Development of kinase inhibitors and activity-based probes

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8 Summary and Future Prospects

Kinases play a role in many diseases including cancer, diabetes and infection diseases. For this reason, kinases are interesting drug targets. Inhibitors for some kinases are already in use as clinical drugs, however due to resistance and side effects, but also to target kinases related to diseases for which there is currently no treatment, research on the discovery of new classes of kinase inhibitors is imperative. To achieve this, not only new inhibitor classes need to be designed and synthesized, but also tools to profile kinases in their physiological context and to determine the selectivity of inhibitors are required. The research in this thesis has focused on the development of more potent AKT1 and FLT3 kinase inhibitors and on the synthesis and application of new chemical tools for the profiling of kinases involved in various types of cancers and other diseases. A brief

description of the function and the role of the kinases subject of study in this thesis is given in **Chapter 1**.

Over the past decades, numerous chemical profiling methods have been developed and used to study the biological functional role of kinases, and to profile the selectivity and targets of kinase inhibitors. In **Chapter 2,** the principles of three chemical profiling methods applied for kinases are reviewed and compared: activity-based protein profiling (ABPP), photoaffinity labeling and affinity-based profiling (AfBP). These three methods formed the common thread throughout this thesis.

H-89, an isoquinolinesulfonamide-based compound, is a well-known, potent PKA inhibitor. Analogues of this compound have been proven to inhibit AKT1 and other kinases, including the oncological target, FLT3. **Chapter 3** deals with the design and synthesis of a new set of isoquinolinesulfonamide-based inhibitors for FLT3 (Figure 1). These compounds all contain the isoquinolinesulfonamide core and variations are at the phenyl moiety. Variations were introduced at the ortho, meta or para position by Suzuki coupling using 34 different bulky (hetero)aromatic boronic acids (leading in total to a focused library of 102 compounds).

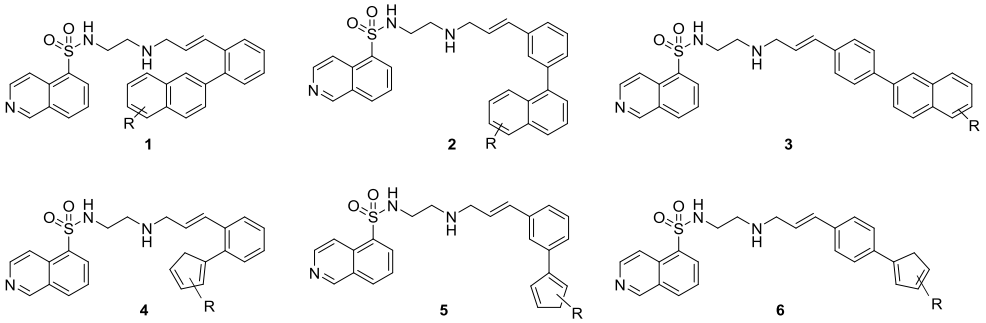


Figure 1. General structures (**1** – **6**) of the library containing 102 isoquinolinesulfonamide-based inhibitors for FLT3.

The inhibitory potency towards PKA, AKT1, AKT2 and FLT3 of the 102 newly synthesized isoquinolinesulfonamides together with 137 isoquinolinesulfonamides previously prepared[1] are discussed in **Chapter 4**. The effect of these 239 compounds on PKA, AKT1, AKT2 and FLT3 was evaluated using a TR-FRET kinase activity assay. The most active AKT1 and AKT2 inhibitors contain a bulky heteroaromatic group at the ortho position (Figure 2). It is clearly that both the kinases AKT1 and AKT2 favor the same kind of compounds, which make it hard to find active and selective inhibitors towards AKT1. The most active PKA inhibitors of the series proved to be compounds **15** and **16**. These compounds have the same conformation and contain an aromatic group at the para position, just like lead compound H-89. Apparently, electron-donating groups at this position are favourable for PKA inhibition.

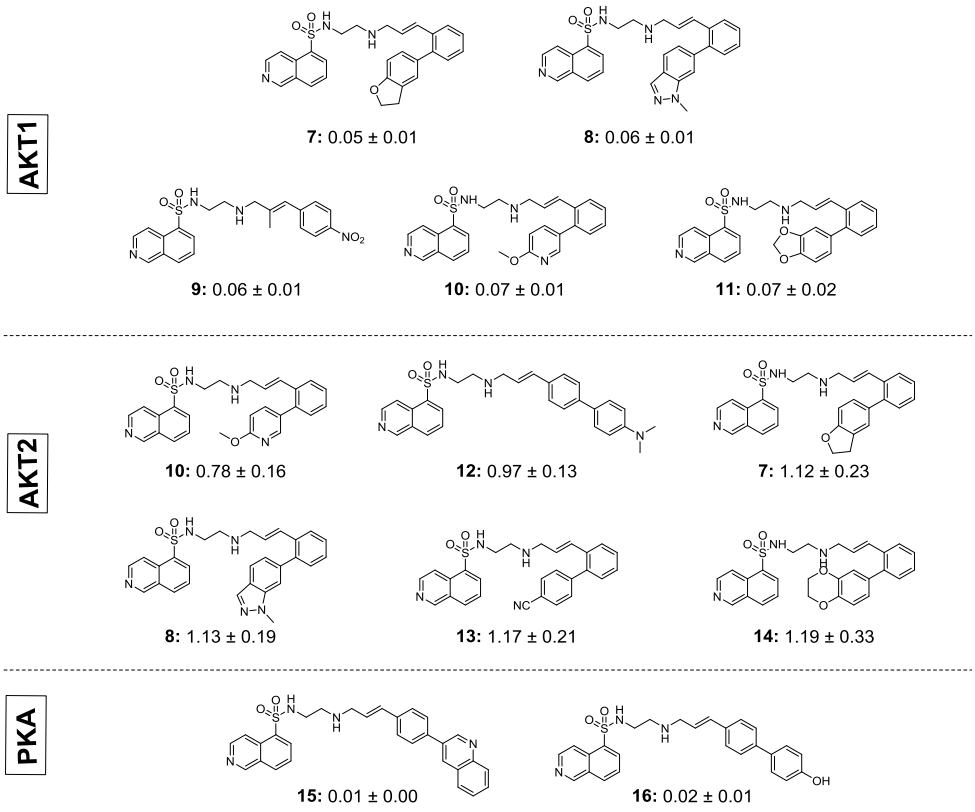


Figure 2. K_i values (μM) given for a selection of AKT1 (**7 – 11**), AKT2 (**7, 8, 10, 12 - 14**) and PKA (**15 - 16**) inhibitors, which are more active than lead compound H-89 based on their K_i values.

Inhibition of both AKT1 and AKT2 has been shown to be lethal in mice, and the ability to inhibit one selectively over the other is therefor of medical importance. The most selective AKT1 over AKT2 inhibitors proved to be compounds **17 – 19** (Figure 3). A methyl substituent at the linker alkene contributes to AKT1 selectivity over AKT2. Next to this, introduction of a para-halogen at the phenyl ring appears to improve the selectivity of AKT1 over AKT2. The configuration of the alkene moiety (E or Z) appears to play a role as well. Compounds **8, 10**, and **20 – 22** (Figure 3) are the most selective AKT1 over PKA inhibitors. These compounds contain aromatic groups having oxygen or nitrogen groups at the ortho position.

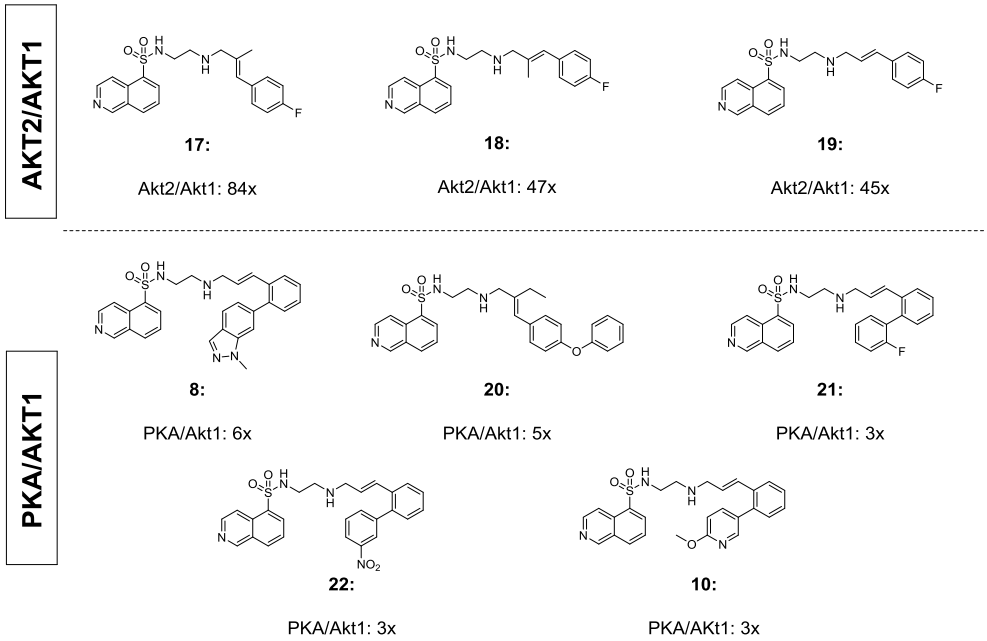


Figure 3. The most selective AKT1 over AKT2 (**17 – 19**) and AKT1 over PKA inhibitors (**8, 10 and 20 – 22**) based on their K_i value differences.

To obtain a more selective and active AKT1 inhibitor, it may be of interest to study the effect of incorporation of a fluorine group at the para position, which is important for selectivity, and a bulky heteroaromatic group at the ortho position, which contributes to enhancement of activity. Suggested compounds featuring these properties are **23** and **24** (Figure 4). Furthermore, a methyl group at the alkene site contributes to activity and selectivity as well, which suggests compounds **25** and **26** as future synthetic targets.

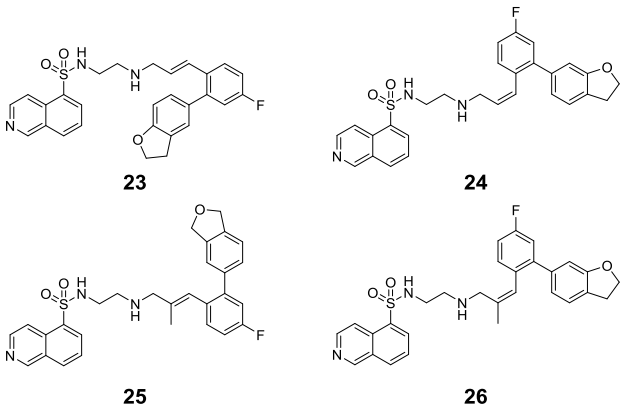


Figure 4. Suggested isoquinolinesulfonamide inhibitors selective for FLT3.

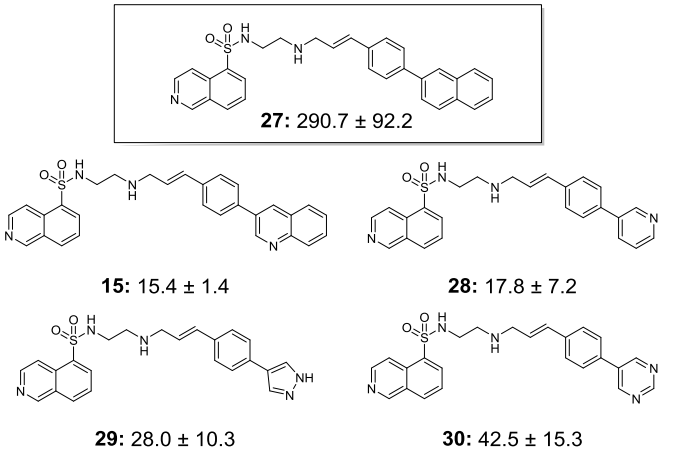


Figure 5. IC_{50} values (nM) given for a selection of FLT3 inhibitors (**15, 28 – 30**), which are more active than lead compound **27** based on their K_I values.

Another kinase studied in this Thesis is FLT3. The most active inhibitors for this kinase are compounds **15**, **28 – 30** (Figure 5). They all have a heteroaromatic group at the para position and appeared to be at least 7 times more active as FLT3 inhibitors than the lead compound (**27**).

Based on these SAR findings, a new set of FLT3 inhibitors (**31 – 36**) can be designed having an electron donating nitrogen-atom containing aromatic group at the para position. Some proposed structural analogues are depicted in Figure 6.

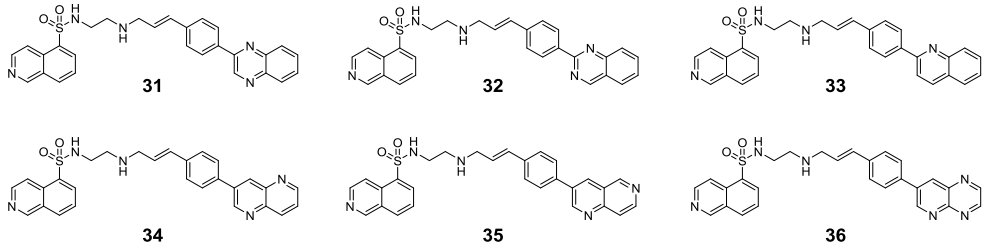


Figure 6. Suggested isoquinolinesulfonamide inhibitors selective for FLT3.

In the second part of this thesis, the synthesis and biological evaluation of chemical tools for profiling of kinases have been described. Three different methods for chemical profiling have been highlighted: ABPP, photo-affinity labeling and AfBP. **Chapter 5** describes the design and synthesis of two types of ABPs for labeling of Bruton's tyrosine kinase (BTK), namely direct (**37** and **38**) and two-step bioorthogonal (**39** – **41**) BTK activity-based probes (Figure 7).

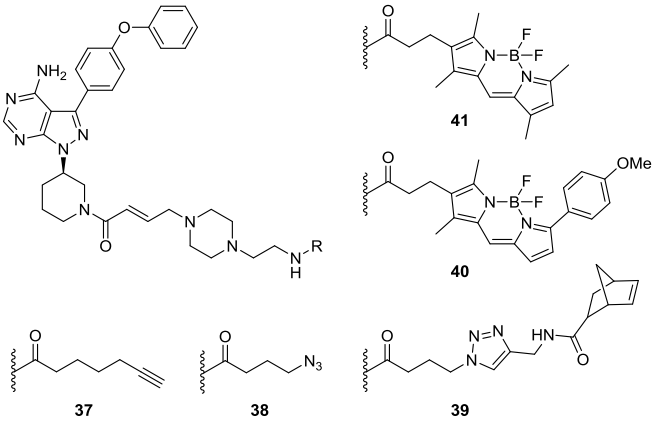
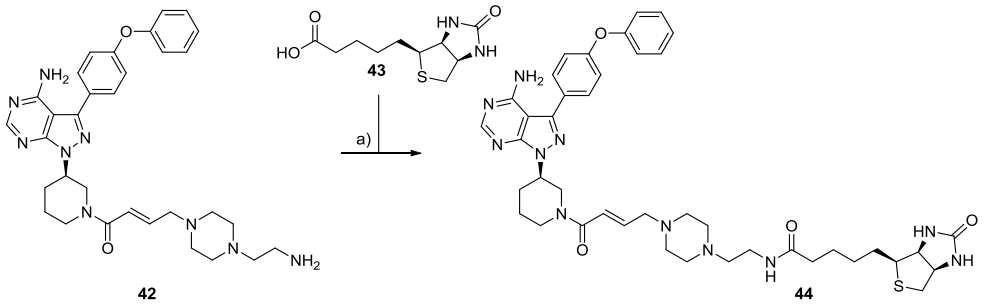


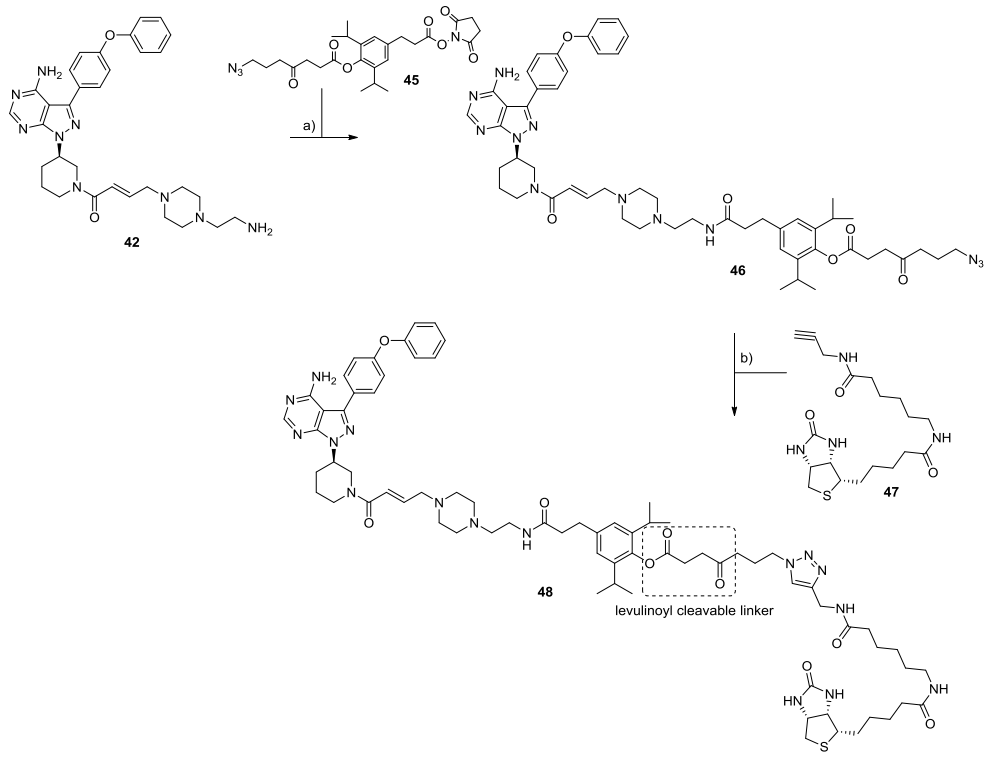
Figure 7. Structures of direct (**37** and **38**) and two-step bioorthogonal (**39 – 41**) BTK activity-based probes.

The core of these probes is based on the covalent inhibitor ibrutinib, which forms a covalent bond by attack of a cysteine thiol in the BTK active site to the acrylamide moiety of ibrutinib. All five probes (**37** – **41**) contain an acryl amide group as does ibrutinib, and all probes were able to label BTK. The direct probes **37** and **38** label BTK in Ramos cell extract and in living Ramos cells. Two-step bioorthogonal labeling of BTK using the probes **39** – **40** was also possible in *in vitro* and *in situ* experiments.

It is not always favorable to perform BTK labeling under fluorescent conditions, for instance when background fluorescence is an issue. In addition, an important method to identify target proteins and to determine the exact site and mechanism of ABP binding is by analysis of labeled proteins using mass spectrometry. In this case, it is usual to label proteins with a biotin-tagged ABP and subsequently enrich them by affinity-purification using streptavidin-beads. To allow for identification of the targets of ibrutinib, ibrutinib-biotin **44** has been synthesized by condensation (HATU, DiPEA) with biotin (**43**) (Scheme 1).



Scheme 1. Synthesis of ibrutinib-biotin **44**. Reagents and conditions: a) **43**, HATU, TEA, DMF, 26.5% after RP-HPLC.



Scheme 2. Synthesis of immobilized dasatinib **3**. Reagents and conditions: a) **45**, DiPEA, DMF, 36% after RP-HPLC; b) **47**, 1M $CuSO_4$, 1M Na L-ascorbate, 23% after RP-HPLC.

A disadvantage of ABPs featuring a biotin moiety is that the release of captured proteins from the beads requires harsh conditions and may result in contamination of the purified sample with endogenous biotinylated proteins. In such situations, an ABP containing a biotin in combination with a cleavable linker may form an attractive alternative. Therefore, ibrutinib-cleavable levulinoyl linker-biotin **48** was designed and synthesized (Scheme 2). This linker can be cleaved by treatment with an excess of hydrazine after protein enrichment.[2]

The synthesis of ibrutinib-cleavable levulinoyl linker-biotin **48** commenced with reacting crude amine **42** with the ester-based levulinoyl linker succinimide **45** to obtain azide **46**. A copper(I)-catalysed click ligation to alkyne-modified biotin **47** was then performed yielding the ibrutinib cleavable levulinoyl linker biotin **48** after HPLC purification. Both biotin probes (**44** and **48**) were able to select and visualize on SDS PAGE BTK from cell extracts. The utility of these probes in comparative and competitive ABPP on this drug target needs to be further investigated, though.

Not all kinases contain a nucleophilic moiety in the active site as BTK. To label these kinases covalently another type of chemical tool is required. **Chapter 6** describes the synthesis and biological evaluation of two diazirine-functionalized photocrosslinking probes (**49** and **50**, Figure 8) for labeling of PKA and AKT1, which are kinases without a nucleophilic group in the active site. Both probes are based on the core of H-89, which is proven to inhibit PKA and AKT1, and contain a diazirine group. Diazirine will bind covalently to the kinase upon UV activation. As well, these probes feature an azide group, which serves as a bioorthogonal ligation handle.

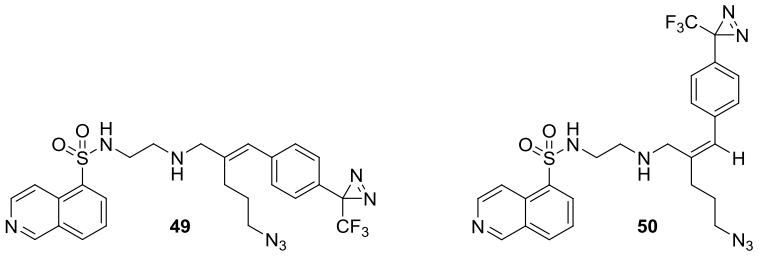
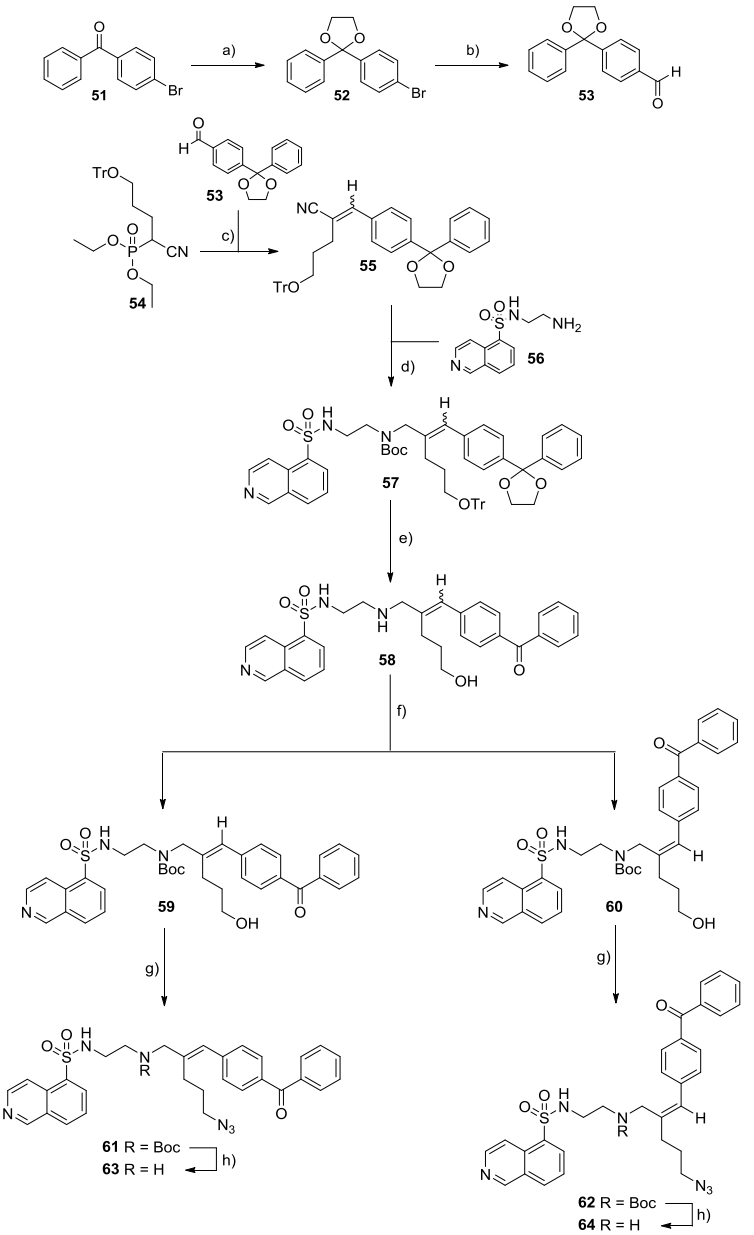


Figure 8. Structures of diazirine-functionalized photocrosslinking probes **49** and **50**.

According to a single point kinase active site-directed competition binding Kinome*scan*™ assay, in which the capability of the probes to inhibit a panel of 111 human kinases was examined, both probes **49** and **50** have a preference to inhibit Ser/Thr kinases including PKA and AKT1. Following this, both photocrosslinking probes were subjected to labeling experiments in which they showed to photocrosslink recombinant PKA and AKT1 efficiently and specifically upon UV activation. Other photocrosslinking groups that have been used in the past include benzophenone moieties. It would be of interest to investigate the benzophenone analogues **63** and **64** (Scheme 3) on their differences with respect to inhibitory potency and labeling efficiency. For the synthesis of both the probes, the benzophenone part can be obtained by protection of bromo-ketone **51** under Dean-Stark conditions giving compound **52**. Compound **52** was in turn lithiated using *n*-BuLi before reaction with DMF to form aldehyde **53**. Next, a Horner-Wadsworth-Emmons (HWE) reaction between benzaldehyde **53** and phosphonate **54** resulted in α-substituted cinnamonitrile **55** as a 1/1 mixture of *E/Z* isomers in 95% yield. Since separation of the isomers in the nitrile-stage was partially successful, and the nitriles were observed to isomerise during the ensuing transimination, the mixture of nitriles was used in the ensuing reaction. Subsequently, *E/Z* cinnamonitrile mixture **55** was used in the four-step-one-pot trans-imination procedure according to Brussee *et al*.[3], which has been described



Scheme 3. Synthesis of benzophenone-functionalized photocrosslinking probes **63** and **64**. Reagents and conditions: a) ethylene glycol, *p*-toluenesulfonic acid monohydrate, benzene, Δ, 71%; b) *n*-BuLi, DMF, THF, -78 °C – RT, 80%; c) NaH, **53**, 0 °C, THF, *E/Z* = 1/1, 95%; d) i) DiBAlH, -78 °C, Et_2O; ii) MeOH, -100 °C; iii) **56**, MeOH, RT; iv) $NaBH_4$, -10 °C to RT; e) THF, HCl, H_2O; f) Boc_2O, TEA, DCM, 0 °C, 6.7% (**59)** and 18% (**60**) after RP-HPLC; g) i) TsCl, DCM, TEA, DMAP; ii) NaN_3, DMF; h) DCM/TFA 1/1, v/v, RT, 44% (**63**) and 38% (**64**) after RP-HPLC.

in detail in Chapter 6, and the crude *E/Z* mixture of trityl protected isoquinolineamide **57** was obtained. The Boc-groups and trityl-groups in **57** were deprotected under acidic conditions to obtain a crude mixture of **58**, and installment of the Boc-group to yield isoquinolinesulfonamide **59** and **60** as crude *E/Z* mixture. HPLC purification allowed separation and isolation of both isomers. Both alcohols **59** and **60** were tosylated followed by substitution using NaN_3 to obtain compounds **61** and **62**. At last, deprotection of the Boc-group furnished the photo-affinity based probes **63** and **64** in 44% and 38% yield after HPLC purification.
Probe **3** has the same double bond geometry as the parent inhibitor H-89 (**1**) and inhibits both PKA and AKT1 with a high potency. A change of the double bond configuration to (*Z*) does not have a significant influence on the inhibition of PKA but for AKT1 a partial loss of potency was observed. In this study, the recombinant kinases in pure form as well as in mixtures with control proteins could be labelled; additionally a variety of control experiments reveal that the probes act in an affinity-based manner on active enzymes and that UV activation is required for labeling have been performed.
A third chemical profiling method for kinases has been described in **Chapter 7**. An affinity-based probe (**65**, Figure 9) has been synthesized to investigate the drug targets of dasatinib in primary CLL. This affinity-based probe is obtained by coupling of the kinase inhibitor and clinical drug, dasatinib to NHS-activated sepharose beads. In this method, the targets are reversibly bound by the probe and are analyzed by SDS-PAGE and/or LC/MS after they are eluted from the immobilized dasatinib probe **65**.

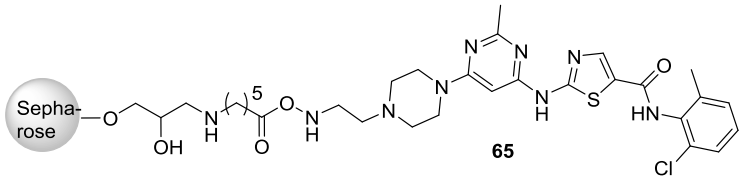
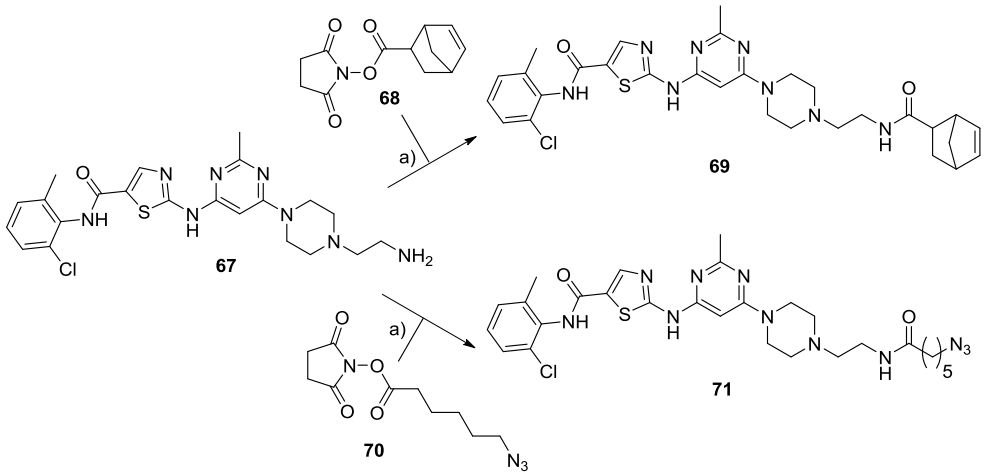


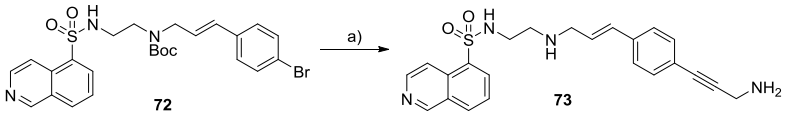
Figure 9. Structure of affinity-based probe **65**, which is based on the structure of dasatinib.

In this study it was found that the important targets of dasatinib in primary CLL were c-Abl and BTK. Furthermore, other identified peptides corresponded to the kinases Lyn, Csk, Fyn, Yes, Src and Lck.
An alternative strategy to identify the targets of dasatinib would be to use dasatinib-based probes containing a ligation handle. Two such two-step probes were designed and synthesized, one featuring a norbornene (**69**) and one containing an azide as ligation handles (**71**). The synthesis of both compounds is depicted in Scheme 4. Both probes were obtained by a reaction between the dasatinib-amine **67** with the corresponding OSu-functionalized tag. In the future, the utility of these probes needs to be studied in detail.



Scheme 4. Synthesis of dasatinib-norbornene **69** and dasatinib-azide **71**. Reagents and conditions: a) **68** or **70**, DiPEA, DMF, **69:** 18%; **71**: 28% after RP-HPLC.

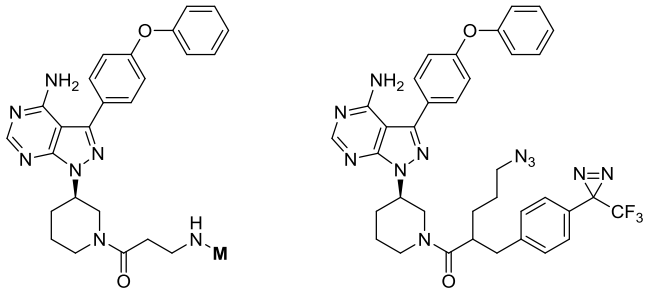
Affinity-based protein profiling can also be useful to study the targets and investigate the drug mechanism of H-89. Therefore, the Boc-protected H-89 **72** was converted into the amine containing isoquinolineamide **73** by a Sonogashira cross-coupling (Scheme 5). This probe can be in turn immobilized by coupling to a NHS-Sepharose bead, which can then be used to isolate the targets of H-89.



Scheme 5. Synthesis of affinity-based probe **73**, based on H-89. Reagents and conditions: a) i) *N*-Boc-propargylamine, pyrrolidine, CuI, $Pd(PPh_3)_4$, DMF; ii) TFA/DCM 1/1 v/v, RT, 34% after RP-HPLC.

In this study, three chemical profiling methods to label kinases have been described including their advantages and disadvantages. To have a true comparison of these three methods, an affinity-based probe, a photoaffinity based probe and an activity-based probe have to be synthesized based on the same small molecule in the future. For example, next to the direct and two-step bioorthogonal ibrutinib probes (**37-41**), an affinity-based probe and a photoaffinity based probe, such as compounds **74** and **75**, respectively should be developed (Figure 10). For affinity-based profiling, the Michael-acceptor moiety has been displaced by an alkyl amine group, which in turn can be immobilized on a matrix. In the case of photoaffinity labeling, the acrylamide group has been displaced by the aryldiazirine, which serves

as the photoaffinity reactive group and by an azide group, which serves as the ligation handle.



74 **75**

Figure 10. Designs of a ibrutinib-based affinity and photoaffinity probe **74** and **75**, respectively. **M** = matrix.

In this thesis, new powerful tools and assays have been developed that unite the fields of synthetic chemistry, protein biochemistry and cell biology for the global analysis of kinase expression and function. The value of chemical profiling as a method for functional proteome analysis has been further highlighted by its application as a screen to evaluate the potency and selectivity of kinase inhibitors. However, even though the numerous considerable advances that has been made to date, chemical approaches for kinome analysis still face significant technical challenges. Currently, 1D or 2D electrophoresis is mostly used to analyze probe-labeled proteins. A disadvantage of this method is the limited resolving power especially in cases where large number of proteins with similar molecular mass has to be analyzed. The use of LC-MS to analyze the large number of proteins overcome this problem due to its higher resolving power, but LC-MS has a much lower sample throughput. So the right strategy has to be chosen depending on the biological question being addressed. In addition, more selective probes are needed to explore the function of a particular kinase and also more general probes are needed that are able to label every single kinase in a cell, which are useful in studies of inhibitor selectivity. In either case, improvement of both the chemical and technical components of chemical profiling has to be made in the near future for a more in-depth analysis of the kinome and the targets of kinase inhibitors. These chemical tools, as developed in this thesis, provide new handles to define the biology of kinases in cells. The exploration of the chemical space around the H-89 building block shows how new and more specific inhibitors can be developed for this important enzyme class. The probes can then be used for profiling the *in vivo* effects.

Experimental

General: Tetrahydrofuran (THF) was distilled over $LiAlH_4$ before use. Acetonitrile (ACN), dichloromethane (DCM), N,N-dimethylformamide (DMF), methanol (MeOH) and trifluoroacetic acid (TFA) were of peptide synthesis grade, purchased at Biosolve, and used as received. All general chemicals (Fluka, Acros, Merck, Aldrich, Sigma) were used as received. Traces of water were removed from reagents used in reactions that require anhydrous conditions by coevaporation with toluene. Solvents that were used in reactions were stored over 4Å molecular sieves, except methanol and acetonitrile, which were stored over 3Å molecular sieves. Molecular sieves were flame dried before use. Unless noted otherwise all reactions were performed under an argon atmosphere. Column chromatography was performed on Silicycle Silia-P Flash Silica Gel, with a particle size of 40 – 63 µm. The eluents toluene and ethyl acetate were distilled prior to use. TLC analysis was conducted on Merck aluminium sheets (Silica gel 60 F254). Compounds were visualized by UV absorption (254 nm), by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in 10% sulphuric acid, a solution of $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in water, or ninhydrin (0.75 g/L) and acetic acid (12.5 mL/L) in ethanol, where appropriate, followed by charring at ca. 150°C. ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker DMX-400 (400 MHz) or a Bruker DMX-600 (600 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane (^{1}H NMR) or $CDCl_3$ (^{13}C NMR) as internal standard. Mass spectra were recorded on a PE/Sciex API 165 instrument equipped with an Electrospray Interface (ESI) (Perkin-Elmer). High-resolution MS (HRMS) spectra were recorded with a Finnigan LTQ-FT (Thermo Electron). IR spectra were recorded on a Shimadzu FTIR-8300 and absorptions are given in cm^{-1}. Optical rotations $[\alpha]_D^{23}$ were recorded on a Propol automatic polarimeter at room temperature. LC-MS analysis was performed on a Jasco HPLC system with a Phenomenex Gemini 3 µm C18 50 x 4.6 mm column (detection simultaneously at 214 and 254 nm), coupled to a PE Sciex API 165 mass spectrometer with ESI. HPLC gradients were 10 → 90%, 0 → 50% or 10 → 50% ACN in 0.1% TFA/H_2O. Chiral HPLC analysis was performed on a Spectroflow 757 system (ABI Analytical Kratos Division, detection at 254 nm) equipped with a Chiralcel OD column (150 x 4.6 mm). The compounds were purified on a Gilson HPLC system coupled to a Phenomenex Gemini 5 µm 250 x 10 mm column and a GX281 fraction collector. The used gradients were either 0 → 30% or 10 → 40% ACN in 0.1% TFA/water, depending on the lipophilicity of the product. Appropriate fractions were pooled, and concentrated in a Christ rotary vacuum concentrator overnight at room temperature at 0.1 mbar.

Ibrutinib-biotin (44)

HATU (1.1 eq., 42 mg, 0.11 mmol) was added to a solution of biotin (2 eq., 50 mg, 0.20 mmol) and TEA (4 eq., 60 µL, 0.4 mmol) in DMF (0.4 mL) and the reaction mixture was allowed to stir for 1 min. A solution of crude amine **42** (42 mg, 0.1 mmol) in DMF (0.3 mL) was added and the resulting mixture was stirred overnight. EtOAc (10 mL) was added and the organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The title compound was obtained after RP-HPLC purification (linear gradient 40% → 60% ACN in H_2O, 0.1% TFA, 15 min) as a white solid (yield: 21.41 mg, 26.5 µmol, 26.5%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.33 (s, 1H), 8.13 (br s, 1H), 7.66 (s, 2H), 7.44 (t, *J* = 8.4 Hz, 2H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 7.8 Hz, 2H), 6.82 (d, *J* = 11.4 Hz, 0.5H), 6.66 – 6.61 (m, 1H), 6.57 – 6.51 (m,

0.5H), 4.75 – 4.70 (m, 1H), 4.56 (d, *J* = 10.8 Hz, 1H), 4.32 (t, *J* = 6.6 Hz, 1H), 4.18 (d, *J* = 10.8 Hz, 1H), 4.13 (s, 1H), 4.06 (d, *J* = 15 Hz, 1H), 3.38 – 3.30 (m, 2H), 3.28 – 3.18 (m, 1H), 3.10 (s, 1H), 3.00 – 2.90 (m, 3H), 2.81 – 2.80 (m, 1H), 2.61 (s, 1H), 2.57 (s, 1H), 2.51 (s, 1H), 2.28 – 2.24 (m, 1H), 2.14 – 2.10 (m, 3H), 1.95 (d, *J* = 13.2 Hz, 1H), 1.61 – 1.58 (m, 2H), 1.53 – 1.45 (m, 4H), 1.26 (br s, 3H), 1.23 (s, 1H), 1.22 (d, *J* = 13.2 Hz, 1H), 1.11 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.81, 163.85, 162.73, 157.34, 156.23, 153.57, 153.26, 144.18, 143.77, 130.18, 127.49, 123.89, 119.02, 97.27, 62.66, 61.06, 59.22, 55.43, 55.13, 52.88, 52.24, 50.38, 49.48, 49.22, 46.32, 45.76, 45.21, 44.77, 41.63, 40.43, 35.10, 31.31, 29.61, 29.44, 28.23, 28.07, 25.09, 24.91, 18.82. HRMS: calculated for $C_{42}H_{53}N_{11}O_4S$ [M+H$^+$]: 808.40027; found: 808.40037.

Ibrutinib Levulinoyl ester-based cleavable linker azide (46)

DiPEA (3.5 eq., 0.12 mL, 0.70 mmol) and levulinoyl ester-based cleavable linker-OSu[2] **45** (2.2 eq., 0.22 g, 0.44 mmol) were added to a solution of crude amine **42** (84 mg, 0.2 mmol) in DMF (0.5 mL). The reaction mixture was stirred overnight before being evaporated. The title compound was obtained after RP-HPLC purification (linear gradient 40% → 60% ACN in H_2O, 0.1% TFA, 15 min) as a white solid (yield: 69.7 mg, 71.0 µmol, 36%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.36 (s, 1H), 8.11 (br s, 1H), 7.66 (s, 2H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.17 (d, *J* = 7.2 Hz, 2H), 7.13 (d, *J* = 9.0 Hz, 2H), 6.98 (s, 2H), 6.84 (d, *J* = 18.0 Hz, 0.5H), 6.69 (d, *J* = 15.0 Hz, 0.5H), 6.65 – 6.59 (m, 0.5H), 6.58 – 6.51 (m, 0.5H), 4.79 – 4.30 (m, 1H), 4.58 (d, *J* = 18.0 Hz, 0.5H), 4.19 (d, *J* = 12.0 Hz, 1H), 4.16 (d, *J* = 12.0 Hz, 0.5H), 3.54 (br s, 1H), 3.37 (br s, 4H), 3.30 (t, *J* = 7.2 Hz, 2H), 3.23 – 3.20 (m, 2H), 3.04 – 2.95 (m, 4H), 2.89 – 2.80 (m, 10H), 2.56 (t, *J* = 7.2 Hz, 3H), 2.41 (s, 3H), 2.26 (t, *J* = 12.0 Hz, 1H), 2.15 – 2.13 (m, 1H), 1.95 – 1.91 (m, 1H), 1.76 – 1.71 (m, 2H), 1.67 – 1.53 (m, 1H), 1.09 (s, 12H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 207.98, 172.27, 171.52, 163.82, 157.31, 157.02, 156.63, 156.20, 153.96, 153.36, 144.13, 143.58, 143.12, 139.93, 138.75, 130.28, 127.36, 124.00, 123.22, 119.15, 97.00, 56.73, 55.12, 53.52, 51.87, 50.22, 50.00, 49.17, 38.60, 36.84, 36.52, 30.67, 27.35, 26.51, 22.58. HRMS: calculated for $C_{54}H_{68}N_{12}O_6$ [M+H$^+$]: 981.53848; found: 981.53856.

Ibrutinib Levulinoyl ester-based cleavable linker biotin (48)

Aqueous $CuSO_4$ (1.0 eq., 24.8 µL, 24.8 µmol, 1M) and aqueous sodium L-ascorbate (1.1 eq., 27.2 µL, 27.2 µmol, 1M) were added to a solution of azide **46** (24 mg, 24 µmol) and biotin-Ahx-propargylamide **47** (1.5 eq., 14.2 mg, 36 µmol) in DMF 0.8 mL. The reaction mixture was stirred at RT overnight and was concentrated *in vacuo*. The title compound was obtained after RP-HPLC purification (linear gradient 45% → 65% ACN in H_2O, 0.1% TFA, 15 min) as a white solid (yield: 7.5 mg, 5.5 µmol, 23%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.33 (s, 1H), 8.21 (t, *J* = 5.4 Hz, 1H), 8.07 (br s, 1H), 7.87 (s, 1H), 7.7.0 (t, *J* = 5.4 Hz, 1H), 7.66 (s, 2H), 7.44 (t, *J* = 7.2 Hz, 2H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.18 (d, *J* = 6.6 Hz, 2H), 7.12 (d, *J* = 7.8 Hz, 2H), 6.98 (s, 2H), 6.82 (d, *J* = 15.0 Hz, 0.5H), 6.66 – 6.61 (m, 1H), 6.58 – 6.51 (m, 0.5H), 6.39 (s, 1H), 6.33 (br s, 1H), 4.79 – 4.68 (m, 1H), 4.58 (d, *J* = 18.0 Hz, 1H), 4.31 – 4.28 (m, 3H), 4.27 (d, *J* = 6.0 Hz, 2H), 4.18 (d, *J* = 6.0 Hz, 1H), 4.13 – 4.11 (m, 1H), 4.05 (t, *J* = 12.0 Hz, 1H), 3.25 – 3.22 (m, 3H), 3.11 – 3.07 (m, 3H), 2.99 (q, J_1 = 7.2 Hz, J_2 = 13.2 Hz, 3H), 2.95 – 2.88 (m, 2H), 2.87 – 2.83 (m, 3H), 2.82 – 2.79 (m, 6H), 2.62 – 2.58 (m, 2H), 2.39 (br s, 2H), 2.31 – 2.22 (m, 1H), 2.15 – 2.10 (m, 1H), 2.07 (s, 5H), 2.04 (t, *J* = 7.8 Hz, 2H), 1.99 (t, *J* = 7.2 Hz, 2H), 1.98 – 1.93 (m, 1H), 1.61 – 1.59 (m, 2H), 1.52 – 1.46 (m, 5H), 1.37 – 1.34 (m, 2H), 1.30 – 1.27 (m, 2H), 1.24 – 1.20 (m, 3H), 1.09 (s, 12H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 207.79, 172.17, 172.00, 171.82, 171.55, 163.86, 162.70, 157.31, 156.22, 145.03, 143.37, 139.79, 138.96, 130.14, 127.52, 126.16, 123.86,

123.64, 122.63, 119.00, 118.02, 117.32, 115.79, 115.36, 97.23, 63.29, 61.05, 59.20, 56.82, 55.39, 52.87, 52.22, 49.52, 49.20, 48.50, 45.78, 45.21, 38.31, 36.87, 36.55, 35.21, 34.12, 30.69, 29.58, 29.37, 28.95, 28.19, 28.02, 27.34, 26.66, 26.12, 25.312, 25.16, 24.91, 24.14, 1.11. HRMS: calculated for $C_{73}H_{98}N_{16}O_9S$ [M+H$^+$]: 1375.74234; found: 1375.74244.

2-(4-Bromophenyl)-2-phenyl-1,3-dioxolane (52)

Ethylene glycol (1.1 eq., 6.13 mL, 110 mmol) and *p*-toluenesulfonic acid monohydrate (0.01 eq., 0.19 g, 1.0 mmol) were added to a solution of (4-bromophenyl)(phenyl)methanone **51** (26.11 g, 100 mmol) in benzene (40 mL) and the reaction mixture was refluxed with a Dean-Stark condenser for 48 hrs. The reaction mixture was poured into aqueous sodium bicarbonate and extracted with Et_2O. The organic layer was washed with aqueous sodium bicarbonate, water and brine before being dried over $MgSO_4$ and concentrated *in vacuo*. The residue was further purified by silica column chromatography (5% → 20% DCM/pentane) to afford the title compound as a white powder (yield: 21.79 g, 71.3 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 7.49 – 7.46 (m, 2H), 7.43 – 7.36 (m, 4H), 7.31 – 7.22 (m, 3H), 3.98 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.53, 141.25, 131.14, 128.11, 127.90, 125.90, 122.09, 108.85.

4-(2-phenyl-1,3-dioxolan-2-yl)benzaldehyde (53)

n-BuLi (1.2 eq., 10.4 mL, 16.60 mmol, 1.6 M in hexanes) was dropwise added to a solution of compound **52** (4.22 g, 13.83 mmol) in THF at -78 °C. The resulting mixture was stirred at -78 °C for 30 min before dropwise addition of DMF (1.5 eq., 1.6 mL, 20.75 mmol). The reaction mixture was allowed to warm up to RT overnight. The mixture was poured into aqueous ammonium chloride (250 mL) and diluted with Et_2O. The layers were separated and the organic layer was washed with brine and water, dried over $MgSO_4$, filtered and evaporated. The obtained material was purified by column chromatography (2% → 10% EtOAc/pentane) and the product was obtained as a white solid (yield: 2.81 g, 11.06 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 9.96 (s, 1H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.51 (d, *J* = 7.2 Hz, 2H), 7.33 – 7.24 (m, 3H), 4.06 – 4.02 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.74, 148.66, 141.28, 135.81, 129.55, 128.23, 126.59, 125.79, 108.75, 64.90.

(*E/Z*)-2-(4-(2-phenyl-1,3-dioxolan-2-yl)benzylidene)-5-(trityloxy)pentanenitrile (55)

To an ice-cold suspension of NaH (1 eq., 0.23 g, 5.77 mmol, 60% mineral oil) in THF (20 mL) a solution of phosphonate **54** (1.25 eq., 3.44 g, 7.21 mmol) in THF (5 mL) was added dropwise and stirred for 30 min. Next, a solution of aldehyde **53** (1.47 g, 5.77 mmol) in THF (5 mL) was added and the reaction mixture was allowed to warm to RT and stirred overnight. The solution was quenched by addition of freshly prepared sat. aq. Na_2HSO_3 (30 mL) and diluted with H_2O (100 mL) and Et_2O (50 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 x 50 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and evaporated. The residue was further purified by column chromatography (80% → 95% toluene/pentane) to afford the title compound as a white solid with an *E/Z* ratio of 1/1 (yield: 3.15 g, 5.46 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 7.59 – 7.50 (m, 10H), 7.43 – 7.37 (m, 12H), 7.29 – 7.09 (m, 26H), 7.08 (s, 1H), 6.78 (s, 1H), 3.95 (s, 8H), 3.12 (d, *J* = 5.2 Hz, 4H), 2.57 (t, *J* = 7.2 Hz, 2H), 2.47 (t, *J* = 7.2 Hz, 2H), 1.91 (d, *J* = 6.0 Hz, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 143.87, 143.78, 143.47, 143.35, 143.00, 141.57, 133.47, 133.23, 128.97, 128.38, 128.22, 128.06, 127.58, 126.77, 126.35, 125.83, 125.10, 120.08, 118.43, 115.36, 110.84, 108.82, 86.43, 86.32, 64.76, 64.72, 62.16, 61.56, 32.96, 29.50, 28.43, 28.24, 26.64. HRMS: calculated for $C_{40}H_{35}NO_3$ $[M+H]^+$ 578.26897; found 578.26883.

***Tert*-butyl (*E*)-(2-(4-benzoylbenzylidene)-5-hydroxypentyl)(2-(isoquinoline-5-sulfonamido)ethyl)carbamate (59)**

A solution of nitrile **55** (8.37 g, 14.49 mmol) in anhydrous Et_2O (75 mL) was cooled to -78 °C. DiBAl-H (2 eq., 29 mL, 29 mmol, 1M solution in hexanes) was added dropwise and the reaction mixture was allowed to warm to 0 °C and stirred for 2h, after which TLC analysis showed complete consumption of starting material. Next, the mixture was cooled to -100 °C followed by rapid addition of MeOH (30 mL). After 5 min a solution of isoquinoline amine **56** (2.5 eq., 13.24 g, 36.25 mmol) in MeOH (10 mL) was added dropwise and the reaction mixture was allowed to stir at RT overnight. Hereafter, the reaction was cooled to -10 °C and $NaBH_4$ (2 eq., 1.10 g, 29.0 mmol) was added and the mixture was allowed to stir for 3h at RT. The reaction mixture was diluted with 0.5 M aq. NaOH (100 mL) and the layers were separated. The aqueous layer was extracted with DCM (3 x 50 mL) and the combined organic phases were washed with H_2O (3 x 50 mL) and brine, dried over $MgSO_4$, filtered and evaporated. The crude product **57** was subjected to the next step without further purification.

The crude product **57** was dissolved in THF (50 mL) and HCl (35 mL) was added. The reaction mixture was stirred for 1h at RT before addition of H_2O (40 mL), the resulting mixture was stirred for 30 min at RT. The mixture was diluted with aqueous NaOH (10 M) to pH14 before addition of $CHCl_3$ (100 mL). The layers were separated and the aqueous layer was extracted with $CHCl_3$ (2x 100 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product **58** was subjected to the next step without further purification.

The residue **58** was dissolved in DCM (75 mL) and was cooled to 0 °C. To this mixture Boc_2O (1.5 eq., 4.74 g, 21.75 mmol) and TEA (4 eq., 8.04 mL, 58.0 mmol) were added and the reaction was allowed to warm to RT and stirred overnight. The reaction mixture was concentrated under reduced pressure and re-dissolved in H_2O (150 mL) and DCM (150 mL). The organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound was obtained after purification by RP-HPLC purification (linear gradient 40% → 60% ACN in H_2O, 0.1% TFA, 15 min) as yellow oil (yield: 0.61 g, 0.97 mmol, 6.7%).

^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 9.31 (s, 1H), 8.56 (s, 1H), 8.48 (d, *J* = 6.0 Hz, 1H), 8.40 (d, *J* = 7.2 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 4H), 7.62 – 7.55 (m, 2H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.02 (t, *J* = 6.8 Hz, 1H), 6.24 (s, 1H), 3.89 (s, 2H), 3.61 (t, *J* = 6.0 Hz, 2H), 3.39 (s, 2H), 3.16 (s, 2H), 2.17 (s, 2H), 1.73 (s, 2H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 210.61, 196.08, 152.81, 144.31, 141.27, 140.18, 137.22, 135.15, 134.35, 133.18, 132.77, 132.16, 130.94, 129.97, 129.61, 128.76, 128.17, 128.02, 125.76, 117.31, 80.37, 69.36, 61.73, 53.71, 46.30, 41.78, 31.51, 27.40. HRMS: calculated for $C_{35}H_{39}N_3O_6S$ $[M+H]^+$ 630.25596; found 630.25533.

***Tert*-butyl(*Z*)-(2-(4-benzoylbenzylidene)-5-hydroxypentyl)(2-(isoquinoline-5-sulfonamido)ethyl)carbamate (60)**

This compound was prepared in the same reaction as **59**. The title compound was obtained after purification by RP-HPLC purification (linear gradient 40% → 60% ACN in H_2O, 0.1% TFA, 15 min) as yellow oil (yield: 1.68 g, 2.67 mmol, 18%). ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 9.31 (s, 1H), 8.60 (s, 1H), 8.36 (d, *J* = 6.0 Hz, 1H), 8.26 (d, *J* = 7.2 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.81 – 7.75 (m, 4H), 7.65 – 7.57 (m, 2H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 1H), 6.56 (s, 1H), 4.07 (s, 2H), 3.67 (t, *J* = 6.0 Hz, 2H), 3.12 (t, *J* = 5.6 Hz, 2H), 2.81 (s, 2H), 2.12 (t, *J* = 7.2 Hz, 2H), 1.80 – 1.77 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.16, 153.09, 144.89, 141.29, 140.38, 137.55, 135.87, 134.37, 133.54, 133.18, 132.61, 131.25, 130.28, 130.07, 129.13, 128.87, 128.44, 126.01, 117.40, 81.15, 62.69, 45.97, 30.34, 28.41, . HRMS: calculated for $C_{35}H_{39}N_3O_6S$ $[M+H]^+$ 630.25596; found 630.25609.

(*E*)-*N*-(2-((5-azido-2-(4-benzoylbenzylidene)pentyl)amino)ethyl)isoquinoline-5-sulfonamide (63)

Alcohol **59** (0.16 g, 0.26 mmol) was dissolved in DCM (1 mL). After addition of TEA (1 eq., 36 µL, 0.26 mmol) and DMAP (0.64 mg, 5 µmol), the resulting mixture was cooled to -20 °C. A solution of TsCl (1 eq., 0.05 g, 0.26 mmol) in DCM (2 mL) was added dropwise and the reaction was stirred at -20 °C for 18h. Subsequently, 0.1 M aq. HCl (10 mL) was added and the layers were separated. The organic layer was washed with 0.1 M HCl (10 mL) and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was subjected to the next step without further purification.

The residue was dissolved in DMF (10 mL). To this was added NaN_3 (20 eq., 0.34 g, 5.2 mmol) and the reaction was stirred at room temperature for 5h before being concentrated. The resulting residue **61** was dissolved in DCM (2.5 mL) and TFA (2.5 mL) was added. The mixture was stirred for 1h at RT before it was co-evaporated with toluene (3x 10 mL). The resulting mixture was purified by RP-HPLC (linear gradient 40% → 60% ACN in H_2O, 0.1% TFA, 15 min) and the title compound was obtained as yellowish oil (yield: 0.06 g, 0.11 mmol, 44%). ^{1}H NMR (600 MHz, MeOD) δ 9.58 (s, 1H), 8.73 – 8.70 (m, 2H), 8.62 (d, *J* = 7.2 Hz, 1H), 8.54 (d, *J* = 7.8 Hz, 1H), 7.95 (t, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 6.6 Hz, 2H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 2H), 7.49 (d, *J* = 8.4, 2H), 3.89 (s, 2H), 3.34 (t, *J* = 6.6 Hz, 2H), 3.28 – 3.24 (m, 4H), 2.50 (t, *J* = 7.8 Hz, 2H), 1.83 – 1.78 (m, 2H). ^{13}C NMR (151 MHz, MeOD) δ 197.92, 153.18, 142.23, 141.73, 138.73, 137.81, 136.28, 135.91, 135.84, 135.75, 135.64, 133.97, 133.74, 133.49, 131.37, 131.12, 130.60, 129.80, 129.51, 128.69, 127.54, 120.31, 53.20, 51.96, 48.26, 28.25, 27.62. HRMS: calculated for $C_{30}H_{30}N_6O_3S$ $[M+H]^+$ 555.21001; found 555.21063.

(*Z*)-*N*-(2-((5-azido-2-(4-benzoylbenzylidene)pentyl)amino)ethyl)isoquinoline-5-sulfonamide (64)

This compound was prepared in the same reaction as **63**. The title compound was obtained after purification by RP-HPLC purification (linear gradient 40% → 60% ACN in H_2O, 0.1% TFA, 15 min) as a yellow oil (yield: 0.08 g, 0.15 mmol, 38%). ^{1}H NMR (600 MHz, MeOD) δ 9.49 (s, 1H), 8.63 – 8.61 (m, 2H), 8.44 (t, *J* = 8.4 Hz, 2H), 7.85 (t, *J* = 7.8 Hz, 1H), 7.78 (t, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 7.2 Hz, 2H), 7.64 (t, *J* = 3.6 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 2H), 6.91 (s, 1H), 3.97 (s, 2H), 3.44 (t, *J* = 6.6 Hz, 2H), 3.08 (t, *J* = 6.0 Hz, 2H), 3.03 (t, *J* = 5.4 Hz, 2H), 2.43 (t, *J* = 7.2 Hz, 2H), 1.93 – 1.88 (m, 2H). ^{13}C NMR (151 MHz, MeOD) δ 197.77, 153.23, 142.51, 141.63, 138.56, 137.69, 136.01, 135.76, 135.62, 135.47, 134.77, 134.29, 133.86, 133.31, 131.52, 131.32, 130.99, 130.48, 129.77, 129.51, 128.95, 128.54, 120.07,

51.92, 47.70, 46.83, 39.54, 32.38, 28.08. HRMS: calculated for $C_{30}H_{30}N_6O_3S$ $[M+H]^+$ 555.21001; found 555.21021.

Dasatinib-norbornene (69)

DiPEA (3 eq., 12 µL, 69 µmol) and 2,5-dioxopyrrolidin-1-yl bicyclo[2.2.1]hept-5-ene-2-carboxylate (**68**) (2 eq., 11 mg, 45 µmol) were added to a solution of Dasatinib-amine **67** (11 mg, 23 µmol) in DMF (2 mL). The reaction mixture was stirred at RT overnight. The title compound was obtained after purification by RP-HPLC purification (linear gradient 20% → 40% ACN in H_2O, 0.1% TFA, 15 min) as a white solid (yield: 2.67 mg, 4.4 µmol, 18%). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.76 (s, 1H), 10.04 (s, 1H), 8.36 (s, 1H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.39 (d, *J* = 9.6 Hz, 2H), 6.28 (s, 1H), 5.32 – 3.87 (m, 10H), 3.35 – 3.21 (m, 2H), 2.66 (s, 6H), 2.56 (s, 3H), 2.35 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ 165.38, 162.45, 162.05, 159.91, 158.93, 157.19, 140.81, 138.84, 133.51, 132.45, 129.07, 128.23, 127.05, 125.94, 83.28, 53.15, 51.26, 41.70, 25.57, 21.09, 18.32. HRMS: calculated for $C_{30}H_{35}ClN_8O_2S$ $[M+H]^+$ 607.22922; found 607.22963.

Dasatinib-azide (71)

This compound was prepared in the same reaction as **69**. However, 2,5-dioxopyrrolidin-1-yl 6-azidohexanoate (**70**) was used instead of the norbornene-succinimide **68**. The title compound was obtained after purification by RP-HPLC purification (linear gradient 20% → 40% ACN in H_2O, 0.1% TFA, 15 min) as a white solid (yield: 4.03 mg, 6.4 µmol, 28%). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.71 (s, 1H), 9.89 (s, 1H), 8.21 (s, 1H), 7.37 (d, *J* = 7.2 Hz, 1H), 7.23 (d, *J* = 10.0 Hz, 2H), 6.10 (s, 1H), 4.44 – 3.51 (m, 6H), 3.31 – 2.83 (m, 4H), 2.51 (s, 2H), 2.40 (s, 3H), 2.20 (s, 3H), 2.07 (t, *J* = 6.4 Hz, 2H), 1.88 (s, 2H), 1.54 – 1.43 (m, 4H), 1.33 – 1.24 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 165.37, 162.05, 159.89, 157.16, 140.81, 138.82, 133.49, 132.43, 129.06, 128.22, 127.03, 50.54, 35.15, 28.03, 25.82, 24.63, 22.32, 21.09, 18.32. HRMS: calculated for $C_{28}H_{36}ClN_{11}O_2S$ $[M+H]^+$ 626.24627; found 626.24685.

(E)-*N*-(2-((3-(4-(3-aminoprop-1-yn-1-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (73)

In separate flasks, a solution of the *N*-Boc-propargylamine (1.5 eq., 93 mg, 0.6 mmol), isoquinolinephenyl bromide **72** (220 mg, 0.4 mmol) and pyrrolidine (3 eq., 0.10 mL, 1.2 mmol) in DMF (0.5 mL), a solution of CuI (0.1 eq., 8 mg, 40 µmol) in DMF (100 µL) and a solution of $Pd(PPh_3)_4$ (0.05 eq., 23 mg, 20 µmol) in DMF (100 µL) were flushed with argon for 1 hr in an ultrasonic bath. To the alkyne mixture were added subsequently the CuI and the $Pd(PPh_3)_4$ solutions and the resulting mixture was stirred at 80 °C overnight. The reaction mixture was filtered over a short silica column and was flushed with DCM/MeOH (1/1 v/v; 10x column volume). The eluate was concentrated and the crude product was subjected to the next step without further purification.

The residue was dissolved in DCM (2 mL) and TFA (2 mL) was added and the reaction mixture was stirred for 2h at RT. The mixture was then co-evaporated with toluene (5x). The title compound was obtained after purification by RP-HPLC purification (linear gradient 25% → 45% ACN in H_2O, 0.1% TFA, 15 min) as a yellow oil (yield: 56,6 mg, 0.13 mmol, 34%). ^{1}H NMR (600 MHz, DMSO-d6) δ 9.60 (s, 1H), 8.75 (s, 1H), 8.70 (s, 1H), 8.61 (d, *J* = 7.2 Hz, 1H), 8.53 (d, *J* = 8.4 Hz, 1H), 7.94 (t, *J* = 7.8 Hz, 1H), 7.47 (s, 4H), 6.85 (d, *J* = 15.6 Hz, 1H), 6.36 – 6.33 (m, 1H), 4.05 (s, 2H), 3.86 (d, *J* = 7.2 Hz, 2H), 3.19 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d6) δ 152.86, 138.88, 137.77, 136.46,

136.04, 135.75, 133.56, 133.22, 133.08, 130.10, 128.81, 128.07, 123.01, 122.83, 122.45, 120.91, 87.36, 82.58, 50.33, 47.62, 40.11, 30.70. HRMS: calculated for $C_{23}H_{24}N_4O_2S$ $[M+H]^+$ 421.16200; found 421.16256.

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