazine}$), 3.18 (m, 2H, 3-, 6-CH*H*$_{piperazine}$), 2.94 (m, 1H, 5-CH*H*$_{piperazine}$), 2.83 (ddd, 1H, *J* = 12.6, 11.9, 3.7 Hz, 5-CH*H*$_{piperazine}$), 2.01 (s, C*H*$_3$CO$_{ethyl\ acetate}$), 1.24 (t, C*H*$_{3,\ ethyl\ acetate}$). ^{13}C NMR (101 MHz, MeOH-d_4): δ = 158.2 (1C, *C*═O), 140.0 (1C, *C*-1$_{phenyl}$), 139.8 (1C, *C*—1$_{Cl\text{-}phenyl}$), 130.0 (2C, *C*-3, -5$_{phenyl}$), 129.5 (2C, *C*-3, -5$_{Cl\text{-}phenyl}$), 129.1 (1C, *C*—4$_{Cl\text{-}phenyl}$), 128.1 (1C, *C*-4$_{phenyl}$), 127.8 (2C, *C*-2, -6$_{phenyl}$), 123.5 (2C, *C*-2, -6$_{Cl\text{-}phenyl}$), 54.7 (1C, *C*-2$_{piperazine}$), 49.2 (1C, *C*-3$_{piperazine}$), 46.2 (1C, *C*-5$_{piperazine}$), 42.1 (1C, *C*-6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3298 (N—H), 2951, 2924 (C—H), 1631 (C═O), 1593 (C-C$_{arom}$).

4.9.33. N-(4-chlorophenyl)-2-phenylpiperazine-1-carboxamide (6d)

Under N_2 atmosphere, *tert*-butyl 3-phenylpiperazine-1-carboxylate (**2a**, 300 mg, 1.14 mmol, 1 eq) was dissolved in dry toluene (3 mL) and 2-chlorophenyl isocyanate (138 μL, 1.14 mmol, 1 eq) was added. The mixture was stirred for 16 h at room temperature. Then, Et_2O x HCl (2 mL) was added and the mixture was stirred overnight. Due to incomplete conversion, more Et_2O x HCl (2 mL) was added and the mixture was stirred for 3 d at room temperature. Then, an aqueous, saturated solution of $NaHCO_3$ (20 mL) was added and the mixture was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by automated flash chromatography (25 g silica column, ethyl acetate: cyclohexane = 1:1 + 1% NEt_3, gradient: 1 cV 0% MeOH, 10 cV 0%–12% MeOH, 5 cV 12% MeOH) to provide 6d as a colorless solid, 181 mg (0.57 mmol, 50%), $C_{17}H_{18}ClN_3O$ (315.80 g/mol). mp: 76 °C. TLC (Silica): 0.48 (ethyl acetate + 5% MeOH). Exact mass (APCI): *m/z* = calcd. for $C_{17}H_{19}ClN_3O$ [M + H^+] 316.1211, found 316.1189. Purity (HPLC, method D): 84%, R_t = 14.4 min ^{1}H NMR (600 MHz, $CHCl_3$-d): δ = 8.16 (dd, *J* = 8.4, 1.6 Hz, 1H, 3-C*H*$_{Cl\text{-}phenyl}$), 7.43–7.36 (m, 4H, 2-, 3-, 5-, 6-C*H*$_{phenyl}$), 7.33–7.29 (m, 1H, 4-C*H*$_{phenyl}$), 7.26 (dd, *J* = 8.0, 1.5 Hz, 2H, 6-C*H*$_{Cl\text{-}phenyl}$), 7.22 (ddd, *J* = 8.6, 7.4, 1.5 Hz, 1H, 4-C*H*$_{Cl\text{-}phenyl}$), 6.96–6.90 (m, 2H, 5-C*H*$_{Cl\text{-}phenyl}$, N*H*$_{urea}$), 5.17 (t, *J* = 4.0 Hz, 1H, 2-C*H*$_{piperazine}$), 4.12 (ddd, *J* = 13.5, 4.4, 2.3 Hz, 1H, 6-C*H*H$_{piperazine}$), 3.53–3.47 (m, 1H, 3-C*H*H$_{piperazine}$), 3.48 (q, diethyl ether 3.38–3.30 (m, 2H, 3-, 6-C*H*H$_{piperazine}$), 3.18–3.13 (m, 1H, 5-C*H*H$_{piperazine}$), 3.04 (td, *J* = 12.1, 4.2 Hz, 1H, 5-CHH$_{piperazine}$), 2.51 (s, 1H, N*H*$_{piperazine}$). Impurities: 3.48 (q, diethyl ether, C*H*$_2$), 1.20 (t, diethyl ether, C*H*$_3$), 0.08 (grease). ^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ = 154.9 (1C, *C*=O), 138.6 (1C, *C*-1$_{phenyl}$), 135.8 (1C, *C*–1$_{Cl\text{-}phenyl}$), 129.4 (1C, *C*-3, -5$_{phenyl}$), 129.0 (1C, *C*–6$_{Cl\text{-}phenyl}$), 128.0 (1C, *C*-4$_{phenyl}$), 127.7 (1C, *C*–4$_{Cl\text{-}phenyl}$), 126.9 (1C, *C*-2, -6$_{phenyl}$), 123.4 (1C, *C*–5$_{Cl\text{-}phenyl}$), 122.6 (1C, *C*–2$_{Cl\text{-}phenyl}$), 121.3 (1C, *C*–3$_{Cl\text{-}phenyl}$), 55.0 (1C, *C*-2$_{piperazine}$), 49.3 (1C, *C*-3$_{piperazine}$), 45.1 (1C, *C*-5$_{piperazine}$), 40.5 (1C, *C*-6$_{piperazine}$). Impurities: 46.8 (diethyl ether, *C*H$_2$), 15.4 (diethyl ether, *C*H$_3$). FTIR (neat): $\underline{\tilde{\nu}}$ (cm^{-1}) = 3294 (C-H$_{arom}$), 2953, 2916 (C-H$_{aliph}$), 1658 (C=O), 1645, 1591, 1514, 1504 (C=C$_{arom}$).

4.9.34. N-(3,4-dimethoxyphenyl)piperazine-1-carboxamide (6e)

N-(*tert*-butoxycarbonyl)-3-phenylpiperazine (**2c**, 250 mg, 1.34 mmol, 1 eq) was dissolved in dry toluene (3 mL) under nitrogen atmosphere. 3,4-Dimethoxyphenyl isocyanate (241 mg, 1.34 mmol, 1 eq) was added and a viscous precipitate forms. More dry toluene (3 mL) was added and the mixture was stirred over night at room temperature. Then the solvent was removed *in vacuo* and the residue was dissolved in MeOH (15 mL). Et_2O x HCl (1 M, 10 mL) was added and the mixture was stirred for 3 d. More Et_2O x HCl (1 M, 10 mL) was added and the mixture was stirred for 1 d. Water (30 mL) and aqueous, concentrated HCl (0.5 mL) was added and the mixture was extracted with ethyl acetate (3 × 30 mL). Then the mixture was basified with a saturated, aqueous solution of K_2CO_3 to pH > 10. The mixture was then extracted with CH_2Cl_2 (6 × 50 mL). The combined organic layers of CH_2Cl_2 were dried over Na_2SO_4 and the solvent was removed *in vacuo* to provide **6e** as a colorless solid, 177 mg (0.68 mmol, 50%), $C_{13}H_{19}N_3O_3$ (265.31 g/mol). mp: 264 °C. TLC (Silica): 0.04 (ethyl acetate+ 10% MeOH + 1% Et_3N). Exact mass (ESI): *m/z* = calcd. for $C_{13}H_{20}N_3O_3$ [M + H^+] 266.1499, found 266.1519. Purity (HPLC, method D): 96%, R_t = 7.8 min ^{1}H NMR (600 MHz, MeOH-d_4, 25 °C): δ = 7.06 (d, *J* = 2.1 Hz, 1H, 6-C*H*$_{dimethoxyphenyl}$), 6.88–6.83 (m, 2H, 2-, 5-C*H*$_{dimethoxyphenyl}$), 3.81 (s, 3H, 3-COC*H*$_{3,dimethoxyphenyl}$), 3.79 (s, 3H, 4- COC*H*$_{3,dimethoxyphenyl}$), 3.50–3.46 (m, 4H, 2-, 6-C*H*$_{2,piperazine}$), 2.85–2.82 (m, 4H, 3-, 5-C*H*$_{2,piperazine}$). ^{13}C NMR (151 MHz, MeOH-d_4, 25 °C): δ = 158.3 (1C, *C*=O), 150.4 (1C, *C*-3$_{dimethoxyphenyl}$), 146.8 (1C, *C*-4$_{dimethoxyphenyl}$), 134.5 (1C, *C*-1$_{dimethoxyphenyl}$), 114.9 (1C, *C*-5$_{dimethoxyphenyl}$), 113.3 (1C, *C*-2$_{dimethoxyphenyl}$), 108.2 (1C, *C*-6$_{dimethoxyphenyl}$), 56.9 (1C, H_3*C*C-4$_{dimethoxyphenyl}$), 56.4 (1C, H_3*C*C-3$_{dimethoxyphenyl}$), 46.4 (2C, *C*-3, -5$_{piperazine}$), 45.7 (1C, *C*-2, -6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3291 (N–H), 1635 (C=O), 1508 (C=C$_{arom}$).

4.9.35. N-(4-(tert-butyl)phenyl)piperazine-1-carboxamid (6f)

tert-Butyl piperazine-1-carboxylate (**2c**, 120 mg, 0.64 mmol, 1 eq) was dissolved in dry toluene (3 mL) under N_2 atmosphere and 4-(*tert*-butyl)phenyl isocyanate was added. The mixture was stirred at room temperature over night. Then, methanol (3 mL) and Et_2O x HCl (1 M, 2 mL) were added and the mixture was again stirred at room temperature over night. Due to incomplete conversion, Dioxane x HCl (4 M, 2 mL) was added and the mixture was again stirred over night. Aqueous solution of sodium hydroxide (2 M, 10 mL) was added and the mixture was extracted with ethyl acetate (3 × 40 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by automated flash chromatography (25 g silica column, ethyl acetate + 10% MeOH + 1% Et_3N) to provide **6f** as a colorless solid, 172 mg, (0.66 mmol, >99%), $C_{15}H_{23}N_3O$ (261.37 g/mol). TLC (Silica): 0.38 (CH_2Cl_2 + 10% MeOH + 1% Et_3N). Exact mass (ESI): *m/z* = calcd. for $C_{15}H_{24}N_3O$ [M + H^+] 262.1914, found 262.1922. Purity (HPLC, method B): 91%, R_t = 15.4 min ^{1}H NMR (600 MHz, MeOH-d_4): δ = 7.33–7.29 (m, 2H, 3-, 5-C*H*$_{t\text{-}butylphenyl}$), 7.27–7.24 (m, 2H, 2-, 6-C*H*$_{t\text{-}butylphenyl}$), 3.65–3.60 (m, 4H, 2-, 6-C*H*$_{2,piperazine}$), 3.07–3.03 (m, 4H, 3-, 5-C*H*$_{2,piperazine}$), 1.93 (s, 1H, N*H*$_{piperazine}$), 1.29 (s, 9H, (C*H*$_3$)$_3$). ^{13}C NMR (151 MHz, MeOH-d_4): δ = 157.9 (1C, *C*=O), 147.5 (1C, *C*-4$_{t\text{-}butylphenyl}$), 137.9 (1C, *C*-1$_{t\text{-}butylphenyl}$), 126.5 (2C, *C*-3, -5$_{t\text{-}butylphenyl}$), 122.1 (1C, *C*-2, -6$_{t\text{-}butylphenyl}$), 45.3 (2C, *C*-3, -5$_{t\text{-}butylphenyl}$), 44.0 (2C, *C*-2, -6$_{t\text{-}butylphenyl}$), 35.1 (1C, *C*(CH$_3$)$_3$), 31.8 (3C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3306 (N–H), 2959 (C-H$_{aliph}$), 1640 (C=O), 1593 (C=C$_{arom}$).

4.9.36. N-(4-fluorophenyl)piperazine-1-carboxamide (6g)

4-Fluorophenyl isocyante (61 μL, 0.54 mmol, 1 eq) was dissolved in toluene (1.5 mL) and *tert*-butyl piperazine-1-carboxylate (**2c**, 100 mg, 0.54 mmol, 1 eq) was added. A white precipitate forms immediately. After stirring overnight at room temperature, water (10 mL) was added and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried with Na_2SO_4 and the solvent was removed *in vacuo*. To the obtained residue, MeOH (4 mL) and Et_2O x HCl (4 mL) were added. The mixture was stirred overnight at room temperature, then more Et_2O x HCl (4 mL) was added. After stirring overnight at room temperature, water (5 mL) and an aqueous solution of $NaHCO_3$ (saturated, 5 mL) were added to the mixture and it was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine, then dried with Na_2SO_4 and the solvent was removed *in vacuo* to provide **6g** as a colorless solid, 80 mg (0.36 mmol, 66%), $C_{11}H_{14}FN_3O$ (223.25 g/mmol). TLC (Silica): R_f = 0.72 (ethyl acetate + 5% MeOH + 1% NEt_3). mp: 200 °C (dec.). Exact mass (APCI): *m/z* = cald. for $C_{11}H_{15}FN_3O$ [M + H^+] 224.1194, found 224.1203. Purity (HPLC, method D): 96%, R_t = 9.5 min ^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ = 7.37–7.24 (m, 2H, 3-, 5-C*H*$_{phenyl}$), 7.07–6.93 (m, 2H, 2-, 6-C*H*$_{phenyl}$), 3.51–3.46 (m, 4H, 2-, 6-C*H*$_{2,piperazine}$), 2.86–2.81 (m, 4H, 3-, 5-C*H*$_{2,piperazine}$). ^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ = 160.4 (d, *J* = 240.7 Hz, 1C, 4-*C*$_{F\text{-}Phenyl}$), 158.1 (1C, *C*=O), 137.0 (d, *J* = 2.8 Hz, 1C, *C*–1$_{F\text{-}phenyl}$), 124.2 (d, *J* = 8.0 Hz, 2C, *C*-2, -6$_{F\text{-}phenyl}$), 116.0 (d, 22.5 Hz, 2C, *C*-3, -4$_{F\text{-}phenyl}$), 46.3 (2C, *C*-3, -6$_{piperazine}$), 45.7 (2C, *C*-2, -6$_{piperazine}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3281, 3242 (C-H$_{arom}$), 2926, 2985, 2849 (C-H$_{aliph}$), 1631 (C=O), 1508, 1417 (C-C$_{arom}$).

4.9.37. 4-[(2-Methylphenyl)amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione (7)

tert-Butyl piperazine-1-carboxylate **2c** (591 mg, 3.17 mmol, 1.5 eq) was dissolved in dry DMF (4 mL) under N_2 atmosphere. 3-Methoxy-4-(-*o*-tolylamino)cyclobut-3-ene-1,2-dione (459 mg, 2.11 mmol, 1 eq) was added. The mixture was stirred overnight at room temperature, then an aqueous, saturated solution of NH_4Cl (10 mL) was added and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried with Na_2SO_4 and the solvent was removed *in vacuo*. The crude intermediate was purified by flash chromatography (cyclohexane:ethyl acetate = 2:1 + 5% methanol, Ø = 4 cm, h = 18 cm, V = 20 mL). The obtained intermediate was dissolved in CH_2Cl_2 (3 mL) and trifluoroacetic acid was added (3 mL). After stirring overnight, further trifluoroacetic acid was added (2 mL) and the mixture was stirred for additional 4 h at room temperature. Then, the mixture was concentrated under reduced pressure and the crude product was purified by automated flash chromatography (120 g kP-C18-HS column, gradient: 3 cV H_2O:acetonitrile = 95:5, 10 cV H_2O:acetonitrile = 95:5 to H_2O:acetonitrile = 20:80, 2 cV H_2O:acetonitrile = 20:80) to provide 7 as a colorless solid, 109 mg (0.40 mmol, 56%), $C_{15}H_{17}N_3O_2$ (271.32 g/mol). mp: 103 °C. TLC (Silica): $R_f = 0.02$ (ethyl acetate + 7.5% MeOH + 1% Et_3N). Exact mass (APCI): m/z = calcd. for $C_{15}H_{18}N_3O_2$ [M + H^+] 272.1394, found 272.1390. Purity (HPLC, method D): 97%, R_t = 9.9 min ^{1}H NMR (400 MHz, MeOH-d_4): δ = 7.27−7.23 (m, 1H, 3-C*H*$_{aminophenyl}$), 7.23−7.13 (m, 2H, 4-, 5-C*H*$_{aminophenyl}$), 7.12 (dd, *J* = 7.6, 1.6 Hz, 1H, 6-C*H*$_{aminophenyl}$), 3.75−3.49 (m, 4H, 2-, 6-C*H*$_{2,piperazine}$), 2.93−2.77 (m, 4H, 3-, 4-C*H*$_{2,piperazine}$), 2.33 (s, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, MeOH-d_4): δ = 185.8 and 184.1 (2C, *C*-1, -2$_{cyclobutene}$), 169.2 (1C, *C*-3$_{cyclobutene}$), 166.7 (1C, *C*-4$_{cyclonutene}$), 138.5 (1C, *C*-1$_{aminophenyl}$), 133.6 (1C, *C*-2$_{aminophenyl}$), 131.9 (1C, *C*-3$_{aminophenyl}$), 127.7 (1C, *C*-5$_{aminophenyl}$), 127.6 (1C, *C*-4$_{aminophenyl}$), 126.0 (1C, *C*-6$_{aminophenyl}$), 49.6 (1C, *C*-2, -6$_{piperazine}$), 46.5 (1C, *C*-3, -4$_{piperazine}$), 17.9 (1C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3221 (N−H), 2978, 2889 (C−H), 1786, 1674, 1570, 1508 (C=O, C−N).

4.9.38. 3-[4-(3,4-Dimethoxybenzoyl)-3-phenylpiperazine-1-yl]-4-[(2-methylphenyl)amino]cyclobut-3-ene-1,2-dione (8a)

To a mixture of 3-methoxy-4-[(2-methylphenyl)amino]cyclobut-3-ene-1,2-dione (**5a**, 67 mg, 0.31 mmol, 1 eq) in MeOH (1.5 mL) was added (3,4-dimethoxyphenyl) (2-phenylpiperazin-1-yl)methanone (**6a**, 100 mg, 0.31 mmol, 1 eq) and trimethylamine (1.5 mL). The mixture was heated to 60 °C for 5 h. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (cyclohexane:ethyl acetate = 2:1 + 7% MeOH + 1% triethylamine, Ø = 2 cm, h = 18 cm, V = 20 mL) and afterwards recrystallized from MeOH/H_2O to provide **8a** as a colorless solid, 85 mg (0.17 mmol, 54%), $C_{30}H_{29}N_3O_5$ (511.58 g/mol). TLC (Silica): $R_f = 0.22$ (cyclohexane:ethyl acetate = 1:1 + 1% NEt_3 + 10% MeOH). mp: 140 °C. Exact mass (APCI): m/z = calcd. for $C_{30}H_{30}N_3O_5$ [M + H^+] 512.2180, found 512.2191. Purity (HPLC, method D): 96%, R_t = 18.7 min ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C): δ = 8.92 (s, 1H, N*H*), 7.45−7.34 (m, 4H, 2-, 3-, 5-, 6-C*H*$_{phenyl}$), 7.31 (t, *J* = 7.1 Hz, 1H, 4-C*H*$_{phenyl}$), 7.21 (t, *J* = 4.5 Hz, 1H, 3-C*H*$_{aminophenyl}$), 7.11 (t, *J* = 4.5 Hz, 2H, 4-, 6-C*H*$_{aminophenyl}$), 7.06−6.95 (m, 4H, 5-C*H*$_{aminophenyl}$, 2-, 5-, 6-C*H*$_{dimethoxyphenyl}$), 5.52 (d, *J* = 4.6 Hz, 1H, 2-C*H*$_{piperazine}$), 4.75 (dd, *J* = 13.7, 3.0 Hz, 1H, 3-CH*H*$_{piperazine}$), 3.98 (d, *J* = 13.7 Hz, 1H, 6-CH*H*$_{piperazine}$), 3.92−3.82 (m, 2H, 3-, 5-, 6-CH*H*$_{piperazine}$), 3.81 (s, 3H, C*H*$_3$OC-3$_{dimethoxyphenyl}$), 3.74 (s, 3H, C*H*$_3$OC-4$_{dimethoxyphenyl}$), 3.48 (td, *J* = 12.3, 3.9 Hz, 1H, 5-CH*H*$_{piperazine}$), 3.26 (t, *J* = 12.5 Hz, 1H, 6-CH*H*$_{piperazine}$), 2.98 (*H*$_2$O), 2.23 (s, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, DMSO-d_6, 100 °C): δ = 184.3 (2C, *C*-3, -4$_{cyclobutenyl}$), 169.7 (1C, *C*=O$_{amide}$), 167.7 (1C, *C*-1$_{cyclobutenyl}$), 165.3 (1C, *C*-2$_{cyclobutenyl}$), 150.2 (1C, *C*-3$_{dimethoxyphenyl}$), 148.6 (1C, *C*-4$_{dimethoxyphenyl}$), 137.4 (1C, *C*-1$_{phenyl}$), 136.7 (1C, *C*-1$_{aminophenyl}$), 131.4 (1C, *C*-2$_{aminophenyl}$), 129.9 (1C, *C*-3$_{aminophenyl}$), 128.2 and 126.1 (4C, *C*-2, -3, -5, -6$_{phenyl}$), 127.5 (1C, *C*-3$_{dimethoxyphenyl}$), 126.8 (1C, *C*-4$_{phenyl}$), 125.6 and 125.1 (2C, *C*-4, -6$_{aminophenyl}$), 124.1 (1C, *C*-5$_{aminophenyl}$), 119.6, 112.0 and 111.4 (3C, *C*-2, -5, -6$_{dimethoxyphenyl}$), 55.5 (2C, *C*H$_3$O), 54.0 (1C, *C*-2$_{piperazine}$), 48.4 (1C, *C*-3$_{piperazine}$), 46.3 (1C, *C*-5$_{piperazine}$), 40.1 (1C, *C*-6$_{piperazine}$), 16.8 (1C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 2978 (N−H), 2341 (C−H), 1790, 1682, 1627 (C=O), 1577 (C−N), 1508, 1458 (C-C$_{arom}$).

4.9.39. 3-[4-(3,4-Dimethoxybenzoyl)-3-phenylpiperazine-1-yl]-4-(quinolin-5-ylamino)cyclobut-3-ene-1,2-dione (8b)

(3,4-Dimethoxyphenyl) (2-phenylpiperazin-1-yl)methanone (**6a**, 100 mg, 0.31 mmol, 1 eq) was dissolved in DMF (3 mL) and MeOH (1 mL). Then, 3-methoxy-4-(quinolin-5-ylamino)cyclobut-1-ene-1,2-dione (**5b**, 78 mg, 0.31 mmol, 1 eq) and Et_3N (2 mL) were added. The mixture was stirred at room temperature for 72 h. Then an aqueous solution of K_2CO_3 (saturated, 10 mL) was added and the mixture was extracted with ethyl acetate (3 × 30 mL). The solvent was removed *in vacuo* and the residue was dispersed in DMF, filtered off and washed with MeOH to provide **8b** as a colorless solid, 28 mg (0.05 mmol, 17%), $C_{32}H_{28}N_4O_5$ (548.60 g/mol). TLC (Silica): $R_f = 0.3$ (ethyl acetate + 5% MeOH). Melting point: 240 °C (dec.). Exact mass (APCI): m/z = calcd. for $C_{32}H_{28}N_4O_5$ [M + H^+] 549.2132, found 549.2135. Purity (HPLC, method D): 99%, R_t = 15.2 min ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C): δ = 9.53 (1H, N*H*), 8.92−8.85 (m, 1H, 2-C*H*$_{quinolinyl}$), 8.47−8.39 (m, 1H, 4-C*H*$_{quinolinyl}$), 7.84 (dt, *J* = 8.5, 1.2 Hz, 1H, 8-C*H*$_{quinolinyl}$), 7.63 (dd, *J* = 8.7, 7.3 Hz, 1H, 7-C*H*$_{quinolinyl}$), 7.50 (dd, *J* = 8.6, 4.2 Hz, 1H, 3-C*H*$_{quinolinyl}$), 7.43−7.35 (m, 4H, 2-, 3-, 5-, 6-C*H*$_{phenyl}$), 7.31 (t, *J* = 7.0 Hz, 1H, 4-C*H*$_{phenyl}$), 7.22−7.15 (m, 1H, 6-C*H*$_{quinolinyl}$), 7.06−6.96 (m, 3H, 2-, 5-, 6-C*H*$_{dimethoxyphenyl}$), 5.53 (d, *J* = 3.7 Hz, 1H, 2-C*H*$_{piperazine}$), 4.80 (d, *J* = 13.9 Hz, 1H, 3-CH*H*$_{piperazine}$), 4.00 (d, *J* = 13.3 Hz, 2H, 5-, 6-C*H*H$_{piperazine}$), 3.88 (dd, *J* = 13.9, 4.4 Hz, 1H, 3-C*H*H$_{piperazine}$), 3.79 (d, *J* = 1.2 Hz, 3H, 4-OC*H*$_{3,dimethoxyphenyl}$), 3.72 (d, *J* = 1.2 Hz, 3H, 3-OC*H*$_{3,dimethoxyphenyl}$), 3.53 (td, *J* = 12.4, 4.1 Hz, 1H, 5-C*H*H$_{piperazine}$), 3.35−3.23 (m, 1H, 6-C*H*H$_{piperazine}$). ^{13}C NMR (101 MHz, DMSO-d_6, 100 °C): δ = 185.0 (2C, *C*-3, -4$_{cyclobutenyl}$), 169.7 (1C, *C*=O$_{arylamide}$), 168.3 (1C, *C*-1$_{cyclobutenyl}$), 165.0 (1C, *C*-2$_{cyclobutenyl}$), 150.0 (2C, *C*-3$_{dimethoxyphenyl}$, *C*-2$_{quinolinyl}$), 148.6 (1C, *C*-4$_{dimethoxyphenyl}$), 147.8 (1C, *C*-8a$_{quinolinyl}$), 137.4 (1C, *C*-1$_{phenyl}$), 131.0 (1C, *C*-4$_{quinolinyl}$), 128.24 (2C, *C*-2, -6$_{quinolinyl}$), 128.16 (1C, *C*-4$_{quinolinyl}$), 126.8 (1C, *C*-4$_{phenyl}$), 126.1 (1C, *C*-3, -5$_{phenyl}$), 125.8 (1C, *C*-8$_{quinolinyl}$), 122.6 (1C, *C*-4a$_{quinolinyl}$), 120.4 (1C, *C*-3$_{quinolinyl}$), 120.2 (1C, *C*-6$_{quinolinyl}$), 119.6, 112.0 and 111.5 (3C, *C*-2, -5, -6$_{phenyl}$), 55.6 (2C, *C*H$_3$), 54.0 (1C, *C*-2$_{piperazine}$), 48.5 (1C, *C*-3$_{piperazine}$), 46.4 (1C, *C*-5$_{piperazine}$), 40.2 (1C, *C*-6$_{piperazine}$).FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3219 (N−H), 3064 (C-H$_{arom}$), 2997, 2927 (C-H$_{aliph}$), 1687, 1606, 1592 (C=O), 1581, 1504, 1487, 1458 (C-C$_{arom}$).

4.9.40. N-(3,4-dimethoxyphenyl)-4-{3,4-dioxo-2-[(2-methylphenyl)amino]cyclobut-1-en-1-yl}-2-phenylpiperazine-1-carboxamide (8c)

1-(3,4-Dimethoxyphenyl)carbamoyl-2-phenylpiperazine **6b** (60 mg, 0.18 mmol, 1 eq) was dissolved in DMF (3 mL). 3-Methoxy-4-(2-methylanilino-1-yl)cyclobut-3-ene-1,2-dione **5a** (38 mg, 0.18 mmol, 1 eq) was added and the mixture was stirred for 3 d at room temperature. Water (5 mL) was then added and a formed colorless precipitate was filtered off. The residue was purified by automated flash chromatography (25 g silica column, cyclohexane, gradient: 3 cV 40% ethyl acetate, 15 cV 40%−100% ethyl acetate, 5 cV 100% ethyl acetate) to provide **8c** as a colorless solid, 38 mg (0.07 mmol, 40%), $C_{30}H_{30}N_4O_5$ (526.59 g/mol). mp: 149 °C. TLC (Silica): $R_f = 0.22$ (ethyl acetate:cyclohexane = 1:1 + 5% MeOH). Exact mass (ESI): m/z = calcd. for $C_{30}H_{29}N_4O_5$ [M-H^+] 525.2143, found 525.2158. Purity (HPLC, method B): 96%, R_t = 12.3 min ^{1}H

NMR (400 MHz, DMSO-d_6, 100 °C): δ = 8.94 (s, 1H, N$H_{cyclobutene}$), 8.26 (s, 1H, N$H_{carbamoyl}$), 7.42–7.32 (m, 4H, 2-, 3-, 5-, 6-CH_{phenyl}), 7.29 (t, J = 7.0 Hz, 1H, 4-CH_{phenyl}), 7.22 (t, J = 4.4 Hz, 1H, 3-C$H_{aminophenyl}$), 7.16 (s, 1H, 2-C$H_{dimethoxyphenyl}$), 7.12 (t, J = 4.2 Hz, 2H, 4-, 6-C$H_{aminophenyl}$), 7.04–6.96 (m, 2H, 5-C$H_{aminophenyl}$, 6-C$H_{dimethoxyphenyl}$), 6.84 (d, J = 8.6 Hz, 1H, 5-C$H_{dimethoxyphenyl}$), 5.55 (d, J = 3.8 Hz, 1H, 2-C$H_{piperazine}$), 4.70 (d, J = 13.7 Hz, 1H, 3-CHH$_{piperazine}$), 4.03 (d, J = 14.0 Hz, 1H, 6-CHH$_{piperazine}$), 3.90 (d, J = 12.7 Hz, 1H, 5-CHH$_{piperazine}$), 3.80 (dd, J = 13.7, 3.9 Hz, 1H, 3-CHH$_{piperazine}$), 3.75 (s, 3H, 3-OC$H_{3,dimethoxyphenyl}$), 3.73 (s, 3H, 4-OC$H_{3,dimethoxyphenyl}$), 3.45 (t, J = 12.0 Hz, 1H, 5-CHH$_{piperazine}$), 3.29–3.18 (m, 1H, 6-CHH$_{piperazine}$), 2.24 (s, 3H, C$H_{3,aminophenyl}$), 1.21 (d, unknown impurity). ^{13}C NMR (101 MHz, DMSO-d_6, 100 °C): δ = 185.3 and 182.7 (2C, C-3, -4$_{cyclobutene}$), 168.6 (1C, C-1$_{cyclobutene}$), 166.3 (1C, C-2$_{cyclobutene}$), 155.8 (1C, C=O), 149.7 (1C, C-3$_{dimethoxyphenyl}$), 145.5 (1C, C-4$_{dimethoxyphenyl}$), 139.3 (1C, C-1$_{phenyl}$), 137.8 (1C, C-1$_{aminophenyl}$), 134.1 (1C, C-1$_{dimethoxyphenyl}$), 132.4 (1C, C-2$_{aminophenyl}$), 130.9 (1C, C-3$_{aminophenyl}$), 129.0 (1C, C-3, -5$_{phenyl}$), 127.5 (1C, C-4$_{phenyl}$), 127.1 (1C, C-2, -6$_{phenyl}$), 126.6 (1C, C-4$_{aminophenyl}$), 126.0 (1C, C-6$_{aminophenyl}$), 125.1 (1C, C-5$_{aminophenyl}$), 114.1 (1C, C-5$_{dimethoxyphenyl}$), 113.3 (1C, C-6$_{dimethoxyphenyl}$), 107.7 (1C, C-2$_{dimethoxyphenyl}$), 57.1 (1C, OCH_3-4$_{dimethoxyphenyl}$), 56.5 (1C, OCH_3-3$_{dimethoxyphenyl}$), 54.0 (1C, C-2$_{piperazine}$), 49.4 (1C, C-3$_{piperazine}$), 47.2 (1C, C-5$_{piperazine}$), 40.00 (1C, C-6$_{piperazine}$), 22.3 (unknown impurity), 17.8 (1C, H$_3$$C-2_{aminophenyl}$).

FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3248 (N–H), 1790, 1678, 1574 (C=O), 1508, 1411 (C-C$_{arom}$).

4.9.41. N-(4-chlorophenyl)-4-{3,4-dioxo-2-[(2-methylaminophenyl)cyclobut-1-en-1-yl]}-2-phenylpiperazine-1-carboxamide (8d)

N-(4-Chlorophenyl)-2-phenylpiperazine-1-carboxamide (**6c**, 73 mg, 0.23 mmol, 1 eq) was dissolved in MeOH (2 mL) and 3-methoxy-4-[(2-methylphenyl)amino]cyclobut-3-ene-1,2-dione (**5a**, 50 mg, 0.23 mmol, 1 eq) was added. The mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the crude product was purified by automated flash chromatography (10 g kP Sil column, ethyl acetate:cyclohexane, gradient: 3 cV 20% ethyl acetate, 10 cV 20–100% ethyl acetate, 10 cV 100% ethyl acetate) to provide **8d** as a light yellow solid, 108 mg (0.22 mmol, 94%), $C_{28}H_{25}ClN_4O_3$ (500.98 g/mol). TLC (Silica): R_f = 0.49 (cyclohexane:ethyl acetate = 2:1 + 1% NEt_3 + 10% MeOH). mp: 167 °C. Exact mass (APCI): m/z = calcd. for $C_{28}H_{26}ClN_4O_3$ [M + H$^+$] 501.1688, found 501.1702. Purity (HPLC, method B): 98%, R_t = 15.1 min ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C): δ = 8.93 (s, 1H, N$H_{cyclobutyl}$), 8.55 (s, 1H, N$H_{carbamoyl}$), 7.55–7.43 (m, 2H, 2-, 6-C$H_{Cl\text{-}phenyl}$), 7.42–7.32 (m, 4H, 2-, 3-, 5-, 6-CH_{phenyl}), 7.32–7.24 (m, 3H, 3-, 5-C$H_{Cl\text{-}phenyl}$, 4-CH_{phenyl}), 7.24–7.19 (m, 1H, 3-C$H_{aminophenyl}$), 7.16–7.08 (m, 2H, 4-, 6-C$H_{aminophenyl}$), 7.02 (t, J = 4.6 Hz, 1H, 5-C$H_{aminophenyl}$), 5.56 (d, J = 3.6 Hz, 1H, 2-C$H_{piperazine}$), 4.70 (d, J = 13.8 Hz, 1H, 3-CH$H_{piperazine}$), 4.05 (dt, J = 13.6 Hz, J = 3.4 Hz, 1H, 6-CH$H_{piperazine}$), 3.90 (d, J = 12.9 Hz, 1H, 5-CH$H_{piperazine}$), 3.81 (dd, J = 13.7, 4.4 Hz, 1H, 3-CH$H_{piperazine}$), 3.46 (td, J = 12.6, 4.0 Hz, 5-CH$H_{piperazine}$), 3.33–3.20 (m, 1H, 6-CH$H_{piperazine}$), 2.96 (H_2O), 2.24 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6, 100 °C): δ = 184.4 and 181.7 (2C, C-3, -4$_{cyclobutenyl}$), 167.6 (1C, C-1$_{cyclobutenyl}$), 165.3 (1C, C-2$_{cyclobutenyl}$), 154.5 (1C, C=O$_{carbamoyl}$), 138.7 (1C, C–1$_{Cl\text{-}phenyl}$), 138.1 (1C, C-1$_{phenyl}$), 136.7 (1C, C-1$_{aminophenyl}$), 131.4 (1C, C-2$_{aminophenyl}$), 129.9 (1C, C-3$_{aminophenyl}$), 128.1 and 126.1 (4C, C-2, -3, -5, -6$_{phenyl}$), 127.6 (2C, C-3, -5$_{Cl\text{-}phenyl}$), 126.6 (1C, C-4$_{phenyl}$), 125.6 (1C, C-6$_{aminophenyl}$), 125.1 (1C, C-4$_{aminophenyl}$), 124.2 (1C, C–5$_{Cl\text{-}phenyl}$), 121.0 (1C, C-2, -6$_{Cl\text{-}phenyl}$), 53.1 (1C, C-2$_{piperazine}$), 48.4 (1C, C-3$_{piperazine}$), 46.2 (1C, C-5$_{piperazine}$), 39.1 (1C, C-6$_{piperazine}$), 16.8 (1C, CH$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3306 (N–H), 3051, 3024, 2920 (C–H), 1790, 1674 (C=O), 1573, 1508 (C=C$_{arom}$).

4.9.42. N-(2-chlorophenyl)-4-{3,4-dioxo-2-[(2-methylphenyl)amino]cyclobut-1-en-1-yl}-2-phenylpiperazine-1-carboxamide (8e)

A round bottom flask was charged with *N*-(4-chlorophenyl)-2-phenylpiperazine-1-carboxamide (**6d**, 73 mg, 0.23 mmol, 1 eq) and MeOH (2 mL). 3-Methoxy-4-[(2-methylphenyl)amino]cyclobut-3-ene-1,2-dione (**5a**, 50 mg, 0.23 mmol, 1 eq) was added to the mixture. After 15 min, a clear solution was formed. The mixture was stirred overnight at room temperature. White precipitate was formed. The mixture was cooled to −20 °C, the solid was filtered off and washed with cold ethyl acetate. The residue was dried under reduced pressure to provide **8e** as a colorless solid, 80 mg (0.16 mmol, 69%), $C_{28}H_{25}ClN_4O_3$ (500.98 g/mol). mp: 210 °C. TLC (Silica): R_f = 0.47 (ethyl acetate:cyclohexane = 1:1 + 5% MeOH). Exact mass (ESI): m/z = calcd. for $C_{28}H_{24}ClN_4O_3$ [M-H$^+$] 499.1542, found 499.1548. Purity (HPLC, method D): 95%, R_t = 20.1 min ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C): δ = 8.90 (s, 1H, N$H_{cyclobutenyl}$), 7.90 (s, 1H, N$H_{carbamoyl}$), 7.65 (dd, J = 8.0, 1.5 Hz, 1H, 6-C$H_{Cl\text{-}phenyl}$), 7.41 (d, J = 4.6 Hz, 5H, 2-, 3-, 5-, 6-CH_{phenyl}, 3-C$H_{Cl\text{-}phenyl}$), 7.34–7.25 (m, 2H, 5-C$H_{Cl\text{-}phenyl}$, 4-CH_{phenyl}), 7.22 (dd, J = 6.2, 2.7 Hz, 1H, 3-C$H_{aminophenyl}$), 7.16–7.07 (m, 3H, 4-C$H_{Cl\text{-}phenyl}$, 4-, 6-C$H_{aminophenyl}$), 7.03 (dd, J = 6.7, 2.6 Hz, 1H, 5-C$H_{aminophenyl}$), 5.50 (t, J = 4.1 Hz, 1H, 2-C$H_{piperazine}$), 4.61 (dd, J = 13.9, 3.6 Hz, 1H, 2-C$H_{piperazine}$), 4.08 (dt, J = 13.7, 3.6 Hz, 1H, 6-CH$H_{piperazine}$), 3.97–3.86 (m, 2H, 3-CH$H_{piperazine}$, 5-CH$H_{piperazine}$), 3.56 (td, J = 12.6, 11.8, 4.0 Hz, 1H, 5-CH$H_{piperazine}$), 3.46–3.34 (m, 1H, 6-CH$H_{piperazine}$), 2.24 (s, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6, 100 °C): δ = 184.4 and 181.6 (2C, C-3, -4$_{cyclobutenyl}$), 167.7 (1C, C-1$_{cyclobutenyl}$), 165.3 (1C, C-2$_{cyclobutenyl}$), 154.5 (1C, C=O$_{carbamoyl}$), 138.1 (1C, C-1$_{phenyl}$), 136.7 (1C, C-1$_{aminophenyl}$), 135.9 (1C, C–1$_{Cl\text{-}phenyl}$), 131.4 (1C, C-2$_{aminophenyl}$), 129.9 (1C, C-3$_{aminophenyl}$), 128.6 (1C, C–3$_{Cl\text{-}phenyl}$), 128.1 (2C, C-3, -5$_{phenyl}$), 127.6 (1C, C–2$_{Cl\text{-}phenyl}$), 126.8 (1C, C–5$_{Cl\text{-}phenyl}$), 126.7 (1C, C-4$_{phenyl}$), 126.1 (2C, C-2, -6$_{phenyl}$), 125.6 (1C, C-4$_{aminophenyl}$), 125.3 (1C, C–6$_{Cl\text{-}phenyl}$), 125.1 (1C, C-6$_{aminophenyl}$), 124.7 (1C, C–4$_{Cl\text{-}phenyl}$), 53.9 (1C, C-2$_{piperazine}$), 48.5 (1C, C-3$_{piperazine}$), 46.2 (1C, C-5$_{piperazine}$), 38.9 (1C, C-6$_{piperazine}$), 16.8 (1C, CH$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3360, 3308 (C-H$_{arom}$), 2922, 2877 (C-H$_{aliph}$), 1676, 1660, 1593 (C=O), 1580 (C–N), 1559, 1504 (C-C$_{arom}$).

4.9.43. N-(3,4-dimethoxyphenyl)-4-{3,4-dioxo-2-[(2-methylphenyl)amino]cyclobut-1-en-1-yl}piperazine-1-carboxamide (8f)

N-(3,4-Dimethoxyphenyl)carbamoylpiperazin (**6e**, 100 mg, 0.38 mmol, 1 eq) was dissolved in DMF (2 mL) and 3-methoxy-4-[(2-methylphenyl)amino]cyclobut-3-ene-1,2-dione (**5a**, 82 mg, 0.38 mmol, 1 eq) was added. The mixture was stirred at room temperature for 3 d. The solvent was removed under reduced pressure and the crude product was purified by automated flash chromatography (25 g silica column, ethyl acetate, gradient: 2 cV 2% MeOH, 20 cV 2%–8% MeOH, 2 cV 8% MeOH) to provide **8f** as a colorless solid, 115 mg (0.26 mmol, 67%), $C_{24}H_{26}N_4O_5$ (450.50 g/mol). mp: 201 °C. TLC (Silica): R_f = 0.15 (ethyl acetate:cyclohexane = 1:1 + 5% MeOH). Exact mass (ESI): m/z = calcd. for $C_{24}H_{25}N_4O_5$ [M-H$^+$] 449.1830, found 449.1842. Purity (HPLC, method D): 98%, R_t = 15.9 min ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.25 (s, 1H, N$H_{cyclobutenyl}$), 8.45 (s, 1H, N$H_{carbamoyl}$), 7.25 (d, J = 6.9 Hz, 1H, 3-C$H_{aminophenyl}$), 7.21 (td, J = 7.6, 1.6 Hz, 1H, 5-C$H_{aminophenyl}$), 7.16–7.12 (m, 2H, 4-C$H_{aminophenyl}$, 2-C$H_{dimethoxyphenyl}$), 7.10 (dd, J = 7.8, 1.3 Hz, 1H, 6-C$H_{aminophenyl}$), 6.95 (dd, J = 8.7, 2.4 Hz, 1H, 6-C$H_{dimethoxyphenyl}$), 6.83 (d, J = 8.7 Hz, 1H, 5-C$H_{dimethoxyphenyl}$), 3.70 (s, 3H, 3-OC$H_{3,dimethoxyphenyl}$), 3.69 (s, 3H, 4-OC$H_{3,dimethoxyphenyl}$), 3.65 (broad, 4H, 3-, 5-C$H_{2,piperazine}$), 3.55–3.51 (m, 4H, 2-, 6-C$H_{,piperazine}$), 2.28 (s, 3H, 2-C$H_{3,aminophenyl}$). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 184.9 and 181.9 (2C, C-3, -4$_{cyclobutenyl}$), 167.4 (1C, C-1$_{cyclobutenyl}$), 165.4 (1C, C-2$_{cyclobutenyl}$), 154.9 (1C, C=O$_{carbamoyl}$), 148.4 (1C, C-3$_{dimethoxyphenyl}$), 144.1 (1C, C-

$4_{dimethoxyphenyl}$), 137.0 (1C, *C*-$1_{aminophenyl}$), 133.8 (1C, *C*-$1_{dimethoxyphenyl}$), 131.7 (1C, *C*-$2_{aminophenyl}$), 130.5 (1C, *C*-$3_{aminophenyl}$), 126.3 (1C, *C*-$5_{aminophenyl}$), 125.6 (1C, *C*-$4_{aminophenyl}$), 124.8 (1C, *C*-$6_{aminophenyl}$), 112.0 (1C, *C*-$4_{dimethoxyphenyl}$), 111.7 (1C, *C*-$6_{dimethoxyphenyl}$), 105.4 (1C, *C*-$1_{dimethoxyphenyl}$), 55.8 (1C, H_3CO-$4_{dimethoxyphenyl}$), 55.3 (1C, H_3CO-$3_{dimethoxyphenyl}$), 46.9 (2C, *C*-3, -$5_{piperazine}$), 43.6 (2C, *C*-2, -$6_{piperazine}$), 17.7 (1C, H_3C-$2_{aminophenyl}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3318, 3294 (N—H), 3210 (C-H_{arom}), 2936 (C-H_{aliph}), 1790, 1659, 1574 (C=O), 1508 (C=C_{arom}).

4.9.44. N-(4-aminophenyl)-4-{3,4-dioxo-2-[(2-methylphenyl) amino]cyclobut-1-en-1-yl}piperazine-1-carboxamide (8g)

N-(4-Aminophenyl)-4-{3,4-dioxo-2-[(2-methylphenyl)amino] cyclobut-1-en-1-yl}piperazine-1-carbox-amide (**8m**, 160 mg, 0.37 mmol) was dissolved in MeOH (30 mL) and Pd/C (100 mg) was added. Hydrogen was applied at a pressure of 1 bar and the mixture was stirred vigorously for 4 h. The solvent was removed *in vacuo* and the crude product was purified by automated flash chromatography (25 g silica column, dichloromethane, gradient: 1 cV 0% MeOH, 10 cV 0%–12% MeOH, 5 cV 12% MeOH). Afterwards, the obtained substance was purified by preparative HPLC (C18): 5 mL/min, gradient (ACN/H_2O) = 30 min (40/60) to (50/50), 2 min (50/50) to (100/0), 5 min (100/0), 5 min (100/0) to (40/60), 10 min (40/60) to provide **8g** as a yellow oil, 16 mg, (0.04 mmol, 11%), $C_{22}H_{23}N_5O_3$ (405.46 g/mol). TLC (Silica): 0.28 (dichloromethane + 10% MeOH + 1% Et_3N). Exact mass (ESI): *m*/*z* = calcd. for $C_{22}H_{22}N_5O_3$ [M-H^+] 404.1728, found 404.1734. Purity (HPLC, method D): 95%, R_t = 5.7 min ^{1}H NMR (600 MHz, MeOH-d_4): δ = 9.21 (s, 1H, N$H_{cyclobutenyl}$), 8.16 (s, 1H, NH_{urea}), 7.25–7.13 (m, 2H, 3-, 6-C$H_{aminophenyl}$), 7.14–7.04 (m, 2H, 4-, 5-C$H_{aminophenyl}$), 7.15–7.07 (m, 2H, 5-, 6-C$H_{aminophenyl}$), 6.99 (d, *J* = 8.7 Hz, 2H, 3-, 5-C$H_{4\text{-}aminophenyl}$), 6.44 (d, *J* = 8.7 Hz, 2H, 2-, 6-C$H_{4\text{-}aminophenyl}$), 4.72 (s, 2H, NH_2), 3.47 (m, 8H, $CH_{2,piperazine}$), 2.25 (s, 3H, CH_3). ^{13}C NMR (151 MHz, MeOH-d_4): δ = δ = 188.1 and 185.5 (2C, *C*-3, -$4_{cyclobutenyl}$), 170.9 and 168.5 (2C, *C*-1, -$2_{cyclobutenyl}$), 155.4 (1C, *C*=$O_{carbamoyl}$), 144.3 (1C, *C*-$4_{4\text{-}aminophenyl}$), 137.3 (1C, *C*-$1_{aminophenyl}$), 131.7 (1C, *C*-$2_{aminophenyl}$), 130.3 and 126.2 (2C, *C*-3, -$6_{aminophenyl}$), 128.9 (1C, *C*-$1_{4\text{-}aminophenyl}$), 125.4 and 124.7 (2C, *C*-4, -$5_{aminophenyl}$), 122.6 (2C, *C*-3, -$5_{4\text{-}aminophenyl}$), 113.8 (2C, *C*-2, -$6_{4\text{-}aminophenyl}$), 46.9 and 43.7 (4C, *C*$H_{2,piperazine}$), 17.7 (1C, H_3C-$2_{aminophenyl}$). ^{13}C signals were determined using gHSQC and gHMBC due to low signal intensity in the carbon spectrum. FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3352, 3314 (N—H), 2924 (C-H_{aliph}), 1790, 1674, 1574 (C=O), 1593 (C=C_{arom}).

4.9.45. N-(4-fluorophenyl)-4-{3,4-dioxo-2-[(2-methylphenyl) amino]cyclobut-1-en-1-yl}piperazine-1-carboxamide (8h)

To *N*-(4-Fluorophenyl)piperazine-1-carboxamide (**6g**, 50 mg, 0.22 mmol, 1 eq) dissolved in DMF (1.5 mL), 4-[(2-methylphenyl) amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione (**5a,** 48 mg, 0.22 mmol, 1 eq) was added. The mixture was stirred overnight at room temperature. Then, brine was added and the mixture it was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The residue was washed with MeOH and ethyl acetate to provide **8h** as a colorless solid, 52 mg (0.13 mmol, 58%), $C_{22}H_{21}FN_4O_3$ (408.16 g/mmol). TLC (Silica): R_f = 0.83 (ethyl acetate + 5% MeOH). Exact mass (APCI): *m*/*z* = calcd. for $C_{22}H_{22}FN_4O_3$ [M + H^+] 409.1670, found 409.1657. Purity (HPLC, method D): 97%, R_t = 17.2 min ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.25 (s, 1H, N$H_{cyclobutenyl}$), 8.65 (s, 1H, N$H_{carbamoyl}$), 7.48–7.42 (m, 2H, 2-, 6-C$H_{F\text{-}phenyl}$), 7.26–7.23 (m, 1H, 3-C$H_{aminophenyl}$), 7.20 (td, *J* = 7.6, 1.6 Hz, 1H, 6-C$H_{aminophenyl}$), 7.14 (td, *J* = 7.4, 1.4 Hz, 1H, 4-C$H_{aminophenyl}$), 7.11–7.05 (m, 3H, 3-, 5-C$H_{F\text{-}phenyl,}$ 5-C$H_{aminonphenyl}$), 3.65 (s, 4H, 3-, 5-C$H_{2,piperazine}$), 3.56–3.51 (m, 4H, 2-, 6-C$H_{2,piperazine}$), 2.28 (s, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 184.8 and 181.9 (2C, *C*-3, -$4_{cyclobutenyl}$), 167.4 and 165.4 (2C, *C*-1, -$2_{cyclobutenyl}$), 157.5 (d, *J* = 237 Hz, *C*–$4_{F\text{-}phenyl}$), 154.8 (1C, *C*=$O_{carbamoyl}$), 137.0 (1C, *C*-$1_{aminophenyl}$), 136.6 (d, *J* = 2.6 Hz, *C*–$1_{F\text{-}phenyl}$), 131.7 (1C, *C*-2 $_{aminophenyl}$), 130.5 (1C, *C*-3 $_{aminophenyl}$), 126.3 (1C, *C*-6 $_{aminophenyl}$), 125.6 (1C, *C*-4 $_{aminophenyl}$), 124.8 (1C, *C*-5 $_{aminophenyl}$), 121.4 (d, *J* = 7.6 Hz, 1C, *C*-2, -$6_{F\text{-}phenyl}$), 114.8 (d, *J* = 22.0 Hz, 1C, *C*-3, -$5_{F\text{-}phenyl}$), 46.9 (2C, *C*-3, -$5_{piperazine}$), 43.6 (2C, *C*-2, -$6_{piperazine}$), 17.7 (1C, CH_3). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3302, 3196 (C-H_{arom}), 1793, 1666, 1589 (C=O), 1578, 1545 (C-C_{arom}).

4.9.46. N-(4-chlorophenyl)-4-{3,4-dioxo-2-[(2-methylphenyl) amino]cyclobut-1-en-1-yl}piperazine-1-carboxamide (8i)

4-[(2-Methylphenyl)amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione **7** (50 mg, 0.18 mmol, 1 eq) was dissolved in toluene (8 mL) and acetonitrile (3 mL). 4-Chlorophenyl isocyanate (28 mg, 0.18 mmol, 1 eq) was added and the mixture was stirred at 50 °C over night. The solvent was removed *in vacuo*, then the residue was dissolved in MeOH (2 mL) and water (2 mL) was added. The mixture was cooled to 0 °C and the resulting precipitate was filtered off. The crude product was purified by flash chromatography (cyclohexane:ethyl acetate = 1:1 + 3% MeOH + 1% Et_3N, Ø = 2 cm, h = 16 cm, V = 20 mL) to provide **8i** as a colorless solid, 48 mg (0.11 mmol, 63%), $C_{22}H_{21}ClN_4O_3$ (424.89 g/mol). TLC (Silica): R_f = 0.74 (ethyl acetate + 5% MeOH + 1% Et_3N) mp: 154 °C. Exact mass (ESI): *m*/*z* = calcd. for $C_{22}H_{20}ClN_4O_3$ [M-H^+] 423.1229, found 423.1262. Purity (HPLC, method D): 97%, R_t = 18.5 min ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.24 (s, 1H, N$H_{cyclobutenyl}$), 8.73 (s, 1H, N$H_{carbamoyl}$), 7.50–7.47 (m, 2H, 2-, 6-C$H_{Cl\text{-}phenyl}$), 7.30–7.27 (m, 2H, 3-, 5-C$H_{Cl\text{-}phenyl}$), 7.26–7.23 (m, 1H, 3-C$H_{aminophenyl}$), 7.21–7.18 (m, 1H, 5-C$H_{aminophenyl}$), 7.14 (dd, *J* = 7.4, 1.5 Hz, 1H, 4-C$H_{aminophenyl}$), 7.10 (dd, *J* = 7.7, 1.4 Hz, 1H, 6-C$H_{aminophenyl}$), 3.66 (br, 4H, 3-, 5-C$H_{2,piperazine}$), 3.55 (br, 4H, 2-, 6-C$H_{2,piperazine}$), 2.28 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.8 and 181.9 (2C, *C*-3, -$4_{cyclobutenyl}$), 167.4 (1C, C-$1_{cyclobutenyl}$), 165.4 (1C, C-2 $_{cyclobutenyl}$), 154.5 (1C, *C*=O), 139.3 (1C, *C*–$1_{Cl\text{-}phenyl}$), 137.0 (1C, *C*-$1_{aminophenyl}$), 131.7 (1C, *C*-$2_{aminophenyl}$), 130.5 (1C, *C*-$3_{aminophenyl}$), 128.2 (2C, *C*-3, -$5_{Cl\text{-}phenyl}$), 126.2 (1C, *C*-$5_{aminophenyl}$), 125.6 (1C, *C*-$4_{aminophenyl}$), 125.5 (1C, *C*–$4_{Cl\text{-}phenyl}$), 124.8 (1C, *C*-$6_{aminophenyl}$), 121.0 (2C, *C*-2, -$6_{Cl\text{-}phenyl}$), 46.9 (2C, *C*-3, -$5_{piperazine}$), 43.6 (2C, *C*-2, -$6_{piperazine}$), 17.6 (1C, CH_3). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3201 (N—H), 3120, 3067 (C-H_{arom}), 2920, 2851 (C-H_{aliph}), 1794, 1670, 1651 (C=O), 1578 (C=O), 1616 (C—N), 1593, 1535 (C-C_{arom}).

4.9.47. N-(4-methylphenyl)-4-{3,4-dioxo-2-[(2-methylphenyl) amino]cyclobut-1-en-1-yl}piperazine-1-carboxamide (8j)

A flask was charged with 4-[(2-methylphenyl)amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione (**7**, 42 mg, 0.16 mmol, 1 eq), then toluene (2 mL) and *p*-tolyl isocyanate (20 μL, 0.16 mmol, 1 eq) were added. The mixture was stirred for 3 d at room temperature. Formed precipitate was filtered off and washed with water (4 mL) and toluene (4 mL). The crude product was purified by flash chromatography (ethyl acetate:cyclohexane = 4:1 + 1% formic acid, Ø = 3 cm, h = 18 cm, V = 20 mL) to provide **8j** as a colorless solid, 45 mg (0.11 mmol, 70%), $C_{23}H_{24}N_4O_3$ (404.47 g/mol). TLC (Silica): R_f = 0.27 (ethyl acetate:cyclohexane = 1:1 + 5% MeOH). mp: 244 °C. Exact mass (ESI): *m*/*z* = calcd. for $C_{23}H_{23}N_4O_3$ [M-H^+] 403.1776, found 403.1790. Purity (HPLC, method D): 97%, R_t = 17.7 min ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.27 (s, 1H, N$H_{cyclobutenyl}$), 8.50 (s, 1H, NH_{urea}), 7.33 (d, *J* = 8.5 Hz, 2H, 2-, 6-C$H_{4\text{-}methylphenyl}$), 7.16 (d, *J* = 7.6 Hz, 1H, 3-C$H_{aminophenyl}$), 7.15–7.07 (m, 2H, 5-, 6-C$H_{aminophenyl}$), 7.04 (d, *J* = 8.3 Hz, 2H, 3-, 5-C$H_{4\text{-}methylphenyl}$), 7.00 (t, *J* = 7.1 Hz, 1H, 4-C$H_{aminophenyl}$), 3.73 (s, 4H, 3-, 5-C$H_{2,piperazine}$), 3.56–3.50 (m, 4H, 2-, 6-C$H_{2,piperazine}$), 2.25 (s, 3H, C$H_{3,\ aminophenyl}$), 2.23 (s, 3H, C$H_{3,4\text{-}methylphenyl}$). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 183.5 (2C, C=$O_{cyclobutenyl}$), 168.3 and 166.5 (2C, *C*-1,

-2$_{cyclobutenyl}$), 154.9 (1C, C=O$_{urea}$), 137.7 (1C, *C*-1$_{4\text{-}methylphenyl}$), 131.2 (1C, *C*-2$_{aminophenyl}$), 130.7 (1C, *C*-4$_{4\text{-}methylphenyl}$), 130.1 (1C, *C*-3$_{aminophenyl}$), 128.7 (2C, *C*-3, -5$_{4\text{-}methylphenyl}$), 126.0 (1C, *C*-5$_{aminophenyl}$), 123.9 (2C, *C*-4, -6$_{aminophenyl}$), 119.9 (2C, *C*-2, -6$_{4\text{-}methylphenyl}$), 46.5 (2C, *C*-3, -5$_{piperazine}$), 43.8 (2C, *C*-2, -6$_{piperazine}$), 20.3 (1C, *C*H$_{3,4\text{-}methylphenyl}$), 17.9 (1C, *C*H$_{3,\ aminophenyl}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3296, 3192, 3124 (C-H$_{arom}$), 2914, 2859 (C-H$_{aliph}$), 1790, 1670, 1602 (C=O), 1574, 1521 (C-C$_{arom}$).

4.9.48. N-phenyl-4-{3,4-dioxo-2-[(2-methylphenyl)amino] cyclobut-1-en-1-yl}piperazine-1-carboxamide (8k)

4-[(2-Methylphenyl)amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione **7** (50 mg, 0.18 mmol, 1 eq) was dissolved in dry DMF (2 mL) and phenyl isocyanate (26 mg, 0.20 mmol, 1.2 eq) was added. The mixture was stirred over night at room temperature. Then, water (8 mL) was added and the mixture was extracted with dichloromethane (3 × 30 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by automated flash chromatography (25 g silica column, ethyl acetate, gradient: 5 cV 0% MeOH, 12 cV 0%–10% MeOH, 5 cV 10% MeOH). Afterwards, the obtained substance was purified by preparative HPLC (C18): 5 mL/min, gradient (ACN/H_2O) = 30 min (40/60) to (50/50), 2 min (50/50) to (100/0), 5 min (100/0), 5 min (100/0) to (40/60), 10 min (40/60) to provide **8k** as a colorless solid, 17 mg, (0.04 mmol, 24%), $C_{22}H_{22}N_4O_3$ (390.44 g/mol). TLC (Silica): 0.33 (ethyl acetate:cyclohexane = 1:1 + 10% MeOH). Exact mass (ESI): *m*/*z* = calcd. for $C_{22}H_{21}N_4O_3$ [M-H$^+$] 389.1619, found 389.1627. Purity (HPLC, method B): 99%, R_t = 10.4 min ^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ = 9.25 (s, 1H, N*H*$_{cyclobutenyl}$), 8.61 (s, 1H, N*H*$_{carbamoyl}$), 7.44 (dd, *J* = 8.7, 1.2 Hz, 2H, 2-, 6-C*H*$_{phenyl}$), 7.26–7.21 (m, 3H, 3-, 5-C*H*$_{phenyl}$, 3-C*H*$_{aminophenyl}$), 7.20 (td, *J* = 7.5, 1.6 Hz, 1H, 5-C*H*$_{aminophenyl}$), 7.12 (t, *J* = 8.4 Hz, 2H, 4-C*H*$_{aminophenyl}$), 7.10 (dd, *J* = 7.8, 1.3 Hz, 1H, 6-C*H*$_{aminophenyl}$), 6.94 (tt, *J* = 7.3, 1.2 Hz, 1H, 4-C*H*$_{phenyl}$), 3.66 (s, 4H, 3-, 5-C*H*$_{2,piperazine}$), 3.55 (s, 4H, 2-, 6-C*H*$_{2,piperazine}$), 2.28 (s, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ = 184.7 and 182.0 (2C, *C*-3, -4$_{cyclobutenyl}$), 167.5 (1C, *C*-1$_{cyclobutenyl}$), 165.5 (1C, *C*-2$_{cyclobutenyl}$), 140.3 (1C, *C*-1$_{phenyl}$), 137.1 (1C, *C*-1$_{aminophenyl}$), 131.7 (1C, *C*-2$_{aminophenyl}$), 130.4 (1C, *C*-3$_{aminophenyl}$), 128.3 (1C, *C*-3, -5$_{phenyl}$), 126.2 (1C, *C*-5$_{aminophenyl}$), 125.5 (1C, *C*-4$_{aminophenyl}$), 124.7 (1C, *C*-6$_{aminophenyl}$), 121.9 (1C, *C*-4$_{phenyl}$), 119.7 (1C, *C*-2, -6$_{phenyl}$), 46.9 (1C, *C*-3, -5$_{piperazine}$), 43.7 (1C, *C*-2, -6$_{piperazine}$), 17.7 (1C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3201 (N–H), 2913 (C-H$_{arom}$), 1794, 1670, 1578 (C=O), 1427 (C-C$_{arom}$).

4.9.49. N-[(4-tert-butyl)phenyl]-4-{3,4-dioxo-2-[(2-methylphenyl) amino]cyclobut-1-en-1-yl}piperazine-1-carboxamide (8l)

A flask was charged with 4-[(2-methylphenyl)amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione **7** (65 mg, 0.24 mmol, 1 eq), then toluene (2 mL) and (4-*tert*-butyl)phenyl isocyanate (42 mg, 0.24 mmol, 1eq) were added. The mixture was stirred overnight at roomtemperature. Then, formed colorless precipitate was filtered off and washed with ethyl acetate (2 mL). The crude product was purified by flash chromatography (ethyl acetate:cyclohexane = 1:1 + 3.5% MeOH, Ø = 3 cm, h = 18 cm, V = 20 mL) to provide **8l** as a colorless solid, 72 mg (0.16 mmol, 67%), $C_{26}H_{30}N_4O_3$ (446.55 g/mol). mp**:** 240 °C (dec.). TLC (Silica): R_f = 0.57 (ethyl acetate:cyclohexane = 1:1 + 5% MeOH). Exact mass (ESI): *m*/*z* = calcd. for $C_{26}H_{29}N_4O_3$ [M-H$^+$] 445.2245, found 445.2269. Purity (HPLC, method D): 98%, R_t = 20.3 min ^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ = 9.28 (1H, N*H*$_{cyclobutenyl}$), 8.54 (1H, N*H*$_{carbamoyl}$), 7.37–7.33 (m, 2H, 2-, 6-C*H*$_{t\text{-}butylphenyl}$), 7.28–7.22 (m, 2H, 3-, 5-C*H*$_{t\text{-}butylphenyl}$), 7.19 (d, *J* = 7.4 Hz, 1H, 3-C*H*$_{aminophenyl}$), 7.15 (t, *J* = 7.4 Hz, 1H, 5-C*H*$_{aminophenyl}$), 7.10 (dd, *J* = 7.9, 1.4 Hz, 1H, 6-C*H*$_{aminophenyl}$), 7.05 (t, *J* = 7.5 Hz, 1H, 4-C*H*$_{aminophenyl}$), 3.83–3.61 (m, 4H, 3-, 5-C*H*$_{2,piperazine}$), 3.56–3.51 (m, 4H, 2-, 6-C*H*$_{2,piperazine}$), 2.26 (s, 3H, C*H*$_{3,tolyl}$), 1.25 (s, 9H, C*H*$_{3,t\text{-}butylphenyl}$). ^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ = 184.00 (2C, *C*-3, -4$_{cyclobutenyl}$), 168.0 and 166.5 (2C, *C*-1, -2$_{cyclobutenyl}$), 154.9 (C=O$_{acetamide}$), 144.2 (1C, *C*-4$_{t\text{-}butylphenyl}$), 139.0 (1C, *C*-1$_{aminophenyl}$), 137.7 (1C, *C*-1$_{t\text{-}butylphenyl}$), 131.4 (1C, *C*-2$_{aminophenyl}$), 130.2 (1C, *C*-3$_{aminophenyl}$), 126.1 (1C, *C*-5$_{aminophenyl}$), 124.9 (1C, *C*-3, -5$_{t\text{-}butylphenyl}$), 124.5 (1C, *C*-4$_{aminophenyl}$), 124.2 (1C, *C*-6$_{aminophenyl}$), 119.5 (1C, *C*-2, -6$_{t\text{-}butylphenyl}$), 46.7 (2C, *C*-3, -5$_{piperazine}$), 43.8 (2C, *C*-2, -6$_{piperazine}$), 33.9 (1C, *C*(CH$_3$)$_3$), 31.3 (1C, *C*H$_{3,t\text{-}butylphenyl}$), 17.8 (1C, *C*H$_{3,aminophenyl}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3379, 3344 (N–H), 2947, 2906, 2862 (C-H$_{aliph}$), 1672, 1660, 1573 (C=O), 1514, 1510, 1460, 1417 (C-C$_{arom}$).

4.9.50. N-(4-nitrophenyl)-4-{3,4-dioxo-2-[(2-methylphenyl) amino]cyclobut-1-en-1-yl}piperazine-1-carboxamide (8 m)

A flask was charged with 4-[(2-Methylphenyl)amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione (**7**, 120 mg, 0.28 mmol, 1 eq) and 4-nitrophenyl isocyanate (59 mg, 0.36 mmol, 1.3 eq) under N_2 atmosphere. Subsequently, dry CH_2Cl_2 (10 mL) and triethylamine (78 μL, 0.56 mmol, 2 eq) were added and the mixture was stirred over night at room temperature. An aqueous, saturated solution of NH_4Cl (10 mL) was added and the mixture was extracted with ethyl acetate:methanol = 6/1 (3 × 30 mL, 3 × 40 mL). The crude product was purified by automated flash chromatography (25 g silica column, CH_2Cl_2, gradient: 2 cV 3% MeOH, 20 cV 3%–5% MeOH, 8 cV 5% MeOH) to provide 8 m as a colorless solid, 120 mg, (0.28 mmol, 98%), $C_{22}H_{21}N_5O_5$ (435.44 g/mol). TLC (Silica): 0.35 (ethyl acetate:cyclohexane = 1:1 + 10% MeOH). Exact mass (ESI): *m*/*z* = calcd. for $C_{22}H_{20}N_5O_5$ [M-H$^+$] 434.1470, found 434.1470. Purity (HPLC, method B): 96%, R_t = 12.2 min ^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ = 9.31 (s, 1H, N*H*$_{carbamoyl}$), 9.26 (s, 1H, N*H*$_{cyclobutenyl}$), 8.16 (d, *J* = 9.3 Hz, 2H, 3-, 5-C*H*$_{nitrophenyl}$), 7.72 (d, *J* = 9.3 Hz, 2H, 2-, 6-CH$_{nitrophenyl}$), 7.25 (dt, *J* = 7.4, 1.1 Hz, 1H, 3-C*H*$_{aminophenyl}$), 7.21 (td, *J* = 7.7, 1.6 Hz, 1H, 5-C*H*$_{aminophenyl}$), 7.14 (td, *J* = 7.5, 1.4 Hz, 1H, 4-C*H*$_{aminophenyl}$), 7.10 (dd, *J* = 7.8, 1.4 Hz, 1H, 6-C*H*$_{aminophenyl}$), 3.67 (s, 4H, 3-, 5-C*H*$_{2,piperazine}$), 3.60 (s, 4H, 2-, 6-C*H*$_{piperazine}$), 2.28 (s, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ = 184.8 and 182.0 (2C, *C*-3, -4$_{cyclobutenyl}$), 167.4 (1C, *C*-1$_{cyclobutenyl}$), 165.4 (1C, *C*-2$_{cyclobutenyl}$), 153.9 (1C, *C*=O$_{carbamoyl}$), 147.2 (1C, *C*-1$_{nitrophenyl}$), 141.0 (1C, *C*-4$_{nitrophenyl}$), 137.0 (1C, *C*-2$_{aminophenyl}$), 131.7 (1C, *C*-2$_{aminophenyl}$), 130.5 (1C, *C*-3$_{aminophenyl}$), 126.3 (1C, *C*-5$_{aminophenyl}$), 125.7 (1C, *C*-4$_{aminophenyl}$), 124.8 (1C, *C*-6$_{aminophenyl}$), 124.7 (2C, *C*-3, -5$_{nitrophenyl}$), 118.4 (2C, *C*-2, -6$_{nitrophenyl}$), 46.9 (1C, *C*-3, -5$_{piperazine}$), 43.8 (1C, *C*-2, -6$_{piperazine}$), 17.7 (1C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3352 (N–H), 2905 (C-H$_{arom}$), 1798, 1682, 1604 (C=O), 1573 (C-C$_{arom}$).

4.9.51. Methyl 4-(4-{3,4-dioxo-2-[(2-methylphenyl)amino] cyclobut-1-en-1-yl}piperazine-1-carboxamido)benzoate (8n)

4-[(2-Methylphenyl)amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione (**7**, 105 mg, 0.39 mmol, 1 eq) was dissolved in dry CH_2Cl_2 (4 mL) and Methyl 4-isocyanatobenzoate (82 mg, 0.46 mmol, 1.2 eq). Triethylamine (100 μL, 0.78 mmol, 2 eq) was added and the mixture was stirred over night at room temperature. The solvent was removed *in vacuo* and the crude substance was purified by automated flash chromatography (25 g silica column, ethyl acetate:cyclohexane = 1:1, gradient: 5 cV 3% MeOH, 20 cV 3%–10% MeOH, 5 cV 10% MeOH) to provide **8n** as a colorless solid, 133 mg, (0.30 mmol, 76%), $C_{24}H_{24}N_4O_5$ (448.48 g/mol). TLC (Silica): 0.19 (ethyl acetate:cyclohexane = 1:1 + 10% MeOH). Exact mass (ESI): *m*/*z* = calcd. for $C_{24}H_{23}N_4O_5$ [M-H$^+$] 447.1674, found 447.1694. Purity (HPLC, method B): 99%, R_t = 11.4 min ^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ = 9.25 (s, 1H, N*H*$_{cyclobutenyl}$), 9.01 (s, 1H, N*H*$_{carboxamidyl}$), 7.86 (d, *J* = 8.9 Hz, 2H, 3-, 5-C*H*$_{benzoyl}$), 7.61 (d, *J* = 8.8 Hz, 2H, 2-, 6-C*H*$_{benzoyl}$), 7.26–7.23 (m, 1H, 3-C*H*$_{aminophenyl}$), 7.20 (td, *J* = 7.5, 1.7 Hz, 1H, 5-C*H*$_{aminophenyl}$), 7.13 (td, *J* = 7.5, 1.4 Hz, 1H, 4-C*H*$_{aminophenyl}$), 7.10 (dd, *J* = 7.8, 1.4 Hz, 1H, 6-C*H*$_{aminophenyl}$), 3.80 (s, 3H, C*H*$_{3,benzoyl}$),

3.78–3.58 (m, 4H, 3-, 5-C$H_{2,piperazine}$), 3.59–3.51 (m, 4H, 2-, 6-C$H_{piperazine}$), 2.28 (s, 3H, C$H_{3,aminophenyl}$). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 184.8 and 182.0 (2C, *C*-3, *C*-4$_{cyclobutenyl}$), 167.5 (1C, *C*-1$_{cyclobutenyl}$), 166.0 (1C, *C*=O$_{benoyl}$), 165.4 (1C, *C*-2$_{cyclobutenyl}$), 154.2 (1C, *C*=O$_{carboxamidyl}$), 145.2 (1C, *C*-1$_{benzoyl}$), 137.1 (1C, *C*-1$_{aminophenyl}$), 131.7 (1C, *C*-2$_{aminophenyl}$), 130.5 (1C, *C*-3$_{aminophenyl}$), 130.0 (2C, *C*-3, -5$_{benzoyl}$), 126.3 (1C, *C*-5$_{aminophenyl}$), 125.6 (1C, *C*-4$_{aminophenyl}$), 124.8 (1C, *C*-6$_{aminophenyl}$), 122.4 (1C, *C*-4$_{benzoyl}$), 118.4 (2C, *C*-2, -6$_{benzoyl}$), 51.8 (1C, *C*H$_{3,benzoyl}$), 46.9 (2C, *C*-3, -5$_{piperazine}$), 43.8 (2C, *C*-2, -6$_{piperazine}$), 17.7 (1C, *C*H$_{3,aminophenyl}$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3345 (N–H), 2947 (C-H$_{arom}$), 1786, 1717, 1670, 1647 (C=O), 1573 (C-C$_{arom}$).

4.9.52. N-cyclohexyl-4-{3,4-dioxo-2-[(2-methylphenyl)amino] cyclobut-1-en-1-yl}piperazine-1-carboxamide (8°)

4-[(2-Methylphenyl)amino]-3-(piperazin-1-yl)cyclobut-1-ene-1,2-dione (**7**, 50 mg, 0.18 mmol, 1 eq) was dissolved in dry DMF (2 mL) and cyclohexyl isocyanate (25 mg, 0.20 mmol, 1.2 eq) was added. The mixture was stirred over night at room temperature. MeOH (5 mL) and silica (1 g) were added and the solvent was removed *in vacuo*. The crude substance was purified by was purified by automated flash chromatography (25 g silica column, ethyl acetate, gradient: 2 cV 0% MeOH, 15 cV 0%–10% MeOH) to provide **8o** as a colorless solid, 26 mg (0.07 mmol, 36%), $C_{22}H_{28}N_4O_3$ (396.49 g/mol). TLC (Silica): 0.30 (ethyl acetate:cyclohexane = 1:1 + 10% MeOH). Exact mass (ESI): *m*/*z* = calcd. for $C_{22}H_{29}N_4O_3$ [M + H$^+$] 397.2234, found 397.2240. Purity (HPLC, method B): 97%, R_t = 17.3 min ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.21 (s, 1H, N*H*$_{cyclobutenyl}$), 7.23 (d, *J* = 7.4 Hz, 1H, 3-C*H*$_{aminophenyl}$), 7.19 (td, *J* = 7.6, 1.3 Hz, 1H, 5-C*H*$_{aminophenyl}$), 7.13 (td, *J* = 7.4, 1.2 Hz, 1H, 4-C*H*$_{aminophenyl}$), 7.08 (d, *J* = 7.8 Hz, 1H, 6-C*H*$_{aminophenyl}$), 6.27 (d, *J* = 7.6 Hz, 1H, N*H*$_{carbamoyl}$), 3.55 (s, 4H, 2-, 6-C$H_{2,piperazine}$), 3.44–3.33 (m, 5H, 1-C*H*$_{cyclohexyl}$, 3-, 5-C$H_{2,piperazine}$), 2.27 (s, 3H, CH_3), 1.79–1.63 (m, 4H, 2-, 3-, 5-, 6-C*H*H$_{cyclohexyl}$), 1.56 (dt, *J* = 12.6, 3.4 Hz, 1H, 4-C*H*H$_{cyclohexyl}$), 1.30–1.00 (m, 5H, 2-, 3-, 4-, 5-, and 6-C*H*H$_{cyclohexyl}$). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 184.9 and 181.8 (2C, *C*-3, -4$_{cyclobutenyl}$), 167.4 (1C, *C*-1$_{cyclobutenyl}$), 165.3 (1C, *C*-2$_{cyclobutenyl}$), 156.4 (1C, *C*=O), 137.0 (1C, *C*-1$_{aminophenyl}$), 131.7 (1C, *C*-2$_{cyclobutenyl}$), 130.4 (1C, *C*-3$_{aminophenyl}$), 126.2 (1C, *C*-5$_{aminophenyl}$), 125.6 (1C, *C*-4$_{aminophenyl}$), 124.8 (1C, *C*-6$_{aminophenyl}$), 49.3 (1C, *C*-1$_{cyclohexyl}$), 46.9 (2C, *C*-2, -5$_{piperazine}$), 43.4 (2C, *C*-2, -6$_{piperazine}$), 33.1 (2C, *C*-2, -6$_{cyclohexyl}$), 25.4 (1C, *C*-4$_{cyclohexyl}$), 25.1 (1C, *C*-3, -5$_{cyclohexyl}$), 17.7 (1C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3372 (N–H), 2924 (C-H$_{arom}$), 1786, 1685, 1636 (C=O), 1581 (C-C$_{arom}$).

4.9.53. N-[4-(tert-butyl)phenyl]-4-[3,4-dioxo-2-(quinolin-5-ylamino)cyclobut-1-en-1-yl]piperazine-1-carboxamid (8p)

N-[4-(*tert*-butyl)phenyl]piperazine-1-carboxamid (**6f,** 128 mg, 0.59 mmol, 1 eq) and 3-methoxy-4-(quinolin-5-ylamino)cyclobut-3-ene-1,2-dione (**5b,** 50 mg, 0.59 mmol, 1 eq) were dissolved in dry DMF (3 mL) under N_2 atmosphere. Triethylamine (165 μL, 1.18 mmol, 2 eq) was added and the mixture was stirred over night at room temperature. Water (20 mL) was added and the mixture was extracted with ethyl acetate (4 × 40 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by automated flash chromatography (25 g silica column, gradient: 2 cV cyclohexane:ethyl acetate = 1:1, 8 cV cyclohexane:ethyl acetate = 1:1 to cyclohexane:ethyl acetate = 0:1, 7 cV cyclohexane:ethyl acetate = 0:1, 3 cV ethyl acetate:methanol = 1/0 to 9/1, 5 cV ethyl acetate:methanol = 9/1) to provide 8p as a colorless solid, 171 mg, (0.35 mmol, 60%), $C_{28}H_{29}N_5O_3$ (483.57 g/mol). TLC (Silica): 0.22 (ethyl acetate:cyclohexane = 1:1 + 10% MeOH). Exact mass (ESI): *m*/*z* = calcd. for $C_{28}H_{28}N_5O_3$ [M-H$^+$] 482.2198, found 482.2183. Purity (HPLC, method B): 97%, R_t = 13.2 min ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.91 (s, 1H, N*H*$_{cyclobutenyl}$), 8.94 (dd, *J* = 4.1, 1.7 Hz, 1H, 2-C*H*$_{aminoquinoline}$), 8.60 (ddd, *J* = 8.5, 1.7, 0.9 Hz, 1H, 4-C*H*$_{aminoquinoline}$), 8.56 (s, 1H, N*H*$_{carbamoyl}$), 7.87 (d, *J* = 8.5 Hz, 1H, 6-C*H*$_{aminoquinoline}$), 7.75 (dd, *J* = 8.4, 7.5 Hz, 1H, 7-C*H*$_{aminoquinoline}$), 7.60 (dd, *J* = 8.6, 4.1 Hz, 1H, 3-C*H*$_{aminoquinoline}$), 7.37–7.33 (m, 2H, 2-, 6-C$H_{t\text{-}butylphenyl}$), 7.33 (dd, *J* = 7.5, 1.1 Hz, 1H, 8-C*H*$_{aminoquinoline}$), 7.27–7.24 (m, 2H, 3-, 5-C*H*$_{aminoquinoline}$), 3.75 (s, 4H, 3-, 5-C$H_{2,piperazine}$), 3.58 (s, 4H, 2-, 6-C$H_{2,piperazine}$), 2.89 (DMF), 2.73 (DMF), 1.25 (s, 9H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 185.6 and 181.7 (2C, *C*-3, -4$_{cyclobutenyl}$), 168.1 (1C, *C*-1$_{cyclobutenyl}$), 165.0 (1C, *C*-2$_{cyclobutenyl}$), 154.9 (1C, *C*=O), 150.7 (1C, *C*-2$_{aminoquinoline}$), 148.1 (1C, *C*-8a$_{aminoquinoline}$), 144.3 (1C, *C*-4$_{t\text{-}butylphenyl}$), 137.6 (1C, *C*-1$_{t\text{-}butylphenyl}$), 134.4 (1C, *C*-5$_{aminoquinoline}$), 132.0 (1C, *C*-4$_{aminoquinoline}$), 129.0 (1C, *C*-7$_{aminoquinoline}$), 126.0 (1C, *C*-6$_{aminoquinoline}$), 125.0 (1C, *C*-3, -5$_{t\text{-}butylphenyl}$), 122.9 (1C, *C*-4a$_{aminoquinoline}$), 119.6 (1C, *C*-2, -6$_{t\text{-}butylphenyl}$), 47.0 (2C, *C*-3, -5$_{piperazine}$), 43.7 (2C, *C*-2, -6$_{piperazine}$), 33.9 (1C, *C*(CH$_3$)$_3$), 31.3 (3C, *C*H$_3$). FTIR (neat): $\tilde{\nu}$ (cm^{-1}) = 3482, 3397, 3183 (N–H), 2949 (C-H$_{aliph}$), 1790, 1658, 1620 (C=O), 1566, 1495 (C=C$_{arom}$).

5. Assays

5.1. Cells

Stable human embryonic kidney (HEK) 293 cells stably expressing human P2X7R (B'SYS GmbH) were maintained in 50/50 mix of Dulbecco's Modified Eagle Medium with F12 medium (DMEM/F12, Thermo Fisher Scientific) supplemented with 9% fetal calf serum (FCS), 1% Penicillin/Streptomycin (10.000 units penicillin and 10 mg streptomycin per mL in 0.9% NaCl, Sigma Aldrich) and G418 (100 μg/mL, Roche) in a tissue culture incubator at 37 °C and 5% CO_2 under humidified conditions. Cells were cultured in cell culture 75 cm^2 flasks and split every 2–4 days (1:3; 1:6; 1:10) once confluent.

The HEK293 cell line stably expressing human P2X4R (B'SYS GmbH) was cultured under same conditions using Puromycin (1.0 μg/ml, Thermo Fisher Scientific) as a selection antibiotic.

Chinese hamster ovary (CHO) cell lines stably expressing human P2X1R, P2X2R or P2X3R (B'SYS GmbH) were cultured under same conditions using Hygromycin (100 μg/ml, Invivogen) as a selection antibiotic.

5.2. YO-PRO1 uptake assay

HEK293 cells expressing P2X7R were seeded into black-walled Nunc 96 well optical bottom plates (Thermo Fisher Scientific) at 2.0–4.0 × 10^4 cells/well and incubated for 24–48 h. Cells were washed with assay buffer (280 mM Sucrose; 5.6 mM KCl; 0.5 mM $CaCl_2$; 10 mM D-Glucose; 10 mM HEPES; 5 mM N-methyl-D-glucamine (pH 7.42)) and were incubated with 2 μmol L^{-1} YO-PRO-1 Iodide (Sigma Aldrich) assay concentration in the presence or absence of 30 min preincubated antagonist at five different concentrations at 37 °C for 2 h. Upon application of final 150 nM BzBzATP (determined EC_{50} value under the assay conditions), uptake of the YO-PRO-1 dye was recorded in 0.2 s intervals by following the fluorescence change using a BertholdTech TriStar [2] plate reader (Software MicroWin Version 5.14, Ex: 485 nm, Em: 535 nm, lamp energy 10%) or a FlexStation® 3 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA, Software SoftMax7 Pro, endpoint protocol, well scan with read pattern "fill scan", ex: 485 nm, em: 535 nm). To obtain the concentration

dependent uptake inhibition by antagonists, the YO-PRO-1 uptake was plotted against concentrations of the compounds reaching from 10^{-5} to 10^{-9} M.

5.3. Ca^{2+} mobilization assay

Fluo-4 NW was prepared according to the manufacturer's directions. HEK293 cells were seeded into black-walled Nunc 96 well plates (Thermo Fisher Scientific) at 2.0–4.0 × 10^4 cells/well and incubated for 24–48 h at 37 °C.

The medium was removed, and the cells were washed using 100 μL HBSS including 20 mM HEPES. Loading with the fluorescent Ca^{2+} indicator Fluo-4 was performed at 37 °C for 1 h followed by loading at room temperature for additional 30 min. The changes of intracellular Ca^{2+} concentrations in presence of five different concentrations of antagonists (10^{-5} to 10^{-9} M) and application of ATP (concentration of determined EC_{50} value) were monitored over 200 s using a FlexStation® 3 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA, Software SoftMax7 Pro, ex: 494 nm, em: 516 nm). The concentration-dependent decrease of Ca^{2+} influx was plotted against the concentrations of the compounds (10^{-5} to 10^{-9} M).

5.4. Data analysis

The inhibition curves from three independent measurements each done in duplicate were fitted to the Hill-equation using GraphPad Prism software version 9.1.0 (GraphPad Software Inc. San Diego, CA, USA). The results represent the mean ± SEM (n = 3).

6. CCR2 assays

6.1. Cell culture

Tango™ CCR2-bla U2OS cells (Invitrogen, Carlsbad, CA) were cultured in McCoy's 5A medium supplemented with 10% (dialyzed) fetal calf serum, 2 mM glutamine, 0.1 mM nonessential amino acids, 25 mM HEPES, 1 mM sodium pyruvate, 200 IU/mL penicillin, 200 μg/mL streptomycin, 100 μg/mL G418, 40 μg/mL hygromycin, and 125 μg/mL zeocin in a humidified atmosphere at 37 °C and 5% CO_2. Cells were subcultured twice a week at a ratio of 1:6 on 10-cm diameter plates by trypsinization. For cell membrane preparation the cells were subcultured in 15-cm diameter plates using medium with dialyzed fetal calf serum. Medium with dialyzed fetal calf serum was also used for β-arrestin recruitment assays.

6.2. Cell membrane preparation

The Tango™ CCR2-bla U2OS cells were collected from 15-cm diameter plates by scraping in 5 mL phosphate-buffered saline and centrifuged at 200 g for 5 min. The pellets were resuspended in ice-cold buffer with 50 mM Tris-HCl (pH 7.4), and 5 mM $MgCl_2$ and homogenized using an UltraTurrax homogenizer (Heidolph Instruments Schwabach, Germany). Membrane fraction was separated from cytosolic fraction by centrifugation in an ultracentrifuge (Beckman Coulter Inc., Fullerton, CA, USA) at 100,000 g for 20 min at 4 °C. The pellet was resuspended in 10 mL ice-cold buffer (50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$), and the homogenization and centrifugation steps were repeated. The membrane pellet was resuspended in 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ and aliquotes of 250 μL were stored at −80 °C. The protein concentration was determined using the Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific, Waltham, MA, USA).

6.3. [^{3}H]CCR2-RA-[R] displacement assay

[^{3}H]CCR2-RA-[*R*] displacement assays on 96-well plates were performed in 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.1% CHAPS assay buffer. CCR2-bla U2OS cell membranes (20 μg per well) were incubated at 25 °C for 2h in the presence of ~6 nM [^{3}H]CCR2-RA-[*R*] (specific activity 41.7 Ci/mmol; Vitrax, Placentia, CA). All compounds were tested at a final concentration of 1 μM. In order to determine the total binding, a control without test compound was included, while non-specific binding was determined in the presence of 10 μM JNJ-2714191. The total assay volume was 100 μL. The final concentration of DMSO was 0.25%. The incubation was terminated by rapid vacuum filtration through GF/B 96-well filter plates (PerkinElmer, Waltham, MA), to separate the bound and free radioligand, using a PerkinElmer Filtermate-harvester (PerkinElmer, Groningen, The Netherlands). Filters were subsequently washed ten times with ice-cold 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.05% CHAPS wash buffer, and finally dried at 55 °C for 30 min. The filter-bound radioactivity was determined by scintillation spectrometry using a Microbeta2® 2450 microplate counter (PerkinElmer, Boston, MA), after addition of 25 μL MicroScint-20 (PerkinElmer, Groningen, The Netherlands) and 3h incubation.

6.4. Tango β-arrestin recruitment assay

U2OS-CCR2b cells, at a density of 10,000 cells per well, were seeded into black-wall, clear-bottom, 384-well assay plates (Corning). Cells were pre-incubated with 1 μM of antagonist for 30 min at room temperature before addition of 5 nM CCL2 (EC_{80} concentration). Cells were then incubated for 16 h at 37 °C and 5% CO_2. After 16h, LiveBLAzer™-FRET B/G substrate (Invitrogen) was added to the cells in the dark (8 μl per well) and incubated for 2 h at room temperature. Fluorescence emission was measured in an EnVision multilabel plate reader (PerkinElmer) at 460 nm and 535 nm, after excitation at 400 nm.

7. Patch-clamp experiments

7.1. Cell preparation

Previously described HEK293 cell line stably expressing human P2X7R were seeded in 1:200 or 1:300 dilution in 2.0 mL sterile round glass coverslips (MatTek Life Science) and incubated at 37 °C and 5% CO_2 overnight.

7.2. Patch-clamp recording

Whole-cell recordings of P2X7 receptor-expressing HEK293 cells were performed at room temperature using borosilicate glass pipettes (GC150TF-10, Clark Electromedical Instruments, Pangbourne, UK) connected to an EPC-10 amplifier (HEKA Electronics, Lambrecht, Germany). The typical electrode resistance was 4–5 MΩ, while series resistance was in the range of 8–15 MΩ. Series resistance compensation of ≥30% was routinely used. Voltage-clamp experiments on cultured cells were controlled by PatchMaster software (HEKA Electronics). Cells were held at a membrane potential of −60 mV (liquid junction potential was not corrected) and transient inward currents were evoked by

application of different concentrations of ATP (1 μM–5 mM) for 20 s. Peak current amplitude was determined. The following recording solutions were used: (1) Extracellular solution (in mM): 147 NaCl, 2 KCl, 10 HEPES, 1 $MgCl_2$, 2 $CaCl_2$, 13 glucose, pH 7.3 with NaOH. (2) Intracellular solution (in mM): 147 NaCl, 10 HEPES, 10 EGTA, pH 7.3 with NaOH. P2X7 receptor antagonists were dissolved in DMSO, added to the standard extracellular solution, and applied for a time period of at least 30 min before HEK cells were challenged with 400 μM ATP. The final DMSO concentration was 0.01% and the solvent was added to all test solutions. A multibarrel application pipette with a tip diameter of ~100 μm was used for test substance application close to the recorded cell. Each cell was tested with a control solution solely containing 0.01% DMSO and challenged 3–5 times with ATP in time intervals of 3 min. For determining the ATP doses response curve cells were challenged with different ATP concentrations. To assess the effects of P2X7 receptor inhibitors 400 μM of ATP was applied. When the same ATP concentration was applied several times, current responses were stable or tended to increase in some cases. The n numbers stated for dose-response profiles represent the number of ATP applications. For each condition, at least three different cells from different culture dishes were recorded. The density of the ATP-induced inward current was calculated by dividing the peak inward current at −60 mV by the membrane capacitance obtained during whole-cell recordings. Results are shown as mean ± SEM. Recordings were analyzed using FitMaster and Origin 7.5 software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We want to thank Elke Naβ for her excellent technical assistance. AJ thanks the German Research Foundation (DFG) for the financial support (JU 2966/2–1). AJ, LV, MH, TB thank the German Research Foundation (DFG) for the financial support (GRK2515/1).

List of abbreviations

CHO	Chinese hamster ovary
cV	column volume
dec	decomposition
DMEM	Dulbecco's modified Eagle's medium
DMF	*N,N*-dimethylformamide;
DMSO	dimethylsulfoxide;
HBSS	Hanks' balanced salt solution
HEK	human embryonic kidney cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
EGTA	ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N'-tetraacetic acid
SAR	structure-activity relationship;
SEM	standard error of the mean
THF	tetrahydrofuran
TM	transmembrane helix

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113838.

References

[1] A. Nicke, H.G. Bäumert, J. Rettinger, A. Eichele, G. Lambrecht, E. Mutschler, G. Schmalzing, P2X1 and P2X3 receptors form stable trimers: a novel structural motif of ligand-gated ion channels, EMBO J. 17 (1998) 3016–3028.
[2] A. Surprenant, F. Rassendren, E. Kawashima, R.A. North, G. Buell, The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7), Science 272 (1996) 735.
[3] R.A. North, Molecular physiology of P2X receptors, Physiol. Rev. 82 (2002) 1013–1067.
[4] T. Kawate, J.C. Michel, W.T. Birdsong, E. Gouaux, Crystal structure of the ATP-gated P2X4 ion channel in the closed state, Nature 460 (2009) 592–598.
[5] J. Wang, Y. Wang, W.-W. Cui, Y. Huang, Y. Yang, Y. Liu, W.-S. Zhao, X.-Y. Cheng, W.-S. Sun, P. Cao, et al., Druggable negative allosteric site of P2X3 receptors, Proc. Natl. Acad. Sci. Unit. States Am. 115 (2018) 4939.
[6] A. Karasawa, T. Kawate, Structural basis for subtype-specific inhibition of the P2X7 receptor, Elife 5 (2016), e22153.
[7] G. Burnstock, A. Nistri, B.S. Khakh, R. Giniatullin, ATP-gated P2X receptors in health and disease, Front. Cell. Neurosci. 8 (2014) 204.
[8] F. Di Virgilio, D. Dal Ben, A.C. Sarti, A.L. Giuliani, S. Falzoni, The P2X7 receptor in infection and inflammation, Immunity 47 (2017) 15–31.
[9] G. Burnstock, G.E. Knight, The potential of P2X7 receptors as a therapeutic target, including inflammation and tumour progression, Purinergic Signal. 14 (2018) 1–18.
[10] E. Adinolfi, M. Capece, F. Amoroso, E. de Marchi, A. Franceschini, Emerging roles of P2X receptors in cancer, Curr. Med. Chem. 22 (2015) 878–890.
[11] L.C. Denlinger, P.L. Fisette, J.A. Sommer, J.J. Watters, U. Prabhu, G.R. Dubyak, R.A. Proctor, P.J. Bertics, Cutting edge: the nucleotide receptor P2X7 contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide, Baltimore, Md, J. Immunol. 167 (1950) 1871–1876. **2001**.
[12] A.E. McCarthy, C. Yoshioka, S.E. Mansoor, Full-length P2X7 structures reveal how palmitoylation prevents channel desensitization, Cell 179 (2019) 659–670, e13.
[13] F. Di Virgilio, G. Schmalzing, F. Markwardt, The elusive P2X7 macropore, Trends Cell Biol. 28 (2018) 392–404.
[14] F. Di Virgilio, P. Chiozzi, S. Falzoni, D. Ferrari, J.M. Sanz, V. Venketaraman, O.R. Baricordi, Cytolytic P2X purinoceptors, Cell Death Differ. 5 (1998) 191–199.
[15] V.B. Mehta, J. Hart, M.D. Wewers, ATP-stimulated release of interleukin (IL)-1beta and IL-18 requires priming by lipopolysaccharide and is independent of caspase-1 cleavage, J. Biol. Chem. 276 (2001) 3820–3826.
[16] C. Clapp, N. Diaz-Lezama, E. Adan-Castro, G. Ramirez-Hernandez, B. Moreno-Carranza, A.C. Sarti, S. Falzoni, A. Solini, F. Di Virgilio, Pharmacological blockade of the P2X7 receptor reverses retinal damage in a rat model of type 1 diabetes, Acta Diabetol. 56 (2019) 1031–1036.
[17] C.B.M. Platania, G. Giurdanella, L. Di Paola, G.M. Leggio, F. Drago, S. Salomone, C. Bucolo, P2X7 receptor antagonism: implications in diabetic retinopathy, Biochem. Pharmacol. 138 (2017) 130–139.
[18] G.L. Romano, R. Amato, F. Lazzara, V. Porciatti, T.-H. Chou, F. Drago, C. Bucolo, P2X7 receptor antagonism preserves retinal ganglion cells in glaucomatous mice, Biochem. Pharmacol. 180 (2020) 114199.
[19] C.B.M. Platania, F. Lazzara, A. Fidilio, C.G. Fresta, F. Conti, G. Giurdanella, G.M. Leggio, S. Salomone, F. Drago, C. Bucolo, Blood-retinal barrier protection against high glucose damage: the role of P2X7 receptor, Biochem. Pharmacol. 168 (2019) 249–258.
[20] D. Ferrari, M. Villalba, P. Chiozzi, S. Falzoni, P. Ricciardi-Castagnoli, F. Di Virgilio, Mouse microglial cells express a plasma membrane pore gated by extracellular ATP, J. Immunol. 156 (1996) 1531.
[21] D. Irnich, R. Burgstahler, P. Grafe, P2 nucleotide receptors in peripheral nerve trunk, Drug Dev. Res. 52 (2001) 83–88.
[22] I.P. Chessell, J.P. Hatcher, C. Bountra, A.D. Michel, J.P. Hughes, P. Green, J. Egerton, M. Murfin, J. Richardson, W.L. Peck, et al., Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain, Pain 114 (2005) 386–396.
[23] D. Avanzato, T. Genova, A. Fiorio Pla, M. Bernardini, S. Bianco, B. Bussolati, D. Mancardi, E. Giraudo, F. Maione, P. Cassoni, et al., Activation of P2X7 and P2Y11 purinergic receptors inhibits migration and normalizes tumor-derived endothelial cells via cAMP signaling, Sci. Rep. 6 (2016) 32602.
[24] A. Giannuzzo, M. Saccomano, J. Napp, M. Ellegaard, F. Alves, I. Novak, Targeting of the P2X7 receptor in pancreatic cancer and stellate cells, Int. J. Canc. 139 (2016) 2540–2552.
[25] J. Xia, X. Yu, L. Tang, G. Li, T. He, P2X7 receptor stimulates breast cancer cell invasion and migration via the AKT pathway, Oncol. Rep. 34 (2015) 103–110.
[26] P. Pellegatti, L. Raffaghello, G. Bianchi, F. Piccardi, V. Pistoia, F. Di Virgilio, Increased level of extracellular ATP at tumor sites: in vivo imaging with plasma membrane luciferase, PloS One 3 (2008) e2599.
[27] J.-H. Park, D.R. Williams, J.-H. Lee, S.-D. Lee, J.-H. Lee, H. Ko, G.-E. Lee, S. Kim, J.-M. Lee, A. Abdelrahman, et al., Potent suppressive effects of 1-piperidinylimidazole based novel P2X7 receptor antagonists on cancer cell migration and invasion, J. Med. Chem. 59 (2016) 7410–7430.
[28] V. Salvestrini, S. Orecchioni, G. Talarico, F. Reggiani, C. Mazzetti, F. Bertolini, E. Orioli, E. Adinolfi, F. Di Virgilio, A. Pezzi, et al., Extracellular ATP induces apoptosis through P2X7R activation in acute myeloid leukemia cells but not in

normal hematopoietic stem cells, Oncotarget 8 (2017) 5895–5908.
[29] J.-H. Park, Y.-C. Kim, P2X7 receptor antagonists: a patent review (2010-2015), Expert Opin. Ther. Pat. 27 (2017) 257–267.
[30] D. Baudelet, E. Lipka, R. Millet, A. Ghinet, Involvement of the P2X7 purinergic receptor in inflammation: an update of antagonists series since 2009 and their promising therapeutic potential, Curr. Med. Chem. 22 (2015) 713–729.
[31] K. Sato, K. Seio, M. Sekine, Squaryl group as a new mimic of phosphate group in modified oligodeoxynucleotides: synthesis and properties of new oligodeoxynucleotide analogues containing an internucleotidic squaryldiamide linkage, J. Am. Chem. Soc. 124 (2002) 12715–12724.
[32] P. Betschmann, B. Bettencourt, D. Donnelly-Roberts, M. Friedman, J. George, G. Hirst, N. Josephsohn, D. Konopacki, B. Li, J. Maull, et al., Synthesis and activity of N-cyanoguanidine-piperazine P2X7 antagonists, Bioorg. Med. Chem. Lett 18 (2008) 3848–3851.
[33] J. Kalliomäki, B. Jonzon, K. Huizar, M. O'Malley, A. Andersson, D.M. Simpson, Evaluation of a novel chemokine receptor 2 (CCR2)-antagonist in painful diabetic polyneuropathy, Scandinavian J. Pain 4 (2013) 77–83.
[34] Molecular Operating Environment (Moe), Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2019, p. 2021, 01.