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Novel anti-tuberculosis agents from MCR libraries

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Abstract—Structure-based design of libraries of multi-component reaction products yields novel potent anti-tuberculosis compounds. Synthesis and preliminary biological results are presented.

Tuberculosis is the world's number one disease amongst infections, killing every year 2 million peoples.[1] More than 2 billion humans (>30%) are infected by *Mycobacterium tuberculosis*, the causing organism of tuberculosis. Often tuberculosis is accompanying AIDS. Whereas tuberculosis is declining in developed countries, it is strongly augmenting in third world countries. Despite the obvious urgentness for new and better treatment options, probably due to the recent tractability of TB in developed countries by current medications and financial reasons, the development of novel drugs is a slow process. However a recent outbreak of extreme resistance tuberculosis (XTR TB) where neither standard drug nor any of at least three of the six classes of more toxic and less-effective backup drugs is effective could potentially prompt more attention to this underdeveloped field in drug discovery and development.[2]

Recently the complete genome of *M. tuberculosis* was published, and novel targets have raised high expectations for new drugs.[3] A host of unusual properties of the infectious agent account for the difficulties in tuberculosis treatment, including a complex cell envelope, colonization of macrophages, slow growth cycle and the ability to remain quiescent, eventually reactivating decades after infection. Current first-line drugs include ethambutol, isoniazid **1**, pyrazinamide **2** and rifampicin and second-line agents include kanamycin, *p*-aminosalicylic acid, ciprofloxacine or cycloserine. However, current drugs suffer from requiring a long treatment time, associated side-effects and a poor efficacy to eradicate dormant pathogens. Moreover the rising incidence of multi-drug-resistant (MDR) tuberculosis could potentially make tuberculosis quickly incurable and an even more dangerous worldwide threat.[4] Generally recognized mechanisms of resistance include, e.g., drug efflux, drug metabolism and target adaptation to the drug. Thus novel approaches in the chemotherapy of tuberculosis are highly needed.

A recent proteome-wide screening for isoniacid targets in *M. tuberculosis* revealed 16 new isoniacid binding proteins beyond NADH-dependent enoyl-ACP reductase (InhA) and the NADPH-dependent dihydrofolate reductase (DfrA).[5] Thus, targeting of multiple enzymes may account for the pleiotropic effects and powerful mycobactericidal properties of INH.

Our approach to the discovery of novel anti-tuberculosis drugs is therefore based upon the pharmacophor of **1** and **2** (Fig. 1), with the pyridine-4-carboxy and pyrazine carboxy groups as essential pharmacophores. Thus we designed two scaffolds based on these scaffolds and produced libraries with 192 compounds each, based on the respective carboxylic acids as starting materials using the Ugi four-component reaction (U-4CR) (Fig. 2).

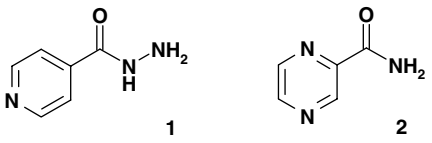

Figure 1. Structural formulas of currently used first-line anti-tuberculosis drugs isoniazid (INH) and pyrazineamide.

Keywords: Tuberculosis; Multi-component reaction; Isocyanide.
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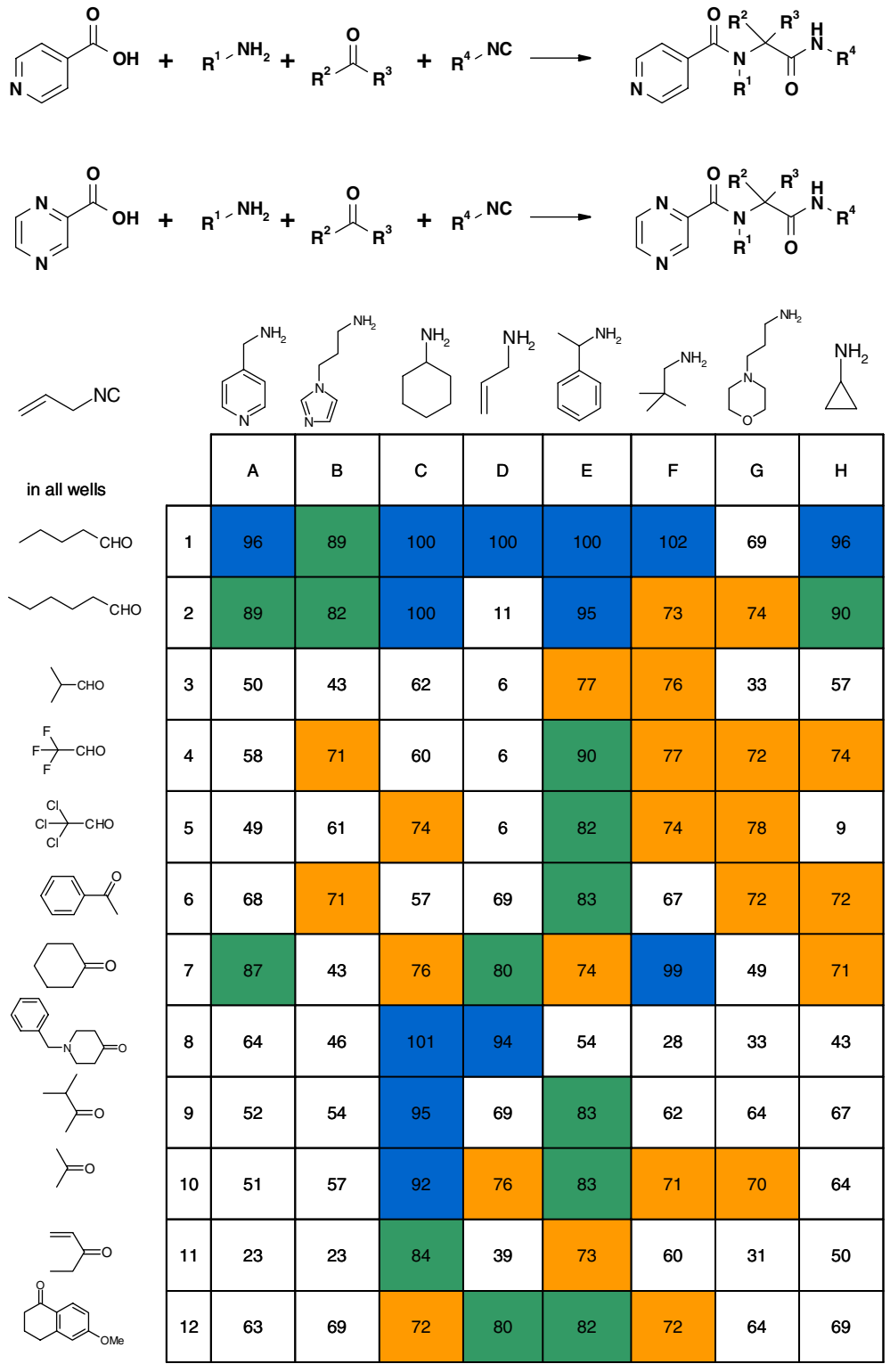
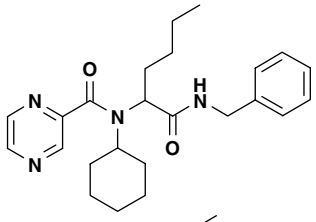
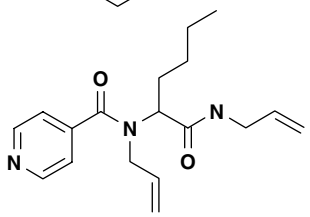
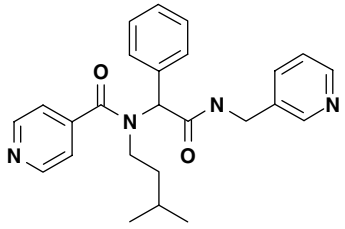
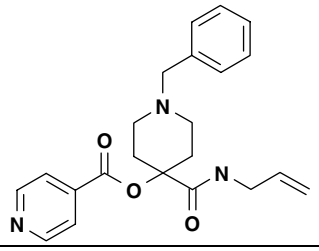


Figure 2. Anti-mycobacterial libraries based on Ugi's 4-CR of nicotinic acid and pyrazine carboxylic acid. The screening results of a 96-well plate of nicotinic acid derived compounds against *Mycobacterium tuberculosis* are shown (>90% growth inhibition (GI), blue; 80–90% GI, green; 70–80% GI, orange determined at a single screening concentration of 12.5 μg/ml).

The synthesis was conveniently performed at room temperature in 96-deep- well polypropylene plates. Analysis of the crude reaction mixtures after 24 h by HPLC–MS revealed the formation of virtually all products, although in different quantities. Moreover it was found that some wells contained considerable amounts of the corresponding Passerini products.[10] Evaporation of the solvent using a GENEVAC yielded the crude products.

The biological evaluation of the libraries was performed in collaboration with the Tuberculosis Antimicrobial

Table 1. Minimum inhibitory concentration (MIC), cytotoxicity and macrophage assay

Compound	Structure	MIC[a] (μg/ml)	IC_{50}[b] (μg/ml)	SI[c]	EC_{90}[d]	EC_{99}[d]	EC_{90}/MIC
3		3.13	27.9	9.9	n.d.	n.d.	n.d.
4		3.13	31.1	9.9	n.d.	n.d.	n.d.
5		3.13	50.3	16.1	>12.5	>12.5	>3.99
6		6.25	>62.5	>10	n.d.	n.d.	n.d.

EC_{90} and EC_{99} represent the concentration effecting 90% and 99% reduction in residual mycobacterial growth after 7 days, compared to untreated controls.

[a] MIC measured minimum inhibitor concentration in serial dilution beginning at 6.25 μg/ml.

[b] IC_{50} cytotoxicity assay on VERO cell line beginning at 10× of MIC.

[c] SI selectivity index is defined as the ratio of the measured IC_{50} to the MIC.

[d] Compounds are screened by serial dilution beginning at 12.5 μg/ml.

Acquisition & Coordinating Facility (TAACF) of the Southern Research Institute.[6]

The following biological screening cascade was used to discover active compounds. First the crude compound libraries were screened at one concentration (12.5 μg/ml) against *M. tuberculosis*. Compounds exhibiting <90% inhibition are not evaluated further. Out of the best ranking compounds we resynthesized and purified several examples.[7] These compounds were rescreened at a lower concentration against *M. tuberculosis* strain H37Rv to determine the actual minimum inhibitory concentration in a broth microdilution Alamar blue assay (MABA). Concurrent with the MIC determination, selected compounds are tested for cytotoxicity. Compounds with a selectivity index >10 are tested for killing of *M. tuberculosis* strain H37Rv in monolayer of mouse peritoneal macrophages at 4-fold concentrations equivalent to 0.25, 1, 4 and 16 times the MIC. Typical compounds and its results from the biological screening cascade are shown in Table 1.

We were delighted to find that a rather high percentage of this library showed anti-tuberculosis activity. One current model of isoniazid activity foresees isoniacid to be an inhibitor of *inhA*-encoded NADH-dependent enoyl-ACP (acyl carrier protein) reductase enzyme.[8] This enzyme is responsible for one step in the biosynthesis of the unusual fatty acids of the *Mycobaterium* sp. It exhibits specificity for long-chain C_{18} and C_{16} enoyl thioester substrates. The mode-of-action postulates the acylation of the C4 of NADH by isoniazid. Thus according to this model isoniazid is a prodrug which becomes activated by the mycobacterial catalase-peroxidase enzyme KatG. The herein described inhibitors are comprised of a nicotinicacyl moiety as in isoniazid. However the bulkiness and the leaving group ability differ very much from the hydrazide isoniazid. Regarding

the recently observed promiscuity of isoniazid and the different chemotypes of the herein described inhibitors one might speculate that other mycobacterial targets are responsible for the observed activity.

In summary, we have introduced design, combinatorial synthesis and first biological evaluation of a novel class of isoniazid derived anti-tuberculosis compounds. Libraries of these compounds were synthesized using isocyanide-based MCRs.[9] Several compounds showed cell-based activities in the same range as the mother compound isoniazid. The synthetic approach used here allows for the rapid and efficient optimization of this compound class. Further studies are needed to fully delineate the biochemical mode of action of representatives of this lead series. The selection of a wider set of starting materials should lead to compounds with improved biological and pharmacokinetic activities.

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References and notes

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5. Argyrou, A.; Jin, L.; Siconilfi-Baez, L.; Angeletti, R. H.; Blanchard, J. S. *Biochemistry* **2006**, *45*, 13947.
6. http://www.taacf.org/.
7. General procedure for compound resynthesis: 5 mmol Amin and 5 mmol aldehyde are placed together without solvent and stirred on rotary evaporator for 4 h at 50 °C. Five millimolar acid and 5 mmol isonitrile are added with 20 ml of methanol. The mixture is stirred at room temperature overnight and the solvent is evaporated in vacuo. The product is purified by silica gel dish chromatography with ethyl acetate as solvent.
   Pyrazine-2-carboxylic acid (1-benzylcarbamoyl-pentyl)-cyclohexyl-amide **3**, $C_{24}H_{32}N_4O_2$ MW: 408.55 g/mol; HPLC–MS (ESI-TOF): t_R = 3.46 min; *m/z* = 409 $[M+H]^+$; 431 $[M+Na]^+$; ^1H NMR ($CDCl_3$, 250 MHz): δ = 0.89–2.28 (m, 19H), 3.19–3.43 (m, 1H), 3.95–4.17 (m, 1H), 4.49 (m, 2H), 7.32 (m, 5H), 8.21–8.94 (m, 3H); ^{13}C NMR ($CDCl_3$, 60 MHz): δ = 13.8, 22.4, 26 .2, 27.9, 29.1, 29.3, 30.0, 30.9, 31.3, 43.7, 58.4, 61.1, 62.0, 63.0, 127.3, 127.8, 128.6, 138.1, 141.0, 142.8, 144.7, 145.5, 146.1, 150.1, 169.5. Due to the cis/trans isomerism at the tertiary amide bond rotation isomers are seen in the carbon NMR spectra.
   N-Allyl-*N*-(1-allylcarbamoyl-pentyl)-isonicotinamide **4**, $C_{18}H_{25}N_3O_2$ MW 315.42 g/mol; HPLC–MS (ESI-TOF): t_R = 3.36 min; *m/z* = 316 $[M+H]^+$; 338 $[M+Na]^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 0.84–0.85 (m, 3H), 1.16–1.29 (m, 4H), 1.77–1.99 (m, 2H), 3.76–4.07 (m, 4H), 4.76–4.86 (m, 1H), 4.99–5.14 (m, 4H), 5.55–5.65 (m, 1H), 5.73–5.79 (m, 1H), 7.19–7.20 (m, 2H), 8.60–8.61 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.1, 22.6, 28.4, 28.6, 41.8, 49.7, 58.6, 116.3, 118.4, 121.1, 133.6, 134.1, 144.0, 150.2, 170.4, 171.2.
   N-(3-Methyl-butyl)-*N*-{phenyl-[(pyridin-3-ylmethyl)-carbamoyl]-methyl}-isonicotinamide **5**, $C_{25}H_{28}N_4O_2$ MW 416.53 g/mol; HPLC–MS (ESI-TOF): t_R = 2.35 min; *m/z* = 417 $[M+H]^+$; 439 $[M+Na]^+$; ^1H NMR ($CDCl_3$, 250 MHz): δ = 0.45–1.32 (m, 11H), 3.17–3.22 (m, 1H), 4.46 (m, 1H), 5.88 (m, 1H), 7.30–7.62 (m, 11H), 8.36–8.61 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 21.7, 25.6, 38.3, 41.0, 46.7, 63.1, 120.7, 123.5, 126.3, 128.8, 128.9, 129.2, 129.6, 133.8, 134.3, 135.5, 144.1, 148.5, 148.8, 149.9, 169.5, 170.1.
   Isonicotinic acid 4-allylcarbamoyl-1-benzyl-piperidin-4-yl ester **6**, $C_{22}H_{25}N_3O_3$ MW 379.46 g/mol; HPLC–MS(ESI-TOF): t_R = 2.69 min; *m/z* = 380 $[M+H]^+$; ^1H NMR ($CDCl_3$, 250 MHz): δ = 2.32–2.76 (m, 8H), 3.53–3.62 (m, 2H), 3.91 (m, 2H), 5.14 (m, 2H), 5.75–5.82 (m, 1H), 7.30–7.35 (m, 5H), 7.82–7.84 (m, 2H), 8.78–8.80 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 32.3, 41.3, 41.9, 48.7, 52.9, 61.9, 62.8, 81.3, 116.5, 122.9, 127.1, 127.3, 128.2, 128.3, 128.8, 128.9, 133.8, 137.1, 137.9, 138.1, 150.7, 163.4, 171.0, 209.2.
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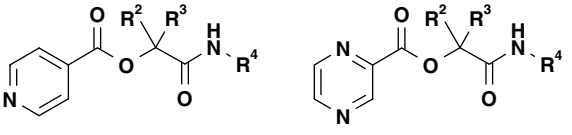
9. Doemling, A. *Chem. Rev.* **2006**, *106*, 17.
10. General formula of the Passerini side-products:



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