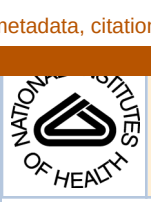

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Selective and potent urea inhibitors of *Cryptosporidium parvum* inosine 5′ monophosphate dehydrogenase

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Abstract

Cryptosporidium parvum and related species are zoonotic intracellular parasites of the intestine. *Cryptosporidium* is a leading cause of diarrhea in small children around the world. Infection can cause severe pathology in children and immunocompromised patients. This waterborne parasite is resistant to common methods of water treatment and therefore a prominent threat to drinking and recreation water even in countries with strong water safety systems. The drugs currently used to combat these organisms are ineffective. Genomic analysis revealed that the parasite relies solely on inosine-5′-monophosphate dehydrogenase (IMPDH) for the biosynthesis of guanine nucleotides. Herein, we report a selective urea-based inhibitor of *C. parvum* IMPDH (*Cp*IMPDH) identified by high throughput screening. We performed a SAR study of these inhibitors with some analogues exhibiting high potency (IC_{50} < 2 nM) against *Cp*IMPDH, excellent selectivity > 1000-fold versus human IMPDH type 2 and good stability in mouse liver microsomes. A subset of inhibitors also displayed potent antiparasitic activity in a *Toxoplasma gondii* model.

Introduction

Cryptosporidium parasites such as *C. parvum* and *C. hominis* are major causes of the 'vicious cycle of diarrhea and malnutrition' that afflicts the developing world[1]. This infection is self-limiting in healthy adult individuals, but can be chronic and fatal in immunocompromised patients and children under 5 years of age[2]. Parasite oocysts are resistant to commonly used methods of water treatment, and contaminated drinking and recreational water are major sources of host to host transmission[3]. While cryptosporidiosis is more prevalent in developing countries[4], the developed world also has a significant disease burden. Fifteen documented *Cryptosporidium* waterborne outbreaks were reported during 2000 in the USA, resulting in enormous medical expenses[5]. Since the oocysts can be obtained with relative ease and the water supply is readily accessed, *C. parvum* has the

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Supporting Information Available: Detailed information for HPLC methods and compound purity are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

potential to be used for bio-terrorism. Consequently, it is considered a class B bio-warfare agent[6]. Neither vaccines nor the approved drug nitazoxanide are effective treatments for cryptosporidiosis in immunocompromised patients, so there is an urgent need for new chemotherapeutic agents.

Genomic analysis revealed that *C. parvum* and *C. hominis* rely on the enzyme inosine 5′-monophosphate dehydrogenase (IMPDH) for the production of guanine nucleotides[2b, 7] (Scheme 1; *Cp*IMPDH will be used to denote the parasite enzyme, which is identical in *C. parvum* and *C. hominis*) [8]. Remarkably, these parasites appear to have obtained their IMPDH gene from an ε-proteobacterium by lateral gene transfer[9]. The bacterial origin of *Cp*IMPDH and the different kinetic properties of these enzymes compared with their human counterparts suggested *Cp*IMPDH-selective inhibitors could be obtained[10]. A high throughput screen for selective inhibitors yielded an urea-based inhibitor with an IC_{50} value of 340 nM against *Cp*IMPDH (Compound **1**, Table 1). Herein, we report a SAR study of **1** with respect to *Cp*IMPDH inhibition and antiparasitic activity and optimization of mouse microsomal stability.

Results and Discussion

High throughput screen

Following our previously described assay protocol [10], we performed a high throughput screen of 85,000 compounds to identify inhibitors of *Cp*IMPDH. This screen targeted the NAD site, which is the most highly diverged region of the active site relative to the human enzymes. This screen yielded 50 compounds that inhibited *Cp*IMPDH but not human IMPDH type 2 (IC_{50} > 5 μM). Six of these compounds were urea-based, of which the most potent was compound **1** (Figure 1) with IC_{50} of 340 nM. Interestingly, two urea-based inhibitors of human IMPDHs, merimepodib and AVN944, have entered clinical trials[11], which further encouraged development of this series

Chemistry

Various derivatives of **1** were prepared following the synthetic method outlined in Scheme 2. Ozonolysis followed by reductive work-up with dimethyl sulfide transformed 3-isopropenyl-α, α-dimethyl benzyl isocyanate **2** into 3-acetyl-α, α-dimethyl benzyl isocyanate **3** in moderate yield. Upon treating **2** and **3** with substituted anilines **4,** the corresponding ureas **5** and **6** were obtained**.** The acetyl group on **6** was converted to the corresponding oxime **7** and methyl oxime **8** in good yields by heating with a slight excess of hydroxylamine hydrochloride and methoxyamine hydrochloride, respectively, in pyridine.[12] Oxime **7** was alkylated in the presence of sodium hydride with 2-chloroethylamine hydrochloride to give amine derivative **9**. Lithium aluminum hydride (LAH) reduction of acetyl derivative **6** gave alcohol **10**. Hydrogenation of isoprenyl derivative **5** with 10% palladium on carbon yielded the saturated urea derivative **11** in good yield.

Scheme 3 outlines the reaction sequence used for the synthesis of a mono-methyl urea derivative. 3-(1-Cyanoethyl)benzoic acid, **12**, was converted into acetyl derivative **13** using a step wise process that involved the conversion of the acid to the corresponding acid chloride followed by treatment with Meldrum's acid in the presence of base and then hydrolytic decarboxylation[13]. Subsequent hydrolysis of nitrile derivative **13** gave substituted hydratropic acid **14,** which was treated with thionyl chloride followed by sodium azide in water to generate acyl azide **15** that was used without isolation. Acyl azide **15** was heated to reflux in benzene under a nitrogen atmosphere to generate isocyanate **16**[14]. Finally, the isocyanate was treated with 4-chloroaniline to give urea derivative **17**.

An analogue of **1** that lacks both methyl groups on the benzylic position was prepared according to the procedure outlined in Scheme 4. 3-Acetylbenzonitrile, **18**, was treated with ethylene glycol[15] to give the ketal protected benzonitrile derivative **19**. LAH reduction generated benzylamine derivative **20,** which was subsequently converted to urea **22** in two steps *via* the intermediate *p*-nitrophenyl carbamate **21**[16]. Removal of the ketal was carried out under acidic conditions to give **23**.

A series of carbamate and amide derivatives were prepared using the methods outlined in Scheme 5. Isocyanate **3** was refluxed with 8N HCl to give benzylamine derivative **24** in good yield. This material was converted to carbamate **25** by treatment with 4-chlorophenyl chloroformate in the presence of base. Amine **24** was also converted to amide **26** in good yield. In addition, **24** was treated with chlorosulfonic acid, followed by exposure to aqueous potassium nitrite and subsequent decomposition with sulfate buffer (pH = 2.8) to give the substituted benzyl alcohol **27**[17]. Upon treatment with 4-chlorophenyl isocyanate, this material was converted to carbamate **28**.

Several biphenyl derivatives were prepared using the methods outlined in Scheme 6. 3-Phenylcyanobenzene, **29**, was allowed to react with 3 equivalents of methyl magnesium bromide in the presence of the Lewis acid catalyst titanium (IV) isopropoxide to give *tert*-alkyamine **30**[18]. Biphenylcyclopropylamine **31** was similarly prepared from **29** by treatment with 2 equivalents of ethyl magnesium bromide and titanium (IV) isopropoxide[19]. Amines **30**, **31** and **32** were converted to corresponding urea derivatives **36**, **37** and **38**, respectively, using a two-step procedure *via* intermediates **33**, **34** and **35**.

Evaluation of enzyme inhibition

IMPDH activity was assayed by monitoring the production of NADH. The IC_{50} values reported in the tables are the average of three independent experiments unless otherwise noted. Nonspecific protein binding was assessed by measuring IC_{50} values in presence of 0.05% fatty acid free bovine serum albumin (BSA)[20]. Our previous experience indicates that the IC_{50} in the presence of BSA is the better predictor of antiparasitic activity[21].

For the SAR study, initial attempts were made to optimize the anilide substituent. Removal of the cyclic urea moiety in **1** yielded compound **5a**, which demonstrated a slight increase in *Cp*IMPDH inhibitory activity with an IC_{50} of 250 nM (Table 1). Replacing the imidazolone with a 4-ClPh or 4-BrPh increased potency by 16-fold (**5b**) and 32-fold (**5c**), respectively. A similar trend has also been observed with other *Cp*IMPDH inhibitor series[22]. However, translocation of the chlorine atom to the 3-position (**5d**) resulted in a 3-fold loss of activity, while the 2-chloro derivative (**5e**) was completely inactive. The electronic nature of the substituent in the 4-position had little effect on inhibitory activity. For example, an electron donating methoxy (**5f)** retained activity comparable to the bromide (**5c**) with IC_{50} of 9 nM. However, sterics did have an effect as illustrated by the 4-*tert*-butyl (**5g**) derivative lacking activity. Introduction of substituents in the 3,4-positions increased activity. For example, 3,4-di-chloro (**5h**), 4-chloro-3-trifluoromethyl (**5i**) and a variety of 3-amido-4-chloro (**5k**, **5l**, and **5m**) substituents increased potency by about 2.5 to 10-fold as compared to **5b**. Again the electronic nature of the 3,4-substitents had little effect as exemplified by 4-chloro-3-methoxy (**5j**) and the 3,4-methylenedioxy (**5n**) analogues, although the latter compound demonstrated significantly weaker activity in the presence of BSA. Given these results, several fused carbo- and heteroaromatic derivatives were examined. The 2-naphthyl derivative (**5o**) retained potent active, while the regioisomer 1-naphthyl was devoid of activity. However, the inhibitory activity of **5o** decreased in presence of BSA. Replacement of the 2-naphthyl with 6- and 7-quinolyl (**5q** and **5r**) resulted in highly potent compounds

that were only modestly reduced in the presence of BSA. The 2- and 3-quinolyl analogues (**5t** and **5s**) were less potent.

Next, the isoprenyl group was examined (Table 2). Saturation of the alkene to the resulting isopropyl (**11**) or removal (**38**) resulted in complete loss of inhibitory activity. Quite surprisingly, replacement of the alkene with an alcohol (**10**) resulted in moderate activity suggesting that polar substituents could be tolerated at this position. Incorporation of the alkene into a phenyl (**36a**) retained potent activity. However, transposing the phenyl (**36b**) to the para-position resulted in complete loss of inhibitory activity. Given the relative importance of the sp^2-hybridized substituent at the 3-position and moderate tolerance of a hydrophilic alcohol in **10**, replacement of the alkene with a ketone was explored. In three cases examined (**6a-c**) good to moderate inhibitory activity was observed.

The SAR of the central portion of **1** was interrogated next (Table 3). First, the importance of the gem-dimethyl was confirmed. For example, the mono-methyl derivative **17** was less active compared to **6a** and elimination of the methyl groups (**23**) resulted in complete loss of activity. Incorporation of the two methyl groups into a cyclopropane (**37**) was also detrimental. Several changes to the urea were examined. For example, N-methylation (**5u**) of the aniline portion of the urea and replacement of the nitrogen with oxygen (**25**) or carbon (**26**) were deleterious. Replacement of the benzylamine nitrogen with oxygen (**28**) was better tolerated, demonstrating only a 2-fold reduction in activity.

Finally, based on the encouraging results obtained with **6a** the ketone functional group was transformed into hydrophilic oximes and O-methyloximes (Table 4). Gratifyingly, the oximes (**7a, 7b**, **7c**, **7d** and **7e**) demonstrated excellent *Cp*IMPDH inhibitory potencies, with $IC_{50} \leq 1$ nM. Likewise, the O-methyloximes **8a**, **8b** and **8c** proved to be quite active with $IC_{50} \leq 5$ nM. O-Ethanolamine **9** also showed promising activity with an IC_{50} value of 20 nM.

The introduction of hydrophilic groups in place of the isoprenyl also resulted in improvements in mouse liver microsomal stability, which was a potential liability for **5k** and **5r** (Table 5). The ketone derivative **6c** was very resistant to oxidative degradation in microsomes. The oximes demonstrated a range of stabilities, with **7c** having a $t_{½}$ of 190 min. While the O-methyloxime **8a** was less stable, the aminoethyleneoxime derivative **9** showed excellent mouse liver microsomal stability with $t_{1/2} > 2$ hours. All of the compounds examined demonstrated excellent mouse plasma stability.

Toxoplasma gondii is an intracellular parasite closely related to *Cryptosporidium* that, unlike *C. parvum*, can be continuously cultured in hTERT immortalized human foreskin fibroblasts. We engineered a *T. gondii* strain (Toxo/*Cp*IMPDH) that relies on *Cp*IMPDH, and is sensitive to *Cp*IMPDH inhibitors[21]. In contrast, the wild type *T. gondii* strain RH (Toxo/WT) contains a typical eukaryotic IMPDH and is resistant to *Cp*IMPDH inhibitors. Therefore, Toxo/WT serves as a proxy for host cell toxicity as well as provides important target validation.

Approximately thirty compounds with good inhibitory activity against *Cp*IMPDH were tested in the *Toxoplasma* model. Eleven compounds had EC_{50} values ≤ 200 nM with selectivity ≥ 100-fold over Toxo/WT. Gratifyingly, **7b**, our best inhibitor in the enzyme assay, also displayed the best antiparasitic activity with EC_{50} of 6 nM and 670-fold selectivity over Toxo/WT. Compounds **7a** and **7e** are also very promising candidates, with EC_{50} values of 10 nM and 58 nM, respectively, and selectivity ≥ 230.

Conclusion

In this study, the SAR of the *Cp*IMPDH inhibitor **1** was investigated. Initially, the anilide portion of the molecule was explored. Several derivatives containing 3,4-di-substitution or 3,4-ring fusion were found to significantly increase inhibitory potency. The aniline nitrogen atom of the urea was necessary for potent activity, while the other nitrogen could be replace with oxygen. The gem-dimethyl group was also found to be best for *Cp*IMPDH inhibition. The 3-isopropenyl substituent could be replaced with a number of hydrophobic or hydrophilic groups, but those with sp^2-hybridization (e.g. phenyl, acetyl, oximes, or O-methyloximes) were best. Eleven compounds displayed potent and selective *in vitro* antiparastic activity, including two that also demonstrated good *in vitro* metabolic stability. These compounds appear to possess the necessary properties for evaluation in an animal model of cryptosporidiosis in order to determine the optimal pharmacological profile necessary for *in vivo* efficacy.

Experimental Section

Chemistry Materials and Methods

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used without further purification. All reactions were performed under nitrogen atmosphere unless otherwise noted. The NMR spectra were obtained using a 400 MHz spectrometer. All ^{1}H NMR spectra are reported in δ units ppm and are reference to tetramethylsilane (TMS) if conducted in $CDCl_3$ or to the central line of the quintet at 2.49 ppm for samples in DMSO-d_6. All chemical shift values are also reported with multiplicity, coupling constants and proton count. All ^{13}C NMR spectra are reported in δ units ppm and are reference to the central line of the triplet at 77.23 ppm if conducted in $CDCl_3$ or to the central line of the septet at 39.5 ppm for samples in DMSO-d_6. Coupling constants (J values) are reported in hertz. Column chromatography was carried out on SILICYCLE SiliaFlash silica gel F60 (40-63 Vm, mesh 230-400). High-resolution mass spectra were obtained using a Q-Tof Ultima mass spectrometer (University of Illinois Urbana-Champaign, Urbana, IL 61801). All melting points were taken in glass capillary tubes and are uncorrected. All test compounds reported in this manuscript had a purity ≥ 95% as determined by high performance liquid chromatography (HPLC) analyses using an instrument equipped with a quaternary pump and a Zorbaxρ SB-C8 column (30 × 4.6 mm, 3.5 μm). UV absorption was monitored at λ = 254 nm. The injection volume was 5 VL. HPLC gradient went from 5 % acetonitrile and 95 % water to 95 % acetonitrile and 5 % water (both solvents contain 0.1% trifluoroacetic acid) over 1.9 min with a total run time of 2.5 min and a flow rate of 3.0 mL/min.

Synthesis of 3-acetyl-α, α-dimethylbenzyl isocyanate (3)

A solution of 3-isopropenyl-α, α-dimethyl benzyl isocyanate (2.04 g, 10.14 mmol) in dichloromethane (40 mL) was cooled to -78 °C and then treated with dry ozone in oxygen until a blue color persist. Excess ozone was flushed off with oxygen. Dimethyl sulfide (0.74 mL, 10.14 mmol) was added to the reaction mixture, which was then stirred overnight at room temperature. Excess Me_2S was removed by evaporated on a water bath placed inside a fume hood. Water (30 mL) was added to the reaction mixture, which was then extracted with dichloromethane. The combined organic layers were washed with brine (30 mL) and dried over anhydrous $MgSO_4$. The mixture was filtered and the filtrate concentrated. The residue was purified by silica gel column chromatography using ethylacetate/ hexane (1: 10) as an eluent to furnish **5b** (1.19 g, 58%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.76 (s, 6H), 2.63 (s, 3H), 7.47 (t, J = 8 Hz, 1H), 7.67 (dd, J_1 = 7.6 Hz, J_2 = 2 Hz, 1H), 7.86 (dt, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 8.04 (t, J = 2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.9, 33.2, 60.9, 124.2, 127.6, 129.0, 129.4, 137.4, 146.6, 198.1.

General procedure for the preparation of urea derivatives 5 : Exemplified for the preparation of 1-(4-chlorophenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5b)

To a solution of 3-isopropenyl α, α-dimethylbenzyl isocyanate **2** (473 mg, 2.35 mmol) in dichloromethane (6 mL) at 0 °C was added 4-chloroaniline (300 mg, 2.35 mmol) in dichloromethane (3 mL). The reaction was stirred until complete consumption of starting materials. The precipitated product was collected by filtration and washed with dichloromethane to give **5b** (852 mg, 80%). mp 234-236 °C Yield 80 %; ^{1}H NMR (DMSO *+d6*, 400 MHz) δ 1.58 (s, 6H), 2.08 (s, 3H), 5.06 (s, 1H), 5.36 (s, 1H), 6.64 (s, 1H), 7.20 (d, *J* = 6.4 Hz, 2H), 7.23-7.32 (m, 5H), 7.47 (s, 1H), 8.55 (s, 1H); ^{13}C NMR (DMSO *+d6*, 100 MHz) δ 22.24, 30.30, 55.0, 113.06, 119.50, 122.35, 123.69, 124.86, 124.95, 128.65, 129.09, 140.11, 140.73, 143.60, 148.95, 154.53; ESI-HRMS for $C_{19}H_{22}N_2OCl$ $(M+H)^+$ calcd. 329.1421, found 329.1418.

1-Phenyl-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5a)

mp 188-190 °C; Yield 81%; ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 1.58 (s, 6H), 2.08 (s, 3H), 5.06 (s, 1H), 5.36 (s, 1H), 6.59 (s, 1H), 6.83 (t, *J* =7.2 Hz, 1H), 7.15 (t, *J* = 8 Hz, 2H), 7.24-7.31 (m, 5H), 7.47 (s, 1H), 8.39 (s, 1H); ^{13}C NMR (DMSO-*d6*, 100 MHz) δ 22.25, 30.39, 54.97, 113.05, 118.00, 121.55, 122.36, 123.65, 124.87, 128.64, 129.28, 140.70, 141.12, 143.62, 149.11, 154.71; ESI-HRMS for $C_{19}H_{23}N_2O$ $(M+H)^+$ calcd. 295.1810, found 389.1815.

1-(4-Bromophenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5c)

Yield 78 %; ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 1.61 (s, 6H), 2.10 (s, 3H), 5.08 (s, 1H), 5.38 (s, 1H), 6.66 (s, 1H), 7.28-7.35 (m, 5H), 7.49 (s, 1H), 8.57 (s, 1H); ^{13}C NMR (DMSO-*d6*, 100 MHz) δ 22.23, 30.29, 55.07, 112.76, 113.06, 119.96, 122.35, 123.70, 124.85, 128.65, 131.97, 140.53, 140.74, 143.62, 148.94, 154.50; ESI-HRMS for $C_{19}H_{22}N_2OBr$ $(M+H)^+$ calcd. 373.0915, found 373.0915.

1-(3-Chlorophenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5d)

Yield 83 %; ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 1.59 (s, 6H), 2.09 (s, 3H), 5.07 (s, 1H), 5.37 (s, 1H), 6.70 (s, 1H), 6.89 (d, *J* = 7.6 Hz, 1H), 7.05 (d, *J* = 7.6 Hz 1H), 7.19 (t, *J* = 8 Hz ,1H), 7.28-7.30 (m, 3H), 7.48 (s, 1H), 7.60 (s, 1H), 8.63 (s,1H) ; ^{13}C NMR (DMSO-*d6*, 100 MHz) δ 22.23, 30.26, 55.08, 113.08, 116.41, 117.32, 121.15, 122.32, 123.71, 124.83, 128.67, 130.88, 133.78, 140.74, 142.63, 143.60, 148.88, 154.44; ESI-HRMS for $C_{19}H_{22}N_2OCl$ $(M+H)^+$ calcd. 329.1421, found 329.1430.

1-(2-Chlorophenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5e)

Yield 65 %; ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 1.62 (s, 6H), 2.1 (s, 3H), 5.08 (s, 1H), 5.38 (s, 1H), 6.91 (t, *J* = 7.2 Hz, 1H), 7.16 (t, *J* = 8.8 Hz, 1H), 7.30-7.32 (m, 3H), 7.37 (dd, 1H, *J* = 7.2 Hz, J2 = 3.2 Hz), 7.49-7.52 (m, 2H), 8.02 (dd, J_1 = 7.8 Hz, *J* = 2 Hz), 8.09 (d, J = 2 Hz, 1H); ^{13} 2 C NMR (DMSO-*d6*, 100 MHz) δ 22.22, 30.29, 55.17, 113.06, 121.08, 121.50, 122.32, 122.86, 123.69, 124.86, 127.99, 128.68, 129.67, 137.38, 140.71, 143.59, 148.92, 154.22; ESI-HRMS for $C_{19}H_{22}N_2OCl$ $(M+H)^+$ calcd. 329.1421, found 329.1430.

1-(4-Methoxyphenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5f)

Yield 85%; ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 1.59 (s, 6H), 2.10 (s, 3H), 3.66 (s, 3H), 5.08 (s, 1H), 5.37 (s, 1H), 6.50 (s, 1H), 6.77 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.29-7.31 (m, 3H), 7.49 (s, 1H), 8.22 (s, 1H); ^{13}C NMR (DMSO-*d6*, 100 MHz) δ 22.23, 30.46, 54.92, 55.74, 113.01, 114.47, 119.64, 122.36, 123.60, 124.88, 128.61, 134.28,

140.67, 143.63, 149.25, 154.40, 154.95; ESI-HRMS for $C_{20}H_{25}N_2O_2$ $(M+H)^+$ calcd. 325.1916, found 325.1919.

1-(4-(*tert*-Butyl)phenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea-(5g)

Yield 87%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.20 (s, 9H), 1.58 (s, 6H), 2.09 (s, 3H), 5.06 (s, 1H), 5.36 (s, 1H), 6.54 (s, 1H), 7.15-7.19 (m, 4H), 7.27-7.30 (m, 3H), 7.48 (s, 1H), 8.31 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.25, 30.45, 31.93, 34.42, 54.95, 113.02, 117.88, 122.37, 123.62, 124.87, 125.84, 128.62, 138.52, 140.70, 143.63, 143.79, 149.19, 154.82; ESI-HRMS for $C_{23}H_{31}N_2O$ $(M+H)^+$ calcd. 351.2436, found 3512433.

1-(3,4-Dichlorophenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5h)

mp 198-200 °C Yield 80 %; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.61 (s, 6H), 2.10 (s, 3H), 5.09 (s, 1H), 5.38 (s, 1H), 6.79 (s, 1H), 7.13 (t, J = 4.8 Hz, 1H), 7.31 - 7.34 (m, 3H), 7.42 (d, J =7.6 Hz ,1H), 7.49 (s, 1H), 7.79 (s, 1H), 8.79 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.23, 30.21, 55.14, 113.03, 118.13, 118.95, 122.31, 123.75, 124.82, 128.69, 131.07, 131.58, 140.76, 141.31, 141.34, 143.58, 148.79, 154.33; ESI-HRMS for $C_{19}H21N_2OCl_2$ $(M+H)^+$ calcd. 363.1031, found 363.1029.

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5i)

Yield 82%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.61 (s, 6H), 2.10 (s, 3H), 5.09 (s, 1H), 5.38 (s, 1H), 6.81 (s, 1H), 7.30-7.33 (m, 3H), 7.42-7.44 (m, 1H), 7.49-7.53 (m, 2H), 8.01 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.22, 30.22, 55.20, 113.11, 116.35, 116.40, 121.90, 122.12, 122.30, 122.78, 123.77, 124.81, 128.70, 132.50, 140.66, 140.78, 143.58, 148.73, 154.36; ESI-HRMS for $C_{20}H_{21}N_2OClF_3$ $(M+H)^+$ calcd. 397.1295, found 397.1296.

1-(4-Chloro-3-methoxyphenyl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5j)

Yield 89%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.59 (s, 6H), 2.09 (s, 3H), 3.73 (s, 3H), 5.07 (s, 1H), 5.37 (s, 1H), 6.66 (s, 1H), 6.72 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 8.8 Hz,1H), 7.28-7.32 (m, 4H), 7.48 (s, 1H), 8.59 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.23, 30.34, 55.03, 56.28, 102.50, 110.59, 112.95, 113.10, 122.33, 123.72, 124.85, 128.70, 130.16, 140.75, 141.47, 143.59, 148.97, 154.53, 155.10; ESI-HRMS for $C_{20}H_{24}N_2O_2Cl$ $(M+H)^+$ calcd. 359.1529, found 359.1532.

2-Chloro-5-(3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)ureido)benzamide (5k)

Yield 72 %; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.57 (s, 6H), 2.06 (s, 3H), 5.04 (s, 1H), 5.34 (s, 1H), 6.65 (s, 1H), 7.22 - 7.27 (m, 5H), 7.44-7.48 (m, 3H), 7.75 (s, 1H), 8.61 (s,1H) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.23, 30.27, 55.08, 113.08, 117.61, 119.67, 121.36, 122.31, 123.71, 124.82, 128.66, 130.33, 137.89, 139.95, 140.73, 143.59, 148.89, 154.45, 168.85; ESI-HRMS for $C_{20}H_{23}N_3O_2Cl$ $(M+H)^+$ calcd. 372.1479, found 372.1485.

2-Chloro-N-methyl-5-(3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)ureido)benzamide (5l)

Yield 72%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.64 (s, 6H), 2.17 (s, 3H), 2.89 (d, J = 4.8 Hz, 3H), 5.05 (s, 1H), 5.32 (s, 1H), 5.97 (s, 1H,), 6.61 (d, J = 4.4 Hz, 1H), 7.10-7.13 (m, 2H), 7.27-7.31 (m, 2H), 7.49 −7.51 (m, 3H), 7.81 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.25, 26.56, 30.26, 55.08, 113.09, 117.76, 119.75, 121.57, 122.31, 123.71, 124.82, 128.66, 130.32, 137.80, 140.01, 140.74, 143.59, 148.90, 167.41, 189.22; ESI-HRMS for $C_{21}H_{25}N_3O_2Cl$ $(M+H)^+$ cacld. 386.1635, found 386.1639.

2-Chloro-N,N-dimethyl-5-(3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)ureido)benzamide (5m)

Yield 72%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.66 (s, 6H), 2.13 (s, 3H), 2.86 (s, 3H), 3.07 (s, 3H), 5.05 (s, 1H), 5.33 (s, 1H), 6.02 (s, 1H,), 6.88 (d, *J* = 2.4 Hz, 1H), 7.11 (d, *J* = 8.8Hz, 2H), 7.25-7.35 (m, 4H), 7.50 (s, 1H), 8.01 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.17, 29.89, 30.16, 34.94, 38.49, 55.13, 112.51, 116.93, 120.92, 121.29, 122.18, 123.81, 124.24, 128.33, 129.97, 135.15, 139.83, 141.27, 143.81, 147.93, 154.48, 169.80; ESI-HRMS for $C_{22}H_{27}N_3O_2Cl$ $(M+H)^+$ calcd. 400.1792, found 400.1787.

1-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl) 3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5n)

Yield 75%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.59 (s, 6H), 2.10 (s, 3H), 4.15 (d, J = 7.6 Hz, 4H), 5.08 (s, 1H), 5.37 (s, 1H), 6.50 (s, 1H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.97 (s, 1H), 7.29-7.31 (m, 3H), 7.49 (s, 1H), 8.22 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.22, 30.42, 54.93, 64.44, 64.82, 107.22, 111.36, 113.0, 117.31, 122.35, 123.60, 124.85, 128.60, 134.89, 138.31, 140.68, 143.62, 143.65, 149.17, 154.81; ESI-HRMS for $C_{21}H_{25}N_2O_3$ $(M+H)+$ calcd. 353.1865, found 353.186.

1-(Naphthalen-2-yl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5o)

mp 201-203 °C; Yield 75%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.64 (s, 6H), 2.11 (s, 3H), 5.08 (s, 1H), 5.39 (s, 1H), 6.72 (s, 1H), 7.27-7.39 (m, 6H), 7.53 (d, *J* = 1.2 Hz, 1H), 7.68 (d, *J* = 8 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.98 (s, 1H), 8.64 (s, 1H) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.23, 30.35, 55.04, 112.83, 113.06, 119.92, 122.37,123.69, 124.12, 124.90, 126.85, 127.38, 128.01, 128.67, 128.89, 129.29, 134.49, 138.73, 140.75, 143.63, 149.09, 154.78; ESI-HRMS for $C_{23}H_{25}N_2O$ $(M+H)^+$ calcd. 345.1967, found 345.1964.

1-(Naphthalen-1-yl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5p)

mp 206-208 °C; Yield 75%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.66 (s, 6H), 2.10 (s, 3H), 5.08 (s, 1H), 5.39 (s, 1H), 7.13 (s, 1H), 7.29-7.38 (m, 4H), 7.50-7.55 (m, 4H), 7.90 (d, *J* = 7.6 Hz, 1H), 7.87 (d, *J* = 8 Hz, 1H), 8.10 (d, *J* = 8.4 Hz, 1H), 8.54 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.24, 30.42, 55.16, 113.05, 116.51, 121.79, 122.40, 123.68, 124.92, 125.80, 126.01, 126.36, 126.52, 128.68, 129.06, 134.33, 135.77, 140.74, 143.61, 149.15, 155.02; ESI-HRMS for $C_{23}H_{25}N_2O$ $(M+H)^+$ calcd. 345.1967, found 345.1976.

1-(2-(3-(Prop-1-en-2-yl)phenyl)propan-2-yl)-3-(quinolin-6-yl)urea (5q)

Yield 81%; 1H NMR (DMSO-d_6, 400 MHz) δ 1.65 (s, 6H), 2.11 (s, 3H), 5.08 (s, 1H), 5.39 (s, 1H), 7.10 (s, 1H), 7.29-7.37 (m, 2H), 7.54 (s, 1H), 7.67-7.74 (m, 2H), 8.04 (d, *J* = 9.2 Hz, 1H), 8.27 (d, *J* = 2 Hz, 1H), 8.33 (s, 1H), 8.57 (d, *J* = 8.4 Hz, 1H), 8.87 (d, *J* = 4 Hz, 1H), 9.39 (s, 1H) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.25, 30.31, 55.14, 112.04, 113.07, 122.37, 122.45, 123.68, 124.87, 125.70, 125.75, 128.65, 130.06, 138.77, 138.79, 140.72, 140.75, 143.58, 145.04, 148.96, 154.68; ESI-HRMS for $C_{22}H_{24}N_3O$ $(M+H)^+$ calcd. 346.1919, found 346.1925.

1-(2-(3-(Prop-1-en-2-yl)phenyl)propan-2-yl)-3-(quinolin-7-yl)urea (5r)

Yield 72%; 1H NMR (DMSO-d_6, 400 MHz) δ 1.64 (s, 6H), 2.11 (s, 3H), 5.08 (s, 1H), 5.39 (s, 1H), 6.79 (s, 1H), 7.28-7.37 (m, 4H), 7.47-7.53 (m, 2H), 7.80 (d, *J* = 8.8 Hz, 1H), 8.03 (d, *J* = 2 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 8.75 (dd, J_1 = 4.4 Hz, J_2=1.6 Hz, 1H), 8.85 (s, 1H) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.23, 30.30, 55.16, 113.09, 113.66, 119.69, 120.23, 122.37, 123.73, 124.88, 128.69, 128.98, 136.03, 140.77, 142.06, 143.61, 148.92, 149.48, 151.27, 154.55; ESI-HRMS for $C_{22}H_{24}N_3O$ $(M+H)^+$ calcd. 346.1919, found 346.1915.

1-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)-3-(quinolin-3-yl)urea (5s)

Yield 69%; 1H NMR (DMSO-d_6, 400 MHz) δ 1.65 (s, 6H), 2.11 (s, 3H), 5.09 (s, 1H), 5.39 (s, 1H), 6.88 (s, 1H), 7.31-7.38 (m, 3H), 7.49-7.55 (m, 3H), 7.79 (d, J = 7.2 Hz, 1H), 7.88 (d, J = 7.4 Hz, 1H), 8.41 (s, 1H), 8.66 (d, J = 2.4 Hz, 1H), 8.91 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.24, 30.25, 55.17, 113.11, 119.44, 122.35, 123.75, 124.89, 127.27, 127.49, 127.81, 128.71, 128.90, 129.10, 134.88, 140.77, 143.60, 143.82, 144.47, 148.88, 154.72; ESI-HRMS for $C_{22}H_{24}N_3O$ $(M+H)^+$ calcd. 346.1919, found 346.1924.

1-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)-3-(quinolin-2-yl)urea (5t)

mp 126-128 °C; Yield 75%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.90 (s, 6H), 2.13 (s, 3H), 5.05 (s, 1H), 5.37 (s, 1H), 6.54 (d, J = 8.8 Hz, 1H), 7.25-7.38 (m, 3H), 7.48 (d, J = 7.6 Hz, 1H), 7.57-7.67 (m, 4H), 7.83 (d, J = 8.8 Hz, 1H), 9.69 (s, 1H), 10.92 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.20, 30.11, 55.62, 113.19, 114.22, 122.47, 123.95, 124.71, 124.84, 124.89, 126.57, 128.42, 128.82, 130.71, 139.03, 140.94, 143.57, 145.55, 148.65, 153.54, 154.10; ESI-HRMS for $C_{22}H_{24}N_3O$ $(M+H)^+$ calcd. 346.1919, found 346.1917.

1-(4-Chlorophenyl)-1-methyl-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (5u)

Yield 56%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.50 (s, 6H), 2.06 (s, 3H), 3.11 (s, 3H), 5.04 (s, 1H), 5.32 (s, 1H), 6.11 (s, 1H), 7.21-7.25 (m, 5H), 7.34-7.40 (m, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.25, 30.28, 37.49, 55.72, 112.90, 122.26, 123.40, 124.81, 127.92, 128.49, 129.50, 129.58, 140.54, 143.64, 144.18, 149.37, 156.06; ESI-HRMS for $C_{20}H_{24}N_2OCl$ $(M+H)^+$ calcd. 343.1577, found 343.1570.

General procedure for the preparation of urea derivatives 6: Exemplified for the preparation of 5-(3-(2-(3-acetylphenyl)propan-2-yl)ureido)-2-chlorobenzamide (6c)

To a solution of 3-acetyl α, α dimethyl isocyanate **3** (118 mg, 0.584 mmol) in THF at room temperature was added 5-amino-2-chlorobenzamide (100 mg, 0.584 mmol). The reaction was heated to 70 °C for 6 h. Volatiles were removed under reduced pressure and the residue was purified by column chromatography using methanol/chloroform as eluent to obtain urea derivative **6c** (156 mg, 72%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.61 (s, 6H), 2.58 (s, 3H), 6.80 (s, 1H), 7.26 (s, 2H), 7.45-7.52 (m, 3H), 7.66 (d, J = 7.2 Hz, 1H), 7.79-7.83 (m, 2H), 7.94 (s, 1H), 8.68 (s, 1H) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.44, 30.19, 54.89, 117.63, 119.72, 121.43, 124.57, 127.12, 129.11, 130.42, 137.24, 137.89, 139.87, 149.54, 154.40, 168.87, 198.76; ESI-HRMS for $C_{25}H_{16}N_3O$ $(M+H)^+$ calcd. 374.1293, found 374.1283.

1-(2-(3-acetylphenyl)propan-2-yl)-3-(4-chlorophenyl)urea (6a)

Yield 85 %; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.57 (s, 6H), 2.52 (s, 3H), 6.71 (s, 1H), 7.17 (d, J = 9.2 Hz, 2H), 7.29 (d, J = 8.8 Hz, 2H), 7.42 (t, J = 7.6 Hz, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.91 (s, 1H), 8.54 (s, 1H) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 29.8, 32.6, 57.2, 121.9, 127.0, 127.4, 129.4, 131.5, 131.55, 132.8, 139.7, 142.4, 152.0, 156.9, 201.1; ESI-HRMS for $C_{18}H_{19}ClN_2O_2$ $(M+H)^+$: calcd. 331.1213, found 331.1214..

1-(2-(3-Acetylphenyl)propan-2-yl)-3-(4-chloro-3 (trifluoromethyl)phenyl)urea (6b)

Yield 69 %; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.62 (s, 6H), 2.51 (s, 3H), 6.91 (s, 1H), 7.42 - 7.53 (m, 3H), 7.68 (d, J = 7.2 Hz, 1H), 7.84 (d, J = 7.2 Hz, 1H), 7.95 (s, 1H), 8.01 (s, 1H), 8.95 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.45, 30.14, 54.99, 116.36, 116.41, 116.47, 121.96, 122.83, 124.56, 127.17, 129.15, 130.42, 132.50, 137.28, 140.58, 149.37, 154.31, 198.71; ESI-HRMS for $C_{19}H_{19}N_2O_2ClF_3$ $(M+H)^+$ calcd. 399.1087, found 399.1089.

General procedure for the preparation of oxime derivatives 7: Exemplified for the preparation of (E) 2 chloro 5-(3-(2-(3-(1-(hydroxyimino)ethyl)phenyl)propan-2-yl)ureido)benzamide (7c)

Hydroxylamine hydrochloride (25 mg, 0.362 mmol) was added to a solution of **56** (100 mg, 0.302 mmol) in 3 mL of pyridine. The reaction solution was heated to 90 °C for 2 hours. The reaction was allowed to cool to room temperature and then the pyridine was removed by evaporation under reduced pressure. The resulting residue was dissolved in methanol and purified by column chromatography eluting with methanol/chloroform to obtained **7c** (88 mg, 85%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.54 (s, 6H), 2.11 (s, 3H), 6.67 (s, 1H), 7.20 - 7.49 (m, 7H), 7.67 (s, 1H), 7.76 (s, 1H), 8.62 (s, 1H), 11.13 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.42, 30.27, 54.98, 117.59, 119.67, 121.38, 122.44, 124.21, 125.87, 128.76, 130.35, 137.33, 137.90, 139.96, 148.95, 153.79, 154.39, 168.90; ESI-HRMS for $C_{19}H_{22}N_4O_3Cl$ $(M+H)^+$ calcd. 389.1380, found 389.1384.

(E)-1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(2-(3-(1-(hydroxyimino)ethyl)phenyl)propan-2-yl)urea (7a)

mp 194-196 °C; Yield 71%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.61 (s, 6H), 2.15 (s, 3H), 6.82 (s, 1H), 7.34 (d, J = 8 Hz, 1H), 7.39 – 7.46 (m, 3H), 7.52 (d, J = 9.2 Hz, 1H), 7.70 (s, 1H), 8.01 (s, 1H), 8.91(s, 1H), 11.17 (bs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.39, 30.19, 55.07, 116.31, 116.37, 116.42, 121.91, 122.44, 122.76, 124.25, 125.84, 128.78, 132.50, 137.36, 140.64, 149.77, 153.74, 154.29; ESI-HRMS for $C_{19}H_{20}N_3O_2ClF_3$ $(M+H)^+$ calcd. 414.1196, found 414.1191.

(E)-1-(4-Chloro-3-nitrophenyl)-3-(2-(3-(1-(hydroxyimino)ethyl)phenyl)propan-2-yl)urea (7b)

mp 183-185 °C**;** Yield 80%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.62 (s, 6H), 2.16 (s, 3H), 6.91 (s, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.41-7.47 (m, 3H), 7.58 (d, J = 8.8 Hz, 1H), 7.71 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 9.04 (s, 1H), 11.18 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.4, 30.13, 55.12, 113.84, 116.26, 122.42, 122.86, 124.27, 125.84, 128.79, 132.28, 137.36, 141.11, 148.14, 148.67, 153.74, 154.12; ESI-HRMS for $C_{18}H_{20}N_4O_4Cl$ $(M+H)^+$ calcd. 391.1173, found 391.1172.

(E)-1-(2-(3-(1-(Hydroxyimino)ethyl)phenyl)propan-2-yl) 3 (naphthalen-2-yl)urea-(7d)

Yield 85 %; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.69 (s, 6H), 2.21 (s, 3H), 6.81 (s, 1H), 7.35-7.50 (m, 6H), 7.72 - 7.80 (m, 4H), 8.05 (s, 1H), 8.70 (s, 1H), 11.22 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.40, 30.32, 54.90, 112.75, 119.86, 122.49, 124.11, 124.16, 125.93, 126.85, 127.37, 128.00, 128.74, 128.90, 129.26, 134.48, 137.32, 138.70, 149.12, 153.77, 154.66; ESI-HRMS for $C_{22}H_{24}N_3O2$ $(M+H)^+$ calcd. 362.1869, found 362.1872.

(E)-1-(2-(3-(1-(Hydroxyimino)ethyl)phenyl)propan-2-yl)-3 (quinolin-7-yl)urea (7e)

mp 164-166 °C; Yield 85 %; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 1.68 (s, 6H), 2.28 (s, 3H), 6.99-7.02 (m, 2H), 7.25 (t, J = 8 Hz, 1H), 7.53 -7.62 (m, 3H), 7.88 (t, J = 9.6 Hz, 2H), 8.16 (s, 1H), 8.63 (s, 1H), 8.77 (d, J = 1.6 Hz, 1H), 9.54 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.41, 30.29, 55.03, 113.61, 119.70, 120.20, 122.50, 123.78, 124.22, 125.93, 128.77, 129.01, 136.04, 137.35, 142.06, 148.97, 149.4, 151.29, 153.77, 154.47; ESI-HRMS for $C_{21}H_{23}N_4O_2$ $(M+H)^+$ calcd. 363.1821, found 363.1825.

General procedure for the preparation of alkoxy oxime derivatives 8: Exemplified for the preparation of (E)-2-chloro-5-(3-(2-(3-(1-(methoxyimino)ethyl)phenyl)propan-2-yl)ureido)benzamide (8b)

Methoxyamine hydrochloride (30 mg, 0.362 mmol) was added to a solution of **6b** (100 mg, 0.302 mmol) in 3 mL of pyridine. The reaction solution was heated to 90 °C for 2 h and then the pyridine was removed by evaporation under reduced pressure. The residue was dissolved in methanol and purified by column chromatography eluting with methanol/chloroform to obtained **8b** (99 mg, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.55 (s, 6H), 2.12 (s, 3H), 3.91 (s, 3H), 6.18 (s, 1H), 6.59 (s, 1H), 6.86 (s, 1H), 7.06 (d, J = 8.8 Hz, 1H), 7.15 (s, 1H), 7.25 (d, J = 6.8 Hz, 1H), 7.33 (d, J = 7.6 Hz, 1H), 7.37 (d, J = 7.6 Hz, 2H), 7.67 (1H, s), 7.97 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.29, 30.23, 54.97, 62.17, 117.68, 119.75, 121.42, 122.77, 124.49, 126.46, 128.83, 130.33, 136.30, 137.89, 139.93, 149.10, 154.43, 155.037, 168.88; ESI-HRMS for $C_{20}H_{24}N_4O_3Cl$ $(M+H)^+$ calcd. 403.1537, found 403.1532.

(E)-1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(2-(3-(1-(methoxyimino)ethyl)phenyl)propan-2-yl)urea (8a)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.62 (s, 6H), 2.1 (s, 3H), 3.90 (s. 3H), 6.84 (s, 1H), 7.36 (dd, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.43-7.48 (m, 3H), 7.52 (dd, J_1 = 8.8 Hz, J_2 = 1.6 Hz), 7.67 (s, 1H), 8.01 (s. 1H), 8.93 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.80, 29.86, 55.30, 62.04, 117.77, 117.82, 117.88, 122.29, 122.87, 124.88, 124.93, 125.63, 128.82, 131.77, 136.96, 137.96, 147.31, 154.63, 154.91; ESI-HRMS for $C_{20}H_{22}N_3O_2F_3Cl$ $(M+H)^+$ calcd. 428.1353, found 428.1353.

(E)-1-(2-(3-(1-(Methoxyimino)ethyl)phenyl)propan-2-yl)-3 (naphthalen-2-yl)urea (8c)

Yield 58%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.65 (s, 6H) 2.19 (s, 3H), 3.90 (s, 3H), 6.78 (s, 1H), 7.30-7.42 (4H, m), 7.48 (d, J = 7.2 Hz, 2H), 7.68-7.72 (m, 2H), 7.76 (d, J = 8.4 Hz, 2H), 8.00 (s, 1H), 8.67 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.30, 30.30, 54.91, 62.16, 112.8, 119.9, 122.8, 124.1, 124.4, 126.5, 126.8, 127.3, 128.0, 128.83, 128.89, 129.28, 134.47, 136.29, 138.68, 149.28, 154.71, 155.06; ESI-HRMS for $C_{23}H_{26}N_3O_2$ $(M+H)^+$ calcd. 376.2025, found. 376.2026.

Preparation of (E)-1-(2-(3-(1-((2-aminoethoxy)imino)ethyl)phenyl)propan-2-yl)-3 (4-chloro-3-nitrophenyl)urea (9)

(E)-1-(4-Chloro-3-nitrophenyl)-3-(2-(3-(1-(hydroxyimino)ethyl)phenyl)propan-2-yl)urea (**7b**, 100 mg, 0.25 mmol) in 3 mL dry DMF was added drop wise at 0 °C to sodium hydride dispersion in mineral oil (15.3 mg, 0.50 mmol). The resulting mixture was stirred at 0 °C for 30 min. 2-Chloroethylamine hydrochloride (29.6 mg, 0.25 mmol) in 2 mL DMF was added and the reaction mixture was stirred at room temperature for 2 h and then the volatiles were removed under reduced pressure. The residue was dissolved in a minimal amount of methanol and purified by column chromatography using methanol/chloroform to obtained **9** (49 mg, 46 %) ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.61 (s, 6H), 2.19 (s, 3H), 2.79 (t, J = 5.6 Hz, 2H), 4.05 (t, J = 5.6 Hz, 2H), 6.95 (s, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.42-7.47 (m, 3H), 7.56 (d, J = 8.8 Hz, 1H), 7.65 (s, 1H), 8.20 (d, J = 2 Hz, 1H), 9 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.3, 30.1, 41.6, 55.4, 76.6, 113.8, 116.2, 122.7, 122.9, 124.5, 126.3, 128.8, 132.2, 136.5, 141.1, 148.1, 148.8, 154.2, 154.9; ESI-HRMS for $C_{20}H_{24}Cl\ N_5O_4$ $(M+H)^+$ calcd. 434.1595, found. 434.1595.

1-(4-chlorophenyl)-3-(2-(3-(1-hydroxyethyl)phenyl)propan-2-yl)urea (10)

A solution of 1-(2-(3-acetylphenyl)propan-2-yl)-3-(4-chlorophenyl)urea (25 mg, 0.07 mmol) in THF was cooled in an ice bath to 0 °C. Lithium aluminum hydride solution (2M in THF, 0.8 equiv) was added drop wise over 5 min and then the reaction was continued for

approximately 1 h at 0 °C until starting material disappeared. The reaction was carefully quenched with a solution of sodium sulfate. The reaction mixture was filtered through a sintered funnel and the supernatant washed with dichloromethane. Combined organic fractions were concentrated under reduced pressure. The residue was purified by column chromatography using chloroform-methanol as a eluent to give **10** (19 mg, 74%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.25 (d, J = 6 Hz, 3H), 1.54 (s, 6H), 4.64 (pent, J = 4.4 Hz, 1H), 5.09 (d, J = 4 Hz, 1H), 6.56 (s, 1H), 7.11-7.19 (m, 5H), 7.28 (s, 1H), 7.31 (d, J = 9.2 Hz, 1H), 8.51 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.78, 30.33, 30.44, 55.06, 68.94, 119.47, 122.42, 123.70, 124.88, 128.28, 129.10, 140.18, 147.68, 148.65, 154.48; ESI-HRMS for $C_{18}H_{22}N_2O_2Cl$ $(M+H)^+$ calcd. 333.1370, found 333.1378.

1-(2-(3-Isopropylphenyl)propan-2-yl)-3-(naphthalen-2-yl)urea (11)

A solution of 1-(naphthalen-2-yl)-3-(2-(3-(prop-1-en-2-yl)phenyl)propan-2-yl)urea (**5o**, 25 mg, 0.072 mmol) in methanol (3 mL) containing a catalytic amount of 10% Pd/C was placed under an atmosphere of hydrogen. After 1h the reaction mixture was filtered through a short silica gel column and concentrated to give **11** (24 mg, 98%).^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.17 (d, J = 4.4 Hz, 6H), 1.60 (s, 6H), 2.84 (hept, J = 6.8 Hz, 1H), 6.66 (s, 1H), 7.03-7.36 (m, 7H), 7.66 (d, J = 8 Hz, 1H), 7.75 (d, J = 8 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.69, 30.42, 34.26, 55.09, 122.75, 118.22, 119.91, 123.01, 123.58, 124.08, 124.31, 126.84, 127.36, 128.01, 128.59, 128.87, 129.25, 134.49, 138.80, 149.00, 154.78; ESI-HRMS for $C_{23}H_{27}N_2O$ $(M+H)^+$ calcd. 347.2123, found 347.2126.

2-(3-Acetylphenyl)propanenitrile (13)

Thionyl chloride (10 mL) was added at 0 °C to 3-(1-cyanoethyl)benzoic acid (**12**, 500 mg, 2.85 mmol). The reaction mixture was heated at 80 °C for 2 h. The excess thionyl chloride was removed to give 3-(1-cyanoethyl)benzoyl chloride, which was used without further purification.

Next, to a solution of Meldrum's acid (408 mg, 2.84 mmol) in dichloromethane (15 mL) at 0 °C was added pyridine (0.457 mL, 5.68 mmol). The resulting mixture was stirred for 15 min and then 3-(1-cyanoethyl)benzoyl chloride (482.5 mg, 2.5 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min and then for 1h at room temperature. The reaction mixture was diluted with dichloromethane and washed with 1N HCl. The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was dissolved in AcOH-H_2O (1:2) and heated at reflux for 4 h. The reaction mixture was diluted with water and extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with $NaHCO_3$ solution and brine, then dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by column chromatography using hexane-ethyl acetate (9:1) as eluent to give **13** (138 mg, 28%).^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.68 (d, J = 7.2 Hz 3H), 2.63 (s, 3H), 3.99 (q, J = 7.2 Hz, 1H), 6.60 (d, J = 7.6 Hz, 1H), 7.53 (d, J = 2.8 Hz, 1H), 7.60 (d, J = 7.2 Hz, 1H), 7.91 (t, J = 2 Hz, 1H), 7.94 (s, 1H).

2-(3-Acetylphenyl) propanoic acid (14)

To a solution of **13** (100 mg, 0.57 mmol) in 2 mL of 1, 4-dioxane was added conc. HCl (1.5 mL) and then the resulting mixture was refluxed for 5 h. After the mixture was allowed to cool to room temperature, the volatiles were removed under reduced pressure. The residue was diluted with water (10 mL) and extracted with dichloromethane (3 × 10 mL). The organic extracts were combined, washed with brine (2 × 10 mL), dried over anhydrous magnesium sulfate, filtered and concentrated to give **14** as a white solid (86 mg, 78% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.57 (d, J = 7.2 Hz, 3H), 2.38 (s, 3H), 3.83 (q, J = 7.2 Hz, 1H), 7.42-7.50 (m, 2H), 7.61 (d, J = 7.6 Hz, 1H), 7.75 (s, 1H).

2-(3-Acetylphenyl)propanoyl azide (15)

Thionyl chloride (2 mL) was added to **14** (86 mg, 0.40 mmol) at 0 °C. The mixture was then heated at 80 °C for 2 h. The reaction mixture was concentrated under reduced pressure. The resulting acid chloride was used without further purification.

A solution of the acid chloride (84 mg, 0.40 mmol) in 3 mL dry acetone was added to a solution of sodium azide (325 mg, 5 mmol) in 2 mL of water at 0 °C over 10 min. The reaction mixture was stirred for 2 h at 25 °C, and then poured into ice and extracted with ether (2 × 10 mL). The organic extracts were combined, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated to give acyl azide **15**, which was used without further purification.

1-(3-(1-Isocyanatoethyl)phenyl) ethanone (16)

Acyl azide **15** (80 mg, 0.42 mmol) was refluxed in benzene (5 mL) of 1.5 h and then the solvent was removed under vacuum to give isocyanate **16** in quantitative yield, which was used without further purification.

1-(1-(3-Acetylphenyl)ethyl)-3-(4-chlorophenyl)urea (17)

Compound **17** (71%) was prepared following the general procedure for **6**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.35 (d, J = 6.8 Hz, 3H), 2.55 (s, 3H), 4.95 (pent, J = 6.8 Hz, 1H), 5.68 (d, J = 7.2 Hz, 1H), 7.12-7.17 (m, 4H), 7.32-7.36 (m, 2H), 7.44 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.84 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.20, 26.97, 49.77, 120.94, 124.96, 127,84, 128.14, 129.14, 129.16, 131.40, 137.47, 137.58, 145.23, 155.23, 199.09; ESI-HRMS for $C_{17}H_{18}N_2O_2Cl$ $(M+H)^+$ calcd. 317.1057, found 317.1060.

3-(2-Methyl-1,-3-dioxolan-2-yl)benzonitrile (19)

To the mixture of ethylene glycol (0.22 mL, 4 mmol), 3-acetylbenzonitrile **18** (300 mg, 2.04 mmol) and benzene (10 mL) in a Dean-Stark apparatus was added a catalytic amount of *p*-TSA (0.1 equiv). The reaction mixture was heated at 110 °C for 4 h. The benzene was removed under reduced pressure and the residue was purified by column chromatography using ethylacetate-hexane as an eluent to give **19** (293 mg, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.62 (s, 4H), 7.59 (t, J = 7.6 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H)), 8.15 (d, J = 8 Hz, 1H), 8.21 (s, 1H).

3-(2-Methyl-1,3-dioxolan 2 yl)phenylmethanamine (20)

A solution of cyano ketal **19** (290 mg, 1.53 mmol) in dry THF (10 mL) was cooled to 0 °C under a nitrogen atmosphere. Then a solution of 2M lithium aluminum hydride (3 mmol) in THF was added over a 10-min period. The reaction mixture was stirred for 1.5 h and then ethyl acetate was added followed by slow addition of water to decompose the excess LAH. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in chloroform, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using chloroform and methanol as an eluent to give **20** (176 mg, yield 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.66 (s, 3H), 3.79 (t, J = 6 Hz, 2H), 3.88 (s, 2H), 4.04 (t, J = 6 Hz, 2H), 7.24-7.42 (m, 4H).

4-Nitrophenyl-3-(2-methyl-1,3-dioxolan 2 yl)benzylcarbamate (21)

To a solution of benzylamine **20** (176 mg, 0.911 mmol) and N,N-diisopropylethylamine (313 cL, 1.8 mmol) in 4 mL of 1:1 CH_2Cl_2/THF was added a solution of 4-nitrophenylchloroformate (366 mg, 1.82 mmol) in 2 mL of 1:1 CH_2Cl_2/THF. After stirring the reaction mixture at room temperature for 24 h, it was diluted with dichloromethane and washed sequentially with saturated $NaHCO_3$, water and brine. The organic layer was dried

over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography eluting with ethylacetate/hexane to give **21** (267 mg, 82%).

1-(4-Chlorophenyl)-3-(3-(2-methyl-1,3-dioxolan-2-yl)benzyl)urea (22)

4-Nitrophenyl-N-benzylcarbamate **21** (267 mg, 0.767 mmol) was added to a solution of 4-chloroaniline (97 mg, 0.767 mmol) and triethylamine in dichloromethane (5 mL). The mixture was stirred at room temperature until starting materials were consumed. The reaction mixture was then diluted with dichloromethane (50 mL) and washed with aq. NaOH, water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated The residue was purified by column chromatography eluting with methanol/chloroform to give **22** (165 mg, 71%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.58 (s, 3H), 3.71 (t, J = 6.8 Hz, 2H), 3.99 (t, J = 6.8 Hz, 2H), 4.36 (d, J = 5.6 Hz, 2H), 6.78 (s, 1H), 7.16-7.18 (m, 4H), 7.23 - 7.26 (m, 1H), 7.35 (d, J = 5.6 Hz, 2H).

1-(3-Acetylbenzyl)-3-(4-chlorophenyl) urea (23)

A 2N HCl solution was added to a solution of **22** (165 mg, 0.479 mmol) in THF (2 mL). The mixture was refluxed for several hours until the starting materials were consumed. The reaction mixture was allowed to cool to room temperature, quenched with solid $NaHCO_3$, and then the volatiles were removed under reduced pressure. The residue was diluted with ethyl acetate and then washed with water. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by chromatography to give **23** (144 mg, 100%)^{1}H NMR (DMSO-d_6, 400 MHz) δ 4.44 (d, J = 5.6 Hz, 2H), 5.37 (t, J = 3.7 Hz, 1H), 6.83 (s, 1H), 7.20-7.26 (5H, m), 7.40 (t, J = 7.6 Hz, 1H), 7.50 (d, J = 7.2 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.85 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.45, 43.13, 119.85, 125.21, 127.11, 127.59, 129.13, 129.37, 132.61, 137.49, 140.07, 141.68, 155.73, 198.58; ESI-HRMS for $C_{16}H_{16}N_2O_2Cl$ $(M+H)^+$ calcd. 303.0900, found 303.0907.

3-Acetyl-α, α-dimethylbenzylamine (24)

3-Acetyl-α, α-dimethylbenzyl isocyanate **3** (1000 mg, 4.92 mmol) in 8N HCl (30 mL) were refluxed for 30 min. The reaction mixture was cooled to 0 °C and then washed with diethyl ether. The aqueous portion was neutralized with a 10% NaOH solution and extracted with diethyl ether (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by chromatography eluting with methanol/chloroform to give **24** as a yellow oil (478 mg, 55%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.57 (s, 6H), 2.61 (s, 3H), 3.72 (s, 2H), 7.43 (t, J = 7.6 Hz, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.82 (d, J =7.6 Hz, 1H), 8.14 (s, 1H).

4-Chlorophenyl (2-(3-acetylphenyl)propan-2-yl)carbamate (25)

4-Chlorophenyl chloroformate (317 cL, 2.24 mmol) in dichloromethane (2 μL) was added to a mixture of 3-acetyl-α, α-dimethylbenzylamine **24** and diisopropylethylamine (390 μL, 2.24 mmol). The reaction mixture was stirred for 2 h. It was then diluted with dichloromethane, washed with 1N HCl, and then brine. The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by column chromatography using hexane/ethyl acetate as eluent to give **25** (594 mg, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.73 (s, 6H), 2.60 (s, 3H), 5.65 (s, 1H), 7.02 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 6 Hz, 2H), 7.44 (t, J = 8 Hz, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 8.07 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.45, 29.79, 55.50, 124.23, 124.55, 127.31, 129.28, 129.55, 129.79, 130.39, 137.30, 148.70, 150.32, 152.97, 198.64; ESI-HRMS for $C_{18}H_{19}NO_3Cl$ $(M+H)^+$ calcd. 332.1053, found 332.1059.

N-(2-(3-Acetylphenyl)propan-2-yl)-2-(4-chlorophenyl)acetamide (26)

4-Chlorophenylacetylchloride (106 mg, 0.56 mmol) in dichloromethane was added to a solution of 3-acetyl-α, α-dimethylbenzylamine **24** (100 mg, 0.56 mmol) and triethylamine (120 μL, 0.86 mmol) in dichloromethane over a period of 5 to 10 min at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The mixture was diluted with dichloromethane (20 mL) and washed with 1N HCl, water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using hexane/ethyl acetate as eluent to give **26** (146 mg, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.61 (s, 6H), 2.53 (s, 3H), 3.46 (s, 2H), 5.68 (s, 1H), 7.19 (d, *J* = 7.2 Hz, 2H), 7.30-7.38 (m, 3H), 7.46 (d, *J* = 8 Hz, 1H), 7.75 (d, *J* =7.2 Hz, 1H), 7.87 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.26, 29.89, 42.54, 55.34, 124.71, 126.76, 128.74, 128.98, 130.23, 131.45, 131.63, 136.22, 137.19, 148.96, 169.54, 198.59; ESI-HRMS for $C_{19}H_{21}NO_2Cl$ $(M+H)^+$ calcd. 330.1261, found 330.1270.

3-Acetyl-α, α-dimethylbenzylalcohol (27)

A solution of 3-acetyl-α α-dimethylbenzylamine (500 mg, 2.82 mmol) in chloroform was cooled to 0 °C. Chlorosulfonic acid (109 mg, 0.94 mmol) was added drop wise over a period of 15 min. During this time a white precipitate formed. Stirring was continued for another 10 min. The reaction mixture was filtered to yield the corresponding substituted N-benzylsulfonic acid (253 mg, 70% with respect to chlorosulfonic acid), which was used without further purification.

The N-benzylsulfonic acid (169 mg, 0.658 mmol) was suspended in 2 mL of water and then KNO_2 (168 mg, 1.97 mmol, 3 equiv) was added. An almost clear solution was immediately formed. To this solution was added 2 mL sulfate buffer (pH = 2.8) and the resulting mixture was stirred for 2 hours. The reaction mixture was diluted with 4 mL water and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with water. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography eluting with ethyl acetate/hexane to obtained **26** (26 mg, ~30%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.55 (s, 6H), 2.55 (s, 3H), 7.37 (t, *J* = 8 Hz, 1H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 8.04 (s, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 26.9, 31.9, 72.5, 124.4, 126.9, 128.6, 129.6, 137.1, 150.0, 198.8.

2-(3-Acetylphenyl) propan-2-yl (4-chlorophenyl)carbamate (28)

To a solution of 3-acetyl-α, α-dimethylbenzylalcohol (**27**, 21 mg, 0.117 mmol) in 2 mL dry benzene was added 4-chlorophenyl isocyante, followed by 17 μL triethyamine. The reaction mixture was heated to 70 °C for 3 h. The reaction mixture was allowed to cool to room temperature and then diluted with ethyl acetate (20 mL), washed with 1N HCl, brine and water. The organic layer was dried over anhydrous magnesium sulfate, filtered and evaporated. The residue was purified by column chromatography eluting with ethyl acetate/ hexane yielding **28** (23 mg, 62%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.84 (s, 6H), 2.60 (s, 3H), 6.71 (s, 1H), 7.20 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.45 (t, *J* = 8.0 Hz), 7.62 (d, *J* = 7.6 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.96, 29.08, 81.73, 119.8, 127.74, 128.90, 129.15, 129.26, 129.92, 136.67, 137.39, 146.71, 151.89, 198.29; ESI-HRMS for $C_{18}H_{18}ClNO_3$ (M+Na)+ calcd. 354.0873, found 354.0872.

3-Phenyl α, α-dimethylbenzylamine (30)

To a room temperature stirring solution of 3-cyanobiphenyl (200 mg, 1.11 mmol) in 5 mL of diethyl ether was added 1M methylmagnesium bromide in butyl ether (3.33 mmol). The reaction mixture was stirred for 30 min and then 326 μL (1.11 mmol) of $(i\text{-}PrO)_4Ti$ was added. The solution became dark-brown and was stirred overnight before being treated with

10% solution NaOH (4 mL). The suspension was filtered to remove precipitated inorganic material, which washed with dichloromethane. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic solutions were washed with water, brine and then dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. The residue was purified by column chromatography eluting with methanol/chloroform to give **30** (133 mg, 57%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.57 (s, 6H), 2.61 (bs, 2H), 7.34 -7.50 (m, 6H), 7.60 (d, *J*=7.2 Hz, 2H), 7.73 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 32.7, 53.0, 123.97, 123.98, 125.4, 127.4, 127.5, 128.9, 141.4, 141.7, 150.4.

3-Phenyl α-cyclopropylbenzylamine (31)

Ethyl magnesium bromide (3M) in diethyl ether (1.841 mmol, 2.2 eq.) was added at −78 °C to a solution of 3-cyanobiphenyl (150 mg, 0.838 mmol) and $Ti(Oi\text{-}Pr)_4$ (0.269 mL, 0.920 mmol) in 5 mL of Et_2O. The yellow solution was stirred for 10 min and then the solution was allowed to warm to room temperature and continued to be stirred for 1 h before BF_3•OEt_2 (0.206 mL, 1.76 mmol) was added. Stirring was continued for an additional hour. The reaction mixture was quenched with 3 mL of 1 N HCl and then washed with ether (15 mL). The aqueous layer was basified with 10% NaOH (10 mL) and extracted with diethyl ether. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with methanol/chloroform to yield **31** (98 mg, 52%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.1 (t, *J* = 4 Hz, 2H), 1.04 (t, *J* = 4 Hz, 2H), 7.25 -7.79 (m, 1H), 7.35-7.46 (m, 5H), 7.52 (s, 1H), 7.58 (d, *J* = 7.2 Hz,1H).

4-Nitrophenyl(2-([1, 1′-biphenyl]-3-yl) propan-2-yl)carbamate (33)

Prepared following the same procedure as **21**. Yield 71%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.62 (s, 6H), 6.81 (d, *J* = 8.8 Hz, 2H), 7.26-7.66 (m, 10H), 8.11 (d, *J* = 8.8 Hz, 2H).

4-Nitrophenyl(1-([1,1′-biphenyl]-3-yl)cyclopropyl)carbamate (34)

Prepared following the same procedure as **21**. Yield 68%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39-1.40 (m, 2H), 1.42-1.45 (m, 2H), 6.89 (d, *J* = 9.2 Hz, 2H), 7.31-7.49 (m, 6H), 7.54-7.58 (m, 3H), 8.16 (d, *J* = 9.2 Hz, 2H).

4-Nitrophenyl (2-phenylpropan-2-yl)carbamate (35)

Prepared folloeing the same procedure as **21**. Yield 62%. ^{1}HNMR ($CDCl_3$, 400 MHz) δ 1.73 (s, 6H), 5.6 (bs, 1H), 6.79 (d, *J* = 8.8 Hz, 2H), 7.23-7.25 (m, 2H), 7.34 (t, *J* = 7.6 Hz, 1H), 8.09 (d, 2H, *J* = 9.2 Hz), 8.16 (d, *J* = 8.8 Hz, 1H).

5-(3-(2-([1, 1′-biphenyl]-3-yl) propan-2-yl)ureido)-2-chlorobenzamide (36a)

Prepared following the same procedure as **22**. Yield 45%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.74 (s, 6H), 5.63 (s, 1H), 5.96 (s, 1H), 6.62 (s, 1H), 7.18 (d, *J* = 8.8 Hz, 1H), 7.28 (d, *J* = 2.4, 1H), 7.31-7.35 (m, 1H), 7.39 -7.48 (m, 6H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.68 (s, 1H), 7.77 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 29.1, 54.9, 118.3, 120.6, 122.8, 123.4, 123.7, 124.8, 126.8, 127.0, 128.5, 130.1, 132.5, 136.0, 141.3, 141.6, 155.1, 171.0; ESI-HRMS for $C_{23}H_{23}N_3O_2Cl$ $(M+H)^+$ calcd. 408.1479, found 408.1485.

1-(2-([1,1′-Biphenyl]-4-yl)propan-2-yl)-3-(4-chlorophenyl)urea (36b)

Synthesized following the synthetic procedure in scheme 6 starting with 4-phenylbenzonitrile. Yield 68%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.73 (s, 6H), 5.53 (s, 1H), 6.76 (d, *J* = 8.8 Hz, 1H), 7.18 (s, 1H), 7.23 (d, *J*= 8.8 Hz, 1H), 7.28 (d, *J* = 7.2 Hz, 1H), 7.35-7.38 (m, 2H), 7.45 -7.53 (m, 6H), 8.06 (d, *J* = 8.8 Hz, 1H), 8.14 (d, *J* = 8.4 Hz,

1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 30.28, 54.79, 116.49, 119.52, 126.07, 126.89, 126.99, 127.18, 129.11, 129.58, 138.48, 140.02, 140.66, 148.20, 154.48; ESI-HRMS for $C_{22}H_{22}N_2OCl$ $(M+H)^+$ calcd. 365.1421, found 365.1428.

5-(3-(1-([1,1′-Biphenyl]-3-yl)cyclopropyl)ureido)-2-chlorobenzamide (37)

Prepared following the same procedure as **22**. Yield 62%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.23 (t, 2H, *J* = 6.8 Hz), 1.30 (t, 2H, *J* = 8 Hz), 7.22 (d, *J* = 7.2 Hz, 2H), 7.28 (d, *J* = 8.8 Hz, 1H), 7.35-7.47 (m, 5H), 7.53 (t, *J* = 2.4 Hz, 2H), 7.61 (d, J = 7.6 Hz, 2H), 7.80 (s, 1H), 8.70 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 19.2, 34.9, 118.1, 121.6, 123.7, 124.6, 124.8, 127.0, 127.4, 128.0, 129.3, 129.5, 130.3, 137.8, 139.8, 140.7, 141.1, 145.5, 155.5, 168.8; ESI-HRMS for $C_{23}H_{20}N_3O_2Cl$ $(M+H)^+$ calcd. 406.1322, found 406.1323.

1-(2-Oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)-3-(2-phenylpropan 2 yl)urea (38)

1.52 (s, 6H), 6.38 (d, *J* = 6 Hz, 1H), 6.56 (*t*, *J* = 7.2 Hz, 1H), 6.66 (t, *J* = 7.2 Hz, 1H), 7.10-7.15 (m, 2H), 7.21-7.25 (m, 2H), 7.31-7.33 (m, 2H), 8.16 (s, 1H), 10.26 (s, 1H), 10.35 (s, 1H)); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 30.4, 54.8, 94.6, 99.9, 108.9, 110.7, 124.5, 125.4, 126.4, 128.5, 130.4, 134.8, 149.2, 154.8, 156.1; ESI-HRMS for $C_{17}H_{18}N_4O_2$ $(M+H)^+$ calcd. 311.1508, found 311.1511.

Biological assays

Determination of IC_{50} values—Inhibition of recombinant *Cp*IMPDH, purified from *E. coli*,[12] was assessed by monitoring the production of NADH by fluorescence at varying inhibitor concentrations (25 pM - 5 cM). IMPDH was incubated with inhibitor for 5 min at room temperature prior to addition of substrates. The following conditions were used: 50 mM Tris-HCl, pH 8.0, 100 mM KCl, 3 mM EDTA, 1 mM dithiothreitol (assay buffer) at 25 °C, 10 nM *Cp*IMPDH, 300 VM NAD and 150 VM IMP. To characterize the non-specific binding of inhibitors, assays were also carried out in the presence of 0.05% BSA (fatty acid free). IC_{50} values were calculated for each inhibitor according to Equation 1 using the SigmaPlot program (SPSS, Inc.):

$$v_i = v_o / (1 + [I] / IC_{50}) \quad \text{(Eq.1)}$$

where ν_i is initial velocity in the presence of inhibitor (I) and ν_o is the initial velocity in the absence of inhibitor. Inhibition at each inhibitor concentration was measured in quadruplicate and averaged; this value was used as ν_i. The IC_{50} values were determined three times; the average and standard deviations are reported.

Determination of antiparasitic activity—Antiparasitic activity was tested by monitoring the growth of a of *T. gondii* strain (Toxo/*Cp*IMPDH) that relies on *Cp*IMPDH. Wild-type *T. gondii* (Toxo/WT) relies on a eukaryotic IMPDH that should be resistant to *Cp*IMPDH inhibitors. Both parasites express yellow fluorescent protein, which allows growth to be easily monitored. Parasites were cultured on hTERT immortalized human foreskin fibroblasts cells in 96 well plates and fluorescence was measured daily with a SpectraMax M22/M2e (Molecular Devices) plate reader (Ex 485, Em 530) for 6-7 days. Growth inhibition was calculated on a day within the exponential growth phase[21].

Stability assays—Mouse microsomal and plasma stability experiments were performed by Cyprotex Discovery (Watertown, MA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Cp	Cryptosporidium parvum
BSA	bovine serum albumin
DIPEA	diisopropylethylamine
hTERT	human telomerase reverse transcriptase
IMP	inosine 5′-monophosphate
IMPDH	inosine 5′-monophosphate dehydrogenase
N.D.	not determined
p-TSA	p-toluenesulfonic acid
TEA	triethylamine
Toxo	Toxoplasma
XMP	xanthosine 5′-monophosphate

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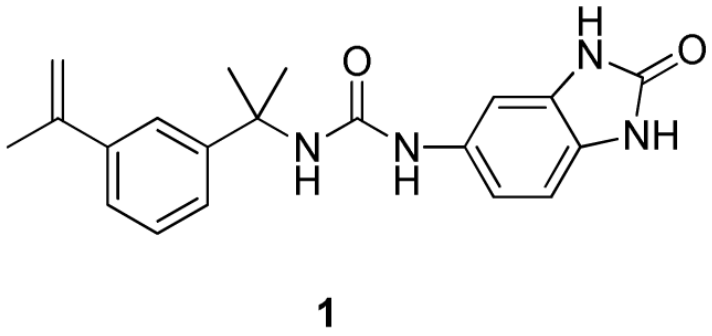

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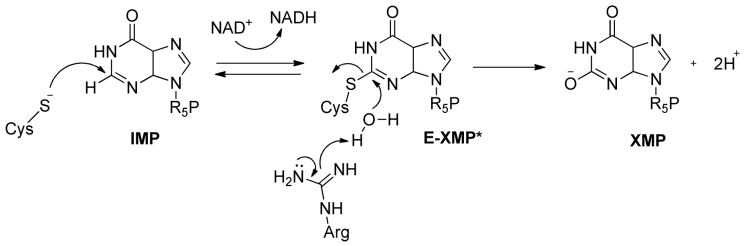
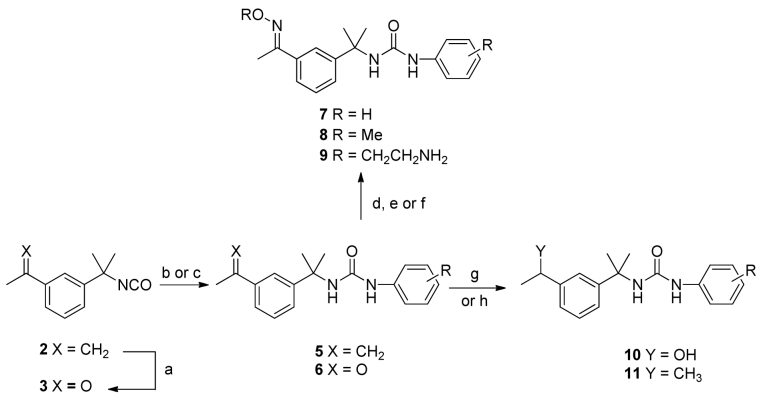
Figure 1.
*Cp*IMPDH inhibitor identified by HTS.



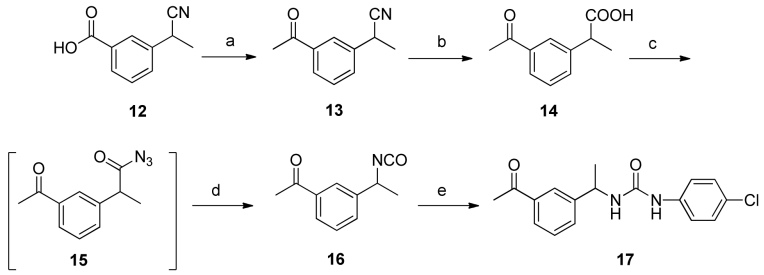
Scheme 1.
IMPDH catalyzed conversion of IMP to XMP. R5P = ribose-5′-phosphate



Scheme 2.

General procedure for preparation of urea derivatives.*

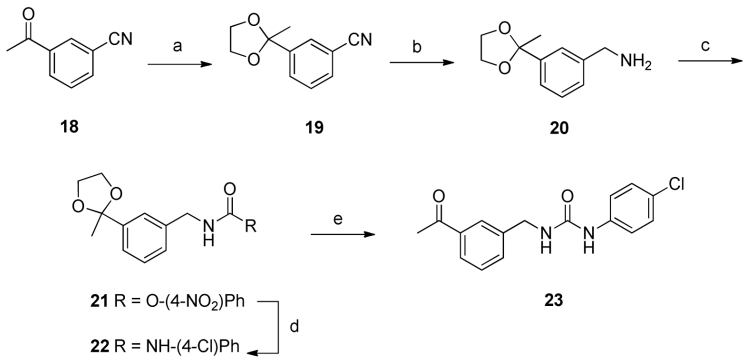
*Reagents and conditions: (a) (i) O_3, DCM, -78 °C; (ii) Me_2S, 10 h, 58%; (b) DCM, R-$PhNH_2$ (**4**), 2-4 h, 70-85%; (c) THF, R-$PhNH_2$ (**4**), 6 h, 70 ^{0}C, 70-75%; (d) NH OH•HCl, pyridine, 2 h, 90 °2 C, 80-85%; (e) NH_2OMe•HCl, pyridine, 2 h, 90 oC, 60-85 %; (f) (i) NaH, THF, 0 oC, 30 min; (ii) Cl(CH) o2 2NH_2•HCl, rt, 10 h, 53%; (g) LAH, THF, 1 h, 0 C, 74%; (h) H_2/Pd-C, MeOH, 1 h, 90%.



Scheme 3.

Preparation of mono-methyl derivative **17**.*

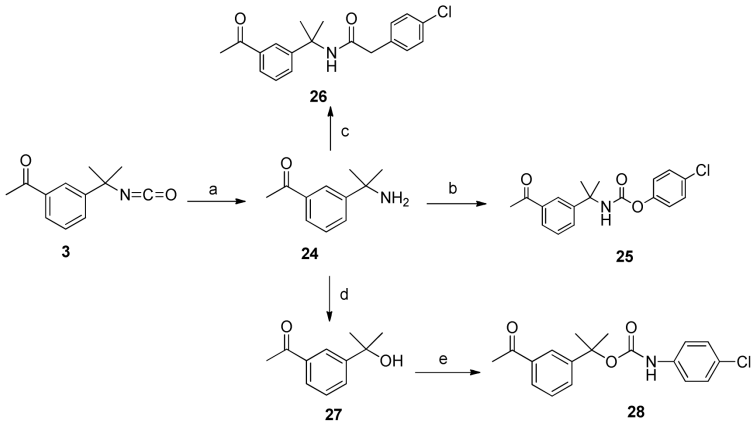
*Reagents and conditions (a) (i) SOCl , reflux, 2 h; (ii) Meldrum's acid, pyridine, DCM, 0-5 ° 2 C, 2 h; iii) AcOH, H_2O, reflux, 4 h, 28%; (b) Conc. HCl, 1,4-dioxane, reflux, 2 h, 78%; (c) (i) $SOCl_2$, reflux; (ii) NaN_3, DCM; (d) benzene, reflux, 1.5 h, 92%; (e) 4-Cl-PhNH_2, THF, reflux, 6 h, 70%.



Scheme 4.

Preparation of benzyl derivative 23.*

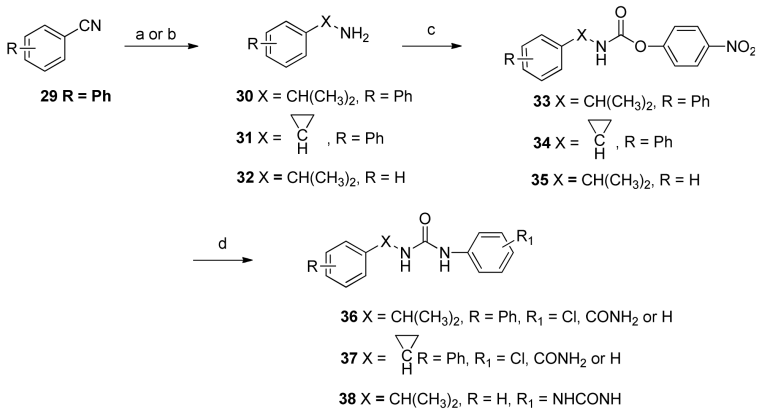
*Reagents and conditions (a) $(CH_2OH)_2$, *p*-TSA, benzene, 4 h, 110 °C, Dean-Stark, 76%; (b) LAH, ether, 0 °C, 1.5 h, 60%; (c) DIPEA, 4-NO_2-PhOCOCl, DCM/THF (1:1), rt, 24 h, 82%; (d) 4-Cl-PhNH_2, DCM, rt, 10 h, 71%; (e) 2N HCl, THF, rt, 2 h, 91%.



Scheme 5.

Preparation of carbamate and amide derivatives.*

*Reagents and conditions (a) 8N HCl, 70 °C, 3 h, 55%; (b) 4-ClPhOCOCl, DIPEA, DCM/THF, rt, 24 h, 81% ; (c) 4-Cl-PhCH COCl, DCM, 0 °C to rt, 80%; (d) (i) ClSO o2 3H, $CHCl_3$, 0 C (ii) KNO_2 (iii) Water, pH = 2.8, 30%; (e) TEA, benzene, 4-Cl-Ph-NCO, 70 °C, 3 h, 62%.



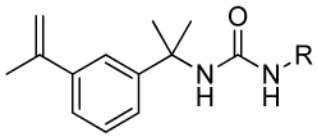
Scheme 6.

Preparation of biphenyl derivatives **36**, **37** and unsubstituted benzylderivative **38**.*

*Reagents and conditions (a) (i) 3 equiv. CH_3MgBr, ether, rt, 30 min; (ii) $Ti(i\text{-}PrO)_4$, 10 h, 57%; (b) (i) 2 equiv. EtMgBr, $Ti(i\text{-}PrO)_4$, -78 °C to rt, 1 h; (ii) 2 equiv. $BF_3{\bullet}OEt_2$, 1 h, 52%; (c) 4-NO_2PhOCOCl, DIPEA, DCM/THF, 10 h; (d) 4-Cl-$PhNH_2$, TEA, DCM, rt, 10 h, 71%.

Table 1

SAR of the anilide. Assays as described in methods. No significant inhibition of human IMPDH type 2 was observed ($IC_{50} \geq 5\ \mu M$).

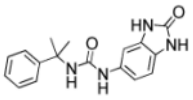
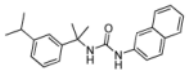
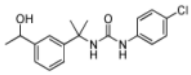
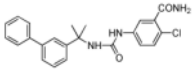
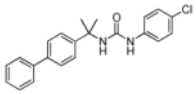
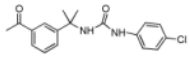
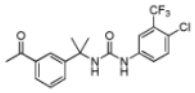
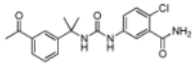


		IC_{50} (nM)	
ID	**R**	**(−) BSA**	**(+) BSA**
5a	Ph	250 ± 20	410 ± 20
5b	4-Cl-Ph	20 ± 7	37 ± 7
5c	4-Br-Ph	10 ± 4	20 ± 10
5d	3-Cl-Ph	70 ± 10	330 ± 30
5e	2-Cl-Ph	>5000 [a]	n.d.
5f	4-OMe-Ph	9 ± 1	21 ± 7
5g	4-*t*Bu-Ph	>5000 [a]	n.d.
5h	3,4-di-ClPh	6 ± 1	50 ± 20
5i	3-CF_3-4-Cl-Ph	4 ± 1	20 ± 6
5j	3-OMe,4-Cl-Ph	1.3 ± 0.2	5 ± 1
5k	3-$CONH_2$,4-Cl-Ph	2.3 ± 0.8	3 ± 1
5l	3-$CONHCH_3$,4-Cl-Ph	7 ± 2	14 ± 3
5m	3-$CON(Me)_2$,4-Cl-Ph	8 ± 1	11.7 ± 0.5
5n	3,4-(OCH_2CH_2O)-Ph	7.2 ± 0.6	110 ± 30
5o	2-Naphthyl	2.1 ± 0.8	40 ± 20
5p	1-Naphthyl	>5000 [a]	n.d.
5q	6-Quinolyl	1.8 ± 0.4	4 ± 1
5r	7-Quinolyl	0.8 ± 0.1	6 ± 2
5s	3-Quinoline	70 ± 10	308 ± 8
5t	2-Quinoline	250 ± 20	1100 ± 300

[a] One determination. n.d., not determined.

Table 2

SAR of the isopropenyl substituent.

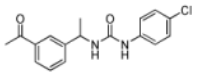
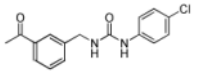
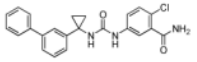
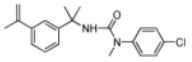
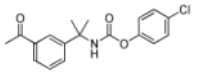
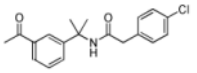
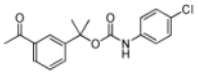
Compound	Structure	IC_{50} (nM)	
		(−) BSA	(+) BSA
38		>5000	n.d.
11		>5000	n.d.
10		80 ± 20	120 ± 10
36a		4 ± 4	14 ± 3
36b		>5000[a]	n.d.
6a		39 ± 10[b]	64 ± 22[b]
6b		9 ± 5	20 ± 2
6c		54 ± 7	52 ± 4

[a]One determination,

[b]Two determinations, n.d., not determined.

Table 3

SAR of the urea moiety.

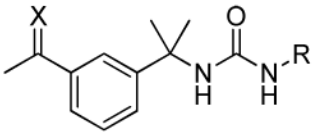
Compound	Structure	IC_{50} (nM)	
		(−) BSA	(+) BSA
17		70 ± 20	110 ± 40
23		~5000[a]	n.d.
37		40 ± 10	90 ± 30
5u		900 ± 400	5000
25		>5000	n.d.
26		>5000	n.d.
28		90 ± 20	120 ± 10

[a] One determination,

[b] n.d., not determined.

Table 4

SAR of oxime and methyloxime analogs



ID	X	R	IC_{50} (nM)	
			(−) BSA	**(+) BSA**
7a	N-OH	3-CF_3,4-Cl-Ph	1.0 ± 0.1	4 ± 2
7b	N-OH	3-NO_2,4-Cl-Ph	0.66 ± 0.08	2.0 ± 0.4
7c	N-OH	3-$CONH_2$, 4-Cl-Ph	5 ± 1	6 ± 2
7d	N-OH	2-Naphthyl	1.0 ± 0.2	2.3 ± 0.6
7e	N-OH	7-Quinolyl	0.9 ± 0.2	1.2 ± 0.3
8a	N-OMe	3-CF_3, 4-Cl-Ph	5 ± 1	18 ± 3
8b	N-OMe	3-$CONH_2$, 4-Cl-Ph	5 ± 2	5 ± 1
8c	N-OMe	2-Naphthyl	1.6 ± 0.1	13 ± 4
9	N-O$(CH_2)_2NH_2$	3-NO_2,4-Cl-Ph	20 ± 3.4	24 ± 17

Table 5

Mouse liver microsome and plasma stability: n.d., not determined.

Compound	Mouse Microsomal Stability ($t_{1/2}$, min)	Mouse Plasma Stability ($t_{1/2}$, min)
5k	25	>120
5r	5.9	>120
6c	>700	>120
7a	88	n.d.
7b	11	n.d.
7c	190	>120
7e	45	>120
8a	33	n.d.
9	121	n.d

Table 6

Antiparasitic activity of selected urea compounds. Assays as described in Methods [21]. Unless otherwise stated all values are the average 3 independent determinations.

Compound	EC_{50} (μM)		Selectivity[a]
	Toxo/WT	Toxo/*Cp*IMPDH	
5a	6 ± 4	0.77 ± 0.06	8
5b	> 25	0.18 ± 0.02	> 140
5c	> 25	0.2 ± 0.01	> 120
5d	0.402 ± 0.002	0.38 ± 0.01	1.0
5g	20 ± 2 [b]	7.2 ± 0.4 [b]	3
5h	4 ± 2	0.22 ± 0.04	20
5j	2.3 ± 0.2 [b]	0.018 ± 0.002 [b]	127
5k	15 ± 3	0.23 ± 0.04	65
5l	19 ± 6	0.18 ± 0.03	100
5m	9 ± 1	0.09 ± 0.03	100
5n	8 ± 1	0.2 ± 0.2	50
5o	> 25	0.20 ± 0.03	> 120
5q	7.6 ± 0.8	0.02 ± 0.01	≥ 80
5r	3.0 ± 0.1 [b]	< 0.2	> 15
5s	2 ± 1	1.1 ± 0.4	2.3
5t	3 ± 1	3.4 ± 0.3	0.9
6b	3.2 ± 0.7	0.2 ± 0.1	16
6c	> 25	0.38 ± 0.05 [b]	> 66
7a	2.7 ± 0.2	0.01 ± 0.01	250
7b	4 ± 2	0.006 ± 0.005	670
7c	> 25	1.9 ± 0.6	> 12
7d	5 ± 4	0.08 ± 0.01	63
7e	14 ± 6	0.058 ± 0.003	230
8a	1.4 ± 0.4	0.016 ± 0.008	86
8b	>25	0.2 ± 0.1	>120
8c	2.3 ± 0.8	0.013 ± 0.009	180
9	10.1 ± 1.9	0.51 ± 0.007	20
10	19 ± 6	0.9 ± 0.4	20
17	8 ± 4	2.3 ± 0.6	4
23	7 ± 5	11 ± 3	0.6
36a	6 ± 3	0.21 ± 0.09	30
37	9 ± 6	0.6 ± 0.2	15

[a] Selectivity is the ratio of EC_{50} Toxo/*Cp*IMPDH to EC_{50} Toxo/WT.

[b] Two determinations.