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Antituberculosis Activity of the Molecular Libraries Screening Center Network Library

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SUMMARY

There is an urgent need for the discovery and development of new antitubercular agents that target novel biochemical pathways and treat drug-resistant forms of the disease. One approach to addressing this need is through high-throughput screening of drug-like small molecule libraries against the whole bacterium in order to identify a variety of new, active scaffolds that will stimulate additional biological research and drug discovery. Through the Molecular Libraries Screening Center Network, the NIAID Tuberculosis Antimicrobial Acquisition and Coordinating Facility tested a 215,110-compound library against *M. tuberculosis* strain H37Rv. A medicinal chemistry survey of the results from the screening campaign is reported herein.

Keywords

MLSCN; TAACF; antitubercular; high-throughput screening; medicinal chemistry analysis; small molecule chemical library; tuberculosis

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CONFLICT OF INTEREST STATEMENT Competing interests: Dr. Goldman is a NIAID staff member who either in the past or currently provides oversight for the project that generated the data used as the basis for this work.

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Tuberculosis (TB) represents one of the top public health concerns worldwide. One-third of the world's population is infected with *Mycobacterium tuberculosis* (*M. tb*), the etiological agent of TB, resulting in 9.2 million new cases and 1.7 million deaths in 2006.¹ Multidrug-resistant TB, defined as resistance to at least the first-line drugs isoniazid and rifampin, and extensively drug-resistant (XDR-) TB, defined as resistance to rifampin, isoniazid, fluoroquinolones, and to at least one of the injectable second-line drugs, have contributed to the resurgence of the disease.²⁻³ Estimates for the global prevalence of drug-resistant TB (including XDR-TB, currently estimated at 40,000 cases per year) are likely a lower bound of the real case burden.⁴ Forty-nine countries have now reported XDR-TB infections as of June 2008, according to the WHO Stop TB Department.⁵ Consequently, the need for novel, more effective drugs is evident. Treatment of active disease needs to be shortened, simplified, and not interfere with the administration of antiretroviral agents. It is also highly desirable to identify new types of TB drugs acting on novel drug targets with no cross-resistance to existing therapeutics.

The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) was established by the National Institute of Allergy and Infectious Diseases (NIAID) in 1994 to allow researchers access to high quality screening services in order to encourage antituberculosis drug discovery research. The functions of the TAACF are described more fully in a recent publication.⁶ Unique to the program is the involvement of medicinal chemists in order to recruit high quality, medicinally relevant compounds into the screening program. More recently, large libraries of drug-like molecules were designed and purchased to supplement those screening samples being donated by the larger research community. Modern high-throughput screening (HTS) systems provide an immensely powerful strategy to identify new lead compounds in a relatively short amount of time. The results of the screening of one such library, a 100,997-compound set obtained by NIAID from ChemBridge, was described in the previous paper in this issue.⁷ Here we summarize the results of a second complementary HTS campaign resulting from a collaboration between the TAACF and an NIH Roadmap initiative, the Molecular Libraries Screening Center Network (MLSCN).

The MLSCN was established in 2005 as a pilot program to assemble a large library of biologically relevant small molecules and make them available through a network of HTS laboratories to researchers worldwide through a competitive assay submission process. Acceptance of the TAACF assay into the MLSCN program made available the unique resources of the NIH Small Molecule Repository (SMR), significantly expanding the spectrum of molecules tested for activity against TB. For this screen, a 215,110-compound library from the SMR was examined for anti-TB activity using the assay described previously,⁷ with the only change to the screening protocol being the elimination of the polyethylene incubator bags, resulting in the identification of a number of novel chemical scaffolds. Moreover, even for classes of compounds identified earlier during testing of the NIAID ChemBridge library,⁷ additional examples emerged that further clarified the structure-activity picture. Since the compounds in the SMR have been examined in scores of diverse assays undertaken by the MLSCN, and the results published on the NIH PubChem website,⁸ another motivation for conducting the MLSCN campaign is the ability to correlate antituberculosis activity of the hits with other biological activities that these compounds may possess, potentially providing information about possible mechanisms of action or toxicity. The raw screening results upon which the structural analysis below is based are now publicly available on PubChem (assay AIDs 1332 and 1626).

MATERIALS AND METHODS

MLSCN TB Compound Selection and Cluster Analysis

A total of 215,110 compounds from the MLSCN SMR library was screened against *M. tb* in a single-dose assay at a concentration of 10 μ M. Of these compounds, 5,839 displayed >80% inhibition of growth of the organism. As described more fully in the previous paper,⁷ growth inhibition was determined by measurement of Alamar blue fluorescence relative to untreated inoculated control wells. Cell viability was determined by luminescence using CellTiter-Glo reagent (Promega). Because of limitations imposed by MLSCN compound resupply rules, only a subset of these hits were able to advance to confirmatory dose-response (D-R) testing. Removal of analogs of known TB drugs, and of compounds with hydrazide, thiono, and other reactive or undesirable functional groups, yielded a set of 3,817 hit compounds; further removal of compounds possessing close similarity to the scaffolds identified as active in a previous HTS campaign⁷ left 3,753 compounds. From this smaller set, a structurally maximally diverse 2,500 compounds were selected on the basis of Tanimoto dissimilarity using a pairwise comparison algorithm in the Tripos SYBYL software package. These 2,500 compounds were evaluated in a D-R assay against *M. tb* and in a cell cytotoxicity assay using VERO cells as previously described.⁷ The D-R assay generated valid TB IC₉₀ data for 2,273 unique compounds, of which 610 compounds possessed TB IC₉₀ values of <10 μ M. In order to identify potentially privileged scaffolds, a clustering analysis was performed on the set of 2,273 compounds using a hierarchical clustering method as implemented in Leadscope. The clustering analysis led to the identification of 22 major scaffolds with significant enrichment ratios for the actives as compared to their distribution in the overall library. Based on activity and selectivity considerations, several scaffolds of interest were identified and are discussed in the following section.

RESULTS AND DISCUSSION

Substituted Quinolones

The screening library contained a small set of 6-fluoroquinolones and 6-fluoronaphthyridinones that are structurally related to established inhibitors of bacterial DNA gyrases and topoisomerase IV.⁹ It is well established that *M. tb* is very sensitive to the newer generation quinolones.¹⁰ Table 1 presents four of the representative actives (**1a-d**) from the screening set that are very similar to known bacterial inhibitors. These compounds have all the hallmarks of newer generation DNA gyrase inhibitors including the 3-COOH group, a 6-F substitution, as well as a basic piperazine moiety at the 7-position.^{9,10}

The table also shows a number of interesting quinolones that diverge from the typical structure associated with DNA gyrase inhibition (**2a-e**), and the basic scaffold shows high, selective activity that should be pursued. Structures **3-5** (see Figures) give other examples from the screen that are related to the compounds given in Table 1. In particular, **3** is an example of a newer generation naphthyridone DNA gyrase inhibitor (cf. gemifloxacin and trovaloxacin). Both **4** and **5** are alternative active amides related to inhibitors in Table 1, although it is not clear if these are prodrugs for a typical 3-COOH quinolone antibiotic or whether they are acting through an alternative mechanism. Quinolone carboxamides have been reported to show antibacterial activity, although the mechanism of action was not clarified.¹¹

Substituted Pyrimidines

The SMR screening set contained over 10,000 variously substituted simple and fused pyrimidines. Approximately 190 of these compounds showed good-to-modest activity in the bacterial growth assay, and only a small number of these samples were deemed both active and selective; for the most part, the active compounds also showed significant toxicity yielding

a selectivity ratio (SI) < 10. Several of the active and selective analogs are depicted in structures **6** and **7** with specific substituents and activity presented in Table 2.

The small numbers of samples in these focused sets do not lend themselves to a thorough structure-activity relationship (SAR) discussion. There are, however, interesting trends in terms of activity/toxicity that are notable. For example, the combination of a 2-(3,5-dimethylpyrazol-1-yl) group with various 4-phenylamino and 4-cycloalkylamino and 5-carboxyethyl substituents (**6a-e**) appears to show higher activity/selectivity ratios than closely related pyrimidines that are substituted with 2-(2-pyridyl), 4-phenylthio, and 5-methoxy groups (data not shown); the latter set being less active and more toxic, yielding selectivity ratios on the order of 1.0. Interestingly, quinazolines with a 2-pyridyl substitution (**7a-c**) as well as a 4-cycloalkyl or 4-arylmethyl group show good activity and selectivity ratios as compared to closely related 2-(2-pyridyl) pyrimidines that are mentioned above. This trend does not hold for other similar 2-substituted quinazolines (2-methyl, 2-cyclobutyl, or 2-(thiophen-2-yl)) that show relatively similar toxicity, but have relatively poor TB IC₉₀ activity values.

Substituted pyrimidines^{12,13} and quinazolines have been reported to show a variety of antibacterial activities including antimycobacterial activity. Derivatives of 2-aryl-3-aminoquinazoline-4(3H)-ones have been reported that show good antibacterial and antitubercular activity.¹⁴⁻¹⁷ There are reports of antibacterial quinazolines that are similar to the hits found in our screen (see structures **8**,¹⁸ **9**,¹⁹ and **10**²⁰).

There is no indication from the literature reports as to a potential target for these compounds, and the small number of actives in each set impacts the ability to define a structure-activity (SAR) profile. The good activity, however, of these drug-like small molecule scaffolds suggests that further work to identify a mode of action and prepare analogs to develop a clear SAR would be worthwhile.

1,3-Diaryl-4-substituted Pyrazoles

Of the seven examples containing scaffold **11**, three had TB IC₉₀ ≤ 10 μM, while only one compound, **11a**, the most active in this cluster, had an SI (defined as CC₅₀/IC₉₀, the ratio of the Vero cell cytotoxicity to the anti-TB activity) of >10 (Table 3). A search of the literature for this specific scaffold did not show any previous reports of TB activity. However, phenylpyrazoles have previously been evaluated for biological activity. For example, a phenylpyrazole has been evaluated as an inhibitor of indoleamine 2,3-dioxygenase, but found not to be active.²¹ Also, a series of 3-(4-phenyloxyphenyl) pyrazoles was studied as a novel class of sodium channel blocker in the rat Chung neuropathy paradigm.²²

1,3,4-Oxadiazoles

1,3,4-oxadiazoles are known from the literature to possess antimycobacterial activity,²³⁻²⁶ and two novel series emerged from this analysis. Of 25 2-substituted thio-5-aryl-1,3,4-oxadiazoles (**12**; Table 4), there were 18 active examples with TB IC₉₀ ≤ 10 μM. D-R testing of these compounds showed that eight had at least modest selectivity with SI > 10. The most active was compound **12a** with SI of 38.

There were four additional 2-alkylthio-5-aryl-1,3,4-oxadiazoles containing the general structure depicted in **13**, all of which had TB IC₉₀ < 4 μM and SI > 10 (Table 5). The most active was compound **13a**. Interestingly, the two most active examples also had the same thiophene-containing 2-alkylthio side chain while the two least active representatives shared the analogous furan-containing 2-alkylthio side chain. Unfortunately, since each compound differed structurally in their respective 5-aryl substituent, it is not possible to definitively conclude whether their differing activities are due to the thiophene-furan modification. A

search of the literature for this specific scaffold did not show any previous reports of TB activity.

2-Carboxamido-1,3,4-oxadiazoles and Related Compounds

The screening set contained 243 acylated 2-amino-1,3,4-oxadiazoles (**14**; Table 6, X = O), of which 44 showed enough activity in the single-dose primary assay to warrant further evaluation in the dose-response format. Of the latter, 20 were confirmed active with a TB IC₉₀ ≤ 20 μM. Though some compounds possessed unacceptable toxicity, many displayed an SI >20. In these compounds the 5-position was invariably substituted, and good activity was observed with a diversity of alkyl, aryl, and heterocyclic groups (**14a-e**).

The closely related 2-amino-1,3,4-thiadiazoles (**14**, X = S; Table 6) were highly represented in the screening set, but were much less active in general. Of 2,277 such compounds, only 23 made the activity cutoff for dose-response screening, but of those, 14 were found active with an TB IC₉₀ ≤ 20 μM. In general the thiadiazoles appeared to be more toxic than their oxygen counterparts, but some were marginally selective (for example, **14f**). The range of 5-substituents was much smaller among the thiadiazole actives, generally restricted to small alkyl or alkylthio moieties.

It should be noted that similar compounds have previously been reported to possess antituberculosis and other antibacterial activities.²⁷

1,2,4-Thiadiazoles

Of the six 3-aryl-5-thioacetamide-substituted 1,2,4-thiadiazoles that were tested in D-R (**15**; Table 7), five possessed TB IC₉₀ < 10 μM. Two compounds (**15a** and **15b**) also displayed SI >10, and thus can be considered potential leads. A search of the literature for this specific scaffold did not identify previous reports of TB activity. However, similar compounds have been reported as bactericidal against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, using a paper-disk diffusion method.²⁸

Tetrahydropyrazolo[1,5-a]pyrimidines

There were a total of 84 compounds containing 5-phenyl-4,5,6,7-tetrahydro[1,5-a]pyrimidine framework in the SMR collection. From this set, seven compounds listed in Table 8 were evaluated in the D-R assay. All of these compounds contained an aryl group at the 5-position and a trifluoromethyl group at the 7-position. In addition, a carboxamide function is present at the 2-position in four compounds (**16a-d**) and at the 3-position in three compounds (**17e-g**). Of these seven compounds, five compounds displayed TB IC₉₀ values < 10 μM. Interestingly, in a recent publication, compounds that are structurally related to **17e-g** have been reported to display weak inhibitory activity against *Staphylococcus aureus* methionyl-tRNA synthetase.²⁹

Low Selectivity Scaffolds

Several classes of compounds were identified that possess good anti-TB activity, but had poor selectivity. In general for these scaffolds, not enough representatives were present in the screening set to fully elucidate the SAR. Consequently, it is possible that through further synthetic work the non-selective toxicities can be disentangled from the desired activity through iterative synthesis/testing, and so these classes should not be dismissed as potential leads. Some of the more interesting of these non-selective scaffolds are discussed below.

3-Phenylpyrazolo[1,5-*a*]pyrimidines

Among the compounds possessing a pyrazolo[1,5-*a*]pyrimidine template, a group of compounds possessing a phenyl substituent at the 3-position and an amine or hydroxyl substituent at the 7-position displayed moderate activity against *M. tb*. Most of these compounds also displayed significant cytotoxicity against VERO cells thus leading to poor SI values (<7.2). One compound in each series did display SI values >10 and both are depicted below (**18,19**).

2,5-Disubstituted Thiazolidin-4-ones

Three of four 4-thiazolidinones possessed TB IC₉₀ of <10 μM (Table 9). Follow-up D-R testing showed that of these three, only **20a** was modestly selective. The literature shows numerous examples of 4-thiazolidinones being studied as antitubercular agents. These include one report that some 2-imino-4-thiazolidinones showed antitubercular activity comparable to streptomycin or phthivazid.³⁰ A few derivatives were reported to inhibit the growth of H37Rv at a concentration of 12.5 μg/mL,³¹ and related analogs were reported by others.³²

4(5)-Phenylacetylimidazole-5(4)-carboxamides

Two compounds from this series, **21a-b** (Table 10), had TB IC₉₀ <10 μM. Since neither had SIs >10 adequate selectivity, additional work is required before assessing the potential of this class of compounds.

Imidazo[1,2-*a*]pyridine-3-amines

The MLSCN library contained 27 imidazo[1,2-*a*]pyridines of generic structure **22a** wherein R₁ is a phenyl or pyridyl ring system. Of these 27 compounds, 12 compounds had the 2-pyridyl ring system (**22b**) as the aryl substituent. In the D-R assay, seven agents from this set displayed TB IC₉₀ values in the range of 1.5–4.4 μM. All of these compounds, however, also displayed significant cytotoxic effects against VERO cells, resulting in poor SI values between 0.4–2.5.

5-Nitrofur-2-carboxamides

Amides derived from 5-nitrofur-2-carboxylic acids have emerged as a class of compounds that display potent antitubercular activity.^{7,33} In conformity with this, a group of such compounds that were present in the MLSCN screening deck were found active. A total of 14 compounds were tested in the D-R assay, resulting in eight compounds possessing TB IC₉₀ <10 μM (**23**; Table 11).

Amides of 3-(Trifluoromethyl)-4-(piperazinylmethyl)aniline

A set of 22 amides derived from 3-(trifluoromethyl)-4-(piperazinylmethyl)aniline by acylation with 3-(acylamino)benzoic acids possessing the generic structure **24** emerged from the assay. Five of these 22 compounds displayed TB IC₉₀ values between 2.8–6.7 μM. These compounds, however, displayed significant cytotoxicities against VERO cells, with SI values in the range of 0.8–4.6.

Individual Compounds

A large number of individual compounds or groups of two or three similar compounds within this library had significant potency and excellent selectivity on the basis of moderate cytotoxicity. Some of these compounds are of structural types that are covered by our initial publication.⁷ Table 12 below provides the structures and activities of several of the most interesting of these compounds.

Acknowledgments

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Abbreviations used in the text

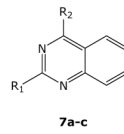
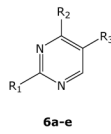
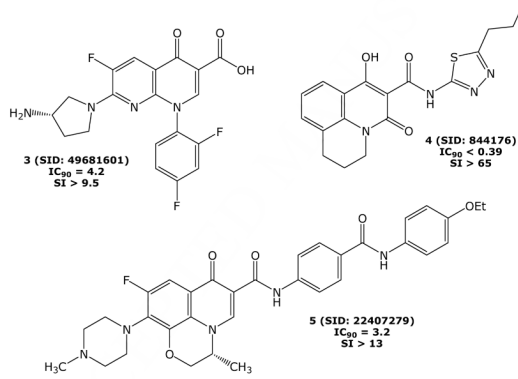
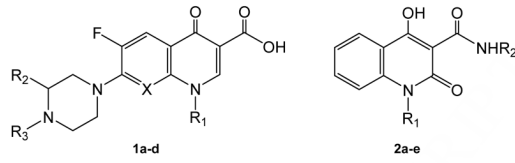
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| NIAID | National Institutes of Allergy and Infectious Disease |
| MLSCN | Molecular Libraries Screening Center Network |
| TAACF | Tuberculosis Antimicrobial Acquisition and Coordinating Facility |
| TB | tuberculosis |
| XDR | extensively drug-resistant |
| WHO | World Health Organization |
| HTS | high-throughput screening |
| NIH | National Institutes of Health |
| SMR | Small Molecule Repository |
| AID | Assay Identifier |
| D-R | dose-response |
| SI | selectivity index |
| SAR | structure-activity relationships |

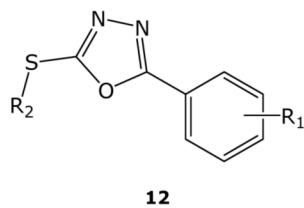
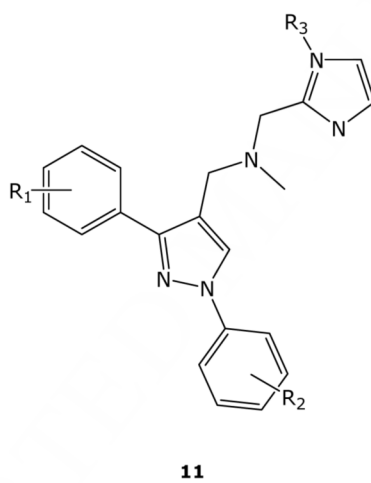
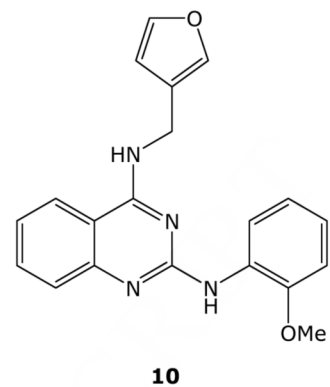
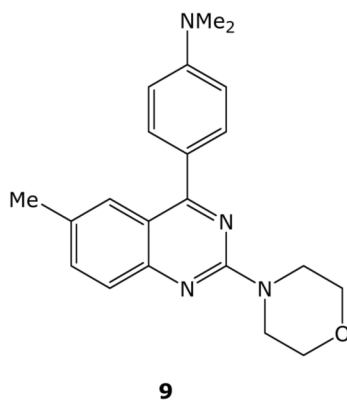
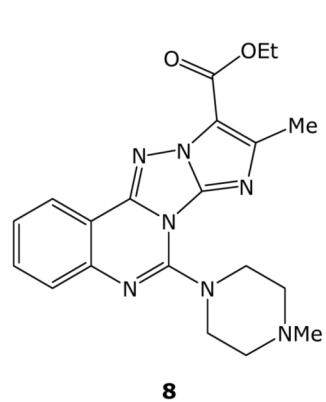
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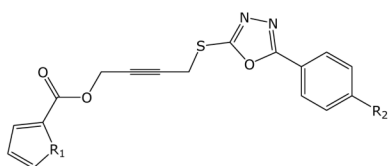
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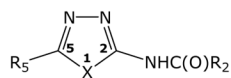
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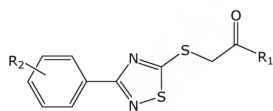




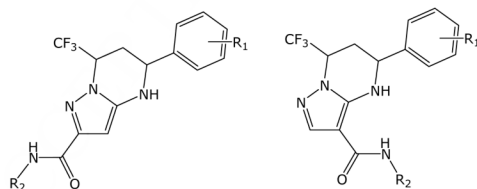
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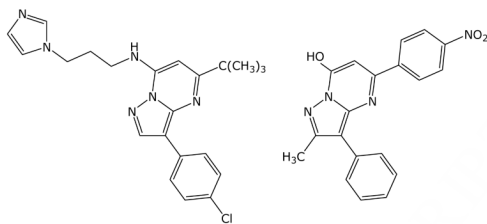


15



16a-d

17e-g

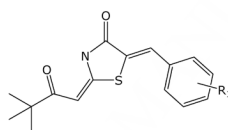


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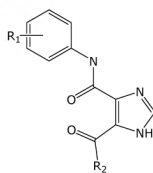
19 (SID 14727639)

IC₉₀ against *M. tb* = 1.4 μM
IC₅₀ against VERO = 22 μM

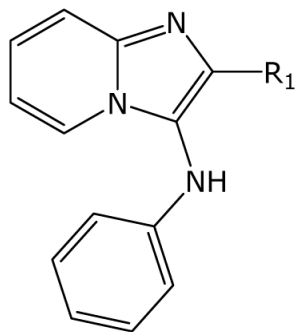
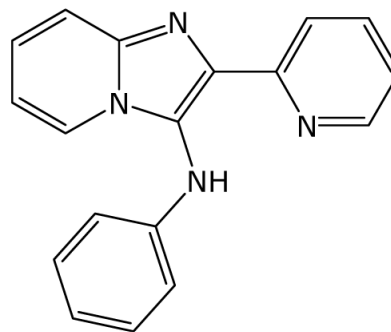
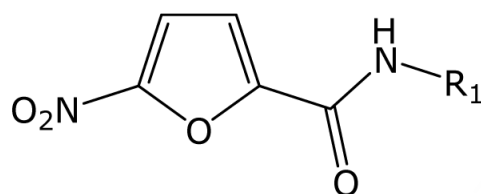
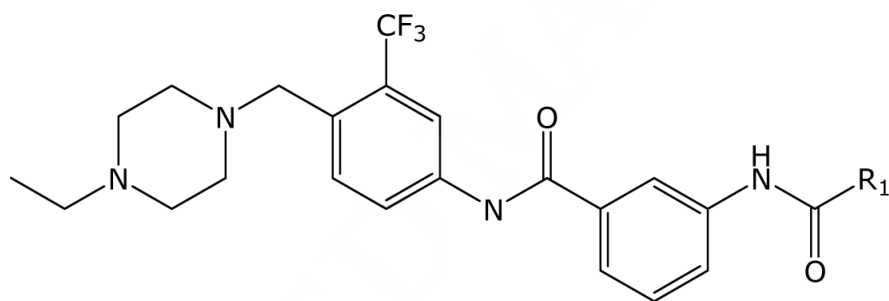
IC₉₀ against *M. tb* = 1.4 μM
IC₅₀ against VERO = 35 μM



20



21

**22a****22b****23****24**

FIGURES.

Table 1

| Cpd. | PubChem/ SID | R ₁ | R ₂ | R ₃ | X | IC ₅₀ ² (μM) | SI ³ |
|-----------|-----------------|----------------------|--|----------------|-----------|---------------------------------------|-----------------|
| 1a | 861394 | cyclopropyl | Me | H | C-methoxy | <0.19 | >204 |
| 1b | 855596 | cyclopropyl | H | ethyl | CH | <0.39 | >102 |
| 1c | 24837296 | cyclopropyl | H | acetyl | CH | 1.6 | >26 |
| 1d | 855614 | ethyl | H | H | CH | 3.1 | >13 |
| 2a | 26660376 | 1-heptyl | 2-pyrazinyl 4- | --- | --- | <0.19 | >12 |
| 2b | 26660314 | 1-hexyl | methylcarboxyethylthiazol- 2-yl | --- | --- | <0.19 | >23 |
| 2c | 24784170 | 3-methyl-1- butyl | methylcarboxyethylthiazol- 2-yl | --- | --- | 0.22 | 38 |
| 2d | 852129 | ethyl | benzimidazol-2-yl | --- | --- | 0.42 | >95 |
| 2e | 24807142 | 1-butyl | 5-iso-propyl-1,3,4- thiadiazol-2-yl | --- | --- | 0.84 | >48 |

¹ PubChem Substance Identifier, see <http://pubchem.ncbi.nlm.nih.gov>

² Antituberculosis activity, defined as the concentration of drug inhibiting 90% growth relative to untreated controls as measured fluorometrically

³ Selectivity index, defined as CC₅₀/IC₉₀

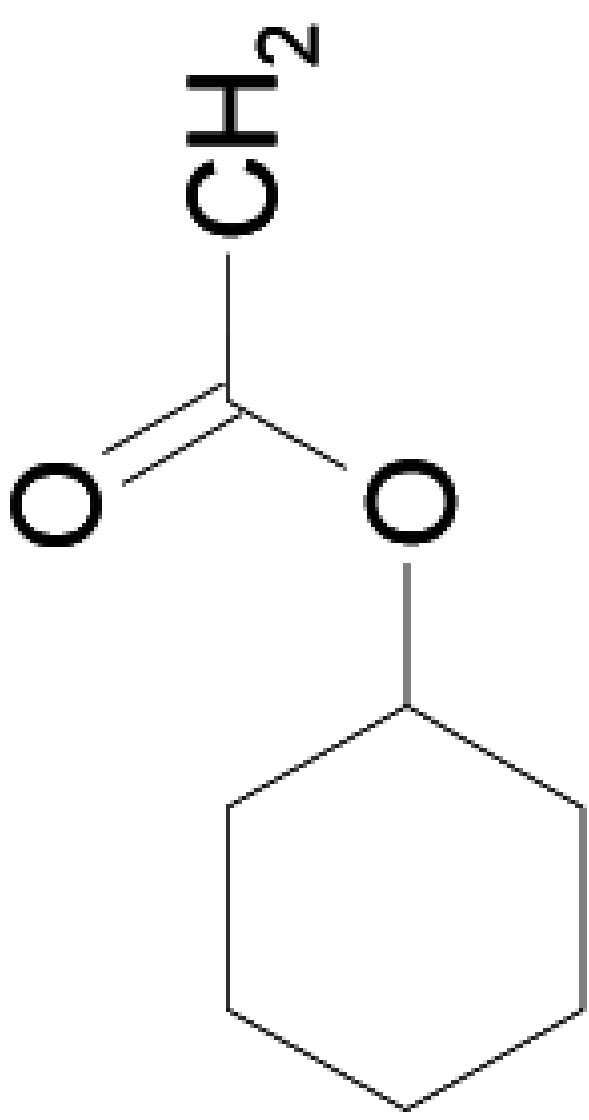
Table 2

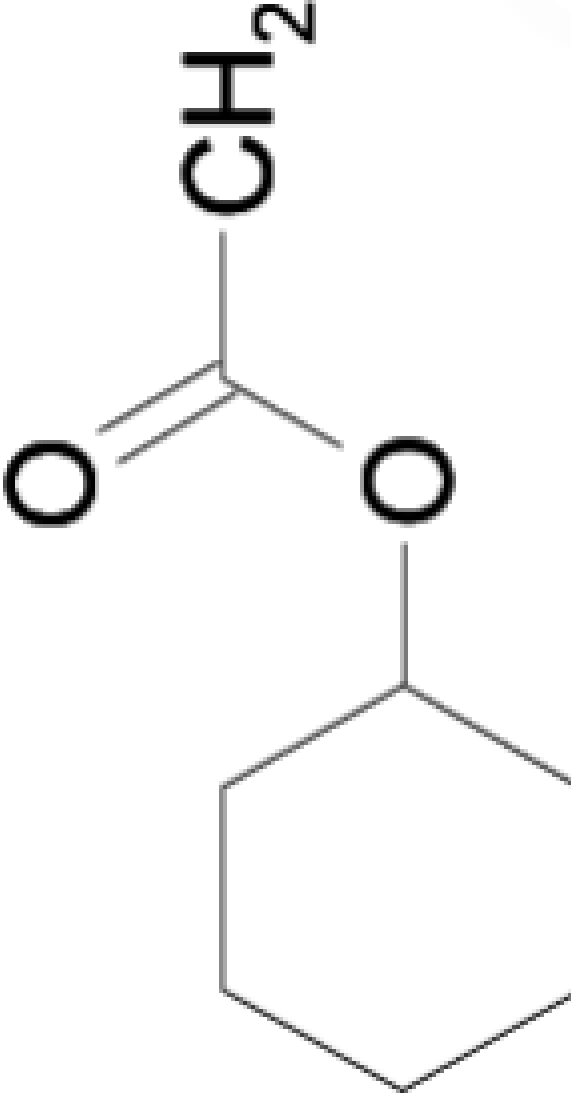
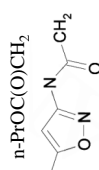
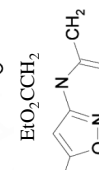
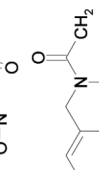







| Cpd. | PubChem SID | R ₁ | R ₂ | R ₃ | IC ₅₀ SI (μM) |
|-----------|-------------|--------------------------|---------------------------------|----------------|--------------------------|
| 6a | 860872 | 3,5-dimethylpyrazol-1-yl | N-(4-dimethylaminophenyl) amino | COOEt | <0.19>120 |
| 6b | 26661470 | 3,5-dimethylpyrazol-1-yl | cyclopentylamino | COOEt | 0.86 18 |
| 6c | 861934 | 3,5-dimethylpyrazol-1-yl | phenethylamino | COOEt | 1.1 >38 |
| 6d | 14741571 | 3,5-dimethylpyrazol-1-yl | N-(4-methoxyphenyl) amino | COOEt | 1.3 >32 |
| 6e | 861838 | 3,5-dimethylpyrazol-1-yl | (S)-(-)-α-methylbenzylamino | COOEt | 2.1 17 |
| 7a | 864349 | 2-pyridyl | N-hexamethylenimino | --- | 0.32 13 |
| 7b | 860397 | 2-pyridyl | N-4-phenylpiperazino | --- | 1.6 16 |
| 7c | 861193 | 2-pyridyl | (S)-(+)-tetrahydrofurfurylamino | --- | 3.2 >12 |

Table 3

| Cpd. | PubChem SID | R ₁ | R ₂ | R ₃ | IC ₅₀ SI (μ M) |
|------|----------------|----------------|----------------|----------------|-----------------------------------|
| 11a | 24798067 | 3-methyl | 4-methyl | ethyl | 3.6 >11 |
| 11b | 24784374 | 3-F | 3-methyl | ethyl | 4.2 >9.6 |
| 11c | 24800363 | 4-F | 4-methyl | ethyl | 6.7 >6.0 |
| 11d | 24783789 | H | 2, 5-dimethyl | ethyl | 10.3 >3.9 |
| 11e | 24800267 | 3-F | 3-methyl | methyl | 12.1 >3.3 |
| 11f | 24800597 | 3-methyl | 4-methyl | H | 13.5 >3.0 |
| 11g | 24800814 | 3-methyl | 4-methyl | methyl | 15.9 >2.5 |

Table 4

| Cpd. | PubChem SID | R ₁ | R ₂ | IC ₅₀ SI (μ M) |
|------|----------------|----------------|--|-----------------------------------|
| 12a | 843463 | 3,4-dimethoxy |  | 1.0 >38 |

| Cpd. | PubChem SID | R ₁ | R ₂ | IC ₉₀ SI (nM) |
|------------|-------------|---------------------------|---|--------------------------|
| 12b | 851931 | 3,4,5-trimethoxy |  | 1.8 >23 |
| 12c | 7972059 | 3,4,5-trimethoxy |  | 1.8 >22 |
| 12d | 24788973 | 4-ethoxy |  | 1.9 >21 |
| 12e | 847334 | 2,4-dimethoxy |  | 2.4 >1 |
| 12f | 4246679 | 3,4-dioxoethylene |  | 2.5 >16 |
| 12g | 3714049 | 2-bromo |  | 3.4 >12 |
| 12h | 24808448 | 4-nitro |  | 3.8 >10 |
| 12i | 24805084 | 3,4,5-trimethoxy |  | 4.4 >9 |
| 12j | 852638 | 2,4-dimethoxy 4-methyl |  | 4.5 >9 |
| 12k | 851695 | benzenesulfonami do |  | 5.3 >8 |
| 12l | 24804266 | 3,4-dimethoxy |  | 5.5 >7 |

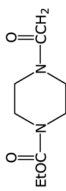
| Cpd. | PubChem SID | R ₁ | R ₂ | IC ₉₀ SI (nM) |
|------------|----------------|------------------------|---|-----------------------------|
| 12m | 848919 | benzenesulfonami do | MeOC(O)CH ₂ | 5.7 >7 |
| 12n | 24837552 | 4-methoxy | | 6.2 >7 |
| 12o | 4246792 | 3,5-dimethoxy |  | 7.0 >6 |
| 12p | 4247581 | 3-methoxy | | 8.5 >5 |
| 12q | 851619 | 2,4-dichloro | i-PrOC(O)CH ₂ | 9.2 >4 |
| 12r | 852877 | 2,4-dichloro | EtOC(O)CH ₂ | 9.4 >4 |

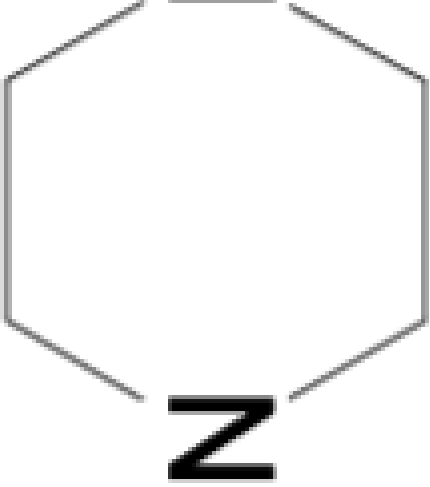
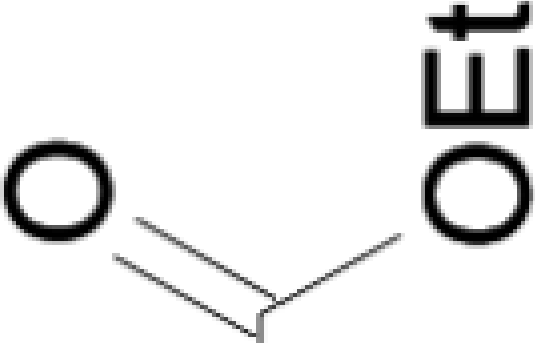
Table 5

| Cpd. | PubChem SID | R ₁ | R ₂ | IC ₅₀ (μ M) | SI |
|------|----------------|----------------|-----------------|--------------------------------|-----|
| 13a | 14735971 | S | NO ₂ | 1.3 | >31 |
| 13b | 14734336 | S | Br | 2.4 | >16 |
| 13c | 14723706 | O methyl | | 3.4 | >12 |
| 13d | 843601 | O | H | 3.9 | >10 |

Table 6

| Cpd. | PubChem SID | R ₂ | R ₅ | IC ₅₀ SI (μM) |
|------|-------------|--------------------|-------------------------------|--------------------------|
| 14a | 14736804 | 2,4-dichlorophenyl | 1,2,3,4-tetrahydronaphth-6-yl | 1.0 >41 |
| 14b | 3715915 | β-naphthyl | methyl | 1.5 >27 |
| 14c | 24816541 | 4-methylphenyl | 4-pyridyl | 7.0 >6 |
| 14d | 7971249 | 3-phenoxyphenyl | 2-thienyl | 2.7 >13 |
| 14e | 17515772 | 4-chlorophenyl | 2,3-dihydro-1,4-dioxin-2-yl | 4.4 >9 |
| 14f | 14743883 | quinolin-2-yl | 3-pentyl | 1.5 24 |

Table 7

| Cpd. | PubChem SID | R ₁ | R ₂ | IC ₅₀ SI (μ M) |
|------------|----------------|---|---|-----------------------------------|
| 15a | 4244687 |  |  | 3-methyl 0.8 >48 |
| 15b | 24816149 | NHCH ₂ CO ₂ Et | H | 3.2 >13 |

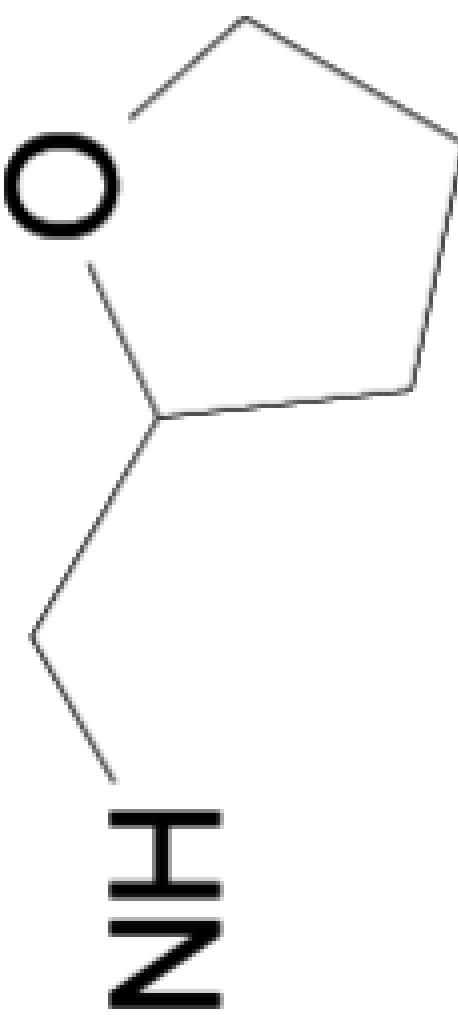
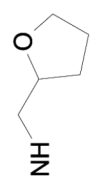
| Cpd. PubChem SID | R ₁ | R ₂ | IC ₉₀ SI (μM) |
|------------------------|--|----------------|-----------------------------|
| 15c 24816198 |  | 3-methyl | 4.2 >10 |
| 15d 24816150 |  | 4-methoxy | 5.2 >8 |
| 15e 24816621 | NHCH ₂ CO ₂ Et | 3-methyl | 8.3 >5 |

Table 8

| Cpd. | PubChem SID | R ₁ | R ₂ | IC ₅₀ SI (μ M) |
|------|----------------|--------------------|----------------------------------|-----------------------------------|
| 16a | 846634 | 4-methyl | 2-furanylmethyl | 1.8 >22 |
| 16b | 858272 | H | n-butyl | 4.1 >10 |
| 16c | 4257312 | 3,4-methylenedioxy | 2-hydroxy-5-chlorophenyl | >100 |
| 16d | 849999 | 3,4-dichlorophenyl | 2-furanylmethyl | >100 |
| 17e | 7976388 | 4-bromo | 2-furanylmethyl | 1.7 >23 |
| 17f | 844088 | 4-methyl | 2-furanylmethyl | 3.6 >11 |
| 17g | 4260736 | 4-bromo | (1,5-dimethylpyrazol-4-yl)methyl | 6.5 >6 |

Table 9

| Cpd. | PubChem SID | R₁ | IC₉₀ (μM) | SI |
|-------------|------------------------|----------------------|--|-----------|
| 20a | 14744789 | 4-ethyl | 3.6 | >11.2 |
| 20b | 24832975 | 4-methoxy-3-hydroxy | 7.6 | 3.3 |
| 20c | 14744896 | 3-methyl | 11.6 | 2.5 |
| 20d | 14744668 | 2-methyl | >100 | |

Table 10

| Cpd. PubChem SID | R ₁ | R ₂ | IC ₅₀ SI (μM) |
|------------------|----------------|----------------|--------------------------|
| 21a | 26732680 | H | 5.6 >7.1 |
| 21b | 24833778 | 4-methyl | 6.4 >6.2 |

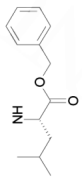
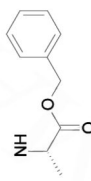
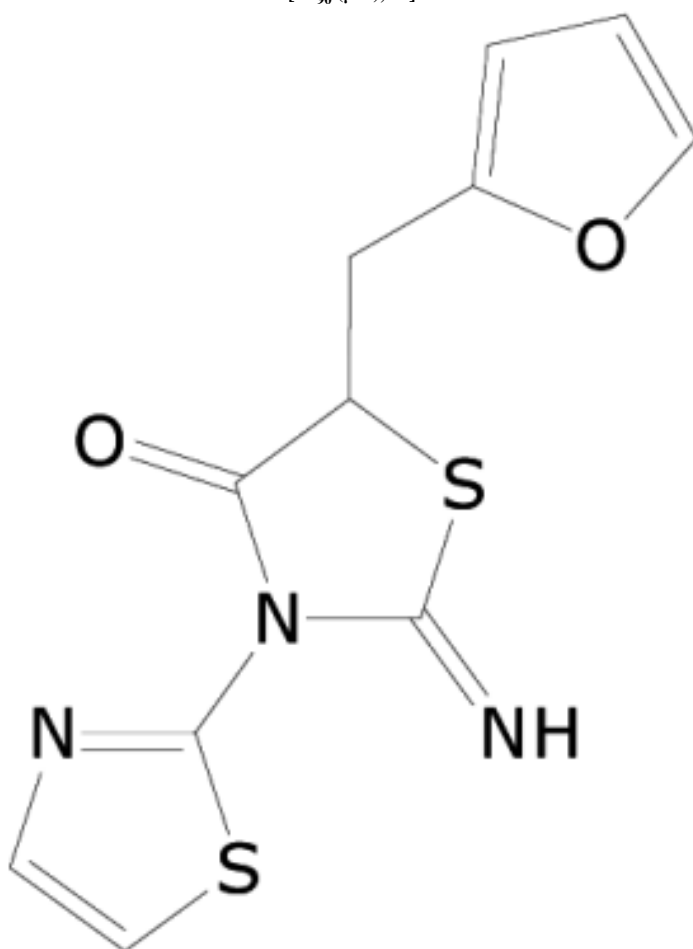
| Cpd. | PubChem SID | R ₁ | R ₂ | IC ₅₀ SI (μM) |
|------------|-------------|----------------|---|--------------------------|
| 21c | 26732664 | 3-methyl |  | >100 |
| 21d | 26732673 | 3-methyl |  | >100 |

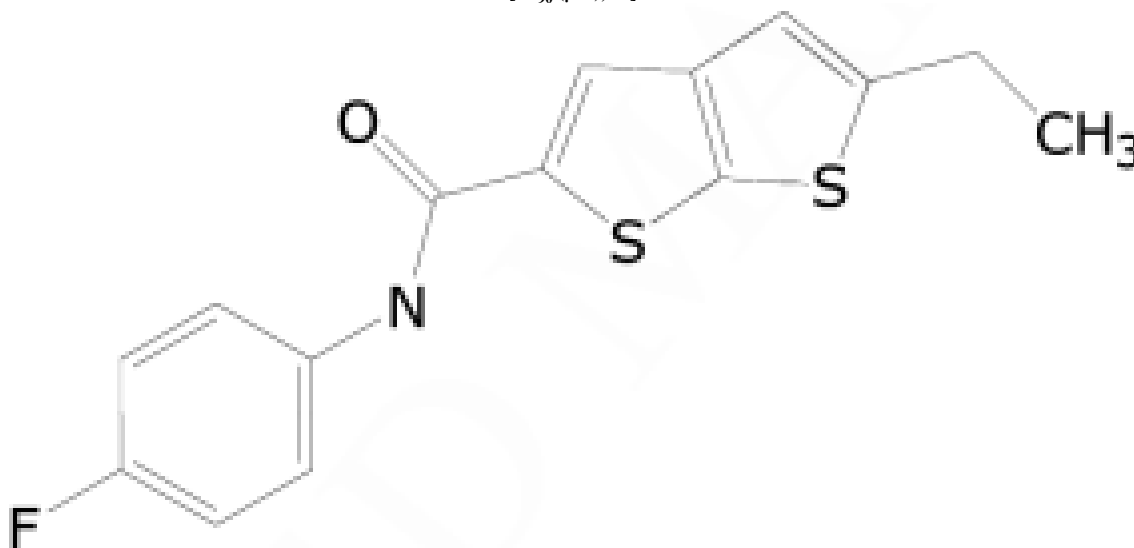
Table 11

| Cpd. | PubChem SID | R ₁ | IC ₉₀ (μM) | SI |
|------------|----------------|--|--------------------------|------|
| 23a | 24808856 | 4-[(4-butanoyl)piperazinyl]-3-chlorophenyl | <0.19 | >9 |
| 23b | 24796288 | 4-[(4-benzoyl)piperazinyl]phenyl | <0.19 | >8 |
| 23c | 24793086 | 4-[4-(4-chlorobenzoyl)piperazinyl]phenyl | <0.19 | >2 |
| 23d | 24809040 | 4-[(4-butanoyl)piperazinyl]phenyl | 2.2 | 1.4 |
| 23e | 17403602 | 4-methylbenzyl | 2.9 | 2.9 |
| 23f | 24808888 | 3-methylphenyl | 6.2 | 0.6 |
| 23g | 24808199 | 5-chloro-2pyridyl | 7.2 | 1.2 |
| 23h | 24789398 | 3,4-dimethylphenyl | 8.6 | >4.6 |

Table 12

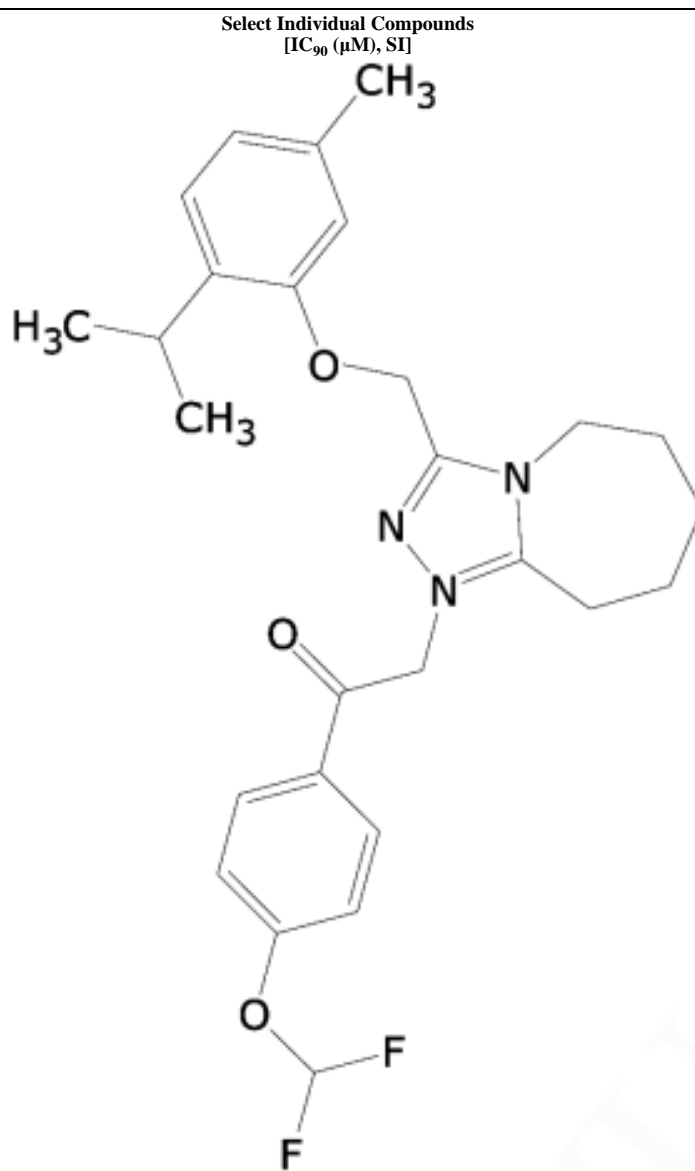
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Select Individual Compounds
[IC₉₀ (μM), SI]



SID: 14718831

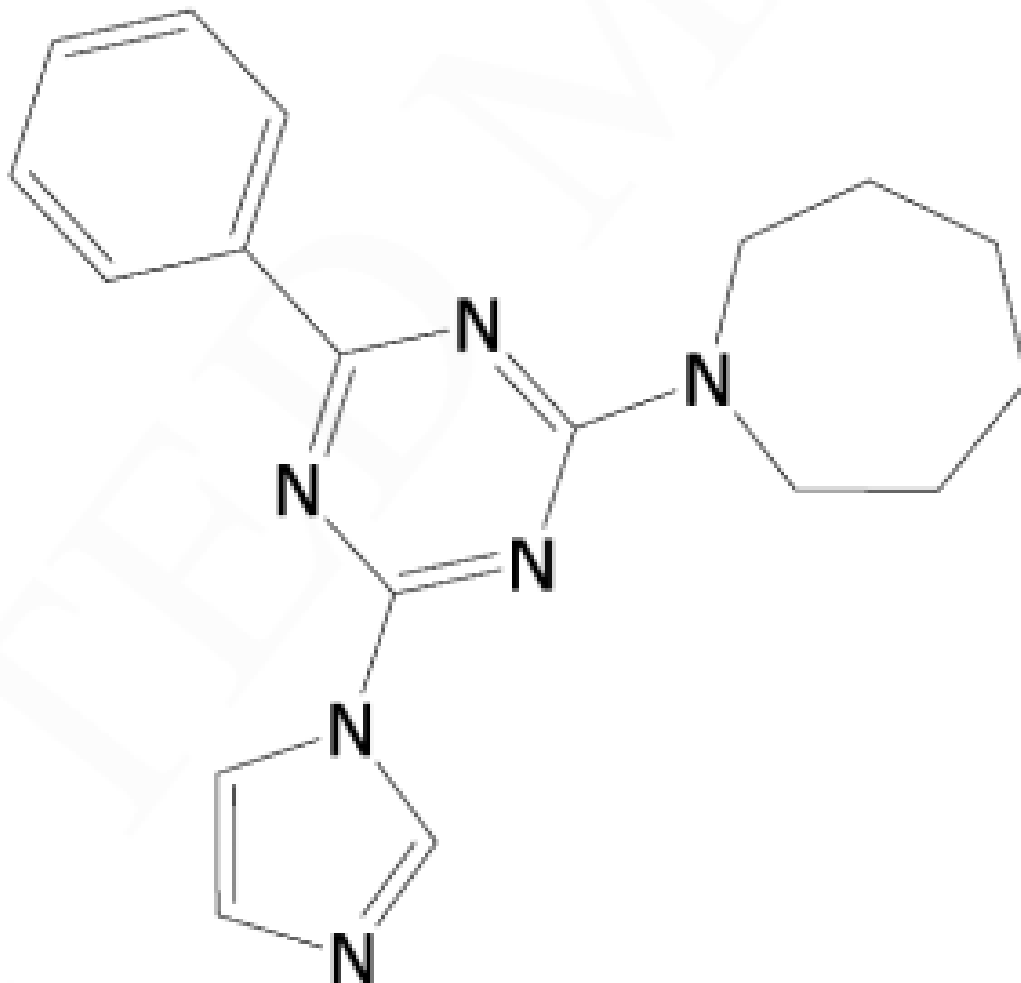
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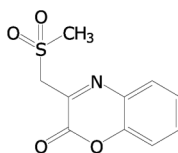
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[IC₉₀ (μM), SI]



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1.9, 16



SID: 850466

2.0, 20