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Promiscuity and selectivity in covalent enzyme inhibition: A systematic study of electrophilic fragments

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ABSTRACT: Covalent ligand-target interactions offer significant pharmacological advantages. However, off-target reactivity of the reactive groups, which usually have electrophilic properties, must be minimized and the selectivity of irreversible inhibitors is a crucial requirement. We therefore performed a systematic study to determine the selectivity of several electrophilic groups that can be used as building blocks for covalently binding ligands. Six reactive groups with modulated electrophilicity were combined with eleven non-reactive moieties, resulting in a small combinatorial library of 72 fragment-like compounds. These compounds were screened against a group of eleven enzyme targets to assess their selectivity and their potential for promiscuous binding to proteins. The assay results showed a considerably lower degree of promiscuity than initially expected, even for those members of the screening collection that contain supposedly highly reactive electrophiles.

INTRODUCTION

Irreversible inhibitors of enzymes exhibit several properties that are beneficial for their clinical use. Non-equilibrium binding of irreversible inhibitors improves their biochemical efficiency[1] and confers robustness against pharmacokinetic liabilities such as fast clearance and binding to serum proteins.[2] Side effects can be minimized, since selective irreversible inhibitors retain their effect after elimination.[3] This allows the creation of rapidly metabolized and excreted drugs that still have high and lasting effectiveness. Covalent inhibitors can also avoid some resistance mechanisms, especially when targeting residues of a target protein that are essential for its proper function.[4] An interruption of metabolic pathways by covalent inhibitors is difficult to overcome by feedback mechanisms, which offers a significant advantage for antibiotic or anticancer drugs. The outstanding pharmacological properties of irreversible enzyme inhibitors are highlighted by such prominent examples as the β-lactam antibiotics,[5] fosfomycin,[6] and proton pump inhibitors.[7] Etacrynic acid is a noteworthy example of a drug that appears to be activated by covalent binding to cysteine and binds to its target as a cysteine-conjugate.[8] Avibactam is a reversible, covalent β-lactamase inhibitor that binds to the active site serine of β-lactamases.[9]

Irreversible enzyme inhibitors contain a chemical functionality, most frequently an electrophile, which is prone to react with (usually nucleophilic) protein residues. It is therefore of extreme importance to tune the reactivity of the electrophilic "warhead" to the intended enzyme target, in order to avoid off-target reactivity. In the examples given above, this requirement is fulfilled nearly to perfection. In the case of β-lactam antibiotics, off-target binding is relevant for the only major side effect, allergic reaction.

Numerous electrophiles have been employed to design covalent target binding into enzyme inhibitors or receptor ligands. Among the more frequently used functional groups are acrylamides and other α,β-unsaturated groups, boronic acids and α-halogen ketones. However, the majority

of drugs with a covalent binding mode were discovered serendipitously and were only retrospectively identified as covalent inhibitors.[10] Only few drugs that contain the abovementioned "classical" electrophiles have entered clinical practice. This prompted us to search for as-yet underexplored chemical functionalities, in order to increase the number of electrophilic warhead options available for the design of covalent target binders. Some of these warheads are described for the first time in detail in the present study.

The present study has two objectives: First, to study the selectivity and reactivity of an extended group of electrophiles towards biological targets; second, to identify electrophilic fragments that covalently modify the target enzymes currently pursued in our laboratory. We therefore studied the binding behavior of six electrophiles (Table 1) towards eleven structurally and functionally diverse enzyme targets (Table 3).

Some of the electrophiles primarily target cysteine residues (**2**-**5**). Chloroacetylamides (**4**) were expected to be more promiscuous due to their (expectedly) high reactivity. The chloroacetylamide group in metazachlor and related herbicides is responsible for the covalent binding of these compounds to an active site cysteine of the very long-chain fatty acid elongase.[11] These herbicides react irreversibly with protein sidechains via nucleophilic substitution of the halogen.

The dimethylsulfoniumacetylamides (**3**) are structurally similar but less reactive. This electrophile was successfully used to target a cysteine in water-soluble tissue transglutaminase inhibitors[12] and modifies the cysteine sulfur by substitution of the dimethylsulfide moiety or by methylation.

A classical motif for covalent inhibitors is the Michael-acceptor acrylamide (**2**) that targets nucleophiles like cysteine[13] and in some cases threonine.[14] Acrylamide groups are present in

numerous natural products and rationally designed, targeted inhibitors and covalent receptor ligands.

Bromodihydroisoxazoles (**5**) are derived from the natural product acivicin,[15] which targets a cysteine sidechain in γ-glutamyltranspeptidase and has been also used to target cysteines in cysteine peptidases.[16] Their binding mode involves nucleophilic replacement of the bromine.

Two reactive groups with preference for serine residues, 2-cyanoacetamides and imidazole-1-carboxamides (carbonylimidazoles, **6**, **7**) were included in this study. 2-Cyanoacetamides (**6**) are widely used in Medicinal Chemistry not only as reactive group but also as a metabolically labile moiety to accelerate degradation. They react with oxygen and sulfur nucleophiles by means of an addition to the triple bond with subsequent hydrolysis to the corresponding ester.[17] Imidazole-1-carboxamides (**7**) have been used to target serines in elastase inhibitors,[18] and form carbamates by substitution of the imidazole. Apart from that, they are rarely used in Medicinal Chemistry.

These electrophiles were linked to eleven amine and aniline scaffolds (Table 2). We chose to link the electrophiles to these scaffolds via amide linkers because of their modulating influence on the reactivity of the adjacent electrophile. The amides have a three orders of magnitude lower reaction rate constant towards GSH when compared to alkyl vinyl ketones.[19] The amide linkers also increase the polarity, when compared to compounds with an all-carbon backbone, which is beneficial for their solubility and prevents aggregation. The scaffolds were chosen to be diverse in size, substitution patterns and electronic effects. Since this work focusses on the covalent rather than the non-covalent part of the intermolecular interaction between target and ligand, relatively small scaffolds were chosen, resulting in fragment-like test compounds. The electronic properties of the aromatic scaffolds range from electron withdrawing (**d**, **e**) to electron donating (**b**, **c**) in order to modulate the reactivity of the electrophiles.

Several of the electrophiles presented here (**3**-**5**, **7**) are somewhat exotic in Medicinal Chemistry, and very few or no information on their binding behavior towards biological nucleophiles is currently available. These "rare" electrophiles were included in the study to specifically assess their usefulness for the design of covalent inhibitors.

The 72 fragment-like substances resulting from the combination of various electrophiles with the non-reactive moieties were assayed for inhibition of eleven enzyme targets, reactivity towards glutathione and stability under assay conditions. Over 900 biochemical measurements were performed by means of reproducible and robust assays that were established previously in the context of drug discovery efforts in our laboratory.[20-23] The enzymes studied in this work are described in Table 3. They represent a wide range of structural recognition motifs, catalytical functions and source organisms. While some similarities exist between DEN and WNV as well as between *hs*MetAP-1 and *E. coli* MetAP, all other enzymes vary considerably in their structure and mechanism.

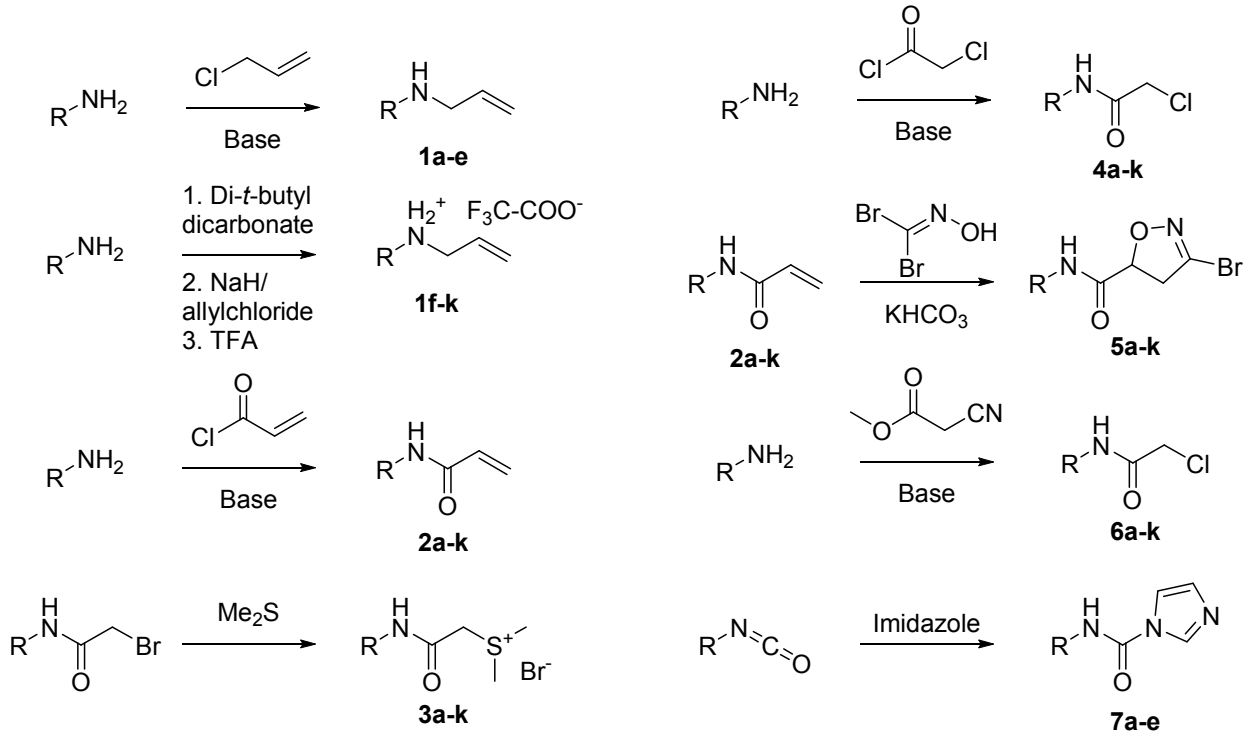
Irreversible inhibitors have been previously described for some of these enzymes, in particular fosfomycin for MurA[6] and fumagillin[24] for the MetAPs. MurA has previously been described to be particularly reactive towards small-molecular electrophiles;[25, 26] therefore we expected this enzyme to be inhibited readily by promiscuous covalent binders. The *E. coli* and human MetAPs contain cysteine residues in the active site, which we also expected to be reactive towards the tested electrophiles.

RESULTS

Chemistry

The allylamines and anilines **1a-k** were synthesized according to previously published procedures.[27-29] The amide/anilide compounds **2a-k**, **3a-k**, **5a-i**, **7a-k**, **9** and **10** were synthesized by amide couplings (Scheme 1, for structures see Table 1, Table 2 and Table 4). Dimethylsulfonium salts (**3a-k)** were obtained by treatment of bromoacetylanilides and -amides with dimethylsulfide following the procedure by Izquierdo *et al.*[30] Bromodihydroisoxazoles (**5a-k)** were obtained by cycloaddition of *in situ* formed bromonitriloxide to acrylanilides and -amides (**2a-k)** following the procedure of Girardin *et al.*;[31] **8** was obtained from styrene in the same fashion. Imidazole-1-carboxamides (**7a-e)** were synthesized by addition of imidazole to the corresponding isocyanates. **11** and **12** were purchased. The five substituted anilines bear *para*-sustituents that range from highly electron-donating (methoxy, **c**) to highly electron-withdrawing (nitro, **d**) substituents, which we expected to have an influence on the reactivity. Aliphatic amines were methyl- (**j)** butyl (**i**) and cyclopentylamine (**h**). Two amines bearing an aromatic residue (benzylamine **f** and phenylethylamine **g**) and norleucine methyl ester (**k**) were also included (Table 1).

The acrylamides (**2**), dimethylsulfonium salts (**3**) and chloroacetylamides (**4**) are considered to be electrophiles that primarily react with cysteine residues, whereas the nitriles (**6**, **9, 10**) as well as imidazole-1-carboxamides (**7**) are considered to have a preference for catalytic serine residues. Bromodihydroisoxazoles (**5**, **8**) are expected to target cysteines, serines, lysines or similar nucleophiles. Allylamines, anilines (**1**), and norleucine methyl ester (**11**) were included as negative controls to identify non-covalent components of inhibitor-enzyme recognition. 4-Nitroacetanilide was included in order to study the possibility of an acyl group transfer by the nitroanilides.



Scheme 1: Synthesis of the compounds. Cf. Table 2 for definitions of group R.

Biochemical data

The inhibition data of the eleven enzymes and the reactivity towards GSH for all compounds are listed in Table 4 and Chart 1.

ESI-MS analysis of covalent inhibitor binding

To examine the formation of covalent enzyme adducts, selected active compounds (inhibition >25%) were incubated with their respective target enzymes for one hour at elevated concentrations (25 µM enzyme, 300 µM inhibitor) and analyzed by ESI-MS. To ensure functionality of this approach, fosfomycin was reacted with MurA and a mass difference of 138 m/z in comparison to an unmodified sample was observed. This difference corresponds to an addition of one molecule of fosfomycin per MurA enzyme.

No covalent adducts of dimethylsulfonium salts **3a**, **3i** and **3k**, as well as of bromodihydroisoxazoles **5d** and **5k** with *E. coli* MurE could be detected. *E. coli* MetAP remained equally unmodified on exposure to **5d**. Co-incubation of MurB and imidazole-1-carboxamide **7e** furnished no adduct.

In addition to the abovementioned experiments we also assessed adduct formation between **2a**, **3a**, **4a**, **5a**, **6a** and **7a** with *E. coli* MetAP, MurE and MurA, in order to study the possibility of binding events without detectable influence on the enzyme activity, but no adduct formation was observed for any of the combinations.

Cell viability assay

A selection of compounds was submitted to cell viability assays. HeLa cells were incubated for 24 h with the compounds of interest at varying concentrations and viability determined with a reaszurin based assay. The EC_{50} values are represented in Table 5. Only compound **4a** had an EC_{50} value below 50 μM.

DISCUSSION

In this work we studied the inhibitory potential of six electrophilic moieties, covering a broad range of reactivity, against several enzymes. Some of the enzymes contain catalytically active serine and cysteine residues that we expected to have an increased nucleophilicity and therefore to be reactive towards the tested electrophiles. The assayed enzymes represent a broad spectrum of species (viral, bacterial, human targets), require different types of cofactors (small proteins, metals) as well as diverse substrates (peptides, proteins, carbohydrates, modified carbohydrates). The electrophiles were selected with respect to their reactivity and suitability for pharmacological

applications. Therefore, highly reactive electrophiles, like aldehydes or trifluoromethylketones that are sometimes used during the target validation and early drug discovery stages, but without conceivable application in approved drugs, were not analyzed in this study. For all six evaluated reactive groups, exemplary applications in Medicinal Chemistry can be found in literature (cf. Table 1). Allylamines/-anilines (**1**) were used as non-reactive references to estimate the part of covalent interaction of the series of electrophiles (**2**-**7**). Depending on the enzyme, the compounds were tested at concentrations of 25 or 50 μM, which is at or above the upper concentration range that is typically used in compound screening. The concentrations were chosen as a "worst case scenario" and to permit the detection of "weak" binders.

The non-reactive controls (**1a-k, 11** and **12**) show little or no inhibition of any of the enzymes and did not react with GSH on a timescale relevant to the assays. Therefore, all significant inhibitory activities can be attributed to the electrophilic moieties in the test compounds.

The acrylanilides and -amides (**2a-k**) are inactive in all enzymatic assays. They were not reactive towards GSH on a timescale relevant for the assays (<5% conversion over 3 h), but formation of GSH adducts was detected by LC-MS after extended incubation times. Therefore, these electrophiles appear to be suitable for the design of targeted covalent inhibitors, which are first anchored to the target protein by non-covalent interactions, followed by the (slow) formation of a covalent bond. The low promiscuity and off-target reactivity of covalent drugs with acrylanilide and acrylamide electrophiles is clearly demonstrated by their inertness towards all tested enzymes. It is highly remarkable that also the unsubstituted acrylamide, under the given experimental conditions, showed only negligible binding to all enzymes and GSH. An earlier report already indicated that acrylamide has a half-live of ~3 h at cellular levels of glutathione (~3 mM).[32]

Dimethylsulfonium salts (**3a-k**) have low unspecific reactivity. Interestingly, **3b-3d** decomposed immediately upon dilution in aqueous media, in reversal of the synthesis. Therefore, these compounds were excluded from the screening. The other dimethylsulfonium salts (**3a**, **3e-k**) are stable under aqueous conditions and were not reactive towards GSH.

Chloroacetylanilides and -amides (**4a-i**) show the most "pronounced" reactivity towards GSH of all tested compounds (k_{GSH} ~ 0.1 $l \cdot mol^{-1} \cdot s^{-1}$), with aqueous half-lives still above 200 h, but virtually no inhibition of any enzyme. The group is therefore a slightly more reactive alternative to the acrylamides (**2a-k**), but retains a surprisingly low random reactivity.

2-Bromodihydroisoxazoles (**5a-k**) show no GSH reactivity and a half-live above 200 h, making them a rather stable electrophilic group. They exhibit limited inhibitory potential in the enzymatic assays and also some promiscuous binding, although not very potent and with a focus on the Mur-enzymes. Substances **5c-e** and **5g-k** are relatively selective inhibitors of MurE. The bromodihydroisoxazoles have been described as cysteine-targeting electrophiles, but *ec*MurE does not contain a solvent-exposed cysteine residue. To pinpoint the reactive residue of *ec*MurE that interacts with this warhead, four of the most active compounds (**5d, 5g, 5i, 5k**) were submitted to docking experiments with MurE (PDB-ID: 1E8C[33]). It was found that in all cases the bromodihydroisoxazole moiety is located in a pocket close to the *m*DAP-binding domain. This pocket contains a lysine (Lys119) that is in a potentially reactive orientation and distance towards the reactive moiety of these compounds (Figure 6). However, a covalent interaction with the MurE protein could not be confirmed by mass spectrometry. Therefore, these compounds are reversible or reversible-covalent inhibitors of MurE.

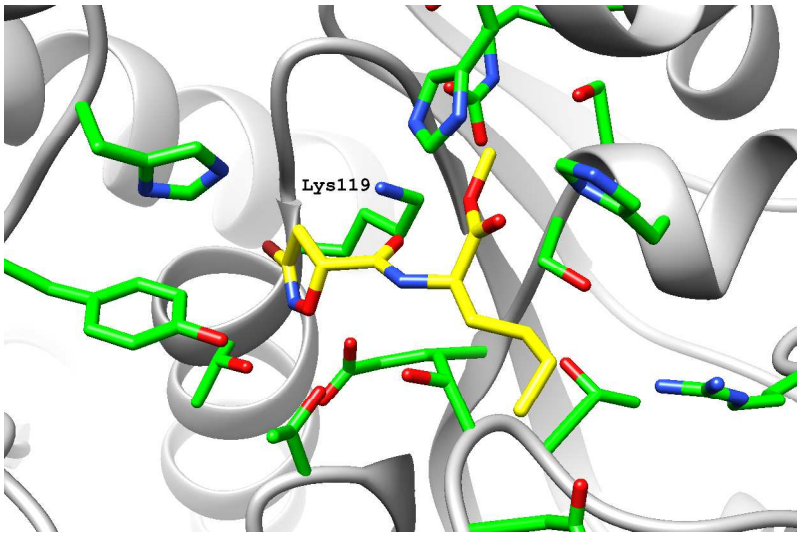


Figure 1: Docked structure of **5k** in the active site of MurE. Lys119, a potential binding partner for the ligand, is located in the center of the graphic. The carbon atoms of the ligand are shown in yellow. PDB code of the target structure: 1E8C.[33] This figure was generated using Chimera.[34]

Cyanoacetanilides and amides (**6a-k**) are not reactive towards GSH, show no substantial inhibition or multi-enzyme reactivity and are stable, with half-lives of over 200 h. Imidazole-1-carboxamides (**7a-k**) show no GSH reactivity and half-lives in the range of days. They show no promiscuous binding to the screened enzymes.

Substances **6a-k** and **7a-k** also show no inhibition of DEN up to 500 µM (data not shown).

Active site serines are largely resistant towards inhibition by all electrophiles studied here. DEN, WNV, THR and MurB, all containing active-site serines with increased nucleophilicity, are neither inhibited by the 2-cyanoacetamides **6a-k** nor the more reactive aminomethylnitriles **9** and **10** and the imidazole-1-carboxamides (**7a-m**).

All compounds that exhibit inhibition of an enzyme were also tested for covalent adduct formation with their corresponding targets, but no adducts were found. This was also extended to all phenyl substituted substances (**2a**-**7a**), because they ranged among the most reactive of their group (cf: Chart 1, **4a**), with *E. coli* MetAP, *E. coli* MurA and *E. coli* MurD. This was done to

search for "unspecific" adducts with residues outside the active site. No adducts could be identified. These findings further emphasize the limited unspecificity of these reactive groups.

Cell toxicity was assessed for all compounds exhibiting significant inhibition of any enzyme and for all phenyl- (**2a**-**7a**) and butyl- (**2i**-**6i**) substituted substances as well as nitriles **9** and **10**. The only substance showing cell toxicity with an EC_{50} value below 50 µM is the chloroacetylanilide **4a**. When compared to **4i**, with equal GSH reactivity and no cytotoxicity below 100 µM, this can not solely be attributed to unspecific reactivity of **4a**, but is probably due to specific interaction with a vital target.

CONCLUSION

In this study, in which biochemical properties of 72 substances were determined in over 900 measurements, we assessed the selectivity of diverse electrophilic groups towards targets with pharmacological relevance.

We found that acryl- and chloroacetylamides/anilides as well as 2-cyanoacetamides and imidazole-1-carboxamides exhibit a surprisingly low off-target reactivity and can therefore be considered as suitable warheads in targeted covalent inhibitors. These inhibitors must recognize their targets by specific, non-covalent interactions that increase the residence time sufficiently to raise the probability of the second, covalent binding event. We will apply these findings in targeted covalent inhibitors that are currently under development in our group.

The 3-bromo-4,5-dihydroisoxazole moiety selectively addresses the antibiotic target enzyme MurE. Therefore, in future drug discovery efforts aimed at the development of antibiotics that interfere with cytoplasmatic peptidoglycan precursor synthesis, this scaffold may be a valuable

fragment for inhibitor design. Apart from that, this reactive moiety did not show any random reactivity with proteins or significant cytotoxicity.

An unexpected but significant consequence of the present study is the relatively low inhibitory potential of the reactive compounds against the analyzed enzymes. Even in cytotoxicity assays and when we looked for inhibitor enzyme adduct formation we did not find any elevated cytotoxicity or unspecific modification of proteins. Particularly in the case of chloroacetylamides/-anilides (**4**) and dimethylsulfonium salts (**3**), which we consider to be among the most reactive in this series, this is a promising results. From these results the following consequences for moderately reactive groups in Medicinal Chemistry can be drawn. Promiscuous reactivity and off-target effects of electrophiles with moderate reactivity may often be overestimated. It also does not appear justified to generally exclude "reactive" moieties from compound libraries for screening purposes, since the non-specific reactivity may turn out to be much inferior than anticipated. A sufficiently close contact of the electrophilic group and the nucleophile of the target and a sufficiently long residence time of the electrophile is required to establish a covalent interaction. In the absence of specific, non-covalent interactions between ligand and target, the covalent binding potential of electrophiles with intermediate reactivity is low.

EXPERIMENTAL SECTION

General. The stock solutions for all assays were prepared in DMSO (10 mM) and stored at -20° C in brown glass vials with a teflon coated lid and further diluted with assay buffer as required. Determination of biochemical data was performed in triplicate with standard deviations typically below 15%.

DEN and WNV overexpression and purification. The dengue virus (serotype 2) and West-Nile Virus NS2B-NS3 proteases were expressed and purified according to the protocol described by Steuer *et al.*[22, 35] For both proteases the cofactor is covalently connected to the protease domain by a glycine-serine linker (GGGGSGGGG).[36]

***E. coli* MetAP overexpression and purification.** *E. coli* MetAP was obtained by overexpression in *E. coli* BL21(DE3) using an Arg-175-Gln mutant kindly provided by Prof. W. T. Lowther and Prof. B. Matthews following their protocol.[24]

***Hs*MetAP-1 overexpression and purification.** The gene for *Hs*MetAP-1 was synthesized with an optimized nucleotide sequence for *E. coli* expression by a commercial supplier and cloned into the pET-28a(+) vector (Novagen) using the BamH1 and Sac1 cloning sites. The enzyme with N-terminal His-tag was overexpressed in *E. coli* BL21(DE3). The enzyme was isolated by nickel affinity chromatography (Chelating Sepharose Fast Flow, Amersham Biosciences; buffer: HEPES 50 mM pH 7.9, 0.5 KCl; imidazole gradient 10-250 mM).

MurA overexpression and purification. Was performed as previously reported.[37] MurB, MurC, MurD, MurE and MurF were obtained analogously.

Thrombin assay. Bovine thrombin was purchased from Sigma-Aldrich. The thrombin assay was performed as a continuous fluorimetric assay using a BMG Labtech Fluostar OPTIMA microtiter fluorescence plate reader and black 96 well V-bottom plates from Greiner Bio-One (Germany). The excitation wavelength was 355 nm and the emission wavelength was 460 nm. The protease was assayed using the substrate Boc-Val-Pro-Arg-AMC, purchased from Bachem (Germany). The final concentrations of the enzyme and substrate were 10 nM and 50 μM, respectively. The inhibitors were preincubated with the enzyme for 15 min at a concentration of 25 μM. The cleavage reaction was initiated by addition of the substrate. The assay buffer

consisted of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl and 0.05% Tween 20.[38] The activity of the enzyme was determined as the slope per second (RFU/s) and monitored for 10 min.

DEN and WNV assays. Were performed as previously reported.[20, 39]

MetAP assay by HPLC. The MetAP HPLC assay was perfomed as an endpoint assay as follows: Into a 96-well-plate (clear PS, U-Bottom from Greiner Bio One) 10 µl inhibitor solution (250 µM), 60 µl master-mix (0.125% w/v BSA, 0.167 M NaCl, 0.167 mM $CoCl_2$ in 50 mM TRIS-buffer at pH 7.5) and 10 µl enzyme (500 nM) were added per well. The content of each well was gently stirred with the pipet tips followed by incubation at 37 °C for 20 min. Then MGMM (20 µl, 2 mM) again followed by gentle stirring and incubation for 15 min (*Hs*MetAP-1) or 20 min (*Ec*MetAP).

This results in a total volume of 100 µl and the following final concentrations: $CoCl_2$: 100 µM; NaCl: 100 mM; BSA: 0.075% (w/v); enzyme: 50 nM; MGMM: 400 µM; inhibitor: 25 µM. The enzyme reactions were stopped by adding 10 µl 4% TFA per well.

The HPLC elution was as follows: The product GMM was quantified by monitoring its UV absorbance at 214 nm. Flow rate 1.0 ml/min at 30°C. Initial conditions: 99% water (0.1% TFA) (solvent A) and 1% acetonitrile (0.1% TFA) (solvent B). These conditions were kept for 0.25 min. From 0.25 min to 2.5 min the gradient was ramped linearly to 60% B. The elution proceeded with 100% B until 3.25 min. The column was then re-equilibrated at initial conditions for 3 min. A ReproSil-Pur ODS 3.5 µm, 50 x 2 mm column from Maisch was used.

MurA Assay. Was performed as previously reported.[37]

MurB, MurC, MurD, MurE, MurF Assays. MurB to MurF reactions were determined in single assays by a LC-MS method, measuring product formation.

The respective NAG-substrates (*N*-acetyl glucosamine) for each Mur enzyme assay were generated by enzymatic conversion by incubating the preceding enzymes at 400 nM with their

required substrates (UNAG 1mM, PEP 1mM, NADPH and amino acids 2 mM, ATP in increasing amounts of 2 mM (MurD substrate), 3 mM (MurE substrate), 4 mM (MurF substrate) for 5 h at 37 °C. Reactions were then terminated by heat denaturation (85 °C for 5 min). NAG-substrate preparations were stored at -20 °C until their use.

For inhibition studies, the respective *E. coli* wild-type enzyme was pre-incubated with inhibitors at 37 °C for 10 min, after which in case of the Mur ligases the NAG-substrate mixtures including the cosubstrate ATP, and in case of *E. coli* MurB NADPH, were added and incubated for another 10 min. Reactions were initiated by the addition of the respective amino acid cosubstrates: L-Ala (MurC), D-Ala (MurD), mDAP (MurE) or dipeptide D-Ala-D-Ala (MurF). MurB assays reactions were initiated with the EP-UNAG-substrate preparation.

For the Mur ligases, final concentrations in the assay were: enzyme 4 nM, ATP and respective NAG-substrates approx. 200 µM, NH_4HCO_3 50 mM, $MgCl_2$ 2 mM (MurD, MurE) / $MgCl_2$ 10 mM (MurC), $MgCl_2$ 5 mM (MurF), BSA 0.07% (w/v), DMSO 1% (v/v).

For MurB, the final concentrations in the assay were: MurB 4 nM, NADPH 200 µM and EP-UNAG substrate approx. 200 µM, NH_4HCO_3 50 mM, KCl 5 mM, BSA 0.07% (w/v), DMSO 1 % (v/v).

Reactions were quenched after 15 min at 37 °C by adding 5 µL of formic acid (10% (v/v)) per 100 µL total reaction volume. A volume of 5 µL was injected onto two coupled RP18-AQ pre-columns (ReproSil-Pur C18-AQ, 3 µm, 3x20 mm, Dr. Maisch, Ammerbuch, Germany) and eluted with a rapid gradient. Solvent A: 50 mM ammonium formate + 0.2% HCOOH, solvent B: 500 mM ammonium formate + 1.6% HCOOH – methanol 10/90 (v/v). HPLC conditions were as follows: 100% A for 0.5 min, gradient to 95% B in 1.0 min. Solvent B was kept at 95% for 0.5 min followed by a gradient to initial conditions in 0.1 min. Initial conditions were maintained until a total analysis time of 7 min. Flow rate was 0.4 mL/min. The HPLC system consisted of an

Agilent 1200 series device (consisting of G1322A degasser, G1312B binary pump, G1367C autosampler, G1316B column oven) coupled to a ESI massspectrometer micrOTOF-Q II (Bruker Daltonik, Bremen, Germany) operating in electrospray negative ionization mode. Product formation of each Mur reaction was quantified as peak area of the monitored EIC trace.

Reactivity towards glutathione.[26] 25 µL of a freshly prepared 10 mM solution of glutathione in water was added to 950 µl of a 50 mM TRIS-maleate-buffer at pH 7.5 and thoroughly mixed. The reaction was started by adddition of 25 µL of the 10 mM compound stock in DMSO (final concentration: 250 µM). 100 µl were transferred into HPLC-vials containing 10 µl of 10% phosphoric acid at 0, 60, 180 min. These aliquots were analysed by RP-HPLC and the decrease of the test compound monitored at a suitable wavelength.

Flow rate 1.0 ml/min at 30 °C. Initial conditions: 99% water (0.1% TFA) (solvent A) and 1% acetonitrile (0.1% TFA) (solvent B). These conditions were kept for 0.25 min. From 0.25 min to 5 min the gradient was ramped linearly to 100% B. The elution proceeded with 100% B until 3.25 min. The column was then re-equilibrated at initial conditions for 3 min. A ReproSil-Pur ODS 3.5 µm, 50 x 2 mm column from Maisch was used.

ESI-MS analysis of covalent inhibitor binding: All experiments were performed in NH_4HCO_3 buffer to ensure ESI-MS compatibility.

Enzymes (25 µM) were incubated for 60 min at 37 °C in NH_4HCO_3 50 mM, pH 7.9 with 300 µM test compound and individual ionic additives (*E. coli* MetAP: $CoCl_2$ 200 µM, *E. coli* MurC: $MgCl_2$ 10 mM, *E. coli* MurD, MurE: $MgCl_2$ 2 mM, *E. coli* MurF: $MgCl_2$ 5 mM). Incubation conditions were analogous to the enzymatic assay procedure: *E. coli* MetAP: incubation of enzyme with inhibitor; *E. coli* MurA: pre-incubation of enzyme with the substrate UNAG 300 µM for 10 min at 37 °C prior to addition of inhibitor; *E. coli* MurB to MurF: pre-incubation of enzyme with inhibitor for 10 min, 37 °C prior to addition of substrates ATP

200 μM, sugar nucleotide substrate approx. 200 μM. To investigate the effects of substrate exposure on the enzyme adduct profile, *E. coli* MurD was incubated without substrates and under simultaneous addition of substrates and inhibitor, respectively. Total incubation volume was 100 μL. Untreated enzymes served as controls. Desalting and removal of excess substrates, non-bound inhibitor and DMSO was performed by spin-desalting using pre-equilibrated (NH_4HCO_3 50 mM) Sephadex G-25-columns, which were prepared according to Weingart.[40] A volume of 20 μL was analyzed by flow injection analysis using an Agilent 1200 series HPLC device coupled to a ESI-MS instrument (mircOTOF-QII, Bruker Daltonik, Bremen) operating in positive ionization mode. Removal of DMSO was required for sufficient MS signal intensity during flow injection analysis. Injection conditions were as follows: no column, isocratic flow of H_2O + 0.1% HCOOH / CH_3CN + 0.1% HCOOH 95/5 (v/v) at 0.3 mL/min. Instrument calibration and external mass calibration at the end of each analysis run were performed with ESI-Tunemix (Fluka) calibration standard. Mass spectra of analytes with multiple, variable charges were deconvoluted using the Maximum Entropy Deconvolution algorithm (Compass DataAnalysis Version 4.0 SP4, Bruker Daltonik).

Docking. The docking experiments were performed using GOLD 5.1 with Hermes [41] on an Intel Core2 Quad CPU Q9450 @ 2.66 GHz running open SuSE 11.0. The X-ray structures were downloaded from the PDB.[42] Ligands and all waters were extracted. Hydrogens were added exhaustively using Hermes. The binding site was set according to the previously extracted ligand plus 6 Å. GoldScore was used as scoring-function. All settings were used in the standard configuration except **for** setting the search option to the "high flexibility" preset. Graphics were created using Chimera.[34]

Cell viability assay. HeLa cells were seeded in triplicate into 96-well plates at 5×10^3 cells in a final volume of 50 μl per well and incubated at 37 °C/5% CO_2 for 24 h. To each well 50 μl of

the compounds as 2x concentrated stock solution in phenol-red free DMEM supplemented with FBS, 100 IU/ml penicillin, 100 µg/ml streptomycin, 1 mm sodium pyruvate, 4 mm l-glutamine were added. After incubation at 37 °C/5% CO_2 for 24 h, the media was replaced with fresh phenol red-free medium containing CellTiter-Blue reagent (100 µl of medium + 20 µl of reagent) per well. Cells were incubated for a further 3 h at 37 °C/5% CO_2, and then the absorbance at 570 nm and 610 nm was measured. After background subtraction, absorbance values were used to calculate the percentage viability, expressed as a percentage of untreated controls which were set as 100% viable.

Chemistry. NMR-Spectra were measured at room temperature (rt) on a Varian Mercury Plus (300 MHz) and a Varian NMR System (500 MHz). The chemical shifts are given relative to TMS ($\delta = 0$ ppm) using the residual solvent peak as internal standard. High resolution mass spectra were measured on a Bruker micrOTOF-Q II with sodium formate as calibrant. All reactions were carried out in standard glassware; temperatures refer to the heating or cooling bath temperatures. All reagents and solvents were unless otherwise stated purchased from Sigma-Aldrich and used as received. MPLC was performed on a Biotage Isolera One using self-packed columns with E60 flash silica gel from SiliCycle. Cyclohexane (technical grade) was purchased from Grüssing and distilled prior to use. Ethyl acetate used for column chromatography was distilled prior to use. Dibromoformaldoxim was prepared following the procedure provided by Wade *et al.*[54] The purity of all compounds was determined to be above 95% unless otherwise stated by either HPLC (on an Agilent 1200 HPLC with DAD on a Reprosil Pur ODS-3 50x2 mm column from Maisch; Gradient: 0-0.5 min 1% B, linear gradient to 100% B at 8 min, hold 100% B for 1 min and re-equilibrate at 1% B for 3 min; A: MeCN + 0.1% TFA. B: Water + 0.1% TFA; detection wavelength was, unless otherwise stated 214 nm) or combustion analysis on a Vario EL from Foss Heraeus. For all compounds known prior to this work ^{1}H-NMR-spectra were used to

verify identity. For all unknown compounds ^{1}H-, ^{13}C- (where needed: 2D NMR-spectra) and high resolution mass spectra were used to verify the identity. Detailed descriptions of the syntheses of compounds known prior to this work can be found in the Supporting Information.

Procedure for the synthesis of dimethylsulfoniumbromide 3a (3b-k were synthesized analogously, analytical data is provided in the Supporting Information).[30] 200 mg of 2-bromo-N-phenylacetamide (2 mmol) were dissolved in 600 µL of dimethylsulfide and stirred in a closed snap-cap vial over night. To the resulting solution 10 ml ether were added and the formed precipitate collected by filtration. The solid was washed repeatedly with ether and dried *in vacuo* to obtain **3a** in 62% (161 mg) yield. **^{1}H-NMR** (500 MHz, DMSO): δ = 7.57 (d, *J* = 7.7 Hz, 2H, 2x $CH_{Ar\text{-}o}$), 7.36 (t, *J* = 7.7 Hz, 2H, 2x $CH_{Ar\text{-}m}$), 7.13 (t, *J* = 7.7 Hz, 1H, $CH_{Ar\text{-}p}$), 4.64 (s, 2H, CH_2), 2.97 (s, 6H, $S(CH_3)_2$) ppm. **^{13}C-NMR** (125 MHz, DMSO): δ = 162.0 (CO), 137.8 ($C_{q\text{-}Ar}$), 129.0 (2x $CH_{Ar\text{-}m}$), 124.4 ($CH_{Ar\text{-}p}$), 119.5 (2x $CH_{Ar\text{-}o}$), 48.2 (CH_2), 24.8 ($S(CH_3)_2$) ppm. **HRMS (ESI)** m/z: $[M]^+$ calcd for $[C_{10}H_{14}NOS]^+$: 196.0791; found: 196.0791.

Procedure for the synthesis of 3-bromo-4,5-dihydroisoxazole 5a (5b-k & 8 were synthesized analogously, analytical data is provided in the Supporting Information).[31] Dibromoformaldoxime (100 mg, 0.493 mmol) and N-phenylacrylamide (**2a**, 87 mg, 0.592 mmol) were dissolved in DMF (1 mL) at 0 °C. An aqueous solution (1.2 mL) of $KHCO_3$ (123 mg, 1.232 mmol) was added to this solution drop-wise during 1 h. The resulting mixture was warmed to rt and stirred for 1h. After addition of ethyl acetate and water, the aqueous layer was separated and extracted three times with ethyl acetate. The combined organic layers were dried and concentrated *in vacuo*. The residue was purified by MPLC to give **5a** in 72% (95 mg) yield. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 8.40 (s, 1H, NH), 7.58 (d, 2H, *J* = 7.5 Hz, H_{Ar}), 7.36 (t, 2H, *J* = 7.5 Hz, H_{Ar}), 7.17 (t, 1H, *J* = 7.5 Hz, H_{Ar}), 5.17 (dd, 1H, *J* = 7.5, 9.8 Hz, CH), 3.68 (dd, 1H, *J* = 9.8, 17.9 Hz, CH_2), 3.61 (dd, 1H, *J* = 7.5, 17.9 Hz, CH_2) ppm. **^{13}C NMR** (75 MHz, $CDCl_3$): δ =

167.56 (CO), 139.17 (C-Br), 136.39($C_{q\ Ar}$), 129.12 (CH_{Ar}), 125.16 (CH_{Ar}), 119.93 (CH_{Ar}), 79.14 (CH), 45.82 (CH_2) ppm. **HRMS (ESI)** (m/z) [M + Na^+] calcd for $[C_{10}H_9O_2N_2BrNa]^+$: 290.9740, found: 297.9709.

ASSOCIATED CONTENT

Supporting Information. Complete analytical data and synthetic procedures; tabular representation of half-lives. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

*Ec*MetAP, methionine aminopeptidase from *E. coli*; *Hs*MetAP-1, methionine-aminopeptidase type 1 from *Homo sapiens*; MurA, UDP-N-acetylglucosamine-1-carboxyvinyltransferase from *E. coli*; MurB, uridine diphospho-N-acetylenolpyruvylglucosamine reductase from *E. coli*; MurC, UDP-N-acetylmuramate-alanine ligase from *E. coli*; MurD, UDP-N-acetylmuramoyl-L-alanine-D-glutamate ligase from *E. coli*; MurE, UDP-N-acetylmuramyl tripeptide synthetase from *E. coli*; MurF, UDP-murNac-tripeptide D-alanyl-D-alanine ligase from *E. coli*; GSH, glutathione; DEN, protease from dengue virus; WNV, protease from West Nile virus; THR, bovine thrombin; mDAP, meso diaminopimelic acid.

Table 1: Electrophiles for covalent protein modification.

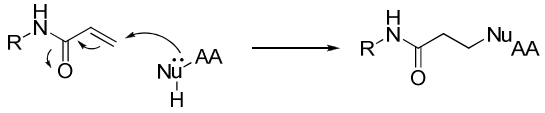
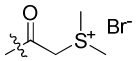
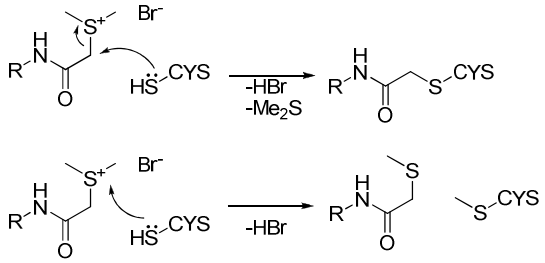
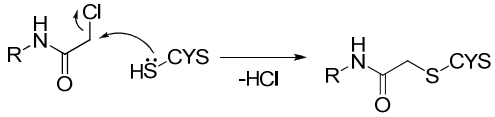
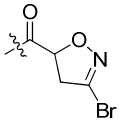
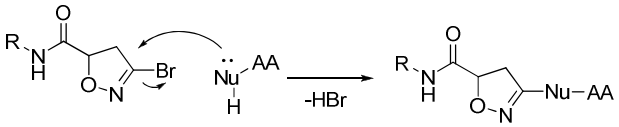
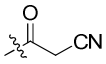
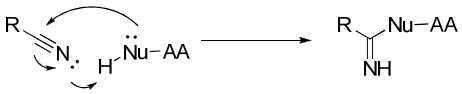
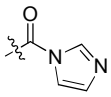
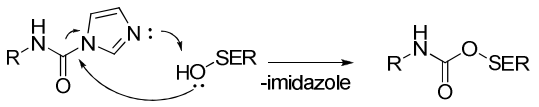
#	Electrophile	Targeted nucleophiles	Example	Binding mode
1		-	-	-
2		Cysteine, Threonine	Afatinib[43] Syringolin A[14]	
3		Cysteine	Transglutaminase inhibitors (M. Griffin *et al.*)[12, 44]	
4		Cysteine	Metazachlor[11]	
5		Cysteine	Bromoacivicin[16, 45, 46]	
6		Serine	NEP-inhibitors (S. S. Ghosh *et al.*)[47]	
		Cysteine	Balicatib[48]	(proposed)
7		Serine	L-norvaline methylester azolide[18]	 (proposed)

Table 2. Non-reactive moieties that were combined with the electrophiles.

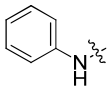
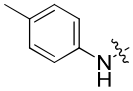
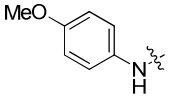
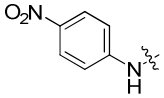
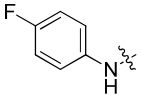
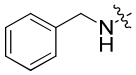
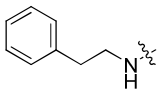
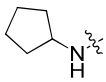
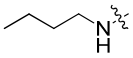
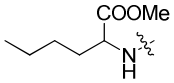
a	b	c	d	e	f
					
g	**h**	**i**	**j**	**k**	
					

Table 3: Target enzymes.

Name	Abbreviation	Type	Native organism	Catalyzed reaction	Function	Nucleophilic residues in or near the active site
Dengue virus protease	DEN	Serine endo-protease	Dengue Virus	Cleavage of viral polyprotein[49]	Production of viral enzymes	SER135[50]
West Nile virus protease	WNV	Serine endo-protease	West-Nile Virus	Cleavage of viral polyprotein	Production of viral enzymes	SER135[51]
Thrombin	THR	Serine endo-protease	*Bos taurus*	Conversion of fibrinogen (coagulation factor I) to fibrin (factor Ia)[52]	Coagulation	SER195[53]
E. coli Methionine aminopeptidase	*ec*MetAP	Metallo exo-protease	*E. coli*	Cleavage of N-terminal methionine[54]	Protein maturation	HIS79
H. sapiens Methionine aminopeptidase type 1	*hs*MetAP-1	Metallo exo-protease	*Homo sapiens*	Cleavage of N-terminal methionine	Protein maturation	HIS231[55]
E. coli MurA	MurA	Transferase	*E. coli*	Transfer of enol pyruvate from PEP to UDP-N-acetylglucosamine	Bacterial murein biosynthesis[56]	CYS115[54]
E. coli MurB	MurB	Oxidoreductase	*E. coli*	Reduction of EP-UDP-N-acetylglucosamine to UDP-Mur-NAc	Bacterial murein biosynthesis	SER229[57]
E. coli MurC	MurC	ATP-dependent ligase	*E. coli*	Ligation of L-Ala and UDP-Mur-NAc	Bacterial murein biosynthesis	-

E. coli MurD	MurD	ATP-dependent ligase	*E. coli*	Ligation of D-Glu and UDP-Mur-NAc-L-Ala	Bacterial murein biosynthesis	CYS413
E. coli MurE	MurE	ATP-dependent ligase	*E. coli*	Ligation of mDAP and UDP-Mur-NAc-L-Ala-D-Glu	Bacterial murein biosynthesis	(LYS391)
E. coli MurF	MurF	ATP-dependent ligase	*E. coli*	Ligation of D-Ala-D-Ala and UDP-Mur-NAc-L-Ala-D-Glu-mDAP	Bacterial murein biosynthesis	CYS371

Table 4: Enzyme inhibition data for compounds **8-12**. n.r. – no reaction, k < 0.1 l·mol^{-1}·s^{-1}; n.i. – no inhibition (<15%); color code: 15-29%. Concentration of test compounds: 25 µM for *ec*MetAP, *hs*MetAP1, THR & MurA-MurF, 50 µM for DEN and WNV.

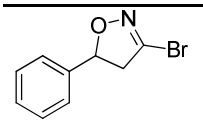
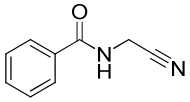
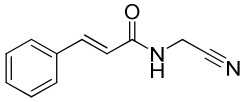
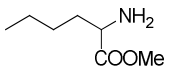
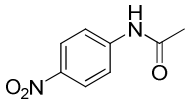
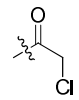
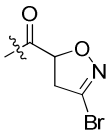
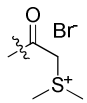
Structure	ID	k_{GSH} [l/(mol*s)]	DEN [%]	WNV [%]	THR [%]	*ec*MetAP [%]	*hs*MetAP1 [%]	MurA [%]	MurB [%]	MurC [%]	MurD [%]	MurE [%]	MurF [%]
	**8**	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	17	n.i.	n.i.
	**9**	n.r.	n.i.	23	n.i.	n.i.	n.i.	n.i.	17	n.i.	n.i.	n.i.	n.i.
	**10**	n.r.	17	18	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	20	n.i.	n.i.
	**11**	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	22	n.i.	18	17	n.i.
	**12**	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

Chart 1: Enzyme inhibition data. n.r. – no reaction, k < 0.1 l·mol^{-1}·s^{-1}; n.i. – no inhibition (<15%); color codes: 15-29%; 30-50%; could not be determined (see text). Concentration of test compounds: 25 µM for *ec*MetAP, *hs*MetAP1, THR & MurA-MurF, 50 µM for DEN and WNV.

Reactive group	Substituent	k_{GSH} [l/(mol·s)]	DEN [%]	WNV [%]	THR [%]	*ec*MetAP [%]	*hs*MetAP1 [%]	MurA [%]	MurB [%]	MurC [%]	MurD [%]	MurE [%]	MurF [%]
 **1**	**a**	n.r.	n.i.	n.i.	n.i.	n.i.	22	16	n.i.	n.i.	n.i.	n.i.	n.i.
	b	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	15
	c	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	20	n.i.	n.i.	n.i.	n.i.
	d	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	20	n.i.	n.i.	n.i.	n.i.
	e	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	17	n.i.	n.i.	n.i.	n.i.
	f	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	g	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	h	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	17	n.i.	n.i.	n.i.	n.i.
	i	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	19	n.i.	n.i.	n.i.	n.i.
	j	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	k	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
 **2**	**a**	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	b	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	c	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	d	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	e	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	f	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	g	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	h	n.r.	n.i.	n.i.	n.i.	n.i.	17	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

Reactive group	Substituent	k_{GSH} [l/(mol·s)]	DEN [%]	WNV [%]	THR [%]	*ec*MetAP [%]	*hs*MetAP1 [%]	MurA [%]	MurB [%]	MurC [%]	MurD [%]	MurE [%]	MurF [%]
 **4**	**a**	0.10	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	b	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	c	0.13	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	d	0.13	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	e	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i..	n.i.
	f	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i..	n.i.
	g	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i..	n.i.
	h	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i..	n.i.
	i	0.11	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	15	n.i.
 **5**	**a**	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	b	n.r.	n.i.	22	n.i.	19	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	c	n.r.	n.i.	21	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	18	n.i.
	d	n.r.	n.i.	n.i.	n.i.	28	n.i.	n.i.	n.i.	n.i.	n.i.	44	n.i.
	e	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	23	21
	f	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	18	n.i.	16	n.i.	15
	g	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	16	n.i.	18	23	21
	h	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	15	16	n.i.
	i	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	27	n.i.
	k	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	39	n.i.
	a	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	23	n.i.

Reactive group	Substituent	k_{GSH} [l/(mol·s)]	DEN [%]	WNV [%]	THR [%]	*ec*MetAP [%]	*hs*MetAP1 [%]	MurA [%]	MurB [%]	MurC [%]	MurD [%]	MurE [%]	MurF [%]
	i	n.r.	n.i.	n.i.	n.i.	n.i.	20	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	k	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Acrylamide		n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	a		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	32	n.i.
	b												
	c												
	d												
	**e**		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	15	n.i.
	f		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
3	**g**		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	h		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	17	n.i.
	i		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	29	n.i.
	j		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	14	n.i.	20	n.i.
	k		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	20	n.i.	25	n.i.

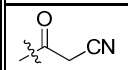
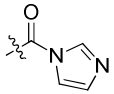
Reactive group	Substituent	k_{GSH} [l/(mol·s)]	DEN [%]	WNV [%]	THR [%]	*ec*MetAP [%]	*hs*MetAP1 [%]	MurA [%]	MurB [%]	MurC [%]	MurD [%]	MurE [%]	MurF [%]
	**b**	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	c	n.r.	n.i.	16	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	15	n.i.
6	**d**	n.r.	n.i.	n.i.	n.i.	n.d.	n.d.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	e	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	f	n.r.	n.i.	18	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	g	n.r.	n.i.	22	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	h	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	16	n.i.
	i	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	j	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	k	n.r.	n.i.	18	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
	**a**	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i..	n.i.
	b	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i..	n.i..	n.i.
	c	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	17	n.i.	n.i..	n.i..	n.i.
7	**d**	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i..	n.i..	n.i.
	e	n.r.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	28	n.i.	n.i..	n.i..	n.i.

Table 5: EC_{50} values of the cell viability assays (HeLa-cells, incubation time: 24 h, resazurin indicator)

Compound	**1a**	**1i**	**2a**	**2i**	Acrylamide	**3a**	**3i**	**4a**	**4i**
EC_{50} [µM]	> 100	> 100	> 100	> 100	> 100	> 100	> 100	20.9	> 100
Compound	**5a**	**5d**	**5i**	**5k**	**6a**	**6i**	**7a**	**9**	**10**
EC_{50} [µM]	> 100	73.4	> 100	> 100	> 100	> 100	> 100	> 100	> 100

Table of Contents graphic

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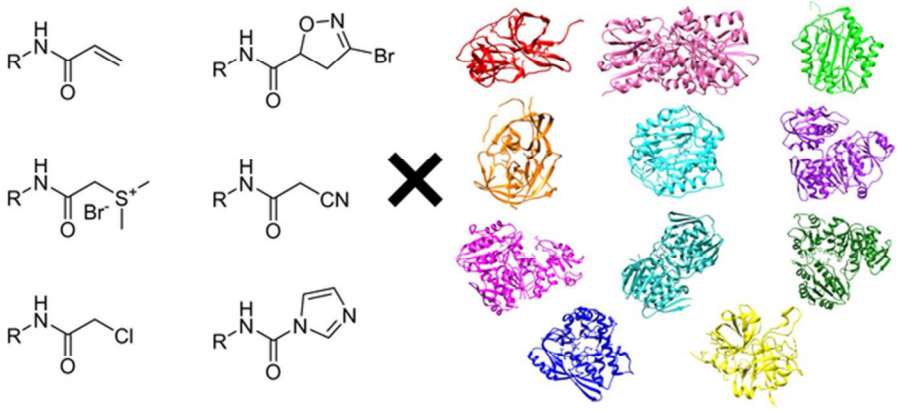

Table of contents graphic
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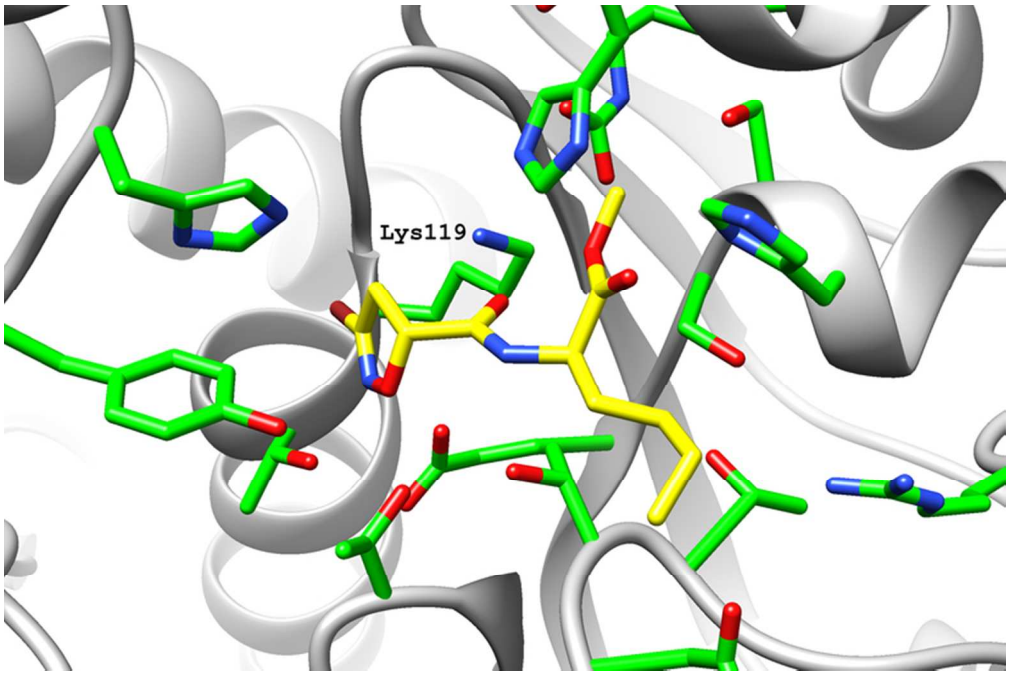

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