Synthesis and Evaluation of Nitrofuranylamides as Novel Antituberculosis Agents

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In an effort to develop new and more potent therapies to treat tuberculosis, a library of compounds was screened for *M. tuberculosis* UDP-Gal mutase inhibition. Nitrofuranylamide **1** was identified as a hit in this screen, possessing good antituberculosis activity. This paper describes the synthesis and evaluation of an expanded set of nitrofuranylamides. We have discovered a number of nitrofuranylamides with submicromolar *M. tuberculosis* MIC values and acceptable therapeutic indexes. The MIC activity did not correlate with UDP-Gal mutase inhibition, suggesting an alternative primary cellular target was responsible for the antituberculosis activity. The compounds were only active against mycobacteria of the tuberculosis complex. On the basis of these results, four compounds were selected for in vivo testing in a mouse model of tuberculosis infection, and of these compounds one showed significant antituberculosis activity.

Introduction

The global burden of tuberculosis (TB) is immense.[1,2] In 1997 there were an estimated 7.96 million new and 16.2 million existing cases with the worldwide mortality rate at 23%. HIV infection is a key risk factor in TB reactivation rates. HIV-infected patients have an elevated risk of primary or reactivated tuberculosis, and such active infectious process may enhance HIV replication and increase the risk of death. An additional major concern is the rise of multidrug-resistant tuberculosis (MDRTB).[3,4] No new effective treatments have been developed since the introduction of Rifampin in 1971,[5] even though there has been significant advances in drug development technologies. Consequently there is an urgent need to develop new, potent, fast-acting antituberculosis drugs with low-toxicity profiles that can be used in conjunction with drugs used to treat HIV infections.[6,7]

The mycobacterial cell wall is a complex and intriguing mixture of unique components, which sets mycobacteria apart from all other known bacteria.[8] Many of the tuberculosis bacilli characteristics, such as its relatively small size, the ability to grow in macrophages, drug resistance, and hydrophilicity are believed to result from components within the ultrastructure of the cell wall.[9] Since many of the structural components of the cell wall are not found in humans, enzymes involved in cell wall biosynthesis have proven to be a very fertile ground for the development of antituberculosis drugs. Isoniazid, ethionamide, and ethambutol are all believed to act against mycobacterial cell wall biosynthesis, validating the enzymes of cell wall biosynthesis as targets for further drug development.[10−13]

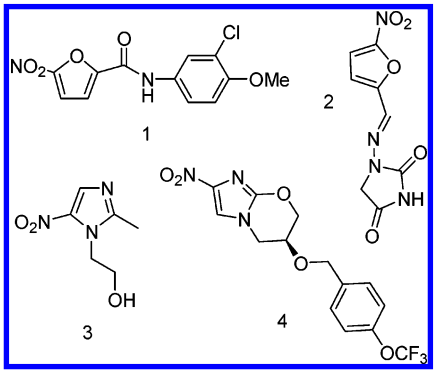

Figure 1. **1**, lead; **2**, nitrofurantoin; **3**, metronidazole; **4**, PA824.

Galactofuranose is an essential component of the mycobacterial cell wall and not found in man; UDP-galactofuranose is biosynthesized from UDP-galactopyranose using the enzyme UDP-galactose mutase (Glf).[14] We have developed a microtiter plate-based screen of this enzyme to discover novel inhibitors as potential new antituberculosis agents.[15] In the course of the screen, nitrofuranylamide **1** (Figure 1) was discovered as an IC_{50} = 12 μg/mL inhibitor of Glf. Noticeably, this compound had good activity against whole cells with an MIC of 1.6 μg/mL.

Nitrofuran antibiotics are known because nitrofurantoin **2** is currently used as a second-line agent to treat urinary tract infections.[16] Nitrofurantoin is moderately active against dormant and growing mycobacteria, possessing an MIC of 12 μg/mL.[17] The nitroimidazole antibiotics, metronidazole **3** and PA824 **4**, are also structurally related. Metronidazole is a widely used antibiotic for the treatment of anaerobic bacterial and protozoan infections but is poorly effective against *M.*

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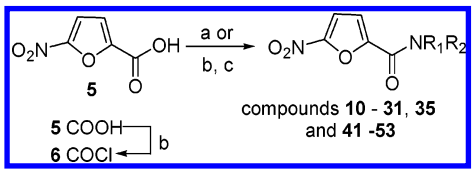
Scheme 1[a]



[a] Reagents: (a) HNR_1R_2, EDCI, DMAP, DMF, room temp, 14 h; (b) $(COCl)_2$, CH_2Cl_2, cat. DMF, 4 h, room temp; (c) HNR_1R_2, CH_2Cl_2, NEt_3, 14 h, room temp or 47 °C (or) HNR_1R_2, DMF, Py, 60° C, 14 h.

tuberculosis, showing activity only against nongrowing mycobacteria.[18] Researchers at Pathogenesis developed the tubercucidal nitroimidazolepyran, PA824, which is active against both growing and dormant tuberculosis strains.[19] PA824 was selected for its potent antitubercular activity, good pharmacokinetics, and low-toxicity profile. PA824 was found to have antibacterial activity limited to mycobacteria of the *Mycobacterium tuberculosis* complex and whose metabolic activation by nitro reduction was required for activity. Importantly, PA824 is not cross-resistant with any currently used TB drugs and was active against both active and dormant bacilli. Genetic studies have demonstrated that PA824 is reductively activated by the *M. tuberculosis* flavin F420 system to an active form, which then targets protein and mycolic acid biosynthesis.[20]

This paper discusses our efforts to develop the structure−activity relationship of **1** with respect to Glf inhibition and antituberculosis activity.

Chemistry

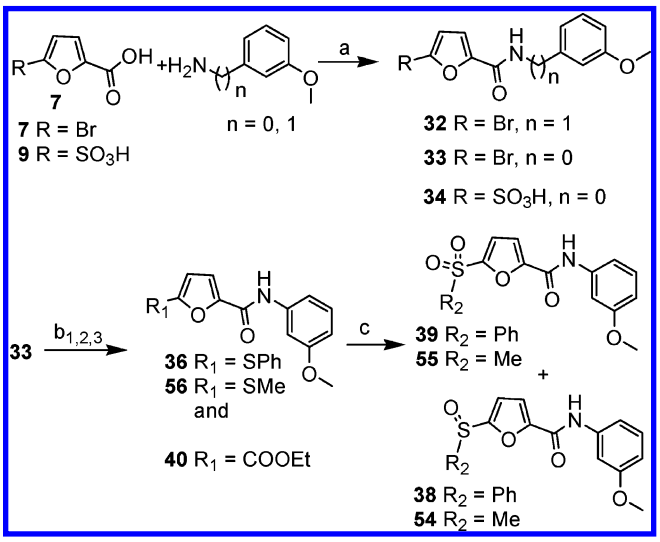
The first objective of this study was to synthesize a variety of nitrofuranylamides and to evaluate the importance of the amide substitution on their Glf inhibitory activity, cytotoxicity, and antituberculosis activity. Second, we planned to investigate the importance of the nitrofunctionality through the synthesis of a number of substituted furanylamides using bioisosteric replacements.

The nitrofuranylamides **10**−**31** and **35** were prepared by reacting the corresponding amines with 5-nitro-2-furancarboxylic acid **5** in the presence of EDCI (Scheme 1) according to the parallel synthesis protocol of Boger.[21] This route was later replaced, when coupling less reactive heteroaromatic amines by using acid chloride **6** to form amides **41**−**53**. This route has proven to be more cost-effective and scalable.

Since 3-substituted anilinylamides showed optimum inhibition of Glf, it was necessary to synthesize an additional analogue, 3-benzyloxyphenylamide **37**, to study the effect of a bulky benzyl group in the 3-position. The required aniline was not available; therefore, amide **37** was synthesized by benzylating the phenolic hydroxyl group of **35** using standard benzylation conditions.

To evaluate the importance of the nitro functionality at the 5-position of the furan ring, other furanylamides were synthesized. The 5-bromofuranylamides, **32** and **33**, were synthesized by reacting 5-bromofurancarboxylic acid with corresponding amines in the presence of EDCI (Scheme 2). The 5-bromo substitution on the furan ring was subsequently used to effect further transformations at the 5-position. Bromofuranyl amide

Scheme 2[a]



[a] Reagents: (a) EDCI, DMAP, DMF, room temp, 14 h; (b_1) PhSH, NaH, 150° C, 12 h; (b_2) NaSMe, THF, room temp, 24 h; (b_3) ClCOOEt, EtMgBr, EtBr, dry THF, 0 °C to room temp; (c) *m*-CPBA, CH_2Cl_2, $NaCO_3$, 0 °C, 3 h.

33 was converted to thioethers **36** and **56** by a nucleophilic substitution of the bromine on the furan ring with thiophenol and methanethiol, respectively. The thioethers **36** and **56** upon oxidation[22] provided sulfoxide and sulfone amides **38**, **54** and **39**, **55** (Scheme 2).

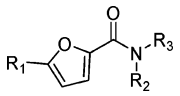
Furanylamide ester **40** was synthesized from furanylamide bromide **33** using standard Grignard chemistry (Scheme 2). Treatment of the bromide **33** with ethylmagnesium bromide followed by reaction of the intermediate with ethyl chloroformate afforded the target ester **40**.[23] The commercially available sodium 5-formylfuran-2-sulfonate **8** was oxidized under the conditions $AgNO_3$ in the presence of aqueous NaOH to give carboxylic acid **9**, which coupled to *m*-anisidine using the standard EDCI coupling protocol to yield sulfonate analogue **34** (Scheme 2).[24]

Results

To develop the structure−activity relationship of **1**, 43 compounds were synthesized and tested for enzymatic inhibition of Glf and for MIC activity against *M. tuberculosis* (Table 1).[15,25] The best inhibitors of Glf were **10** and **11**, both of which are 3-substituted anilinylnitrofuranamides. In general, substituted nitrofuranylbenzylamides, were less active than the analogous anilinylamides with the exception of 2-chlorobenzylamide. The nitrofuranyl group was shown to be required for optimum Glf activity. Overall, we were unable to significantly improve on the Glf inhibition of the hit compound **1**.

The MIC activity of the series showed a strong structure−activity relationship with the nitro group being required for activity in most cases with exception of **38** and **39** that showed modest activity. Nitrofuranylanilinyl, benzylamides, and phenethylamides all had significant activity, with increased activity compared to saturated cyclohexylamide **15** and adamantylamide amide **16**. Heteroaromatic substitutions such as pyridines **44**−**46**, pyrazole **47**, pyrazine **48**, and furfuryl amide **18** were all less active than the corresponding aniline amide **17**. Tertiary amides **41** and **42** were less

Table 1. Structures and Activities of Substituted Furan Amides



compd	R_1	HNR_2R_3	TB MIC (μg/mL)	Glf IC_{50} (μmol/mL)	toxicity IC_{50}	selectivity index[a]	CLogP[b]	solubility[c]	protein binding[d]
10	NO_2	3-chloroaniline	0.8	15	6.86	8.6	2.804	−3.52	68.45
11	NO_2	3-bromoaniline	1.6	11	15.37	9.6	2.954	−3.59	68.91
12	NO_2	3-fluoroaniline	0.8	28	9.29	11.6	2.234	−3.30	66.61
13	NO_2	3-anisidine	0.8	42	18.63	23.3	1.975	−3.39	63.60
14	NO_2	4-anisidine	0.4	42	21.44	53.6	1.975	−3.44	63.09
15	NO_2	cyclohexylamine	3.1	67	9.08	2.9	2.243	−3.00	64.78
16	NO_2	adamantylamine	3.1	49	9.43	3.0	2.871	−3.57	74.18
17	NO_2	aniline	0.8	22	2.19	2.7	2.001	−3.18	67.46
18	NO_2	furfurylamine	6.25	58	4.08	0.7	1.356	−2.92	62.01
19	NO_2	4-aminobenzonitrile	0.8	101	3.53	4.4	1.342	−4.46	75.07
20	NO_2	4-methoxybenzylamine	0.1	115	16.36	163.6	2.099	−3.72	65.28
21	NO_2	2-chlorobenzylamine	1.6	15	11.75	7.3	2.893	−3.36	68.69
22	NO_2	2,4-dimethoxybenzylamine	0.4	34	5.38	13.5	2.188	−3.65	59.13
23	NO_2	3,4-dimethoxybenzylamine	0.2	23	18.17	90.9	1.838	−3.77	63.68
24	NO_2	3,4,5-trimethoxybenzylamine	0.8	62	23.59	29.5	1.480	−4.12	65.57
25	NO_2	1-amino-1,2,3,4-tetrahydronaphthalene	3.1	32	9.03	2.9	3.153	−3.77	77.18
26	NO_2	1-aminoindane	3.1	72	10.28	3.3	2.594	−3.65	77.02
27	NO_2	phenethylamine	1.6	82	25.23	15.7	2.309	−3.76	75.35
28	NO_2	4-methoxyphenethylamine	0.8	17	20.45	25.6	2.228	−4.19	71.20
29	NO_2	(*S*)-1-phenylethylamine	1.6	38	25.63	16.0	2.489	−3.64	74.10
30	NO_2	(*R*)-1-phenylethylamine	3.1	39	23.59	7.6	2.489	−3.44	71.13
31	NO_2	3,4-dimethoxyphenethylamine	0.4	53	27.44	68.6	1.967	−4.31	69.68
35	NO_2	3-aminophenol	1.6	78	12.5	7.8	1.334	−3.20	60.94
37	NO_2	3-benzyloxyaniline	12.5	28			3.743	−5.15	87.00
38	SOPh	3-anisidine	6.25		65	10.4	2.376	−4.25	61.04
39	SO_2Ph	3-anisidine	6.25	23	25.85	4.1	2.736	−4.11	51.50
41	NO_2	*N*-methylaniline	3.12	64	23.75	7.6	1.457	−2.94	66.83
42	NO_2	*N*-methyl-4-anisidine	6.25	138	25.25	4.0	1.406	−3.15	60.45
43	NO_2	2,3-dihydroindole	0.8		7.93	9.9	3.249	−3.52	75.02
44	NO_2	2-aminopyridine	3.12	53	10.8	3.5	1.051	−3.12	56.21
45	NO_2	3-aminopyridine	6.25	48	12.83	2.1	1.051	−2.83	55.33
46	NO_2	4-aminopyridine	3.12	43	6.86	2.2	1.051	−2.92	55.36
47	NO_2	3-aminopyrazole	6.25	162	7.29	1.2	0.601	−2.97	48.70
48	NO_2	2-aminopyrazine	6.25	162			0.286	−2.90	47.18
49	NO_2	2-aminomethylpyridine	0.8	119			0.683	−3.02	57.31
50	NO_2	2-amino-4-methoxybenzothiazole	1.6	67			2.801	−3.96	74.30
51	NO_2	4-amino-6-methoxypyrimidine	1.6				1.226	−3.33	53.13
52	NO_2	2-methoxybenzylamine	1.6	99			2.099	−3.30	66.45
53	NO_2	2,3-dimethoxybenzylamine	1.2	142			1.838	−3.46	64.36

[a] Selectivity index is expressed as the ratio (toxicity IC_{50})/(TB MIC). [b] CLogP was calculated using the ChemDraw Ultra, version 7, software by Cambridge Soft. [c] Solub is log [1/Soly], where solubility is expressed in mol/L at 25 °C, as determined by the VolSurf ADME prediction program.[26] [d] The protein binding is expressed as percent protein bound, as determined by VolSurf.

active than their corresponding secondary amides **14** and **17**. The most active series was the methoxy-substituted benzylamides with a range of relative activities: 4-methoxybenzyl **20** > 3,4-dimethoxybenzyl **23** > 2,4-dimethoxybenzyl **22** > 3,4,5-trimethoxybenzyl **24** > 2,3 dimethoxybenzyl **53** > 2-methoxybenzyl **52**. The activity of this series shows a clear preference for 4-methoxy-substituted systems. Compounds in the methoxybenzyl series showed the highest therapeutic index, principally due to their low MIC values. To determine if the activity of the nitrofuranylamides was exclusive to mycobacteria of the tuberculosis complex, 20 different nitrofuranylamides were tested against a panel of other representative organisms: *Mycobacterium smegmatis*, *Staphylococcus epidermis*, or *Escherichia coli* in MIC assays, which were all inactive.

Added to Table 1 are the predictive pharmacokinetic values of CLogP, solubility, and protein binding. These values were used to help determine which compounds were to advance toward in vivo testing. Volsurf was used to predict the solubility and protein binding.[26] The predicted protein binding values for this series is acceptable, but the data suggest that solubility may be a problem. After examining the MIC, selectivity index, Glf inhibition, CLogP, calculated solubility, and protein binding data for the compounds, **10**, **12**, **13**, **20**, and **23** were selected for in vivo testing.

Animal Testing. Maximum tolerated dosing (MTD) was performed on the selected compounds. An escalating dose of drug (100, 300, and 500 mg/kg) was given to mice by oral gavage. Compounds **10**, **12**, and **23** showed no effect or adverse reactions at the maximum dose. **13** showed some pathology at 500 mg/kg. We were unable to test **20** because of solubility problems in creating a suitable formulation.

Subsequently, the four compounds were tested for efficacy against *M. tuberculosis* at a dose (see Table 2) lower than the MTD in infected C57BL/6 interferon-γ gene-depleted mice (see below).[27] The results of the experiment are presented in Table 2.

Discussion

The good MIC activity of some of the nitrofuranylamides has lead us to explore their use as antituber-

Table 2. Determination of Viable *M. tuberculosis* in Spleens and Lungs of Infected Mice after an 8-Day Drug Treatment Regimen (SEM = Standard Error)

compd	lungs ± SEM (log CFU)	spleen ± SEM (log CFU)
untreated controls	8.0 ± 0.17	6.7 ± 0.16
isoniazid (25 mg/kg)	5.5 ± 0.21	3.2 ± 0.21
metronidazole (150 mg/kg)	8.5 ± 0.18	6.9 ± 0.04
10 (300 mg/kg)	8.3 ± 0.21	7.3 ± 0.12
12 (300 mg/kg)	8.4 ± 0.24	7.0 ± 0.11
13 (150 mg/kg)	8.0 ± 0.3	7.2 ± 0.07
23 (300 mg/kg)	6.5 ± 0.25	6.0 ± 0.19

culosis agents. We were able to develop compounds with submicromolar *M. tuberculosis* MIC activity and most importantly were able to demonstrate in vivo activity for one compound in this class. The importance of this result becomes clear when it is compared to clinical compounds tested in the same rapid mouse model; levofloxacin administered at 100 mg/kg gives a reduction in CFU of 1.5 log in the lungs,[28] rifampin at 20 mg/kg a reduction of 2.5 log CFU, and ethambutol at 150 mg/kg reduces the CFU in the lungs by 2 log. This is a highly significant result, because few compounds, especially those from new compound classes, have shown the in vivo activity of **23**.[29] The inactivity of **10**, **12**, and **13** may have been due to their poor bioavailablity.

The MIC activities for the best compounds in the series are close to that of the front-line antituberculosis agents isoniazid and ethambutol. The nitrofuranylamides are structurally related to currently used antibiotics, and they have an acceptable therapeutic index. The relatively high IC_{50} values in contrast with the exceptionally low MIC values suggest that MIC activity must originate from a different primary mode of action. Also, the lack of activity against nontuberculosis mycobacteria is not consistent with Glf being the primary target. As with PA824, we have found that their activity is restricted to mycobacteria of the *M. tuberculosis* complex with no appreciable activity against *M. smegmatis*, *S. epidermis*, or *E. coli*, a property that we believe is desirable for the development of new tuberculosis treatments with a narrow spectrum of activity. Studies are currently ongoing to explore the mode of action of these compounds and to see if they are activated in a way similar to that of PA824. How these compounds inhibit Glf is currently being evaluated through cocrystal trials, and it is hoped that with a better structural knowledge it will be possible to further develop a new generation of Glf inhibitors. In summary, we have described a new and interesting set of nitrofuranylamides with potent antituberculosis activity. The compounds are easily synthesized, enabling facile development of this series, and are unlikely to be cross-resistant with any clinically used antituberculosis drugs. Further development of this series includes an assessment of the mutagenic potential, steps to increase the bioavailability of this series, and an investigation into the mechanism of action.

Experimental Section

All the anhydrous solvents and starting materials were purchased from Aldrich Chemical Co. (Wilwaukee, WI). All reagent grade solvents used for chromatography were purchased from Fisher Scientific (Suwanee, GA), and flash column chromatography silica cartridges were obtained from Biotage Inc. (Lake Forest, VA). The reactions were monitored by thin-layer chromatography (TLC) on precoated Merck 60 F_{254} silica gel plates and visualized using UV light (254 nm). Biotage FLASH 25+ column chromatography system was used to purify mixtures. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 (300 and 75 MHz for ^{1}H and ^{13}C NMR, respectively) or Varian INOVA-500 (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively) spectrometer. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak or internal standard (tetramethylsilane), and coupling constants (J) are reported in hertz (Hz) (s = singlet, bs = broad singlet, d = doublet, dd = double dublet, bd = broad doublet, ddd = double doublet of dublet, t = triplet, tt = triple triplet, q = quartet, m = multiplet). Mass spectra were recorded on a Bruker Esquire ESI-MS instrument. The yields quoted are unoptimized. Purity of the final products was confirmed before testing by analytical HPLC using an Alltech platinum C-18 reverse-phase column (4.5 mm × 150 mm) and an H_2O (0.1% TFA) to acetonitrile 0−100% linear gradient at a flow rate of 1.0 mL min^{-1} and UV detection at 254 nm.

General Procedure for Preparation of Amides 10−30, 32, 33, and 35. 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol, 1 equiv) and amine (1.9 mmol, 1 equiv) in DMF (5 mL) were treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) 98% (730 mg, 3.8 mmol, 2 equiv) followed by *N*,*N*-dimethylaminopyridine (DMAP) (582 mg, 4.7 mmol, 2.5 equiv), and the resulting solution was stirred for 14 h at 25 °C. The reaction mixture was poured into ethyl acetate (75 mL), washed with 10% aqueous HCl (2 × 50 mL), and washed with 10% aqueous $NaHCO_3$ (3 × 50 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated followed by flash column purification with petroleum ether and ethyl acetate system to provide the corresponding amides.

5-Nitrofuran-2-carboxylic Acid (3-Chlorophenyl)-amide (10). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and *m*-chloroaniline (202 μL, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 432 mg of product (85% yield). TLC: R_f = 0.82 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (1H, ddd, J = 7.8 Hz, 2.0 Hz, 1.0 Hz), 7.35 (1H, t, J = 7.8 Hz), 7.44 (2H, q, J = 3.8 Hz), 7.54 (1H, ddd, J = 7.8 Hz, 2.0 Hz, 1.0 Hz), 7.84 (1H, t, J = 2.0 Hz), 8.27−8.33 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): δ 112.0, 116.5, 117.8, 116.5, 120.0, 125.1, 129.7, 134.4, 136.9, 146.8, 153.4. ESI-MS, *m*/*z*: 265 (M − 1). Anal. ($C_{11}H_7ClN_2O_4$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (3-Bromophenyl)-amide (11). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and *m*-bromoaniline (306 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 469 mg of product (79% yield). TLC: R_f = 0.82 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 (1H, t, J = 7.7 Hz), 7.36 (1H, t, J = 1.4 Hz), 7.43 (2H, q, J = 9.6 Hz, 3.8 Hz), 7.6 (1H, ddd, J = 7.7 Hz, 2.1 Hz, 1.2 Hz), 7.98 (1H, t, J = 2.1 Hz), 8.23−8.3 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): δ 112.0, 116.5, 118.3, 122.8, 127.9, 129.9, 137.2, 140.7, 146.9, 153.5, 158.9. ESI-MS, *m*/*z*: 310.8 (M − 1). Anal. ($C_{11}H_7BrN_2O_4$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (3-Fluorophenyl)-amide (12). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and *m*-fluoroaniline (184 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 429 mg of product (89% yield). TLC: R_f = 0.82 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.9−6.98 (1H, m), 7.33−7.4 (2H, m), 7.44 (2H, q, J = 3.8 Hz), 7.64−7.7 (1H, m), 8.3−8.4 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): δ 107.6, 112.0, 115.1, 116.5, 129.9, 137.3, 146.9, 153.4, 160.8, 164.0. ESI-MS, *m*/*z*: 248.8 (M − 1). Anal. ($C_{11}H_7FN_2O_4$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (3-Methoxyphenyl)-amide (13). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and *m*-anisidine (214 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 450 mg of product (90% yield). TLC: R_f = 0.75 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.79 (1H, ddd, J = 8.4 Hz, 2.8 Hz, 1.2 Hz), 7.19 (1H, ddd, J = 8.4 Hz, 2.16 Hz, 0.7 Hz), 7.32 (1H, t, J = 8.4 Hz), 7.39−7.45 (2H, m), 8.22−8.28 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): 54.8, 105.6, 110.8, 112.0, 112.1, 116.2, 129.3, 137.0, 147.3, 153.4, 159.7. ESI-MS, *m*/*z*: 260.8 (M − 1).

5-Nitrofuran-2-carboxylic Acid (4-Methoxyphenyl)-amide (14). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and *p*-anisidine (234 mg, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 425 mg of product (85% yield). TLC: R_f = 0.7 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.95 (1H, d, J = 8.9 Hz), 7.38 (1H, d, J = 3.9 Hz), 7.44 (1H, d, J = 3.9 Hz), 7.6 (1H, d, J = 8.9 Hz), 8.15−8.21 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): δ 112.1, 113.8, 115.9, 121.6, 128.8, 147.5, 153.2, 156.7, 157.1. ESI-MS, *m*/*z*: 260.9 (M − 1). Anal. ($C_{12}H_{10}N_2O_5$) C, H, N.

5-Nitrofuran-2-carboxylic Acid Phenylamide (17). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and aniline (152 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 376 mg of product (85% yield). TLC: R_f = 0.75 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24 (1H, tt, J = 7.9 Hz, 0.8 Hz), 7.39−7.48 (4H, m), 7.7 (2H, dd, J = 8.4 Hz, 0.8 Hz), 8.22−8.28 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): δ 112.1, 116.2, 119.9, 125.0, 128.7, 135.8, 147.3, 153.4. ESI-MS, *m*/*z*: 230.8 (M − 1). Anal. ($C_{11}H_8N_2O_4$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (4-Cyanophenyl)amide (19). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 4-aminobenzonitrile (225 mg, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 441 mg of product (90% yield). TLC: R_f = 0.62 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52 (1H, d, J = 3.9 Hz), 7.61 (1H, d, J = 3.9 Hz), 7.76 (1H, d, J = 8.9 Hz), 7.98 (1H, d, J = 8.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 106.2, 113.2, 117.2, 118.7, 120.5, 133.1, 142.0, 147.1, 151.8, 154.8. ESI-MS, *m*/*z*: 255.8 (M − 1). Anal. ($C_{12}H_7N_3O_4$) C, H, N.

5-Nitrofuran-2-carboxylic Acid 4-Methoxybenzyl-amide (20). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 4-methoxybenzylamine (248 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 448 mg of product (85% yield). TLC: R_f = 0.55 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (3H, s), 4.58 (2H, d, J = 5.8 Hz), 6.82−6.92 (1H, bs), 6.92 (2H, d, J = 8 Hz), 7.27−7.32 (3H, m), 7.38 (1H, d, J = 3.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.5, 54.7, 111.8, 113.7, 115.4, 128.5, 128.9, 147.5, 155.5, 158.8. ESI-MS, *m*/*z*: 275.6 (M − 1).

5-Nitrofuran-2-carboxylic Acid 2-Chlorobenzylamide (21). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 2-chlorbenzylamine (230 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 454 mg of product (85% yield). TLC: R_f = 0.72 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.75 (2H, d, J = 6.2 Hz), 7.68−7.09 (1H, bs), 7.27−7.32 (3H, m), 7.38 (1H, d, J = 3.9 Hz), 7.41−7.49 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): 40.9, 111.8, 115.6, 126.6, 128.9, 129.2, 129.8, 133.2, 133.9, 147.2, 155.6. ESI-MS, *m*/*z*: 278.8 (M − 1). Anal. ($C_{12}H_9ClN_2O_4$) C, H, N.

5-Nitrofuran-2-carboxylic Acid 2,4-Dimethoxybenzyl-amide (22). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 2,4-dimethoxybenzylamine (286 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 508 mg of product (87% yield). TLC: R_f = 0.50 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (3H, s), 3.91 (3H, s), 4.57 (2H, d, J = 5.8 Hz), 6.45−6.53 (2H, m), 7.02−7.12 (1H, bs), 7.24 (1H, s), 7.27 (1H, d, J = 2.5 Hz), 7.36 (1H, d, J = 2.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.5, 54.8, 54.9, 98.1, 103.6, 111.8, 115.1, 117.0, 130.2, 147.8, 155.3, 158.1, 160.4. ESI-MS, *m*/*z*: 304.8 (M − 1). Anal. Calcd. for $C_{14}H_{14}N_2O_6$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.13; H, 4.58; N, 8.90.

5-Nitrofuran-2-carboxylic Acid 3,4-Dimethoxybenzyl-amide (23). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 3,4-dimethoxy-benzylamine (289 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 526 mg of product (90% yield). TLC: R_f = 0.3 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.9 (6H, s) 4.19 (2H, d, J = 6.5 Hz), 6.8−6.97 (3H, m), 7.31 (1H, d, J = 3.4 Hz), 7.39 (1H, d, J = 3.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.9, 55.3, 55.4, 110.8, 111.0, 111.8, 115.4, 119.9, 129.1, 147.5, 148.3, 148.7, 155.5. ESI-MS, *m*/*z*: 205.0 (M − 1). Anal. ($C_{14}H_{14}N_2O_6$) C, H, N.

5-Nitrofuran-2-carboxylic Acid 3,4,5-Trimethoxybenz-ylamide (24). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 3,4,5-trimethoxybenzylamine (326 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 532 mg of product (83% yield). TLC: R_f = 0.80 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (75 MHz, $CDCl_3$): δ 3.87 (3H, s), 3.89 (6H, s), 4.58 (2H, d, J = 5.8 Hz), 6.59 (2H, s), 6.86−6.93 (1H, bs), 7.32 (1H, d, J = 4.2 Hz), 7.4 (1H, d, J = 4.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.4, 55.6, 60.2, 104.8, 111.8, 115.5, 132.1, 147.4, 153.0, 155.5. ESI-MS, *m*/*z*: 335.0 (M − 1). Anal. ($C_{15}H_{16}N_2O_7$) C, H, N.

5-Nitrofuran-2-carboxylic Acid Phenethylamide (27). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and phenethylamine (239 μL, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 402 mg of product (81% yield). TLC: R_f = 0.70 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (75 MHz, $CDCl_3$): δ 2.95 (2H, t, J = 7.5 Hz), 3.72 (2H, q, J = 13.8 Hz, 7.5 Hz), 6.81−6.92 (1H, bs), 7.21−7.38 (7Hs, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 35.0, 40.2, 111.8, 115.2, 126.3, 128.1, 128.2, 137.5, 147.5, 155.6. ESI-MS, *m*/*z*: 258.8 (M − 1).

5-Nitrofuran-2-carboxylic Acid [2-(4-Methoxyphenyl)-ethyl]amide (28). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 4-methoxyphenethylamine (279 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 443 mg of product (80% yield). TLC: R_f = 0.6 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.9 (2H, t, J = 7.1 Hz), 3.68 (2H, q, J = 7.1 Hz), 3.81 (3H, s), 6.67−6.76 (1H, bs), 6.88 (2H, d, J = 8.6 Hz), 7.16 (2H, d, J = 8.6 Hz), 7.25 (1H, d, J = 3.8 Hz), 7.36 (1H, d, J = 3.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 34.1, 40.4, 54.7, 111.8, 113.6, 115.2, 129.1, 129.5, 147.5, 155.7, 157.9. ESI-MS, *m*/*z*: 288.8 (M − 1). Anal. ($C_{14}H_{14}N_2O_5$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (1-Phenylethyl)amide (29). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and

1-(*S*)-phenylethylamine (245 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 422 mg of product (85% yield). TLC: $R_f = 0.75$ (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65 (3H, d, $J = 7.2$ Hz), 5.32 (1H, quin, $J = 7.2$ Hz), 6.8−6.92 (1H, bd, $J = 7.2$ Hz), 7.24−7.45 (7H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.9, 48.6, 111.9, 115.5, 125.7, 127.3, 128.3, 141.4, 147.5, 154.8. ESI-MS, *m*/*z*: 258.8 (M − 1). Anal. Calcd. for $C_{13}H_{12}N_2O_4$: C, 60.0; H, 4.65; N, 10.76. Found: C, 59.54; H, 4.68; N, 10.66.

5-Nitrofuran-2-carboxylic Acid [2-(3,4-Dimethoxyphenyl)ethyl]amide (31). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 2,4-dimethoxyphenethylamine (319 μL, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 550 mg of product (90% yield). TLC: $R_f = 0.75$ (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.89 (2H, t, $J = 7.3$ Hz), 3.69 (2H, q, $J = 7.3$ Hz), 3.86 (3H, s), 3.88 (3H, s), 6.7−6.87 (4H, m), 7.24 (1H, d, $J = 4$ Hz), 7.35 (1H, d, $J = 4$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 34.5, 40.3, 55.3, 55.4, 111.0, 111.3, 111.8, 115.2, 120.1, 130.1, 147.4, 147.5, 148.7, 155.6. ESI-MS, *m*/*z*: 318.9 (M − 1). Anal. ($C_{15}H_{16}N_2O_6$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (3-Hydroxyphenyl)-amide (35). 5-Nitro-2-furancarboxylic acid (300 mg, 1.9 mmol) and 3-aminophenol (208 mg, 1.9 mmol) in DMF (5 mL) were treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mixture was stirred for 14 h at room temperature and worked up as explained in the general procedure to afford 331 mg of product (70% yield). TLC: $R_f =$ 0.50 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, CD_3OD): δ 5.09 (1H, ddd, $J = 8.0$ Hz, 2.5 Hz, 1.0 Hz), 5.59 (1H, ddd, $J = 8.0$ Hz, 2.0 Hz, 1.0 Hz), 5.64 (1H, t, $J = 8.0$ Hz), 5.78 (1H, t, $J = 2.0$ Hz), 5.9 (1H, d, J, = 4.0 Hz), 6.45 (1H, d, $J = 4.0$ Hz), 6.45 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 110.2, 114.2, 114.3, 118.3, 131.5, 140.1, 150.1, 157.2, 159.4. ESI-MS, *m*/*z*: 247.2 (M − 1). Anal. ($C_{11}H_8N_2O_5$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (3-Benzyloxyphenyl)-amide (37). Compound **35** (150 mg, 0.6 mmol) was dissolved in dry THF (5 mL) and K_2CO_3 (167 mg, 1.2 mmol) followed by benzyl bromide (146 μL, 1.2 mmol). The reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with 30 mL of ethyl acetate and washed with H_2O (25 mL) brine (25 mL). The ethyl acetate was dried and concentrated. The crude product was purified with flash column chromatography using 15% ethyl acetate in petroleum ether to afford 147 mg of product (72% yields). TLC: $R_f = 0.82$ (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.16 (2H, s), 6.9 (1H, dd, $J = 8.3$, 2.4, 1.0 Hz), 7.23 (1H, dd, $J = 8.1$, 2.0, 0.8 Hz), 7.35 (1H, t, $J = 8$ Hz), 7.39 (1H, dt, $J = 7.0$, 2.5 Hz), 7.42−7.44 (4H, m), 7.5−7.52 (2H, m), 7.56 (1H, t, $J = 2.2$ Hz), 8.26−8.3 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): δ 69.5, 106.5, 111.8, 112.1, 112.2, 116.2, 126.9, 127.5, 128.0, 129.4, 136.1, 136.9, 147.2, 153.3, 158.9. ESI-MS, *m*/*z*: 337.6 (M − 1).

5-Benzenesulfinylfuran-2-carboxylic Acid (3-Methoxyphenyl)amide (38) and 5-Benzenesulfonylfuran-2-carboxylic Acid (3-Methoxyphenyl)amide (39). A mixture of compound **36** (0.1 g, 0.3 mmol) and $NaHCO_3$ (0.116 g, 1.3 mmol) in CH_2Cl_2 (5 mL) at 0° C was treated with *m*-chloroperbenzoic acid (0.116 g, 0.67 mmol) and stirred at for 3 h. The reaction mixture was quenched with diluted aqueous NH_4OH solution (5 mL) and diluted with CH_2Cl_2 (30 mL). The organic layer was washed with diluted aqueous NH_4OH solution (30 mL), water (30 mL), and brine (30 mL) and dried over Na_2SO_4. The organic solution was concentrated in a vacuum followed by flash column purification with petroleum ether and ethyl acetate in 5:1 ratio, which afforded 31 mg of **38** and 38 mg of **39** in 30% and 35% yields, respectively.

Compound 38. TLC: $R_f = 0.30$ (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.87 (3H, s), 6.78 (1H, dd, $J = 8$ Hz, 2.5 Hz), 6.81 (1H, d, $J = 3.5$ Hz), 7.15 (1H, dd, $J = 8$ Hz, 1.5 Hz), 7.29 (1H, d, $J = 3.5$ Hz), 7.32 (1H, d, $J = 8$ Hz), 7.42 (1H, t, $J = 2$ Hz), 7.64 (3H, t, $J = 3$ Hz), 7.82 (2H, dd, $J = 6$ Hz, 3.5 Hz, 2.5 Hz), 8.2 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 54.8, 105.3, 110.4, 111.7, 115.1, 116.5, 124.4, 129.0, 129.2, 131.5, 137.4, 140.3, 150.7, 154.3, 159.6. ESI-MS, *m*/*z*: 340.6 (M − 1).

Compound 39. TLC: $R_f = 0.70$ (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.85 (3H, s), 6.79 (1H, dd, $J = 8$ Hz, 2.2 Hz, 1.7 Hz), 7.18 (1H, dd, $J = 7.4$ Hz, 1.5 Hz), 7.28−7.34 (3H, m), 7.4 (1H, t, $J = 2.5$ Hz), 7.62 (2H, t, $J = 8$ Hz), 7.72 (1H, t, $J = 7$ Hz), 8.09 (2H, d, $J = 7$ Hz), 8.22 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 54.8, 105.5, 110.6, 112.0, 115.2, 118.4, 127.4, 129.0, 129.3, 133.8, 137.2, 138.6, 150.3, 150.6, 154.0, 159.6. ESI-MS, *m*/*z*: 356.5 (M − 1). Anal. ($C_{18}H_{15}NO_5S$) C, H, N.

Preparation of 5-Nitrofuran-2-carbonyl Chloride (6). 5-Nitro-furan-2-carboxylic acid (942 mg, 6 mmol) in DCM (10 mL) was treated with oxalyl chloride (1.05 mL, 12 mmol) followed by 2 drops of DMF and stirred at room temperature for 4 h. The reaction mixture was concentrated in a vacuum to obtain acid chloride, and the crude was used in further reactions without purification.

General Procedure for Preparation of Amides 6. Method 1. 5-Nitrofuran-2-carbonyl chloride (526 mg, 3 mmol) in DMF (5 mL) was added to amine (3 mmol) in pyridine (5 mL), and the reaction was carried out at 60 °C. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with 10% aqueous $NaHCO_3$ (2 × 50 mL), water (2 × 50 mL), and brine (2 × 50 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated followed by flash column purification to provide the corresponding amides.

Method 2. 5-Nitrofuran-2-carbonyl chloride (930 mg, 5.3 mmol) in CH_2Cl_2 (10 mL) was added to the amine (5.3 mmol, 1 equiv) in Et_3N (3 mL), and the mixture was stirred for 14 h at 47 °C. Reaction was followed by TLC. After completion of reaction, 100 mL of ethyl acetate was added and the mixture was washed with 10% aqueous $NaHCO_3$ (2 × 50 mL), water (2 × 50 mL), and brine (2 × 50 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated followed by flash column purification to provide the corresponding amides.

(2,3-Dihydroindol-1-yl)-(5-nitrofuran-2-yl)methanone (43). 5-Nitrofuran-2-carbonyl chloride (526 mg, 3 mmol) in CH_2Cl_2 (5 mL) was added to a mixture of indoline (336 μL, 3 mmol), *N*,*N*-diisopropylethylamine (1.04 mL, 6 mmol) in CH_2Cl_2 (20 mL), followed by DMAP (2 mg), and the reaction mixture was stirred for 14 h at room temperature. The reaction mixture was worked up as explained in method 2 to yield 600 mg (77%) of compound **43**. TLC: $R_f = 0.42$ (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.23 (2H, t, $J = 8.30$ Hz), 4.46 (2H, t, $J = 8.30$ Hz), 7.11 (1H, dt, $J = 0.7$ Hz, 7.5 Hz), 7.23 (1H, t, $J = 7.8$ Hz), 7.32 (1H, d, $J = 7.5$ Hz), 7.51 (1H, d, $J = 4.1$ Hz), 7.82 (1H, d, $J = 3.9$ Hz), 8.0−8.2 (1H, bs). ^{13}C NMR (75 MHz, DMSO): δ 27.9, 48.9, 112.9, 117.0, 118.2, 124.7, 124.9, 127.0, 132.5, 142.2, 147.9, 151.3, 154.8. ESI-MS, *m*/*z*: 281.9 (M + 23). Anal. ($C_{13}H_{10}N_2O_4$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (Pyridin-2-ylmethyl)-amide (49). 5-Nitrofuran-2-carbonyl chloride (930 mg, 5.3 mmol) in CH_2Cl_2 (10 mL) was added to 2-aminomethylpyridine (0.54 mL, 5.3 mmol) in Et_3N (3 mL) and stirred for 14 h at 47 °C. The reaction was followed as explained in method 2 to yield 1.12 g (85%) of product **49**. TLC: $R_f = 0.11$ (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.83 (2H, d, $J = 5.3$ Hz), 7.32 (1H, d, $J = 3.9$ Hz), 7.38 (1H, d, $J = 3.6$ Hz), 7.39−7.42 (1H, m), 7.48 (1H, d, $J = 7.8$ Hz), 7.86 (1H, dt, $J = 1.7$ Hz, 7.8 Hz), 8.10−8.22 (1H, bs), 8.65 (1H, d, $J = 4.8$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.7, 111.7, 115.3, 121.5, 122.1, 136.4, 147.6, 148.7, 154.7, 155.7. ESI-MS, *m*/*z*: 248.1 (M + 1). IR: 1670, 3305 cm^{-1}. Anal. ($C_{11}H_9N_3O_4$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (4-Methoxybenzothiazol-2-yl)amide (50). 5-Nitrofuran-2-carbonyl chloride (667 mg, 3.8 mmol) in CH_2Cl_2 (5 mL) was added to 2-amino-4-methoxybenzothiazole (684 mg, 3.8 mmol) followed by pyridine (5 mL), and the reaction mixture was stirred for 14 h at room

temperature. The reaction was followed as explained in method 2 to yield 480 mg (29%) of compound **50**. TLC: R_f = 0.37 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, CD_3OD): δ 4.03 (3H, s), 7.04 (1H, d, J = 8.0 Hz), 7.30 (1H, t, J = 8.0 Hz), 7.44 (1H, d, J = 8.0 Hz), 7.53 (1H, d, J = 3.6 Hz), 7.59 (1H, d, J = 3.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.3, 106.5, 111.5, 113.0, 117.7, 125.1. ESI-MS, *m/z*: 318.1 (M − 1). IR: 1561, 1701 cm^{-1}. Anal. ($C_{13}H_9N_3O_5S$) C, H, N.

5-Nitrofuran-2-carboxylic Acid (6-Methoxypyrimidin-4-yl)amide (51). 5-Nitrofuran-2-carbonyl chloride (667 mg, 3.8 mmol) in CH_2Cl_2 (5 mL) was added to 4-amino-6-methoxypyrimidine (475 mg, 3.8 mmol) followed by pyridine (5 mL), and the reaction mixture was stirred for 14 h at 50 °C. The reaction was followed as explained in method 2 to yield 550 mg (40%) of compound **51**. TLC: R_f = 0.53 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.04 (3H, s), 7.44 (2H, q, J = 3.6 Hz), 7.65 (1H, d, J = 0.9 Hz), 8.58 (1H, d, J = 0.9 Hz), 8.82−8.88 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): δ 53.7, 95.6, 111.7, 117.3, 145.9, 154.1, 156.1, 157.2, 170.8. ESI-MS, *m/z*: 263.0 (M − 1).

5-Nitrofuran-2-carboxylic Acid 2-Methoxybenzylamide (52). 5-Nitrofuran-2-carbonyl chloride (877 mg, 5 mmol) in CH_2Cl_2 (10 mL) was added to 2-methoxybenzylamine (0.646 mL, 5 mmol) in Et_3N (3 mL), and the reaction mixture was stirred for 14 h at room temperature. The reaction was followed as explained in method 2 to yield 685 mg (49%) of compound **52**. TLC: R_f = 0.53 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.94 (3H, s), 4.65 (2H, d, J = 5.86 Hz), 6.96 (2H, dd, J = 7.3 Hz, 14.4 Hz), 7.12−7.2 (1H, bs), 7.26 (1H, d, J = 3.66 Hz), 7.31−7.38 (3H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.9, 54.8, 109.9, 111.8, 115.1, 120.1, 124.5, 128.7, 129.3, 147.8, 155.4, 157.0. ESI-MS, *m/z*: 299.3 (M+23). IR: 1676, 3307 cm^{-1}. Anal. ($C_{13}H_{12}N_2O_5$) C, H, N.

5-Nitrofuran-2-carboxylic Acid 2,3-Dimethoxybenzylamide (53). 5-Nitrofuran-2-carbonyl chloride (877 mg, 5 mmol) in CH_2Cl_2 (10 mL) was added to 2,3-dimethoxy benzylamine (0.734 mL, 5 mmol) in Et_3N (3 mL), and the reaction mixture was stirred for 14 h at room temperature. The reaction was followed as explained in method 2 to yield 830 mg (54% yield) of compound **53**. TLC: R_f = 0.48 (1:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.90 (3H, s), 3.95 (3H, s), 4.65 (2H, d, J = 6.10 Hz), 6.95 (2H, ddd, J = 1.4 Hz, 8.0 Hz, 15.1 Hz), 7.03−7.09 (2H, m), 7.26 (1H, d, J = 3.6 Hz), 7.35 (1H, d, J = 3.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.9, 55.2, 60.1, 111.8, 115.2, 120.8, 123.6, 130.0, 146.7, 147.6, 152.1, 155.5. ESI-MS, *m/z*: 329.1 (M + 23). IR: 1676, 3323 cm^{-1}. Anal. ($C_{14}H_{14}N_2O_6$) C, H, N.

5-Methylsulfanylfuran-2-carboxylic Acid (3-Methoxyphenyl)amide (56). To the mixture of compound **33** (0.5 g, 1.68 mmol) in tetrahydrofuran (10 mL) was added $NaSCH_3$ (236 mg, 3.37 mmol) at room temperature, and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was diluted with ethyl acetate (75 mL) and washed with water (50 mL) followed by brine (50 mL). The organic fraction was dried over Na_2SO_4 and concentrated in a vacuum to provide 330 mg (74% yields) of product **56**. TLC: R_f = 0.0.75 (3:1 petroleum ether/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.58 (3Hs, s), 3.89 (3H, s), 6.55 (1H, d, J = 3.4 Hz), 6.76 (1H, ddd, J = 0.7 Hz, 2.4 Hz, 8.3 Hz), 7.16 (1H, ddd, J = 0.7 Hz, 1.9 Hz, 8.0 Hz), 7.26 (1H, d, J = 3.4 Hz), 7.32 (1H, t, J = 4.1 Hz), 7.49 (1H, t, J = 2.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.3, 54.7, 105.1, 110.0, 111.6, 114.3, 116.3, 129.1, 138.0, 148.4, 150.9, 155.0, 159.6. ESI-MS, *m/z*: 286.0 (M + 23).

MIC Determinations against *M. tuberculosis* H37Ra. The MIC of the nitrofuranylamides against *M. tuberculosis* H37Ra was determined by the microbroth dilution method using microtiter plates.[25] *M. tuberculosis* was grown in Middlebrook 7H9 medium to an OD_{650} of 0.4−0.6 and a dilution made to an OD_{650} of 0.01. An amount of 100 μL of these cells was then added to a microtiter well containing serial dilutions of the nitrofuranyl amides. The cells were then incubated at 37 °C for 8 days and visually examined for growth. MIC_{90} was visually determined for wells with greater than 90% inhibition of growth using isoniazid as a positive control on every plate.

Determination of Glf IC_{50} Values. The IC_{50} of compound **1** was determined in the forward direction as described.[15] The Glf IC_{50} for the remaining compounds was determined in the reverse direction using UDP-6-[^{3}H]Galf prepared as previously described[30] except that it was used directly after HPLC purification without desalting. The crude soluble *M. tuberculosis* Glf expressed in *E. coli* was prepared as described.[15,31] Prior to use, the enzyme was activated by adding 37 μL of 14 mg/mL crude enzyme extract to 2.2 mL of 25 mM MOPS containing 10 mM NADH and 0.115 mM FAD and incubating at 37 °C for 30 min. The inhibitors were tested at 100, 50, 25, 12.5, and 6.3 μM, and their IC_{50} were calculated from the resulting data. Thus, each microtiter plate well contained 1 μL of DMSO (along with the varying amounts of inhibitor), 625 nmol of MOPS, pH 7.2, 25 nmol of $MgCl_2$, 110 nmol of NADH, 1.2 nmol of FAD, 2.3 μg of protein, and 20 pmol (1.4 nCi) of UDP-6-[^{3}H]Galf in 25 μL. The inhibitors in 1 μL of DMSO were added first followed by a 14 μL aliquot containing all the remaining components except for the enzyme. The reaction was started by adding 10 μL of the enzyme mix to each well. The reaction time was chosen empirically so that the control reactions converted approximately 50% of the UDP-Galf to UDP-Galp and was typically 5−20 min. The conversion of UDP-Galf to UDP-Galp was monitored by one of two methods. The method used for each compound is indicated in Table 1. For conversions monitored by HPLC the reaction was terminated by the addition of 200 μL of 95% ethanol. The small amount of precipitated protein was removed by centrifugation, and the supernatant was analyzed for the ratio of UDP-Galp/UDP-Galf by HPLC as described.[31] For conversion monitored by the microtiter filter plate assay, the enzymatic reactions were terminated by the addition of 5 μL of 200 mM sodium *m*-periodate in 250 mM sodium acetate, pH 4.4. The neutral tritiated formaldehyde produced from UDP-6-[^{3}H]Galf by periodate was separated from the acidic tritiated product produced from UDP-6-[^{3}H]Galp and counted as described. [15]

Cytoxicity Assay. Cytotoxicity was assessed against VERO cells (CCL-81, American Type Culture Collection) by exposing monolayers in 96-well plates to 3-fold dilutions of test compounds for 72 h. Cell viability was measured using the CellTiter96 aqueous nonradioactive cell proliferation assay (Promega Corp, Madison, WI), which determines the extent of reduction of a tetrazolium dye by measuring the absorbance of the product at 490 nm.[32] Untreated cells and cells lysed with sodium dodecyl sulfate were used to determine 0% and 100% inhibition, respectively.

Maximum Tolerated Dose Assay. Three healthy mice were given orally one single dose of the compound and were observed at regular times for any adverse effects. Three different concentrations are tested: 100, 300, and 500 mg/kg. The last dose was 2−5 times the dose used for efficacy testing of the compound in mice. After 7 days of observation the mice were sacrificed and the organs were studied by gross necropsy. In the case of abnormalities, the organs were fixed in formalin and further analyzed by extensive pathology analysis.

GKO Mouse Model. Mice were infected via low-dose aerosol to reproducibly deliver *M. tuberculosis* in the alveolar regions of the lungs in low numbers to mimic the realistic disease in humans.[28] Treatment was initiated 18 days postinfection for 8 daily treatments of one single dose (at 300 mg/kg). Bacterial load was determined 28 days postinfection in lungs and spleens of the mice by serial dilution of the tissue homogenates on nutrient Middlebrook 7H11 agar plates (GIBCO BRL, Gaithersburg, MD). The plates were incubated at 37 °C in ambient air for 4 weeks prior to the counting of viable *M. tuberculosis* colonies (CFU). The viable counts were converted to logarithms, which were then evaluated by multiple-comparison analysis of variance by a one-way Dunnett test using the Sigma Stat program. Differences were considered significant at the 95% level of confidence.

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Supporting Information Available: Experimental procedure, analytical data, and bioassay values for the remaining compounds in the series (**9**, **15**, **16**, **18**, **25**, **26**, **30**, **32**−**34**, **36**, **40**−**42**, **44**−**48**, **54**, and **55**). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dye, C.; Scheele, S.; Dolin, P.; et al. Global Burden of Tuberculosis. Estimated Incidence, Prevalence, and Mortality by Country. *J. Am. Med. Assoc.* **1999**, *282* (7), 677−686.
(2) Murray, J. F. Tuberculosis and HIV Infection: A Global Perspective. *Respiration* **1998**, *65* (5), 335−342.
(3) Schraufnagel, D.; Abubaker, J. Global Action Against Multidrug-Resistant Tuberculosis. *J. Am. Med. Assoc.* , **2000**, *283* (1), 54−54.
(4) Portaels, F.; Rigouts, L.; Bastian, I. Addressing Multidrug-Resistant Tuberculosis in Penitentiary Hospitals and in the General Population of the Former Soviet Union. *Int. J. Tuberc. Lung D* **1999**, 3 (7), 582−588.
(5) Burman, W. J.; Jones, B. E.. Treatment of HIV-Related Tuberculosis in the Era of Effective Antiretroviral Therapy. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 7−12.
(6) Burman, W. J.; Gallicano, K.; Peloquin, C. Therapeutic Implications of Drug Interactions in the Treatment of HIV-Related Tuberculosis. *Clin. Infect. Dis.* **1999**, *28*, 419−430.
(7) Duncan, K. Progress in TB Drug Development and What Is Still Needed. *Tuberculosis* **2003**, *83* (1−3), 201−207.
(8) Brennan, P. J.; Nikaido, H. The Envelope of Mycobacteria. *Annu. Rev. Biochem.* **1995**, *64*, 29−63.
(9) Lee, R. E.; Brennan, P. J.; Besra, G. S. Mycobacterium Tuberculosis Cell Envelope. *Curr. Top. Microbiol.* **1996**, *215*, 1−27.
(10) Banerjee, A.; Dubnau, E.; Quemard, A.; Balasubramanian, V.; Um, K. S.; Wilson, T.; Collins, D.; Delisle, G.; Jacobs, W. R.; Inh, A. A Gene Encoding A Target for Isoniazid and Ethionamide in Mycobacterium-tuberculosis. *Science* **1994**, *263* (5144), 227−230.
(11) Mdluli, K.; Slayden, R. A.; Zhu, Y. Q.; Ramaswamy, S.; Pan, X.; Mead, D.; Crane, D. D.; Musser, J. M.; Barry, C. E. Inhibition of a Mycobacterium Tuberculosis Beta-Kethyl Acetateyl ACP Synthase by Isoniazid. *Science* **1998**, *280* (5369), 1607−1610.
(12) Takayama, K.; Kilburn, J. O. Inhibition of Synthesis of Arabinogalactan by Ethambutol in *Mycobacterium Smegmatis*. *Antimicrob. Agents Chemother.* **1989**, *33*, 1493−1499.
(13) Lee, R. E.; Mikusova, K.; Brennan, P. J.; Besra, G. S. Synthesis of the Mycobacterial Arabinose Donor β-D-Arabinofuranosyl-1-monophosphoryldecaprenol, Development of a Basic Arabinosyl Transferase Assay, and Identification of Ethambutol as an Arabinosyl Transferase Inhibitor. *J. Am. Chem. Soc.* **1995**, *117*, 11829−11832.
(14) Pan, F. M.; Jackson, Y.; Ma; McNeil, M. R. Determination That Cell Wall Galactofuran Synthesis Is Essential for Growth of Mycobacteria. *J. Bacteriol.* **2001**, *183*, 3991−3998.
(15) Scherman, M. S.; Winans, K.; Bertozzi, C. R.; Stern, R.; Jones, V. C.; McNeil, M. R. Drug Targeting *Mycobacterium tuberculosis* Cell Wall Synthesis: Development of a Microtiter Plate-Based Screen for UDP-Galactopyranose Mutase and Identification of an Inhibitor from a Uridine-Based Library. *Antimicrob. Agents Chemother.* **2003**, *47*, 378−382.
(16) Guay, D. R. An Update on the Role of Nitrofurans in the Management of Urinary Tract Infections. *Drugs* **2001**, *61*, 353−364.
(17) Murugasu-Oei, B.; Dick, T. Bactericidal Activity of Nitrofurans against Growing and Dormant Mycobacterium Bovis BCG. *J Antimicrob. Chemother.* **2000**, *46*, 917−919.
(18) Brooks, J. V.; Furney, S. K.; et al. Metronidazole Therapy in Mice Infected with Tuberculosis. *Antimicrob. Agents Chemother.* **1999**, *43*, 1285−1288.
(19) Stover, C. K.; Warrener, P.; VanDevanter, D. R.; Sherman, D. R.; Arain, T. M.; Langhorne, M. H.; Anderson, S. W.; Towell, J. A.; Yuan, Y.; McMurray, D. N.; Kreiswirth, B. N.; Barry, C. E.; Baker, W. R. A Small-Molecule Nitroimidazopyran Drug Candidate for the Treatment of Tuberculosis. *Nature* **2000**, *405*, 962−966.
(20) Choi, K. P.; Bair, T. B.; Bae, Y. M.; Daniels, L. Use of Transposon Tn5367 Mutagenesis and a Nitroimidazopyran-Based Selection System To Demonstrate a Requirement for fbiA and fbiB in Coenzyme F(420) Biosynthesis by *Mycobacterium Bovis* BCG. *J. Bacteriol.* **2001**, *183*, 7058−7066.
(21) Boger, D. L.; Dechantsreiter, M. A.; Ishii, T.; Fink, B. E.; Hedrick, M. P. Assessment of Solution-Phase Positional Scanning Libraries Based on Distamycin A for the Discovery of New Bioactive DNA Binding Agents and Developemnt of a Rapid, High-Throughput Screen for Determinating Relative DNA Binding Affinity or DNA Binding Sequence Selectivity. *J. Am. Chem. Soc.* **2000**, *122*, 6382−6394.
(22) Hart, D. J.; Li, J.; Wu, W.; Kozikowski, A. P. Applications of Organosulfur Chemistry to Organic Synthesis: Total Synthesis of (+)-Himbeline and (+)-Himbacine. *J. Org. Chem.* **1997**, *62*, 5023−5033.
(23) Cella, J. A. 5-Substituted-2-furoic Acids as Latent Dienes for the Preparation of Aryl Ethers and Thioethers via the Diels−Alder Reaction. *J. Org. Chem.* **1988**, *53*, 2099−2103.
(24) Saldabol, N. O.; Slavinska, V.; Popelis, J.; Mazeika, I. Synthesis and Conversion of 5-methyl-2-nitro-2-furanaldehyde. *Latv. Kim. Z.* **2000**, *3*, 73−77.
(25) Lee, R. E., Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M.; Barry, C. E., III. Combinatorial Lead Optimization of [1,2]-Diamines Based on Ethambutol as Potential Antituberculosis Preclinical Candidates. *J. Comb. Chem.* **2003**, *5*, 172−187.
(26) *Volsurf*; Tripos Inc. (1699 South Hanley Road, St. Louis, MO, 63144).
(27) Lenaerts, A. J.; Gruppo, V. J.; Brooks, V.; Orme I. M. Rapid in Vivo Screening of Experimental Drugs for Tuberculosis Using Gamma Interferon Gene-Disrupted Mice. *Antimicrob. Agents Chemother.* **2003**, *47*, 783−785.
(28) Kelly, B. P.; Furney, S. K.; Jessen, M. T.; Orme, I. M. Low-Dose Aerosol Infection Model for Testing Drugs for Efficacy against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **1996**, *40*, 2809−2812.
(29) Orme, I. Search for New Drugs for Treatment of Tuberculosis. Tuberculosis Drug Screening Program. *Antimicrob. Agent Chemother.* **2001**, *45*, 1943−1946.
(30) Lee, R. E.; Monsey, D.; Weston, A.; Duncan, K.; Rithner, C.; McNeil, M. R. Enzymatic Synthesis of UDP-Galactofuranose and Assays for UDP-Galactopyranosyl Mutase Based on HPLC and on Linked Enzymatic Production of NADH. *Anal. Biochem.* **1996**, *242*, 1−7.
(31) Weston, A.; Stern, R. J.; Lee, R. E.; Nassau, P. M.; Monsey, D.; Martin, S. L.; Scherman, M. S.; Besra, G. S.; Duncan, K.; McNeil, M. R. Biosynthetic Origin of Mycobacterial Cell Wall Galactofuranosyl Residues. *Tuber. Lung Dis.* **1998**, *78*, 123−131.
(32) Mangalindan, G. C.; Talaue, M. T.; Cruz, L. J.; Franzblau, S. G.; Adams, L. B.; Richardson, A. D.; Ireland, C. M.; Concepcion, G. P. Agelasine F from a Philippine Agelas sp. Sponge Exhibits in Vitro Antituberculosis Activity. *Planta Med.* **2000**, *66*, 364−365.

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