Published in final edited form as:

Bioorg Med Chem. 2014 January 1; 22(1): 406–418. doi:10.1016/j.bmc.2013.11.011.

Synthesis of 3-(3-Aryl-pyrrolidin-1-yl)-5-aryl-1,2,4-triazines That Have Antibacterial Activity and Also Inhibit Inorganic Pyrophosphatase

Wei Lv[†], Biplab Banerjee[†], Katrina L. Molland[‡], Mohamed N. Seleem[§], Adil Ghafoor[§], Maha I. Hamed[§], Baojie Wan[#], Scott G. Franzblau[#], Andrew D. Mesecar[‡], and Mark Cushman^{†,*}

[†]Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, and The Purdue University Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907, United States

[‡]Department of Biological Sciences, and The Purdue University Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907, United States

[§]Department of Comparative Pathobiology, Purdue University College of Veterinary Medicine, Purdue University, West Lafayette, Indiana 47907, United States

[#]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, United States

Abstract

Inorganic pyrophosphatases are potential targets for the development of novel antibacterial agents. A pyrophosphatase-coupled high-throughput screening assay intended to detect *o*-succinyl benzoic acid coenzyme A (OSB CoA) inhibitors led to the unexpected discovery of a new series of novel inorganic pyrophosphatase inhibitors. Lead optimization studies resulted in a series of 3-(3-aryl-pyrrolidin-1-yl)-5-aryl-1,2,4-triazine derivatives that were prepared by an efficient synthetic pathway. One of the tetracyclic triazine analogues **22h** displayed promising antibiotic activity against a wide variety of drug-resistant *Staphylococcus aureus* strains, as well as activity vs. *Mycobacterium tuberculosis* and *Bacillus anthracis*, at a concentration that was not cytotoxic to mammalian cells.

Keywords

Inorganic pyrophosphatase; inhibitors; antibacterial agents; 3-(3-aryl-pyrrolidin-1-yl)-5-aryl-1,2,4-triazine

1. Introduction

Inorganic pyrophosphatases (PPases) are essential metal-dependent enzymes that catalyzes the hydrolysis of inorganic pyrophosphate (PPi) to phosphate.¹ They have been categorized into three structurally and mechanistically distinct families of soluble PPases in addition to

© 2013 Elsevier Ltd. All rights reserved.

*To whom Correspondence should be addressed: Mark Cushman. Phone: 765-494-1465. Fax: 765-494-6790. cushman@purdue.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

membrane-integral PPases that couple pyrophosphate hydrolysis to H^+ and/or Na^+ transport.² The energy released from pyrophosphate hydrolysis can be used to drive energetically unfavorable reactions to completion.³ PPases play essential roles in many biological processes, including lipid metabolism, calcium absorption, bone formation, and DNA synthesis.^{4–7} PPases are widely expressed in most microorganisms and they have therefore been considered to be possible targets for the discovery of antimicrobial agents.⁸ In general, catalysis involves coordination of the pyrophosphate by three or four metal ions and does not proceed through a covalent enzyme-phosphate intermediate.^{9–14} The enzyme and associated metal ions increase the nucleophilicity of the substrate water molecule by facilitating deprotonation, while increasing the electrophilicity of one of the two phosphorus atoms and also making the attached phosphate into a better leaving group through coordination with the pyrophosphate oxygens. The crystal structures of a wide variety of inorganic PPases are available in the Protein Data Bank, including the recently determined structures of two sodium-pumping membrane-integral pyrophosphatases.^{13,15}

A number of small inorganic substances have been reported to inhibit inorganic pyrophosphatase, including fluoride,^{16–23} calcium,^{24–27} arsenate,²⁸ phosphonate,²⁹ phosphate,³⁰ and imidodiphosphates.³¹ Substituted methane derivatives can be added to the list, including diphosphonates^{21,32–39} and tetranitromethane.⁴⁰ Covalent inhibitors include *N,N'*-dicyclohexylcarbodiimide,⁴¹ 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide,⁴² fluorescein 5'-isothiocyanate,⁴³ and *N*-ethylmaleimide.^{42,44} Finally, various amines inhibit inorganic PPase, including adenine nucleotides,⁴⁵ nucleotidyl sulfates,⁴⁶ amino acid esters,⁴⁷ acylspermidine derivatives,⁴⁸ and phosphoethanolamine.⁴⁹ In general, antimicrobial activity has not been reported in the publications that list PPase inhibitory activity for these substances. One exception is the reported inhibition of streptococcal growth by several diphosphonates at 2–5 millimolar concentration levels, but in that case inhibition of bacterial growth was ascribed to magnesium chelation instead of PPase inhibition.³⁷

Our efforts to identify *o*-succinyl benzoic acid coenzyme A (OSB-CoA) synthetase inhibitors by high-throughput screening using a PPase-coupled assay⁵⁰ led unexpectedly to the discovery that compound **12a** (Scheme 2) is a weak inhibitor of *Mycobacterium tuberculosis* PPase (45% inhibition at 100 μ M, IC_{50} 148 μ M). This result is noteworthy because in contrast to many of the reported inorganic pyrophosphatase inhibitors, **12a** is not a substrate analogue and it is also not ionic at physiological pH. Compound **12a** was subsequently found to inhibit the growth of *Mycobacterium tuberculosis* with a MIC of approximately 60 μ M. These results suggested that it might be possible to design and synthesize a series of **12a** analogues to optimize the PPase inhibitory activity and the antimicrobial activity. A number of 1,2,4-triazine-6-ones,⁵¹ 1,3,5-triazines,^{52–56} and annulated 1,2,4- and 1,2,3-triazines⁵⁷ have previously been reported to possess antimicrobial activity.

Although compound **12a** was discovered to be a PPase inhibitor due to its presence in a high-throughput screening library, it has not been reported in the chemical literature. In order to provide enough material for biological testing and confirm the structure, a synthesis of compound **12a** was devised from simple starting materials. The route is quite versatile and has been used to make 3-(3-aryl-pyrrolidin-1-yl)-5-aryl-1,2,4-triazine analogues. The PPase inhibitory activities and antibacterial activities of the resulting compounds against *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Bacillus* species were determined. These efforts were eventually rewarded through the synthesis of compound **22h**, which was active against a wide range of drug-resistant *Staphylococcus aureus* strains.

2. Results and Discussion

2.1 Synthesis

2.1.1 Synthesis of the Triazinyl Sulfone 5—The general procedure reported by Hajbi et al.⁵⁸ was used to prepare the triazinyl sulfone **5**. The oxidation of 2-acetyl-5-methylfuran (**1**) by selenium dioxide proceeded selectively on the ketone methyl group to afford a mixture of furyl glyoxal **2** and its covalent monohydrate. Without purification, the furyl glyoxal **2** was condensed with *S*-methylthiosemicarbazide hydrogen iodide (**3**)⁵⁹ to regioselectively provide the triazinyl methyl sulfide **4**.^{60,61} The oxidation of **4** with *meta*-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane proceeded selectively on the sulfur atom to afford the triazinyl sulfone **5**.

2.1.2 Synthesis of the Analogues 12a–m—The succinic acids **9a–e** are key intermediates that were synthesized based on the procedure reported by Breukelman et al.⁶² Starting from the corresponding benzaldehydes **6a–e**,⁶³ the Knoevenagel condensation with diethyl malonate was performed to afford the diesters **7a–e**. Michael addition with KCN in hot ethanol, followed by hydrolysis and decarboxylation gave the nitrile esters **8a–e**. Hydrolysis of **8a–e** under acidic or basic conditions produced the succinic acids **9a–e**. The succinimides **10a–e** were synthesized by treating **9a–e** with urea in refluxing xylene. Further reduction of **10a–i** with borane in THF provided the pyrrolidines **11a–i** (**10f–i** were commercially available). When the pyrrolidines **11a–i** were reacted with the triazine sulfone **5** in dry DMF, the tetracyclic compounds **12a–i** were obtained in good yield. Hydrogenation of compounds **12b–e** afforded the reduction products **12j–m**.

2.1.3 Synthesis of the Adenine Analogue 17—Intermediate **15** was prepared by the procedure reported by Kocalka et al. (Scheme 3).⁶⁴ The *N*-Boc group of **15** was removed smoothly in 20% TFA in dichloromethane. Treatment of the crude product **16** with the triazine sulfone **5** in the presence of triethylamine provided the desired product **17** in high yield (Scheme 3).

2.1.4 Synthesis of Analogues 22a–h and 29a–d—Oxidation of the acetophenones **18a–f** with selenium dioxide proceeded smoothly to provide a mixture of glyoxals **19a–f** and their covalent monohydrate forms (Scheme 4). Without purification, the crude products **19a–f** were condensed with *S*-methylthiosemicarbazide hydrogen iodide (**3**) to regioselectively afford the triazinyl methyl sulfides **20a–f** in good yields. Then, *m*-CPBA was added to convert **20a–f** to the corresponding sulfones **21a–f**. The oxidation proceeded smoothly, but the product triazine sulfones **21a–f** were poorly soluble in most organic solvents (including chloroform, dichloromethane, ethyl acetate and acetone) and therefore could not be purified by column chromatography. The crude products were therefore used for the next step. The coupling reactions of **21a–f** with pyrrolidine **11a** were carried out in dry DMF to afford **22a–f** in good yields (Scheme 4). The nitro compounds **22e–f** were converted to the amines **22g–h** by catalytic hydrogenation.

Compounds **29a–c** were synthesized starting from the boronic acids **23a–c**. The Suzuki coupling with the bromide **24** furnished the bicyclic products **25a–c** in high yield. The same procedures used to make **22a–f** were also employed to synthesize **29a–c** (Scheme 5).

Compound **29d** was synthesized through a similar route starting from 4-bromoacetophenone (**30**) and 3-nitrophenylboronic acid (**23a**) as described in Scheme 6.

2.2 MtPPase Inhibitory Activity and Antimicrobial Activity

The inhibitory activities of the triazine analogues **12a–c**, **12f–m**, **17**, **22a–h**, and **29a–d** were determined against inorganic pyrophosphatase from *Mycobacterium tuberculosis* (MtPPase). The percentage inhibition of MtPPase catalytic activity in the presence of a 100 μ M concentration of the triazine analogues was initially measured. Only those compounds with more than 35% inhibition were subjected to IC₅₀ determination (Table 1). The results indicate that replacing the 3-chloro substituent on the phenyl ring of **12a** with a hydroxy group (**12k–m**) abolished the MtPPase inhibitory activity, while other substituents including 3-OBn (**12c**), 3-F (**12g**), 3-CF₃ (**12h**), 3-NH₂ (**12j**) were tolerated better in this position. Replacing the furan ring with a substituted phenyl ring usually afforded more potent inhibitors (**22a–h**) with IC₅₀ values ranging from 33 μ M to 118 μ M. The 4-bromo compound **22c** had the best inhibitory activity, with an IC₅₀ of 33 μ M. Two of the compounds with an additional aromatic ring attached to the furan ring (**29a** and **29c**) had poor solubility in DMSO and therefore could not be tested. On the other hand, the trifluoromethyl compound **29b** had good inhibitory activity against MtPPase with an IC₅₀ of 38 μ M, while the nitrated biphenyl compound **29d** was inactive.

All of the prepared triazine analogues were evaluated for their antimicrobial activities against the *Mycobacterium tuberculosis* strain H₃₇Rv (replicating phenotype, tested by the MABA method)^{65,66} and *Mycobacterium tuberculosis* strain H₃₇Rv-CV-lux AB (nonreplicating persistent phenotype, tested by the LORA method).^{67,68} Most of the compounds with PPase inhibitory activities also exhibit good to moderate antibacterial activities against *Mycobacterium tuberculosis* (MIC ranging from 5.7 μ M to 78 μ M). Compound **22b** (MIC 15 μ M) was the most potent compound when tested in the MABA assay, while **12j** (MIC 47 μ M) had the highest potency in the LORA assay.

The antibacterial activities against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus* species were also tested. The 3-amino compound (**22g**) inhibited the growth of MRSA with a MIC of 30 μ M (Table 2), while the remaining compounds were all inactive (no obvious inhibition at 50 μ M). Five compounds (**12k**, **22a**, **22b**, **22g** and **22h**) showed moderate antimicrobial activities against *Bacillus anthracis* and *Bacillus subtilis*, with MIC values ranging from 16 μ M to 128 μ M (Table 2).

The compound **22h** with the most potent antibacterial activity was then tested against a wide variety of antibiotic-resistant clinical isolates (MRSA, VISA, and VRSA) and the results are listed in Table 3. Activity was observed at the MIC 16 μ M potency level against all of the drug-resistant strains except for the ciprofloxacin-resistant, oxacillin-resistant, and linezolid-resistant NRS37 strain, against which activity was detected at MIC 8 μ M.

Several of the inorganic pyrophosphatase inhibitors were assayed against the HeLa human tumor cell line and the murine macrophage J774.A1 cell line in order to determine the potential toxic effect in mammalian cells. MTS cell viability assays conducted on compounds **22g**, **22a**, **22h**, **12k**, and **22b** at 64 μ M concentration showed no significant decrease in percentage mean absorption relative to the DMSO control (Figure 1), indicating that the compounds are not toxic in vitro at this concentration, which is four times that of the MIC displayed by **22h** in most of the drug-resistant *S. aureus* strains.

3. Conclusion

High-throughput screening in a pyrophosphatase-coupled enzyme assay intended to detect *o*-succinyl benzoic acid coenzyme A (OSB CoA) inhibitors led to the unexpected discovery of the novel inorganic PPase inhibitor **12a**. The structure of **12a** was confirmed by an efficient synthesis that also allowed access to an array of structural analogues. The inorganic

PPase inhibitory potencies of the series were determined and the antimicrobial activities were optimized vs. *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Bacillus* species. The most active compound **22h** displayed promising antibiotic activity against a wide variety of drug-resistant *Staphylococcus aureus* strains at concentrations that were not cytotoxic in two different mammalian cell lines. In general, there is a lack of correlation between the enzyme inhibitory activities and the antibacterial activities vs. *M. tuberculosis* (Table 1). For example, at the extremes, based on its inactivity as an enzyme inhibitor, the phenol **12k** should not have antibiotic activity yet it does, and the enzyme inhibitor **29b** should have antibacterial activity and it does not. These results bring into question whether inhibition of inorganic pyrophosphatase is in fact responsible for the antibacterial activity. On the other hand, the results of this study are significant because many of the compounds are structurally novel inorganic pyrophosphatase inhibitors, and also because the series presents a new antimicrobial scaffold for further development.

4. Experimental Section

4.1 Inorganic Pyrophosphatase Inhibition Assay

All chemicals used were of the highest available purity and were ordered from Sigma-Aldrich. The enzyme MtPPase was expressed and purified by a published protocol.⁶⁹ Enzyme concentration was determined using absorbance at 280 nm and an extinction coefficient of 30940 L M⁻¹cm⁻¹.⁷⁰ MtPPase was stored at -80 °C in buffer containing 20 mM Tris pH 8, 150 mM NaCl, and 10% v/v glycerol. For testing the inhibition of MtPPase by the synthesized compounds, a malachite green assay was used. Malachite green stock solution was prepared according to Baykov's procedure.⁷¹ The final assay composition consisted of 50 mM Tris-HCl pH 7.5, 1 mM DTT, 100 mM NaCl, 1 mM MgCl₂, 0.2 mg/ml BSA, 0.13% Triton X-100, 5 μM PPI, 13.3 μM OSB,⁷² 170.5 μM CoA, and 50 nM MtPPase.

Enzyme kinetic assays were performed in clear, flat-bottomed 96-well plates (Fisherbrand Cat. No. 12566601) with a total reaction volume of 100 μL. A 10 mM stock solution in DMSO was made for each compound and a volume appropriate for the intended final concentration was added to 60 μL substrate solution and mixed for 2 min by shaking. To initiate the reaction, an aliquot of 20 μL enzyme solution was added and the mixture was allowed to react for 6 min. The reaction was stopped by the addition of 20 μL malachite green development solution followed by 10 min of shaking, and then absorbance of each well at 612 nm was measured on a Synergy 5 plate reader (Biotek). In the case of negative control wells, no MtPPase was included in the enzyme solution. In the case of positive control wells, an equivalent volume of DMSO lacking inhibitor was added in the first step.

Kinetic data at each inhibitor concentration were collected in triplicate, and the percent inhibition was calculated according to the following formula: %I = (1 - (Abs_{sample} - Abs_{(-) control}) / (Abs_{(+) control} - Abs_{(-) control})) * 100 with error represented by the standard deviation of three data points.⁷³ IC₅₀ values were calculated according to the formula: %I = %max[I] / (IC₅₀ + [I]) in the Enzyme Kinetics module of SigmaPlot.

4.2 Chemistry

Melting points were determined using capillary tubes with a Mel-Temp apparatus and are uncorrected. The nuclear magnetic resonance (¹H and ¹³C NMR) spectra were recorded using Bruker ARX300 spectrometer (300 MHz) with a QNP probe or Bruker DX-2 spectrometer (500 MHz) with a BBO probe. IR spectra were obtained using a Perkin-Elmer 1600 series FTIR spectrometer. High-resolution mass spectra were recorded on a double-focusing sector mass spectrometer with magnetic and electrostatic mass analyzers. HPLC analyses were performed on a Waters 1525 binary HPLC pump/Waters 2487 dual λ

absorbance detector system using a 5 μ M C₁₈ reverse phase column. Purities of biologically important compounds were 95%. For purities estimated by HPLC, the major peak accounted for 95% of the combined total peak area when monitored by a UV detector at 254 nm. Elemental analyses were performed at Midwest Microlab LLC (Indianapolis, IN) and the determined values are within 0.4% of the calculated values.

4.2.1. General Procedure for the Synthesis of Triazinyl Methyl Sulfides 4, 20a–f and 27a–d—Solutions of selenium dioxide (808 mg, 7.28 mmol) in dioxane (8 mL) and water (0.8 mL) were stirred at 50–55 °C until the solids completely dissolved, and then they were combined with solutions of the ketone (**1**, **18a–f**, **25a–d**, 2.44 mmol) in dioxane (2 mL). The mixtures were heated at reflux for 5 h. After cooling down, the black solids were filtered off, and the solvents were evaporated. The residues were partially dissolved in ethyl acetate (10 mL), and the white solids were filtered off. The filtrates were evaporated and the residues were dissolved in ethanol (10 mL), and then aqueous solutions of *S*-methylthiosemicarbazide hydrogen iodide (**3**, 582 mg, 2.50 mmol) and sodium bicarbonate (553 mg, 6.58 mmol) in water (2 mL) were added. The solutions were heated at reflux for 2 h. After cooling down, the solvents were evaporated and the residues were dissolved in water (20 mL) and extracted with dichloromethane (3 $\times$ 20 mL). The organic layers were combined and dried over Na₂SO₄. Evaporation of the solvents afforded the desired products, which were pure enough for the next reaction.

4.2.2. 5-(5-Methylfuran-2-yl)-3-(methylthio)-1,2,4-triazine (4)—Yellow solid (54%): mp 91–93 °C. IR (film) 1593, 1539, 1483 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.09 (s, 1 H), 7.33 (d, *J* = 3.5 Hz, 1 H), 6.22 (d, *J* = 3.5 Hz, 1 H), 2.62 (s, 3 H), 2.41 (s, 3 H); ¹³C NMR (125 MHz, CDCl₃) δ 173.0, 158.4, 147.0, 145.8, 140.0, 118.2, 110.0, 14.0, 13.7; CIMS *m/z* 208 (MH⁺). Anal. Calcd for C₉H₉N₃OS: C, 52.16; H, 4.38; N, 20.27. Found: C, 52.09; H, 4.34; N, 20.11.

4.2.3. 3-(Methylthio)-5-phenyl-1,2,4-triazine (20a)⁶¹—Yellow solid (82.6%): mp 92–94 °C (lit.⁶¹ 97–99 °C). ¹H NMR (500 MHz, CDCl₃) δ 9.30 (s, 1 H), 8.07 (dd, *J* = 1.5, 8.5 Hz, 2 H), 7.52–7.46 (m, 3 H), 2.65 (s, 3 H); ¹³C NMR (125 MHz, CDCl₃) δ 173.5, 154.3, 141.8, 132.9, 132.5, 129.2, 127.5, 13.7.

4.2.4. 5-(3,4-Dimethoxyphenyl)-3-(methylthio)-1,2,4-triazine (20b)⁷⁴—Yellow solid (46.7%): mp 147–149 °C (lit.⁷⁴ 150–152 °C). ¹H NMR (300 MHz, CDCl₃) δ 9.29 (s, 1 H), 7.73–7.68 (m, 2 H), 6.97 (d, *J* = 8.3 Hz, 1 H), 3.97 (s, 3 H), 3.95 (s, 3 H), 2.70 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 173.2, 153.9, 153.1, 149.7, 141.6, 125.4, 121.4, 110.1, 109.7, 56.1, 56.0, 13.9.

4.2.5. 5-(4-Bromophenyl)-3-(methylthio)-1,2,4-triazine (20c)⁷⁴—Yellow solid (67.2%): mp 149–151 °C (lit.⁷⁴ 153–154 °C). ¹H NMR (300 MHz, CDCl₃) δ 9.32 (s, 1 H), 7.98 (m, 2 H), 7.64 (m, 2 H), 2.69 (s, 3 H).

4.2.6. 5-(3,4-Methylenedioxyphenyl)-3-(methylthio)-1,2,4-triazine (20d)—Brown solid (64.9%): mp 154–157 °C. IR (film) 2903, 1537, 1488, 1441, 1277, 1246, 1147, 1034 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.23 (s, 1 H), 7.67 (dd, *J* = 1.7, 8.2 Hz, 1 H), 7.64 (d, *J* = 1.7 Hz, 1 H), 6.92 (d, *J* = 8.2 Hz, 1 H), 6.07 (s, 2 H), 2.68 (s, 3 H); ¹³C NMR (125 MHz, CDCl₃) δ 173.3, 153.7, 151.6, 148.8, 141.4, 127.0, 123.0, 108.8, 107.2, 102.0, 13.8; EIMS *m/z* 247 (M⁺); HREIMS *m/z* calcd for C₁₁H₉N₃O₂S (M⁺) 247.0416, found 247.0418.

4.2.7. 3-(Methylthio)-5-(3-nitrophenyl)-1,2,4-triazine (20e)—Yellow solid (60.0%): mp 133–135 °C. IR (film) 3090, 1526, 1495, 1348, 1242 cm⁻¹; ¹H NMR (300 MHz, CDCl₃)

δ 9.46 (s, 1 H), 8.97 (s, 1 H), 8.48 (d, $J = 7.8$ Hz, 1 H), 8.41 (m, 1 H), 7.77 (t, $J = 8.0$ Hz, 1 H), 2.72 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 174.2, 152.2, 148.9, 141.5, 135.0, 133.2, 130.6, 126.9, 122.5, 13.9; EIMS m/z 248 (M^+); HREIMS m/z calcd for $\text{C}_{10}\text{H}_8\text{N}_4\text{O}_2\text{S}$ (M^+) 248.0368, found 248.0368.

4.2.8. 3-(Methylthio)-5-(4-nitrophenyl)-1,2,4-triazine (20f) ⁷⁵—Yellow solid (67.0%): mp 179–181 °C (lit.⁷⁵ 222–223 °C). ^1H NMR (300 MHz, CDCl_3) δ 9.45 (s, 1 H), 8.41 (d, $J = 8.8$ Hz, 2 H), 8.34 (d, $J = 8.8$ Hz, 2 H), 2.76 (s, 3 H).

4.2.9. 3-(Methylthio)-5-(5-(3-nitrophenyl)furan-2-yl)-1,2,4-triazine (27a)—Yellow solid (74.3%): mp 190–192 °C. IR (film) 1593, 1521, 1491, 1349, 1269, 1248 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 9.32 (s, 1 H), 8.58 (s, 1 H), 8.21 (d, $J = 7.9$ Hz, 1 H), 8.11 (d, $J = 7.5$ Hz, 1 H), 7.66 (t, $J = 8.0$ Hz, 1 H), 7.58 (d, $J = 3.7$ Hz, 1 H), 7.06 (d, $J = 3.7$ Hz, 1 H), 2.70 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 173.5, 155.5, 149.1, 148.7, 145.5, 140.0, 130.7, 130.2, 130.0, 123.5, 119.4, 118.6, 110.4, 13.9; ESIMS m/z 315 (MH^+); HRESIMS m/z calcd for $\text{C}_{14}\text{H}_{11}\text{N}_4\text{O}_3\text{S}$ (MH^+) 315.0552, found 315.0556.

4.2.10. 3-(Methylthio)-5-(5-(2-(trifluoromethyl)phenyl)furan-2-yl)-1,2,4-triazine (27b)—Yellow solid (91.5%): mp 114–116 °C. IR (film) 1577, 1539, 1312, 1261, 1127, 1035 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 9.27 (s, 1 H), 7.80 (t, $J = 7.0$ Hz, 2 H), 7.65 (t, $J = 7.6$ Hz, 1 H), 7.57–7.50 (m, 2 H), 6.90 (d, $J = 3.6$ Hz, 1 H), 2.69 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 173.4, 155.3, 149.0, 145.9, 140.1, 132.0, 130.3, 129.3, 127.9, 127.4, 127.1, 127.0, 125.5, 121.9, 118.1, 113.0, 13.8; EIMS m/z 337 (M^+); HREIMS m/z calcd for $\text{C}_{15}\text{H}_{10}\text{F}_3\text{N}_3\text{OS}$ (M^+) 337.0497, found 337.0496.

4.2.11. Methyl 3-(5-(3-(Methylthio)-1,2,4-triazin-5-yl)furan-2-yl)benzoate (27c)—Yellow solid (95%): mp 168–170 °C. IR (film) 3120, 1725, 1537, 1281, 1247, 753 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 9.29 (s, 1 H), 8.38 (s, 1 H), 8.01 (d, $J = 7.7$ Hz, 1 H), 7.94 (dd, $J = 7.7, 1.0$ Hz, 1 H), 7.54–7.51 (m, 2 H), 6.94 (d, $J = 3.7$ Hz, 1 H), 3.95 (s, 3 H), 2.67 (s, 3 H); ^{13}C NMR (125 MHz, CDCl_3) δ 173.3, 166.3, 157.2, 148.3, 145.7, 140.0, 130.9, 130.0, 129.4, 129.1, 128.6, 125.6, 118.7, 109.3, 52.3, 13.8; EIMS m/z 327 (M^+); HREIMS m/z calcd for $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_3\text{S}$ (M^+) 327.0678, found 327.0681.

4.2.12. 3-(Methylthio)-5-(3'-nitrobiphenyl-4-yl)-1,2,4-triazine (27d)—Yellow solid (87.3%): mp 197–199 °C. IR (film) 1535, 1508, 1481, 1345, 1240, 745 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 9.44 (s, 1 H), 8.53 (t, $J = 1.9$ Hz, 1 H), 8.32–8.26 (m, 3 H), 7.99 (d, $J = 7.8$ Hz, 1 H), 7.83 (d, $J = 8.5$ Hz, 2 H), 7.69 (d, $J = 7.8$ Hz, 1 H), 2.76 (s, 3 H); ^{13}C NMR (125 MHz, CDCl_3) δ 173.8, 153.6, 148.7, 142.6, 141.7, 141.3, 133.1, 133.0, 130.0, 128.4, 128.0, 122.9, 122.0, 13.9; EIMS m/z 324 (M^+); HREIMS m/z calcd for $\text{C}_{16}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ (M^+) 324.0681, found 324.0679.

4.2.13. 5-(5-Methylfuran-2-yl)-3-(methylsulfonyl)-1,2,4-triazine (5)—The triazinyl methyl sulfide **4** (208 mg, 1 mmol) was dissolved by anhydrous dichloromethane (5 mL) and the solution was cooled to 0 °C. Then 77% *m*-CPBA (450 mg, 2 mmol) was added and the mixture was stirred under argon at room temperature for 3.5 h. The solution was filtered, and solvent was evaporated. The residue was purified by column chromatography using 40% ethyl acetate-hexane to afford the product as yellow solid (160 mg, 66%): mp 125–127 °C. IR (film) 1596, 1542, 1535, 1319, 1140 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 9.51 (s, 1 H), 7.63 (d, $J = 3.6$ Hz, 1 H), 6.35 (dd, $J = 3.6, 0.8$ Hz, 1 H), 3.45 (s, 3 H), 2.47 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 166.3, 161.0, 147.9, 146.1, 145.7, 122.3, 111.5, 39.5, 14.3; EIMS m/z 239 (M^+); HREIMS m/z calcd for $\text{C}_9\text{H}_9\text{N}_3\text{O}_3\text{S}$ (M^+) 239.0365, found 239.0361. Anal. Calcd for $\text{C}_9\text{H}_9\text{N}_3\text{O}_3\text{S}$: C, 45.18; H, 3.79; N, 17.56. Found: C, 45.33; H, 3.71; N, 17.40.

4.2.14. General Procedure for the Synthesis of Methylsulfonyltriazines 21a–f and 28a–d—The triazinyl methyl sulfides (**20a–f**, **27a–d**, 0.247 mmol) were dissolved in dichloromethane (2 mL) and the solutions were cooled to 0 °C. *m*-CPBA (77%, 125 mg, 0.558 mmol) was added in portions and the mixtures were stirred under argon at room temperature for 3 h. The solvents were evaporated and the residues were purified by silica gel column chromatography using 50% ethyl acetate in hexane and then 15% ethyl acetate in dichloromethane. Evaporation of the solvents provided the crude products, which were used in the next reaction without further purification.

4.2.15. General Procedure for the Synthesis of 7a–e—Mixtures of diethyl malonate (4.65 g, 29.0 mmol), the appropriate benzaldehyde (**6a–e**, 29.6 mmol), and piperidine (300 μ L) in toluene (25 mL) were stirred under reflux for 5 h using a Dean-Stark trap. After cooling down, the reaction mixtures were washed sequentially with aqueous 1 M HCl, saturated aqueous sodium bicarbonate, water (each for 25 mL), and dried over sodium sulfate. Removal of the solvents gave thick yellow oils, which were used in the next step without further purification.

4.2.16. General Procedure for the Synthesis of 8a–e—The crude oils **7a–e** (26.6 mmol) were dissolved in absolute ethanol (35 mL) and a solution of potassium cyanide (1.75 g, 26.8 mmol) in water (5 mL) was added. The mixtures were stirred at 70 °C overnight, filtered and acidified by 1 M HCl to pH < 1. Solvents were evaporated and the residues were dissolved in ethyl acetate (40 mL), washed with brine (25 mL) and dried over sodium sulfate. Concentration of the organic layer afforded the products, which were used in the next step without purification.

4.2.17. General Procedure for the Synthesis of 9a–b—The crude esters **8a–b** (6.4 mmol) were added to concentrated hydrochloric acid (10 mL), and the mixtures were heated to reflux overnight. After the mixtures were cooled down, the solid cakes were collected by filtration and broken up. The products were washed with cold water and dried at 60 °C overnight.

4.2.18. 2-(3-Chlorophenyl)succinic Acid (9a) ⁷⁶—White solid (79.2%): mp 163–165 °C (lit.⁷⁶ 161–162 °C). ¹H NMR (300 MHz, CD₃OD) δ 7.33 (s, 1 H), 7.31–7.23 (m, 3 H), 4.02 (dd, *J* = 9.6, 5.4 Hz, 1 H), 3.08 (dd, *J* = 17.1, 9.9 Hz, 1 H), 2.64 (dd, *J* = 17.1, 5.4 Hz, 1 H).

4.2.19. 2-(3-Nitrophenyl)succinic Acid (9b) ^{77,78}—Brown solid (73.2%): mp 212–215 °C (lit.⁷⁸ 216–218 °C). ¹H NMR (500 MHz, methanol-*d*₄) δ 8.21–8.20 (m, 1 H), 8.15–8.13 (m, 1 H), 7.75–7.73 (m, 1 H), 7.58 (t, *J* = 8.0 Hz, 1 H), 4.18 (dd, *J* = 9.3, 5.9 Hz, 1 H), 3.13 (dd, *J* = 17.0, 9.3 Hz, 1 H), 2.72 (dd, *J* = 17.0, 5.9 Hz, 1 H).

4.2.20. General Procedure for the Synthesis of 9c–e—The crude nitrile esters **8c–e** (25.3 mmol) were dissolved in absolute ethanol (55 mL), potassium hydroxide (7.81 g, 139 mmol) was added, and the mixtures were heated to reflux for 4 h. After cooling down, the solvents were evaporated, the residues were dissolved in water (50 mL), and 3 M HCl was added to make the pH < 1. The solid precipitates were collected by filtration and dried at 90 °C overnight.

4.2.21. 2-[3-(Benzyloxy)phenyl]succinic Acid (9c)—Yellow solid (91.6%): mp 137–139 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.46–7.30 (m, 5 H), 7.21 (t, *J* = 8.0 Hz, 1 H), 6.97 (s, 1 H), 6.89–6.85 (m, 2 H), 5.05 (s, 2 H), 3.82 (dd, *J* = 9.5, 5.2 Hz, 1 H), 2.94 (dd, *J* = 16.6, 9.5 Hz, 1 H), 2.45 (dd, *J* = 16.6, 5.2 Hz, 1 H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 174.5,

173.7, 159.2, 142.6, 138.0, 130.3, 129.4, 128.8, 128.7, 120.9, 115.4, 113.6, 70.1, 47.7, 38.0; negative ion ESIMS m/z 299 ($M - H^+$)⁻; negative ion HRESIMS m/z calcd for $C_{17}H_{15}O_5$ ($M - H^+$)⁻ 299.0920, found 299.0926.

4.2.22. 2-[3,4-Bis(benzyloxy)phenyl]succinic Acid (9d)—Yellow solid (90%): mp 176–179 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.47–7.29 (m, 10 H), 7.03–6.95 (m, 2 H), 6.80–6.77 (m, 1 H), 5.08 (s, 4 H), 3.81 (dd, J = 10.5, 4.6 Hz, 1 H), 2.93 (dd, J = 16.8, 10.5 Hz, 1 H), 2.45 (dd, J = 16.8, 4.6 Hz, 1 H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 175.0, 173.7, 149.1, 148.4, 138.2, 138.1, 132.5, 129.3, 128.7, 128.4, 121.2, 115.4, 115.2, 71.2, 71.0, 47.2, 38.5; negative ion ESIMS m/z 405 ($M - H^+$)⁻; negative ion HRESIMS m/z calcd for $C_{24}H_{21}O_6$ ($M - H^+$)⁻ 405.1338, found 405.1341.

4.2.23. 2-(3-(Benzyloxy)-4-methoxyphenyl)succinic Acid (9e)—Yellow solid (92%): mp 159–162 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.46–7.32 (m, 4 H), 7.06–7.05 (m, 1 H), 6.88–6.81 (m, 3 H), 5.01 (s, 2 H), 3.76 (dd, J = 9.9, 5.3, 1 H), 3.72 (s, 3 H), 2.92 (dd, J = 16.6, 9.9 Hz, 1 H), 2.41 (dd, J = 16.6, 5.3 Hz, 1 H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 174.9, 173.8, 149.0, 148.5, 138.0, 133.6, 129.3, 128.9, 128.8, 120.9, 114.3, 113.0, 71.0, 56.5, 47.2, 38.1; negative ion ESIMS m/z 329 ($M - H^+$)⁻; negative ion HRESIMS m/z calcd for $C_{18}H_{17}O_6$ ($M - H^+$)⁻ 329.1025, found 329.1031.

4.2.24. General Procedure for the Synthesis of 10a–e—Mixtures of the succinic acid (**9a–e**, 10.4 mmol) and urea (2.54 g, 42.3 mmol) in xylene (35 mL) were heated at reflux overnight, and then concentrated to dryness in vacuo. The crude products were purified by silica gel column chromatography, eluting with hexane-ethyl acetate 1:1.

4.2.25. 3-(3-Chlorophenyl)pyrrolidine-2,5-dione (10a) ⁷⁹—Light yellow solid (55%): mp 120–122 °C (lit. ⁷⁹ 119–121 °C). ¹H NMR (500 MHz, CDCl₃) δ 8.98 (s, 1 H, -NH), 7.33–7.29 (m, 2 H), 7.26–7.25 (m, 1 H), 7.15–7.13 (m, 1 H), 4.06 (dd, J = 5.5, 10.0 Hz, 1 H), 3.24 (dd, J = 10.0, 18.5 Hz, 1 H), 2.85 (dd, J = 5.5, 18.5 Hz, 1 H); ¹³C NMR (125 MHz, CDCl₃) δ 177.5, 176.0, 138.3, 135.0, 130.4, 128.3, 127.6, 125.6, 46.7, 37.9.

4.2.26. 3-(3-Nitrophenyl)pyrrolidine-2,5-dione (10b) ⁷⁸—Light yellow oil (78%). IR (film) 3310, 1780, 1710, 1520, 1340, 890 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 9.20 (s, 1 H, -NH), 8.17–8.14 (m, 2 H), 7.63–7.53 (m, 2 H), 4.24 (dd, J = 9.6, 5.4 Hz, 1 H), 3.30 (dd, J = 18.6, 9.9 Hz, 1 H), 2.90 (dd, J = 18.6, 5.7 Hz, 1 H); ¹³C NMR (CDCl₃, 75 MHz) δ 180.4, 179.1, 149.8, 140.9, 135.3, 131.2, 124.0, 123.7, 48.1, 38.8.

4.2.27. 3-(3-(Benzyloxy)phenyl)pyrrolidine-2,5-dione (10c)—Orange solid (66%): mp 113–115 °C. IR (film) 3220, 1775, 1708, 1584, 1349, 1180 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 8.91 (brs, 1 H), 7.44–7.37 (m, 4 H), 7.35–7.31 (m, 1 H), 7.29 (t, J = 8.0 Hz, 1 H), 6.94–6.91 (m, 1 H), 6.88–6.87 (m, 1 H), 6.84 (d, J = 7.9 Hz, 1 H), 5.05 (s, 2 H), 4.03 (dd, J = 9.6, 5.0 Hz, 1 H), 3.20 (dd, J = 18.6, 9.6 Hz, 1 H), 2.85 (dd, J = 18.6, 5.0 Hz, 1 H); ¹³C NMR (125 MHz, CDCl₃) δ 178.1, 176.4, 159.2, 138.1, 136.5, 130.2, 128.6, 128.0, 127.5, 119.8, 114.4, 114.0, 70.0, 47.2, 38.1, 30.9; CIMS m/z 282 (MH⁺); HREIMS m/z calcd for $C_{17}H_{15}NO_3$ (MH⁺) 281.1052, found 281.1055.

4.2.28. 3-(3,4-Bis(benzyloxy)phenyl)pyrrolidine-2,5-dione (10d)—Yellow solid (57%): mp 125–127 °C. IR (film) 3246, 1777, 1712, 1513, 1259, 1012, 696 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.20 (s, 1 H), 7.43–7.26 (m, 10 H), 6.91 (d, J = 8.2 Hz, 1 H), 6.82 (s, 1 H), 6.75 (d, J = 8.2 Hz, 1 H), 5.15 (s, 4 H), 3.97 (dd, J = 9.5, 5.0 Hz, 1 H), 3.18 (dd, J = 18.6, 9.6 Hz, 1 H), 2.77 (dd, J = 18.6, 5.0 Hz, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 177.7, 175.8, 149.3, 148.8, 137.0, 129.5, 128.5, 127.9, 127.4, 127.2, 120.3, 115.2, 114.4, 71.5,

71.2, 46.7, 38.1; ESIMS m/z 410 (MNa^+); HRESIMS m/z calcd for $C_{24}H_{21}NO_4Na$ (MNa^+) 410.1368, found 410.1371.

4.2.29. 3-(3-(Benzyloxy)-4-methoxyphenyl)pyrrolidine-2,5-dione (10e)—Yellow solid (50%): mp 169–171 °C. IR (film) 3247, 1778, 1713, 1516, 1258, 1140, 1020, 698 cm^{-1} ; 1H NMR (500 MHz, $DMSO-d_6$) δ 11.23 (s, 1 H, -NH), 7.41 (d, $J = 7.3$ Hz, 2 H), 7.36 (t, $J = 7.3$ Hz, 2 H), 7.30 (t, $J = 7.2$ Hz, 1 H), 6.97 (d, $J = 1.5$ Hz, 1 H), 6.89 (d, $J = 8.3$ Hz, 1 H), 6.77 (dd, $J = 8.2, 1.5$ Hz, 1 H), 5.04–4.97 (m, 2 H), 3.99 (dd, $J = 9.5, 5.6$ Hz, 1 H), 3.70 (s, 3 H), 3.03 (dd, $J = 18.0, 9.5$ Hz, 1 H), 2.67 (dd, $J = 18.0, 5.6$ Hz, 1 H); EIMS m/z 311 (M^+); HRESI-TOFMS m/z calcd for $C_{18}H_{17}NO_4Na$ (MNa^+) 334.1055, found 334.1063.

4.2.30. General Procedure for the Synthesis of 11a–i—Solutions of **10a–i** (0.124 mmol) in dry tetrahydrofuran (5 mL) were stirred under argon at 0 °C, and 1 M BH_3 -tetrahydrofuran (6 mL) was added dropwise. The solutions were stirred at 0 °C for 30 min and then heated to reflux for 3 h. After cooling down, 3 M HCl was added cautiously until pH < 1, and white solids precipitated. The solvents were evaporated and the white solids were washed twice with ether (3 mL) and then dried in vacuo. The white solids were combined with 5 M NaOH (3 mL) and the mixtures were extracted with ethyl acetate (10 mL). The organic layers were dried over Na_2SO_4 , filtered and dried to give the products as pale yellow oils that were used in the next step without further purification.

4.2.31. General Procedure for the Synthesis of 12a–i, 22a–f, and 29a–d—Mixtures of the corresponding methylsulfonyl triazine (**5**, **21a–f**, or **28a–d**, 0.654 mmol) and the crude pyrrolidines (**11a–i**, 0.917 mmol) were dissolved in dry DMF (5 mL). The solutions were stirred under argon at room temperature overnight. Solvents were evaporated, and the residues were further purified by silica gel column chromatography, eluting with hexane-ethyl acetate 7:3.

4.2.32. 3-[3-(3-Chlorophenyl)pyrrolidin-1-yl]-5-(5-methylfuran-2-yl)-1,2,4-triazine (12a)—Dark brown oil (65%). IR (film) 2943, 2873, 1551, 1527, 1478, 1081, 784 cm^{-1} ; 1H NMR (500 MHz, acetone- d_6) δ 8.81 (s, 1 H), 7.42 (s, 1 H), 7.36–7.34 (m, 3 H), 7.28–7.26 (m, 1 H), 6.32 (s, 1 H), 4.30–3.40 (m, 5 H), 2.50–2.40 (m, 1 H), 2.39 (s, 3 H), 2.25–2.15 (m, 1 H); ^{13}C NMR (125 MHz, acetone- d_6) δ 159.1, 156.9, 148.3, 146.5, 144.4, 134.1, 133.8, 130.1, 127.2, 126.6, 125.6, 116.0, 109.2, 52.3, 46.0, 43.2, 32.5, 12.9; ESIMS m/z 341 (MH^+); HRESIMS m/z calcd for $C_{18}H_{18}ClN_4O$ (MH^+) 341.1169, found 341.1172; HPLC purity: 95.8% (C-18 reverse phase, MeOH- H_2O , 90:10), 96.0% (C-18 reverse phase, MeOH- H_2O , 85:15).

4.2.33. 5-(5-Methylfuran-2-yl)-3-(3-(3-nitrophenyl)pyrrolidin-1-yl)-1,2,4-triazine (12b)—Light brown oil (51%). IR (film) 2943, 2873, 1551, 1510, 1462, 1345, 978 cm^{-1} ; 1H NMR ($CDCl_3$, 300 MHz) δ 8.85 (s, 1 H), 8.19–8.11 (m, 2 H), 7.64 (d, $J = 7.8$ Hz, 1 H), 7.52 (t, $J = 8.1$ Hz, 1 H), 7.25 (d, $J = 3.6$ Hz, 1 H), 6.21 (d, $J = 3.6$ Hz, 1 H), 4.30–4.20 (m, 1 H), 4.16–3.92 (m, 1 H), 3.85–3.55 (m, 3 H), 2.60–2.50 (m, 1 H), 2.43 (s, 3 H), 2.30–2.10 (m, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.1, 157.1, 148.4, 148.2, 147.1, 143.7, 134.9, 133.3, 129.6, 122.1, 121.9, 116.3, 109.4, 52.3, 46.2, 43.4, 32.7, 29.6, 14.0; ESIMS m/z 352 (MH^+); HRESIMS m/z calcd for $C_{18}H_{17}N_5O_3$ (MH^+) 352.1409, found 352.1405. HPLC purity: 96% (MeOH- H_2O , 90:10).

4.2.34. 3-(3-(3-(Benzyloxy)phenyl)pyrrolidin-1-yl)-5-(5-methylfuran-2-yl)-1,2,4-triazine (12c)—Brown oil (67%). IR (film) 2872, 1553, 1526, 1478, 1020, 696 cm^{-1} ; 1H NMR (500 MHz, $CDCl_3$) δ 8.82 (s, 1 H), 7.43 (d, $J = 7.1$ Hz, 2 H), 7.39–7.35 (m, 2 H), 7.33–7.30 (m, 1 H), 7.27–7.23 (m, 2 H), 6.95–6.94 (m, 1 H), 6.91 (d, $J = 7.6$ Hz, 1 H), 6.86

(dd, $J = 8.1, 2.4$ Hz, 1 H), 6.19 (d, $J = 3.0$ Hz, 1 H), 5.05 (s, 2 H), 4.3–3.4 (brs, 5 H), 2.42 (brs, 1 H), 2.41 (s, 3 H), 2.13 (brs, 1 H); ^{13}C NMR (125 MHz, CDCl_3) δ 159.1, 159.0, 156.8, 148.3, 147.0, 143.3, 136.9, 134.5, 129.6, 128.5, 127.9, 127.4, 119.7, 116.0, 114.0, 112.7, 109.3, 69.9, 52.5, 46.3, 43.7, 32.7, 14.0; ESIMS m/z 413 (MH^+); HRESIMS m/z calcd for $\text{C}_{25}\text{H}_{25}\text{N}_4\text{O}_2$ (MH^+) 413.1978, found 413.1971.

4.2.35. 3-(3-(3,4-Bis(benzyloxy)phenyl)pyrrolidin-1-yl)-5-(5-methylfuran-2-yl)-1,2,4-triazine (12d)—Brown oil (61%). IR (film) 2871, 1553, 1526, 1478, 1257, 1019 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 8.84 (s, 1 H), 7.46–7.44 (m, 4 H), 7.38–7.33 (m, 4 H), 7.32–7.28 (m, 2 H), 7.25 (s, 1 H), 6.92–6.90 (m, 2 H), 6.82 (dd, $J = 8.2, 1.7$ Hz, 1 H), 6.20 (d, $J = 3.0$ Hz, 1 H), 5.16 (s, 2 H), 5.15 (s, 2 H), 4.30–3.35 (brs, 5 H), 2.43 (s, 3 H), 2.39 (brs, 1 H), 2.06 (brs, 1 H); ^{13}C NMR (125 MHz, CDCl_3) δ 159.1, 156.9, 148.9, 148.4, 147.8, 147.0, 137.3, 137.2, 135.0, 134.5, 128.4, 127.7, 127.3, 127.2, 119.9, 116.0, 115.2, 114.4, 109.3, 71.4, 71.3, 52.6, 46.3, 43.3, 33.1, 30.8, 14.0; ESIMS m/z 519 (MH^+); HRESIMS m/z calcd for $\text{C}_{32}\text{H}_{31}\text{N}_4\text{O}_3$ (MH^+) 519.2396, found 519.2393.

4.2.36. 3-(3-(3-(Benzyloxy)-4-methoxyphenyl)pyrrolidin-1-yl)-5-(5-methylfuran-2-yl)-1,2,4-triazine (12e)—Dark brown oil (64.9%). IR (film) 2936, 2872, 1553, 1515, 1478, 1255, 1020, 697 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 8.82 (s, 1 H), 7.43–7.41 (m, 2 H), 7.36–7.32 (m, 2 H), 7.29–7.27 (m, 1 H), 7.23 (s, 1 H), 6.86–6.84 (m, 3 H), 6.20 (d, $J = 2.8$ Hz, 1 H), 5.14 (s, 2 H), 4.3–3.4 (brs, 5 H), 3.87 (s, 3 H), 2.42 (s, 3 H), 2.37 (brs, 1 H), 2.05 (brs, 1 H); ^{13}C NMR (125 MHz, CDCl_3) δ 159.0, 156.9, 148.5, 148.3, 148.1, 147.0, 137.0, 134.4, 134.1, 128.4, 127.7, 127.2, 119.6, 116.0, 113.4, 111.8, 109.2, 71.1, 56.0, 52.6, 46.3, 43.2, 33.0, 14.0; ESIMS m/z 443 (MH^+); HRESIMS m/z calcd for $\text{C}_{26}\text{H}_{27}\text{N}_4\text{O}_3$ (MH^+) 443.2083, found 443.2093.

4.2.37. 5-(5-Methylfuran-2-yl)-3-(3-phenylpyrrolidin-1-yl)-1,2,4-triazine (12f)—Dark brown liquid (63%). IR (film) 2941, 2871, 1605, 1527, 1297, 1039 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 8.81 (s, 1 H), 7.35–7.21 (m, 6 H), 6.18 (d, $J = 2.7$ Hz, 1 H), 4.35–4.12 (m, 1 H), 4.10–3.85 (m, 1 H), 3.80–3.60 (m, 2 H), 3.58–3.46 (m, 1 H), 2.46–2.36 (m, 1 H), 2.40 (s, 3 H), 2.25–2.05 (m, 1 H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 159.1, 156.9, 148.3, 147.0, 141.5, 134.5, 128.6 (2C), 127.0 (2C), 126.7, 116.0, 109.3, 52.6, 46.3, 43.7, 32.9, 14.0; ESIMS m/z 307 (MH^+); HRESIMS m/z calcd for $\text{C}_{18}\text{H}_{19}\text{N}_4\text{O}$ (MH^+) 307.1559, found 307.1561; HPLC purity: 96%. (C-18 reverse phase, $\text{MeOH-H}_2\text{O}$, 85:15).

4.2.38. 3-(3-(3-Fluorophenyl)pyrrolidin-1-yl)-5-(5-methylfuran-2-yl)-1,2,4-triazine (12g)—Dark brown liquid (88%). IR (film) 2943, 2873, 1606, 1589, 1527, 1478, 785 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 8.82 (s, 1 H), 7.56–7.40 (m, 4 H), 7.25 (d, $J = 3.1$ Hz, 1 H), 6.20 (d, $J = 3.0$ Hz, 1 H), 4.32–4.21 (m, 1 H), 4.13–3.84 (m, 1 H), 3.80–3.65 (m, 1 H), 3.63–3.56 (m, 2 H), 2.52–2.44 (m, 1 H), 2.40 (s, 3 H), 2.23–2.01 (m, 1 H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 164.5, 161.2, 159.0, 156.9, 144.2, 134.6, 130.0, 122.7, 116.1, 114.1, 113.8, 113.4, 109.3, 52.3, 46.2, 43.4, 32.7, 14.0; ESIMS m/z 325 (MH^+); HRESIMS m/z calcd for $\text{C}_{18}\text{H}_{18}\text{FN}_4\text{O}$ (MH^+) 325.1465, found 325.1463; HPLC purity: 97% (C-18 reverse phase, $\text{MeOH-H}_2\text{O}$, 90:10).

4.2.39. 5-(5-Methylfuran-2-yl)-3-(3-(3-(trifluoromethyl)phenyl)pyrrolidin-1-yl)-1,2,4-triazine (12h)—Dark brown liquid (85%). IR (film) 2944, 2874, 1606, 1529, 1328, 1121 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 8.83 (s, 1 H), 7.58–7.42 (m, 4 H), 7.24 (d, $J = 3.3$ Hz, 1 H), 6.19 (d, $J = 2.7$ Hz, 1 H), 4.37–4.20 (m, 1 H), 4.15–3.88 (m, 1 H), 3.83–3.68 (m, 1 H), 3.67–3.56 (m, 2 H), 2.53–2.44 (m, 1 H), 2.41 (s, 3 H), 2.26–2.02 (m, 1 H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 159.0, 157.0, 148.2, 147.0, 142.5, 134.6, 131.0, 130.4, 129.0, 123.9, 123.8, 123.6, 116.2, 109.3, 52.4, 46.2, 43.5, 32.8, 13.9; ESIMS m/z 375

(MH⁺); HRESIMS *m/z* calcd for C₁₉H₁₈F₃N₄O (MH⁺) 375.1433, found 375.1439; HPLC purity: 98% (C-18 reverse phase, MeOH-H₂O, 85:15).

4.2.40. 3-(1-(5-(5-Methylfuran-2-yl)-1,2,4-triazin-3-yl)pyrrolidin-3-yl)benzonitrile (12i)—Dark brown liquid (93%). IR (film) 2962, 2890, 2243, 1600, 1552, 1438, 852 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.75 (s, 1 H), 7.53–7.47 (m, 3 H), 7.40–7.36 (m, 1 H), 7.18 (d, *J* = 3.3 Hz, 1 H), 6.15 (d, *J* = 3.0 Hz, 1 H), 4.30–4.10 (m, 1 H), 4.08–3.82 (m, 1 H), 3.75–3.60 (m, 1 H), 3.57–3.43 (m, 2 H), 2.44–2.35 (m, 1 H), 2.36 (s, 3 H), 2.13–2.04 (m, 1 H); ¹³C NMR (CDCl₃, 75 MHz) δ 159.0, 157.0, 148.1, 147.0, 143.2, 134.7, 131.6, 130.7, 130.4, 129.4, 118.7, 116.3, 112.5, 109.4, 52.2, 46.1, 43.2, 32.6, 14.0; ESIMS *m/z* 332 (MH⁺); HRESIMS *m/z* calcd for C₁₉H₁₈N₅O (MH⁺) 332.1511, found 332.1507; HPLC purity: 96% (C-18 reverse phase, MeOH-H₂O, 85:15).

4.2.41. 3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-5-phenyl-1,2,4-triazine (22a)—Yellow oil (30.1%). IR (film) 1535, 1478, 1309, 1018 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.03 (s, 1 H), 8.12 (d, *J* = 7.2 Hz, 2 H), 7.55–7.49 (m, 3 H), 7.31–7.18 (m, 4 H), 4.30 (brs, 1 H), 4.10 (brs, 1 H), 3.90–3.45 (brs, 3 H), 2.48 (brs, 1 H), 2.17 (brs, 1 H); ¹³C NMR (125 MHz, CDCl₃) δ 159.4, 155.2, 143.5, 136.3, 134.5, 134.4, 131.7, 129.8, 128.9, 127.3, 126.9, 125.2, 52.4, 46.4, 43.4, 32.9; ESIMS *m/z* 336.9 (MH⁺); HRESIMS *m/z* calcd for C₁₉H₁₈N₄Cl (MH⁺) 337.1220, found 337.1224; HPLC purity: 98.7% (C-18 reverse phase, MeOH-H₂O, 95:5), 99.4% (C-18 reverse phase, MeOH-H₂O, 90:10).

4.2.42. 3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-5-(3,4-dimethoxyphenyl)-1,2,4-triazine (22b)—Yellow oil (84.9%). IR (film) 2937, 1531, 1508, 1478, 1276 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.98 (s, 1 H), 7.71–7.68 (m, 2 H), 7.31–7.18 (m, 4 H), 6.96 (d, *J* = 8.6 Hz, 1 H), 4.4–3.4 (brs, 5 H), 3.96 (s, 3 H), 3.95 (s, 3 H), 2.48 (brs, 1 H), 2.17 (brs, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 159.4, 154.8, 152.3, 149.4, 143.7, 136.1, 134.4, 129.9, 127.4, 127.0, 125.3, 121.0, 111.0, 109.6, 56.0, 55.9, 52.4, 46.1, 43.5, 32.6; ESIMS *m/z* 397 (MH⁺); HRESIMS *m/z* calcd for C₂₁H₂₂N₄O₂Cl (MH⁺) 397.1431, found 397.1429; HPLC purity: 99.09% (C-18 reverse phase, MeOH-H₂O, 90:10), 99.11% (C-18 reverse phase, MeOH-H₂O, 85:15).

4.2.43. 5-(4-Bromophenyl)-3-(3-(3-chlorophenyl)pyrrolidin-1-yl)-1,2,4-triazine (22c)—Yellow glass (72%). IR (film) 1573, 1530, 1479, 1071, 1008 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.97 (s, 1 H), 7.96 (d, *J* = 8.5 Hz, 2 H), 7.61 (d, *J* = 8.5 Hz, 2 H), 7.34–7.16 (m, 4 H), 4.4–3.4 (brs, 5 H), 2.48 (brs, 1 H), 2.16 (brs, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 159.3, 154.2, 143.4, 135.7, 134.4, 133.3, 132.3, 129.9, 128.8, 127.3, 127.0, 126.6, 125.3, 52.5, 46.5, 43.4, 32.9; ESIMS *m/z* 415 (MH⁺); HRESIMS *m/z* calcd for C₁₉H₁₇N₄BrCl (MH⁺) 415.0325, found 415.0319; HPLC purity: 97.80% (C-18 reverse phase, MeOH-H₂O, 90:10), 97.24% (C-18 reverse phase, MeOH-H₂O, 95:5).

4.2.44. 5-(3,4-Methylenedioxyphenyl)-3-(3-(3-chlorophenyl)pyrrolidin-1-yl)-1,2,4-triazine (22d)—Yellow solid (67.6%): mp 169–171 °C. IR (film) 2874, 1537, 1530, 1501, 1487, 1439 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.93 (s, 1 H), 7.66–7.63 (m, 2 H), 7.30–7.17 (m, 4 H), 6.92–6.89 (m, 1 H), 6.05 (s, 2 H), 4.4–3.4 (brs, 5 H), 2.48 (brs, 1 H), 2.16 (brs, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 159.3, 154.6, 150.9, 148.6, 143.6, 135.9, 134.4, 129.9, 128.6, 127.3, 127.0, 125.3, 122.4, 108.6, 107.2, 101.8, 52.4, 46.3, 43.5, 32.8; ESIMS *m/z* 381 (MH⁺); HRESIMS *m/z* calcd for C₂₀H₁₈N₄O₂Cl (MH⁺) 381.1118, found 381.1121; HPLC purity: 98.45% (C-18 reverse phase, MeOH-H₂O, 90:10), 96.79% (C-18 reverse phase, MeOH-H₂O, 95:5).

4.2.45. 3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-5-(3-nitrophenyl)-1,2,4-triazine (22e)—Yellow oil (54.1%). IR (film) 2872, 1542, 1525, 1478, 1348, 1082 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 9.06 (s, 1 H), 8.92 (s, 1 H), 8.42–8.34 (m, 2 H), 7.70 (t, J = 8.0 Hz, 1 H), 7.29–7.18 (m, 4 H), 4.4–3.5 (brs, 5 H), 2.50 (brs, 1 H), 2.19 (brs, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 159.3, 152.7, 148.8, 143.3, 136.5, 135.6, 134.5, 132.9, 130.1, 130.0, 127.3, 127.1, 126.0, 125.3, 122.2, 52.6, 46.7 (46.2), 43.4, 32.9 (32.4); ESIMS m/z 382 (MH^+); HRESIMS m/z calcd for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_2\text{Cl}$ (MH^+) 382.1071, found 382.1078; HPLC purity 97.10% (C-18 reverse phase, MeOH- H_2O , 90:10), 98.31% (C-18 reverse phase, MeOH- H_2O , 85:15).

4.2.46. 3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-5-(4-nitrophenyl)-1,2,4-triazine (22f)—Yellow oil (47.4%). IR (film) 2873, 1542, 1478, 1346, 1070, 858 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 9.07 (s, 1 H), 8.35 (d, J = 8.5 Hz, 2 H), 8.28 (d, J = 8.5 Hz, 2 H), 7.31–7.18 (m, 4 H), 4.4–3.4 (brs, 5 H), 2.51 (brs, 1 H), 2.22 (brs, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 159.3, 152.8, 149.7, 143.3, 140.6, 136.0, 134.5, 130.0, 128.3, 127.3, 127.1, 125.3, 124.1, 52.6, 46.2, 43.4, 32.9, 32.4; ESIMS m/z 382 (MH^+); HRESIMS m/z calcd for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_2\text{Cl}$ (MH^+) 382.1071, found 382.1075; HPLC purity: 95.06% (C-18 reverse phase, MeOH- H_2O , 90:10).

4.2.47. 3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-5-(5-(3-nitrophenyl)furan-2-yl)-1,2,4-triazine (29a)—Light yellow solid (63.5%): mp 206–208 $^\circ\text{C}$. IR (film) 2924, 1552, 1524, 1477, 1347 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 9.02 (s, 1 H), 8.58 (d, J = 1.8 Hz, 1 H), 8.21–8.18 (m, 1 H), 8.12–8.09 (m, 1 H), 7.65 (t, J = 8.0 Hz, 1 H), 7.47 (d, J = 3.6 Hz, 1 H), 7.32–7.19 (m, 4 H), 7.02 (d, J = 3.6 Hz, 1 H), 4.3–3.4 (brs, 5 H), 2.50 (brs, 1 H), 2.18 (brs, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 159.1, 154.4, 150.5, 148.7, 146.5, 143.5, 134.6, 131.1, 130.1, 129.9, 129.8, 127.3, 127.1, 125.3, 123.0, 119.2, 116.6, 110.0, 52.5, 46.5, 43.5, 32.7; ESIMS m/z 448 (MH^+); HRESIMS m/z calcd for $\text{C}_{23}\text{H}_{19}\text{ClN}_5\text{O}_3$ (MH^+) 448.1176, found 448.1182; HPLC purity: 97.5% (C-18 reverse phase, MeOH- H_2O , 90:10), 95.6% (C-18 reverse phase, MeOH).

4.2.48. 3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-5-(5-(2-(trifluoromethyl)phenyl)furan-2-yl)-1,2,4-triazine (29b)—Yellow solid (80.8%): mp 131–133 $^\circ\text{C}$. IR (film) 2873, 1530, 1478, 1312, 1130 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 8.96 (s, 1 H), 7.80 (d, J = 8.4 Hz, 2 H), 7.63 (t, J = 7.6 Hz, 1 H), 7.50 (t, J = 7.6 Hz, 1 H), 7.43 (d, J = 3.5 Hz, 1 H), 7.30–7.17 (m, 4 H), 6.87 (d, J = 3.5 Hz, 1 H), 4.4–3.4 (brs, 5 H), 2.46 (brs, 1 H), 2.13 (brs, 1 H); ^{13}C NMR (125 MHz, CDCl_3) δ 159.0, 154.0, 150.3, 146.7, 143.6, 134.7, 134.4, 131.9, 130.1, 129.8, 128.8, 128.2, 127.3, 127.1, 126.9, 126.8, 126.6 (CF_3), 125.2, 124.8 (CF_3), 122.6 (CF_3), 120.5 (CF_3), 116.0, 112.6, 52.3, 46.3, 43.4, 32.9; ESIMS m/z 471 (MH^+); HRESIMS m/z calcd for $\text{C}_{24}\text{H}_{19}\text{N}_4\text{OCIF}_3$ (MH^+) 471.1200, found 471.1193; HPLC purity: 97.9% (C-18 reverse phase, MeOH- H_2O , 90:10), 95.5% (C-18 reverse phase, MeOH- H_2O , 95:5).

4.2.49. Methyl 3-(5-(3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-1,2,4-triazin-5-yl)furan-2-yl)benzoate (29c)—Light yellow solid (66%): mp 169–171 $^\circ\text{C}$. IR (film) 1722, 1526, 1477, 1297, 1248 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 9.01 (s, 1 H), 8.41 (s, 1 H), 8.03–7.95 (m, 2 H), 7.53 (t, J = 7.8 Hz, 1 H), 7.44 (d, J = 3.6 Hz, 1 H), 7.31–7.18 (m, 4 H), 6.93 (d, J = 3.6 Hz, 1 H), 4.4–3.5 (brs, 5 H), 3.97 (s, 3 H), 2.48 (m, 1 H), 2.15 (brs, 1 H); ^{13}C NMR (125 MHz, CDCl_3) δ 166.4, 159.1, 156.1, 149.7, 146.7, 143.6, 134.6, 134.4, 130.9, 129.8, 129.5, 129.0, 128.4, 127.3, 127.0, 125.5, 125.2, 116.6, 108.7, 52.4, 52.3, 46.3, 43.4, 32.8; ESIMS m/z 461 (MH^+); HRESIMS m/z calcd for $\text{C}_{25}\text{H}_{22}\text{ClN}_4\text{O}_3$ (MH^+) 461.1380, found 461.1374; HPLC purity: 95.8% (C-18 reverse phase, MeOH- H_2O , 90:10).

4.2.50. 3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-5-(3'-nitrobiphenyl-4-yl)-1,2,4-triazine (29d)—Yellow oil (56.2%). IR (film) 2925, 1530, 1477, 1349, 1308 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 9.06 (s, 1 H), 8.47 (s, 1 H), 8.24–8.23 (m, 3 H), 7.95 (d, $J = 7.6$ Hz, 1 H), 7.75 (d, $J = 7.7$ Hz, 2 H), 7.64 (t, $J = 8.0$ Hz, 1 H), 7.31–7.19 (m, 4 H), 4.4–3.5 (brs, 5 H), 2.50 (brs, 1 H), 2.17 (brs, 1 H); ^{13}C NMR (125 MHz, CDCl_3) δ 159.4, 154.3, 148.7, 143.5, 141.7, 141.5, 136.1, 134.6, 134.4, 132.9, 130.0, 128.1, 127.7, 127.3, 127.0, 125.2, 122.7, 121.9, 53.4, 46.5, 46.1 (conformer), 43.4, 32.9, 32.4 (conformer); ESIMS m/z 458 (MH^+); HRESIMS m/z calcd for $\text{C}_{25}\text{H}_{21}\text{ClN}_5\text{O}_2$ (MH^+) 458.1384, found 458.1389; HPLC purity: 95.6% (C-18 reverse phase, MeOH- H_2O , 90:10).

4.2.51. General Procedure for the Synthesis of 12j–m, 22g–h—Palladium on carbon (10%, 42.4 mg) was added to a solution of **12b–e**, **22e–f** (0.243 mmol) in methanol (6 mL) and ethyl acetate (1.5 mL). The reaction mixture was vigorously stirred at room temperature under atmospheric pressure of hydrogen overnight. The catalyst was removed by filtration and the filtrate was concentrated and further purified by silica gel column chromatography, eluting with hexane-ethyl acetate (1:1).

4.2.52. 3-(1-(5-(5-Methylfuran-2-yl)-1,2,4-triazin-3-yl)pyrrolidin-3-yl)aniline (12j)—Yellow oil (26%). IR (film) 3490, 3310, 2920, 2870, 1540, 1505, 854 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 8.83 (s, 1 H), 7.23 (d, $J = 3.3$ Hz, 1 H), 7.13 (t, $J = 7.8$ Hz, 1 H), 6.72 (d, $J = 7.8$ Hz, 1 H), 6.64–6.57 (m, 2 H), 6.21 (d, $J = 2.7$ Hz, 1 H), 4.40–3.90 (m, 2 H), 3.85–3.52 (m, 2 H), 3.50–3.41 (m, 1 H), 2.44–2.33 (m, 1 H), 2.44 (s, 3 H), 2.29–2.08 (m, 1 H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 159.1, 156.9, 148.3, 147.0, 146.6, 142.8, 134.4, 129.5, 117.2, 116.0, 113.7, 113.5, 109.3, 52.5, 46.3, 43.7, 32.8, 14.0; ESIMS m/z 322 (MH^+); HRESIMS m/z calcd for $\text{C}_{18}\text{H}_{19}\text{N}_5\text{O}$ (MH^+) 322.1668, found 322.1664; HPLC purity: 97% (C-18 reverse phase, MeOH- H_2O , 85:15).

4.2.53. 3-(1-(5-(5-Methylfuran-2-yl)-1,2,4-triazin-3-yl)pyrrolidin-3-yl)phenol (12k)—Yellow foam (64.6%). IR (film) 3127, 2966, 2946, 2874, 1602, 1555, 1533, 1478, 1453 cm^{-1} ; ^1H NMR (300 MHz, methanol- d_4) δ 8.77 (s, 1 H), 7.39 (d, $J = 3.3$ Hz, 1 H), 7.14 (t, $J = 7.8$ Hz, 1 H), 6.79 (d, $J = 7.8$ Hz, 1 H), 6.76 (s, 1 H), 6.66 (d, $J = 7.9$ Hz, 1 H), 6.32 (d, $J = 2.8$ Hz, 1 H), 4.13–4.07 (m, 1 H), 3.91–3.87 (m, 1 H), 3.69–3.49 (m, 3 H), 2.43 (s, 3 H), 2.41 (brs, 1 H), 2.18–2.15 (m, 1 H); ^{13}C NMR (125 MHz, methanol- d_4) δ 158.8, 157.6, 157.2, 147.8, 143.0, 133.6, 129.1, 117.8, 116.8, 113.4, 113.2, 109.2, 52.3, 46.0, 43.7, 32.3, 12.3; ESIMS m/z 323 (MH^+); HRESIMS m/z calcd for $\text{C}_{18}\text{H}_{19}\text{N}_4\text{O}_2$ (MH^+) 323.1508, found 323.1514; HPLC purity: 95.2% (C-18 reverse phase, MeOH- H_2O , 90:10); 96.7% (C-18 reverse phase, MeOH, 100).

4.2.54. 4-(1-(5-(5-Methylfuran-2-yl)-1,2,4-triazin-3-yl)pyrrolidin-3-yl)benzene-1,2-diol (12l)—Yellow foam (33%). ^1H NMR (300 MHz, methanol- d_4) δ 8.73 (s, 1 H), 7.36 (d, $J = 3.4$ Hz, 1 H), 6.75–6.70 (m, 2 H), 6.65–6.61 (m, 1 H), 6.30 (d, $J = 3.1$ Hz, 1 H), 4.05 (brs, 1 H), 3.88 (brs, 1 H), 3.62 (brs, 1 H), 3.47–3.31 (brs, 2 H), 2.41 (s, 3 H), 2.35 (brs, 1 H), 2.09 (brs, 1 H); ^{13}C NMR (125 MHz, methanol- d_4) δ 158.7, 157.6, 147.8, 147.7, 144.9, 143.6, 133.5, 132.8, 117.8, 116.8, 114.9, 113.6, 109.2, 52.6, 46.0, 42.8, 32.6, 12.3; ESIMS m/z 339 (MH^+); HRESIMS m/z calcd for $\text{C}_{18}\text{H}_{19}\text{N}_4\text{O}_3$ (MH^+) 339.1457, found 339.1453; HPLC purity: 95.2% [C-18 reverse phase, MeOH (with 1% TFA)- H_2O , 70:30].

4.2.55. 2-Methoxy-5-(1-(5-(5-methylfuran-2-yl)-1,2,4-triazin-3-yl)pyrrolidin-3-yl)phenol (12m)—Brown oil (54%). ^1H NMR (300 MHz, CDCl_3) δ 8.81 (s, 1 H), 7.23 (d, $J = 3.0$ Hz, 1 H), 6.90 (d, $J = 1.7$ Hz, 1 H), 6.83–6.78 (m, 2 H), 6.20 (d, $J = 3.0$ Hz, 1 H), 4.3–3.4 (brs, 5 H), 3.88 (s, 3 H), 2.43 (s, 3 H), 2.40 (brs, 1 H), 2.10 (brs, 1 H); ^{13}C NMR (125

MHz, CDCl₃) δ 159.0, 156.8, 148.3, 147.0, 145.6, 145.3, 134.8, 134.4, 118.3, 116.0, 113.3, 110.6, 109.2, 55.9, 52.6, 46.4, 43.1, 32.9, 14.0; ESIMS m/z 353 (MH⁺); HRESIMS m/z calcd for C₁₉H₂₁N₄O₃ (MH⁺) 353.1614, found 353.1617; HPLC purity: 95.2% (C-18 reverse phase, MeOH-H₂O, 90:10).

4.2.56. 3-(3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-1,2,4-triazin-5-yl)aniline (22g)—

Light yellow glass (37.8%). IR (film) 3337, 3217, 1535, 1477, 1327 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.97 (s, 1 H), 7.46–7.43 (m, 2 H), 7.30–7.17 (m, 5 H), 6.86–6.83 (m, 1 H), 4.4–3.4 (brs, 7 H), 2.48 (brs, 1 H), 2.16 (brs, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 159.4, 155.4, 147.1, 143.6, 136.6, 135.5, 134.4, 129.9, 127.4, 127.0, 125.3, 118.4, 117.5, 113.3, 52.5, 46.4, 43.5, 32.8; ESIMS m/z 352 (MH⁺); HRESIMS m/z calcd for C₁₉H₁₉N₅Cl (MH⁺) 352.1329, found 352.1325; HPLC purity: 97.3% (C-18 reverse phase, MeOH-H₂O, 90:10), 98.0% (C-18 reverse phase, MeOH-H₂O, 85:15).

4.2.57. 4-(3-(3-(3-Chlorophenyl)pyrrolidin-1-yl)-1,2,4-triazin-5-yl)aniline (22h)—

Yellow solid (68.6%): mp 179–181 °C. IR (film) 3332, 3211, 1605, 1522, 1477, 1313, 1294 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 8.91 (s, 1 H), 7.96 (d, J = 8.5 Hz, 1 H), 7.30–7.17 (m, 4 H), 6.72 (d, J = 8.5 Hz, 1 H), 4.4–3.4 (brs, 7 H), 2.45 (brs, 1 H), 2.14 (brs, 1 H); ¹³C NMR (125 MHz, CDCl₃) δ 159.3, 154.9, 150.2, 143.7, 135.8, 134.3, 129.8, 129.0, 127.3, 126.9, 125.2, 123.6, 114.6, 52.3, 46.2, 43.4, 32.7; ESIMS m/z 352 (MH⁺); HRESIMS m/z calcd for C₁₉H₁₉N₅Cl (MH⁺) 352.1329, found 352.1330; HPLC purity: 97.5% (C-18 reverse phase, MeOH-H₂O, 90:10), 98.8% (C-18 reverse phase, MeOH-H₂O, 85:15).

4.2.58. 9-(1-(5-(5-Methylfuran-2-yl)-1,2,4-triazin-3-yl)pyrrolidin-3-yl)-9H-purin-6-amine (17)—

A solution of **15**⁶⁴ (80 mg, 0.26 mmol) in 20% (v/v) TFA in CH₂Cl₂ (5 mL) was stirred for one day. The solvent was evaporated, the crude oil was mixed with triethylamine (200 μ L, 1.43 mmol), and then a solution of **5** (39.7 mg, 0.166 mmol) in dry DMF (2 mL) was added. The mixture was stirred under argon at room temperature for 3 days. Solvents were evaporated and the residue was further purified by silica gel preparative TLC, eluting with MeOH-CH₂Cl₂, 1:9, to provide the product as light yellow solid (33.2 mg, 56.4%): mp 173–175 °C. IR (KBr) 3326, 3178, 2924, 1648, 1598, 1532, 1509, 1478, 1252 cm⁻¹; ¹H NMR (300 MHz, CDCl₃ and methanol-*d*₄) δ 8.63 (s, 1 H), 8.06 (s, 1 H), 7.76 (s, 1 H), 7.12 (d, J = 3.3 Hz, 1 H), 6.06 (d, J = 3.0 Hz, 1 H), 5.19–5.15 (m, 1 H), 4.04–4.02 (m, 2 H), 3.78–3.73 (m, 2 H), 2.50–2.41 (m, 2 H), 2.23 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃ and methanol-*d*₄) δ 158.9, 157.7, 155.4, 152.4, 149.1, 147.6, 147.4, 138.1, 134.8, 118.9, 117.3, 109.5, 53.6, 50.8, 44.4, 30.8, 13.5; ESIMS m/z 386 (MNa⁺); HRESIMS m/z calcd for C₁₇H₁₇N₉ONa (MNa⁺) 386.1454, found 386.1452; HPLC purity: 99.1% (C-18 reverse phase, MeOH-H₂O, 90:10).

4.2.59. General Procedure for the Synthesis of 25a–b and 25d—Mixtures of the aryl bromides **24** or **30** (2.65 mmol), the boronic acids **23a** or **23b** (2.23 mmol), tetrabutylammonium bromide (796 mg, 2.47 mmol), and K₂CO₃ (2.9 g, 21 mmol) were suspended in distilled water (10 mL) under argon for 20 min. Then Pd(OAc)₂ (9.2 mg, 0.04 mmol) was added, and the resulting suspensions were heated to 70 °C and stirred under argon for 3 h. After cooling down, the solutions were diluted with distilled water (10 mL) and extracted with CH₂Cl₂ (3 $\times$ 15 mL). The combined organic layers were dried over Na₂SO₄ and the solvents were evaporated. The crude residues were further purified by silica gel column chromatography, eluting with hexane-ethyl acetate 8:2, to provide the products **25a–b** or **25d**.

4.2.60. 1-(5-(3-Nitrophenyl)furan-2-yl)ethanone (25a)⁸⁰—Yellow solid (32.5%): mp 112–113 °C (lit.⁸⁰ 116–117 °C). ¹H NMR (300 MHz, CDCl₃) δ 8.55 (t, J = 1.9 Hz, 1 H),

8.19–8.15 (m, 1 H), 8.10–8.07 (m, 1 H), 7.61 (t, $J = 8.0$ Hz, 1 H), 7.28 (d, $J = 3.7$ Hz, 1 H), 6.93 (d, $J = 3.7$ Hz, 1 H), 2.54 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 186.4, 154.6, 152.6, 148.6, 130.9, 130.3, 130.1, 123.4, 119.6, 119.2, 109.3, 26.1.

4.2.61. 1-(5-(2-(Trifluoromethyl)phenyl)furan-2-yl)ethanone (25b)⁸⁰—Light yellow oil (49.6%). ^1H NMR (300 MHz, CDCl_3) δ 7.71–7.67 (m, 2 H), 7.54–7.50 (m, 1 H), 7.43–7.39 (m, 1 H), 7.18 (dd, $J = 1.1, 3.6$ Hz, 1 H), 6.72 (d, $J = 3.6$ Hz, 1 H), 2.44 (s, 3 H).

4.2.62. 1-(3'-Nitrobiphenyl-4-yl)ethanone (25d)⁸¹—White crystals (96.5%): mp 109–110 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.43 (t, $J = 1.9$ Hz, 1 H), 8.22–8.18 (m, 1 H), 8.05 (d, $J = 8.3$ Hz, 2 H), 7.95–7.91 (m, 1 H), 7.69 (d, $J = 8.3$ Hz, 2 H), 7.63 (t, $J = 8.0$ Hz, 1 H), 2.63 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 197.4, 148.7, 142.9, 141.4, 136.7, 133.1, 130.0, 129.1, 128.9, 127.3, 122.8, 122.0, 26.7.

4.2.63. Methyl 3-(5-Acetylfuran-2-yl)benzoate (25c)⁸²—A mixture of 1-(5-bromofuran-2-yl)ethanone (**24**, 1.57 g, 8.3 mmol), 3-(methoxycarbonyl)phenylboronic acid (**23c**, 1.71 g, 9.5 mmol), (2-biphenyl)dicyclohexylphosphine (0.29 g, 0.83 mmol) and K_3PO_4 (7.10 g, 33.5 mmol) was suspended in toluene (36 mL) under argon for 20 min. Then $\text{Pd}(\text{OAc})_2$ (114.5 mg, 0.51 mmol) was added, and the resulting suspension was heated to 90 °C and stirred under argon overnight in a sealed tube. After cooling down, the solvent was evaporated. The crude residue was further purified by silica gel column chromatography, eluting with hexane-ethyl acetate 8:2, to provide the product as light yellow solid (1.00 g, 49%): mp 117–118 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.38 (t, $J = 1.5$ Hz, 1 H), 8.01–7.93 (m, 2 H), 7.49 (t, $J = 7.8$ Hz, 1 H), 7.25 (d, $J = 3.6$ Hz, 1 H), 6.84 (d, $J = 3.6$ Hz, 1 H), 3.93 (s, 3 H), 2.52 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 186.3, 166.4, 156.4, 152.1, 130.9, 129.9, 129.6, 129.0, 128.9, 125.9, 119.3, 108.2, 52.3, 26.0.

4.3 *M. tuberculosis* MABA and LORA Assay

Detailed experimental procedures for *M. tuberculosis* MABA and LORA assay can be found in our previous published papers.⁸³

4.4 Activity against MRSA and *Bacillus* Strains

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of PPase inhibitors were tested in triplicate against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Staphylococcus aureus* (VRSA), and *Bacillus* strains [*Bacillus anthracis* Sterne strain (34F2) and *Bacillus subtilis*] in the micro dilution broth method, using Muller Hinton broth (MHB) and 96 well-plates according to Clinical and Laboratory Standards (CLSI).⁸⁴ The bacterial cultures were incubated at 37 °C for 20 h.

4.5 Cytotoxicities of Inorganic Pyrophosphatase Inhibitors In Vitro

Inorganic pyrophosphatase inhibitors were assayed at concentrations of 8 μM , 16 μM , 32 μM , and 64 μM against human tumor cell line HeLa and murine macrophage cell line (J774.A1) to determine the potential toxic effect in mammalian cells.⁸⁵ Briefly, $\sim 2 \times 10^4$ cells suspended in 200 μL of DMEM supplemented with 10% fetal bovine serum (FBS), L-glutamine, NaHCO_3 , pyridoxine-HCl, and 45,000 mg/L glucose and preserved with 1% penicillin-streptomycin solution were seeded in 96-well plates and incubated at 37 °C in a 5% CO_2 atmosphere. The cells were cultured for 24 h (60% confluency) before the assays. The cells were further incubated with 8 μM , 16 μM , 32 μM , and 64 μM of compounds **22g**, **22a**, **22h**, **12k**, **22b** and DMSO as a control for 2 h. The culture media was discarded, and the cells in each well were washed with PBS and 100 μL of cell culture media were added

prior to addition of the assay reagent MTS3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (Promega). The plates were incubated for 4 h at 37 °C in a humidified 5% CO₂ atmosphere. The absorbance at 490 nm was recorded using a 96-well Elisa plate reader (SoftMax ProInc., USA). Corrected absorbance readings (actual absorbance readings for each treatment subtracted from background absorbance) were taken using a kinetic ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA). The quantity of viable cells after treatment with each compound was expressed as a percentage of the control, DMSO.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was made possible by the National Institutes of Health (NIH) through support with Research Grant UO1AI077949. This work was supported in part by an award from the Floss Endowment, provided to the department of Medicinal Chemistry and Molecular Pharmacology.

References

1. Harold FM. Bacteriol Rev. 1966; 30:772. [PubMed: 5342521]
2. Kajander T, Kellosalo J, Goldman A. FEBS Lett. 2013; 587:1863. [PubMed: 23684653]
3. Heinonen, JK. Biological Role of Inorganic Pyrophosphate. Kluwer Academic Publishers, Inc; Boston: 2001.
4. Orimo H, Ohata M, Fujita T. Endocrinology. 1971; 89:852. [PubMed: 4327778]
5. Poole KES, Reeve J. Curr Opin Pharmacol. 2005; 5:612. [PubMed: 16181808]
6. Ko KM, Lee W, Yu JR, Ahnn J. FEBS Lett. 2007; 581:5445. [PubMed: 17981157]
7. Usui Y, Uematsu T, Uchihashi T, Takahashi M, Takahashi M, Ishizuka M, Doto R, Tanaka H, Komazaki Y, Osawa M, Yamada K, Yamaoka M, Furusawa K. Journal of Dent Res. 2010; 89:504. [PubMed: 20332330]
8. Rodina EV, Vainonen LP, Vorobyeva NN, Kurilova SA, Sitnik TS, Nazarova TI. Biochemistry-Moscow. 2008; 73:897. [PubMed: 18774936]
9. Ahn S, Milner AJ, Futterer K, Konopka M, Ilias M, Young TW, White SA. J Mol Biol. 2001; 313:797. [PubMed: 11697905]
10. Merckel MC, Fabrichniy IP, Salminen A, Kalkkinen N, Baykov AA, Lahti R, Goldman A. Structure. 2001; 9:289. [PubMed: 11525166]
11. Heikinheimo P, Lehtonen J, Baykov A, Lahti R, Cooperman BS, Goldman A. Structure. 1996; 4:1491. [PubMed: 8994974]
12. Baykov AA, Kasho VN, Bakuleva NP, Rea PA. FEBS Lett. 1994; 350:323. [PubMed: 8070586]
13. Lin SM, Tsai JY, Hsiao CD, Huang YT, Chiu CL, Liu MH, Tung JY, Liu TH, Pan RL, Sun YJ. Nature. 2012; 484:399. [PubMed: 22456709]
14. Salminen T, Kapyla J, Heikinheimo P, Kankare J, Goldman A, Heinonen J, Baykov AA, Cooperman BS, Lahti R. Biochemistry. 1995; 34:782. [PubMed: 7827037]
15. Kellosalo J, Kajander T, Kogan K, Pokharel K, Goldman A. Science. 2012; 337:473. [PubMed: 22837527]
16. Baykov AA, Tamviloslado JJ, Avaeva SM. Biochem Biophys Acta. 1979; 569:228. [PubMed: 476127]
17. Baykov AA, Alexandrov AP, Smirnova IN. Arch Biochem Biophys. 1992; 294:238. [PubMed: 1312806]
18. Baykov AA, Artjukov AA, Avaeva SM. Biochem Biophys Acta. 1976; 429:982. [PubMed: 5150]
19. Baykov AA, Bakuleva NP, Nazarova TI, Avaeva SM. Biochem Biophys Acta. 1977; 481:184. [PubMed: 843537]

20. Baykov AA, Artjukov AA, Avaeva SM. *Biochem Biophys Acta*. 1977; 481:195. [PubMed: 843538]
21. Baykov AA, Dubnova EB, Bakuleva NP, Evtushenko OA, Zhen RG, Rea PA. *FEBS Lett*. 1993; 327:199. [PubMed: 8392953]
22. Samygina VR, Moiseev VM, Rodina EV, Vorobyeva NN, Porov AN, Kurilova SA, Nazarova TI, Avaeva SM, Bartunik HD. *J Mol Biol*. 2007; 366:1305. [PubMed: 17196979]
23. Pohjanjoki P, Fabrichniy IP, Kasho VN, Cooperman BS, Goldman A, Baykov AA, Lahti R. *J Biol Chem*. 2001; 276:434. [PubMed: 11031269]
24. Baykov AA, Volk SE, Unguryte A. *Arch Biochem Biophys*. 1989; 273:287. [PubMed: 2549870]
25. Avaeva SM, Vorobyeva NN, Kurilova SA, Nazarova TI, Polyakov KM, Rodina EV, Samygina VR. *Biochemistry (Moscow, Russ Fed)*. 2000; 65:373.
26. Davidson AM, Halestrap AP. *Biochem J*. 1989; 258:817. [PubMed: 2543362]
27. Maeshima M. *Eur J Biochem*. 1991; 196:11. [PubMed: 1848180]
28. Eaton RH, Moss DW. *Biochem J*. 1967; 102:917. [PubMed: 16742510]
29. Martin H, Grant BR, Stehmann C. *Pestic Biochem and Physio*. 1998; 61:65.
30. Grigorieva OV, Mit'kevich VA, Sklyankina VA, Avaeva SM. *Russ J Bioorg Chem*. 2001; 27:27.
31. Zyryanov AB, Lahti R, Baykov AA. *Biochemistry-Moscow*. 2005; 70:908. [PubMed: 16212547]
32. Smirnova IN, Kudryavtseva NA, Komissarenko SV, Tarusova NB, Baykov AA. *Arch Biochem Biophys*. 1988; 267:280. [PubMed: 2848451]
33. Gordon-Weeks R, Parmar S, Davies TGE, Leigh RA. *Biochem J*. 1999; 337:373. [PubMed: 9895279]
34. Kelly SJ, Feldman F, Sperow JW, Butler LG. *Biochemistry*. 1973; 12:3338. [PubMed: 4354609]
35. Woltgens JHM, Bervoets TJM, Devries W. *J Periodont Res*. 1977; 12:462. [PubMed: 145485]
36. Kotsikourou E, Song YC, Chan JMW, Faelens S, Tovian Z, Broderick E, Bakalara N, Docampo R, Oldfield E. *J Med Chem*. 2005; 48:6128. [PubMed: 16162013]
37. Hsu MT, Sturr MG, Curran TM, Marquis RE. *Oral Microbiol Immunol*. 1995; 10:47. [PubMed: 7644273]
38. Zhen RG, Baykov AA, Bakuleva NP, Rea PA. *Plant Physiol*. 1994; 104:153. [PubMed: 12232069]
39. Yatsimirsky KB, Manorik PA, Gaivoronskaya GG, Lopatina OI, Davidenko NK, Komissarenko SV. *Dopovidi Akademii Nauk Ukrainskoi Rsr Seriya B-Geologichni Khimichni Ta Biologichni Nauki*. 1983; 11:55.
40. Yang SJ, Jiang SS, Tzeng CM, Kuo SY, Hung SH, Pan RL. *Biochem Biophys Acta*. 1996; 1294:89. [PubMed: 8639720]
41. Zhen RG, Kim EJ, Rea PA. *J Biol Chem*. 1997; 272:22340. [PubMed: 9268385]
42. GordonWeeks R, Steele SH, Leigh RA. *Plant Physiol*. 1996; 111:195. [PubMed: 12226285]
43. Yang SJ, Jiang SS, Van RC, Hsiao YY, Pan RL. *Biochem Biophys Acta*. 2000; 1460:375. [PubMed: 11106777]
44. Kim EJ, Zhen RG, Rea PA. *J Biol Chem*. 1995; 270:2630. [PubMed: 7852329]
45. Nordlie RC, Lardy HA. *Biochem Biophys Acta*. 1961; 53:309. [PubMed: 14480309]
46. Shoyab M, Marx W. *Arch Biochem Biophys*. 1971; 146:71. [PubMed: 4335488]
47. Burobin AV, Lomonova MV, Sklyankina VA, Avaeva SM. *Bioorg Khim*. 1997; 23:104. [PubMed: 9157843]
48. Hirono M, Ojika M, Mimura H, Nakanishi Y, Maeshima M. *J Biochem*. 2003; 133:811. [PubMed: 12869538]
49. Kuznetsov AV, Sklyankina VA, Avaeva SM. *Bioorganicheskaya Khimiya*. 1979; 5:1396.
50. Tian Y, Suk DH, Cai F, Crich D, Mesecar AD. *Biochemistry*. 2008; 47:12434. [PubMed: 18973344]
51. Dawane BS, Kadam SN, Shaikh BM. *Pharm Lett*. 2010; 2:126.
52. Chohan ZH, Farooq MA. *J Chem Soc Pak*. 1995; 17:14.
53. Chohan ZH, Pervez H, Rauf A, Khan KM, Maharvi GM, Supuran CT. *J Enzyme Inhib Med Chem*. 2004; 19:161. [PubMed: 15449731]

54. Sawant RL, Ramdin SS, Rathi SR. *Int J PharmTech Res.* 2012; 4:1096.
55. Patel RV, Kumari P, Rajani DP, Pannecouque C, De Clercq E, Chikhaliya KH. *Future Med Chem.* 2012; 4:1053. [PubMed: 22709250]
56. Sun XB, Cao ZB, Sun YY. *Ind Eng Chem Res.* 2009; 48:607.
57. Aly AA. *Curr Org Chem.* 2009; 13:1758.
58. Hajbi Y, Suzenet F, Khouili M, Lazar S, Guillaumet G. *Tetrahedron.* 2007; 63:8286.
59. Wu W, Sil D, Szostak ML, Malladi SS, Warshakoon HJ, Kimbrell MR, Cromer JR, David SA. *Bioorg Med Chem.* 2009; 17:709. [PubMed: 19064323]
60. Gullo, JMH.; WP; Wayner, RJ.; Moser, RE. Diamond Shamrock Corporation; U.S: 1979.
61. Alphonse FA, Suzenet F, Keromnes A, Lebreton B, Guillaumet G. *Synthesis-Stuttgart.* 2004:2893.
62. Breukelman SP, Meakins GD, Roe AM. *J Chem Soc, Perkin Trans 1.* 1985:1627.
63. Chang CY, Kuo SC, Lin YL, Wang JP, Huang LJ. *Bioorg Med Chem Lett.* 2001; 11:1971. [PubMed: 11454460]
64. Kocalka P, Pohl R, Rejman D, Rosenberg I. *Tetrahedron.* 2006; 62:5763.
65. Collins LA, Franzblau SG. *Antimicrob Agents Chemother.* 1997; 41:1004. [PubMed: 9145860]
66. Falzari K, Zhu ZH, Pan DH, Liu HW, Hongmanee P, Franzblau SG. *Antimicrob Agents and Chemother.* 2005; 49:1447. [PubMed: 15793125]
67. Jayaprakash S, Iso Y, Wan B, Franzblau SG, Kozikowski AP. *ChemMedChem.* 2006; 1:593. [PubMed: 16892398]
68. Bate AB, Kalin JH, Fooksman EM, Amorose EL, Price CM, Williams HM, Rodig MJ, Mitchell MO, Cho SH, Wang YH, Franzblau SG. *Bioorg Med Chem Lett.* 2007; 17:1346. [PubMed: 17188865]
69. Tammenkoski M, Benini S, Magretova NN, Baykov AA, Lahti R. *J Bio.* 2005; 280:41819.
70. Gasteiger, E.; Hoogland, C.; Gattiker, A.; Duvaud, S.; Wilkins, MR.; Appel, RD.; Bairoch, A. *The Proteomics Protocols Handbook.* Walker, JM., editor. Humana Press; 2005. p. 571
71. Baykov AA, Evtushenko OA, Avaeva SM. *Anal Biochem.* 1988; 171:266. [PubMed: 3044186]
72. Leete E, Bodem GB. *J Am Chem Soc.* 1976; 98:6321.
73. Pegan SD, Tian Y, Sereshon V, Mesecar AD. *Comb Chem High Throughput Screen.* 2010; 13:27. [PubMed: 20201823]
74. Heilman WP, Heilman RD, Scozzie JA, Wayner RJ, Gullo JM, Ariyan ZS. *J Pharm Sci.* 1980; 69:282. [PubMed: 7381702]
75. Pellegatti L, Vedrenne E, Leger JM, Jarry C, Routier S. *Tetrahedron.* 2010; 66:4383.
76. Citterio A, Cominelli A, Bonavoglia F. *Synthesis-Stuttgart.* 1986:308.
77. Kamlet MJ. *J Am Chem Soc.* 1955; 77:4896.
78. Lange J, Lapszewicz J. *Acta Poloniae Pharmaceutica.* 1978; 35:289. [PubMed: 696359]
79. Lapszewicz J, Lange J, Mazur W. *Acta Poloniae Pharmaceutica.* 1981; 38:269.
80. Obushak ND, Gorak YI, Matiichuk VS, Lytvyn RZ. *Russ J Org Chem.* 2008; 44:1689.
81. Hebel A, Haag R. *J Org Chem.* 2002; 67:9452. [PubMed: 12492353]
82. Haddach M, Michaux J, Schwaebé MK, Pierre F, O'Brien SE, Borsan C, Tran J, Raffaele N, Ravula S, Drygin D, Siddiqui-Jain A, Darjania L, Stansfield R, Proffitt C, Macalino D, Streiner N, Bliesath J, Omori M, Whitten JP, Anderes K, Rice WG, Ryckman DM. *ACS Med Chem Lett.* 2012; 3:135.
83. Zhao Y, Bacher A, Illarionov B, Fischer M, Georg G, Ye QZ, Fanwick PE, Franzblau SG, Wan B, Cushman M. *J Org Chem.* 2009; 74:5297. [PubMed: 19545132]
84. NCCLS Approved Standard M7-A9. 9. NCCLS; Wayne, Pa: 2012. NCCLS: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.
85. Mosmann T. *J Immunol Methods.* 1983; 65:55. [PubMed: 6606682]

Figure 1. MTS assays showing the percent mean absorbance at 490 nm after incubating J774A.1 cells (A) and HeLa cells (B) with compounds **22g**, **22a**, **22h**, **12k**, **22b**, and DMSO at 64 μ M for 2 hours. DMSO was used as a control.

Scheme 1.

Reagents and conditions: (a) SeO₂, dioxanes, H₂O; (b) NaHCO₃, EtOH, H₂O; (c) *m*-CPBA, CH₂Cl₂.

Scheme 2.

Reagents and conditions: (a) diethyl malonate, piperidine, toluene; (b) KCN, EtOH, H₂O; (c) HCl, H₂O or KOH, EtOH; (d) urea, xylene; (e) BH₃, THF; (f) dry DMF; (g) H₂, Pd/C, MeOH, AcOEt.

Scheme 3.

Reagents and conditions: (a) MsCl, pyridine, DCM; (b) adenine, Cs₂CO₃, DMSO; (c) TFA, DCM; (d) NEt₃, DMF.

Scheme 4.

Reagents and conditions: (a) SeO₂, dioxanes, H₂O; (b) **3**, NaHCO₃, EtOH, H₂O; (c) *m*-CPBA, CH₂Cl₂; (d) dry DMF, (e) H₂, Pd/C, MeOH, AcOEt.

Scheme 5.

Reagents and conditions: (a) Pd(OAc)₂, K₂CO₃, (tBu)₄N⁺ Br⁻, H₂O; (b) Pd(Ph₃P)₄, K₃PO₄, Toluene; (c) SeO₂, dioxanes, H₂O; (d) **3**, NaHCO₃, EtOH, H₂O; (e) *m*-CPBA, CH₂Cl₂; (f) dry DMF.

Scheme 6.

Reagents and conditions: (a) $\text{Pd}(\text{OAc})_2$, K_2CO_3 , $(t\text{Bu})_4\text{N}^+ \text{Br}^-$, H_2O ; (b) SeO_2 , dioxanes, H_2O ; (c) **3**, NaHCO_3 , EtOH , H_2O ; (d) *m*-CPBA, CH_2Cl_2 ; (e) dry DMF.

Table 1

The Inhibitory Activity against MtPPase and the Minimum Inhibitory Concentration (MIC) against *Mycobacterium tuberculosis*.

Compound	%I (100 μ M)	IC ₅₀ (μ M)	MIC (μ M)	
			MABA ^c	LORA ^d
12a	45.6 $\pm$ 3.9	147.5 $\pm$ 69	61.0 $\pm$ 0.5	58.8 $\pm$ 1.8
12b	N.T. ^a		> 16	> 16
12c	73.2 $\pm$ 1.2	63.2 $\pm$ 6.2	N.T. ^e	N.T. ^e
12f	19.7 $\pm$ 1.4		40.5 $\pm$ 10.6	54.7 $\pm$ 0.9
12g	21.3 $\pm$ 1.6		38.9 $\pm$ 11.1	47.9 $\pm$ 5.5
12h	37.8 $\pm$ 0.7	354.5 $\pm$ 79	52.4 $\pm$ 6.7	57.6 $\pm$ 0.0
12i	10.3 $\pm$ 1.0		64.4 $\pm$ 1.3	50.3 $\pm$ 2.0
12j	33.4 $\pm$ 0.7		70.1 $\pm$ 11.2	47.1 $\pm$ 3.1
12k	N.A. ^b		60.3 $\pm$ 1.3	58.9 $\pm$ 1.8
12l	N.A. ^b		> 128	> 128
12m	5.0 $\pm$ 0.0		> 128	> 128
17	N.A. ^b		> 128	> 128
22a	50.0 $\pm$ 2.2	101.5 $\pm$ 20	32.0 $\pm$ 1.2	53.6 $\pm$ 2.1
22b	58.8 $\pm$ 0.7	53.2 $\pm$ 7.4	15.4 $\pm$ 0.0	127.4
22c	53.0 $\pm$ 1.0	33.3 $\pm$ 3.6	30.7 $\pm$ 1.0	77.7 $\pm$ 11.5
22d	N.T. ^a		> 16	> 16
22e	39.4 $\pm$ 0.4	118.2 $\pm$ 16.4	> 128	> 128
22f	30.3 $\pm$ 0.9		31.2 $\pm$ 1.2	> 128
22g	53.4 $\pm$ 0.2	57.4 $\pm$ 7.1	29.7 $\pm$ 0.6	56.3 $\pm$ 0.1
22h	46.6 $\pm$ 0.0	88.1 $\pm$ 11.1	5.7 $\pm$ 2.2	> 128
29a	N.T. ^a		> 16	> 16
29b	67.7 $\pm$ 2.3	38.3 $\pm$ 9.7	> 128	> 128
29c	N.T. ^a		> 16	> 16
29d	N.A. ^b		> 128	> 128
rifampin			0.08 $\pm$ 0.02	0.24 $\pm$ 0.01
isoniazid			0.45 $\pm$ 0.03	> 256
nitroimidazopyran			0.44 $\pm$ 0.02	6.83 $\pm$ 0.79

^aThe inhibitory activity could not be tested because of poor compound solubility in DMSO (< 100 μ M).

^bNot active. No detectable inhibition.

^cMABA = microplate Alamar Blue assay.

^dLORA = low oxygen recovery assay (luciferase readout).

^eNot submitted for testing.

Table 2

The Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentrations (MBC) against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus* species.

Compound	MRSA (MIC ^a , μM)	<i>Bacillus anthracis</i> (MIC/MBC, μM)	<i>Bacillus subtilis</i> (MIC/MBC, μM)
12k	> 50	64/128	128/128
22a	> 50	16/128	16/>128
22b	> 50	16/>128	16/>128
22g	30	32/128	32/>128
22h	16/>256	16/128	
Vancomycin	0.5	<1/<1	<1/<1

^aThe highest value of triplicate MIC determinations is reported.

Table 3

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Compound **22h** Against *S. aureus* MRSA, VISA and VRSA Clinical Isolates

Strain name	NARSA ^a Strain ID	Origin	Phenotypic Properties	MIC ^b /MBC (μM)
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) ATCC 4330	-	USA	Resistant to methicillin and oxacillin	16/>256
Vancomycin-intermediate <i>Staphylococcus aureus</i> (VISA) ATCC 700699	NRS1	Japan	Resistant to aminoglycosides and tetracycline (minocycline) glycopeptide-intermediate	16/>256
Vancomycin-intermediate <i>Staphylococcus aureus</i> (VISA) HIP07256	NRS19	USA	Resistant to oxacillin, tetracycline and ciprofloxacin. glycopeptide- intermediate	16/>256
Vancomycin-intermediate <i>Staphylococcus aureus</i> (VISA) LIM 3	NRS37	France	Resistant to aminoglycosides, tetracycline, and ciprofloxacin. glycopeptide-intermediate	16/>256
Linezolid-resistant <i>Staphylococcus aureus</i> SA LinR12	NRS119	USA	Resistant to ciprofloxacin, oxacillin and linezolid	8/>256
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) USA 100	NRS382	USA	Resistant to ciprofloxacin, clindamycin, erythromycin, and oxacillin	16/>256
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) USA 200	NRS383	USA	Resistant to ciprofloxacin, clindamycin, erythromycin, gentamicin, and oxacillin	16/>256
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) USA 300	NRS384	USA	Resistant to erythromycin, oxacillin, and tetracycline	16/>256
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) USA 500	NRS385	USA	Resistant to ciprofloxacin, clindamycin, erythromycin, gentamicin, oxacillin, tetracycline, and trimethoprim	16/>256
Vancomycin-resistant <i>Staphylococcus aureus</i> (VRSA)	VRS10	USA	Resistant to oxacillin, ciprofloxacin, clindamycin, erythromycin, gentamicin, and vancomycin	16/>256

^aNARSA = Network on Antimicrobial Resistance in *Staphylococcus aureus*.

^bThe highest value of triplicate MIC determinations is reported.