Published in final edited form as:
Tuberculosis (Edinb). 2012 January ; 92(1): 72–83. doi:10.1016/j.tube.2011.05.005.

High Throughput Screening of a Library Based on Kinase Inhibitor Scaffolds Against *Mycobacterium Tuberculosis* H37Rv

Robert C. Reynolds[a,*], **Subramaniam Ananthan**[a,c], **Ellen Faaleolea**[b,d], **Judith V. Hobrath**[a,e], **Cecil D. Kwong**[a,f], **Clinton Maddox**[a,g], **Lynn Rasmussen**[a,h], **Melinda I. Sosa**[a,i], **Elizabeth Thammasuvimol**[b,j], **E. Lucile White**[a,k], **Wei Zhang**[a,l], and **John A. Secrist III**[a,m]

[a]Southern Research Institute, 2000 Ninth Avenue South, Birmingham, AL 35205, USA

[b]Southern Research Institute, 431 Aviation Way, Frederick, MD 21701, USA

Summary

Kinase targets are being pursued in a variety of diseases beyond cancer, including immune and metabolic as well as viral, parasitic, fungal and bacterial. In particular, there is a relatively recent interest in kinase and ATP-binding targets in *Mycobacterium tuberculosis* in order to identify inhibitors and potential drugs for essential proteins that are not targeted by current drug regimens. Herein, we report the high throughput screening results for a targeted library of approximately 26,000 compounds that was designed based on current kinase inhibitor scaffolds and known kinase binding sites. The phenotypic data presented herein may form the basis for selecting scaffolds/compounds for further enzymatic screens against specific kinase or other ATP-binding targets in *Mycobacterium tuberculosis* based on the apparent activity against the whole bacteria *in vitro*.

Keywords

TAACF; Antitubercular; High-throughput screening methods; Medicinal chemistry analysis; Designed kinase inhibitor library

There is a critical need for the development of new drugs to treat tuberculosis due to the recent and rapid appearance of numerous single, multiple, and extensively drug-resistant forms of the disease. In response to the potential for a significant public health crisis need, the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) was established in 1994 by the National Institutes of Health through NIAID. Over 16 years,

[*]Corresponding author. Tel.: +1 205 581 2454; fax: +1 205 581 2447.
reynolds@SouthernResearch.org (R.C. Reynolds), ananthan@SouthernResearch.org (S. Ananthan), faaleolea@SouthernResearch.org (E. Faaleolea), hobrath@SouthernResearch.org (J.V. Hobrath), kwong@SouthernResearch.org (C.D. Kwong), maddox@SouthernResearch.org (C. Maddox), rasmussen@SouthernResearch.org (L. Rasmussen), sosa@SouthernResearch.org (M.I. Sosa), tham@SouthernResearch.org (E. Thammasuvimol), white@SouthernResearch.org (E.L. White), zhangw@SouthernResearch.org (W. Zhang), secrist@SouthernResearch.org (J.A. Secrist III).
[c]Tel.: +1 205 581 2822; fax: +1 205 581 2726.
[d]Tel.: +1 301 228 2197; fax: +1 301 694 7223.
[e]Tel.: +1 205 581 2761.
[f]Tel.: +1 205 581 2746.
[g]Tel.: +1 205 581 2802.
[h]Tel.: +1 205 581 2259.
[i]Tel.: +1 205 581 2440.
[j]Tel.: +1 301 694 3232x240.
[k]Tel.: +1 205 581 2344.
[l]Tel.: +1 205 581 2361.
[m]Tel.: +1 205 581 2442.

scores of organizations and scientists worldwide participated in this important effort, utilizing robust and readily accessible *in vitro* and *in vivo* TAACF screens against virulent *Mycobacterium tuberculosis* (Mtb) H37Rv as well as other relevant mycobacterial strains with the purpose of identifying and advancing new leads for tuberculosis drug discovery.[1] More recently, the TAACF and the Molecular Libraries programs (an NIH Roadmap initiative) initiated the high throughput screening (HTS) of large, medicinally relevant chemical libraries in order to provide the tuberculosis drug research community with high quality screening data for a large number of diverse compounds against Mtb H37Rv. As part of the HTS campaign, and in order to stimulate community-wide research and drug design against new targets in the bacillus, these data have been deposited in PubChem and are reported in two recent publications.[2,3] Herein, we report data for the remaining large, but focused library screened under the auspices of the TAACF program.

The discovery of highly effective, and oftentimes very selective, inhibitors of serine, threonine, and tyrosine protein kinases has led to the field of kinomics and a renaissance in small molecule drug discovery to treat cancer.[4] Kinases have become one of the most intensely pursued protein targets, and inhibitors of approximately 30 distinct kinase targets are being pursued in clinical trials, primarily for the treatment of cancer.[5] In particular, factors that drive selectivity of inhibitors, especially ATP-competitive compounds, are being critically evaluated and optimized.[5–9] Additionally, the extensive knowledge base that has developed for this class[10,11] has stimulated research into how these proteins regulate cellular processes with potential application to other therapeutic areas.[12] For example selective, ATP-competitive drug design has extended into a variety of clinical areas including immunological diseases,[12,13] CNS disorders,[12,14] and metabolic[12,15] as well as infectious diseases (bacterial,[16,17] viral,[18,19] and parasitic[20,21]). The kinome and associated signaling pathways (tubercular and human response to infection) that are crucial for the survival and growth of the mycobacterial pathogen in the host are beginning to receive considerable attention as potential areas for new antitubercular drug discovery.[22–25] Among these, the eukaryotic-like signal transduction pathways driven by the serine/threonine protein kinases (Pkns) in Mtb have received substantial interest.[26–30] Other ATP and nucleotide binding proteins are also beginning to be explored as new drug targets in tuberculosis.[31]

For this study we pursued a focused library supplied by Life Chemicals, Inc. that was designed around specific kinase targets as well as commercially available scaffolds related to known broad kinase inhibitors (General Kinase and Sharp Focused Libraries - http://www.lifechemicals.com/services/diversity). Kinase-specific inhibitors were selected by screening the Life Chemicals database against CDK2, GSK3, PKB, SRC and EGFR. Two protein structures were used for SRC. Candidate structures were filtered according to accepted medicinal chemistry parameters for oral bioavailability; compounds with molecular weights higher than 500, more than seven flexible bonds and more than five hydrogen bond donors or acceptors were discarded. Most compounds with phenol substituents and nitro groups were also discarded. Compounds that passed these filters and that were among the top scoring 5,000 for each kinase were considered for further analysis. Each potential inhibitor was matched against a general kinase inhibitor pharmacophore proposed by Traxler and Furet.[32] These authors proposed that ATP competitive inhibitors bind to the hinge region of kinases via hydrogen bonding (acceptor and/or donor) and to two hydrophobic regions. Thus, compounds that did not satisfy this general pharmacophore were also discarded. Specific libraries were designed in a Sharp Focused Kinase Library set to target FGFR1K, JAK2, PDK-1, PI3K and PKA kinases. The Life Chemicals approach used Sharp Focusing: each molecule's interaction was measured with only a single target in a protein family. The target's X-ray structural data was thus incorporated. Next, after preprocessing, the molecules were individually docked. Flexible molecular docking was the core of the Life Chemicals approach. After docking the ligand, a re-scoring algorithm was applied. This re-

scoring procedure involved correcting the final summation of interaction energies (the score) according to the ligand's structural features. Finally, each docking complex was scanned for key contacts: H-bonds formed between the ligand and critical amino acid residues in the protein's active site. This detailed analysis of each docked protein-ligand complex was central to the design approach.

A total of 25,671 compounds from the Life Chemicals kinase-like inhibitor library was screened against Mtb in a single-dose assay at a concentration of 10 μg/mL. All Life Chemicals library samples are checked for purity and authenticity by ^{1}H NMR and LC-MS prior to library shipment. The minimum acceptable and supplied purity is 90% with an average purity over the library of 96%. These samples were used as is for screening without further quality control or purification. Of these compounds, 1,329 were deemed active based on their ability to inhibit growth of the organism by 85% or more. The 1,329 active compounds were next evaluated in a dose response (DR) format against Mtb and in a cell cytotoxicity assay using Vero cells as previously described.[2] This assay identified compounds that target Mtb and not host cell kinases. The Mtb DR assay involved measurement of Alamar Blue fluorescence relative to untreated inoculated control wells.[33] The quality of the screen was high and consistent with the standars previously described.[2] In brief the Z′-value for the screen was 0.77 ± 0.04. Control wells on each plate contained amikacin at 0.13 μg/mL, its IC_{50} concentration, which inhibited Mtb growth from 33%–60%. Cell viability/compound toxicity after compound exposure was determined by luminescence using CellTiter-Glo reagent (Promega) in order to identify relatively non-toxic compounds. Hyamine was used during validation and had an IC_{50} value of 30 μM. Of the 1,329 compounds evaluated in the DR format, 584 (≥80% viability at all test concentrations) compounds possessed TB IC_{90} values of <10 μg/mL. In order to identify potentially privileged scaffolds, a clustering analysis was performed on the set of 584 compounds using a hierarchical clustering method as implemented in Leadscope. The clustering analysis led to the identification of 26 major scaffolds and two minor 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 sections.

Potent and non-cytotoxic compounds (IC_{90} < 10 μg/mL and selectivity SI > 10) that resulted from this screening effort include a large number of carboxamide-oxadiazole containing scaffold series totaling approximately half of all such actives (95 compounds). The remaining non-cytotoxic and potent compounds include a set of structurally diverse core scaffolds represented by analog series of varying sizes as well as a set of singleton samples. Table 1 summarizes active core scaffolds, rank ordered starting with the most promising/highest activity oxadiazole-containing scaffolds downward. For each active series, several logistical values are listed including the total number of analogs screened and the number of members with IC_{90} < 10 μg/mL as well as other properties such as selectivity (SI), median IC_{90}, Lipinski violations, etc. The summary of active scaffolds in Table 1 focuses specifically on scaffold series rather than singleton structures for several reasons including the greater likelihood of utility in series represented by larger number of actives and a potential for SAR comparisons within the data set. This decision does not, however, imply that the singleton set may not contain new and interesting lead samples that could be pursued for new antitubercular leads and drugs.

Data are presented in Figures 1–6 and Tables 2–10. Where specific structures are listed, a chronological number is given for reference within the document as well as an identifier from the PubChem database (PubChem SID) and a Life Chemicals compound identifier (Corp ID). The latter numbers are given in order to allow ready access to the PubChem screening data (http://pubchem.ncbi.nlm.nih.gov/; Assay ID: 2842) and direct ordering

information from Life Chemicals should the reader be interested in following up on any compound screening information presented herein.

2-Acylaminothiopene-3-carboxamide and related compounds

In the library of compounds evaluated, there were a relatively large number (1,376 out of 25,671) of amide derivatives of 2-aminothiophene-3-carboxylic acids. Most of these compounds also have a fused ring system such as a tetra or pentamethylene or a tetrahydropyridine system at the 4–5 positions of the thiophene moiety. The number of compounds possessing the 2-acylaminothiophene moiety with a primary amide (**1**, Figure 1) in the screened set was 487. Of these, in the primary assay, 41 compounds displayed >86% inhibition of the growth of Mtb at 10 μg/mL. From this group, confirmed dose-response data were available for 32 compounds. Most of these compounds, however, displayed either poor IC_{90} values or displayed significant cytotoxicity against Vero cells thus yielding poor SI values. Only two compounds, **2** and **3** (Figure 1) emerged as compounds with moderate to good activity against Mtb coupled with greater than 10-fold selectivity index values.

Pyrazolo[1,5-a]pyrimidines

In the screening set, there were 37 compounds possessing the pyrazolo[1,5-a]pyrimidine framework. Most of these compounds possessed an amino substituent at the 7-position, an aryl group at the 3-position and an alkyl, primarily a methyl group, at the 5-position. The inhibition potencies of these compounds in the primary assay covered the entire range from 100% to 0%. Of these 37 compounds, 11 compounds displayed > 85% inhibition in the primary assay. Five of these 11 compounds (**4a**–**e**) that displayed activity in the dose response assay against Mtb without attendant cytotoxicity against Vero cells (SI about or greater than 10) are presented in Table 2.

Tetrahydrobenzo[1,4]diazepin-2-ones

There were 48 compounds within the evaluated set that were 4-phenyl or substituted phenyl 1,3,4,5-tetrahydrobenzo[e][1,4]diazepinones acylated on the nitrogen at the 4-position with various acyl groups (**5**). In the primary screen, 20 displayed inhibition potencies > 85%. In the dose response assay, however, only five compounds displayed IC_{90}s in the range of 5–10 μg/mL. Most of these compounds also displayed significant cytotoxicity against Vero cells, leading to modest SI values in the range of 2.7 to 7.9. Compound **6** gave the highest selectivity (Figure 2).

Substituted 1,2,3-benzotriazin-4(3*H*)-ones

There were 14 1,2,3-benzotriazin-4(3*H*)-ones, and, of these, five compounds had IC_{90}s ≤ 10 μg/mL. Only one of these compounds gave an SI of >10 after cytotoxicity screening. The most active sample, **7a** (Table 3), gave an SI of >28 resulting from its IC_{90} of 1.4 μg/mL and cytoxicity of >40 μg/mL. The three most active and selective examples of this class all contained R_1 = aryl. It is notable that this class contains a labile ester linkage that may serve as a prodrug form of the 1,2,3-benzotriazine-4(3*H*)-one core heterocycle.

A search of the literature did not reveal any compounds of this general structure with tuberculosis activity or kinase activity. Some 1,2,3-benzotriazin-4(3H)-ones have been prepared and evaluated as metalloproteinase inhibitors.[34] Similar compounds were also among derivatives reported as compounds with potential chemotherapeutic use.[35] Structurally similar 3-aryl-1,2,3-benzotriazin-4(3H)-ones were also found to have antimycobacterial activity that was weaker than that of some corresponding 2-aryl-2*H*-1,3-benzoxazine-2,4-(3*H*)-diones.[36] Similar compounds have also been shown to exhibit

antimicrobial and marine antifouling activity in industrial and commercial applications.[37] An analog, azinphosmethyl (Figure 3), to which tufted apple budmoth larvae (*P. idaeusalis*) are susceptible, has been reported.[38]

Substituted benzopyran-2-ones

The 2*H*-1-benzopyran-2-ones (commonly referred to as coumarins) **8a**–**i** shown in Table 4 have consistent activity, low cytotoxicity as measured in Vero cells, and consequently high Selectivity Index values. These compounds, with alkoxy ester groups at C-7 of the coumarin ring are very similar to a class that we presented in an earlier publication.[2] The background for this class and the anti-tuberculosis activity are presented therein. In the kinase library examined, there were 11 compounds that possessed the core structure represented by a coumarin ring with a 3-phenyl moiety and a 7-carbomethoxymethoxy group. Of these, the nine compounds in Table 4 possessed significant activity. It is very clear that this class of compounds has consistent activity and warrants expanded research efforts in the search for new compounds with new mechanisms of action.

2-Aminobenzothiazoles

In general, the benzothiazole core is only poorly represented in the antibacterial literature, but is more commonly seen in the kinase inhibitor literature. The reported compounds inhibited a variety of kinases including SHP-2,[39] JNK kinases,[40–42] ROCK-II,[43] and FLT-3.[44] A smaller subset of reported compounds contained the 2-aminobenzothiazole moiety and has been reported to inhibit P38α MAP kinase,[45] Raf-1,[46] LCK,[47] and others.[48] While some similarities to the current screening set exist, it must be emphasized that beyond the library selection criteria it is not clear that these “kinase-like” inhibitor sets will affect any of these kinases, nor is it clear that structurally related kinases or specific, related ATP binding sites exist in Mtb that could be the target of active samples from the screening set.

There were several small clusters of active compounds enriched in the 2-aminobenzothiazole core. The great majority of samples include a 2-aryl- or 2-heteroarylbenzothiazole core. One notable singleton (non-clustered hit) that contained a 2-pyrrolidine, but not a 2-heteroaryl substituent, is given in Figure 4 (structure **9**), suggesting that alternative 2-substituents on the benzothiazole core should be explored for activity.

There were a small number (three – see general structure **10**, Figure 4) of 2-phenylamino-substituted benzothiazoles of limited diversity that gave modest IC_{90} values and showed significant toxicity and poor overall selectivities. Other, heteroaryl substitutions include 2-amine-linked thiazoles, benzothiazoles, and tetrahydrobenzothiazoles, but, for the most part, the small numbers of compounds did not lend themselves to a clear structure activity relationship (SAR) pattern, were modestly active and did not show significant selectivity. Examples of 2-aminobenzothiazoles that showed good to high activity and some degree of selectivity are presented in Tables 5–7.

There were three examples of 4-(2-pyridyl)-2-aminothiazoles of the type depicted by **11**, not a sufficient number to allow SAR discussion. On the other hand, modest differences in substitution in terms of electron withdrawing potency and substitution position can apparently have a significant impact on toxicity and the resulting selectivity (see **11a** and **11b** in Table 5). These effectors could alter hydrogen bonding and/or chelation ability for this class, but more information is needed to determine what requirements are necessary for optimal potency and selectivity. Two other examples of the class are given by **12** and **13** (Fig. 5), again both showing significant differences in activity and selectivity with relatively modest structural alterations.

Another cluster of five compounds contains the 2-aminobenzothiazole core linked to a coumarin-3-carboxylic acid as an acylhydrazide. The majority of these (4/5 – see Table 6) compounds showed modest activity (> 1.0 μg/mL) and modest selectivities (>9.5 to >19). Again, there was not sufficient information to ascertain a clear SAR pattern. Additionally, the acylhydrazide linkage can be labile in *M. tuberculosis* (e.g. isonicotinic acid hydrazide, the active antitubercular drug INH), and there is a distinct possibility that these compounds may act similarly as prodrugs. Testing against INH resistant strains that lack the activation enzyme (e.g. catalase/peroxidase) may shed some light on the mechanism of these compounds and yield important information relevant to their potential value as INH resistance is now commonplace. Two other structurally related active samples (**15** and **16**, Figure 6) that have similar activities and selectivities are shown below.

The remaining active cluster that contains the 2-aminobenzothiazole core showed significant activity (many IC_{90}s < 1.0 μg/mL) and good selectivities (as high as >83). Overall, there were 50 representatives in the cluster with 18 of these giving significant activity and selectivity as shown in Table 7. The active and selective samples in the cluster all contain a furan-2- (**17a–j**) or thiophene-2- (**18a–e**) carboxylic acid amide linkage with a small number of similar actives of structure **19** (**a–c**) that contain a 3-(2-thienyl)acrylic acid amide linkage. Within the cluster of 50 compounds, there were a significant number of samples that contained a substituted benzoic acid, phenylacetic acid, or 2-phenoxyacetic acid amide linkage, but these compounds were, for the most part, significantly less active (IC_{90} range 1.6 to 10 μg/mL) and selectivities ranging from 0.9 to 4.0. Overall, while there were a greater number of analogs within this particular set than with the other 2-aminobenzothiazoles, a distinct SAR pattern was not clear, and, from the variety of substitutions screened, it was a clear indication that a larger diversity set, as well as specific examples for comparison analysis, need to be explored. The identification of a specific target or targets would help in profiling the activity of this compound class, and preliminary animal screening of an active analog to ascertain bioavailability and activity in an efficacy model would help prioritize the class.

Substituted 2-benzylidenebenzofuran-3(2*H*)-ones

These compounds, also known as aurones, have demonstrated some antibacterial or antifungal activity as reported in several citations (see below). Aurones as originally identified are yellow naturally occurring pigments derived from plants.[49] They are flavonoid compounds, thus identifying them with a class of compounds with significant biological activity. The synthetic compounds **20a–c** in Table 8 demonstrated significant activity as evidenced by their IC_{90} and SI values. The kinase-like inhibitor library contained a total of 63 compounds with the base aurone structure, and only these three showed reproducible anti-TB activity.

A series of aurones was prepared through the oxidative cyclization of 2′-hydroxychalcones, and these compounds were found to have moderate activity against both *Staphylococcus aureus* and *Escherichia coli*.[50] Several patents have focused on either the antibacterial activity or the inhibition of bacterial chorismate synthase, an enzyme shown to be essential for bacterial viability. In one case,[51] a series of aurones was prepared and evaluated for inhibition of *Streptococcus pneumoniae* chorismate synthase. A series of (2*Z*)-6,7-dihydroxybenzylidenebenzofuran-3(2*H*)-ones with various substitutions on the phenyl ring was prepared, and data demonstrating significant inhibition of this enzyme were presented.[52] In another patent, aurones substituted both on the benzofuranone ring and the phenyl ring were found to significantly inhibit the growth of *Streptococcus aureus* KLE820 at 10 μg/mL.[49] Finally, a substituted aurone demonstrated modest inhibition of glucan synthase, possibly suggesting the existence of antifungal activity.[53]

Thus, this class of compounds appears to have significant biological activity, and some enzyme targets have been suggested as potential leads for new antibacterial discovery. In the case of tuberculosis, however, only a fraction of the compounds had activity, and a molecular target still remains to be identified. The key question of selectivity would also need to be carefully considered, but there is clear potential for new drug discovery in the aurone class of compounds.

Substituted 2-(benzimidazol-2-yl)acrylonitriles

The core scaffold was present in 20 compounds in the target library. Two compounds (**21a,b**) from that group are shown in Table 9 and were found to have reproducible activity. All the compounds had R_1 as an aryl or heteroaryl moiety, and some cytotoxicity was seen for three of the four compounds subjected to follow-up assays, the exception being compound **21a**. It is therefore difficult to draw any solid conclusions as to the value of these compounds as potential leads.

This general class of compounds can be found in a series of literature references that mention antibacterial activity.[54–58] Specific antibacterial activity was reported several times,[54–57]though when cytotoxicity data was reported,[54] it was clear that this series, with R_1 (see structure in Table 9) as a variety of heteroaryl moieties, had significant cytotoxicity and little, if any, selectivity. In the one report that focuses on specific enzyme inhibition,[58] two compounds with R_1 as a furanyl salicylate demonstrated reasonable inhibition of the *Yersinia pestis* tyrosine phosphatase YopH (2 μM and 14 μM). This enzyme is of potential therapeutic interest because *Y. pestis* strains lacking the protein are avirulent. No data against the organism itself, however, was presented. Other types of compounds in the library evaluated in this report[58] were more potent inhibitors of the enzyme, and there have been no further reports on the two compounds containing a benzimidazolyl acrylonitrile unit. A series of compounds closely related to the title compounds but with a benzotriazole moiety rather than a benzimidazole moiety have been reported.[59–60] These compounds were initially found to have some antitubercular activity, but investigation of the cytotoxicity profile indicates that in fact, there was no selectivity and that the compounds were significantly cytotoxic.

To summarize this small series, some selectivity has been seen, but because of the cytotoxicity profile of the series in general, it is critical that any exploration of SAR with *M. tuberculosis* or any other bacteria be accompanied by a careful examination of the cytotoxic effects of the new compounds.

1,3,4-Oxadiazoles

There are numerous literature examples of the 2,5-disubstituted-1,3,4-oxadiazole system that have shown biological activities including anticancer (apoptosis induction, mitotic arrest, kinase inhibition, cell proliferation arrest etc.), anti-inflammatory, antifungal, antiviral, and antibacterial. In particular, 1,3,4-oxadiazoles are known from the literature to possess potent antibacterial or antimycobacterial activity.[61–75] Among the evaluated compounds, one series in particular, the 2-carboxamido-1,3,4-oxadiazoles, showed high activity and good selectivity for a number of samples.

The library evaluated contains 1,045 compounds that share a 2-carboxamido-1,3,4-oxadiazole core scaffold (**22**, Table 10). Approximately half (522 compounds) of these samples contained R_2 = phenyl and R_5 = phenyl/pyridyl. Of these, 91 compounds have potencies $IC_{90} < 10$ μg/mL, out of which 18 hits also show cytotoxicity > 40 μg/mL. Within this set of 91 hits, potent IC_{90} and high SI values were associated with specific substituent groups at R_2 and R_5 compared to inactive compounds. For example, R_5 = pyridyl appears

favored over phenyl (e.g. **22a** is potent and relatively non-toxic while six close analogs of **22a** all containing a substituted phenyl for R_5 are inactive). In another typical example, substituting phenyl for the pyridyl group in **22b** rendered this compound inactive. 1,2,3,4-Tetrahydronaphth-6-yl at R_5 also appears favored, **22c** being a representative of a series of such compounds. The tetrahydronaphthyl may be substituted for R_2 as well as in compound **22d**, yielding active and selective analogs. R_2 substitutions that were associated with potent and non-toxic hits are: 4-*t*-butyl (**22e** and **22f**), 3-or 4-*O*-*n*-butyl (**22g** and **22h**), 3-*O*-phenyl (**22i**). Dimethyl substitutions on the R_5 ring were also well tolerated yielding potent analogs, such as compounds **22j** and **22k**, although these 16 compounds display a range of toxicities from relatively non-toxic compounds with SI > 97 (**22l**) to toxic analogs such as **22m** with SI < 1.0. In general, using a selectivity index of 15 as the criteria between non-toxic and toxic compounds, half of this series were relatively active and non-toxic compounds. Certain substitutions were not favorable on R_2/R_5 such as 3,4,5-tri-OMe substituents on R_5, rendering all 18 such analogs inactive. In another such case, out of 43 analogs containing benzodioxin at R_5, there were only two hits that were potent, and both these samples were also considered relatively toxic. Further, all 53 members of the analog series with a *p*-sulfonyl-amine substitution on R_2 were either inactive or highly toxic. Of the remaining 523 carboxamido-oxadiazole compounds, 336 have R_5 = phenyl/pyridyl and R_2 a group other than phenyl. The following R_2 atom groups were associated with potent analog series. A furyl or thiophenyl at R_2 is present in 26 potent analogs out of 58 such compounds in the library, for example **22n**, **22o**, and **22p**. A few thiophen-3-yl analogs were also active (**22q**). Alkyl groups at R_2 were well represented among potent and non-toxic active hits and 19 out of 43 such compounds had IC_{90}s < 10 μg/mL (see **22r** – **22v** in Table 10). Analog series with the most optimal R_2 alkyls (e.g. isopropyl or *t*-butyl) appeared sensitive to R_5 phenyl substitutions (e.g. -OMe at any position tends to decrease activity). Further R_2 groups that were tolerated though under-represented among actives were benzothiazol-2-yl (**22w**), thiophene-2-ethenyl (**22×**), dihydro-1,4-dioxin-5-yl (**22y**), and cyclohexyl (**22z**). Examples of unfavorable R_2 groups (largely associated with inactivity) were methylpyrrolidine-2,5-dione and phenylsulfonylpiperidin-4-yl. Out of the remaining 187 oxadiazoles in the screened set, 176 belong to one of the following two analog series: R_5 = dihydro-1,4-dioxin-5-yl (36 compounds) and R_5 = furan/thiophen-2-yl (140 compounds). Potent, nontoxic hits within the latter series showed preference for R_2 = phenyl where R_2 substituents include O-phenyl (**22aa**), 4-*O*-*n*-butyl (**22ab**), or 4-*t*-butyl (**22ac**), and these examples were associated with the most potent hits and are analogous to the R_5 = phenyl/pyridyl. Further preferred R_2 groups are furyl, thiophenyl, benzothiazolyl and others (e.g. **22ad**, **22ae**, **22af**, **22ag**). In the R_5 series (R_5 = dihydro-1,4-dioxin-5-yl, 36 compounds) the only potent analogs in that specific grouping were those containing a phenyl at R_2 (e.g. **22ah** and **22ai**).

Conclusions

Herein, we report the screening of a designed set of kinase-type inhibitors to identify compounds and scaffolds that show significant antitubercular activity *in vitro*. Although the specific molecular targets of the active compounds have not been identified per se, these active compounds and their basic core scaffolds may serve as a useful basis set for the community of tuberculosis drug design researchers to probe activities of these actives in order to identify new targets, potentially crucial kinase and ATP-binding targets, as well as others. Hopefully, this publicly available data will stimulate new drug design programs and the development of new agents to treat tuberculosis.

Acknowledgments

This work was supported by contracts N01-AI-95364 (JAS) and N01-AI-15449 (ELW). The complete data sets for the HTS campaign will be deposited in PubChem. The authors thank Dr. Scott Franzblau who generously provided us with his protocol for the Alamar Blue *M. tuberculosis* assay at the beginning of this program. The authors are appreciative of the assistance of Sara McKellip and Alka Mehta in running the H37Rv screen, Anna Manouvakhova for assistance in the data analysis and Shuang Feng for the statistical analyses. We gratefully acknowledge Drs. Robert Goldman and Barbara Laughon from NIAID, whose support, oversight and input were crucial to the success of the TAACF program and generation of the results reported herein.

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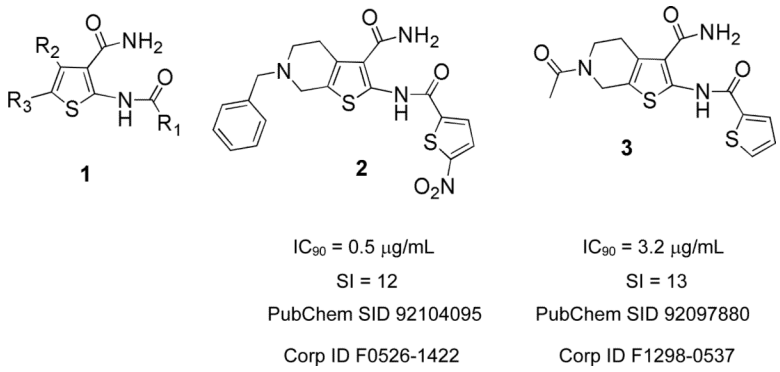


Figure 1.

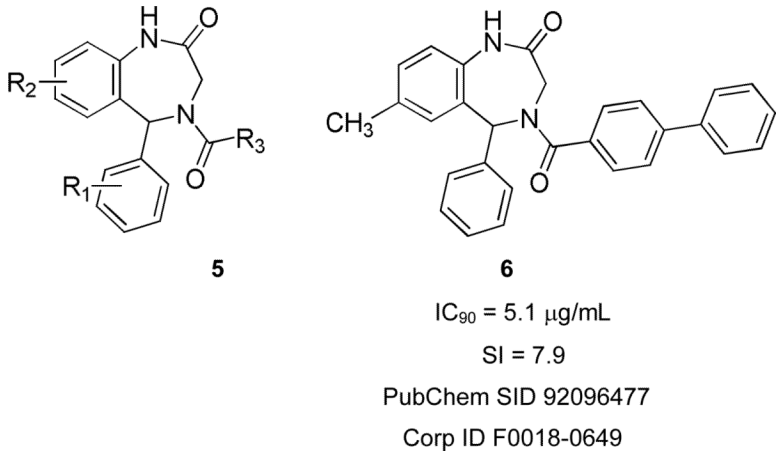
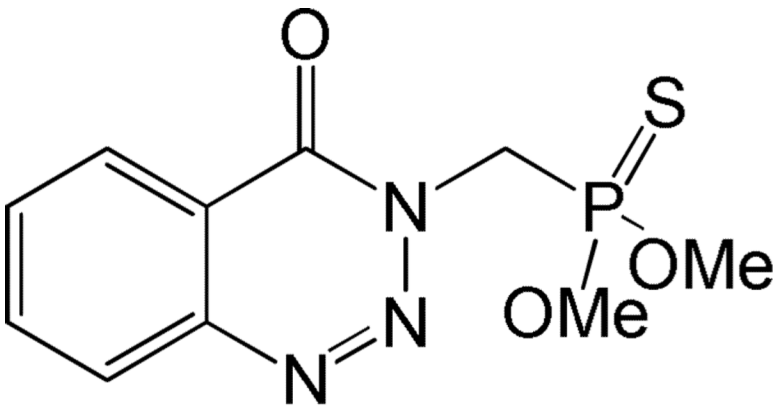
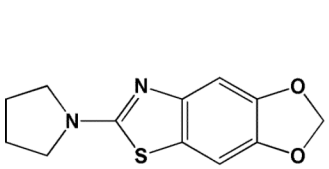


Figure 2.



Azinphosmethyl

Figure 3.



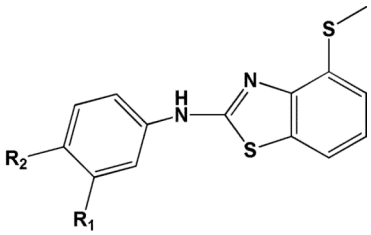
9

IC_{90} = <0.2 μg/mL

SI ≥ 200

PubChem SID 92101561

Corp ID F0647-0334

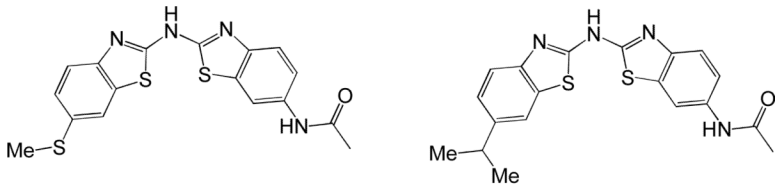


10

IC_{90} range 2-7 μg/mL

SI range 1-3

Figure 4.



12

IC_{90} = <0.2 μg/mL

SI ≥ 172

PubChem SID 92107784
Corp ID F2020-0076

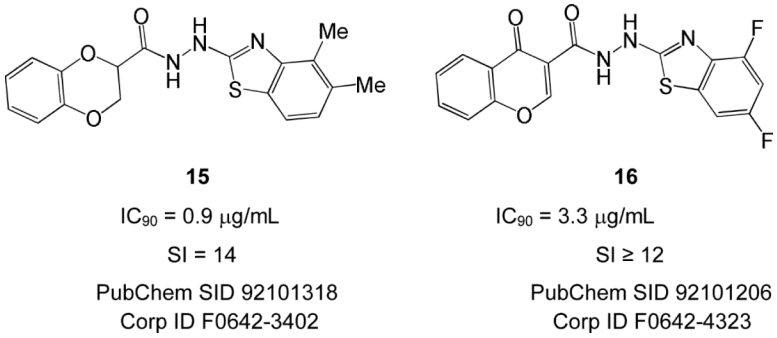
13

IC_{90} = 2.0 μg/mL

SI = 8.8

PubChem SID 92101217
Corp ID F0646-2888

Figure 5.



15

IC_{90} = 0.9 μg/mL

SI = 14

PubChem SID 92101318

Corp ID F0642-3402

16

IC_{90} = 3.3 μg/mL

SI ≥ 12

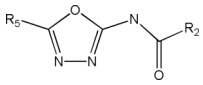
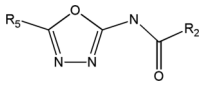
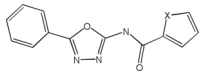
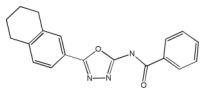
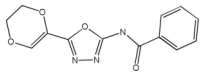
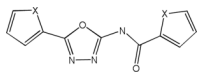
PubChem SID 92101206

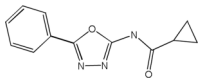
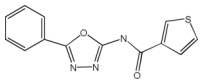
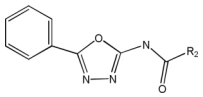
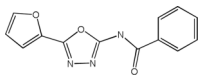
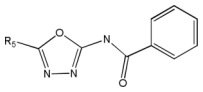
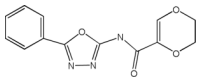
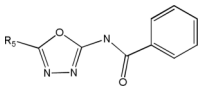
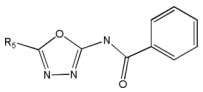
Corp ID F0642-4323

Figure 6.

Table 1

Summary of core structures reported in this study with the following numbers/values given for each scaffold series: N1: total number of members within the screened kinase library, N2: number of members with inhibition > 85%, N3: number having $IC_{90} \leq 100$ μg/mL, N4: number having $IC_{90} < 10$ μg/mL, N5: number having $IC_{90} < 10$ μg/mL and selectivity index SI ≥ 10, V6: highest potency IC_{90} value in the series, V7: median IC_{90} that includes those that display at least some activity: $IC_{90} \leq 100$ μg/mL (number of such compounds is N3), V8: highest selectivity SI within the series, V9: median SI (computed for members with $IC_{90} \leq 100$ μg/mL), N10: average number of Lipinski rules violations for each series. Table numbers and examples are shown under each core structure. Core structures are listed in the order of ranking from most desirable/best scaffold clusters toward worst; 2-Carboxamido-oxadiazole scaffolds are listed/ranked first, followed by the ranking of all other scaffolds.

Core Structure	N1	N2	N3	N4	N5	V6	V7	V8	V9	N10
 R_2/R_5 = any atom group; Table 10	1045	512	356	211	95	<0.2	7	>158	3.5	0.025
 R_2/R_5 = Ph/pyridine Table 10, **22a – m**	525	269	173	93	30	<0.2	8.6	>98	2.4	0.04
 X = O or S; Table 10, **22n – p**	58	35	30	26	21	<0.2	1.9	103	17	0.03
 Table 10, **22c**	28	23	11	8	5	<0.2	4.5	>78	9	0.13
 Table 10, **22ah, ai**	20	12	12	9	6	0.44	4.2	60	10	0
 X = O or S; Table 10, **22ad, ae**	5	4	3	3	3	0.85	3.1	33	13	0

Core Structure	N1	N2	N3	N4	N5	V6	V7	V8	V9	N10
 Table 10, **22u**	8	5	5	4	3	1.1	1.5	>38	23	0
 Table 10, **22q**	8	5	4	3	3	2.2	3.5	>98	11	0
 R_2 = Ethyl, *i*-propyl, *t*-butyl, *n*-butyl; Table 10, **22r – t, v**	29	22	22	15	8	<0.2	6.4	>158	6.2	0
 Table 10, **22aa - ac**	44	25	13	11	6	<0.2	3.3	>74	6	0
 R_5 = Pyridine; Table 10, **22a, b, e, f, h**	66	32	21	10	5	0.84	11.6	>48	2.6	0
 Table 10, **22y**	9	7	7	5	1	<0.2	7.3	>84	5	0
 R_5 = 2, 4-diMe-Ph; Table 10, **22g, j**	56	37	23	12	6	<0.2	9.1	30	1.8	0
	62	44	34	15	5	<0.2	12.5	>98	1.8	0

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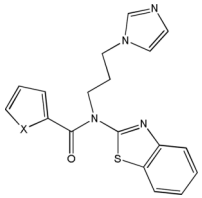
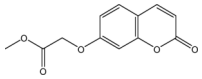
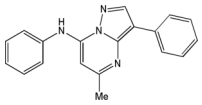
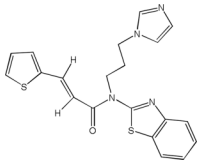
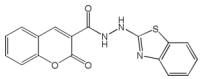
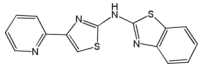
Core Structure	N1	N2	N3	N4	N5	V6	V7	V8	V9	N10
R_5 = 2, 5-diMe-Ph; Table 10, **22k, l, m**										
 X = O or S; Table 7, **17** and **18**	28	27	27	26	14	<0.2	1.7	>83	12	0
 Table 4, 8	21	12	11	11	8	0.85	2.5	>47	16	0
 Table 2, 4	14	6	6	5	5	1.7	3	18	12	0.17
 Table 7, **19**	4	3	3	3	3	0.24	0.8	>28	26	0
 Table 6, **14**	9	6	6	5	4	1.8	3.6	>19	10	0
 Table 5, 11	4	4	4	4	1	<0.2	0.9	>200	0.6	0

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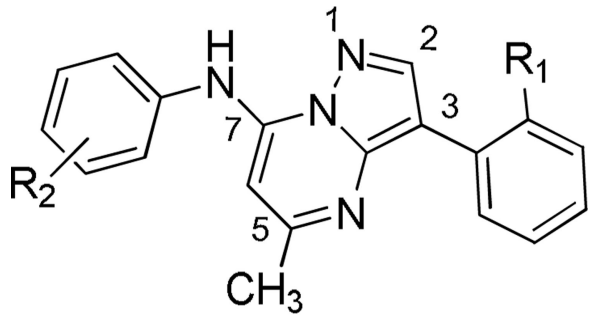
Core Structure	N1	N2	N3	N4	N5	V6	V7	V8	V9	N10
3, 7	14	5	5	5	1	1.4	4.3	>28	8	0
	63	4	4	3	3	1.7	3.4	17	12	0

Table 8, **20**

Table 2

Antitubercular activities of representative pyrazolo(1,5-α)pyrimidines

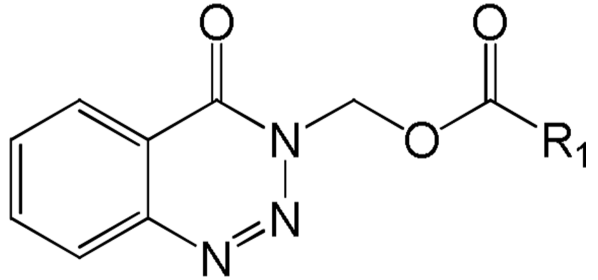


4

Compd	PubChem SID	Corp ID	R_1	R_2	IC_{90} (Mg/mL)	SI
4a	92111969	F3250-0549	OMe	3-Cl	1.7	19
4b	92112276	F3305-0318	H	3-OMe	1.8	10
4c	92112234	F3305-0297	H	3,5-diMe	2.7	>15
4d	92112194	F3305-0296	H	3-Cl-4-Me	3.4	>12
4e	92112074	F3305-0290	H	3,4-diMe	3.4	>11

Table 3

Antitubercular activities of representative 1,2,3-benzotriazin-4(3H)-ones

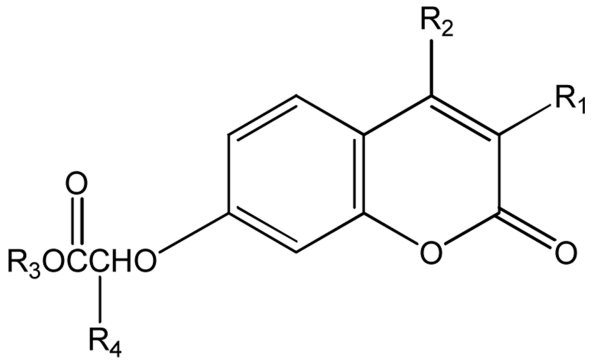


7

Compd	PubChem SID	Corp ID	R_1	IC_{90} (μg/mL)	SI
7a	92114592	F0624-0059	Ph	1.4	>28
7b	92114830	F0624-0044	2-thienyl	4.3	>9
7c	92114632	F0624-0067	2-furyl	4.8	>8
7d	92114552	F0624-0058	Me	3.3	7
7e	92114590	F0624-0008	$(Ph)_2CH$-	8.9	5

Table 4

Antitubercular activities of benzopyran-2-ones

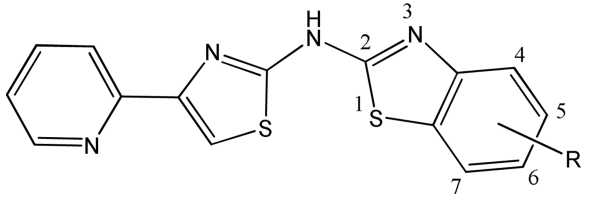


8

Compd	PubChem SID	Corp ID	R_1	R_2	R_3	R_4	TB IC_{90}	SI
8a	92094833	F1862-0221	Ph	Me	$CH_2CH{=}CH_2$	H	0.85	>47
8b	92106801	F1862-0596	4-OCH_3Ph	H	Me	Me	1.1	>37
8c	92106681	F1862-0220	Ph	H	$CH_2CH{=}CH_2$	H	1.2	>33
8d	92094889	F1862-0422	3,4-diOCH_3Ph	H	Et	H	2.2	>18
8e	92094928	F1862-0433	3,4-diOCH_3Ph	Me	Et	H	2.3	>17
8f	92106721	F1862-0224	4-ClPh	H	$CH_2CH{=}CH_2$	H	2.5	>16
8g	92094809	F1862-0176	Ph	H	iPr	H	3.0	>34
8h	92094857	F1862-0226	4-OCH_3Ph	H	$CH_2CH{=}CH_2$	H	3.6	>11
8i	92106841	F1862-0598	3,4-diOCH_3Ph	H	Me	Me	6.7	>6

Table 5

Antitubercular activities of representative substituted 2-aminobenzothiazoles

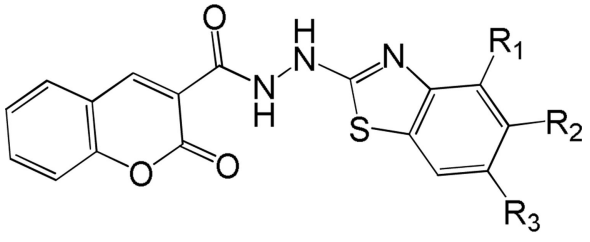


11

Compd	PubChem SID	Corp ID	R	IC_{90} (μg/mL)	SI
11a	92101369	F0646-1672	4-F	<0.20	>200
11b	92101304	F0646-3492	6-OMe	<0.20	0.4

Table 6

Antitubercular activities of representative substituted 2-aminobenzothiazoles

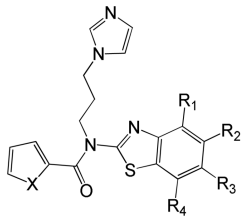


14

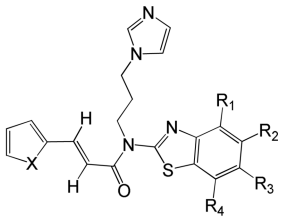
Compd	PubChem SID	Corp ID	R_1	R_2	R_3	IC_{90} (μg/mL)	SI
14a	92100993	F0642-1292	H	H	Me	1.7	11
14b	92101200	F0642-3484	Me	H	Me	2.2	>19
14c	92114593	F0642-0059	Me	H	H	2.9	>14
14d	92101198	F0642-3347	Me	Me	H	4.2	>9.5

Table 7

Antitubercular activities of representative substituted 2-aminobenzothiazoles



17 and **18**

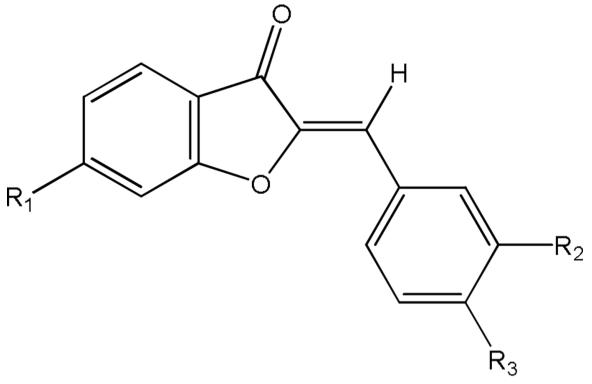


19

Compd	PubChem SID	Corp ID	X	R_1	R_2	R_3	R_4	IC_{90} (μg/mL)	SI
17a	92111140	F2971-0818	O	H	H	Me	H	0.48	>83
17b	92111108	F2972-0561	O	Me	Me	H	H	0.58	>69
17c	92111228	F2972-0644	O	Me	Cl	H	H	0.23	43
17d	92111094	F2971-0196	O	F	H	H	H	3.1	>13
17e	92111131	F2972-0760	O	Me	H	Cl	H	<0.2	>32
17f	92111060	F2971-0775	O	H	H	Br	H	0.74	21
17g	92111254	F2971-0283	O	Me	H	H	H	0.83	20
17h	92111342	F2972-0023	O	F	H	F	H	0.75	16
17i	92111098	F2971-0615	O	H	H	Cl	H	0.89	12
17j	92111172	F2971-0027	O	H	H	H	H	4.3	>9.4
18a	92111056	F2971-0362	S	Et	H	H	H	0.3	28
18b	92111348	F2972-0702	S	OMe	H	H	Cl	1.7	>23
18c	92111302	F2972-0017	S	F	H	F	H	2.4	>17
18d	92111186	F2972-0412	S	OMe	H	H	Me	2.4	>17
18e	92111068	F2972-0553	S	Me	Me	H	H	0.5	15
19a	92111268	F2972-0654	S	Me	Cl	H	H	0.2	26
19b	92111136	F2971-0382	S	Et	H	H	H	0.8	11
19c	92111252	F2971-0039	S	H	H	H	H	1.4	>28

Table 8

Antitubercular activities of benzylidenebenzofuranones

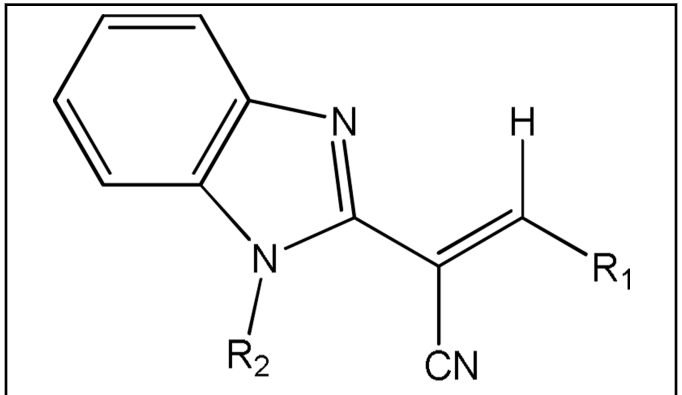


20

Compd	PubChem SID	Corp ID	R_1	R_2	R_3	TB IC_{90}	SI
20a	92392487	F1860-0087	OEt	OCH_2CO_2Me	H	1.7	17
20b	92094851	F1860-0579	OMe	H	OCH_2CO_2Et	3.3	>12
20c	92094708	F1860-0257	OMe	OCH_2CO_2Et	H	3.5	>11

Table 9

Antitubercular activities of bezimidazolylacrylonitriles

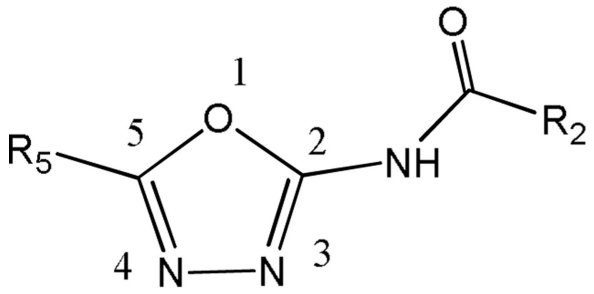


21

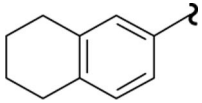
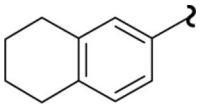
Compd	PubChem SID	Corp ID	R_1	R_2	TB IC_{90}	SI
21a	92112720	F3097-4582	NHPh	H	3.3	>12
21b	92117267	F0862-0053	4-NO_2Ph	H	6.1	4.4

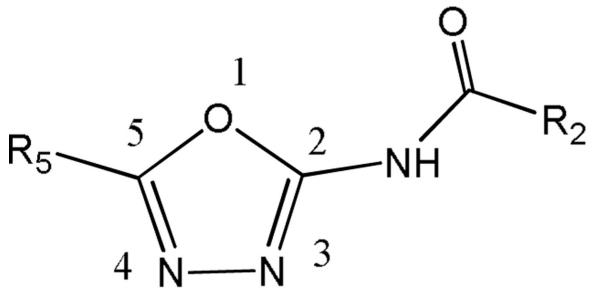
Table 10

Compounds 22a – ai

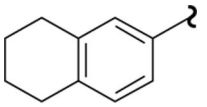
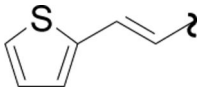


22

Compd	PubChem SID	Corp ID	R_2	R_5	IC_{90}	SI
22a	92099271	F0608-0888	4-OEt-Ph	pyridin-2-yl	1.0	36
22b	92114527	F0608-0814	3-F-Ph	pyridin-2-yl	3.5	>12
22c	92099223	F0608-0617	2-F-Ph		0.93	>43
22d	92104898	F1374-0277		2-OMe-Ph	8.0	>5
22e	92099195	F0608-1018	4-*t*-butyl-Ph	pyridin-3-yl	0.84	>48
22f	92099718	F0608-1152	4-*t*-butyl-Ph	pyridin-4-yl	1.2	290
22g	92108663	F2518-0218	3-O-butyl-Ph	2,4-diMe-Ph	2.6	>15
22h	92099397	F0608-1094	4-O-butyl-Ph	pyridin-4-yl	3.4	>12
22i	92104730	F1374-0081	3-OPhe-Ph	2-Cl-Ph	0.86	>47
22j	92108625	F2518-0235	2-Me-Ph	2,4-diMe-Ph	2.5	>16
22k	92108793	F2518-0422	2,5-diMe-Ph	2,5-diMe-Ph	2.6	>16
22l	92108926	F2518-0386	naphthalen-1-yl	2,5-diMe-Ph	0.41	>97
22m	92109070	F2518-0292	3-Me-Ph	2,5-diMe-Ph	3.4	0.98



22

Compd	PubChem SID	Corp ID	R_2	R_5	IC_{90}	SI
22n	92116091	F1374-0870	furan-2-yl	Ph	2.8	>14
22o	92114407	F0608-0567	furan-2-yl		0.87	>46
22p	92108739	F2518-0186	2-Cl-thiophen-5-yl	2,4-diMe-Ph	2.0	>20
22q	92108962	F2518-0360	2,5-diCl-thiophen-3-yl	2,5-diMe-Ph	2.2	>18
22r	92108843	F2518-0522	*t*-butyl	4-Br-Ph	<0.2	>54
22s	92108803	F2518-0521	*n*-Bu	4-Br-Ph	2.3	>17
22t	92109081	F2518-0520	i-Pr	4-Br-Ph	1.2	>32
22u	92104729	F1374-0488	cyclopropyl	4-OMe-Ph	1.06	>38
22v	92108502	F2518-0231	Et	2,4-diMe-Ph	<0.2	>160
22w	92108619	F2518-0183	benzothiazol-2-yl	2,4-diMe-Ph	1.76	17
22x	92116089	F1374-0365		3-OMe-Ph	3.55	>11

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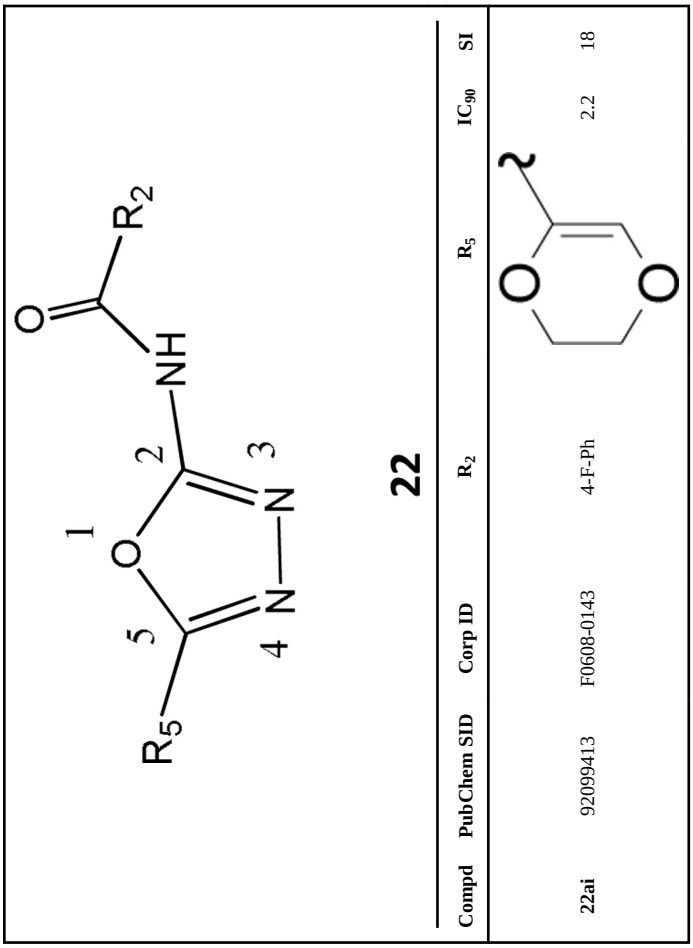
Compd	PubChem SID	Corp ID	R_2	R_5	IC_{90}	SI
22y	92108951	F2518-0415		2,5-diMe-Ph	<0.2	>86
22z	92104893	F1374-0773	cyclohexyl	4-Cl-Ph	5.6	>7
22aa	92099270	F0608-0491	4-OPhe-Ph	furan-2-yl	<0.2	>75
22ab	92099422	F0608-0424	4-O-butyl-Ph	furan-2-yl	0.8	>50
22ac	92099468	F0608-0482	4-*t*-butyl-Ph	furan-2-yl	1.2	>34
22ad	92104859	F1374-0975	furan-2-yl	thiophen-2-yl	3.4	>12
22ae	92099302	F0608-0420	thiophen-2-yl	furan-2-yl	3.1	>13
22af	92104899	F1374-0978	benzothiazol-2-yl	thiophen-2-yl	3.0	4.6
22ag	92104866	F1374-1138	Ph-ethyl	2-Cl-thiophen-5-yl	2.3	15
22ah	92099217	F0608-0206			0.44	60



22

Compd	PubChem SID	Corp ID	R_2	R_5	IC_{90}	SI
22ai	92099413	F0608-0143	4-F-Ph		2.2	18