ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Thiazolotriazoles As Anti-infectives: Design, Synthesis, Biological Evaluation and *In Silico* Studies

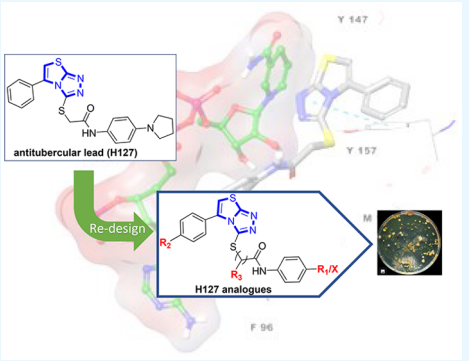
Umadevi Kizhakke Purakkel, Ganji Praveena, Valli Y. Madabhushi, Surender Singh Jadav, Reddy Shetty Prakasham, Saiprasad Goud Dasugari Varakala, Dharmarajan Sriram, Ewan W. Blanch, and Subashani Maniam*

Cite This: *ACS Omega* 2024, 9, 8846−8861

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The rational design of novel thiazolo[2,3-*c*][1,2,4]triazole derivatives was carried out based on previously identified antitubercular hit molecule H127 for discovering potent compounds showing antimicrobial activity. The designed compounds were screened for their binding efficacies against the antibacterial drug target enoyl-[acyl-carrier-protein] reductase, followed by prediction of drug-likeness and ADME properties. The designed analogues were chemically synthesized, characterized by spectroscopic techniques, followed by evaluation of antimicrobial activity against bacterial and fungal strains, as well as antitubercular activity against *M. tuberculosis* and *M. bovis* strains. Among the synthesized compounds, five compounds, **10**, **11**, **35**, **37** and **38**, revealed antimicrobial activity, albeit with differential potency against various microbial strains. Compounds **10** and **37** were the most active against *S. mutans* (MIC: 8 μg/mL), while compounds **11** and **37** showed the highest activity against *B. subtillis* (MIC: 16 μg/mL), whereas compounds **10**, **11** and **37** displayed activities against *E. coli* (MIC: 16 μg/mL). Meanwhile, compounds **10** and **35** depicted activities against *S. typhi* (MIC: 16 μg/mL) and compound **10** showed antifungal activity against *C. albicans* (MIC: 32 μg/mL). The current study has identified two broad-spectrum antibacterial hit compounds (**10** and **37**). Further structural investigation on these molecules is underway to enhance their potency.



INTRODUCTION

Heterocycles have a significant role in drug design and optimization.[1] In medicinal chemistry, *N*-heterocycles are important scaffolds as the dual nature of nitrogen (to accept or donate proton) can lead to beneficial ligand receptor interactions.[2] In 2019, the FDA approved 48 new drugs, in which 27 of the 32 small molecules contain *N*-heterocycles, justifying their relevance.[3] *N*-Heterocycles such as thiazole and triazole are privileged scaffolds in drug discovery programs, with diverse pharmacological activity.[4,5] Similarly, thiazole or triazole fused with other heterocycles have been investigated against various diseases.[6,7] The condensed heterocycle of thiazole and triazole is termed as thiazolotriazole and exists in three isomeric forms: thiazolo[2,3-*c*][1,2,4]triazole, thiazolo-[3,2-*b*][1,2,4]triazole and isothiazolo[3,2-*c*][1,2,4]triazole.[8] A wide range of pharmacological activities like anticancer,[9−11] antimicrobial,[12−17] anti-inflammatory,[18] analgesic,[18] antidiuretic,[19] and COX_2 inhibition[19] were reported for thiazolotriazoles; major efforts in research were focused on antimicrobial drug design, with the [3,2-*b*] ring system being widely investigated when compared to the [2,3-*c*] ring system (Figure 1); however, the proven or suggested mode of action or drug targets are not well established for any of the thiazolotriazole-based antimicrobials. In antitubercular drug research, the thiazolotriazoles have not been widely investigated (Figure 1).

Our group had earlier performed phenotypic screening of chemically diverse compounds from an internal database and identified 12 hits against *M. tuberculosis* H37Rv. H127 (Figure 1) was one of the identified hits, having a thiazolo[2,3-*c*][1,2,4]triazole (TTz) scaffold with an MIC of 12.5 μM against *M. tuberculosis* H37Rv. The drug target or mode of action has not been established for H127.[21]

Antimicrobial resistance is an ongoing threat to human health.[22] Specifically, the bacterial resistance acquired by chromosomal mutations and by various other bacterial mechanisms has resulted in the emergence of monodrug/multidrug/extensive-drug resistance to highly efficient antibiotics.[23,24] To combat this existing and emerging antimicrobial resistance, as well as the drug toxicity and long treatment durations associated with many antimicrobial medications,

Received: August 25, 2023
Revised: December 3, 2023
Accepted: December 14, 2023
Published: February 19, 2024

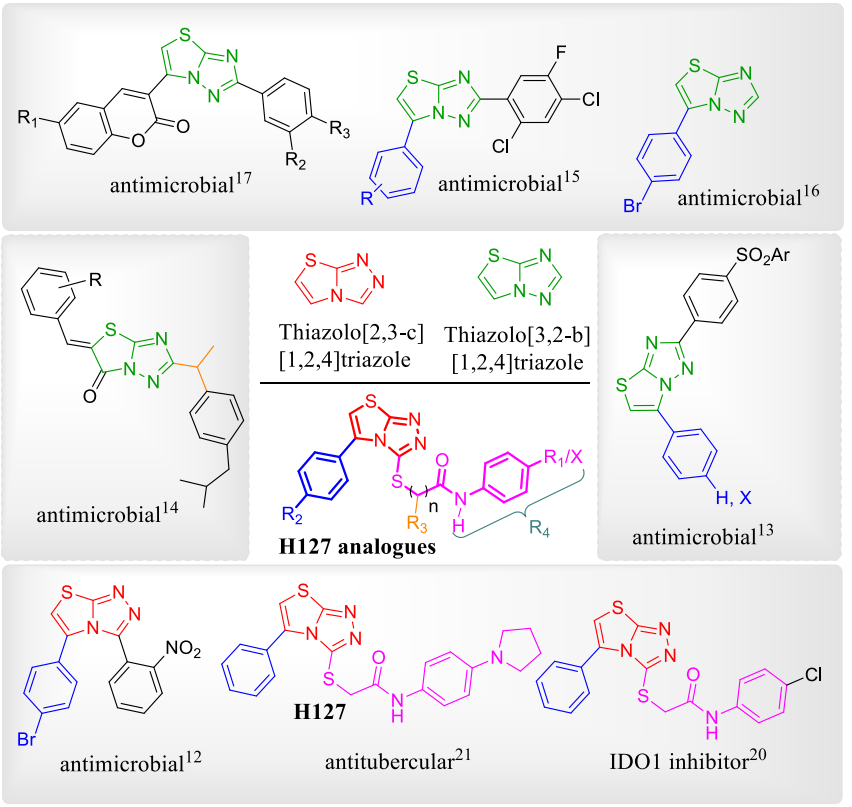


Figure 1. Representative biologically active thiazolotriazoles. Reprinted with permission from [12]. Copyright [2013] John Wiley & Sons. Reprinted with permission from [13]. Copyright [2009] Elsevier. Reprinted with permission from [14]. Copyright [2010] De Gruyter. Reprinted with permission from [15]. Copyright [2009] Elsevier. Reprinted with permission from [16]. Copyright [2015] John Wiley & Sons. Reprinted with permission from [17]. Copyright [2012] NIScPR Publications. Reproduced with permission from [20]. Copyright [2020] American Chemical Society. Reprinted with permission from [21]. Copyright [2015] PLOS.

potent novel compounds with robust safety and remarkable chemotherapeutic efficacy are in high demand.[25,26]

In view of the existing challenges, H127 was chosen as a potential hit for antimicrobial studies. Here, we report the design, synthesis, and biological evaluation of H127 analogues. The individual, as well as the combined, effects of the stated modifications (Figure 2(a) and (b)) on the structure−activity relationship (SAR) with respect to antibacterial and antitubercular activity were evaluated and can serve as a guide for future discovery and development of antimicrobials based on the TTz scaffold. *In silico* studies were conducted to predict the pharmacokinetics and drug target of the investigated compounds.

RESULTS AND DISCUSSION

Rational Design and *In Silico* Analysis. Analogues of H127 (**1**) were designed (Table 1) based on structural modifications as shown in Figure 2(a) and (b). The TTz core was kept unchanged, and variations were introduced at the lateral chain attached to the triazole and the aryl group attached to thiazole ring of TTz. Initially, the modulation of *N*-amide substituents (R_4 and X) was our focus (series 1: compounds **2**−**4**). The relevance of 4-(pyrrolidin-1-yl) aniline with respect to biological activity was assessed by introducing groups (R_4) like an aliphatic amine (butylamine) and an amino acid (L-alanine methyl ester), resulting in compounds **2** and **3**, respectively. Also, to investigate whether a halogen atom (X) serves better than pyrrolidine, compound **4** was designed.

Second, the diversity of *N*-heterocycles (R_1) was explored by replacing the pyrrolidine with heterocycles such as piperidine, piperazine, morpholine, thiomorpholine, and 1*H*-imidazol-1-yl and with an aliphatic amine, diethylamine (series 2: compounds **5**−**11**). These pharmacophoric units are prevalent in the fluoroquinolone, oxazolidinone and nitroimidazole classes of antibiotics, with the structural unit having a significant role in inhibiting bacteria.[27−29] In addition to diverse pharmacophoric applications, they also exhibit favorable pharmacokinetics and target interactions, making them commonly employed pharmacophores in medicinal chemistry.[30−34]

Next, the modification of the carbon linker attached between the thiotriazole and the amide group (n and R_3) was carried out (series 3: compounds **12**−**23**). The modification of the carbon chain of a biologically active molecule significantly affects biological activity as seen from the literature.[35,36] In this series, the impact of variation of alkyl chain length (n) and steric factors (R_3) along with R_1 modulation were assessed. Presumably, the additional methylene group provides flexibility for internal rotations (compounds **12**−**16**), while the methyl (compounds **17**−**21**) and cyclobutane (compounds **22** and **23**) groups cause restricted molecular rotations.

Finally, for investigating the electronic effects of phenyl substituents (R_2), electron donating and withdrawing groups, such as OMe, CF_3 and F, were incorporated into the phenyl ring attached to the TTz heterocycle (series 4: compounds **24**−**40**). Incorporation of F and CF_3 to a biologically active molecule may enhance its pharmacological effect and

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

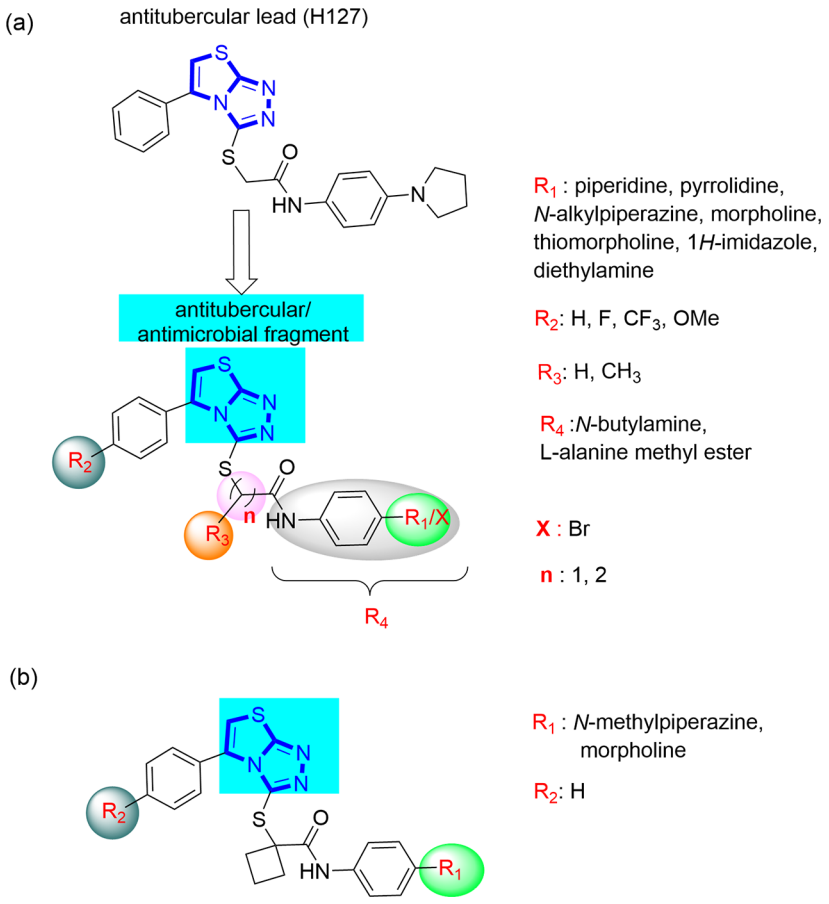


Figure 2. (a, b) Design of H127 analogues (compounds **2**–**40**).

physicochemical properties.[37] Additionally, OMe, CF_3 and F moieties incorporated into TTz were reported to show activity against various bacterial strains.[13,15,16,38] In this series, for each R_2 substituent, R_1 was also varied. All compounds were subjected to docking studies to understand ligand receptor interactions.

For the docking studies, the antibacterial drug target, enoyl-[acyl-carrier-protein] reductase (FabI) of *S. aureus* (PDB: 4FS3), was chosen for the designed H127 analogues to understand ligand–receptor interactions. The enoyl-[acyl-carrier-protein] reductase, FabI, catalyzes the last step of fatty acid synthesis in bacteria.[39] FabI is present in Gram-positive bacteria, Gram-negative bacteria, mycobacteria and protozoa; many compounds are reported to target this enzyme, making it a promising target for antimicrobial agents.[40−42] Heterocyclic compounds containing thiazole and thiadiazole moiety were found to inhibit enoyl-[acyl-carrier-protein] reductase enzymes.[43,44] Thiazolotriazoles (compounds **2**–**40**) were designed and synthesized based on a hit compound, H127, which showed promising activity against *M. tuberculosis* while the mode of action or specific drug targets for thiazolotriazole-based antimicrobials have not been reported until now in the literature. The structural similarities of synthesized thiazolotriazoles and H127, as well as pharmacophoric considerations, led to FabI being the chosen putative target for ligand–receptor interactions. The docking validation by redocking cocrystal ligands was performed, and the root-mean-square deviation (RMSD) of the redocked ligands was found to be less than 1 Å. The dock score and binding energy of the compounds against the proposed target was determined (see Figure S1, Supporting Information). Compounds **10**, **11**, **37**, **20**, and **4** were observed to be the best with the lowest binding energies of −63.83, −63.11, −62.55, −60.89, and −60.67 kcal/mol, respectively. The docking image of compound **10**, having the highest binding affinity, is presented in Figure 3 where the molecule was interacting through F96 and Y157 amino acid residues at the binding site.

Physicochemical Properties. Lipinski's rule of five is considered as the benchmark for determining whether a compound or drug may be orally bioavailable. Those compounds that violate more than one criterion of this rule are less likely to be successful in drug development due to permeability or solubility issues.[45] In view of this, the drug likenesses of the designed compounds were determined *in silico*. Most of the compounds presented here satisfy this rule (Table 1). Absorption–distribution–metabolism–excretion (ADME) parameters are significant in determining the safety profile of a drug.[46] The ADME parameters for H127 analogues were studied using *in silico* techniques. The compounds exhibit favorable ADME properties as the parameters studied here are within the range (see Table S1, Supporting Information).

Chemistry. Based on the promising results from the docking and physicochemical property analysis, the 40 compounds (Table 1) including the parent compound, H127 (**1**), were synthesized following the synthetic routes depicted in Schemes 1(a), 1(b), and 2. Appropriate commercially available phenacyl bromides, **I(a–d)**, were treated with thiosemicarbazide, **II**, to give the corresponding hydrazines, **III(a–d)**, and were converted to thiols, **IV(a–d)**, using CS_2 and KOH under reflux, followed by acidification. Esters, **V(a–**

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

Table 1. Synthesized Compounds and Their Predicted (*In Silico*) Physicochemical Properties Using Schrödinger QikProp Software[a]

Cpd[b]	n	R_1/X	R_2	R_3/ cyclobutane	R_4	MW	HBD	HBA	Log *Po*/w	RO5
1	1	pyrrolidine	-	H	-	435.56	1	5.5	5.5	1
2	1	-	H	H	-NH-$(CH_2)_3$-CH_3	449.58	1	5.5	5.4	1
3	1	-	H	H	L-alanine methyl ester	464.6	1	7.5	4.46	0
4	1	Br	H	H		451.56	1	7.2	4.66	0
5	1	piperidine	H	H	-	467.62	1	6	5.53	1
6	1	*N*-methylpiperazine	H	H	-	437.57	1	5.5	5.58	1
7	1	morpholine	H	H	-	478.62	1	7.5	4.88	0
8	1	thiomorpholine	H	H	-	432.51	1	6	4.79	0
9	1	*N*,*N*-diethylamine	H	H	-	465.58	1	6.25	4.93	0
10	1	*N*-ethylpiperazine	H	H	-	479.61	1	6.25	5.36	1
11	1	1*H*-imidazol-1-yl	H	H	-	494.62	1	8.25	4.6	0
12	2	pyrrolidine	H	H	-	481.58	1	7.95	4.18	0
13	2	piperidine	H	H	-	497.64	1	6.75	5.62	1
14	2	*N*-methylpiperazine	H	H	-	467.6	1	6.25	5.52	1
15	2	morpholine	H	H	-	453.55	1	5.5	5.74	1
16	2	thiomorpholine	H	H	-	467.57	1	5.5	5.63	1
17	1	pyrrolidine	H	methyl	-	482.59	1	7.5	4.73	0
18	1	piperidine	H	methyl	-	469.55	1	7.2	4.89	0
19	1	*N*-methylpiperazine	H	methyl	-	485.61	1	6	5.62	1
20	1	morpholine	H	methyl	-	455.56	1	5.5	5.81	1
21	1	thiomorpholine	H	methyl	-	503.55	1	5.5	6.47	2
22	1	*N*-methylpiperazine	H	cyclobutane	-	517.58	1	5.5	6.71	2
23	1	morpholine	H	cyclobutane	-	532.6	1	7.5	5.45	2
24	1	pyrrolidine	OMe	H	-	519.55	1	7.2	5.62	2
25	1	piperidine	OMe	H	-	535.61	1	6	6.71	2
26	1	*N*-methylpiperazine	OMe	H	-	449.58	1	5.5	5.28	1
27	1	morpholine	OMe	H	-	463.61	1	5.5	5.73	1
28	1	thiomorpholine	OMe	H	-	478.62	1	7.5	4.82	0
29	1	*N*,*N*-diethylamine	OMe	H	-	465.58	1	7.2	4.25	0
30	1	pyrrolidine	CF_3	H	-	481.64	1	6	6.04	1
31	1	piperidine	CF_3	H	-	449.58	1	5.5	5.46	1
32	1	*N*-methylpiperazine	CF_3	H	-	463.61	1	5.5	5.39	1
33	1	morpholine	CF_3	H	-	478.62	1	7.5	4.96	0
34	1	thiomorpholine	CF_3	H	-	465.58	1	7.2	5.24	1
35	1	pyrrolidine	F	H	-	481.64	1	6	5.96	1
36	1	piperidine	F	H	-	504.66	1	7.5	5.13	2
37	1	*N*-methylpiperazine	F	H	-	491.62	1	7.2	5.28	1
38	1	morpholine	F	H	-	445.35	1	4.5	5.19	1
39	1	thiomorpholine	F	H	-	346.46	1	4.5	3.21	0
40	1	*N*,*N*-diethylamine	F	H	-	376.44	0.25	5.75	3.09	0

[a]MW: molecular weight, HBD: hydrogen bond donor, HBA: hydrogen bond acceptor, RO5: number of violations of Lipinski rule. [b]Compound is abbreviated as Cpd.

g) were synthesized by alkylation of thiols, **IV(a−d)**, with different bromoalkyl esters, and further hydrolysis of **V(a−g)** gave the corresponding acids, **VI(a−g)**. The nitro compounds **VII(i−p)** upon reduction gave anilines, **VIII(i−p)**.[47] The coupling of acids, **VI(a−g)**, with anilines, **VIII(i−p)**, afforded the target compounds **1**, **5−40** (Scheme 1(a) and (b)). Compounds **2−4** were prepared by coupling acid, **VI(a)**, with *N*-butylamine, L-alanine methyl ester and 4-bromoaniline as shown in Scheme 2. All synthesized compounds (Table 1) were characterized by ^{1}H and ^{13}C NMR (see Figures S8−S130), IR and HRMS. The purity of each compound was determined by HPLC or LCMS. The analogues synthesized were then subjected to biological studies.

Biological Studies. *Antimicrobial Activity.* All compounds were screened against two Gram-positive bacterial strains: *Bacillus subtilis* (*B. subtilis*, MTCC 736) and *Streptococcus mutans* (*S. mutans*, MTCC 497); two Gram-negative bacterial strains: *Escherichia coli* (*E. coli*, MTCC 40) and *Salmonella typhi* (*S. typhi*, MTCC 3216); and a fungal strain *Candida albicans* (*C. albicans*, MTCC 1637) to determine their antimicrobial activity. The cultures were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. Initially, the zones of inhibition of the compounds were determined by the agar well diffusion method (Table 2), and those having an inhibition range of 14 mm or greater were selected for dose response tests. The minimum inhibitory concentration (MIC) of these compounds (Table 2) was determined by the broth dilution method against the mentioned bacterial and fungal strains. The results were compared with the standard drugs fluconazole and streptomycin.

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

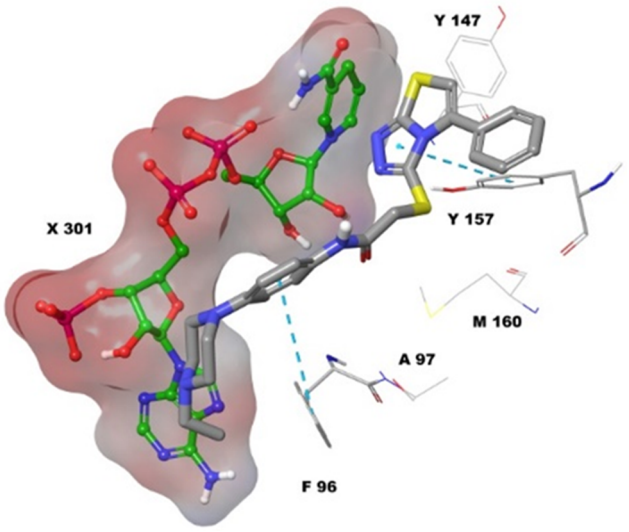


Figure 3. Docked pose obtained for compound **10** in the active site of FabI enzyme of *S. aureus* (PDB: 4FS3). Carbon atoms of the ligand are presented in gray, nitrogen in blue, sulfur in yellow and oxygen in red. For the enzyme, only the main interacting amino acids are presented, as well as 3′NADPH is presented on the surface. Dashed blue lines represent $\pi-\pi$ interactions.

Initially, the parent compound (H127) was tested for antimicrobial activity and was inactive against all strains. From the first series, compounds **2** and **4** showed a considerable zone of inhibition, but their MIC values were not significant. In the second series (compounds **5**−**11**), the compounds containing *N*-ethylpiperazine (**10**, MIC: 8−32 μg/mL) and 1*H*-imidazol-1-yl (**11**, MIC: 16−32 μg/mL) were active against the tested bacteria; with the highest activity being exhibited by compound **10** against *S. mutans* (8 μg/mL). The antibacterial activity of the compounds from the third series (compounds **12**−**23**) was insignificant. From the fourth series (compounds **24**−**40**), none of the OMe derivatives (compounds **24**−**29**) were active. With respect to CF_3 derivatives (compounds **30**−**34**), the one having a pyrrolidine group (compound **30**) showed activity against all bacteria (MIC: 64 μg/mL). All fluoro derivatives (compounds **35**-**40**) except compound **36** were active; the MIC values range from 8 to 128 μg/mL. Among them, compound **37** having an *N*-methylpiperazine showed the highest activity against *S. mutans* (MIC: 8 μg/mL). With respect to antifungal activity, compound **10** showed the highest activity (MIC: 32 μg/mL), while the others had MIC values ranging from 64 to 128 μg/mL.

Out of the 40 synthesized compounds, ten of them exhibited antimicrobial activity (Table 2). From these results, it is evident that compounds **10**, **11**, **35**, **37** and **38** exhibited significant antimicrobial activity. Compounds **10** and **37** showed the highest activity and were most potent against *S. mutans*, with MIC values of 8 μg/mL in both cases, which is double the concentration of the standard drug streptomycin (MIC: 4 μg/mL). For other strains, the most potent compounds were **11** and **37** against *B. subtillis* (MIC: 16 μg/mL); **10**, **11** and **37** against *E. coli* (MIC: 16 μg/mL); **10** and **35** against *S. typhi* (MIC: 16 μg/mL); and **10** against *C. albicans* (MIC: 32 μg/mL).

The activity results agree well with the docking studies that the most potent compounds have the lowest binding energies. The docking of compounds against FabI of *S. aureus* indicates that Tyr157 and Phe96 were the essential residues at the 4FS3 active site involved in $\pi-\pi$ interactions with the triazole and benzene ring attached to the amide groups, respectively (Figure 3, see Figures S2−S6, Supporting Information) and may be responsible for the activity recorded. The active compounds were oriented at the active site such that the TTz heterocycle points toward the tyrosine residues enabling $\pi-\pi$ interactions. The antimicrobial activity of compounds investigated here is expected to be due to the inhibition of FabI associated with bacterial fatty acid biosynthesis. The inactive compounds have a different orientation at the 4FS3 active site, making π bonding difficult (see Figure S7, Supporting Information). This might be the reason for the loss of antibacterial potency.

Antitubercular Activity. All H127 analogues were screened *in vitro* against BCG and H37Rv strains of *M. bovis* and *M. tuberculosis*, respectively, for evaluation of antimycobacterial activity. The results were compared with standard drugs, isoniazid (inh) and rifampicin (rif). Compounds **6**, **7**, **8** and **37** inhibited the growth of *M. bovis* by 59.4, 60.06, 74.85 and 67.18%, respectively (Figure 4), at a concentration of 30 μM, and for the remaining compounds, the inhibition was less than 50% (see Table S2, Supporting Information). For *M. tuberculosis*, only compounds **6** and **37** showed some activity (MIC: 50 μM); others had MIC values greater than 100 μM. Overall, the H127 analogues were far less potent than the parent compound **1**; hence, they are less likely to be antitubercular agents.

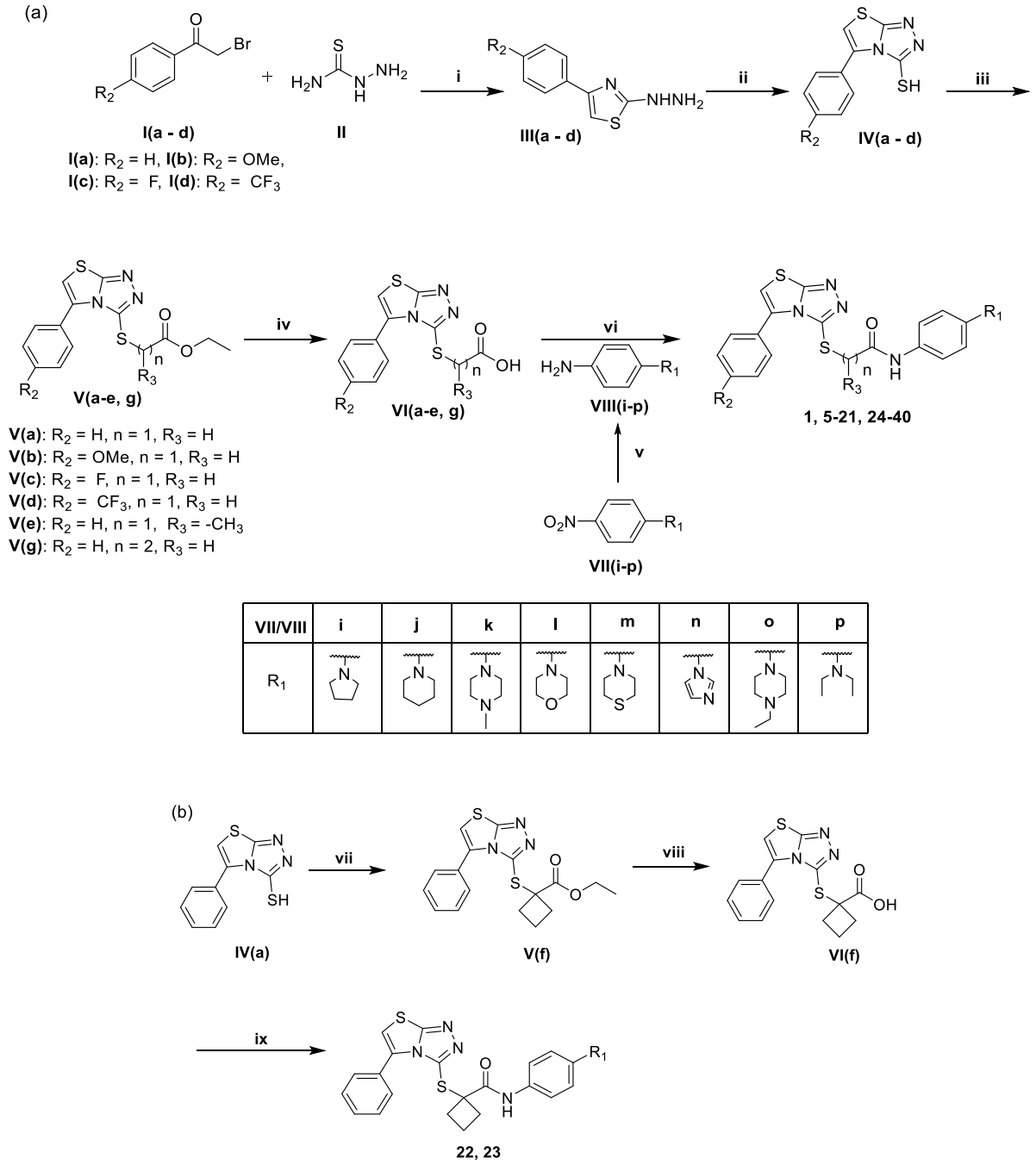
Antiproliferative Activity. All H127 analogues (compounds **2**−**40**) were screened *in vitro* against various cancer cell lines by resazurin assay. The percentage inhibition of compounds was determined at 10 μM against five cancer cell lines. The compounds possess very low percentages of inhibition against the tested cell lines, indicating that their anticancer activities are not significant (see Table S3, Supporting Information).

Structure−Activity Relationships. Based on the biological activity of compounds **1**−**40**, a structure−activity relationship (SAR) responsible for antimicrobial and antitubercular activity is derived (Figure 5). For antimicrobial activity, an increase in linker chain length (n = 2) and R_3 modifications did not have a positive impact on activity. Some positive activity was rendered by compounds with R_4 as butylamine and X as bromine atom. Electron donating groups (such as OMe) were not tolerated, while hydrogen and electron withdrawing groups (particularly 4-fluoro) were preferred as R_2 substituents. Regarding R_1 substituents, only *N*-methyl or ethyl piperazines, pyrrolidine, morpholine, and 1*H*-imidazol-1-yl moieties render activity in the presence of the appropriate R_2 group. Of these, the *N*-alkyl piperazines were found to be the best. Thiomorpholine and diethylamine groups also exhibit activity, but to a much lesser extent than the previously mentioned R_1 substituents. In general, the enhanced antibacterial activity is attributed due to *N*-ethylpiperazine and *N*-methylpiperazine with phenyl or fluorophenyl groups (compounds **10** and **37**).

For antitubercular activity (*M. tuberculosis*), the parent compound was found to be better than the analogues. The R_1, R_2, R_3, R_4, n and X modulations failed to deliver potent compounds. R_1 as pyrrolidine, R_2 and R_3 as H, R_4 as 4-pyrrolidinylaniline, and n = 1 are the structural requirements for optimal antitubercular activity when both the parent compound and its analogues are considered (Figure 5). The possible reasons for the reduced antitubercular activity of the analogues might be due to the electronic effect of R_2

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

Scheme 1. (a) Synthesis of Compounds 1, 5−21, and 24−40[a]



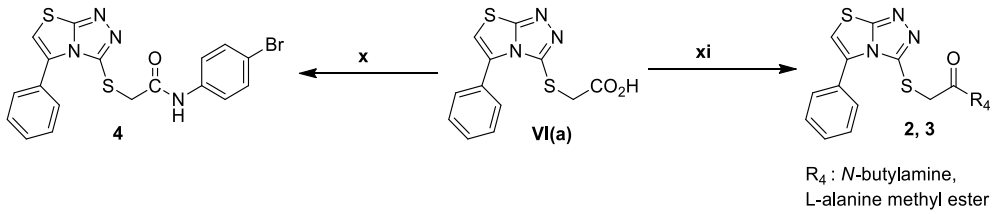
[a](i) MeOH, 70 °C, 2 h, yield: 80−90%. (ii) (a) CS_2, KOH, MeOH, 60 °C, 4 h; (b) 1 N HCl, yield: 30−40%. (iii) bromoalkylester, K_2CO_3, acetone, 60 °C, 4 h, yield: 70−80%. (iv) (a) aq. KOH, EtOH, 80 °C, 1 h; (b) 1 N HCl. For **VI(g)**: dioxane, 1 N HCl, 100 °C, overnight, yield: 80−90%. (v) Pd/C, MeOH, EtOAc (1:1), H_2, RT, yield: 50−60%. (vi) **VIII(i−p)**, EDC·HCl, HOBT, DIPEA, DMF, RT, 12 h, yield: up to 80%. (b) Synthesis of compounds **22** and **23**. (vii) Ethyl 1-bromocyclobutanecarboxylate, K_2CO_3, acetone, 60 °C, 4 h, yield: 78%. (viii) Aq. KOH, EtOH, 80 °C, 1 h, 1 N HCl; yield: 69%. (ix) **VIII(k,l)**, EDC·HCl, HOBT, DIPEA, DMF, RT, 12 h, yield: 70−75%.

substituents influencing the polarity and interaction of the TTz heterocycle at the active site. The aromaticity or basicity of the R_1 substituents might also have an impact on the antitubercular activity.

The study performed here describes synthesis and biological evaluation of 40 compounds designed based on previously reported antitubercular hit, H127. *In vitro* antimicrobial screening identified ten compounds that were active against the investigated strains; five of them (compounds **10**, **11**, **35**, **37** and **38**) showed antimicrobial activity, and two of them (compounds **10** and **37**) exhibited noteworthy activity against *S. mutans*. The antimicrobial activity of the compounds investigated here is proposed to be due to the inhibition of FabI associated with bacterial fatty acid biosynthesis. Docking studies suggest the active compounds were binding at the 4FS3 active site through amino acid residues Tyr157 and Phe96. An

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

Scheme 2. Synthesis of Compounds 2−4[a]



[a](x) 4-Bromoaniline, EDC·HCl, HOBT, DIPEA, DMF, RT, 12 h, yield: 60%; (xi) R_4, EDC·HCl, HOBT, DIPEA, DMF, RT, 12 h, yield: 60−75%.

Table 2. Antimicrobial Activity of Synthesized Compounds

	MIC[a] (μg/mL) and zone of inhibition[b] (mm)				
	Gram +ve species		Gram −ve species		Fungi
Cpd[c]	*B. subtilis*	*S. mutans*	*E. coli*	*S. typhi*	*C. albicans*
2	128 (14)	128 (15)	128 (14)	128 (15)	64 (16)
4	128 (14)	128 (15)	128 (15)	128 (14)	128 (15)
10	32 (18)	8 (26)	16 (21)	16 (20)	32 (18)
11	16 (19)	16 (19)	16 (19)	32 (18)	64 (17)
30	64 (16)	64 (16)	64 (17)	64 (17)	128 (15)
35	32 (18)	16 (21)	32 (18)	16 (20)	64 (16)
37	16 (19)	8 (24)	16 (21)	32 (18)	64 (16)
38	64 (17)	16 (20)	32 (18)	32 (18)	64 (16)
39	64 (17)	64 (16)	128 (15)	128 (15)	64 (16)
40	128 (15)	64 (16)	128 (15)	64 (16)	128 (14)
Std[d]	4 (30)	4 (30)	4 (30)	4 (30)	8 (24)

[a]Expressed outside parentheses. [b]Expressed inside parentheses. [c]Compound is abbreviated as Cpd. [d]Streptomycin for bacterial and fluconazole for fungal strains.

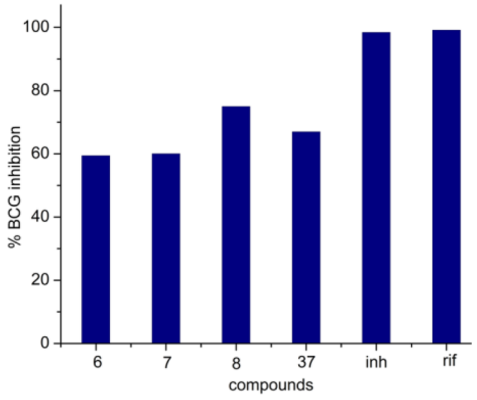


Figure 4. Percentage inhibition of compounds against BCG.

alternate enoyl reductase encoding gene, FabK, is present in some bacterial species, including *streptococcus*.[39,48,49] FabK is also involved in fatty acid synthesis.[50] At this stage, it is difficult to justify whether the activity of the compounds is solely due to FabI inhibition or an additional target is also involved, and this should be validated by enzyme studies, which are not conducted here. At this stage, we could only propose that the active compounds target FabI of enoyl-[acyl-carrier-protein] reductase, which is associated with bacterial fatty acid biosynthesis. When compared to H127, the antitubercular activity of analogues was significantly less.

CONCLUSION

In summary, the design, synthesis and biological evaluation of 2-[(5-arylthiazolo[2,3-*c*][1,2,4]triazol-3-yl)thio]-*N*-[(4-substituted)aryl]acetamides have been described here. All synthesized compounds were characterized by ^{1}H and ^{13}C NMR, IR and HRMS. *In vitro* screening was performed against Gram-positive bacteria, Gram-negative bacteria and fungal strains to determine antimicrobial activity. Antimycobacterial activity was assessed against *M. tuberculosis* H37Rv and *M. bovis* BCG strains for antitubercular activity. Five compounds, **10**, **11**, **35**, **37**, and **38**, showed notable antimicrobial activity, of which **10** and **37** were most potent against *S. mutans* (MIC: 8 μg/mL); **11** and **37** were most potent against *B. subtillis* (MIC: 16 μg/mL); **10**, **11** and **37** were most potent against *E. coli* (MIC: 16 μg/mL); **10** and **35** were most potent against *S. typhi* (MIC: 16 μg/mL); and **10** was most potent against *C. albicans* (MIC: 32 μg/mL). The antitubercular activities of the H127 analogues were not significant when compared to the parent compound **1**. The investigated compounds are expected to show drug-like characteristics as indicated from *in silico* ADME studies. The active compounds might be targeting the enoyl-[acyl-carrier-protein] reductase (FabI) involved in bacterial fatty acid synthesis, as shown in the docking studies. Thus, the current study has identified two broad-spectrum antimicrobial hit compounds (**10** and **37**) based on the TTz scaffold. Further structural investigation on these molecules is underway for enhancing their potency.

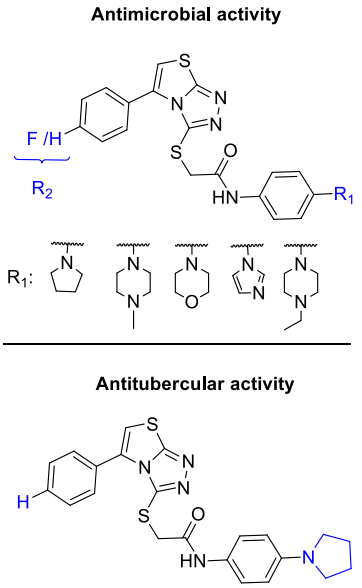


Figure 5. SAR representation of compounds with enhanced antimicrobial and antitubercular activity.

EXPERIMENTAL SECTION

Molecular Docking Protocol. The three-dimensional protein template structure with accession number 4FS3 (1.80

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

Å resolution) was taken as the target for the current set of antimicrobial agents (https://www.rcsb.org/structure/4fs3). The obtained protein template was prepared for docking by following the protein preparation and minimization protocol of Schrödinger's LLC. The chain C with cocrystal ligand AFN-1252 was employed as the binding site for the incoming ligands. The ligands were sketched using 2D-sketcher and converted into 3D form and prepared by LigPrep module. The binding of all ligands was performed by the extra precision (XP) docking protocol, and the resulting poses were analyzed by Maestro 13.1.

Typically, the binding affinity of the ligands in the pocket in this program depends on the scoring and searching algorithms implemented in the GLIDE module. The search algorithm generates combinations of protein ligand poses, studies rotatable bonds, conformational spaces, binding pattern and optimal protein−ligand interaction. The second scoring algorithm identifies better binding affinity to protein by determining energy, calculates free energy, and ranks the poses according to free energy by rescoring phenomenon. The OPLS force field in the GLIDE module performs the functions of whole search and scoring related sorting of docked ligands in the binding pocket.

Chemistry. All starting materials and reagents used were reagent grade and were either commercially available or prepared using literature protocols as described in the relevant sections. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 and visualized by UV light. Silica gel of 100−200 mesh size was used to perform column chromatography using appropriate eluents. NMR spectra were recorded on 300, 400, or 500 MHz spectrometers for ^{1}H NMR, 75, 100, or 125 MHz spectrometers for ^{13}C NMR, and 376 MHz spectrometer for ^{19}F NMR. $CDCl_3$, DMSO-d_6, CD_3OD or a mixture of deuterated solvents was used to record the NMR spectra, and chemical shift values were assigned with respect to TMS or deuterated solvents. Chemical shift values are expressed in parts per million (ppm), and coupling constants (J) are expressed in hertz (Hz). The multiplicity of NMR signals is expressed as s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet or if multiplicity is not resolved. Melting points were recorded using Stuart SMP3 and Electro thermal IA9100 apparatus and were uncorrected. HRMS spectra were recorded using ESI-TOF techniques. IR spectra were recorded on PerkinElmer-Frontier or Bruker-alpha spectrometers. Physicochemical properties of the compounds were investigated using the QikProp program. Molecular docking studies were performed using the Schrödinger suite of software.

General Procedure for Synthesis of Hydrazines (III(a−d)). All hydrazinylthiazoles were synthesized from commercially available phenacyl bromides and thiosemicarbazide according to the procedure reported by Bhat and Holla.[51] Data collected were compared with the literature.[52−55]

General Procedure for Synthesis of Thiols (IV(a−d)). All thiols were synthesized according to literature reports.[56]

*5-Phenylthiazolo[2,3-c][1,2,4]triazole-3-thiol (**IV(a)**).* White solid (4 g, yield: 40%); mp 221−224 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.05 (s, 1H), 7.66−7.58 (m, 2H), 7.48−7.38 (m, 3H), 7.23 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 161.1, 154.6, 133.6, 130.9, 129.8, 127.6, 127.4, 115.5; ESIMS: m/z 234 $[M + H]^+$.

*(4-Methoxyphenyl)thiazolo[2,3-c][1,2,4]triazole-3-thiol (**IV(b)**).* White solid (3.5 g, yield: 35%); mp 250−253 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.02 (s, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.12 (s, 1H), 6.96 (d, J = 8.8 Hz, 2H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.1, 160.6, 154.6, 133.6, 132.5, 119.7, 114.3, 113.0, 55.7; ESIMS: m/z 263 $[M+]^+$.

*5-(4-Fluorophenyl)thiazolo[2,3-c][1,2,4]triazole-3-thiol (**IV(c)**).* White solid (4 g, yield: 40%); mp 238−241 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.05 (s, 1H), 7.70−7.64 (m, 2H), 7.29−7.21 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3 (d, J = 246.5 Hz), 161.0, 154.5, 133.4 (d, J = 8.6 Hz), 132.5, 123.9 (d, J = 2.7 Hz), 115.7, 114.7, 114.6 (d, J = 21.9 Hz), 114.4; ESIMS: m/z 252 $[M + H]^+$.

*5-(4-(Trifluoromethyl)phenyl)thiazolo[2,3-c][1,2,4]-triazole-3-thiol (**IV(d)**).* White solid (2.5 g, yield: 25%); mp 218−222 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.12 (s, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.40 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.9, 154.6, 131.9, 131.7, 131.4, 129.9 (q, J = 31.8 Hz), 124.6 (q, J = 270 Hz), 124.3 (d, J = 3.4 Hz), 117.5; ESIMS: m/z 302 $[M + H]^+$.

General Procedure for Synthesis of Esters (V(a−g)). 5-(Phenyl/4-substituted-phenyl)thiazolo[2,3-c][1,2,4]triazole-3-thiol (**IV(a-d)**, 25 mmol) was taken in a round-bottom flask to which dry acetone and K_2CO_3 (50 mmol) were added and stirred well. To this alkyl bromoacetates (25 mmol) were added carefully, and the reaction mixture was refluxed for 5−9 h. After completion of the reaction, the reaction mixture was brought to room temperature and filtered through Celite to remove K_2CO_3. The filtrate was concentrated. The residue was purified by normal column chromatography using ethyl acetate and hexane as eluents.

*Ethyl 2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-acetate (**V(a)**).* White solid (650 mg, yield: 79%); mp 122−125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57−7.47 (m, 5H), 6.75 (s, 1H), 4.15 (q, J = 7.1 Hz, 2H), 3.91 (s, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.1, 158.9, 141.4, 131.3, 130.6, 129.6, 128.6, 127.4, 113.7, 62.0, 35.1, 14.1; IR (cm^{-1}): 1735; HRMS: calcd. for $C_{14}H_{14}N_3O_2S_2$ $[M + H]^+$, 320.0527; found, 320.0522.

*Ethyl 2-((5-(4-Methoxyphenyl)thiazolo[2,3-c][1,2,4]-triazol-3-yl)thio)acetate (**V(b)**).* White solid (3 g, yield: 75%); mp 112−115 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 6.67 (s, 1H), 4.16 (q, J = 7.1 Hz, 2H), 3.92 (s, 2H), 3.88 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.2, 161.3, 158.8, 141.4, 131.2, 119.4, 114.0, 113.0, 62.0, 55.5, 35.0, 14.08; IR (cm^{-1}): 1712; HRMS: calcd. for $C_{15}H_{16}N_3O_3S_2$ $[M + H]^+$, 350.0633; found, 350.0630.

*Ethyl 2-((5-(4-Fluorophenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetate (**V(c)**).* White solid (4.1 g, yield: 73%); mp 142−145 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56−7.51 (m, 2H), 7.23−7.17 (m, 2H), 6.75 (s, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.94 (s, 2H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.1, 164.1 (d, J = 251.6 Hz), 158.7, 141.3, 131.8 (d, J = 8.6 Hz), 130.2, 123.4 (d, J = 2.9 Hz), 115.9 (d, J = 22.1 Hz), 114.1, 62.1, 35.2, 14; IR (cm^{-1}): 1736; HRMS: calcd. for $C_{14}H_{13}FN_3O_2S_2$ $[M + H]^+$, 338.0433; found, 338.0434.

*Ethyl 2-((5-(4-(Trifluoromethyl)phenyl)thiazolo[2,3-c]-[1,2,4]triazol-3-yl)thio)acetate (**V(d)**).* White solid (2.2 g, yield: 71%); mp 174−177 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, J = 8.0 Hz, 2H), 7.71 (d, J = 8.0 Hz, 2H), 6.87 (s, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.97 (s, 2H), 1.25 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.9, 158.8, 141.3, 133.0−132.0 (q, J = 33 Hz), 130.8, 130.0, 129.8, 125.6 (d, J =

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

4.0 Hz, Ar−C), 125.0 (q, J = 271.0 Hz, $\underline{C}F_3$), 115.4, 62.1, 35.3, 14.0; ^{19}F NMR (377 MHz, $CDCl_3$): δ −62.89 (CF_3); IR (cm^{-1}): 1735; HRMS: calcd. for $C_{15}H_{13}N_3O_2S_2F_3$ $[M + H]^+$, 388.0401; found, 388.0395.

*Ethyl 2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-propanoate (**V(e)**).* White solid (3.5 g, yield: 81%); mp 130−133 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55−7.46 (m, 5H), 6.78 (s, 1H), 4.08−4.03 (m, 3H), 1.40 (d, J = 7.5 Hz, 3H), 1.15 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 153.9, 135.7, 126.5, 125.2, 124.5, 123.2, 122.1, 108.8, 56.4, 40.0, 12.2, 8.7; IR (cm^{-1}): 1704; HRMS: calcd. For $C_{15}H_{16}N_3O_2S_2$ $[M + H]^+$, 334.0684; found, 334.0689.

*Ethyl 1-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-cyclobutanecarboxylate, (**V(f)**).* White solid (1.2 g, yield: 78%); mp 92−95 °C; ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.57−7.44 (m, 5H), 6.77 (s, 1H), 4.04 (q, J = 7.1 Hz, 2H), 2.69−2.60 (m, 2H), 2.25−2.16 (m, 2H), 2.07−1.96 (m, 1H), 1.92−1.82 (m, 1H), 1.13 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 159.2, 140.5, 132.1, 130.3, 129.8, 128.4, 127.5, 114.1, 61.7, 55.0, 32.4, 16.2, 13.9; IR (cm^{-1}): 1725; HRMS: calcd. for $C_{17}H_{18}N_3O_2S_2$ $[M + H]^+$, 360.0840; found, 360.0854.

*Ethyl 3-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-propanoate (**V(g)**).* White solid (2.9 g, yield: 67%); mp 97−100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57−7.46 (m, 5H), 6.75 (s, 1H), 4.10 (q, J = 7.1 Hz, 2H), 3.31 (t, J = 6.9 Hz, 2H), 2.80 (t, J = 6.9 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 158.8, 142.3, 131.4, 130.5, 129.7, 128.5, 127.4, 113.7, 60.8, 34.4, 28.1, 14.2; IR (cm^{-1}): 3200, 1750; HRMS: calcd. for $C_{15}H_{16}N_3O_2S_2$ $[M + H]^+$, 334.0684; found, 334.0693.

General Procedure for Synthesis of Acids (VI(a−f)). To a stirred solution of appropriate esters, (**V(a-f)**, 60 mmol) in ethanol, KOH (180 mmol) was added, and the reaction mixture was heated at reflux for 1 h. The reaction was brought to room temperature and acidified with conc. HCl (pH = 1). The solid obtained was filtered off, washed thoroughly with water and diethyl ether, and dried to obtain the required product.

*2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetic Acid (**VI(a)**).* White solid (1.74 g, yield: 82%); mp 227−230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.65−7.61 (m, 2H), 7.58−7.51 (m, 3H), 7.37 (s, 1H), 3.88 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.7, 158.6, 141.6, 130.8, 130.8, 130.1, 128.9, 127.9, 115.8, 35.3; IR (cm^{-1}): 1738, 2700−2000; HRMS: calcd. for $C_{12}H_{10}N_3O_2S_2$ $[M + H]^+$, 292.0214; found, 292.0211.

*2-((5-(4-Methoxyphenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)acetic Acid (**VI(b)**).* White solid (2.0 g, yield: 90%); mp 248−251 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.84 (s, 1H), 7.55 (d, J = 6.8 Hz, 2H), 7.27 (s, 1H), 7.08 (d, J = 6.8 Hz, 2H), 3.88 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.7, 161.2, 158.5, 141.6, 131.7, 130.7, 120.0, 114.9, 114.3, 55.9, 35.2; IR (cm^{-1}): 1704, 2700−2300; HRMS: calcd. for $C_{13}H_{12}N_3O_3S_2$ $[M + H]^+$, 322.0320; found, 322.0314.

*2-((5-(4-Fluorophenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)acetic Acid (**VI(c)**).* White solid (3.15 g, yield: 84%); mp 224−227 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.86 (s, 1H), 7.75−7.67 (m, 2H), 7.46−7.33 (m, 3H), 3.86 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.7, 163.7 (d, J = 247.7 Hz), 158.5, 141.5, 132.8 (d, J = 8.7 Hz), 129.8, 124.4 (d, J = 2.5 Hz), 116.2, 115.9 (d, J = 22.0 Hz), 35.5; IR (cm^{-1}): 2800−2300, 1736, 2485; HRMS: calcd. for $C_{12}H_9FN_3O_2S_2$ $[M + H]^+$, 310.0120; found, 310.0117.

*2-((5-(4-(Trifluoromethyl)phenyl)thiazolo[2,3-c][1,2,4]-triazol-3-yl)thio)acetic Acid (**VI(d)**).* White solid (2.1 g, yield: 87%); mp 227−230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.84 (s, 1H), 7.95−7.87 (m, 4H), 7.52 (s, 1H), 3.88 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 158.7, 141.5, 132.0, 131.2−130.3 (q, J = 32 Hz), 131.0, 129.4 (Ar−C), 125.8 (q, J = 270.0 Hz, $\underline{C}F_3$), 125.7 (d, J = 4.0 Hz, Ar−C), 117.6, 35.7; ^{19}F NMR (377 MHz, DMSO-d_6): δ −61.25 (CF_3); IR (cm^{-1}): 2700−2200, 1737; HRMS: calcd. for $C_{13}H_9N_3O_2F_3S_2$ $[M + H]^+$, 360.0088; found, 360.0083.

*2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-propanoic Acid (**VI(e)**).* White solid (1.5 g, yield: 82%); mp 180−183 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.81 (s, 1H), 7.67−7.49 (m, 5H), 7.40 (s, 1H), 3.81 (q, J = 7.1 Hz, 1H), 1.23 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.0, 158.6, 139.8, 130.8, 130.1, 129.9, 128.2, 127.4, 115.7, 45.0, 17.4; IR (cm^{-1}): 2500−2400, 1718; HRMS: calcd. for $C_{13}H_{12}N_3O_2S_2$ $[M + H]^+$, 306.0371; found, 306.0375.

*1-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-cyclobutane Carboxylic Acid (**VI(f)**).* White solid (0.70 g, yield: 69%); mp 231−234 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.60 (s, 1H), 7.67−7.57 (m, 2H), 7.57−7.46 (m, 3H), 7.39 (s, 1H), 2.48−2.40 (m, 2H), 2.06−1.93 (m, 2H), 1.84−1.67 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.4, 159.3, 140.0, 131.7, 130.5, 128.7, 128.0, 116.3, 54.8, 32.0, 15.8; IR (cm^{-1}): 2000−2300, 1720; HRMS: calcd. for $C_{15}H_{14}N_3O_2S_2$ $[M + H]^+$, 332.0527; found, 332.0534.

Synthesis of Acid (VI(g)). The ester, **V(g)** (1 mmol), was taken in a round-bottom flask to which 1:1 1,4-dioxane/1 N HCl (10 mL) were added and heated at reflux for 12 h. After that, dioxane was removed under reduced pressure. The resulting solid was used for the coupling reaction. White solid (1.5 g, yield: 82%); mp 210−213 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.32 (s, 1H), 7.62−7.59 (m, 2H), 7.57−7.50 (m, 3H), 7.36 (s, 1H), 3.10 (t, J = 6.9 Hz, 2H), 2.59 (t, J = 6.9 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 158.7, 141.9, 131.0, 130.7, 130.3, 128.7, 128.0, 115.9, 34.3, 28.6; IR (cm^{-1}): 3220, 2400−2200, 1700; HRMS calcd. for $C_{13}H_{12}N_3O_2S_2$ $[M + H]^+$, 306.0371; found, 306.0378.

General Procedure for Synthesis of Amides (1−40). To a stirred solution of appropriate 2-((5-phenylthiazolo[2,3-*c*][1,2,4]triazol-3-yl)thio)acetic acids (**VI(a−g)**, 0.33 mmol) in anhydrous DMF (5 mL), EDC·HCl (0.5 mmol), DIPEA (1.0 mmol) and HOBT (0.5 mmol) were added. To this, appropriate amines (**VIII(i−p)**/butylamine/4-bromoaniline/L-alanine methyl ester) were added, and the reaction mixture was stirred at room temperature overnight under nitrogen. After completion of the reaction, ice cold water was added, and the reaction mixture was extracted three times with ethyl acetate. The combined organic layer was washed with water followed by brine solution and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure, and the crude compound obtained was purified by column chromatography using 100−200 mesh silica gel and dichloromethane and methanol as solvents.

*2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-N-(4-(pyrrolidin-1-yl)phenyl)acetamide (**1**).* White solid (108 mg, yield: 80%); mp 191−194 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.59 (s, 1H), 7.58−7.47 (m, 5H), 7.38 (d, J = 8.8 Hz, 2H), 6.78 (s, 1H), 6.47 (d, J = 8.8 Hz, 2H), 3.85 (s, 2H), 3.27−3.19 (m, 4H), 2.01−1.94 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$):

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

δ 165.7, 158.9, 145.4, 143.5, 131.3, 130.8, 129.6, 128.8, 127.0, 126.9, 121.6, 113.9, 111.6, 47.8, 36.5, 25.4; IR (cm^{-1}): 3250, 1669; HRMS: calcd. For $C_{22}H_{22}N_5OS_2$ $[M + H]^+$, 436.1266; found, 436.1262.

*N-Butyl-2-((5-phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)acetamide (**2**).* White solid (90 mg, yield: 76%); mp 108−111 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 7.62−7.45 (m, 6H), 6.78 (s, 1H), 3.68 (s, 2H), 3.23−3.19 (m, 2H), 1.50−1.43 (m, 2H), 1.35−1.24 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 166.7, 158.6, 141.9, 131.0, 130.7, 130.1, 128.8, 127.9, 115.8, 39.1, 37.0, 31.4, 19.9, 14.1; IR (cm^{-1}): 3291, 1665; HRMS: calcd. for $C_{16}H_{19}ON_4S_2$ $[M + H]^+$, 347.0994; found, 347.0987.

*Methyl(2-((5-phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-acetyl)-L-alaninate (**3**).* White solid (182 mg, yield: 70%); mp 154−157 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, *J* = 6.6 Hz, 1H), 7.58−7.47 (m, 5H), 6.78 (s, 1H), 4.49 (quint, *J* = 7.2 Hz, 1H), 3.76 (d, *J* = 7.2 Hz, 2H), 3.70 (s, 3H), 1.39 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 167.8, 158.9, 142.8, 131.3, 130.8, 129.6, 128.7, 127.1, 113.9, 52.4, 48.6, 35.6, 17.9; IR (cm^{-1}): 3247, 1238, 1670; HRMS: calcd. for $C_{16}H_{17}N_4O_3S_2$ $[M + H]^+$, 377.0742; found, 377. 0741.

*N-(4-Bromophenyl)-2-((5-phenylthiazolo[2,3-c][1,2,4]-triazol-3yl)thio)acetamide (**4**).* White solid (130 mg, yield: 60%); mp 249−252 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H), 7.66−7.60 (m, 2H), 7.59−7.46 (m, 7H), 7.37 (s, 1H), 3.98 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.1, 158.7, 141.7, 138.6, 132.1, 130.9, 130.8, 130.1, 128.9, 127.9, 121.5, 115.9, 115.6, 37.9; IR (cm^{-1}): 3241,1659; HRMS: calcd. for $C_{18}H_{14}ON_4BrS_2$ $[M + H]^+$, 444.97869; found, 444.97641.

*2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-N-(4-(piperidin-1-yl)phenyl)acetamide (**5**).* White solid (191 mg, yield: 81%); mp 206−209 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 9.79 (s, 1H), 7.59−7.46 (m, 5H), 7.42 (d, *J* = 8.9 Hz, 2H), 6.86 (d, *J* = 8.9 Hz, 2H), 6.78 (s, 1H), 3.86 (s, 2H), 3.09−3.04 (m, 4H), 1.71−1.66 (m, 4H), 1.57−1.51(m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 159.0, 149.3, 143.5, 131.3, 130.9, 130.3, 129.6, 128.8, 127.0, 120.8, 117.1, 114.0, 51.2, 36.5, 25.9, 24.2; IR (cm^{-1}): 3247, 1673; HRMS: calcd. for $C_{23}H_{24}N_5OS_2$ $[M + H]^+$, 450.1422; found, 450.1417.

*N-(4-(4-Methylpiperazin-1-yl)phenyl)-2-((5-phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**6**).* White solid (153 mg, yield: 75%); mp 196−199 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 9.84 (s, 1H), 7.57−7.41 (m, 7H), 6.84 (d, *J* = 8.5 Hz, 2H), 6.78 (s, 1H), 3.85 (s, 2H), 3.17−3.09 (m, 4H), 2.61−2.52 (m, 4H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 159.0, 148.2, 143.5, 131.3, 130.9, 130.8, 129.6, 128.8, 127.0, 120.9, 116.6, 114.0, 55.1, 49.6, 46.1, 36.6; IR (cm^{-1}): 3252, 1675; HRMS: calcd. for $C_{23}H_{25}N_6OS_2$ $[M + H]^+$, 465.1531; found, 465.1524.

*N-(4-Morpholinophenyl)-2-((5-phenylthiazolo[2,3-c]-[1,2,4]triazol-3-yl)thio)acetamide (**7**).* White solid (240 mg, yield: 59%); mp 237−240 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H), 7.66−7.62 (m, 2H),7.59−7.49 (m, 3H), 7.40−7.35(m, 3H), 6.88 (d, *J* = 9.1 Hz, 2H), 3.96 (s, 2H), 3.74−3.65 (m, 4H), 3.08−2.97 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.5, 158.6, 147.9, 141.9, 131.5, 131.0, 130.7, 130.2, 128.9, 128.0, 120.7, 115.9, 66.6, 49.3, 37.9; IR (cm^{-1}): 3254, 1670; HRMS: calcd. for $C_{22}H_{22}N_5O_2S_2$ $[M + H]^+$, 452.1215; found, 452.1216.

*2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-N-(4-thiomorpholinophenyl)acetamide (**8**).* White solid (220 mg, yield: 69%); mp 211−214 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.01 (s, 1H), 7.65−7.62 (m, 2H),7.59−7.50 (m, 3H), 7.38−7.34 (m, 3H), 6.86 (d, *J* = 9.1 Hz, 2H), 3.95 (s, 2H), 3.44−3.40 (m, 4H), 2.67−2.63 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.2, 158.6, 147.6, 141.9, 131.3, 131.0, 130.7, 130.2, 128.9, 127.9, 120.8, 117.3, 115.8, 52.0, 37.9, 26.2; IR (cm^{-1}): 3252, 1678; HRMS: calcd. for $C_{22}H_{22}N_5OS_3$ $[M + H]^+$, 468.0986; found, 468.0991.

*N-(4-(Diethylamino)phenyl)-2-((5-phenylthiazolo[2,3-c]-[1,2,4]triazol-3-yl)thio)acetamide (**9**).* White solid (185 mg, yield: 51%); mp 185−188 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 7.58−7.46 (m, 5H), 7.37 (d, *J* = 9.0 Hz, 2H), 6.78 (s, 1H), 6.60 (d, *J* = 9.0 Hz, 2H), 3.85 (s, 2H), 3.29 (q, *J* = 7.0 Hz, 4H), 1.10 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 158.9, 145.1, 143.5, 131.3, 130.9, 129.6, 128.8, 127.1, 127.0, 121.6, 113.9, 112.5, 44.6, 36.4, 12.5; IR (cm^{-1}): 3240, 1670; HRMS: calcd. for $C_{22}H_{24}N_5OS_2$ $[M + H]^+$, 438.1422; found, 438.1432.

*N-(4-(4-Ethylpiperazin-1-yl)phenyl)-2-((5-phenylthiazolo-[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**10**).* White solid (180 mg, yield: 73%); mp 207−210 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 9.82 (s, 1H), 7.59−7.42 (m, 7H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.78 (s, 1H), 3.85 (s, 2H), 3.19−3.12 (m, 4H), 2.63−2.56 (m, 4H), 2.47 (q, *J* = 7.2 Hz, 2H), 1.12 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 159.0, 148.3, 143.5, 131.3, 130.9, 130.7, 129.6, 128.8, 127.0, 120.9, 116.6, 114.0, 52.8, 52.4, 49.6, 36.5, 12.0; IR (cm^{-1}, $CHCl_3$): 3250, 1673; HRMS: calcd. for $C_{24}H_{27}ON_6S_2$ $[M + H]^+$, 479.1682; found, 479.1659.

*N-(4-(1H-Imidazol-1-yl)phenyl)-2-((5-phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**11**).* White solid (179 mg, yield: 60%); mp 252−255 °C; ^1H NMR (500 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.18 (s, 1H), 7.65−7.64 (m, 2H), 7.64−7.63 (m, 2H), 7.59−7.51 (m, 6H), 7.38 (s, 1H), 7.08 (s, 1H), 4.01 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.1, 158.7, 141.7, 138.0, 135.9, 135.8, 132.8, 131.0, 130.8, 130.1, 128.9, 127.9, 121.3, 120.6, 118.5, 115.9, 37.9; IR (cm^{-1}): 3251, 1677; HRMS: calcd. for $C_{21}H_{17}ON_6S_2$ $[M + H]^+$, 433.0899; found, 433.0876.

*3-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-N-(4-(pyrrolidin-1-yl)phenyl)propanamide (**12**).* White solid (110 mg, yield: 50%); mp 226−229 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 9.55 (s, 1H), 7.62−7.57 (m, 2H), 7.55−7.50 (m, 1H), 7.49−7.43 (m, 2H), 7.35 (s, 1H), 7.31 (d, *J* = 9.0 Hz, 2H), 6.46 (d, *J* = 9.0 Hz, 2H), 3.23−3.13 (m, 6H), 2.62 (t, *J* = 6.8 Hz, 2H), 1.97−1.89 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3, 144.8, 142.0, 132.7, 131.1, 130.6, 130.3, 128.7, 128.4, 127.9, 121.3, 115.8, 111.9, 48.0, 36.2, 29.3, 25.4; IR (cm^{-1}): 3228, 1666; HRMS: calcd. for $C_{23}H_{24}N_5OS_2$ $[M + H]^+$, 450.1422; found, 450.1422.

*3-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-N-(4-(piperidin-1-yl)phenyl)propanamide (**13**).* White solid (98 mg, yield: 52%); mp 196−199 °C; ^1H NMR (400 MHz, DMSO-d_6); δ 9.67 (s, 1H), 7.61−7.56 (m, 2H), 7.55−7.50 (m, 1H), 7.48−7.42 (m, 2H), 7.38−7.32 (m, 3H), 6.84 (d, *J* = 9.0 Hz, 2H), 3.19 (t, *J* = 6.7 Hz, 2H), 3.07−3.00 (m, 4H), 2.64 (t, *J* = 6.7 Hz, 2H), 1.64−1.56 (m, 4H), 1.53−1.46 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.6, 158.7, 148.4, 142.0, 131.3, 131.1, 130.6, 130.3, 128.7, 127.9, 120.6, 116.7, 115.8, 50.6, 36.3, 29.3, 25.8, 24.3; IR (cm^{-1}): 3264, 1641; HRMS: calcd. for $C_{24}H_{26}N_5OS_2$ $[M + H]^+$, 464.1579; found, 464.1583.

N-(4-(4-Methylpiperazin-1-yl)phenyl)-3-((5-phenylthiazolo[2,3-c][1,2,4]triazol-3yl)thio)propanamide

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

*(**14**)*. White solid (110 mg, yield: 47%); mp 215−218 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (s, 1H), 7.56−7.51 (m, 1H), 7.50−7.46 (m, 4H), 7.45 (d, J = 9.0 Hz, 2H), 6.88 (d, J = 9.0 Hz, 2H), 6.76 (s, 1H), 3.32 (t, J = 6.9 Hz, 2H), 3.18−3.14 (m, 4H), 2.83 (t, J = 6.9 Hz, 2H), 2.60−2.57 (m, 4H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.8, 158.9, 148.1, 142.8, 131.6, 130.9, 130.6, 129.7, 128.6, 127.2, 121.1, 116.7, 113.9, 55.1, 49.6, 46.1, 37.7, 29.3; IR (cm^{-1}): 3250, 1660; HRMS: calcd. for $C_{24}H_{27}ON_6S_2$ [M + H]$^+$, 479.1682; found, 479.1668.

*N-(4-Morpholinophenyl)-3-((5-phenylthiazolo[2,3-c]-[1,2,4]triazol-3-yl)thio)propanamide (**15**)*. White solid (70 mg, yield: 46%); mp 249−252 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.70 (s, 1H), 7.61−7.57 (m, 2H), 7.55−7.50 (m, 1H), 7.48−7.43 (m, 2H), 7.38 (d, J = 9.1 Hz, 2H), 7.35 (s, 1H), 6.86 (d, J = 9.1 Hz, 2H), 3.74−3.69 (m, 4H), 3.20 (t, J = 6.7 Hz, 2H), 3.05−3.00 (m, 4H), 2.65 (t, J = 6.7 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.7, 158.7, 147.6, 142.0, 131.8, 131.1, 130.6, 130.3, 128.7, 127.9, 120.6, 115.9, 115.8, 66.6, 49.4, 36.3, 29.2; IR (cm^{-1}): 3255, 1671; HRMS: calcd. for $C_{23}H_{24}N_5O_2S_2$ [M + H]$^+$, 466.1371; found, 466.1380.

*N-(4-Thiomorpholinophenyl)-3-((5-phenylthiazolo[2,3-c]-[1,2,4]triazol-3-yl)thio)propanamide (**16**)*. White solid (102 mg, yield: 43%); mp 229−232 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.70 (s, 1H), 7.62−7.56 (m, 2H), 7.55−7.50 (m, 1H), 7.48−7.42(m, 2H), 7.37 (d, J = 9.1 Hz, 2H), 7.35 (s, 1H), 6.85 (d, J = 9.1 Hz, 2H), 3.44−3.38 (m, 4H), 3.20 (t, J = 6.7 Hz, 2H), 2.68−2.62 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.7, 158.7, 147.4, 142.0, 131.7, 131.1, 130.6, 130.3, 128.7, 127.9, 120.8, 117.4, 115.8, 52.1, 36.3, 29.2, 26.3; IR (cm^{-1}): 3268, 1676; HRMS: calcd. for $C_{23}H_{24}N_5OS_3$ [M + H]$^+$, 482.1143, found, 482.1154.

*2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-N-(4-(pyrrolidin-1-yl)phenyl)propanamide (**17**)*. White solid (220 mg, yield: 57%); mp 253−256 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.74 (s, 1H), 7.62−7.58 (m, 2H), 7.55−7.46 (m, 3H), 7.37 (s, 1H), 7.28 (d, J = 9.0 Hz, 2H), 6.46 (d, J = 9.0 Hz, 2H), 4.20 (q, J = 6.9 Hz, 1H), 3.19−3.15 (m, 4H), 1.95−1.91 (m, 4H), 1.34 (d, J = 6.9 Hz, 3H); IR (cm^{-1}): 3225, 1663; ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3, 159.0, 145.2, 131.3, 130.7, 130.6, 130.4, 128.7, 128.0, 127.7, 121.6, 116.1, 111.9, 47.9, 47.8, 25.4, 19.1; HRMS: calcd. for $C_{23}H_{24}N_5OS_2$ [M + H]$^+$, 450.1422; found, 1417.

*2-((5-Phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-N-(4-(piperidin-1-yl)phenyl)propanamide (**18**)*. White solid (237 mg, yield: 62%); mp 205−208 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.84 (s, 1H), 7.62−7.58 (m, 2H), 7.56−7.45 (m, 3H), 7.38 (s, 1H), 7.31 (d, J = 9.1 Hz, 2H), 6.85 (d, J = 9.1 Hz, 2H), 4.21 (q, J = 7.2 Hz, 1H), 3.08−3.02 (m, 4H), 1.63−1.56 (m, 4H), 1.53−1.47 (m, 2H), 1.33 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.6, 159.0, 148.7, 141.0, 131.3, 130.7, 130.6, 130.3, 128.7, 128.0, 120.9, 116.6, 116.1, 50.5, 47.7, 25.8, 24.3, 19.1; IR (cm^{-1}): 3236, 1674; HRMS: calcd. for $C_{24}H_{26}N_5OS_2$ [M + H]$^+$, 464.1579; found, 464.1579.

*N-(4-(4-Methylpiperazin-1-yl)phenyl)-2-((5-phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)propanamide (**19**)*. White solid (235 mg, yield: 70%); mp 188−191 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.86 (s, 1H), 7.61−7.58 (m, 2H), 7.55−7.45 (m, 3H), 7.38 (s, 1H), 7.33 (d, J = 9.1 Hz, 2H), 6.86 (d, J = 9.1 Hz, 2H), 4.20 (q, J = 6.8 Hz, 1H), 3.10−3.03 (m, 4H), 2.47−2.42 (m, 4H), 2.22 (s, 3H), 1.33 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7, 158.8, 148.0, 143.6, 131.4, 131.0, 130.8, 129.5, 128.7, 127.0, 120.7, 116.7, 113.9, 55.1, 49.6, 46.1, 43.8, 16.3; IR (cm^{-1}): 3242, 1668; HRMS: calcd. for $C_{24}H_{27}N_6OS_2$ [M + H]$^+$, 479.1688; found, 479.1693.

*N-(4-Morpholinophenyl)-2-((5-phenylthiazolo[2,3-c]-[1,2,4]triazol-3-yl)thio)propanamide (**20**)*. White solid (89 mg, yield: 62%); mp 211−214 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1H), 7.57−7.46 (m, 7H), 6.84 (d, J = 8.9 Hz, 2H), 6.77 (s, 1H), 4.51 (q, J = 7.3 Hz, 1H), 3.87−3.82 (m, 4H), 3.11−3.06 (m, 4H), 1.50 (d, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.7, 159.0, 147.97, 140.9, 131.3, 131.3, 130.6, 130.4, 128.70, 128.0, 120.9, 116.1, 115.8, 66.6, 49.3, 47.7, 19.0; IR (cm^{-1}): 3231, 1671; HRMS: calcd. for $C_{23}H_{24}N_5O_2S_2$ [M + H]$^+$, 466. 1371; found, 466.1374.

*N-(4-Thiomorpholinophenyl)-2-((5-phenylthiazolo[2,3-c]-[1,2,4]triazol-3-yl)thio)propanamide (**21**)*. White solid (167 mg, yield: 74%); mp135−138 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.88 (s, 1H), 7.63−7.58 (m, 2H), 7.57−7.46 (m, 3H), 7.39 (s, 1H), 7.35 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.1 Hz, 2H), 4.22 (q, J = 7.2 Hz, 1H), 3.46−3.41 (m, 4H), 2.69−2.64 (m, 4H), 1.34 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7, 158.8, 148.3, 143.6, 131.4, 131.3, 130.8, 129.5, 128.8, 127.0, 120.8, 117.9, 114.0, 52.7, 43.8, 27.0, 16.3; IR (cm^{-1}): 3244, 1679; HRMS: calcd. for $C_{23}H_{24}N_5OS_3$ [M + H]$^+$, 482.1143; found, 482.1140.

*N-(4-(4-Methylpiperazin-1-yl)phenyl)-1-((5-phenylthiazolo[2,3-c][1,2,4]triazol-3-yl)thio)-cyclobutanecarboxamide (**22**)*. White solid (200 mg, yield: 71%); mp 151−154 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 7.54−7.47 (m, 3H), 7.45−7.40 (m, 2H), 7.35 (d, J = 9.1 Hz, 2H), 7.18(s, 1H), 6.93 (d, J = 9.1 Hz, 2H), 3.22−3.17 (m, 4H), 2.70−2.60 (m, 6H), 2.38 (s, 3H), 2.17−2.09 (m, 2H), 2.01−1.89 (m, 1H), 1.81−1.71 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.0, 159.6, 147.8, 139.9, 131.8, 131.3, 130.5, 130.4, 128.6, 127.9, 121.5, 116.5, 115.9, 56.5, 55.0, 48.9, 46.1, 31.9, 15.4; IR (cm^{-1}): 3252, 1675; HRMS: calcd. for $C_{26}H_{29}ON_6S_2$ [M + H]$^+$, 505.1838; found, 505.1827.

*N-(4-Morpholinophenyl)-1-((5-phenylthiazolo[2,3-c]-[1,2,4]triazol-3-yl)thio)cyclobutanecarboxamide (**23**)*. White solid (220 mg, yield: 74%); mp 210−213 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.33 (s, 1H), 7.57−7.47 (m, 7H), 6.86 (d, J = 9.0 Hz, 2H), 6.77 (s, 1H), 3.86−3.83 (m, 4H), 3.10−3.07 (m, 4H), 2.97−2.91 (m, 2H), 2.29−2.23 (m, 2H), 2.12−2.02 (m, 1H), 1.95−1.87 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 158.6, 147.9, 142.6, 131.8, 131.6, 130.7, 129.7, 128.7, 127.2, 120.6, 116.4, 114.1, 66.9, 54.9, 50.0, 32.0, 16.2; IR (cm^{-1}): 3250, 1670; HRMS: calcd. for $C_{25}H_{26}O_2N_5S_2$ [M + H]$^+$, 492.1522; found, 492.1497.

*2-((5-(4-Methoxyphenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-(pyrrolidin-1-yl)phenyl)acetamide (**24**)*. White solid (291 mg, yield: 67%); mp 206−209 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 7.43−7.36 (m, 4H), 6.99 (d, J = 8.4 Hz, 2H), 6.70 (s, 1H), 6.47 (d, J = 8.8 Hz, 2H), 3.88 (s, 3H), 3.86 (s, 2H), 3.27−3.20 (m, 4H), 2.01−1.94 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.8, 161.1, 158.5, 145.1, 141.9, 131.8, 130.9, 127.8, 121.3, 120.0, 114.9, 114.2, 111.9, 55.8, 47.9, 37.9, 25.4; IR (cm^{-1}): 3245, 1666; HRMS: calcd. for $C_{23}H_{24}N_5O_2S_2$ [M + H]$^+$, 466.137; found, 466.1364.

*2-((5-(4-Methoxyphenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-(piperidin-1-yl)phenyl)acetamide (**25**)*. White solid (245 mg, yield: 83%); mp 225−228 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.00 (s, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 9.0 Hz, 2H), 7.27 (s, 1H), 7.05 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 9.0 Hz, 2H), 3.96 (s, 2H), 3.82 (s, 3H),

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

3.07−3.0 (m, 4H), 1.62−1.56 (m, 4H), 1.52−1.46 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9, 165.9, 163.3, 153.4, 146.6, 136.5, 135.7, 135.6, 125.4, 124.8, 121.4, 119.7, 119.0, 60.6, 55.3, 42.6, 30.5, 29.0; IR (cm^{-1}): 3245, 1666; HRMS: calcd. for $C_{24}H_{26}N_5O_2S_2$ [M + H]$^+$, 480.1528; found, 480.1540.

*2-((5-(4-Methoxyphenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-(4-methylpiperazin-1-yl)phenyl)acetamide (**26**).* White solid (187 mg, yield: 75%); mp 202−205 °C; ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 7.51 (d, *J* = 8.8 Hz, 2H), 7.38 (d, *J* = 8.8 Hz, 2H), 7.07 (s, 1H), 7.00 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 3H), 3.79 (s, 2H), 3.23−3.13 (m, 4H), 2.73−2.68 (m, 4H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.2, 161.1, 158.5, 147.7, 141.9, 131.8, 131.3, 130.9, 120.6, 120.0, 116.2, 115.0, 114.2, 55.8, 54.9, 48.8, 46.0, 37.9; IR (cm^{-1}): 3245, 1669; HRMS: calcd. for $C_{24}H_{27}N_6O_2S_2$ [M + H]$^+$, 495.1637; found, 495.1645.

*2-((5-(4-Methoxyphenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-morpholinophenyl)acetamide (**27**).* White solid (330 mg, yield: 78%); mp 243−246 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.24 (s, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 6.85 (d, *J* = 8.70 Hz, 2H), 3.94 (s, 2H), 3.80 (s, 3H), 3.71−3.66 (m, 4H), 3.03−2.97 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.0, 165.9, 163.3, 152.6, 146.6, 136.5, 136.3, 135.6, 125.4, 124.8, 120.6, 119.7, 119.0, 71.32, 60.6, 54.1, 42.6; IR (cm^{-1}): 3245, 1661; HRMS: calcd. for $C_{23}H_{24}N_5O_3S_2$ [M + H]$^+$, 482.1321; found, 482.1330.

*2-((5-(4-Methoxyphenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-thiomorpholinophenyl)acetamide (**28**).* White solid (268 mg, yield: 72%); mp 224−227 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, 1H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.37 (d, *J* = 8.9 Hz, 2H), 7.27 (s, 1H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.9 Hz, 2H), 3.96 (s, 2H), 3.82 (s, 3H), 3.44−3.39 (m, 4H), 2.63−2.68 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.2, 161.1, 158.5, 147.6, 141.9, 131.8, 131.3, 130.9, 120.8, 120.0, 117.3, 115.0, 114.2, 55.8, 52.0, 37.8, 26.2; IR (cm^{-1}): 3242, 1669; HRMS: calcd. for $C_{23}H_{24}N_5O_2S_3$[M + H]$^+$, 498.1092; found, 498.1103.

*N-(4-(Diethylamino)phenyl)-2-((5-(4-methoxyphenyl)-thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**29**).* White solid (320 mg, yield: 70%); mp 208−211 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 9.2 Hz, 2H), 7.27 (s, 1H), 7.06 (d, *J* = 8.0 Hz, 2H), 6.59 (d, *J* = 9.2 Hz, 2H), 3.94 (s, 2H), 3.82 (s, 3H), 3.27 (q, *J* = 7.0 Hz, 4H), 1.04 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.8, 161.1, 158.5, 144.6, 141.9, 131.8, 130.9, 128.0, 121.4, 120.0, 114.9, 114.2, 112.4, 55.8, 44.2, 37.9, 12.9; IR (cm^{-1}): 3245, 1664; HRMS: calcd. for $C_{23}H_{26}N_5O_2S_2$ [M + H]$^+$, 468.1528; found, 468.1536.

*N-(4-(Pyrrolidin-1-yl)phenyl)-2-((5-(4-(trifluoromethyl)-phenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**30**).* White solid (185 mg, yield: 66%); mp 227−230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.83 (s, 1H), 7.91−7.85 (m, 4H), 7.52 (s, 1H), 7.29 (d, *J* = 9.0 Hz, 2H), 6.46 (d, *J* = 9.0 Hz, 2H), 3.92 (s, 2H), 3.17 (t, *J* = 6.5 Hz, 4H), 1.95−1.90 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 158.8, 145.1, 141.8, 132.0, 131.1, 130.7 (q, 32 Hz), 129.6, 125.8 (q, *J* = 270.0 Hz, $\underline{C}F_3$), 127.9, 125.6 (d, *J* = 4.0 Hz), 121.2, 117.6, 111.8, 47.9, 38.4, 25.4; ^{19}F NMR (377 MHz, $CDCl_3$): δ −62.96; IR (cm^{-1}): 3214, 1633; HRMS: calcd. for $C_{23}H_{21}N_5OS_2F_3$ [M + H]$^+$, 504.1140; found, 504.1141.

*N-(4-(Piperidine-1-yl)phenyl)-2-((5-(4-(trifluoromethyl)-phenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**31**).* White solid (200 mg, yield: 69%); mp 204−207 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.93 (s, 1H), 7.90−7.84 (m, 4H), 7.52 (s, 1H), 7.32 (d, *J* = 9.0 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 2H), 3.92 (s, 2H), 3.06−3.03 (m, 4H), 1.63−1.56 (m, 4H), 1.53−1.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 158.9, 149.3, 143.3, 132.9 (q, *J* = 33 Hz), 130.4, 130.1, 129.9, 129.7, 125.8 (d, *J* = 3 Hz), 124.9 (q, *J* = 271.0 Hz, CF_3), 120.8, 117.0, 115.6, 51.1, 36.6, 25.8, 24.2; ^{19}F NMR (377 MHz, $CDCl_3$) δ −62.95; IR (cm^{-1}): 3103, 1654; HRMS: calcd. for $C_{24}H_{23}N_5OF_3S_2$ [M + H]$^+$, 518.1296; found, 518.1287.

*N-(4-(4-Methylpiperazin-1-yl)phenyl)-2-((5-(4-(trifluoromethyl)phenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)acetamide (**32**).* White solid (190 mg, yield: 64%); mp 196−199 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.96 (s, 1H), 7.87 (m, 4H), 7.52 (s, 1H), 7.33 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 3.93 (s, 2H), 3.08−3.03 (m, 4H), 2.47−2.42 (m, 4H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 158.8, 147.8, 141.7, 132.0, 131.1, 130.7 (q, *J* = 32 Hz), 129.6, 125.8 (q, *J* = 271 Hz, CF_3), 125.6 (d, *J* = 3 Hz), 123.1, 120.6, 117.7, 116.1, 55.0, 48.9, 46.1, 38.4; ^{19}F NMR (377 MHz, $CDCl_3$) δ −62.95; IR (cm^{-1}): 3278, 1661; HRMS: calcd. for $C_{24}H_{24}N_6OF_3S_2$ [M + H]$^+$, 533.1405; found, 533.1364.

*N-(4-Morpholinophenyl)-2-((5-(4-(trifluoromethyl)-phenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**33**).* White solid (220 mg, yield: 76%); mp 250−253 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.97 (s, 1H), 7.87 (m, 4H), 7.52 (s, 1H), 7.36 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 3.93 (s, 2H), 3.73−3.70 (m, 4H), 3.04−3.01 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.1, 158.8, 147.8, 141.7, 132.0, 131.5, 130.7 (q, *J* = 32 Hz), 131.1, 129.6, 125.8 (q, *J* = 270.0 Hz, $\underline{C}F_3$), 125.6 (d, *J* = 3 Hz), 120.6, 117.6, 115.9, 66.5, 49.3, 38.4; ^{19}F NMR (377 MHz, $CDCl_3$): δ −62.95; IR (cm^{-1}): 3245, 1674; HRMS: calcd. for $C_{23}H_{21}N_5O_2S_2F_3$ [M + H]$^+$, 520.1089; found, 520.1114.

*N-(4-Thiomorpholinophenyl)-2-((5-(4-(trifluoromethyl)-phenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**34**).* White solid (207 mg, yield: 69%); mp 224−227 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 1H), 7.91−7.84 (m, 4H), 7.52 (s, 1H), 7.34 (d, *J* = 9.1 Hz, 2H), 6.86 (d, *J* = 9.1 Hz, 2H), 3.92 (s, 2H), 3.44−3.39 (m, 4H), 2.68−2.63 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.1, 158.8, 147.6, 141.7, 132.0, 131.3, 130.8 (q, *J* = 32 Hz), 131.12, 129.60, 125.8 (q, *J* = 270.0 Hz, $\underline{C}F_3$), 125.6 (d, *J* = 3 Hz), 120.8, 117.7, 117.3, 52.02, 38.3, 26.2; ^{19}F NMR (377 MHz, $CDCl_3$): δ −62.95; IR (cm^{-1}): 3161, 1649; HRMS: calcd. for $C_{23}H_{21}N_5OS_3F_3$ [M + H]$^+$, 536.0860; found, 536.0861.

*2-((5-(4-Fluorophenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-(pyrrolidin-1-yl)phenyl)acetamide (**35**).* White solid (186 mg, yield: 65%); mp 227−230 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.54 (s, 1H), 7.50−7.46 (m, 2H) 7.37 (d, *J* = 8.9 Hz, 2H), 7.23−7.16 (m, 2H), 6.77 (s, 1H), 6.47 (d, *J* = 8.9 Hz, 2H), 3.87 (s, 2H), 3.26−3.19 (m, 4H), 2.0−1.94 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5, 164.1 (d, *J* = 252.1 Hz), 158.7, 145.4, 143.3, 131.8 (d, *J* = 8.6 Hz), 130.1, 126.8, 123.0 (d, *J* = 2.9 Hz), 121.5, 116.0 (d, *J* = 22.1 Hz), 114.3, 111.6, 47.8, 36.4, 25.4; IR (cm^{-1}): 3252, 1665; HRMS: calcd. for $C_{22}H_{21}FN_5OS_2$ [M + H]$^+$, 454.1172; found, 454.1175.

*2-((5-(4-Fluorophenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-(piperidin-1-yl)phenyl)acetamide (**36**).* White

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

solid (180 mg, yield: 72%); mp 198−201 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.96 (s, 1H), 7.74−7.67 (m, 2H), 7.39−7.31 (m, 5H), 6.85 (d, J = 8.8 Hz, 2H), 3.92 (s, 2H), 3.07−3.03 (m, 4H), 1.63−1.56 (m, 4H), 1.53−1.46 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.8, 167.4 (d, J = 247.3 Hz), 162.3, 152.4, 145.5, 136.6 (d, J = 8.3 Hz), 134.6, 133.7, 128.13 (d, J = 2.6 Hz), 124.4, 120.4, 119.9, 119.6 (d, J = 22.0 Hz), 54.2, 41.8, 29.5, 28.1; IR (cm^{-1}): 3267, 1675; HRMS: calcd. for $C_{23}H_{23}FN_5OS_2$ [M + H]$^+$, 468.1328; found, 468.1329.

*2-((5-(4-Fluorophenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-(4-methylpiperazin-1-yl)phenyl)acetamide (**37**).* White solid (210 mg, yield: 69%); mp 188−191 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.79 (s, 1H), 7.50−7.45 (m, 2H), 7.43 (d, J = 9.0 Hz, 2H), 7.23−7.15 (m, 2H), 6.84 (d, J = 9.0 Hz, 2H), 6.78 (s, 1H), 3.87 (s, 2H), 3.16−3.11 (m, 4H), 2.58−2.55 (m, 4H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 164.2 (d, J = 252.3 Hz), 158.8, 148.1, 143.3, 131.7 (d, J = 8.7 Hz), 130.7, 130.1, 122.9 (d, J = 2.9 Hz), 120.8, 116.6, 116.0 (d, J = 22.1 Hz), 114.4, 55.0, 49.5, 46.1, 36.4; IR (cm^{-1}): 1672, 3257; HRMS: calcd. for $C_{23}H_{24}FN_6OS_2$ [M + H]$^+$, 483.1437; found, 483.1440.

*2-((5-(4-Fluorophenyl)thiazolo[2,3-c][1,2,4]triazol-3-yl)-thio)-N-(4-morpholinophenyl)acetamide (**38**).* White solid (195 mg, yield: 73%); mp 244−247 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.00 (s, 1H), 7.76−7.66 (m, 2H), 7.41−7.30 (m, 5H), 6.88 (d, J = 8.8 Hz, 2H), 3.93 (s, 2H), 3.74−3.68 (m, 4H), 3.09−2.97 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9, 168.4 (d, J = 247.8 Hz), 163.3, 152.6, 146.5, 137.6 (d, J = 5.6 Hz), 136.2, 134.7, 129.2 (d, J = 2.1 Hz), 125.4, 120.8 (d, J = 21.3 Hz), 120.6, 120.5, 71.3, 54.1, 42.8; IR (cm^{-1}): 1675, 3259; HRMS: calcd. for $C_{22}H_{21}FN_5O_2S_2$ [M + H]$^+$, 470.1121; found, 470.1123.

*N-(4-(Thiomorpholino)phenyl)-2-((5-(4-fluorophenyl)-thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**39**).* White solid (245 mg, yield: 73%); mp 206−209 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.99 (s, 1H), 7.74−7.68 (m, 2H), 7.40−7.31 (m, 5H), 6.86 (d, J = 9.0 Hz, 2H), 3.93 (s, 2H), 3.46−3.39 (m, 4H), 2.69−2.62 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.2, 163.7 (d, J = 247.4 Hz), 158.6, 147.6, 141.7, 132.8 (d, J = 8.7 Hz), 131.3, 129.9, 124.4 (d, J = 2.5 Hz), 120.8, 117.3, 116.1 (d, J = 21.6 Hz), 115.7, 52.0, 38.1, 26.2; IR (cm^{-1}): 1675, 3263; HRMS: calcd. for $C_{22}H_{21}FN_5OS_3$ [M + H]$^+$, 486.0892; found, 486.0901.

*N-(4-(Diethylamino)phenyl)-2-((5-(4-fluorophenyl)-thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetamide (**40**).* White solid (200 mg, yield: 69%); mp 187−190 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.54 (s, 1H), 7.51−7.45 (m, 2H), 7.36 (d, J = 9.0 Hz, 2H), 7.23−7.16 (m, 2H), 6.77 (s, 1H), 6.60 (d, J = 9.0 Hz, 2H), 3.87 (s, 2H), 3.29 (q, J = 7.2 Hz, 4H), 1.10 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.6, 164.2 (d, J = 252.4 Hz), 158.8, 145.1, 143.3, 131.8 (d, J = 8.7 Hz), 130.2, 127.0, 123.0 (d, J = 3.0 Hz), 121.6, 116.1 (d, J = 22.2 Hz), 114.4, 112.4, 44.6, 36.4, 12.5; IR (cm^{-1}): 3247, 1663; HRMS: calcd. for $C_{22}H_{23}FN_5OS_2$ [M + H]$^+$, 456.1328; found, 456.1335.

Synthesis of VIII(i−p). Nitro compounds (**VII(i−p)**) were synthesized according to literature reports.[57] Nitro compounds were purified by recrystallization, and the data were compared with literature reports. Nitro compounds on reduction gave anilines, **VIII(i−p)**.[47] Anilines, **VIII(i−p)**, were used for the coupling reactions without any purification (**VIII(n)** and **VIII(p)** were obtained commercially).

***In Vitro* Antimicrobial Assay.** All compounds were evaluated for their *in vitro* antimicrobial activity by the agar well diffusion method against two Gram-positive bacterial strains (*B. subtillis* and *S. mutans*), two Gram-negative bacterial strains (*E. coli* and *S. typhi*) and fungi (*C. albicans*). In brief, Mueller−Hinton agar (MHA) medium was prepared, autoclaved and poured in a sterile Petri plate under sterile environment. After solidification of agar plates, 50 μL (106 CFU mL^{-1}) of test cultures were inoculated and spread uniformly by sterile cotton swabs, and 8 mm wells were made on an agar plate using a sterile cork borer. A 100 μg mL^{-1} stock solution of test compounds and standard drugs streptomycin sulfate for bacterial strains and fluconazole as a standard for fungal strains were prepared using DMSO as a solvent. Standard drugs were used as positive controls, and DMSO was used as a negative control. The test compounds (100 μL), standard drug and DMSO were loaded individually in separate wells. The plates were incubated at 37 °C for 18 h, 48 h for fungal culture at 28 °C, and the zone of inhibition was measured by using a calibrated scale and expressed in mm. All the experiments were carried out in triplicate, and mean values were considered for data representation.[58]

MIC values were defined as the lowest concentration of each compound which can inhibit any visible bacterial growth. The assays were performed by the broth dilution method. The different dilutions (2, 4, 8, 16, 32, 64, 128, and 256 μg mL^{-1} in DMSO) of compounds, standard drugs streptomycin sulfate and fluconazole were prepared and transferred to the test cultures which were grown in Mueller−Hinton broth. The tubes were incubated in a shaking incubator fat 37 °C for 12 to 16 h and subsequently checked for the growth of bacteria by measuring their absorbance at 560 nm. The turbidity of each tube is measured with respect to the control tube.

***In Vitro* MABA Assay.** The MICs of compounds against H37Rv were determined by MABA assay. For the *in vitro* MTB MABA assay,[59,60] the inoculum was prepared from fresh Lowenstein−Jensen (LJ) medium resuspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a OD590 1.0, and diluted 1:20; 100 μL was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at 4-fold, the final highest concentration tested. Serial 2-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 μL of 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags, and incubated at 37 °C in normal atmosphere. After 7 days incubation, 30 μL of alamarBlue solution was added to each well, and the plate was reincubated overnight. A change in color from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in color.

***In Vitro M. bovis* Assay.** The antimycobacterial activity of synthesized compounds was tested against the *M. bovis* strain, a good surrogate model using growth inhibition assay by the turbidometry method.[61] The compounds were dissolved in the required volume of DMSO to have 10 mM stock concentrations and further diluted to achieve a working concentration of 1.5 mM. The inoculum for the assay was prepared by reviving a glycerol stock in Middlebrook 7H9 broth supplemented with 0.1% Tween 80 and 0.2% glycerol. At

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

the time of inoculation, 10% ADS was added to the media, and the culture was incubated in a shaker incubator at 37 °C and 200 rpm. When the optical density (O.D.) of the inoculums reached approximately 0.8, a secondary inoculum was inoculated and subsequently incubated. This was incubated until the O.D. of the inoculum reached approximately 0.7, following which the inoculums were diluted by 1:1000. In a 96-well microtiter plate, a 2 μL aliquot of the 1.5 mM dilution of compound was added to each well in triplicate, to which 98 μL of inoculum dilution was added, making the final concentration of compound 30 μM. To minimize media evaporation during assay incubation, 340 μL of sterile water was added to each well of the peripheral rows of the 96-well plate. To each plate, a set of controls was added to better ascertain the activity of the compounds. These included DMSO, which was taken as a growth control, media control (blank), rifampicin and isoniazid as positive controls of inhibition of *M. bovis*. Bacterial growth was assessed after 168 h of incubation by measuring turbidity absorbance at 600 nm using TECAN Infinite 200 PRO. Absorbance is considered directly proportional to the increase in growth of bacteria; thus, this measures growth of bacteria in each well. Percentage inhibition was determined against DMSO.

Resazurin Assay for Anticancer Activity. Anticancer activities of the compounds were determined by resazurin assay.[62,63] This assay is a quantitative fluorometric method used for determination of cell viability. Resazurin is a blue dye used as an oxidation−reduction indicator in cell viability assays and is itself weakly fluorescent until it is irreversibly reduced to the pink colored and highly red fluorescent resorufin. Resazurin is effectively reduced in mitochondria in the presence of NADH dehydrogenases. NADH is the reductant that converts resazurin to resorufin. The reduction of resazurin correlates with the number of live cells. The cells were plated in 96-well plates at a density of 1 × 104 cells in 100 μL of medium per well of the 96-well plate. Cells were treated with 10 μM concentration of test compounds for 48 h. After the incubation period the supernatant was aspirated, and cell monolayers were washed with Dulbecco's phosphate-buffered saline (DPBS). The assay was terminated with the addition of 50 μL (50 μg/mL) of resazurin dye for 1 h. The O.D. was measured at 560 nm for resorufin since resazurin exhibits an absorption peak at 590 nm using a Tecan infinite M200 pro Multimode reader.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06324.

Docking studies, ADME parameters, chemistry and biological studies (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Subashani Maniam − *Applied Chemistry and Environmental Science, STEM College, RMIT University, Melbourne, Victoria 3001, Australia;* orcid.org/0000-0002-0272-864X; Email: subashani.maniam@rmit.edu.au

Authors

Umadevi Kizhakke Purakkel − *Applied Chemistry and Environmental Science, STEM College, RMIT University, Melbourne, Victoria 3001, Australia; Organic Synthesis and Process Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India*

Ganji Praveena − *Organic Synthesis and Process Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India*

Valli Y. Madabhushi − *Organic Synthesis and Process Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India*

Surender Singh Jadav − *Department of Natural Products and Medicinal Chemistry, CSIR-Indian Institute of Chemical Technology Tarnaka, Hyderabad 500037, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India;* orcid.org/0000-0002-5397-8956

Reddy Shetty Prakasham − *Organic Synthesis and Process Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India*

Saiprasad Goud Dasugari Varakala − *Department of Pharmacy, Birla Institute of Technology & Science Pilani, Hyderabad Campus, Hyderabad 500078, India*

Dharmarajan Sriram − *Department of Pharmacy, Birla Institute of Technology & Science Pilani, Hyderabad Campus, Hyderabad 500078, India*

Ewan W. Blanch − *Applied Chemistry and Environmental Science, STEM College, RMIT University, Melbourne, Victoria 3001, Australia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c06324

Author Contributions

U.K.P.: synthesis, data curation, analysis, and draft writing; G.P. and R.S.P.: antimicrobial assay, data curation, analysis, and draft writing; S.S.J.: in silico studies and draft writing; S.G.D.V. and D.S.: MABA assay; Y.V.M.: conceptualization, supervision, and data curation; E.W.B. and S.M.: conceptualization, data curation supervision, writing, review, and editing.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the discussion and contribution of Dr. Prathama S. Mainkar towards this project. The authors are thankful to the Royal Melbourne Institute of Technology (RMIT) and Indian Institute of Chemical Technology (IICT) for providing research facilities and the RMIT-IICT joint Ph.D. program for the funding provided to U.K.P. The authors thank the National Mol Bank of CSIR-IICT for performing anticancer and *M. bovis* assays.

■ ABBREVIATIONS

M. tuberculosis, *Mycobacterium tuberculosis*; *M. bovis*, *Mycobacterium bovis*; TTz, thiazolo[2,3-*c*][1,2,4]triazole; *B. subtilis*, *Bacillus subtilis*; *S. mutans*, *Streptococcus mutans*; *E. coli*, *Escherichia coli*; *S. typhi*, *Salmonella typhi*; *C. albicans*, *Candida albicans*; Cpd, compound

■ REFERENCES

(1) Gomtsyan, A. Heterocycles in drugs and drug discovery. *Chem. Heterocycl. Compd.* **2012**, *48* (1), 7−10.

(2) Kerru, N.; Gummidi, L.; Maddila, S.; Gangu, K. K.; Jonnalagadda, S. B. A Review on Recent Advances in Nitrogen-

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

Containing Molecules and Their Biological Applications. *Molecules* **2020**, *25* (8), 1909.
(3) de la Torre, B. G.; Albericio, F. The Pharmaceutical Industry in 2019. An Analysis of FDA Drug Approvals from the Perspective of Molecules. *Molecules* **2020**, *25* (3), 745.
(4) Petrou, A.; Fesatidou, M.; Geronikaki, A. Thiazole Ring-A Biologically Active Scaffold. *Molecules* **2021**, *26* (11), 3166.
(5) Kumar, S.; Khokra, S. L.; Yadav, A. Triazole analogues as potential pharmacological agents: a brief review. *Future J. Pharm. Sci.* **2021**, *7* (1), 106.
(6) Kharb, R.; Sharma, P. C.; Yar, M. S. Pharmacological significance of triazole scaffold. *J. Enzyme Inhib. Med. Chem.* **2011**, *26* (1), 1−21.
(7) Chhabria, M. T.; Patel, S.; Modi, P.; Brahmkshatriya, P. S. Thiazole: A Review on Chemistry, Synthesis and Therapeutic Importance of its Derivatives. *Curr. Top. Med. Chem.* **2016**, *16* (26), 2841−2862.
(8) El-Sherif, H.; Mahmoud, A.; Sarhan, A.; Hozien, Z.; Habib, O. One pot synthesis of novel thiazolo [3, 2-b][1, 2, 4] triazoles: a useful synthetic application of the acidified acetic acid method. *J. Sulphur Chem.* **2006**, *27* (1), 65−85.
(9) El-Sherief, H. A. M.; Youssif, B. G. M.; Abbas Bukhari, S. N.; Abdelazeem, A. H.; Abdel-Aziz, M.; Abdel-Rahman, H. M. Synthesis, anticancer activity and molecular modeling studies of 1,2,4-triazole derivatives as EGFR inhibitors. *Eur. J. Med. Chem.* **2018**, *156*, 774−789.
(10) Gomaa, H. A. M.; El-Sherief, H. A. M.; Hussein, S.; Gouda, A. M.; Salem, O. I. A.; Alharbi, K. S.; Hayallah, A. M.; Youssif, B. G. M. Novel 1,2,4-triazole derivatives as apoptotic inducers targeting p53: Synthesis and antiproliferative activity. *Bioorg. Chem.* **2020**, *105*, 104369.
(11) Rostom, S. A. F.; Badr, M. H.; Abd El Razik, H. A.; Ashour, H. M. A. Structure-based development of novel triazoles and related thiazolotriazoles as anticancer agents and Cdc25A/B phosphatase inhibitors. Synthesis, in vitro biological evaluation, molecular docking and in silico ADME-T studies. *Eur. J. Med. Chem.* **2017**, *139*, 263−279.
(12) Kumar, P.; Kumar, A.; Makrandi, J. Synthesis and Evaluation of Bioactivity of Thiazolo [3, 2-b]-[1, 2, 4]-triazoles and Isomeric Thiazolo [2, 3-c]-[1, 2, 4]-triazoles. *J. Heterocycl. Chem.* **2013**, *50* (5), 1223−1229.
(13) Barbuceanu, S. F.; Almajan, G. L.; Saramet, I.; Draghici, C.; Tarcomnicu, A. I.; Bancescu, G. Synthesis, characterization and evaluation of antibacterial activity of some thiazolo[3,2-b][1,2,4]-triazole incorporating diphenylsulfone moieties. *Eur. J. Med. Chem.* **2009**, *44* (11), 4752−4757.
(14) Kumsi, M.; Poojary, B.; Lobo, P. L.; Kumari, N. S.; Chullikana, A. Synthesis, characterization and biological studies of some bioactive thiazolotriazole derivatives. *Z. Naturforsch B* **2010**, *65* (12), 1509−1515.
(15) Karthikeyan, M. S. Synthesis, analgesic, anti-inflammatory and antimicrobial studies of 2, 4-dichloro-5-fluorophenyl containing thiazolotriazoles. *Eur. J. Med. Chem.* **2009**, *44* (2), 827−833.
(16) Krishnaraj, T.; Muthusubramanian, S. Synthesis of 6-Aryl-4H-imidazo[1,2-b][1,2,4]triazoles and 6-Aryl-thiazolo[3,2-b][1,2,4]-triazoles. *J. Heterocycl. Chem.* **2015**, *52* (5), 1314−1320.
(17) Jakhar, K.; Makrandi, J. K. A green synthesis and antibacterial activity of 2-aryl 5-coumarin −3-yl-thiazolo[3,2-b][1,2,4]triazoles. *Indian J. Chem.* **2012**, *51B*, 1511−1516.
(18) Sarigol, D.; Uzgoren-Baran, A.; Tel, B. C.; Somuncuoglu, E. I.; Kazkayasi, I.; Ozadali-Sari, K.; Unsal-Tan, O.; Okay, G.; Ertan, M.; Tozkoparan, B. Novel thiazolo[3,2-b]-1,2,4-triazoles derived from naproxen with analgesic/anti-inflammatory properties: Synthesis, biological evaluation and molecular modeling studies. *Bioorg. Med. Chem.* **2015**, *23* (10), 2518−2528.
(19) Gaware, V. M.; Dighe, N. S.; Pattan, S. R.; Shinde, H. V.; Musmade, D. S.; Chavan, P. A.; Patel, P. Thiazolo-Triazole a nucleus possessing range of pharmacological activities: a review. *Der Pharm. Lett.* **2010**, *2* (2), 35−40.
(20) Peng, Y.-H.; Liao, F.-Y.; Tseng, C.-T.; Kuppusamy, R.; Li, A.-S.; Chen, C.-H.; Fan, Y.-S.; Wang, S.-Y.; Wu, M.-H.; Hsueh, C.-C.; et al. Unique Sulfur-Aromatic Interactions Contribute to the Binding of Potent Imidazothiazole Indoleamine 2,3-Dioxygenase Inhibitors. *J. Med. Chem.* **2020**, *63* (4), 1642−1659.
(21) Kotapalli, S. S.; Nallam, S. S. A.; Nadella, L.; Banerjee, T.; Rode, H. B.; Mainkar, P. S.; Ummanni, R. Identification of New Molecular Entities (NMEs) as Potential Leads against Tuberculosis from Open Source Compound Repository. *PLoS One.* **2015**, *10* (12), No. e0144018.
(22) WHO. *Antimicrobial resistance factsheet*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (accessed on 7/30/2023).
(23) Prestinaci, F.; Pezzotti, P.; Pantosti, A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health* **2015**, *109* (7), 309−318.
(24) Reygaert, W. C. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol.* **2018**, *4* (3), 482−501.
(25) Jackson, N.; Czaplewski, L.; Piddock, L. J. V. Discovery and development of new antibacterial drugs: learning from experience? *J. Antimicrob. Chemother.* **2018**, *73* (6), 1452−1459.
(26) Miethke, M.; Pieroni, M.; Weber, T.; et al. Towards the sustainable discovery and development of new antibiotics. *Nat. Rev. Chem.* **2021**, *5* (10), 726−749.
(27) Vitaku, E.; Smith, D. T.; Njardarson, J. T. Analysis of the Structural Diversity, Substitution Patterns, and Frequency of Nitrogen Heterocycles among U.S. FDA Approved Pharmaceuticals. *J. Med. Chem.* **2014**, *57* (24), 10257−10274.
(28) Poce, G.; Cocozza, M.; Consalvi, S.; Biava, M. SAR analysis of new anti-TB drugs currently in pre-clinical and clinical development. *Eur. J. Med. Chem.* **2014**, *86*, 335−351.
(29) Beena; Rawat, D. S. Antituberculosis Drug Research: A Critical Overview. *Med. Res. Rev.* **2013**, *33* (4), 693−764.
(30) Li Petri, G.; Raimondi, M. V.; Spanò, V.; Holl, R.; Barraja, P.; Montalbano, A. Pyrrolidine in Drug Discovery: A Versatile Scaffold for Novel Biologically Active Compounds. *Top. Curr. Chem. (Z)* **2021**, *379* (5), 34.
(31) Siwach, A.; Verma, P. K. Synthesis and therapeutic potential of imidazole containing compounds. *BMC Chem.* **2021**, *15* (1), 12.
(32) Sharma, A.; Wakode, S.; Fayaz, F.; Khasimbi, S.; Pottoo, F. H.; Kaur, A. An Overview of Piperazine Scaffold as Promising Nucleus for Different Therapeutic Targets. *Curr. Pharm. Des.* **2020**, *26* (35), 4373−4385.
(33) Abdelshaheed, M. M.; Fawzy, I. M.; El-Subbagh, H. I.; Youssef, K. M. Piperidine nucleus in the field of drug discovery. *Future J. Pharm. Sci.* **2021**, *7* (1), 188.
(34) Kourounakis, A. P.; Xanthopoulos, D.; Tzara, A. Morpholine as a privileged structure: A review on the medicinal chemistry and pharmacological activity of morpholine containing bioactive molecules. *Med. Res. Rev.* **2020**, *40* (2), 709−752.
(35) Cancemi, P.; Buttacavoli, M.; D'Anna, F.; Feo, S.; Fontana, R. M.; Noto, R.; Sutera, A.; Vitale, P.; Gallo, G. The effects of structural changes on the anti-microbial and anti-proliferative activities of diimidazolium salts. *New J. Chem.* **2017**, *41* (9), 3574−3585.
(36) Nasompag, S.; Dechsiri, P.; Hongsing, N.; Phonimdaeng, P.; Daduang, S.; Klaynongsruang, S.; Camesano, T. A.; Patramanon, R. Effect of acyl chain length on therapeutic activity and mode of action of the CX-KYR-NH2 antimicrobial lipopeptide. *Biochim. Biophys. Acta, Biomembr. 10, Part A* **2015**, *1848*, 2351−2364.
(37) Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. Fluorine in medicinal chemistry. *Chem. Soc. Rev.* **2008**, *37* (2), 320−330.
(38) Kar, S. S.; Thomas, A. C. Strategically Placed Trifluoromethyl Substituent in the Realm of Antitubercular Drug Design. *Curr. Drug Ther.* **2019**, *14* (2), 114−123.
(39) Moir, D. T. Identification of inhibitors of bacterial enoyl-acyl carrier protein reductase. *Curr. Drug Targets Infect. Disord.* **2005**, *5* (3), 297−305.

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861

(40) Zitko, J.; Doležal, M. Enoyl acyl carrier protein reductase inhibitors: an updated patent review (2011 −2015). *Expert. Opin. Ther. Pat.* **2016**, *26* (9), 1079−1094.
(41) Massengo-Tiassé, R. P.; Cronan, J. E. Diversity in enoyl-acyl carrier protein reductases. *Cell. Mol. Life Sci.* **2009**, *66* (9), 1507−1517.
(42) Rana, P.; Ghouse, S. M.; Akunuri, R.; Madhavi, Y. V.; Chopra, S.; Nanduri, S. FabI (enoyl acyl carrier protein reductase) - A potential broad spectrum therapeutic target and its inhibitors. *Eur. J. Med. Chem.* **2020**, *208*, 112757.
(43) Doğan, H.; Doğan, Ş. D.; Gündüz, M. G.; Krishna, V. S.; Lherbet, C.; Sriram, D.; Şahin, O.; Sarıpınar, E. Discovery of hydrazone containing thiadiazoles as Mycobacterium tuberculosis growth and enoyl acyl carrier protein reductase (InhA) inhibitors. *Eur. J. Med. Chem.* **2020**, *188*, 112035.
(44) Saito, J.; Yamada, M.; Watanabe, T.; Iida, M.; Kitagawa, H.; Takahata, S.; Ozawa, T.; Takeuchi, Y.; Ohsawa, F. Crystal structure of enoyl-acyl carrier protein reductase (FabK) from Streptococcus pneumoniae reveals the binding mode of an inhibitor. *Protein Sci.* **2008**, *17* (4), 691−699.
(45) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23* (1), 3−25.
(46) Gleeson, M. P.; Hersey, A.; Hannongbua, S. In-silico ADME models: a general assessment of their utility in drug discovery applications. *Curr. Top. Med. Chem.* **2011**, *11* (4), 358−381.
(47) Hu, G.; Wang, C.; Xin, X.; Li, S.; Li, Z.; Zhao, Y.; Gong, P. Design, synthesis and biological evaluation of novel 2, 4-diaminopyrimidine derivatives as potent antitumor agents. *New J. Chem.* **2019**, *43* (25), 10190−10202.
(48) Kim, T. O.; Im, D. W.; Jung, H. Y.; Kwon, S. J.; Heo, Y. S. Purification, crystallization and preliminary X-ray diffraction analysis of enoyl-acyl carrier protein reductase (FabK) from Streptococcus mutans strain UA159. *Acta Crystallogr. Sect. F. Struct. Biol. Cryst. Commun.* **2012**, *68* (3), 292−294.
(49) Marrakchi, H.; Dewolf, W. E.; Quinn, C.; West, J.; Polizzi, B. J.; So, C. Y.; Holmes, D. J.; Reed, S. L.; Heath, R. J.; Payne, D. J.; et al. Characterization of Streptococcus pneumoniae enoyl-(acyl-carrier protein) reductase (FabK). *Biochem. J.* **2003**, *370* (3), 1055−1062.
(50) Yao, J.; Ericson, M. E.; Frank, M. W.; Rock, C. O. Enoyl-Acyl Carrier Protein Reductase I (FabI) Is Essential for the Intracellular Growth of Listeria monocytogenes. *Infect. Immun.* **2016**, *84* (12), 3597−3607.
(51) Bhat, K. S.; Holla, B. S. Novel three component synthesis of 1,2,4-triazolo[3,4-b]thiazoles and their antimicrobial activity. *Phosphorus Sulfur Silicon Relat. Elem.* **2004**, *179* (6), 1019−1026.
(52) Rudrapal, M.; Satyanandam, R. S.; Swaroopini, T. S.; Lakshmi, T. N.; Jaha, S. K.; Zaheera, S. Synthesis and antibacterial activity of some new hydrazones. *Med. Chem. Res.* **2013**, *22* (6), 2840−2846.
(53) Yefidoff-Freedman, R.; Chen, T.; Sahoo, R.; Chen, L.; Wagner, G.; Halperin, J. A.; Aktas, B. H.; Chorev, M. 3-Substituted Indazoles as Configurationally Locked 4EGI-1 Mimetics and Inhibitors of the eIF4E/eIF4G Interaction. *Chem. Bio. Chem.* **2014**, *15*, 595−611.
(54) Franco, P. H. C.; Vieira, J. G.; Ramos, C. A. d. O.; Johann, S.; de Oliveira, R. B.; César, I. C. Stability-indicating method for the novel antifungal compound RI76: Characterization and in vitro antifungal activity of its active degradation product. *Biomed. Chromatogr.* **2021**, *35*, No. e5014.
(55) Lohrey, L.; Uehara, T. N.; Tani, S.; Yamaguchi, J.; Humpf, H.-U.; Itami, K. 2,4- and 2,5-Disubstituted Arylthiazoles: Rapid Synthesis by C-H Coupling and Biological Evaluation. *Eur. J. Org. Chem.* **2014**, *2014* (16), 3387−3394.
(56) Potts, K. T.; Husain, S. 1,2,4-Triazoles. XXVII. Synthesis of the thiazolo [2,3-c]-s-triazole and the thiazolo[3,2-b]-s-triazole systems. *J. Org. Chem.* **1971**, *36* (1), 10−13.
(57) Abdel-Atty, M. M.; Farag, N. A.; Kassab, S. E.; Serya, R. A. T.; Abouzid, K. A. M. Design, synthesis, 3D pharmacophore, QSAR, and docking studies of carboxylic acid derivatives as Histone Deacetylase inhibitors and cytotoxic agents. *Bioorg. Chem.* **2014**, *57*, 65−82.
(58) Linday, M. E. *Practical Introduction to Microbiology*; E. & F. N. Spon, Ltd.: U.K., 1962.
(59) Collins, L.; Franzblau, S. G. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. *Antimicrob. Agents Chemother.* **1997**, *41* (5), 1004−1009.
(60) Krishna, V. S.; Zheng, S.; Rekha, E. M.; Guddat, L. W.; Sriram, D. Discovery and evaluation of novel Mycobacterium tuberculosis ketol-acid reductoisomerase inhibitors as therapeutic drug leads. *J. Comput. Aided Mol. Des.* **2019**, *33* (3), 357−366.
(61) Ramesh, R.; Shingare, R. D.; Kumar, V.; Anand, A.; B, S.; Veeraraghavan, S.; Viswanadha, S.; Ummanni, R.; Gokhale, R.; Srinivasa Reddy, D. Repurposing of a drug scaffold: Identification of novel sila analogues of rimonabant as potent antitubercular agents. *Eur. J. Med. Chem.* **2016**, *122*, 723−730.
(62) Sharma, N.; Arya, G.; Kumari, R. M.; Gupta, N.; Nimesh, S. Evaluation of Anticancer activity of Silver Nanoparticles on the A549 Human lung carcinoma cell lines through Alamar Blue Assay. *Bio. Protoc.* **2019**, *9* (1), No. e3131.
(63) Kamiloglu, S.; Sari, G.; Ozdal, T.; Capanoglu, E. Guidelines for cell viability assays. *Food Frontiers.* **2020**, *1* (3), 332−349.

https://doi.org/10.1021/acsomega.3c06324
ACS Omega 2024, 9, 8846−8861