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# THE SYNTHESIS OF NOVEL 2,4,6-TRISUBSTITUTED 1,3,5-TRIAZINES: A SEARCH FOR POTENTIAL MURF ENZYME INHIBITORS

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**Abstract** – A series of new 2,4,6-trisubstituted 1,3,5-triazines, possessing a variety of substituents (–OH, –SH, –OMe, –Cl, –HNR, –SR and amino acid moieties), were synthesized and evaluated for the inhibition of the bacterial peptidoglycan biosynthesis enzyme MurF. Ethoxycarbonyl isothiocyanate successfully reacted with a variety of amidines, enabling an approach to 6-substituted-4-thioxo-1,3,5-triazin-2-ones. Also, a representative set of 2-thio-, 2-amino-, and 2-oxo-substituted 1,3,5-triazines was synthesized by the $S_NAr$ reaction, employing 2,4,6-trichloro-1,3,5-triazine and 2-chloro-4,6-dimethoxy-1,3,5-triazine as the starting materials. One compound displayed notable inhibitory activity against MurF from Escherichia coli.

## INTRODUCTION

1,3,5-Triazines (or *s*-triazines) constitute a class of heterocyclic compounds that have been well known for a long time, and still represent the object of considerable interest, mainly due to their applications in different fields, such as the production of polymeric photostabilisers[1] and herbicides.[2] Triazine herbicides act as inhibitors of photosynthesis in plants by interrupting the light-driven flow of electrons from water to $NADP^+$.[3] Some 1,3,5-triazines also display other important biological properties, of which it is worth mentioning antineoplastic activity[4] and antimalarial activity.[5, 6] 1,3,5-Triazines have also been

recognized as antibacterial compounds.[7] Recently, Maeda *et al.* described a series of novel 4,2-di(substituted)amino-1,2-dihydro-1,3,5-triazine derivatives that were evaluated for their antiseptic properties by MIC and MBC tests against Gram-positive and Gram-negative bacteria.[8]

Peptidoglycan is an essential bacterial cell-wall polymer and its biosynthesis provides a unique and selective target for antibacterial chemotherapy.[9] Peptidoglycan biosynthesis consists of 10 biosynthetic transformations, each of them requiring a specific enzyme.[10] These enzymes include MurA, MurB, MurC, MurD, MurE, MurF, MraY, MurG, and the transglycosylase and transpeptidase families of enzymes. As a part of our efforts to identify new, small-molecule inhibitors of the intracellular steps of peptidoglycan biosynthesis, the virtual high-throughput screening (VHTS) of the National Cancer Institute "Diversity Set" bank of compounds was performed. We identified the triazine derivative NSC 209931 (Figure 1) as a promising inhibitor of MurF ($IC_{50}$ = 63 μM).[11]

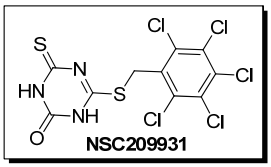
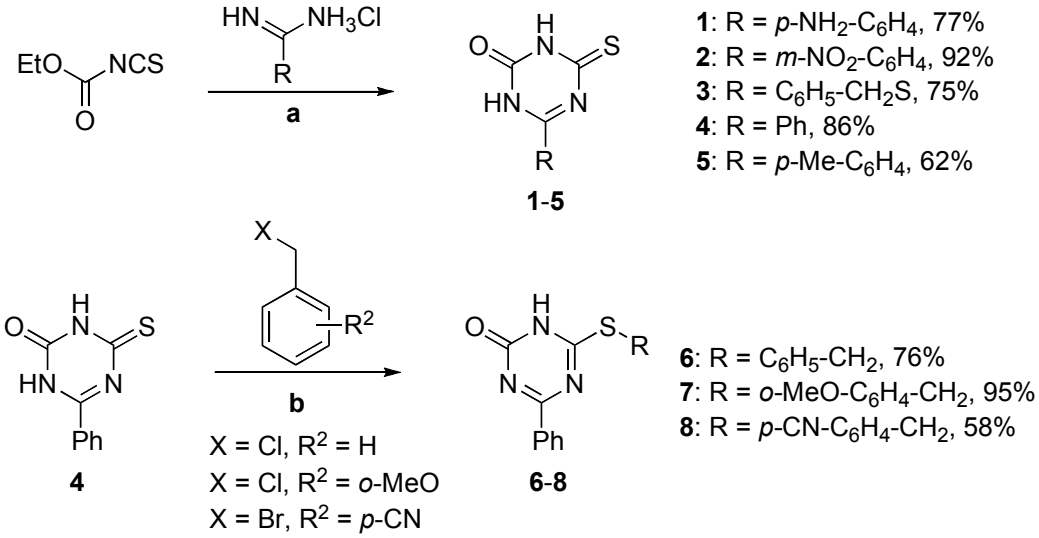



Figure 1. VHTS hit NSC 209931; $IC_{50}$ (MurF, *E. coli*) = 63 μM.

MurF belongs to the family of ATP-dependent Mur ligases and catalyzes the formation of the peptide bond between UDP-Mur*N*Ac-tripeptide and D-Ala-D-Ala, to form the final soluble peptidoglycan biosynthesis precursor UDP-Mur*N*Ac-pentapeptide.[10a] There are only a few known inhibitors of MurF. The pseudo-tripeptide and pseudo-tetrapeptide aminoalkylphosphinic acids were developed as simplified transition-state analogues.[12] A series of small-molecule cyanothiophenes were developed by Abbott Laboratories[13, 14] and thiazolylaminopyrimidines were discovered by Johnson & Johnson.[15] However, none of these showed significant antibacterial activity. In order to find new inhibitors of MurF as potential antibacterial lead compounds, we initiated the synthesis of a series of 4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-one derivatives related to our VHTS hit compound NSC 209931.

## RESULTS AND DISCUSSION

The 4-thioxo-1,3,5-triazin-2(1*H*)-one derivatives **1**–**8** were prepared from appropriate amidines and ethoxycarbonyl isothiocyanate.[16] Independent of the nature of the substituent of the amidine, the reactions of ethoxycarbonyl isothiocyanate with 1 equiv of amidines in the presence of 2 M NaOH proceeded smoothly in toluene at room temperature to give the desired 6-substituted-4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-ones **1**–**5** (Scheme 1).

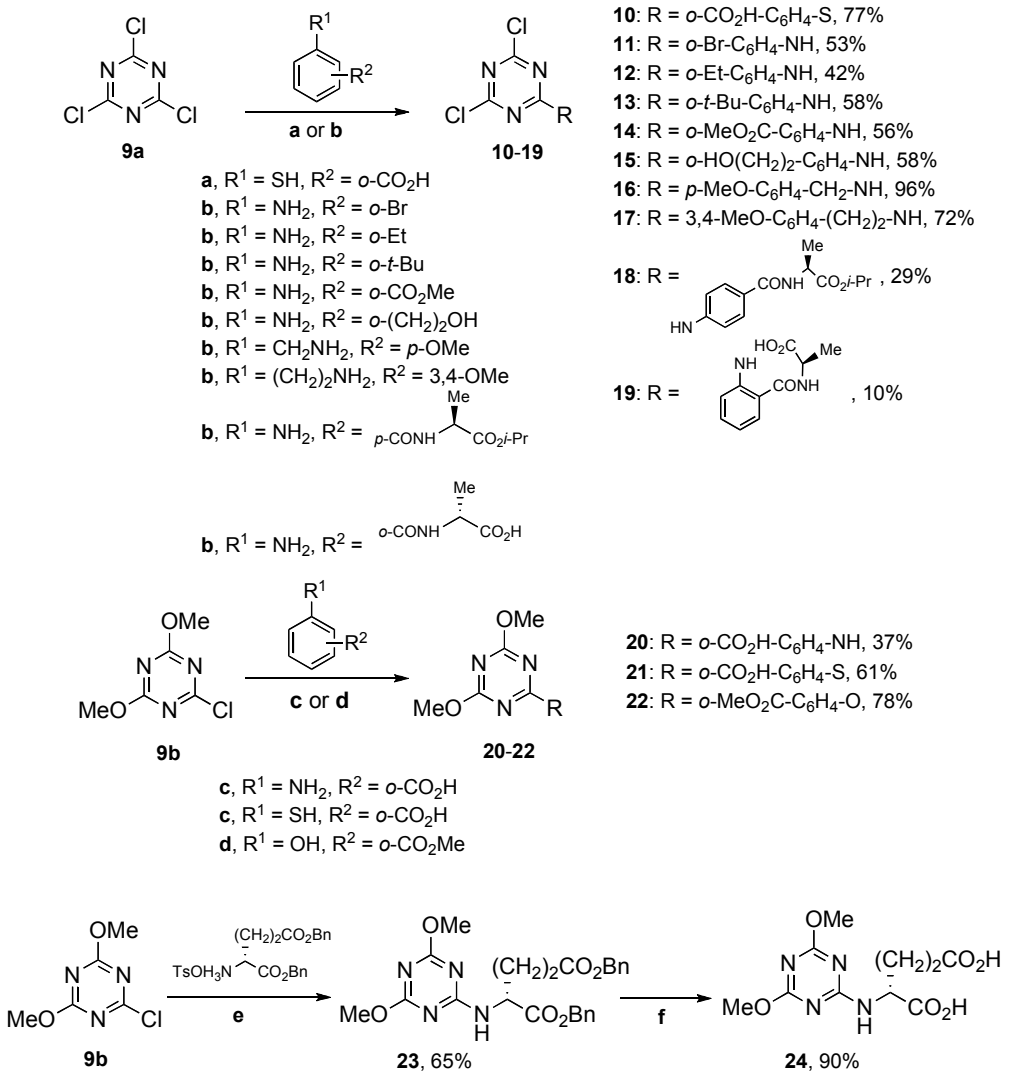



Scheme 1. Synthesis of potential inhibitors of MurF based on 4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-one core. Reagents and conditions: (a) 2 M NaOH, toluene, $H_2O$, 25 °C, 1h; (b) 2 M NaOH, EtOH, 25 °C, 1 h.

Furthermore, product **4** (Scheme 1) was readily benzylated in a basic EtOH solution at room temperature, providing 4-thiobenzyl derivatives **6**–**8** in good yields. However, compounds **1**–**8** did not show any significant inhibitory activity against MurF from *Escherichia coli.*

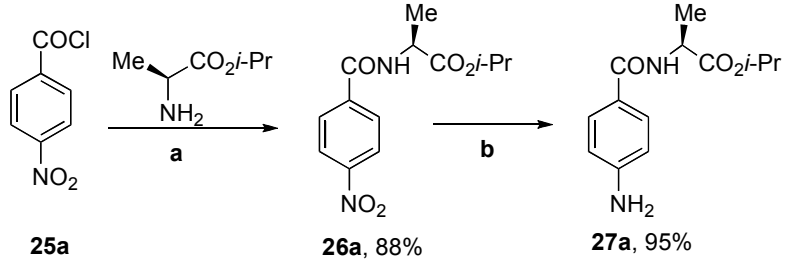
In a second approach, we focused our efforts on the nucleophilic substitution of the chloro substituents in the 1,3,5-triazine derivatives **9a** and **9b** (Scheme 2). We reasoned that the use of trichloro-1,3,5-triazine (**9a**) and dimethoxychloro-1,3,5-triazine (**9b**) as a core scaffold might give us access to multifunctional architectures of the triazine derivatives. Moreover, in the light of the known sequential reactivity of the three chlorine atoms in the trichloro-triazine backbone, such a scaffold is well known to provide a high synthetic variability, useful for combinatorial synthesis.[17] With these objectives in mind, series of 2,4,6-trichloro-1,3,5-triazine (**9a**) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (**9b**) derivatives were prepared, as outlined in Scheme 2.

Reacting the 2,4,6-trichloro-1,3,5-triazine (**9a**) at 0 °C with 2-mercaptobenzoic acid resulted in the displacement of one chlorine, yielding the corresponding 1,3,5-triazinethiobenzoic acid **10** in a good yield. The substitution of the chlorine by the 2-substituted primary anilines and benzyl amines was achieved at –15 °C in MeCN, giving products **11**−**17,** with high purity and in reasonable yields. As the replacement of the chloro substituent in **9a** appeared straightforward, we decided to investigate its $S_NAr$ displacement with aminobenzoic acids. The products of the nucleophilic substitution, (4,6-dichloro-1,3,5-triazin-2-yl)aminobenzoic acids, could serve us as valuable substrates for the secondary modifications on the carboxylic functionality. Unfortunately, we were unsuccessful in all our attempts to prepare such (4,6-dichloro-1,3,5-triazin-2-yl)aminobenzoic acids in a one-step process.



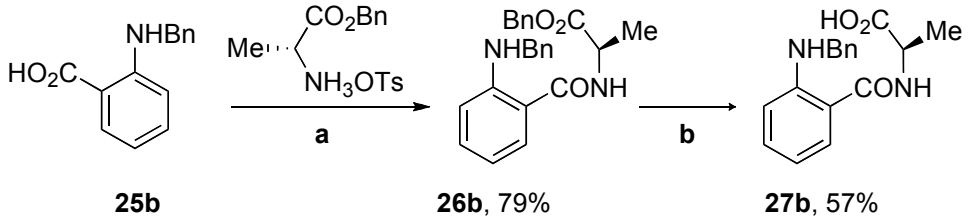

Scheme 2. Synthesis of potential inhibitors of MurF based on triazine core. Reagents and conditions: (a) $H_2O$, MeCN, 0 °C, 1 h; (b) $Et_3N$, MeCN, –15 °C, 1 h; (c) MeCN, $Et_3N$, 25 °C, 2 h; (d) *t*BuOK, MeCN, 25 °C, 2 h; (e) $K_2CO_3$, MeCN, 2 days; (f) $H_2$, 60 psi, Pd-C, MeOH.

Nevertheless, we overcame this problem by applying the reverse synthetic strategy. As outlined in Scheme 3, 4-nitrobenzoyl chloride was reacted with isopropyl L-alaninate, yielding **26a**, which was subsequently reduced to the amino product **27a** in an excellent yield.




Scheme 3. Preparation of isopropyl *N*-(4-aminobenzoyl)- L-alaninate **27a**. Reagents and conditions: (a) $Et_3N$, MeCN, 0 °C, then 25 °C, 14 h; (b) MeOH, $H_2$, 1atm, Pd-C.

Alternatively, amide-bond formation was achieved between the *N*-benzyl-protected 2-aminobenzoic acid **25b** and the D-alanine benzyl ester *p*-toluenesulfonate salt, using EDC and HOBt as the coupling reagents. The hydrogenolysis ($H_2$, Pd-C) of **26b** resulted in the formation of the desired aniline derivative, *N*-(2-aminobenzoyl)-D-alanine (**27b**) (Scheme 4).



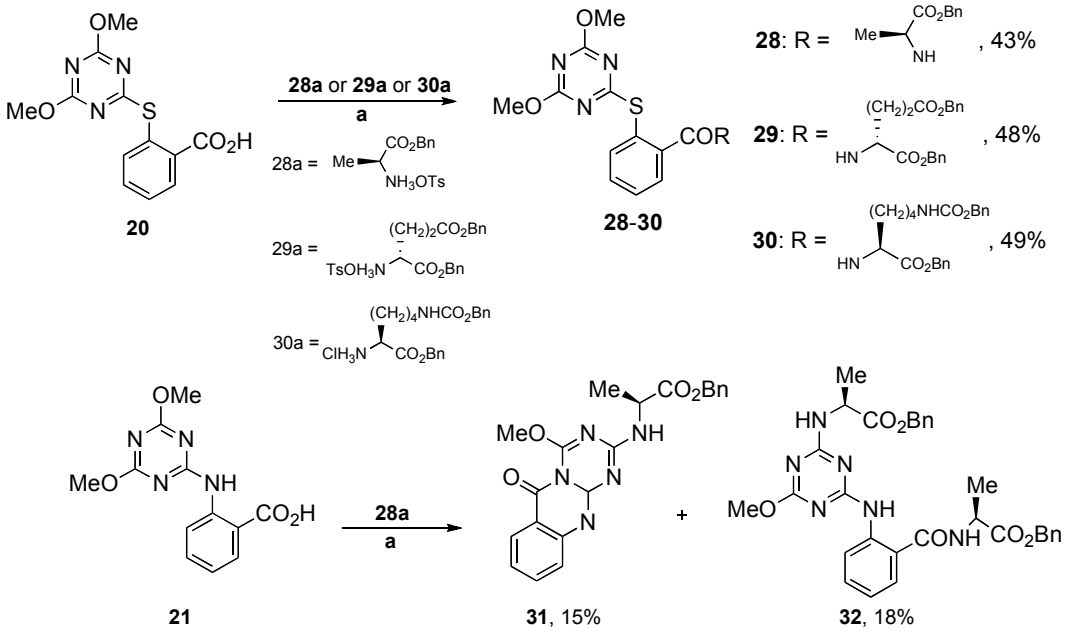

**25b** **26b**, 79% **27b**, 57%

Scheme 4. Preparation of *N*-(2-aminobenzoyl)- D-alanine **27b**. Reagents and conditions: (a) EDC·HCl, HOBt, $Et_3N$, DMF, 0 °C, 24 h; (b) $H_2$, 60 psi, Pd-C.

Finally, target compounds **18** and **19** were obtained in low yields, 20% and 10%, respectively, by the treatment of 2,4,6-trichloro-1,3,5-triazine with **27a** and **27b** (Scheme 2).

We attempted to hydrolyse the chloro substituents in products **10**–**19** under basic or acidic conditions. Different reaction conditions (a variation of the reaction temperature and the concentration of the acid or base) were investigated without any success. In most cases, complex reaction mixtures were formed, from which it was not possible to isolate any identifiable products. We proceeded with the synthesis of the dimethoxy analogues **20**−**24**, which were derived from 2-chloro-4,6-dimethoxy-1,3,5-triazine (**9b**). In this case, **9b** was successfully reacted with 2-aminobenzoic acid and 2-mercaptobenzoic acid, giving the corresponding 4,6-dimethoxy-1,3,5-triazine derivatives **20** and **21**, respectively, in good yields. The same procedure (MeCN, r.t.) was also used for the synthesis of the methyl ester analogue **22**. Furthermore, the treatment of **9b** with C-protected D-Glu provided the amino acid derivative **23**, which was converted by catalytic hydrogenation ($H_2$, 10% Pd-C) to the target compound **24** (Scheme 2) in an excellent yield.

The 1,3,5-triazin-2-yl amino- and thiobenzoic acid derivatives **20** and **21** were further modified, as shown in Scheme 5. In the course of these SAR studies, we postulated that the introduction of an amino acid side-chain in **20** and **21** might have an impact on the inhibitory activity of such derivatives. Thus, the dimethoxy-1,3,5-triazinethiobenzoic acid derivative **21** was coupled to the D-alanine benzyl ester *p*-toluenesulfonate salt (**28a**), the D-glutamic acid dibenzyl ester *p*-toluenesulfonate salt (**29a**), and the $N^6$-carbobenzyloxy-L-lysine benzyl ester hydrochloride (**30a**) to produce the amido derivatives **28**, **29** and **30**.

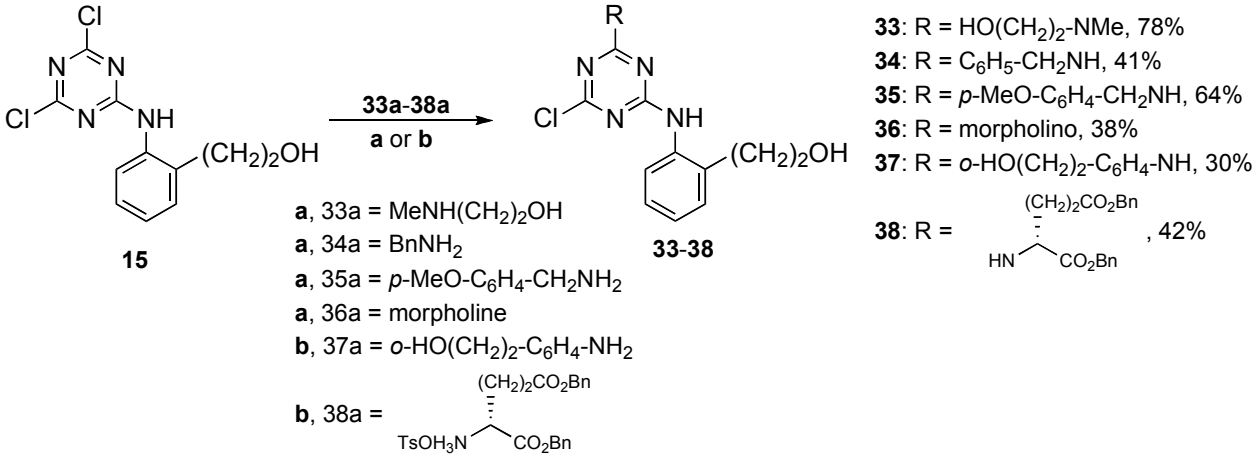



Scheme 5. Synthesis of potential inhibitors of MurF based on triazine core. Reagents and conditions: (a) EDC·HCl, HOBt, $Et_3N$, DMF, 0 °C, 24 h.

The attempted hydrogenolysis of the benzyl groups was unsuccessful. Furthermore, the controlled basic hydrolysis (1 M LiOH, 25 °C) turned out to be problematic as well, resulting in the formation of the complex reaction mixtures. When the amino analogue **20** was reacted with the D-alanine benzyl ester *p*-toluenesulfonate salt (**28a**), two products were isolated in low yields (Scheme 5, products **31** and **32**). Product **31** resulted from the $S_NAr$ substitution of one of the methoxy groups in the dimethoxy-1,3,5-triazine **20** with the D-alanine benzyl ester *p*-toluenesulfonate salt and the simultaneous cyclization of the EDC-activated carboxylate with the 1,3,5-triazine ring. The structure of **31** was confirmed by HMQC and HMBC NMR spectra. The isolated product **32** indicates that unlike the thio derivatives **21**, the 2-amino substituted 4,6-dimethoxy-1,3,5-triazines **20** are subject to the $S_NAr$ substitution reaction of the methoxy group under the applied reaction condition for the amide-bond formation.

Target compounds **2**–**8**, **10**–**24**, and **28**–**32** were tested for the inhibitory activity of the MurF enzyme from *E. coli* using the Malachite green assay, which detects the free phosphate liberated during the reaction.[18] To avoid any possible non-specific (promiscuous) inhibition, all the compounds were tested in the presence of the detergent (Triton X-114, 0.005%).[19] The results were obtained as the residual activities (RAs) of the enzyme in the presence of 500 μM concentrations of each compound. Based on the results we can conclude that all of the tested compounds are poor inhibitors of MurF, with the RA values being between 60 and 90%. The only exception is compound **15,** with an RA value of 41%, and for which the $IC_{50}$ was determined to be 450 μM.

On the basis of the results presented above, we used compound **15** as a starting point for further structural modifications. Several analogues of **15** were prepared via the $S_NAr$ displacement of chlorine in the 1,3,5-triazine ring with nitrogen nucleophiles such as aniline, morpholine, D-glutamic acid dibenzyl ester *p*-toluenesulfonate salt, and 2-(methylamino)ethanol (Scheme 6).




Scheme 6. Derivatization of 15. Reagents and conditions: (a) $Et_3N$, MeCN, rt, 3 h; (b) $K_2CO_3$, acetone, 50 °C, 24 h.

All the corresponding compounds were isolated in reasonable yields and with high purity. Unfortunately, when tested on MurF they did not show any significant increase in potency against MurF, having RA values >60% in all cases.

To locate the possible binding orientation of inhibitor **15** within the active site of MurF, a docking experiment was performed using the FlexX software.[20] The predicted binding pose of compound **15** is presented in Figure 2.

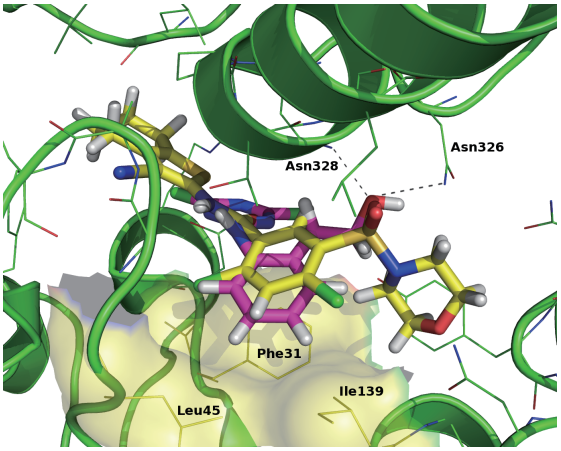



Figure 2. Docking of compound **15** (magenta) into the active site of MurF (green). The co-crystallized cyanothiophene inhibitor is also shown (yellow). Amino acid residues that form interactions with compound **15** are labelled (polar Asn326 and Asn328; hydrophobic Phe31, Leu45 and Ile139, which are also presented as a yellow surface).

In this docking pose, the hydroxy group of the inhibitor forms hydrogen bonds with Asn326 and Asn328, while the phenyl ring forms hydrophobic interactions with Phe31, Leu45 and Ile139. Phe31 also forms π-stacking interactions with the inhibitor's phenyl and 1,3,5-triazine rings. It should also be noted that compound **15** is well aligned with the co-crystallized cyanothiophene inhibitor.[14] The hydroxy group of compound **15** is aligned with the sulfonylamide group of the co-crystallized inhibitor, forming similar interactions. In addition, the phenyl ring of compound **15** and that of the co-crystallized inhibitor sit in the hydrophobic pocket formed by Phe31, Leu45 and Ile139.

Since it is known that tautomerism occurs in 1,3,5-triazines when they are substituted by hydroxy, sulfanyl, or amino functionality,[21] it should be noted that unlike the 4-amino-substituted 1,3,5-triazines, the 2,4-diamino substituted derivatives **33**−**38** exist in tautomeric equilibria, as is evident from the $^{1}$H and $^{13}$C NMR spectra recorded in DMSO and $CDCl_3$ solutions at 25 °C. For this reason, some of the NMR spectra were recorded at elevated temperatures for reasons of clarity. For most of the compounds possessing the symmetric 2,4-dichloro-substituted 1,3,5-triazine ring, we have observed in the $^{13}$C NMR spectra three distinct resonances (between 150–170 ppm) for the triazine carbons. On the other hand, the 2,4-dimethoxy derivatives **20**−**22** and **28**−**30**, display the symmetric pattern in the $^{13}$C NMR spectra, having only two resonances in the region of 168–172 ppms.

## CONCLUSION

In summary, based on encouraging results with the VHTS hit NSC209931, a series of new 1,3,5-triazines were designed and synthesized as putative MurF inhibitors. Ethoxycarbonyl isothiocyanate successfully reacted with a variety of amidines, enabling an approach to the 6(4)-substituted-4-thioxo-1,3,5-triazin-2-ones. Also, a representative set of 2-thio-, 2-amino-, and 2-oxo-substituted 1,3,5-triazines was synthesised by the $S_NAr$ reaction, employing 2,4,6-trichloro-1,3,5-triazine (**9a**) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (**9b**) as the starting materials. All the synthesised compounds were tested for their inhibitory activity of the MurF enzyme from *E. coli;* however, only compound **15** turned out to display significant inhibitory activity, with an $IC_{50}$ value of 450 μM.

## EXPERIMENTAL

**General methods.** Solvents and starting compounds were obtained from commercial sources (Fluka, Sigma and Aldrich). Light petroleum refers to the fraction with the boiling point 40–60 °C. TLC was carried out on Fluka silica-gel TLC-cards. All mps were determined on a hot-stage apparatus and are uncorrected. IR spectra were recorded on a BioRad FTS 3000MX instrument. NMR spectra were recorded on a Bruker Avance 300 DPX spectrometer at 302 K. Chemical shifts are reported in δ ppm, referenced to an internal TMS standard for $^{1}$H NMR, chloroform–*d* (δ 77.0), DMSO–$d_6$ (δ 39.5) for $^{13}$C

NMR. Microanalyses were performed on a Perkin-Elmer 2400 series II CHNS/O analyser. Mass spectra and high-resolution mass measurements were performed on a VG-Analytical Autospec EQ instrument. **Hardware and software.** The docking experiment was made on a computer workstation with four dual-core Opteron processors, 16GB of RAM and 1.2TB of hard-drive space running the Fedora 7 operating system. FlexX 3.1.2 from BioSolveIT GmbH[20] was used for the active-site preparation, the docking of the compound **15** and the scoring. PyMol from DeLano Scientific was used for preparation of the graphical representation of the docking results.

**General procedure for the preparation of 6-substituted-4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-ones 1-5.** To a solution of corresponding amidine (1.0 mmol) in $H_2O$ (3 mL), toluene (5 mL) was added and the mixture stirred vigorously. After 5 min, ethoxycarbonyl isothiocyanate (1.4 mmol) in toluene (2 mL) and NaOH (2 M, 4 mL) were simultaneously added over a period of 10 minutes. Additional NaOH (2 M, 2 mL) was added after 15 min and then the reaction mixture was stirred for 1 h at room temperature. The phases were then separated and the organic layer washed with NaOH (2 M, 6 mL). The combined alkaline phases were acidified to pH 1–2 with $H_2SO_4$, and in this way the precipitate was formed, which was then filtered off. Pure products were obtained by crystallization from the corresponding solvent.

**6-(4-Aminophenyl)-4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-one (1):** Crystallization from MeCN gave 170 mg (77%) of yellow crystals. mp 283.0–287.0 °C. $R_f$ 0.35 (MeOH-$CH_2Cl_2$ = 1/5). IR (KBr): 3442, 3338, 3020, 2891, 1720, 1633, 1601, 1577, 1519, 1490, 1322, 1184, 969, 830, 764, 601 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 6.34 (br s, 2H, PhN$\underline{H_2}$), 6.62 (d, $J$ = 9.0 Hz, 2H, -$\underline{Ph}$), 7.95 (d, $J$ = 9.0 Hz, 2H, -$\underline{Ph}$), 12.38 (br s, 1H, -N$\underline{H}$-), 12.59 (br s, 1H, -N$\underline{H}$-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 113.0, 114.7, 131.3, 150.3, 154.7, 158.9, 183.8. MS (EI) *m/z* (%): 220 ($M^+$, 100), 161 (30), 119 (100), 69 (22), 57 (30). HRMS calcd for $C_9H_8N_4OS$: 220.0419. Found: 220.0234. Anal. Calcd for $C_9H_8N_4OS$: C, 49.08; H, 3.66; N, 25.44. Found: C, 48.79; H, 3.68; N, 25.69.

**6-(3-Nitrophenyl)-4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-one (2)[22]:** Crystallization from MeCN gave 461 mg (92%) of orange crystals. mp 99.0–105.0 °C. mp 74 °C.[22] $R_f$ 0.18 (MeOH-$CH_2Cl_2$ = 1/5). IR (KBr): 3414, 3210, 3107, 1678, 1617, 1528, 1449, 1388, 1350, 1278, 1224, 1150, 918, 702, 578 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 7.86 (t, $J$ = 8.0 Hz, 1H, -$\underline{Ph}$), 8.45–8.54 (m, 2H, -$\underline{Ph}$), 8.92 (t, $J$ = 2.0 Hz, 1H, -$\underline{Ph}$), 12.89 (br s, 1H, -N$\underline{H}$-), 13.47 (br s, 1H, -N$\underline{H}$-). MS (ESI) *m/z*: 249 $(M-H)^-$. HRMS calcd for $C_9H_5N_4O_3S$: 249.0082. Found: 249.0078.

**6-(Benzylthio)-4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-one (3)[16]:** Crystallization from MeCN gave 1.87 g (75%) of pale white crystals. mp 226.0–228.0 °C. mp 230.0–234.0 °C.[16] $R_f$ 0.44 (MeOH-$CH_2Cl_2$ = 1/5). IR (KBr): 3415, 3140, 3031, 2912, 1655, 1528, 1391, 1239, 1207, 1145, 1031, 882, 701, 614, 577

$cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 4.38 (s, 2H, -C$\underline{H_2}$S-), 7.24–7.46 (m, 5H, -$\underline{Ph}$), 12.52 (br s, 1H, -N$\underline{H}$), 13.58 (br s, 1H, -N$\underline{H}$-). MS (ESI) *m/z*: 274 $(M+Na)^+$. HRMS calcd for $C_{10}H_9N_3ONaS_2$: 274.0085. Found: 274.0088.

**6-Phenyl-4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-one (4)[16]:** Crystallization from EtOH gave 1.76 g (86%) of yellow crystals. mp 244.0–248.0 °C. mp 246.0–248.0 °C.[16] $R_f$ 0.17 (MeOH-$CH_2Cl_2$ = 1/20). IR (KBr): 3421, 3062, 2936, 1678, 1601, 1551, 1385, 1320, 1231, 1164, 941, 816, 766, 696 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 7.52–7.61 (m, 2H, -$\underline{Ph}$), 7.64–7.72 (m, 1H, -$\underline{Ph}$), 8.07–8.14 (m, 2H, -$\underline{Ph}$), 12.74 (br s, 1H, -N$\underline{H}$-), 13.18 (br s, 1H, -N$\underline{H}$-). MS (ESI) *m/z*: 206 $(M+H)^+$. HRMS calcd for $C_9H_8N_3OS$: 206.0388. Found: 206.0380.

**6-*p*-Tolyl-4-thioxo-3,4-dihydro-1,3,5-triazin-2(1*H*)-one (5)[16]:** Crystallization from EtOH gave 135 mg (62%) of white crystals. mp 245.0–247.5 °C. mp 246.0–247.0 °C.[16] $R_f$ 0.63 (MeOH-$CH_2Cl_2$ = 1/5). IR (KBr): 3414, 3124, 3051, 2936, 1645, 1615, 1589, 1549, 1397, 1328, 1233, 1169, 952, 832, 783, 732, 626, 564 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 2.40 (s, 3H, -C$\underline{H_3}$), 7.37 (d, $J$ = 8.0 Hz, 2H, -$\underline{Ph}$), 8.04 (d, $J$ = 8.0 Hz, 2H, -$\underline{Ph}$), 12.67 (br s, 1H, -N$\underline{H}$-), 13.09 (br s, -N$\underline{H}$-). MS (EI) *m/z* (%): 219 ($M^+$, 68), 160 (30), 144 (43), 118 (100), 91 (24). HRMS calcd for $C_{10}H_9N_3OS$: 219.0466. Found: 219.0471.

**General procedure for the preparation of benzylated derivatives 6–8.** To a solution of compound **4** (1.0 mmol) in EtOH (8 mL) and NaOH (2 M, 5 mL), the corresponding benzyl halide (1.1 mmol) was slowly added. The reaction mixture was stirred for 1 h at room temperature. After the reaction was complete, $H_2O$ (10 mL) was added, followed by the addition of $H_2SO_4$ (2 M, 5 mL). The precipitate formed was filtered off and the pure product obtained by crystallization from the corresponding solvent.

**6-Phenyl-4-(benzylthio)-1,3,5-triazin-2(3*H*)-one (6)[23]:** Crystallization from dioxane gave 224 mg (76%) of white crystals. mp 220.0–222.0 °C. mp 229.0 °C.[23] $R_f$ 0.45 (MeOH-$CH_2Cl_2$ = 1/20). IR (KBr): 3414, 3078, 3025, 2939, 1668, 1538, 1494, 1423, 1312, 1276, 1236, 1167, 1099, 1008, 848, 778, 711, 696 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 4.45 (s, 2H, -SC$\underline{H_2}$-), 7.23–7.38 (m, 3H, -$\underline{Ph}$), 7.42–7.50 (m, 2H, -$\underline{Ph}$), 7.52–7.62 (m, 2H, -$\underline{Ph}$), 7.64–7.73 (m, 1H, -$\underline{Ph}$), 8.21 (d, $J$ = 6.5 Hz, 2H, -$\underline{Ph}$), 12.96 (br s, 1H, -N$\underline{H}$CO-). MS (ESI) *m/z*: 294 $(M-H)^-$. HRMS calcd for $C_{16}H_{12}N_3OS$: 294.0701. Found: 294.0707.

**6-Phenyl-4-(2-methoxybenzylthio)-1,3,5-triazin-2(3*H*)-one (7):** Crystallization from MeCN gave 308 mg (95%) of a white foamy solid. mp 224.5–226.0 °C. $R_f$ 0.47 (MeOH-$CH_2Cl_2$ = 1/20). IR (KBr): 3414, 2944, 1672, 1550, 1494, 1420, 1315, 1274, 1252, 1168, 1097, 1026, 850, 751, 710, 624 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 3.85 (s, 3H, -OC$\underline{H_3}$), 4.40 (s, 2H, -SC$\underline{H_2}$-), 6.90 (dt, $J$ = 7.5, 1.0 Hz, 1H, -$\underline{Ph}$), 7.03 (d, $J$ = 8.0 Hz, 1H, -$\underline{Ph}$), 7.29 (dt, $J$ = 7.5, 1.5 Hz, 1H, -$\underline{Ph}$), 7.43 (dd, J = 7.5, 1.5 Hz, 1H, -$\underline{Ph}$), 7.54–7.62 (m, 2H, -$\underline{Ph}$), 7.64–7.73 (m, 1H, -$\underline{Ph}$), 8.21 (d, $J$ = 7.0 Hz, 2H, -$\underline{Ph}$), 12.91 (br s, 1H, -N$\underline{H}$CO-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 29.3, 55.6, 111.0, 120.4, 124.4, 128.6 (2C), 128.9 (2C), 129.1, 130.3,

133.5, 154.7, 157.2, 164.6. MS (ESI) *m/z*: 326 $(M+H)^+$. HRMS calcd for $C_{17}H_{16}N_3O_2S$: 326.0963. Found: 326.0960. Anal. Calcd for $C_{17}H_{15}N_3O_2S$: C, 62.75; H, 4.65; N, 12.91. Found: C, 62.53; H, 4.72; N, 12.94.

**6-Phenyl-4-(4-cyanobenzylthio)-1,3,5-triazin-2(3*H*)-one (8):** Crystallization from MeCN gave 185 mg (58%) of a white foamy solid. mp 229.5–232.5 °C. $R_f$ 0.26 (MeOH-$CH_2Cl_2$ = 1/20). IR (KBr): 3549, 3413, 3080, 2940, 2225, 1671, 1532, 1493, 1423, 1311, 1272, 1168, 1100, 1008, 944, 847, 776, 709, 684, 554 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 4.52 (s, 2H, -SC*H*$_2$-), 7.53–7.61 (m, 2H, -*Ph*), 7.64–7.72 (m, 3H, -*Ph*), 7.77–7.84 (m, 2H, -*Ph*), 8.18 (d, $J$ = 7.0 Hz, 2H, -*Ph*), 12.99 (br s, 1H, -N*H*CO-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 33.5, 110.0, 118.7, 128.6, 128.9, 129.9 (2C), 132.4 (2C), 133.7, 143.7, 154.6, 164.5. MS (ESI) *m/z*: 321 $(M+H)^+$. HRMS calcd for $C_{17}H_{13}N_4OS$: 321.0810. Found: 321.0825. Anal. Calcd for $C_{17}H_{12}N_4OS$: C, 63.73; H, 3.78; N, 17.49. Found: C, 63.50; H, 3.85; N, 17.52.

**Procedure for the preparation of 2-[(4,6-dichloro-1,3,5-triazin-2-yl)thio]benzoic acid 10.** Thiosalicylic acid (308 mg, 2.0 mmol) was added to an ice-cooled solution of 2,4,6-trichloro-1,3,5-triazine (369 mg, 2.0 mmol) in MeCN (20 mL). The reaction mixture was allowed to react for 1 h at 0 °C and then $H_2O$ (5 mL) was added. The white solid formed was filtered off and washed with $H_2O$ (5 mL) and MeCN (5 mL), yielding a pure product, 465 mg (77%). mp 140.0–146.0 °C. $R_f$ 0.70 (MeOH). IR (KBr): 3067, 1989, 1882, 1664, 1686, 1585, 1524, 1467, 1278, 1260, 846, 745, 560 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 7.55–7.66 (m, 2H, -*Ph*), 7.70–7.77 (m, 1H, -*Ph*), 7.88–7.94 (m, 1H, -*Ph*), 11.17 (br s, 1H, -$CO_2$*H*). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 125.7, 129.9, 130.4, 131.8, 135.3, 137.2, 151.8, 166.9, 169.1, 169.2. MS (EI) *m/z* (%): 301 ($M^+$, 8), 256 (100), 195 (35), 136 (45). HRMS calcd for $C_{10}H_5N_3O_2SCl_2$: 300.9479. Found: 300.9480.

**General procedure for the preparation of 2-arylamino-4,6-dichloro-1,3,5-triazines 11–19.** To a stirred, cooled solution (–15 °C) of 2,4,6-trichloro-1,3,5-triazine (1.0 mmol) in dry MeCN (10 mL), the corresponding substituted aniline (1.0 mmol) was added over a period of 5 min, followed by the addition of $Et_3N$ (1.3 mmol). The reaction mixture was stirred at –15 °C for 1 h. After the reaction was complete (monitored by TLC), the solvent was removed under reduced pressure, then $H_2O$ (10 mL) was added to give solid crude products, which were filtered off and subsequently purified by silica gel radial chromatography.

***N*-(2-Bromophenyl)-2-amino-4,6-dichloro-1,3,5-triazine (11)** [24]: Purification on $SiO_2$ (EtOAc-petroleum ether = 3/5) gave 170 mg (53%) of a brown solid. mp 155.5–157.0 °C. mp 158.5–159.0 °C.[24] $R_f$ 0.61 (EtOAc-petroleum ether = 3/5). IR (KBr): 3366, 1582, 1541, 1519, 1385, 1314, 1251, 1226, 1166, 1025, 870, 793, 760, 606 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 7.26–7.35 (m, 1H, -*Ph*), 7.42–7.52 (m, 2H, -*Ph*), 7.74 (dd, $J$ = 8.5, 1.0 Hz, 1H, -*Ph*), 10.96 (br s, 1H, -N*H*-).

***N*-(2-Ethylphenyl)-2-amino-4,6-dichloro-1,3,5-triazine (12)[24]:** Purification on $SiO_2$ (EtOAc-petroleum ether = 3/5) gave 225 mg (42%) of a white solid. mp 109.0–113.0 °C. mp 119.5–120.5 °C.[24] $R_f$ 0.57 (EtOAc-petroleum ether = 3/5). IR (KBr): 3237, 3109, 2968, 1596, 1577, 1546, 1512, 1391, 1317, 1225, 1169, 1018, 835, 797, 615 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 1.11 (t, $J$ = 7.5 Hz, 3H, -$CH_2C\underline{H}_3$), 2.56 (q, $J$ = 7.5 Hz, 2H, -$C\underline{H}_2CH_3$), 7.21–7.36 (m, 4H, -*Ph*), 10.69 (br s, 1H, -N*H*-).

***N*-(2-*Tert*-butylphenyl)-2-amino-4,6-dichloro-1,3,5-triazine(13):** Purificationon $SiO_2$(EtOAc-petroleum ether = 3/5) gave 345 mg (58%) of a white solid. mp 154.5–159.5 °C. $R_f$ 0.62 (EtOAc-petroleum ether = 3/5). IR (KBr): 3230, 3107, 2967, 1609, 1591, 1549, 1512, 1390, 1211, 1171, 1019, 845, 801, 756, 664 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 1.29 (s, 9H, -$C(C\underline{H}_3)_3$), 7.17 (dd, $J$ =, 7.5, 2.0 Hz, 1H, -*Ph*), 7.21–7.34 (m, 2H, -*Ph*), 7.48 (dd, $J$ = 8.0, 2.0 Hz, 1H, -*Ph*), 10.65 (br s, 1H, -N*H*-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 30.7, 34.8, 126.9, 127.2, 128.0, 130.6, 134.2, 146.3, 165.6, 168.8, 169.4. MS (ESI) *m/z*: 297 $(M+H)^+$. HRMS calcd for $C_{13}H_{15}N_4Cl_2$: 297.0674. Found: 297.0682. Anal. Calcd for $C_{13}H_{14}N_4Cl_2$: C, 52.54; H, 4.75; N, 18.85. Found: C, 52.41; H, 4.89; N, 18.93.

**Methyl 2-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzoate (14) [25] :** Purification on $SiO_2$ (EtOAc-petroleum ether = 3/5) gave 1.35 g (56%) of a white solid. mp 196.0–197.5 °C. mp 192.0–194.0 °C.[25] $R_f$ 0.59 (EtOAc-petroleum ether = 3/5). IR (KBr): 3140, 2958, 2338, 1686, 1619, 1566, 1508, 1458, 1428, 1388, 1316, 1247, 1168, 1090, 820, 795, 759, 699 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 3.78 (s, 3H, -$CO_2C\underline{H}_3$), 7.35–7.45 (m, 1H, -*Ph*), 7.63–7-75 (m, 2H, -*Ph*), 7.86–7.93 (m, 1H, -*Ph*), 11.19 (br s, 1H, -N*H*-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 52.8, 125.4, 126.3, 130.8, 133.5, 133.7, 136.2, 154.6, 164.6, 166.8, 167.0.

***N*-[2-(2-Hydroxyethyl)phenyl]-2-amino-4,6-dichloro-1,3,5-triazine (15):** Purification on $SiO_2$ (EtOAc-petroleum ether = 3/5) gave 165 mg (58%) of a white solid. mp 110.5–114.5 °C. $R_f$ 0.39 (EtOAc-petroleum ether = 3/5). IR (KBr): 3266, 2942, 2884, 1616, 1549, 1456, 1394, 1231, 1018, 825, 794, 761, 612 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 2.73 (t, $J$ = 7.0 Hz, 2H, -$C\underline{H}_2CH_2OH$), 3.58 (t, J = 7.0 Hz, 2H, -$CH_2C\underline{H}_2OH$), 4.90 (br s, 1H, -$CH_2CH_2O\underline{H}$), 7.20–7.40 (m, 4H, -*Ph*), 10.67 (br s, 1H, -N*H*-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 34.5, 61.3, 126.5, 126.6, 127.0, 130.3, 134.4, 135.2, 165.0, 168.8, 169.6. MS (ESI) *m/z*: 285 $(M+H)^+$. HRMS calcd for $C_{11}H_{11}N_4OCl_2$: 285.0310. Found: 285.0317. Anal. Calcd for $C_{11}H_{10}N_4OCl_2$: C, 46.34; H, 3.54; N, 19.65. Found: C, 46.82; H, 3.80; N, 19.70.

***N*-(4-Methoxybenzyl)-2-amino-4,6-dichloro-1,3,5-triazine (16):** Purification on $SiO_2$ (EtOAc-hexane = 3/5) gave 273 mg (96%) of a white solid. mp 113.0–115.0 °C. $R_f$ 0.52 (EtOAc-hexane = 3/5). IR (KBr): 3551, 3414, 3266, 1640, 1551, 1514, 1404, 1324, 1236, 1170, 1104, 1032, 848, 795 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 3.81 (s, 3H, -$OC\underline{H}_3$), 4.59 (d, $J$ = 6.0 Hz, 2H, -$NHC\underline{H}_2Ph$), 6.26 (br s, 1H, -$N\underline{H}CH_2$-), 6.88 (d, $J$ = 8.5 Hz, 2H, -*Ph*), 7.23 (d, $J$ = 8.5 Hz, 2H, -*Ph*). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 43.4, 55.0, 113.7, 128.7, 129.2, 158.4, 165.2, 168.5, 169.4. MS (ESI) *m/z*: 285 $(M+H)^+$. HRMS calcd for

$C_{11}H_{11}N_4OCl_2$: 285.0310. Found: 285.0315. Anal. Calcd for $C_{11}H_{10}N_4OCl_2$: C, 46.34; H, 3.54; N, 19.65. Found: C, 46.16; H, 3.61; N, 19.73.

***N*-(3,4-Dimethoxyphenethyl)-2-amino-4,6-dichloro-1,3,5-triazine (17):** Purification on $SiO_2$ (EtOAc-hexane = 3/5) gave 1.187 g (72%) of a yellowish solid. mp 121.5–122.0 °C. $R_f$ 0.32 (EtOAc-hexane = 3/5). IR (KBr): 3479, 3414, 3332, 2945, 1611, 1516, 1433, 1324, 1236, 1156, 1140, 1098, 1020, 838, 626 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 2.86 (t, $J$ = 7.0 Hz, 2H, -NHCH$_2$C$\underline{H}_2$Ph), 3.75 (q, J = 6.5 Hz, 2H, -NHC$\underline{H}_2$CH$_2$Ph), 3.87 (s, 3H, -OC$\underline{H}_3$), 3.88 (s, 3H, -OC$\underline{H}_3$), 5.91 (br s, 1H, -N$\underline{H}$CH$_2$CH$_2$Ph), 6.69–6.76 (m, 2H, -$\underline{Ph}$), 6.82 (d, $J$ = 8.0 Hz, 1H, -$\underline{Ph}$). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 33.6, 42.3, 55.3, 55.4, 111.8, 112.5, 120.5, 131.0, 147.3, 148.5, 165.1, 168.3, 169.3. MS (ESI) *m/z*: 329 $(M+H)^+$. HRMS calcd for $C_{13}H_{15}N_4O_2Cl_2$: 329.0572. Found: 329.0577. Anal. Calcd for $C_{13}H_{14}N_4O_2Cl_2$: C, 47.43; H, 4.29; N, 17.02. Found: C, 47.15; H, 4.41; N, 16.88.

**Isopropyl *N*-{4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzoil}-L-alaninate (18):** Purification on $SiO_2$ (EtOAc-hexane = 3/5) gave 67 mg (29%) of a white solid. mp 228.0–234.0 °C. $R_f$ 0.20 (EtOAc-hexane = 3/5). $[\alpha]_D^{20}$ +39.2 (*c* 0.39, DMSO). IR (KBr): 3418, 3273, 1732, 1651, 1608, 1565, 1499, 1381, 1216, 824, 794, 624 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 1.19 (t, $J$ = 7.0 Hz, 6H, -CH(C$\underline{H}_3$)$_2$). 1.39 (d, $J$ = 7.0 Hz, 3H, -C$\underline{H}_3$), 4.39 (m, 1H, -C$\underline{H}$(CH$_3$)$_2$), 4.91 (sim m, 1H, -NHC$\underline{H}$-), 7.71 (d, $J$ = 8.0 Hz, 2H, -$\underline{Ph}$), 7.91 (d, J = 8.5 Hz, 2H, -$\underline{Ph}$), 8.67 (br d, J = 6.5 Hz, 1H, -N$\underline{H}$CH-), 11.30 (br s, 1H, -N$\underline{H}$-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 16.6, 21.4, 21.4, 48.4, 67.6, 119.7, 120.5, 128.2, 129.8, 163.8, 165.5, 165.5, 172.1, 172.1. MS (ESI) *m/z*: 398 $(M+H)^+$. HRMS calcd for $C_{16}H_{18}N_5O_3Cl_2$: 398.0787. Found: 398.0805. Anal. Calcd for $C_{16}H_{17}N_5O_3Cl_2$: C, 48.25; H, 4.30; N, 17.59. Found: C, 48.32; H, 4.29; N, 17.37.

***N*-{2-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzoyl}-D-alanine (19):** Purification on $SiO_2$ (EtOAc-petroleum ether-AcOH = 10/50/1) gave 35 mg (10%) of a white solid. mp 165.0–167.0 °C. $R_f$ 0.22 (EtOAc-petroleum ether-AcOH = 10/50/1). IR (KBr): 3374, 3072, 2988, 1721, 1649, 1602, 1572, 1528, 1503, 1382, 1318, 1246, 1225, 1173, 873, 195, 612 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 1.38 (d, $J$ = 7.5 Hz, 3H, -C$\underline{H}_3$), 4.29–4.44 (m, 1H, -NHC$\underline{H}$-), 7.34 (t, $J$ = 7.5 Hz, 1H, -$\underline{Ph}$), 7.61 (t, $J$ = 7.5 Hz, 1H, -$\underline{Ph}$), 7.80 (d, $J$ = 7.5 Hz, 1H, -$\underline{Ph}$), 7.97 (d, $J$ = 8.0 Hz, 1H, -$\underline{Ph}$), 8.88 (br d, $J$ = 7.0 Hz, 1H, -N$\underline{H}$CH-), 11.51 (br s, 1H, -N$\underline{H}$-), 12.58 (br s, 1H, -CO$_2\underline{H}$). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 17.6, 49.1, 124.6, 124.8, 125.0, 129.2, 132.2, 137.7, 164.8, 168.2, 168.3, 174.6, 174.7. MS (ESI) *m/z*: 354 $(M-H)^-$. HRMS calcd for $C_{13}H_{10}N_5O_3Cl_2$: 354.0161. Found: 354.0167.

**General procedure for the preparation of 2-arylamino-4,6-dimethoxy-1,3,5-triazine and of 2-arylthio-4,6-dimethoxy-1,3,5-triazine 20–21.** To a stirred solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (3.0 mmol) in dry MeCN (15 mL), 2-aminobenzoic acid (or 2-thiobenzoic acid) (3.0

mmol) and $Et_3N$ (4.0 mmol) were added. The reaction mixture was stirred at room temperature for 2 h. After the reaction was complete (monitored by TLC), the solvent was removed under reduced pressure to obtain solid crude products.

**2-[(4,6-Dimethoxy-1,3,5-triazin-2-yl)amino]benzoic acid (20):** The title compound was obtained after the crude material was suspended in a mixture of $H_2O$ (25 mL) and EtOH (25 mL), filtered off and washed with the same mixture yielding an off-white solid, 306 mg (37%). mp 154.5–156.0 °C. $R_f$ 0.67 (MeOH). IR (KBr): 3318, 3022, 2965, 2556, 1674, 1607, 1562, 1494, 1459, 1377, 1350, 1260, 1132, 810, 750 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 3.93 (s, 6H, 2 × -OC*H*$_3$), 7.15 (ddd, $J$ = 8.0, 7.5, 1.0 Hz, 1H, -*Ph*), 7.63 (ddd, $J$ = 8.5, 7.5, 1.5 Hz, 1H, -*Ph*), 8.01 (dd, $J$ = 8.0, 1.5 Hz, 1H, -*Ph*), 8.62 (dd, $J$ = 8.5, 1.0 Hz, 1H, -*Ph*), 11.16 (br s, 1H, -N*H*-), 13.60 (br s, 1H, -$CO_2$*H*). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 54.6 (2C), 116.9, 120.6, 122.1, 131.1, 133.8, 140.5, 165.7, 169.4 (2C), 171.9. MS (EI) *m/z* (%): 276 ($M^+$, 15), 257 (9), 231 (100), 159 (16). HRMS calcd for $C_{12}H_{12}N_4O_4$: 276.0859. Found: 276.0860. Anal. Calcd for $C_{12}H_{12}N_4O_4$: C, 52.17; H, 4.38; N, 20.28. Found: C, 52.29; H, 4.43; N, 19.99.

**2-[(4,6-Dimethoxy-1,3,5-triazin-2-yl)thio]benzoic acid (21):** The title compound was obtained after the crude material was suspended in HCl (1 M, 15 mL), filtered off and washed with HCl (1 M, 5 mL) yielding a white solid, 533 mg (61%). mp 180.0–182.0 °C. $R_f$ 0.70 (MeOH). IR (KBr): 3016, 2968, 2667, 1679, 1561, 1532, 1347, 1298, 1192, 1011, 1042, 941, 808, 743 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 3.83 (s, 6H, 2 × -OC*H*$_3$), 7.53–7.65 (m, 2H, -*Ph*), 7.73–7.80 (m, 1H, -*Ph*), 7.86–7.93 (m, 1H, -*Ph*), 13.06 (br s, 1H, -$CO_2$*H*). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 54.9 (2C), 127.0, 129.6, 130.1, 131.5, 135.6, 136.6, 167.3, 170.6 (2C), 184.3. MS (EI) *m/z* (%): 293 ($M^+$, 4), 276 (1), 248 (100), 176 (32). HRMS calcd for $C_{12}H_{11}N_3O_4S$: 293.0470. Found: 293.0480. Anal. Calcd for $C_{12}H_{11}N_3O_4S$: C, 49.14; H, 3.78; N, 14.33. Found: C, 48.83; H, 3.73; N, 14.12.

**Procedure for the preparation of methyl 2-[(4,6-dimethoxy-1,3,5-triazin-2-yl)oxy]benzoate 22.** [26] To a stirred solution of methyl 2-hydroxybenzoate (182 mg, 1.2 mmol) in dry MeCN (5 mL), potassium *tert*-butoxide (123 mg, 1.1 mmol) was added. The reaction mixture was stirred at room temperature for 5 min and then 2-chloro-4,6-dimethoxy-1,3,5-triazine (176 mg, 1.0 mmol) was added. The mixture was stirred for 2 hours at room temperature and then diluted with $H_2O$ (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (2 × 20 mL) and dried over $Na_2SO_4$. The solvent was evaporated and the residue was purified by silica gel radial chromatography (EtOAc-petroleum ether = 1/1) to yield the pure product, 227 mg (78 %) as a white solid. mp 69.0–73.5 °C. mp 64.0–68.0 °C.[26] $R_f$ 0.11 (EtOAc-petroleum ether = 1/1). IR (KBr): 3430, 3029, 3012, 2953, 2613, 1722, 1584, 1561, 1472, 1363, 1271, 1214, 1123, 1097, 1082, 814, 738, 664, 449 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 3.76 (s, 3H, -$CO_2$C*H*$_3$), 3.98 (s, 6H, 2 × -OC*H*$_3$), 7.22 (dd, $J$ = 8.0, 1.0 Hz,

1H, -*Ph*), 7.35 (ddd, *J* = 7.5, 7.5, 1.0 Hz, 1H, -*Ph*), 7.59 (ddd, *J* = 8.0, 7.5, 1.5 Hz, 1H, -*Ph*), 8.03 (dd, *J* = 8.0, 1.5 Hz, 1H, -*Ph*). MS (ESI) *m/z*: 292 $(M+H)^+$. HRMS calcd for $C_{13}H_{14}N_3O_5$: 292.0933. Found: 292.0943.

**Procedure for the preparation of dibenzyl *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)-D-glutamate 23.** To a stirred solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (176.0 mg, 1.0 mmol) in dry MeCN (10 mL), D-glutamic acid dibenzyl ester *p*-toluenesulfonate salt (520 mg, 1.0 mmol) and $K_2CO_3$ (414 mg, 3.0 mmol) were added. The mixture was allowed to react at ambient temperature for 2 days. After the reaction was complete (monitored by TLC), $H_2O$ (10 mL) was added and the aqueous layer extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (2 × 20 mL) and dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel radial chromatography (EtOAc-petroleum ether = 3/5) to yield the pure product, 307 mg (65%) as a yellowish oil. $R_f$ 0.32 (EtOAc-petroleum ether =1/1). $[\alpha]_D^{20}$ –3.6 (*c* 1.06, $CH_2Cl_2$). IR (NaCl-plates): 3354, 3257, 3150, 2955, 1738, 1577, 1550, 1482, 1466, 1378, 1362, 1192, 1165, 819, 698 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.03–2.57 (m, 4H, 2 × -$CO_2CH_2$Ph), 3.85 (s, 3H, -$OCH_3$), 3.91 (s, 3H, -$OCH_3$), 4.78–4.88 (sim m, 1H, -NHC*H*-), 5.07 and 5.11 (AB, $J_{AB}$ = 12.5 Hz, 2H, -$CO_2CH_2$Ph-), 5.15 and 5.19 (AB, $J_{AB}$ = 12.0 Hz, 2H, -$CO_2CH_2$Ph-), 6.10 (br d, *J* = 8.0 Hz, 1H, -N*H*CH-), 7.27–7.41 (m, 10H, 2 × -*Ph*). $^{13}C$ NMR (75.5 MHz, $CDCl_3$): $\delta$ 27.3, 30.1, 53.2, 54.7 (2C), 66.5, 67.3, 128.2, 128.3, 128.3, 128.5, 128.5, 128.6, 135.1, 135.6, 167.8, 171.5, 172.2, 172.2, 172.4. MS (ESI) *m/z*: 467 $(M+H)^+$. HRMS calcd for $C_{24}H_{27}N_4O_6$: 467.1931. Found: 467.1950. Anal. Calcd for $C_{24}H_{26}N_4O_6$: C, 61.79; H, 5.62; N, 12.01. Found: C, 61.78; H, 5.85; N, 11.82.

**Procedure for the preparation of *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)-D-glutamic acid 24.** To a solution of compound **23** (416 mg, 0.9 mmol) in MeOH (20 mL), 10% Pd-C (105 mg) was added, and the mixture was hydrogenated for 7 h at room temperature and 60 psi. The suspension was then filtered through a pad of celite and washed with MeOH (50 mL). The solvent was removed under reduced pressure. The residue was treated with $CH_2Cl_2$ (5 mL), filtered off and washed with $CH_2Cl_2$ (10 mL) yielding the pure product, 230 mg (90%) as a white solid. mp 151.0–154.0 °C. $R_f$ 0.59 (MeOH). $[\alpha]_D^{20}$ +7.9 (*c* 0.29, DMSO). IR (KBr): 3246, 3154, 2961, 1561, 1737, 1704, 1589, 1554, 1479, 1385, 1348, 1192, 1108, 813 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 1.82–2.15 (m, 2H, -$CH_2CH_2CO_2H$), 2.34 (t, *J* = 7.5 Hz, 2H, -$CH_2CH_2CO_2H$ ), 3.80 (s, 3H, -$OCH_3$), 3.84 (s, 3H, -$OCH_3$), 4.29–4.41 (m, 1H, -NHC*H*-), 8.08 (br d, *J* = 7.5 Hz, 1H, -N*H*-), 12.09 (br s, 1H, -$CO_2H$). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 26.9, 31.2, 54.2, 55.0, 55.1, 168.6, 172.6, 172.6, 174.3, 174.6. MS (ESI) *m/z*: 285 $(M-H)^-$. HRMS calcd for $C_{10}H_{13}N_4O_6$: 285.0835. Found: 285.0840.

**Procedure for the preparation of (*S*)-isopropyl 2-(4-nitrobenzamido)propanoate 26a.** To a stirred, cooled solution (–10 °C) of 4-nitrobenzoyl chloride (1.00 g, 5.39 mmol) in dry MeCN (25 mL), (*S*)-isopropyl 2-aminopropanoate hydrochloride (0.903 g, 5.39 mmol) was added, followed by the slow addition of $Et_3N$ (1.361 g, 13.48 mmol). The reaction mixture was stirred at –10 °C for 1 h and then allowed to reach 25 °C. The stirring was continued at this temperature for an additional 14 h. After the reaction was complete (monitored by TLC), the solvent was removed under reduced pressure, $H_2O$ (50 mL) was added and the resulting mixture extracted with $CH_2Cl_2$ (3 × 25 mL). The combined organic phases were washed with brine (2 × 20 mL) and dried over $Na_2SO_4$. The solvent was then removed under reduced pressure to yield the pure product, 1.33 g (88%) as a white solid. mp 154.0–159.0 °C. $R_f$ 0.30 (EtOAc-petroleum ether = 3/5). $[\alpha]_D^{20}$ +36.7 (*c* 2.00, $CH_2Cl_2$) IR (KBr): 3315, 2986, 1746, 1645, 1603, 1543, 1349, 12251179, 1018 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): 1.17 (d, *J* = 7.5 Hz, 3H, *i*-*Pr*), 1.20 (d, *J* = 7.5 Hz, 3H, *i*-*Pr*), 1.37 (d, *J* = 8.0 Hz, 3H, -(NH)CHC*H*$_3$), 4.51 (pent, *J* = 7.0 Hz, 1H, -NHC*H*-), 4.91 (sept, *J* = 7.5 Hz, 1H, -C*H*-*i*Pr), 5.62 (br s, 2H, -N*H*$_2$), 8.13 (AA'BB', *J* = 8.5 Hz, 2H, -*Ph*), 8.35 (AA'BB', *J* = 8.5 Hz, 2H, -*Ph*), 9.14 (br d, J = 6.5 Hz, 1H, -N*H*-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): $\delta$ 16.8, 21.6, 53.0, 67.9, 123.5, 128.9, 139.3, 149.1, 164.7, 172.3. MS (ESI) *m/z*: 281 $(M+H)^+$. HRMS calcd for $C_{13}H_{17}N_2O_5$: 281.1137. Found: 281.1140.

**Procedure for the preparation of (*S*)-isopropyl 2-(4-aminobenzamido)propanoate 27a.** To a solution of compound **26a** (1.00 g, 3.57 mmol) in MeOH (20 mL), 10% Pd-C (200 mg) was added, and the mixture was hydrogenated for 24 h at room temperature and 1 bar. The solution was then filtered through a pad of celite and washed with MeOH (50 mL). The solvent was removed under reduced pressure, yielding the pure product, 849 mg (95%) as a white solid. mp 148.0–148.5 °C. $R_f$ 0.15 (EtOAc-petroleum ether = 3/5). $[\alpha]_D^{20}$ +40.6 (*c* 2.54, $CH_2Cl_2$). IR (KBr): 3449, 3352, 2986, 1741, 1634, 1513, 1501, 1299, 1215, 1182, 1107. $^1H$ NMR (300 MHz, DMSO–$d_6$): 1.17 (d, *J* = 7.5 Hz, 3H, *i*-*Pr*), 1.20 (d, *J* = 7.5 Hz, 3H, *i*-*Pr*), 1.37 (d, *J* = 8.0 Hz, 3H, -(NH)CHC*H*$_3$), 4.38 (pent, *J* = 7.0 Hz, 1H, -NHC*H*-), 4.91 (sept, *J* = 7.5 Hz, 1H, -C*H*-*i*Pr), 5.62 (br s, 2H, -N*H*$_2$), 6.57 (AA'BB', *J* = 8.5 Hz, 2H, -*Ph*), 7.64 (AA'BB', *J* = 8.5 Hz, 2H, -*Ph*) 8.21 (br d, *J* = 6.5 Hz, 1H, -N*H*CH-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$): 16.8, 21.47, 21.53, 48.3, 67.6, 112.5, 120.5, 129.1, 151.8, 166.4, 172.7. MS (ESI) *m/z*: 250 $(M+H)^+$. HRMS calcd for $C_{13}H_{19}N_2O_3$: 251.1396. Found: 251.1385. Anal. Calcd for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.52; N, 11.19. Found: C, 62.38; H, 7.48; N, 11.30.

**Procedure for the preparation of benzyl *N*-[2-(benzylamino)benzoyl]-D-alaninate 26b.** To a stirred ice-cooled solution of the 2-(benzylamino)benzoic acid **25b** (725 mg, 3.2 mmol) and the *D*-alanine benzyl ester *p*-toluenesulfonate salt (1.23 g, 3.5 mmol) in DMF (8 mL), HOBt×$H_2O$ (582 mg, 3.8 mmol) and

$Et_3N$ (960 mg, 9.5 mmol) were slowly added, followed by the addition of EDC (790 mg, 4.1 mmol) after 5 min. The reaction mixture was stirred for 1 h at 0 °C and then at room temperature for 24 h. After the reaction was complete EtOAc (50 mL) was added and then the reaction mixture was washed consecutively with HCl (1 M, 3 × 20 mL), satd aq $NaHCO_3$ (3 × 20 mL), brine (2 × 20 mL) and then dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel radial chromatography (EtOAc-petroleum ether = 1/7) to yield the pure product, 980 mg (79%) as a yellowish oil. $R_f$ 0.34 (EtOAc-petroleum ether = 1/3). $[\alpha]_D^{20}$ –19.1 (*c* 1.94, $CH_2Cl_2$). IR (NaCl-plates): 3354, 3030, 2937, 1740, 1638, 1580, 1518, 1452, 1200, 1169, 747, 697 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.51 (d, $J$ = 7.0 Hz, 3H, -CH(C*H*$_3$)$CO_2$-), 4.39 (d, $J$ = 4.5 Hz, 2H, -NHC*H*$_2$Ph), 4.78 (m, 1H, -NHC*H*-), 5.18 and 5.24 (AB, $J_{AB}$ = 12.0 Hz, 2H, -$CO_2$C*H*$_2$Ph), 6.53-6.70 (m, 3H, -*Ph*), 7.18-7.26 (m, 2H, -N*H*-, and -*Ph*), 7.27-7.39 (m, 9H, -*Ph*), 7.41 (dd, $J$ = 8.0, 1.5 Hz, 1H, -*Ph*), 8.03 (br s, 1H, PhN*H*$CH_2$Ph). $^{13}C$ NMR (75.5 MHz, $CDCl_3$): $\delta$ 18.6, 47.1, 48.3, 67.2, 112.2, 114.5, 115.0, 127.0, 127.1, 127.5, 128.1, 128.4, 128.6, 128.6, 133.1, 135.4, 139.1, 149.6, 169.2, 173.1. MS (ESI) *m/z*: 389 $(M+H)^+$. HRMS calcd for $C_{24}H_{25}N_2O_3$: 389.1865. Found: 389.1850.

**Procedure for the preparation of *N*-(2-aminobenzoyl)-D-alanine 27b.** To a solution of the compound **26b** (980 mg, 2.5 mmol) in MeOH (20 mL), 10% Pd-C (204 mg) was added, and the mixture was hydrogenated for 6 h at room temperature and 60 psi. The suspension was then filtered through a pad of celite and washed with MeOH (50 mL). The solvent was removed under reduced pressure. The residue was treated with $Et_2O$ (10 mL), filtered off and washed with $Et_2O$ (5 mL) yielding the pure product, 237 mg (57%) as a white solid. mp 129.5–132.0 °C. $R_f$ 0.54 ($CH_2Cl_2$-MeOH-AcOH = 30/10/1). $[\alpha]_D^{20}$ –30.0 (*c* 0.51, DMSO). IR (KBr): 3466, 3395, 3363, 3096, 2988, 1717, 1651, 1613, 1583, 1522, 1526, 1212, 1182, 845, 756, 528 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$): $\delta$ 1.35 (d, $J$ = 7.5 Hz, 3H, -C*H*$_3$), 4.22–4.36 (m, 1H, -NHC*H*-), 6.31 (br s, 2H, PhN*H*$_2$), 6.46–6.55 (m, 1H, -*Ph*), 6.68 (dd, $J$ = 8.0, 1.0 Hz, 1H, -*Ph*), 7.08–7.17 (m, 1H, -*Ph*), 7.53 (dd, $J$ = 8.0, 1.5 Hz, 1H, -*Ph*), 8.23 (br d, $J$ = 7.0 Hz, 1H, -CON*H*-). MS (ESI) *m/z*: 209 $(M+H)^+$. HRMS calcd for $C_{10}H_{13}N_2O_3$: 209.0926. Found: 209.0930.

**General procedure for the preparation of amido derivatives 28–32.** To a stirred, ice-cooled solution of the compound **20** (or **21**) (1.0 mmol) and a corresponding C-protected amino acid derivative (1.1 mmol) in DMF (3 mL), HOBt×$H_2O$ (1.2 mmol) and $Et_3N$ (3.2 mmol) were slowly added, followed by the addition of EDC (1.3 mmol) after 5 min. The reaction mixture was stirred for 1 h at 0 °C and then at room temperature for 24 h. After the reaction was complete, EtOAc (20 mL) was added and then the reaction mixture was washed consecutively with HCl (1 M, 3 × 10 mL), satd aq $NaHCO_3$ (3 × 10 mL), brine (2 × 10 mL) and then dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the residue

was purified by silica gel radial chromatography.

**Benzyl *N*-{2-[(4,6-dimethoxy-1,3,5-triazin-2-yl)thio]benzoyl}-L-alaninate (28):** Purification on $SiO_2$ (EtOAc-petroleum ether = 3/5) gave 194 mg (43%) of a colourless oil. $R_f$ 0.31 (EtOAc-petroleum ether = 1/1). $[\alpha]_D^{20}$ +11.7 (*c* 1.05, $CH_2Cl_2$). IR (NaCl-plates): 3585, 3323, 2941, 1740, 1661, 1541, 1456, 1352, 1296, 1045, 815, 752, 698 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.34 (d, $J$ = 7.0 Hz, 3H, -C$\underline{H}_3$), 3.90 (s, 6H, 2 × -OC$\underline{H}_3$), 4.73 (dq, $J$ = 7.0, 7.0 Hz, 1H, -NHC$\underline{H}$-), 5.09 and 5.13 (AB, $J_{AB}$ = 12.5 Hz, 2H, -$CO_2C\underline{H}_2$Ph), 7.22 (br d, $J$ = 7.0 Hz, 1H, -CON$\underline{H}$CH-), 7.27–7.41 (m, 5H, -$\underline{Ph}$), 7.42–7.57 (m, 2H, -$\underline{Ph}$), 7.63 (dd, $J$ = 7.5, 1.0 Hz, 1H, -$\underline{Ph}$), 7.68 (dd, $J$ = 7.5, 1.5 Hz, 1H, -$\underline{Ph}$). $^{13}C$ NMR (75.5 MHz, $CDCl_3$): $\delta$ 18.3, 48.6, 55.3 (2C), 67.1, 124.2, 128.1, 128.4, 128.6, 129.2, 130.7, 135.3 (2C), 137.3, 142.0, 167.3, 171.4 (2C), 172.3, 184.8. MS (EI) *m/z* (%): 454 ($M^+$, 2), 320 (1), 276 (88), 248 (100), 159 (23), 91 (40). HRMS calcd for $C_{22}H_{22}N_4O_5S$: 454.1311. Found: 454.1320. Anal. Calcd for $C_{22}H_{22}N_4O_5S$: C, 58.14; H, 4.88; N, 12.33. Found: C, 57.87; H, 5.05; N, 12.34.

**Dibenzyl *N*-{2-[(4,6-dimethoxy-1,3,5-triazin-2-yl)thio]benzoyl}-D-glutamate (29):** Purification on $SiO_2$ (EtOAc-petroleum ether = 3/5) gave 288 mg (48%) of a colourless oil. $R_f$ 0.36 (EtOAc-petroleum ether = 1/1). $[\alpha]_D^{20}$ –4.5 (*c* 1.45, $CH_2Cl_2$). IR (NaCl-plates): 3323, 2947, 1738, 1665, 1542, 1504, 1456, 1353, 1296, 1165, 1106, 1046, 815, 750, 698 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.88–2.50 (m, 4H, -($C\underline{H}_2$)$_2$-), 3.87 (s, 6H, 2 × -OC$\underline{H}_3$), 4.80 (sim m, 1H, -NHC$\underline{H}$-), 5.01–5.13 (m, 4H, -$CO_2C\underline{H}_2$Ph), 7.27–7.38 (m, 11H, -N$\underline{H}$-, and 2 × -$\underline{Ph}$), 7.42–7.57 (m, 2H, -$\underline{Ph}$), 7.59–7.68 (m, 2H, -$\underline{Ph}$). $^{13}C$ NMR (75.5 MHz, $CDCl_3$): $\delta$ 27.6, 29.9, 52.0, 55.3 (2C), 66.4, 67.3, 124.1, 128.2, 128.2, 128.3, 128.5, 128.5, 128.6, 129.3, 130.7, 130,8, 135.1, 135.8, 137.5, 142.0, 167.8, 171.2, 171.4 (2C), 172.2, 184.8. MS (ESI) *m/z*: 603 $(M+H)^+$. HRMS calcd for $C_{31}H_{31}N_4O_7S$: 603.1913. Found: 603.1914. Anal. Calcd for $C_{31}H_{30}N_4O_7S$: C, 61.78; H, 5.02; N, 9.30. Found: C, 61.96; H, 5.28; N, 9.53.

**Benzyl $N^6$-[(benzyloxy)carbonyl]-$N^2$-{2-[(4,6-dimethoxy-1,3,5-triazin-2-yl)thio]benzoyl}-L-lysinate (30):** Purification on $SiO_2$ (EtOAc-petroleum ether = 3/5) gave 157 mg (49%) of a colourless oil. $R_f$ 0.23 (EtOAc-petroleum ether = 1/1). $[\alpha]_D^{20}$ +2.2 (*c* 1.01, $CH_2Cl_2$). IR (NaCl-plates): 3320, 2942, 1719, 1660, 1542, 1504, 1547, 1353, 1597, 1106, 1046, 815, 735, 698 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.08–1.51 (m, 4H, -$CH_2C\underline{H}_2C\underline{H}_2CH_2$NH-), 1.52–1.92 (m, 2H, -$C\underline{H}_2CH_2CH_2CH_2$NH-), 2.99–3.19 (m, 2H, -$CH_2CH_2CH_2C\underline{H}_2$NH-), 3.88 (s, 6H, 2 × -OC$\underline{H}_3$), 4.74 (sim m, 1H, -CONHC$\underline{H}$-), 4.95 (br s, 1H, -N$\underline{H}CO_2CH_2$Ph), 5.03–5.16 (m, 4H, -NHCO$_2$C$\underline{H}_2$Ph), 7.19 (br d, $J$ = 7.5 Hz, 1H, -N$\underline{H}$CO-), 7.27–7.41 (m, 10H, 2 × -$\underline{Ph}$), 7.41–7.55 (m, 2H, -$\underline{Ph}$), 7.62 (dd, $J$ = 7.5, 2.0 Hz, 1H, -$\underline{Ph}$), 7.67 (dd, $J$ = 7.0, 2.0 Hz, 1H, -$\underline{Ph}$). $^{13}C$ NMR (75.5 MHz, $CDCl_3$): $\delta$ 22.2, 29.3, 32.2, 40.7, 52.5, 55.4 (2C), 66.5, 67.1, 124.0, 128.0, 128.0, 128.4, 128.5, 128.5, 128.6, 129.4, 130.7, 130.8, 135.2, 136.7, 137.4, 142.1, 156.4, 167.7, 171.4 (2C), 171.7, 184.9. MS (ESI) *m/z*: 646 $(M+H)^+$. HRMS calcd for $C_{33}H_{36}N_5O_7S$: 646.2335. Found: 646.2354. Anal. Calcd for $C_{33}H_{35}N_5O_7S$: C, 61.38; H, 5.46; N, 10.85. Found: C, 61.18; H, 5.63; N, 10.83.

**Benzyl *N*-(4-methoxy-6-oxo-6*H*-[1,3,5]triazino[2,1-b]quinazolin-2-yl)-L-alaninate (31):** Purification on $SiO_2$ (EtOAc-petroleum ether = 1/3) gave 60 mg (15%) of a yellowish oil. Rf 0.20 (EtOAc-petroleum ether = 1/1). $[\alpha]_D^{20}$ +85.2 (*c* 1.26, $CH_2Cl_2$). IR (NaCl-plates): 3160, 2982, 2952, 1737, 1691, 1639, 1579, 1548, 1470, 1380, 1326, 1194, 1134, 777, 700, 618 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.62 (d, $J$ = 7.0 Hz, 3H, -C*H*$_3$), 4.05 (s, 3H, -OC*H*$_3$), 4.88 (dq, $J$ = 7.0, 7.0 Hz, 1H, -NHC*H*-), 5.25 (s, 2H, -$CO_2$C*H*$_2$Ph), 7.30–7.42 (m, 6H, -*Ph*), 7.64 (d, $J$ = 8.0 Hz, 1H, -*Ph*), 7.76 (ddd, $J$ = 8.5, 7.0, 1.5 Hz, 1H, -*Ph*), 8.21 (dd, $J$ = 8.0, 1.0 Hz, 1H, -*Ph*), 11.36 (br d, $J$ = 6.5 Hz, 1H, -N*H*-). $^{13}C$ NMR (75.5 MHz, $CDCl_3$): $\delta$ 17.6, 50.8, 55.5, 67.4, 117.5, 125.1, 126.6, 127.5, 128.2, 128.4, 128.6, 135.1, 136.5, 148.4, 148.8, 156.0, 164.6, 164.9, 171.3. MS (ESI) *m/z*: 406 $(M+H)^+$. HRMS calcd for $C_{21}H_{20}N_5O_4$: 406.1515. Found: 406.1520.

**Benzyl (2*S*)-2-({2-[(4-{[(1*S*)-2-(benzyloxy)-1-metil-2-oxoethyl]amino}-6-methoxy-1,3,5-triazin-2-yl)-amino]benzoyl}amino)propanoate (32):** Purification on $SiO_2$ (EtOAc-petroleum ether = 1/3) gave 103 mg (18%) of a yellowish oil. Rf 0.66 (EtOAc-petroleum ether = 1/1). $[\alpha]_D^{20}$ + 31.6 (*c* 0.50, $CH_2Cl_2$). IR (NaCl-plates): 3064, 3034, 1986, 1742, 1669, 1633, 1589, 1545, 1450, 1306, 1157, 750, 697 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.49 (d, $J$ = 7.0 Hz, 3H, -C*H*$_3$), 1.59 (d, $J$ = 7.0 Hz, 3H, -C*H*$_3$), 3.63 (s, 3H, -OC*H*$_3$), 4.39 (dq, $J$ = 7.0, 7.0 Hz, 1H, -NHC*H*-), 4.65–4.77 (m, 1H, -CONHC*H*-), 5.20 (s, 2H, -$CO_2$C*H*$_2$Ph), 5.25 (s, 2H, $CO_2$C*H*$_2$Ph), 7.27–7.41 (m, 12H, -Ph), 7.58 (ddd, $J$ = 8.5, 7.0, 1.5 Hz, 1H, -*Ph*), 8.18 (dd, $J$ = 8.0, 1.5 Hz, 1H, -*Ph*), 8.85 (br s, 1H, -ArN*H*Ph), 11.24 (br d, $J$ = 6.0 Hz, 1H, -N*H*-), 11.41 (br d, $J$ = 6.0 Hz, 1H, -N*H*-). $^{13}C$ NMR (75.5 MHz, $CDCl_3$): $\delta$ 18.2, 19.0, 50.2, 51.0, 54.2, 66.7, 67.5, 119.1, 123.5, 125.3, 126.5, 128.2 (2C), 128.3, 128.4, 128.5, 128.6, 134.1, 135.2, 135.7, 149.4, 153.1, 158.7, 160.2, 163.4, 172.6, 173.2. MS (ESI) *m/z*: 585 $(M+H)^+$. HRMS calcd for $C_{31}H_{33}N_6O_6$: 585.2462. Found: 585.2470.

**General procedure for the preparation of 2,6-diamino-4-chloro-1,3,5-triazines 33–36.** To a stirred solution of the compound **15** (1.0 mmol) in dry MeCN (10 mL), the corresponding amino derivative (1.1 mmol) was added, followed by the addition of $Et_3N$ (1.0 mmol) after 5 min. The reaction mixture was stirred at room temperature for 3 h. After the reaction was complete (monitored by TLC), the solvent was removed under reduced pressure to obtain crude products.

***N*$^2$-[2-(2-Hydroxyethyl)phenyl]-2-amino-4-chloro-6-((2-hydroxyethyl)(methyl)amino)-1,3,5-triazine (33):** The title compound was obtained after the crude material was suspended in $H_2O$ (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine (2 × 20 mL), dried over $Na_2SO_4$ and subsequently the solvent was removed under reduced pressure. The oily residue was treated with a mixture of hexane (8 mL) and EtOAc (3 mL) and left to stand at room temperature for 1 h. The white precipitate formed was filtered off and washed with hexane (5 mL) to yield 180 mg (78%) of a white solid. mp 144.5–145.0 °C. $R_f$ 0.35 (EtOAC). IR (KBr): 3403, 3277, 2901, 1606, 1578, 1528,

1455, 1412, 1392, 1239, 1205, 1049, 988, 795, 763, 615 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$, 80 °C): $\delta$ 2.78 (t, $J$ = 6.5 Hz,, 2H, -C$\underline{H_2}$$CH_2OH$), 3.06 (s, 3 H, -N(C$\underline{H_3}$)-), 3.50–3.62 (m, 4H, -N($CH_3$)$CH_2$C$\underline{H_2}$OH and -$CH_2$C$\underline{H_2}$OH), 3.66 (t, $J$ = 6.5 Hz, 2H, -N($CH_3$)C$\underline{H_2}$$CH_2OH$), 4.45 (br s, 1H, -O$\underline{H}$), 4.78 (br s, 1H, -O$\underline{H}$), 7.12 (dt, $J$ = 7.5, 1.5 Hz, 1H, -$\underline{Ph}$), 7.21 (dt, $J$ = 7.5, 1.5 Hz, 1H, -$\underline{Ph}$), 7.26 (dd, $J$ = 7.5, 1.5 Hz, 1H, -$\underline{Ph}$), 7.52 (d, $J$ = 7.5 Hz, 1H, -$\underline{Ph}$), 9.20 (br s, 1H, -N$\underline{H}$-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$, 80 °C): $\delta$ 34.4, 34.8, 50.3, 58.0, 61.4, 124.6, 125.1, 125.5, 129.4, 133.9, 136.0, 163.9, 164.4, 167.9. MS (ESI) *m/z*: 324 $(M+H)^+$. HRMS calcd for $C_{14}H_{19}N_5O_2Cl$: 324.1227. Found: 324.1233. Anal. Calcd for $C_{14}H_{18}N_5O_2Cl$: C, 51.93; H, 5.60; N, 21.63. Found: C, 51.89; H, 5.69; N, 21.34.

***N*$^2$-[2-(2-Hydroxyethyl)phenyl]-2-amino-4-chloro-6-benzylamino-1,3,5-triazine (34):** The title compound was obtained after the crude material was suspended in $H_2O$ (10 mL), filtered off and washed with $H_2O$ (5 mL) obtaining a white solid, which was subsequently purified by silica gel radial chromatography (EtOAc-petroleum ether = 1/3). A white solid, 145 mg (41%). mp 159.0–160.0 °C. $R_f$ 0.24 (EtOAc-petroleum ether = 3/5). IR (KBr): 3258, 3123, 2937, 2874, 1612, 1577, 1531, 1452, 1389, 1354, 1246, 1103, 1047, 990, 800, 756, 697, 610 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$, mixture of tautomers): $\delta$ 2.87 (t, $J$ = 5.5 Hz, 2H, -C$\underline{H_2}$$CH_2OH$), 3.90–4.00 (m, 2H, -$CH_2$C$\underline{H_2}$OH), 4.52 and 4.61 (d, $J$ = 5.5 Hz, 2H, -NHC$\underline{H_2}$Ph), resonance for –OH missing, 5.53 and 5.59 (br s, 1H, -N$\underline{H}$-), 7.07–7.38 (m, 8H, 2 × -$\underline{Ph}$), 7.56–7.72 (m, 1H, -$\underline{Ph}$), 8.46 and 8.63 (br s, 1H, -N$\underline{H}$-). $^{13}C$ NMR (75.5 MHz, $CDCl_3$, mixture of tautomers): $\delta$ 29.7, 45.0, 64.5, 125.0 in 125.1 (1C), 127.4, 127.5, 127.7 in 127.8 (1C), 128.6, 128.7, 130.3 in 130.4 (1C), 132.8, 136.3 in 136.4 (1C), 137.9, 164.5, 165.9, 166.0. MS (ESI) *m/z*: 356 $(M+H)^+$. HRMS calcd for $C_{18}H_{19}N_5OCl$: 356.1278. Found: 356.1292. Anal. Calcd for $C_{18}H_{18}N_5OCl$: C, 60.76; H, 5.10; N, 19.68. Found: C, 60.89; H, 5.00; N, 19.72.

***N*$^2$-[2-(2-Hydroxyethyl)phenyl]-2-amino-4-chloro-6-(4-methoxybenzylamino)-1,3,5-triazine (35):** The title compound was obtained after the crude material was suspended in $H_2O$ (10 mL), filtered off and washed with $H_2O$ (5 mL), obtaining a white solid, which was then purified by silica gel radial chromatography (EtOAc-hexane = 3/5). A white solid, 249 mg (64%). mp 138.5–139.0 °C. $R_f$ 0.23 (EtOAc-petroleum ether = 3/5). IR (KBr): 3546, 3413, 3260, 2934, 1612, 1577, 1537, 1513, 1456, 1395, 1275, 1248, 1175, 1032, 987, 799, 752, 608, 595 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$, 80 °C): $\delta$ 2.74 (t, $J$ = 6.5 Hz, 2H, -C$\underline{H_2}$$CH_2OH$), 3.58–3.68 (m, 2H, -$CH_2$C$\underline{H_2}$OH), 3.72 (s, 3H, -OC$\underline{H_3}$), 4.28 (d, $J$ = 6.5 Hz, 2H, -NHC$\underline{H_2}$Ph), 4.72 (t, $J$ = 4.5 Hz, 1H, -$CH_2CH_2$O$\underline{H}$), 6.83 (d, $J$ = 7.5 Hz, 2H, -$\underline{Ph}$), 7.02–7.30 (m, 5H, -$\underline{Ph}$), 7.41 (d, $J$ = 7.5 Hz, 1H, -$\underline{Ph}$), 8.11 (br s, 1H, -N$\underline{H}$$CH_2$-), 8.22 (br s, 1H, -N$\underline{H}$-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$, 80 °C): $\delta$ 34.4, 42.8, 54.7, 61.4, 113.4, 124.9, 125.6, 125.7, 128.1, 128.3, 129.6, 130.6, 134.3, 136.0, 158.0, 164.4, 165.3. MS (ESI) *m/z*: 408 $(M+Na)^+$. HRMS calcd for $C_{19}H_{20}N_5O_2ClNa$: 408.1203. Found: 408.1183. Anal. Calcd for $C_{19}H_{20}N_5O_2Cl$: C, 59.14; H, 5.22; N, 18.15. Found: C, 59.30; H, 5.03; N, 17.91.

***N*-[2-(2-Hydroxyethyl)phenyl]-2-amino-4-chloro-6-morpholino-1,3,5-triazine (36):** The title compound was obtained after the crude material was suspended in a mixture of $H_2O$ (10 mL) and EtOH (4 mL), filtered off and washed with the same mixture, obtaining an off-white solid, which was subsequently purified by silica gel radial chromatography (EtOAc-petroleum ether = 3/5). A white solid, 126 mg (38%). mp 171.5–176.0 °C. $R_f$ 0.19 (EtOAc-petroleum ether = 3/5). IR (KBr): 3329, 2961, 1895, 1605, 1574, 1536, 1446, 1406, 1285, 1243, 1117, 798, 757, 537 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.89 (t, $J$ = 5.5 Hz, 2H, -C*H*$_2$CH$_2$OH), 3.62–3.87 (m, 8H, morpholino-C*H*$_2$), 3.92–4.03 (m, 2H, -CH$_2$C*H*$_2$OH), resonance for –OH missing, 7.09–7.17 (m, 1H, -*Ph*), 7.18–7.29 (m, 2H, -*Ph*), 7.74 (dd, $J$ = 8.0, 1.0 Hz, 1H, -*Ph*), 8.67 (br s, 1H, -N*H*-). $^{13}C$ NMR (75.5 MHz, $CDCl_3$): $\delta$ 34.8, 43.9, 64.5, 66.6, 124.6, 125.2, 126.8, 130.4, 132.9, 136.7, 164.2, 164.5, 164.6. MS (ESI) *m/z*: 336 $(M+H)^+$. HRMS calcd for $C_{15}H_{19}N_5O_2Cl$: 336.1227. Found: 336.1235.

**General procedure for the preparation of 2,6-diamino-4-chloro-1,3,5-triazines 37–38.** To a stirred solution of the compound **15** (1.0 mmol) in acetone (8 mL), the corresponding amino derivative (1.1 mmol) and $K_2CO_3$ (3.0 mmol) were added. The reaction mixture was refluxed for 24 h at 50 °C. After the reaction was complete (monitored by TLC), $H_2O$ was added and the resulting mixture extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (2 × 20 mL) and dried over $Na_2SO_4$. The solvent was then removed under reduced pressure to obtain crude products, which were subsequently purified by silica gel radial chromatography.

**2,6-Bis-[2-(2-hydroxyethyl)phenylamino]-4-chloro-1,3,5-triazine (37):** Purification on $SiO_2$ (EtOAc-hexane = 1/1) gave 117 mg (30%) of a white solid. mp 170.5–172.0 °C. $R_f$ 0.53 (EtOAc). IR (KBr): 3313, 3245, 2889, 1617, 1596, 1528, 1454, 1390, 1231, 1073, 995, 801, 748, 610, 586 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO–$d_6$, 80 °C): $\delta$ 2.45–2.78 (m, 4H, 2 × -C*H*$_2$CH$_2$OH), 3.46–3.75 (m, 4H, 2 × -CH$_2$C*H*$_2$OH), 5.03 (br s, 2H, 2 × -CH$_2$CH$_2$O*H*), 7.06–7.61 (m, 8H, 2 × -*Ph*), 9.56 (br s, 2H, 2 × -N*H*-). $^{13}C$ NMR (75.5 MHz, DMSO–$d_6$, 80 °C): $\delta$ 34.6, 61.6, 125.3, 125.8, 126.0, 129.8, 134.3, 136.0, 164.8, 168.6. MS (ESI) *m/z*: 386 $(M+H)^+$. HRMS calcd for $C_{19}H_{21}N_5O_2Cl$: 386.1384. Found: 386.1401. Anal. Calcd for $C_{19}H_{20}N_5O_2Cl$: C, 59.14; H, 5.22; N, 18.15. Found: C, 59.38; H, 5.39; N, 17.99.

**Dibenzyl *N*-(4-chloro-2-{[2-(2-hydroxyethyl)phenyl]amino}-1,3,5-triazin-6-yl)-D-glutamate (38):** Purification on $SiO_2$ (EtOAc-petroleum ether = 1/3) gave 243 mg (42%) of a colourless oil. $R_f$ 0.42 (EtOAc-petroleum ether = 3/5). $[\alpha]_D^{20}$ –0.8 (*c* 1.46, $CH_2Cl_2$). IR (NaCl-plates): 3324, 3034, 2950, 2881, 1737, 1572, 1522, 1455, 1385, 1245, 1170, 989, 805, 752, 734, 698 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$, mixture of tautomers): $\delta$ 1.97–2.53 (m, 4H, -NHCH(C*H*$_2$C*H*$_2$CO$_2$CH$_2$Ph)CO$_2$CH$_2$Ph), 2.84 (t, $J$ = 5.0 Hz, 2H, -C*H*$_2$CH$_2$OH), 3.86–3.98 (m, 2H, -CH$_2$C*H*$_2$OH), resonance for –OH missing, 4.58 in 4.85 (m, 1H, -NHC*H*-), 4.99–5.24 (m, 4H, -CO$_2$C*H*$_2$Ph), 5.90 in 6.03 (br d, $J$ = 7.0 Hz, 1H, -N*H*-), 7.01–7.40 (m, 13H,

-*Ph*), 7.53–7.71 (m, 1H, -*Ph*), 8.63 in 8.79 (br s, 1H, -N*H*-). $^{13}$C NMR (75.5 MHz, $CDCl_3$, mixture of tautomers): $\delta$ 27.6, 30.1, 34.8, 52.3, 64.5, 66.6, 67.4, 124.9 and 125.1 (1C), 125.5 and 125.7 (1C), 128.2, 128.3, 128.3, 128.4, 128.5, 128.6, 128.7 (2C), 130.4, 135.1 in 135.2 (1C), 135.6 in 135.7 (1C), 136.2, 164.3, 165.4, 165.8, 171.2, 172.3. MS (ESI) *m/z*: 576 $(M+H)^+$. HRMS calcd for $C_{30}H_{31}N_5O_5Cl$: 576.2014. Found: 576.1993.

**Biochemical evaluation.** The inhibitory activity of the compounds against MurF from *E. coli* was tested for their ability to inhibit the addition of D-Ala-D-Ala to UDP-Mur*N*Ac-L-Ala-γ-D-Glu-*meso*-$A_2$pm. The detection of orthophosphate generated during the reaction was based on the colorimetric Malachite green method[18] with slight modifications in a mixture (final volume, 50 µl) containing 50 mM Hepes, pH 8.0, 50 mM $MgCl_2$, 100 µM UDP-Mur*N*Ac-L-Ala-γ-D-Glu-*meso*-$A_2$pm, 600 µM D-Ala-D-Ala, 500 µM ATP, purified MurF from *E.coli* [27] (diluted with 50 mM Hepes, 1 mM dithiothreitol), and 500 µM of the tested compound dissolved in DMSO. The final concentration of DMSO was 5% (v/v). The mixture was incubated at 37 °C for 20 min and then quenched with 100 µl of Biomol® reagent. The absorbance was measured after 5 min at 650 nm. All the experiments were run in duplicates. The residual activity (RA) was calculated with respect to a similar assay without the inhibitor. The $IC_{50}$ value was determined by measuring the residual activity at seven different inhibitor concentrations and represents the concentration of the inhibitor for which RA is 50%.

**Docking.** The crystal structure of MurF (PDB entry: 2AM1)[13] was used for our docking experiment. The active site was defined as the area within a distance of 5Å around the co-crystallized inhibitor (2,4-dichloro-*N*-(3-cyano-4,5,6,7-tetrahydrobenzothiophen-2yl)-5-(morpholine-4-sulfonyl)-benzamide). The docking and scoring were made with the default parameters of the FlexX program. The docking program was validated by the removal and subsequent re-docking of the co-crystallized inhibitor. FlexX successfully replicated the conformation from the crystal structure (Figure 3).

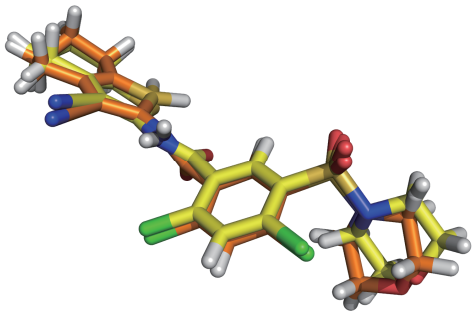


Figure 3. Validation of FlexX docking. Conformation of co-crystallized inhibitor (yellow) and the same compound re-docked with FlexX (orange). The structure of the enzyme is not shown for reasons of clarity.

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