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Identification, Synthesis, and Pharmacological Evaluation of Tetrahydroindazole Based Ligands as Novel Antituberculosis Agents

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The resurgence of tuberculosis (TB), the incidence of drug-resistant strains of *Mycobacterium tuberculosis* (MTB), and the coinfection between TB and HIV have led to serious infections, high mortality, and a global health threat, resulting in the urgent search for new classes of antimycobacterial agents. Herein, we report the identification of a novel class of tetrahydroindazole based compounds as potent and unique inhibitors of MTB. Compounds **6a**, **6m**, and **6q** exhibited activity in the low micromolar range against replicating *Mycobacterium tuberculosis* (R-TB) phenotype, with minimum inhibitory concentrations (MICs) of 1.7, 1.9, and 1.9 μM, respectively, while showing no toxicity to Vero Ccells. Moreover, studies aimed to assess the in vitro metabolic stability of **6a** and **6m** in mouse liver microsomes and in vivo pharmacokinetic profiles in plasma levels gave satisfactory results. This research suggests that tetrahydroindazole based anti-TB compounds can serve as a promising lead scaffold in developing new drugs to combat tuberculosis infections.

Introduction

Tuberculosis (TB[a]) is an airborne infectious disease that often remains in its latent form. However, immunosuppressive diseases, such as AIDS, have pushed TB to rank among the top five deadliest diseases in the world, especially in developing countries. About one-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB), leading to an estimated 1.7 million deaths in 2006.[1] Moreover, the lack of health care, the coinfection between MTB and HIV (TB/HIV), and the incidence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis strains have further aggravated the mortality and spread of this disease. Drug-susceptible TB can be cured by using a 6-month treatment regimen encompassing a combination of three or four drugs, comprising isoniazid, rifampin (**1**), and pyrazinamide, with or without ethambutol (Figure 1). However, this long-term treatment is accompanied by challenges including the operational hurdles of ensuring uninterrupted drug availability and patient compliance for the full duration of therapy as well as toxicity of prolonged drug therapy. In addition, the emergence of MDR and XDR strains of MTB coupled with the fact that no new TB drug classes have been introduced over the past 40 years raises the realistic problem of essentially untreatable forms of TB spreading further in human populations.[2] Therefore, an immediate need exists for new anti-TB drugs that are able not only to shorten the long treatment regimen but also to counter drug resistant forms of TB and that can be used along with the current AIDS/HIV retroviral treatments.

To identify new chemical scaffolds for our TB drug design program, we screened a diverse 50 000 compounds library (NOVACore Chembridge) against MTB which led to the identification of a tetrahydroindazole scaffold (Figure 2) as one of the hit series.[3] Approximately 30 compounds with this scaffold were found to be active against replicating *Mycobacterium tuberculosis* (R-TB) using the microplate Alamar blue assay[4] (MABA) MICs ranging from 0.6 to 30 μM. In addition, most of these representative compounds did not exhibit cytotoxicity against Vero cells.[5] All members of this series of compounds are predicted to achieve oral bioavailability by passing Lipinski's "rule of 5" filters[6] and by applying Medchem I and II filters.[7] In light of these data, we thought that a concerted chemistry−biology effort should be made to improve upon the lead candidate, with the aim to refine this molecule for use in the treatment of TB. Compounds **6a** and **6e** with screening MICs of 0.6 and 1.0 μM, respectively, were identified to be the most potent compounds in this tetrahydroindazole series and served as the lead compounds for further structure−activity relationship (SAR) studies (Figure 2). Both **6a** and **6e** contain the *N*-aryltetrahydroindazole core linked to another aromatic heterocycle via an amide linker at position C4.

Herein, we report the synthesis of the tetrahydroindazole based analogues and their activities against MTB by using the MABA testing protocol. The selected compounds were further screened for cytotoxicity against Vero cells. The SAR

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[a] Abbreviations: MTB, *Mycobacterium tuberculosis*; TB, tuberculosis; R-TB: replicating *Mycobacterium tuberculosis*; MDR, multidrug-resistant; XDR, extensively drug-resistant; SAR, structure−activity relationship; MIC, minimum inhibitory concentration; IC_{50}, half maximal inhibitory concentration; MABA, microplate Alamar blue assay; HTS, high throughput screening; SI, selectivity index; $T_{1/2}$, half-life; CL'_{int}, intrinsic clearance; CL_h, hepatic plasma clearance; AUC, area under the curve.

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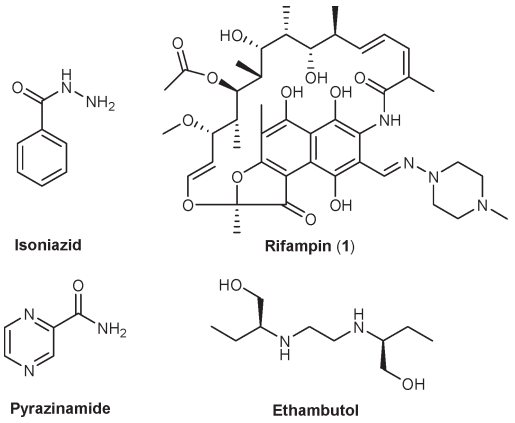


Figure 1. Regimen drugs for TB.

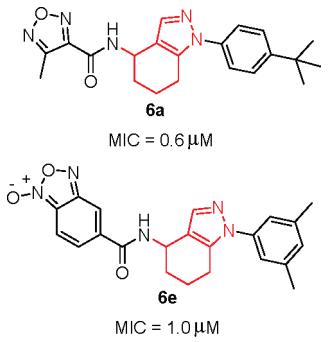


Figure 2. Lead structures from the high throughput screening (HTS) campaigns.

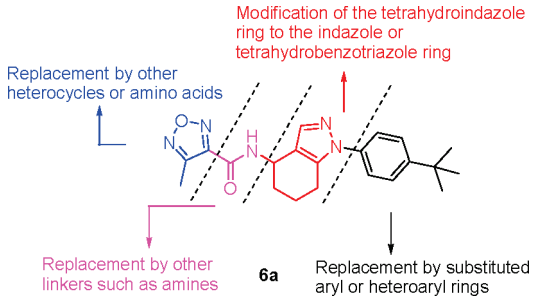


Figure 3. Strategies to modify the lead **6a**.

studies encompassed modifications of the furazan ring, the *N*-aryl group, the amide linker, and the tetrahydroindazole core (Figure 3). In addition, the liver microsomal stability and oral exposure of the two most potent compounds stemming from this series were characterized.

Chemistry

The synthetic routes used for the preparation of the hit compounds and their analogues are described in Scheme 1. 1,3-Cyclohexanedione (**2**) was treated with dimethylformamide−dimethylacetal (DMF−DMA) to give compound **3**, which was subjected to AcOH-catalyzed cyclocondensation with various monosubstituted hydrazines to afford the keto compounds **4a**−**k**.[8] Next reductive amination of **4a**−**k** gave 4-aminotetrahydroindazoles **5a**−**k**. The target compounds **6a**−**x** were prepared readily by an amide coupling of crude **5a**−**k** with appropriate carboxylic acids.

Compound **6u** was reduced to the amino derivatives **7** and **8** by palladium-catalyzed hydrogenation in DMF−acetone and DMF−EtOAc, respectively (Scheme 2). Compound **8** was also coupled with CF_3COOH, *t*-BuCOCl, or 4-methylfurazan-3-carboxylic acid to form the corresponding amides **9a**−**c**.

4-*tert*-Butylaniline (**10**) was converted into the corresponding azide **11**, which in turn was refluxed with 2-cyclohexen-1-one (**12**) in xylene to afford **13**. Next the intermediate **13** was converted by a reductive amination reaction to form intermediate **14**, which was further coupled with the 4-methylfurazan-3-carboxylic acid to give the final product **15** (Scheme 3).

Treatment of the intermediate **5a** with 3-bromomethyl-4-methylfurazan (**16**) in the presence of potassium carbonate in DMF afforded the final compound **17** as shown in Scheme 4.

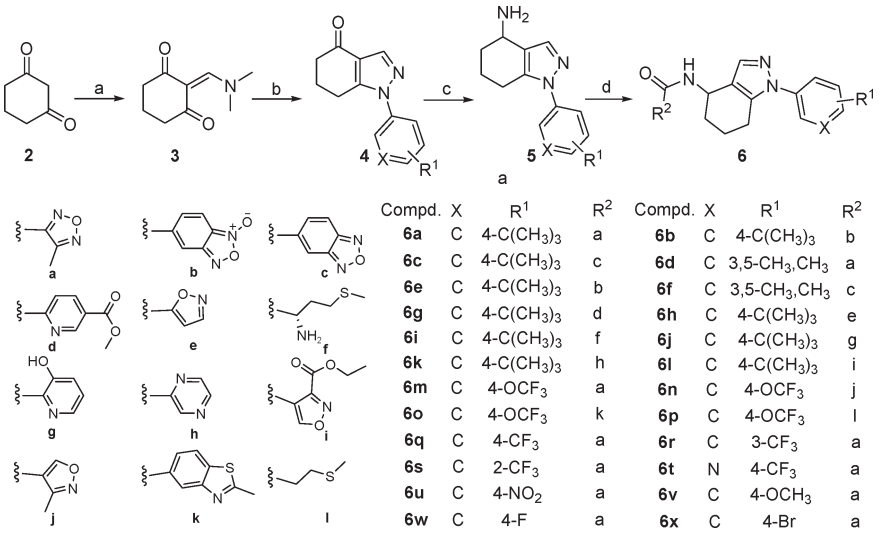
A further modification we investigated focused on the replacement of the tetrahydroindazole core scaffold with an aromatic indazole ring (**22a**−**d**, Scheme 5). Compounds **22a**−**d** were synthesized starting from 2-methyl-3-nitroaniline (**18**). Treatment of **18** with sodium nitrite gave 4-nitroindazole (**19**),[9] which in turn was coupled with aryl bromides to give **20a**,**b**.[10] Next, the nitro group in **20a**,**b** was reduced with Fe and NH_4Cl to give 4-aminoindazole intermediates **21a**,**b**. The final compounds **22a**−**d** were obtained by coupling **21a**,**b** with the appropriate carboxylic acids as described previously.

Results and Discussion

All of the synthesized derivatives were evaluated for their ability to inhibit the growth of MTB strain $H_{37}Rv$ by the MABA. The possible toxicity of the synthesized compounds was assessed in Vero cells. The anti-TB drug **1** was used as a reference compound. The lead compound **6a**, bearing a novel tetrahydroindazole scaffold, is first reported here. The synthesized compounds were found to cover a broad range of activity with MICs ranging from submicromolar to $> 128\,\mu M$ with some interesting SAR trends (Table 1). The activities of the newly resynthesized, lead compounds **6a** and **6e** were consistent with those determined in the original HTS assay, with MICs of 1.7 and 1.9 μM, respectively. Compounds **6a**, **6b**, **22a**, and **22b** were found to be the most active compounds in the series with MICs of 1.7, 1.0, 0.4, and 0.7 μM, respectively. However, compound **6b**, bearing a benzoxadiazole *N*-oxide moiety, and the indazole derivatives **22a** and **22b**, proved to have some toxicity in the Vero cell assay, with IC_{50} values of 29.0, 4.5, and 3.0 μM, respectively. The reduction of the *N*-oxide moiety of **6b** to the benzoxadiazole derivative **6c** (MIC $> 128\,\mu M$) led to a complete loss of activity. The same trend was also observed for derivatives **6e** and **6f**, which differed from the analogues **6b** and **6c**, respectively, by having a 3,5-dimethylphenyl group attached to the tetrahydroindazole nitrogen in place of the 4-*tert*-butylphenyl group. Somewhat unexpectedly, derivative **6c**, while showing a fairly good structural similarity to the lead **6a**, was completely devoid of activity.

The first stage of our chemical modifications focused on the *N*-aryl group of **6a**, as related analogues in the HTS library containing the same scaffold were found to show reasonably good MIC values and no cytotoxicity. We thus assembled a small library of analogues (**6a**, **6c**, **6d**, **6f**, **6m**, **6q**, **6r**−**x**, **7**, **8**, and **9a**−**c**) employing the chemistry methods discussed above. The sharp difference observed in activity between **6a** and **6d**

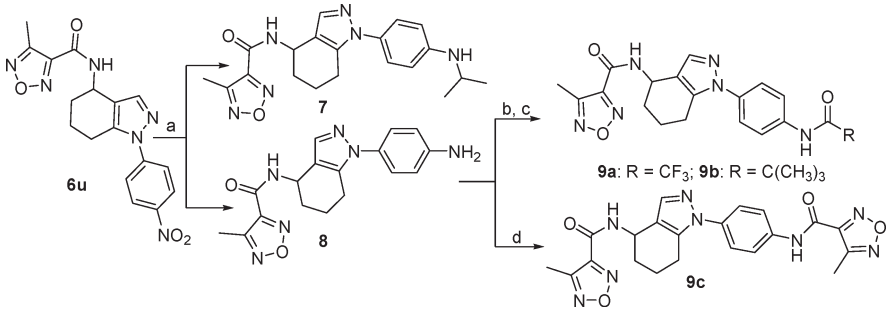
Scheme 1[a]



Compd.	X	R^1	R^2	Compd.	X	R^1	R^2
6a	C	4-$C(CH_3)_3$	a	**6b**	C	4-$C(CH_3)_3$	b
6c	C	4-$C(CH_3)_3$	c	**6d**	C	3,5-CH_3,CH_3	a
6e	C	4-$C(CH_3)_3$	b	**6f**	C	3,5-CH_3,CH_3	c
6g	C	4-$C(CH_3)_3$	d	**6h**	C	4-$C(CH_3)_3$	e
6i	C	4-$C(CH_3)_3$	f	**6j**	C	4-$C(CH_3)_3$	g
6k	C	4-$C(CH_3)_3$	h	**6l**	C	4-$C(CH_3)_3$	i
6m	C	4-OCF_3	a	**6n**	C	4-OCF_3	j
6o	C	4-OCF_3	k	**6p**	C	4-OCF_3	l
6q	C	4-CF_3	a	**6r**	C	3-CF_3	a
6s	C	2-CF_3	a	**6t**	N	4-CF_3	a
6u	C	4-NO_2	a	**6v**	C	4-OCH_3	a
6w	C	4-F	a	**6x**	C	4-Br	a

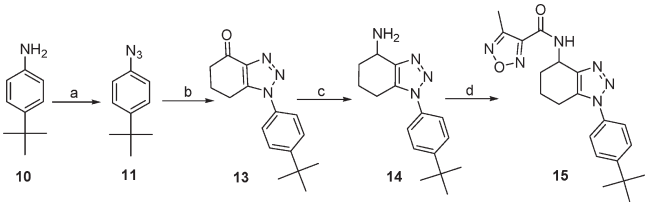
[a] Reagents and conditions: (a) DMF, DMA, reflux, 2 h; (b) $R^1ArNHNH_2$, MeOH, NaOH, AcOH, reflux, 3 h; (c) NH_4Ac, $NaBH_3CN$, 4 Å molecule sieves, 2-propanol, 75 °C, 1 day; (d) R^2COOH, EDC, HOBt, DMAP, CH_2Cl_2, room temp, overnight.

Scheme 2[a]



[a] Reagents and conditions: (a) Pd/C, H_2, DMF−acetone or DMF−EtOAc, room temp, overnight; (b) CF_3COOH, EDC, HOBt, DMAP, CH_2Cl_2, room temp, overnight; (c) *t*-BuCOCl, DMAP, pyridine, room temp, overnight; (d) 4-methylfurazan-3-carboxylic acid, EDC, HOBt, DMAP, CH_2Cl_2, room temp, overnight.

Scheme 3[a]



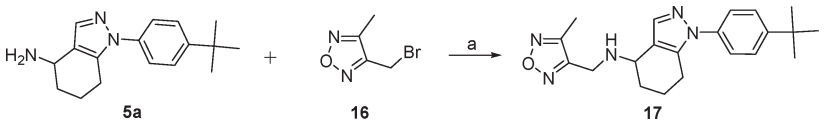
[a] Reagents and conditions: (a) (i) $NaNO_2$, HCl, H_2O; (ii) NaN_3, H_2O; 0 °C to room temp; (b) xylene, **12**, 145 °C, 2 h; (c) NH_4Ac, $NaBH_3CN$, 4 Å molecule sieves, 2-propanol, 75 °C, 1 day; (d) 4-methylfurazan-3-carboxylic acid, EDC, HOBt, DMAP, CH_2Cl_2, room temp, overnight.

led us to hypothesize that the presence of a para substituent on the *N*-aryl group, rather than ortho or meta substitution, might best improve upon the antimycobacterial potency. To test this hypothesis, compounds **6q**−**s** containing different trifluoromethyl substitution patterns were prepared. **6q**, with a *p*-trifluoromethyl group, had an MIC comparable to that of the lead **6a** (1.9 μM vs 1.7 μM), while showing a much better MIC than the meta- or ortho-analogues **6r**−**s**. In addition, replacement of the *p*-trifluoromethyl group by the *p*-trifluoromethoxy group did not affect the potency at all, for derivatives **6m** and **6q** had the same MIC. Moreover, both **6m** and **6q** were not toxic to Vero cells with $IC_{50} > 128$ μM. On the basis of these observations, several additional para subtituted derivatives (**6t**−**x**, **7**, **8**, and **9a**−**c**) were prepared and tested. Unfortunately, none of the analogues **6t**−**x**, the anilines **7** and 8, and the amides **9a**−**c** were found to possess comparable activity, which may be due to any of a number of possibilities. For example, they may not dock appropriately into the active site pocket of the yet unknown target or not efficiently permeate the cell wall of MTB.

The second set of structural modifications was performed keeping intact either the *N*-[1-(4-*tert*-butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]amide or *N*-[1-(4-trifluoromethoxylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]amide moiety while replacing the furazan ring with other heterocycles (**6g**−**l** and **6n**−**p**). Although compounds **6g**,**h**, **6j**−**l**, and **6n**−**p** failed to exhibit improved activity, the methionine amide **6i** showed an MIC of 4.8 μM, only 3-fold higher than **6a**. These preliminary results suggest that while the furazan is important for good anti-TB activity, it is not essential and can likely be replaced by simpler appendages.

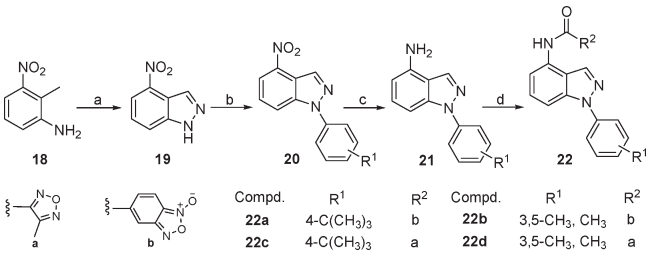
Some exploratory modifications of the amide linker and the tetrahydroindazole ring were also carried out. The replacement of the 4,5,6,7-tetrahyro-1*H*-indazole moiety of compound **6a** by a 4,5,6,7-terahyro-1*H*-benzotriazole to give compound **15** resulted in a 4.5-fold decrease in potency

Scheme 4[a]



[a] Reagents and conditions: (a) K_2CO_3, DMF, room temp, 2 h.

Scheme 5[a]



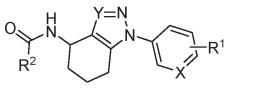
[a] Reagents and conditions: (a) $NaNO_2$, AcOH, room temp; (b) R^1ArBr, $Pd_2(dba)_3$, Xphos, *t*-BuOK, room temp; (c) Fe, NH_4Cl, 90 °C; (d) R^2COOH, BOP, DMAP, Et_3N, room temp.

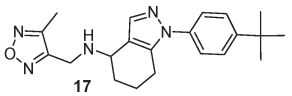
(MIC = 7.7 μM). The reduction of the amide **6a** led to the amine **17**, which showed a substantially reduced activity (MIC = 38.6 μM). The substitution of the tetrahydroindazole with the planar indazole (Table 2) resulted in derivatives **22a**, **b**, with increased activities (0.4 and 0.7 μM, respectively); however, their potential as drug candidates was limited by the toxicity in the Vero cell assay.

Metabolism and Pharmacokinetic Study

The in vivo pharmacokinetic profile of a compound is related to the potential for the drug to elicit the desired pharmacodynamic response and support the desired dose regimen. In vitro studies are usually conducted to predict the pharmacokinetic properties of a drug candidate, with the aim of rank ordering and prioritizing the compounds with the best potential for such desired pharmacokinetic profile. The oxidative hepatic clearance, one of the major contributors to the systemic clearance of the drug, may be predicted by deriving an intrinsic clearance from the linear rate of metabolism of the compound of interest in liver microsomes. The scaled intrinsic clearance, when compared to the liver plasma flow for the same species, predicts the extent for the potential of drug to undergo first pass hepatic metabolism followed by rapid systemic clearance. In vitro incubations in liver microsomes of the most promising compounds, **6a** and **6m**, resulted in in vitro half-lives ($T_{1/2}$) of 27 and 102 min, respectively (Table 3). The shorter in vitro half-life of **6a** (approximately 4-fold) was probably attributed to potential oxidation of the *tert*-butyl group (Figure 4). The in vivo intrinsic clearance (CL'_{int}) in hepatocytes, reflecting **6a** and **6m** depletion, was estimated by scaling up of the in vitro $T_{1/2}$,[11,12] while the hepatic plasma clearance (CL_h) was predicted using the non-restricted well-stirred liver model and with the assumption that rat protein binding was similar in blood and microsomes.[13] For **6a**, the predicted hepatic plasma clearance was approximately 70% of the mouse liver plasma flow. This value suggests that **6a** is likely to display borderline acceptable pharmacokinetic characteristics with a significant first pass hepatic metabolism extraction and high systemic clearance rate that could limit oral bioavailability.[12] For **6m**, however, the predicted plasma clearance was approximately 40% of the

Table 1. In Vitro Activity against MTB H37Rv

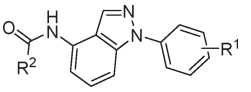




Compd.	Structure X	Y	R^1	R^2	MIC (μM) MABA[a,b]	IC_{50} (μM) Vero cells	SI (MABA)
1			–		0.06	122	2033
6a	C	C	4-$C(CH_3)_3$		1.7	> 128	> 75.3
6b	C	C	4-$C(CH_3)_3$		1.0	29.0	29.0
6c	C	C	4-$C(CH_3)_3$		> 128		
6d	C	C	3,5-CH_3, CH_3		>128		
6e	C	C	3,5-CH_3, CH_3		1.9		
6f	C	C	3,5-CH_3, CH_3		>128		
6g	C	C	4-$C(CH_3)_3$		7.3		
6h	C	C	4-$C(CH_3)_3$		123.9		
6i	C	C	4-$C(CH_3)_3$		4.8		
6j	C	C	4-$C(CH_3)_3$		26.9		
6k	C	C	4-$C(CH_3)_3$		57.7		
6l	C	C	4-$C(CH_3)_3$		> 128		
6m	C	C	4-OCF_3		1.9	> 128	> 67.4
6n	C	C	4-OCF_3		48.9		
6o	C	C	4-OCF_3		>128		
6p	C	C	4-OCF_3		88.3		
6q	C	C	4-CF_3		1.9	>128	> 67.4
6r	C	C	3-CF_3		15.4		
6s	C	C	2-CF_3		>128		
6t	N	C	4-CF_3		>128		
6u	C	C	4-NO_2		>128		
6v	C	C	4-OCH_3		>128		
6w	C	C	4-F		30.7		
6x	C	C	4-Br		64.6		
7	C	C	4-$NHCH(CH_3)_2$		>128		
8	C	C	4-NH_2		>128		
9a	C	C	4-$NHCOCF_3$		127.5		
9b	C	C	4-$NHCOC(CH_3)_3$		110.0		
9c	C	C	4-NHCO-(3-CH_3)-furazan		44.9		
15	C	N	4-$C(CH_3)_3$		7.7		
17			–		38.6		

[a] Microplate Alamar blue assay. [b] MTB strain $H_{37}Rv$.

Table 2. In Vitro activity against MTB H37Rv



Compd.	Structure		MIC (μM)	IC_{50} (μM)	SI
	R^1	R^2	MABA[a,b]	Vero cells	(MABA)
1	–		0.06	122	2033
22a	4-C$(CH_3)_3$		0.4	1.8	4.5
22b	3,5-CH_3, CH_3		0.7	16.1	23.0
22c	4-C$(CH_3)_3$		> 128		
22d	3,5-CH_3, CH_3		116.9		

[a] Microplate Alamar blue assay. [b] MTB strain H_{37}Rv.

Table 3. Metabolic Stability Parameters of **6a** and **6m** in Mouse Microsomes

compd	$T_{1/2}$ [a] (min)	CL'_{int} [b] ((mL/min)/kg)	CL_h [c] ((mL/min)/kg)
6a	27	200	34
6m	102	54	23

[a] Half-life. [b] Intrinsic clearance. [c] Hepatic plasma clearance.

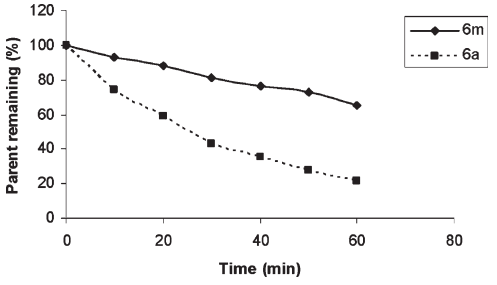


Figure 4. In vitro metabolism by mouse liver microsomes of **6a** and **6m**.

mouse liver plasma flow. This suggested a compound with better potential for acceptable pharmacokinetic characteristics, predicting moderate first pass hepatic extraction and prolongation of systemic clearance. That is, the calculated CL'_{int} and CL_h rates of **6m** suggest adequate oral bioavailability and sustained levels in plasma, both superior to those of **6a**.

In order to evaluate the in vivo pharmacokinetic profile of **6a** and **6m**, both compounds were administered per os to female Balb/c at the dosage of 50 mg/kg. The absorption was rapid for both derivatives, and the maximum concentration (C_{max}) in plasma was reached in 0.5 h after dosing. As predicted from the in vitro data, **6m** had overall higher systemic exposures. The C_{max} of **6m** was found to be 9.5 μM, which was 6.5-fold higher than that of **6a** (C_{max} = 1.5 μM), while the area under the curve (AUC) of **6m** (AUC = 53.5 μM·h) from 0 to 24 h was approximately 15-fold higher than that of **6a** (AUC = 3.6 μM·h) (Figure 5 and Table 4). Compared to the lead compound **6a**, the plasma concentration levels in mouse of the analogue **6m** were always higher than its MIC value (in vitro) for the 24 h period.

In summary, these results indicate that compound **6m** is metabolically more stable, resulting in higher systemic

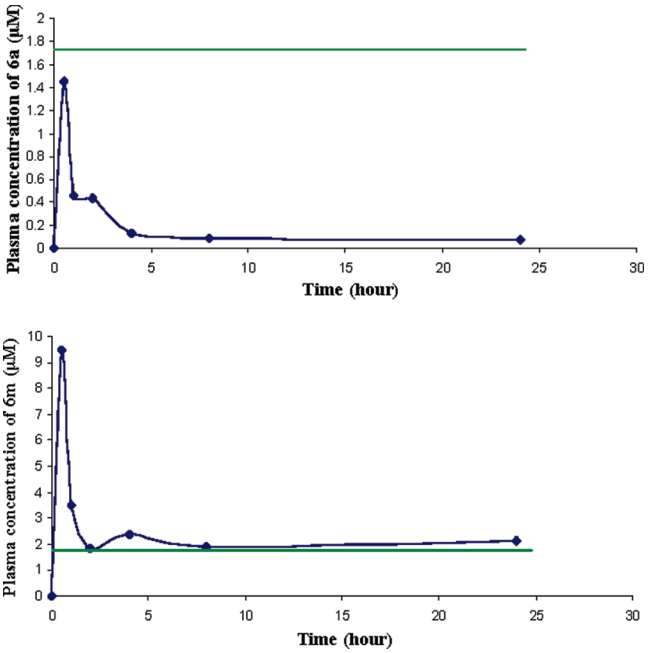


Figure 5. Pharmacokinetic properties of **6a** and **6m** in plasma levels. The straight green line marks the in vitro MIC level.

Table 4. Pharmacokinetic properties of **6a** and **6m**

compd	C_{max} [a] (μM)	T_{max} [b] (h)	AUC [c] (μM·h)
6a	1.5	0.5	3.6
6m	9.5	0.5	53.5

[a] Maximum concentration. [b] Time to reach C_{max}. [c] Area under the curve.

exposures in vivo than compound **6a**. Since both compounds possess comparable potency and the presence of protein does not limit the distribution of either compound to the bacterial cell, the improved metabolic stability and PK profile of **6m** should allow for longer drug coverage at lower doses possibly resulting in an increased safety margin.

Conclusion

A series of structurally unique tetrahydroindazole analogues were identified to be novel anti-TB agents through in

vitro MABA assays, with little toxicity being observed for a number of these compounds against Vero cells. Herein, we have developed a synthesis route to generate this novel chemical scaffold and to prepare a number of analogues in order to develop preliminary SAR information. We have found that a tetrahydroindazole core linked to a furazan ring via an amide linker at position C4 and bearing a para-substituted aryl group on the indazole nitrogen is optimal for activity. The most potent compounds in the series, **6a**, **6m**, and **6q**, exhibited micromolar activity against R-TB, with MABA MICs of 1.7, 1.9, and 1.9 μM, respectively. The compounds **6g**, **6i**, and **15** also displayed reasonably good activity against R-TB. In addition, these compounds failed to show any toxicity against Vero cells at 128 μM and therefore have a good selectivity index against the bacteria. The newly synthesized analogue **6m** is more druglike than the corresponding parent compound **6a** in terms of possessing better in vitro metabolic stability and in vivo pharmacokinetic characteristics. These data together suggest that tetrahydroindazole based compounds may represent promising lead candidates against TB that are worthy of further investigation.

Experimental Section

Chemistry. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer at 300/400 MHz and 75/100 MHz, respectively, with TMS as an internal standard. HRMS experiments were performed on Q-TOF-2TM (Micromass). TLC was performed with Merck 60 F254 silica gel plates. Preparative TLC was performed with Analtech 1000 μm silica gel GF plates. Column chromatography was performed using Merck silica gel (40−60 mesh). HPLC was carried out on an ACE AQ columns (100 mm × 4.6 mm or 250 mm × 10 mm), with detection at 210, 240, 254, 280, or 300 nm on a Shimadzu SPD-10A VP detector, flow rate of 2.0−3.5 mL/min, from 10% acetonitrile in water to 100% acetonitrile with 0.05% TFA. All purities are predicted to be greater than 95.0% by HPLC.

General Procedures for the Synthesis of Compounds 4a−k, 5a−k, 6a−x, 7, 8, 9a−c, 11, 13, 14, 15, 17, 19, 20a,b, 21a,b, and 22a−d. Method A. To a solution of 2-dimethylaminomethylenecyclohexane-1,3-dione (**3**) (1.67 g, 10.0 mmol) in methanol (60 mL) and water (10 mL) were added 4-*tert*-butylphenylhydrazine hydrochloride (2.0 g, 10.0 mmol) and sodium hydroxide (0.4 g, 10.0 mmol). The resulting mixture was heated at reflux for 2 h and concentrated under reduced pressure. Then to the residue were added AcOH (60 mL) and water (30 mL) and the corresponding mixture was heated to 110 °C for 1.5 h. On completion of the reaction (TLC, eluent hexanes/EtOAc, 1:1), the solution was concentrated under reduced pressure. The residue was diluted with EtOAc (100 mL) and washed thoroughly with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (hexanes/EtOAc, 1:1) of the crude mixture afforded **4a** (2.6 g, 96%).

Method B. A solution of **4a** (3.5 g, 13.0 mmol) in 2-propanol (200 mL) was treated, with vigorous stirring, with ammonium acetate (10.0 g, 130.0 mmol). After complete dissolution, molecular sieves (4 Å, 5.0 g) and $NaBH_3CN$ (4.1 g, 65.0 mmol) were added and the reaction mixture was stirred for 1 day at 70 °C. On completion of the reaction (TLC, eluent hexanes/EtOAc, 1:1), the solution was concentrated under reduced pressure. The residue was diluted with EtOAc (200 mL) and washed thoroughly with 2 M aqueous NaOH (20 mL) and saturated brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. Without further purification, crude compound **5a** was directly used in next step.

Method C. To a solution of 4-methylfurazan-3-carboxylic acid (1.3 g, 10.1 mmol), HOBt (2.1 g, 15.5 mmol), and EDC (3.0 g, 15.5 mmol) in CH_2Cl_2 (150 mL) were added **5a** (2.7 g, 10.1 mmol), DMAP (122.0 mg, 1.0 mmol), and molecular sieves (4 Å, 1.0 g). Then the resulting solution was stirred at room temperature overnight. On completion of the reaction (TLC, eluent hexanes/EtOAc, 1:1), the solution was concentrated under reduced pressure. The residue was diluted with EtOAc (100 mL) and washed thoroughly with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (hexanes/EtOAc, 2:1) of the crude mixture afforded **6a** (2.3 g, 61%).

Method D. To a solution of **6b** (50.0 mg, 0.12 mmol) in ethanol (5 mL) was added triethyl phosphite (99.6 mg, 0.6 mmol), and the resulting solution was heated at reflux overnight. On completion of the reaction (TLC, eluent hexanes/EtOAc, 2:1), the solution was concentrated under reduced pressure. Silica gel chromatography (hexanes/EtOAc, 2:1) of the crude mixture afforded **6c** (35.0 mg, 71%).

Method E. To a 10 mL round-bottom flask was added **19** (163.0 mg, 1.0 mmol), 1-bromo-4-*tert*-butylbenzene, tris[μ-[(1,2-η:4,5-η)-(1*E*,4*E*)-1,5-diphenyl-1,4-pentadien-3-one]]dipalladium (Pd_2dba_3, 22.8 mg, 0.25 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos, 42.4 mg, 0.1 mmol), and potassium *tert*-butoxide (177.2 mg, 1.5 mmol). The flask was equipped with a rubber septum sealed condenser and was degassed and filled with argon three times. Toluene (3 mL) was added, and the mixture was heated to 80 °C for 20 h. The resulting mixture was cooled to room temperature and was repartitioned between water (10 mL) and EtOAc (15 mL). The aqueous phase was washed with EtOAc (15 mL × 2). The combined organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (hexanes/EtOAc, 25:1) to afford **20a** as a yellow solid (139.0 mg, 47%).

Method F. To a mixture of **20a** (210.0 mg, 0.7 mmol), iron powder (846.0 mg, 10.5 mmol), and ammonium chloride (42.3 mg, 0.76 mmol) was added a mixture of ethanol and water (1:4, 15 mL). The resulting suspension was refluxed overnight and was allowed to cool to room temperature. The solvents were removed under reduced pressure. The residue was redissolved in 20% triethylamine in EtOAc (5 mL), filtered through a silica gel pad, and washed with 20% triethylamine in EtOAc (5 mL × 5). The combined filtrate was dried over Na_2SO_4 and concentrated to give crude **21a** as a gray oil (180.0 mg, 97%).

Method G. A mixture of **21a** (71.0 mg, 0.27 mmol), 1-oxybenzo[1,2,5]oxadiazole-5-carboxylic acid (99.0 mg, 0.54 mmol), benzotriazole-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 237.0 mg), and *N*,*N*-dimethylpyridin-4-amine (DMAP, 3.0 mg) was dissolved in dry dichloromethane (5 mL). Triethylamine (183 μL) was added, and the mixture was stirred under a N_2 atmosphere at room temperature overnight. The resulting mixture was diluted with dichloromethane (20 mL) and washed with 10% aqueous $KHSO_4$ (10 mL), saturated $NaHCO_3$ (10 mL), and brine (10 mL). The organic phase was dried over Na_2SO_4 and concentrated, and the residue was purified by column chromatography on silica gel (hexanes/EtOAc, 5:1) to afford **22a** as a yellow solid (24.0 mg, 21%).

1-(4-*tert*-Butylphenyl)-1,5,6,7-tetrahydroindazol-4-one (4a). 4a was synthesized by method A by using 4-*tert*-butylphenylhydrazine hydrochloride as a starting material. Yield 96%. ^{1}H NMR ($CDCl_3$) δ 8.07 (s, 1H), 7.52 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 2.98 (t, J = 6.4 Hz, 2H), 2.56 (t, J = 6.4 Hz, 2H), 2.30−2.05 (m, 2H), 1.37 (s, 9H).

1-(3,5-Dimethylphenyl)-1,5,6,7-tetrahydroindazol-4-one (4b). 4b was synthesized by method A by using 3,5-dimethylphenylhydrazine hydrochloride as a starting material. Yield 86%. ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H), 7.12 (s, 2H), 7.07 (s, 1H), 2.98 (t, J = 6.4 Hz, 2H), 2.56 (t, J = 6.4 Hz, 2H), 2.40 (s, 6H), 2.25−2.05 (m, 2H).

1-(4-Trifluoromethoxylphenyl)-1,5,6,7-tetrahydroindazol-4-one (4c). 4c was synthesized by method A by using 4-trifluoromethoxylphenylhydrazine hydrochloride as a starting material. Yield 93%. ^{1}H NMR ($CDCl_3$) δ 8.09 (s, 1H), 7.65−7.50

(m, 2H), 7.38 (d, J = 8.0 Hz, 2H), 3.00 (t, J = 6.4 Hz, 2H), 2.57 (t, J = 6.4 Hz, 2H), 2.28−2.12 (m, 2H).

1-(4-Trifluoromethylphenyl)-1,5,6,7-tetrahydroindazol-4-one (4d). 4d was synthesized by method A by using 4-trifluoromethylphenylhydrazine hydrochloride as a starting material. Yield 64%. ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 3.09 (t, J = 6.4 Hz, 2H), 2.52 (t, J = 6.4 Hz, 2H), 2.28−2.12 (m, 2H).

1-(3-Trifluoromethylphenyl)-1,5,6,7-tetrahydroindazol-4-one (4e). 4e was synthesized by method A by using 3-trifluoromethylphenylhydrazine hydrochloride as a starting material. Yield 100%. ^{1}H NMR ($CDCl_3$) δ 8.11 (s, 1H), 7.83 (s, 1H), 7.80−7.60 (m, 3H), 3.03 (t, J = 6.4 Hz, 2H), 2.58 (t, J = 6.4 Hz, 2H), 2.30−2.15 (m, 2H).

1-(2-Trifluoromethylphenyl)-1,5,6,7-tetrahydroindazol-4-one (4f). 4f was synthesized by method A by using 2-trifluoromethylphenylhydrazine hydrochloride as a starting material. Yield 48%. ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H), 7.95−7.85 (m, 1H), 7.78−7.66 (m, 2H), 7.46−7.41 (m, 1H), 2.63 (t, J = 6.4 Hz, 2H), 2.55 (t, J = 6.4 Hz, 2H), 2.22−2.12 (m, 2H).

1-(5-Trifluoromethylpyridin-2-yl)-1,5,6,7-tetrahydroindazol-4-one (4g). 4g was synthesized by method A by using 5-trifluoromethylpyridin-2-ylhydrazine hydrochloride as a starting material. Yield 48%. ^{1}H NMR ($CDCl_3$) δ 8.74 (s, 1H), 8.24−8.13 (m, 1H), 8.13−8.03 (m, 2H), 3.51 (t, J = 6.4 Hz, 2H), 2.57 (t, J = 6.4 Hz, 2H), 2.30−2.15 (m, 2H).

1-(4-Nitrophenyl)-1,5,6,7-tetrahydroindazol-4-one (4h). 4h was synthesized by method A by using 4-nitrophenylhydrazine hydrochloride as a starting material. Yield 38%. ^{1}H NMR ($CDCl_3$) δ 8.41 (d, J = 8.8 Hz, 2H), 8.15 (s, 1H), 7.78 (d, J = 8.8 Hz, 2H), 3.10 (t, J = 6.4 Hz, 2H), 2.61 (t, J = 6.4 Hz, 2H), 2.35−2.15 (m, 2H).

1-(4-Methoxylphenyl)-1,5,6,7-tetrahydroindazol-4-one (4i). 4i was synthesized by method A by using 4-methoxyphenylhydrazine hydrochloride as a starting material. Yield 80%. ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H), 7.43 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 3.89 (s, 3H), 2.93 (t, J = 6.4 Hz, 2H), 2.56 (t, J = 6.4 Hz, 2H), 2.30−2.10 (m, 2H).

1-(4-Fluorophenyl)-1,5,6,7-tetrahydroindazol-4-one (4j). 4j was synthesized by method A by using 4-fluorophenylhydrazine hydrochloride as a starting material. Yield 76%. ^{1}H NMR ($CDCl_3$) δ 8.07 (s, 1H), 7.55−7.45 (m, 2H), 7.30−7.15 (m, 2H), 2.95 (t, J = 6.0 Hz, 2H), 2.57 (t, J = 6.0 Hz, 2H), 2.27−2.12 (m, 2H).

1-(4-Bromophenyl)-1,5,6,7-tetrahydroindazol-4-one (4k). 4k was synthesized by method A by using 4-bromophenylhydrazine hydrochloride as a starting material. Yield 84%. ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H), 7.65 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.8 Hz, 2H), 2.98 (t, J = 6.0 Hz, 2H), 2.57 (t, J = 6.0 Hz, 2H), 2.28−2.15 (m, 2H).

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6a). 6a** was synthesized by method C by using crude **5a** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 61%. ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H), 7.48 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 8.8 Hz, 2H), 7.04 (d, J = 7.2 Hz, 1H), 5.44−5.22 (m, 1H), 3.00−2.54 (m, 2H), 2.67 (s, 3H), 2.26−2.04 (m, 1H), 2.04−1.82 (m, 3H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 156.4, 151.4, 150.1, 147.8, 139.3, 137.8, 136.6, 125.7, 122.6, 116.7, 42.5, 34.3, 30.9, 29.3, 22.7, 19.7, 8.8; HRMS (ESI) calculated for $C_{21}H_{25}N_5O_2$ [M + H]$^+$ 380.2081, found 380.2072. HPLC purity: 99.2%.

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-2,1,3-benzoxadiazole-5-carboxamide, 1-Oxide (6b). 6b** was synthesized by method C by using crude **5a** and 1-oxybenzo[1,2,5]-oxadiazole-5-carboxylic acid as starting materials. Two-step overall yield 55%. ^{1}H NMR ($CDCl_3$) δ 8.05−7.60 (m, 2H), 7.60−7.45 (m, 1H), 7.56 (s, 1H), 7.45−7.35 (m, 2H), 7.35−7.25 (m, 2H), 7.25−7.10 (m, 1H), 5.35−5.20 (m, 1H), 2.83−2.57 (m, 2H), 2.23−2.05 (m, 1H), 2.00−1.75 (m, 3H), 1.33 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 164.3 (br), 152.6 (br), 150.6, 139.9, 138.1, 136.7, 135.0 (br), 131.5 (br), 126.1, 122.8, 118.2 (br), 117.7, 114.2 (br), 112.5 (br), 43.5, 34.6, 31.3, 29.7, 23.1, 20.3; HRMS (ESI) calculated for $C_{24}H_{25}N_5O_3$ [M + H]$^+$ 432.2030, found 432.2017. HPLC purity: 98.7%.

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-2,1,3-benzoxadiazole-5-carboxamide (6c). 6c** was synthesized by method D by using **6b** as a starting material. Yield 71%. ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H), 8.00−7.82 (m, 2H), 7.63 (s, 1H), 7.47 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 8.8 Hz, 2H), 6.57 (d, J = 6.8 Hz, 1H), 5.40−5.30 (m, 1H), 2.90−2.60 (m, 2H), 2.25−2.05 (m, 1H), 2.05−1.75 (m, 3H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 164.4, 150.2, 148.9, 148.4, 139.4, 137.7, 137.6, 137.6, 130.0, 125.7, 122.5, 117.2, 116.8, 115.2, 43.1, 34.3, 30.9, 29.4, 22.8, 19.8; HRMS (ESI) calculated for $C_{24}H_{25}N_5O_2$ [M + H]$^+$ 416.2081, found 416.2067. HPLC purity: 97.2%.

***N*-[1-(3,5-Dimethylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6d). 6d** was synthesized by method C by using crude **5b** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 61%. ^{1}H NMR ($CDCl_3$) δ 7.58 (s, 1H), 7.25 (d, J = 7.2 Hz, 2H), 7.05 (s, 2H), 6.98 (s, 1H), 5.35−5.20 (m, 1H), 2.85−2.55 (m, 2H), 2.64 (s, 3H), 2.35 (s, 6H), 2.20−2.00 (m, 1H), 2.00−1.80 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.5, 151.4, 147.8, 139.5, 138.8, 138.6, 137.7, 128.8, 120.9, 116.8, 42.5, 29.3, 22.6, 20.8, 19.7, 8.7; HRMS (ESI) calculated for $C_{19}H_{21}N_5O_2$ [M+H]$^+$ 352.1768, found 352.1759. HPLC purity: 95.9%.

***N*-[1-(3,5-Dimethylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-2,1,3-benzoxadiazole-5-carboxamide, 1-Oxide (6e). 6e** was synthesized by method C by using crude **5b** and 1-oxybenzo[1,2,5]-oxadiazole-5-carboxylic acid as starting materials. Two-step overall yield 77%. ^{1}H NMR ($CDCl_3$) 8.10−7.60 (m, 2H), 7.76 (d, J = 8.0 Hz, 1H), 7.60−7.40 (m, 1H), 7.58 (s, 1H), 7.04 (s, 2H), 7.00 (s, 1H), 5.30−5.10 (m, 1H), 2.80−2.55 (m, 2H), 2.31 (s, 6H), 2.20−2.00 (m, 1H), 2.00−1.70 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 164.3 (br), 152.6 (br), 139.6, 138.6, 137.6, 135.0 (br), 131.5 (br), 128.8, 120.8, 117.5 (br), 117.4, 114.2 (br), 112.5 (br), 43.0, 29.2, 22.6, 20.7, 19.8; HRMS (ESI) calculated for $C_{22}H_{21}N_5O_3$ [M + H]$^+$ 404.1717, found 404.1711. HPLC purity: 96.1%.

***N*-[1-(3,5-Dimethylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-2,1,3-benzoxadiazole-5-carboxamide (6f). 6f** was synthesized by method D by using **6e** as a starting material. Yield 80%. ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 1H), 7.95−7.75 (m, 2H), 7.68 (d, J = 7.6 Hz, 1H), 7.54 (s, 1H), 6.97 (s, 2H), 6.93 (s, 1H), 5.35−5.15 (m, 1H), 2.80−2.55 (m, 2H), 2.30 (s, 6H), 2.20−2.00 (m, 1H), 2.00−1.70 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 165.0, 148.8, 148.4, 139.6, 138.7, 138.6, 137.7, 137.5, 130.3, 128.8, 120.8, 117.4, 116.3, 115.4, 43.1, 29.3, 22.7, 20.8, 19.9; HRMS (ESI) calculated for $C_{22}H_{21}N_5O_2$ [M + H]$^+$ 388.1768, found 388.1761. HPLC purity: 95.2%.

6-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl-carbamoyl]nicotinic Acid Methyl Ester (6g). 6g was synthesized by method C by using crude **5a** and pyridine-2,5-dicarboxylic acid 5-methyl ester as starting materials. Two-step overall yield 56%. ^{1}H NMR ($CDCl_3$) δ 9.15−9.05 (m, 1H), 8.55−8.40 (m, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.30−8.15 (m, 1H), 7.63 (s, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.8 Hz, 2H), 5.45−5.25 (m, 1H), 3.97 (s, 3H), 2.90−2.60 (m, 2H), 2.25−2.05 (m, 1H), 2.05−1.75 (m, 3H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 164.7, 162.3, 152.5, 149.9, 148.9, 139.2, 138.2, 138.0, 136.7, 127.6, 125.7, 122.6, 121.5, 117.7, 52.3, 42.3, 34.3, 30.9, 29.6, 22.8, 20.0; HRMS (ESI) calculated for $C_{25}H_{28}N_4O_3$ [M + H]$^+$ 433.2234, found 433.2227. HPLC purity: 97.5%.

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-isoxazole-5-carboxamide (6h). 6h** was synthesized by method C by using crude **5a** and isoxazole-5-carboxylic acid as starting materials. Two-step overall yield 32%. ^{1}H NMR ($CDCl_3$) δ 8.33 (d, J = 1.6 Hz, 1H), 7.63 (s, 1H), 7.48 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 1.6 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 5.40−5.25 (m, 1H), 2.90−2.65 (m, 2H), 2.25−2.05 (m, 1H), 2.00−1.80 (m, 3H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.5, 154.7, 150.7, 150.1, 139.3, 137.9, 136.7, 125.7, 122.6, 116.9, 106.1, 42.4, 34.3, 30.9, 29.3, 22.7, 19.7; HRMS (ESI) calculated

for $C_{21}H_{24}N_4O_2$ $[M - H]^-$ 363.1826, found 363.1837. HPLC purity: 96.7%.

(*S*)-2-Amino-*N*-[1-(4-*tert*-butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylsulfanylbutyramide Trifluoroacetate (6i). 6i was synthesized by method C by using crude **5a** and Boc-Met-OH as starting materials and then treated with TFA. Three-step overall yield 71%. ^{1}H NMR ($CDCl_3$) δ 8.90−8.35 (m, 4H), 8.35−8.20 (m, 1H), 8.10−7.95 (m, 1H), 7.61 (s, 1H), 7.60 (s, 1H), 7.50−7.35 (m, 4H), 7.30−7.10 (m, 4H), 5.10−4.90 (m, 2H), 4.30−4.05 (m, 2H), 2.80−2.35 (m, 8H), 2.25−2.05 (m, 4H), 2.05−1.80 (m, 8H), 1.85−1.50 (m, 4H), 1.32 (s, 9H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.1, 168.0, 162.0, 161.7, 151.0, 150.5, 140.5, 140.0, 137.9, 137.8, 136.5, 136.0, 126.2, 126.1, 123.1, 122.8, 117.9, 117.7, 53.1, 52.9, 43.1, 43.0, 34.7, 34.6, 31.2, 30.5, 30.3, 29.3, 29.2, 29.1, 22.9, 22.7, 20.3, 20.2, 14.8, 14.8; HRMS (ESI) calculated for $C_{22}H_{32}N_4OS$ $[M + H]^+$ 401.2370, found 401.2369. HPLC purity: 95.6%.

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-3-hydroxypyridine-2-carboxamide (6j). 6j** was synthesized by method C by using crude **5a** and 3-hydroxypyridine-2-carboxylic acid as starting materials. Two-step overall yield 36%. ^{1}H NMR ($CDCl_3$) δ 12.25 (s, 1H), 8.21 (d, J = 7.2 Hz, 1H), 8.10−7.95 (m, 1H), 7.66 (s, 1H), 7.49 (d, J = 7.2 Hz, 2H), 7.42 (d, J = 7.2 Hz, 2H), 7.40−7.25 (m, 2H), 5.40−5.25 (m, 1H), 2.90−2.65 (m, 2H), 2.25−2.10 (m, 1H), 2.05−1.85 (m, 3H), 1.37 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.2, 157.9, 150.4, 139.6, 139.5, 138.4, 137.1, 131.5, 128.6, 126.1, 126.1, 123.0, 117.7, 42.2, 34.6, 31.3, 29.9, 23.2, 20.3; HRMS (ESI) calculated for $C_{23}H_{26}N_4O_2$ $[M + H]^+$ 391.2129, found 391.2136. HPLC purity: 95.0%.

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-pyrazine-2-carboxamide (6k). 6k** was synthesized by method C by using crude **5a** and pyrazine-2-carboxylic acid as starting materials. Two-step overall yield 71%. ^{1}H NMR ($CDCl_3$) δ 9.55−9.40 (m, 1H), 8.80−8.65 (m, 1H), 8.55−8.40 (m, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.62 (s, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 5.45−5.25 (m, 1H), 2.90−2.65 (m, 2H), 2.25−2.10 (m, 1H), 2.05−1.80 (m, 3H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.0, 150.0, 146.9, 144.1, 144.1, 142.1, 139.2, 137.9, 136.7, 125.7, 122.5, 117.6, 42.2, 34.3, 30.9, 29.6, 22.8, 19.9; HRMS (ESI) calculated for $C_{22}H_{25}N_5O$ $[M + H]^+$ 376.2132, found 376.2131. HPLC purity: 95.9%.

5-[1-(4-*tert*-Butylphenyl)-*1H*-4,5,6,7-tetrahydroindazol-4-yl-carbamoyl]isoxazole-3-carboxylic Acid Ethyl Ester (6l). 6l was synthesized by method C by using crude **5a** and isoxazole-3,5-dicarboxylic acid 3-ethyl ester as starting materials. Two-step overall yield 55%. ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.30 (s, 1H), 6.92 (d, J = 8.0 Hz, 1H), 5.40−5.25 (m, 1H), 4.47 (q, J = 6.8 Hz, 2H), 2.90−2.65 (m, 2H), 2.25−2.05 (m, 1H), 2.00−1.80 (m, 3H), 1.43 (t, J = 6.8 Hz, 3H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 164.9, 159.0, 157.2, 154.4, 150.5, 139.7, 138.2, 137.0, 126.1, 122.9, 117.1, 107.5, 62.6, 43.0, 34.6, 31.3, 29.6, 23.1, 20.1, 14.1; HRMS (ESI) calculated for $C_{24}H_{28}N_4O_4$ $[M - H]^-$ 435.2038, found 435.2058. HPLC purity: 96.8%.

***N*-[1-(4-Trifluoromethoxylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6m). 6m** was synthesized by method C by using crude **5c** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 76%. ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.10−6.90 (m, 1H), 5.45−5.20 (m, 1H), 2.95−2.60 (m, 2H), 2.70 (s, 3H), 2.30−2.05 (m, 1H), 2.05−1.80 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.5, 151.4, 147.7, 147.5, 139.5, 138.5, 137.7, 124.2, 121.4, 120.0 (q, J = 256 Hz), 117.6, 42.3, 29.2, 22.8, 19.7, 8.7; HRMS (ESI) calculated for $C_{18}H_{16}F_3N_5O_3$ $[M + H]^+$ 408.1278, found 408.1280. HPLC purity: 98.0%.

***N*-[1-(4-Trifluoromethoxylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-3-methylisoxazole-4-carboxamide (6n). 6n** was synthesized by method C by using crude **5c** and 3-methylisoxazole-4-carboxylic acid as starting materials. Two-step overall yield 81%. ^{1}H NMR ($CDCl_3$) δ 8.71 (s, 1H), 7.55 (s, 1H), 7.45 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 8.8 Hz, 2H), 6.57 (d, J = 7.2 Hz, 1H), 5.25−5.10 (m, 1H), 2.80−2.60 (m, 2H), 2.45 (s, 3H), 2.20−2.00 (m, 1H), 1.95−1.75 (m, 2H), 1.75−1.55 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 160.0, 158.1, 158.0, 147.4, 139.4, 138.4, 137.5, 123.8, 121.4, 121.3, 120.0 (q, J = 256 Hz), 118.7, 118.3, 115.7, 42.3, 29.4, 22.8, 20.0, 10.4; HRMS (ESI) calculated for $C_{19}H_{17}F_3N_4O_3$ $[M + H]^+$ 407.1326, found 407.1322. HPLC purity: 99.3%.

***N*-[1-(4-Trifluoromethoxylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-2-methylbenzothiazole-5-carboxamide (6o). 6o** was synthesized by method C by using crude **5c** and 2-methylbenzothiazole-5-carboxylic acid as starting materials. Two-step overall yield 36%. ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.95−7.80 (m, 2H), 7.69 (s, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H), 6.57 (d, J = 7.2 Hz, 1H), 5.45−5.25 (m, 1H), 2.85 (s, 3H), 2.95−2.60 (m, 2H), 2.30−2.05 (m, 1H), 2.05−1.75 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 168.7, 166.6, 153.1, 147.8, 139.8, 139.0, 138.1, 132.7, 124.6, 124.4, 123.6, 121.7, 121.6, 120.6, 120.3 (q, J = 256 Hz), 119.1 43.0, 29.8, 23.3, 20.3, 20.2; HRMS (ESI) calculated for $C_{23}H_{19}F_3N_4O_2S$ $[M - H]^-$ 471.1108, found 471.1128. HPLC purity: 96.0%.

***N*-[1-(4-Trifluoromethoxylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-3-methylsulfanylpropionamide (6p). 6p** was synthesized by method C by using crude **5c** and 3-methylsulfanylpropionic acid as starting materials. Two-step overall yield 67%. ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 1H), 7.52 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 8.8 Hz, 2H), 6.00 (d, J = 7.2 Hz, 1H), 5.25−5.05 (m, 1H), 2.84 (t, J = 7.2 Hz, 2H), 2.85−2.65 (m, 2H), 2.50 (t, J = 7.2 Hz, 2H), 2.15 (s, 3H), 2.20−2.00 (m, 1H), 2.00−1.80 (m, 2H), 1.80−1.65 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 170.6, 147.7, 139.6, 138.9, 138.1, 124.3, 121.7, 119.2, 42.5, 36.5, 30.0, 29.8, 23.3, 20.3, 15.7; HRMS (ESI) calculated for $C_{18}H_{20}F_3N_3O_2S$ $[M - H]^-$ 398.1156, found 398.1158. HPLC purity: 99.8%.

***N*-[1-(4-Trifluoromethylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6q). 6q** was synthesized by method C by using crude **5d** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 30%. ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 8.4 Hz, 2H), 7.68 (s, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.0 Hz, 1H), 5.40−5.25 (m, 1H), 3.00−2.75 (m, 2H), 2.67 (s, 3H), 2.25−2.10 (m, 1H), 2.05−1.85 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.5, 151.4, 147.7, 142.0, 139.6, 139.0, 128.6 (q, J = 33 Hz), 126.1 (q, J = 4 Hz), 123.4 (q, J = 270 Hz), 122.5, 118.1, 42.3, 29.1, 23.1, 19.8, 8.7; HRMS (ESI) calculated for $C_{18}H_{16}F_3N_5O_2$ $[M + H]^+$ 392.1329, found 392.1326. HPLC purity: 98.9%.

***N*-[1-(3-Trifluoromethylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6r). 6r** was synthesized by method C by using crude **5e** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 62%. ^{1}H NMR ($CDCl_3$) δ 7.85−7.75 (m, 1H), 7.75−7.64 (m, 1H), 7.65 (s, 1H), 7.62−7.55 (m, 2H), 7.07 (d, J = 7.6 Hz, 1H), 5.40−5.20 (m, 1H), 2.90−2.70 (m, 2H), 2.64 (s, 3H), 2.25−2.05 (m, 1H), 2.05−1.80 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.5, 151.4, 147.7, 139.6, 139.5, 138.8, 131.4 (q, J = 33 Hz), 129.5, 125.7, 123.4 (q, J = 4 Hz), 123.2 (q, J = 270 Hz), 119.6 (q, J = 33 Hz), 118.0, 42.3, 29.1, 22.8, 19.7, 8.7; HRMS (ESI) calculated for $C_{18}H_{16}F_3N_5O_2$ $[M + H]^+$ 392.1329, found 392.1328. HPLC purity: 95.7%.

***N*-[1-(2-Trifluoromethylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6s). 6s** was synthesized by method C by using crude **5f** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 59%. ^{1}H NMR ($CDCl_3$) δ 7.85−7.75 (m, 1H), 7.75−7.55 (m, 2H), 7.60 (s, 1H), 7.40−7.30 (m, 1H), 7.08 (d, J = 8.0 Hz, 1H), 5.40−5.20 (m, 1H), 2.63 (s, 3H), 2.50−2.28 (m, 2H), 2.20−2.00 (m, 1H), 1.95−1.75 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.5, 151.4, 147.8, 141.7, 137.8, 136.5 (q, J = 2 Hz), 132.4, 129.7, 129.4, 127.8 (q, J = 31 Hz), 127.1 (q, J = 5 Hz), 122.4 (q, J = 272 Hz), 115.9, 42.4, 29.4, 20.8, 19.4, 8.7; HRMS (ESI) calculated for $C_{18}H_{16}F_3N_5O_2$ $[M + H]^+$ 392.1329, found 392.1319. HPLC purity: 98.9%.

***N*-[1-(5-Trifluoromethylpyridin-2-yl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6t). 6t** was synthesized by method C by using crude **5g** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 45%. ^{1}H NMR ($CDCl_3$) δ 8.66 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.67 (s, 1H), 7.05 (d, J = 7.2 Hz, 1H), 5.40−5.20 (m, 1H), 3.40−3.10 (m, 2H), 2.65 (s, 3H), 2.25−2.05 (m, 1H), 2.05−1.80 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.5, 155.1, 151.4, 147.7, 144.6 (q, J = 4 Hz), 142.0, 140.0, 135.3 (q, J = 4 Hz), 123.2 (q, J = 32 Hz), 123.1 (q, J = 270 Hz), 119.4, 113.8, 42.3, 29.0, 24.9, 19.6, 8.7; HRMS (ESI) calculated for $C_{17}H_{15}F_3N_6O_2$ $[M - H]^-$ 391.1136, found 391.1155. HPLC purity: 99.9%.

***N*-[1-(4-Nitrophenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6u). 6u** was synthesized by method C by using crude **5h** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 37%. ^{1}H NMR ($CDCl_3$) δ 8.36 (d, J = 8.8 Hz, 2H), 7.80−7.70 (m, 3H), 7.01 (d, J = 8.0 Hz, 1H), 5.40−5.35 (m, 1H), 3.00−2.80 (m, 2H), 2.68 (s, 3H), 2.25−2.10 (m, 1H), 2.10−1.90 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.9, 151.8, 148.1, 145.8, 144.5, 140.3, 140.2, 125.0, 122.4, 119.4, 42.6, 29.4, 23.9, 20.2, 9.1; HRMS (ESI) calculated for $C_{17}H_{16}N_6O_4$ $[M - H]^-$ 367.1160, found 367.1177. HPLC purity: 99.8%.

***N*-[1-(4-Methoxylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6v). 6v** was synthesized by method C by using crude **5i** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 45%. ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.10−6.95 (m, 3H), 5.40−5.25 (m, 1H), 3.87 (s, 3H), 2.80−2.60 (m, 2H), 2.68 (s, 3H), 2.20−2.08 (m, 1H), 2.00−1.85 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 158.8, 156.8, 151.8, 148.2, 139.7, 137.9, 132.7, 125.0, 116.9, 114.3, 55.6, 42.9, 29.7, 22.8, 20.0, 9.1; HRMS (ESI) calculated for $C_{18}H_{19}N_5O_3$ $[M + H]^+$ 354.1561, found 354.1561. HPLC purity: 99.3%.

***N*-[1-(4-Fluorophenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6w). 6w** was synthesized by method C by using crude **5j** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 62%. ^{1}H NMR ($CDCl_3$) δ 7.60 (s, 1H), 7.50−7.40 (m, 2H), 7.20−7.10 (m, 2H), 7.06 (d, J = 7.2 Hz, 1H), 5.35−5.25 (m, 1H), 2.85−2.65 (m, 2H), 2.65 (s, 3H), 2.20−2.05 (m, 1H), 2.00−1.80 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 161.6 (d, J = 246 Hz), 156.8, 151.8, 148.1, 139.8, 138.4, 135.7 (d, J = 3 Hz), 125.2 (d, J = 9 Hz), 117.5, 116.1 (d, J = 22 Hz), 42.8, 29.6, 22.9, 20.1, 9.1; HRMS (ESI) calculated for $C_{17}H_{16}FN_5O_2$ $[M - H]^-$ 340.1215, found 340.1231. HPLC purity: 98.7%.

***N*-[1-(4-Bromophenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (6x). 6x** was synthesized by method C by using crude **5k** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 25%. ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1H), 7.56 (d, J = 8.8 Hz, 2H), 7.40−7.20 (m, 3H), 5.35−5.20 (m, 1H), 2.85−2.60 (m, 2H), 2.61 (s. 3H), 2.20−2.00 (m, 1H), 2.00−1.80 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.6, 151.4, 147.8, 139.5, 138.4, 138.0, 132.0, 124.3, 120.6, 117.6, 42.3, 29.1, 22.7, 19.7, 8.7; HRMS (ESI) calculated for $C_{17}H_{16}BrN_5O_2$ $[M - H]^-$ 400.0415, found 400.0435. HPLC purity: 96.9%.

***N*-{1-[4-(2-Propanyl)aminophenyl]-1*H*-4,5,6,7-tetrahydroindazol-4-yl}-4-methylfurazan-3-carboxamide (7).** To a solution of **6u** (105.0 mg, 0.28 mmol) in EtOAc (20 mL) and acetone (10 mL) was added Pd/C (10.5 mg) in a thick-walled glass bottle. The bottle was degassed with N_2 and charged with H_2 (50 psi), and then the mixture was stirred at room temperature overnight. On completion of the reaction (TLC, eluent hexanes/EtOAc, 2:1), the solution was concentrated under reduced pressure. Silica gel chromatography (hexanes/EtOAc, 2:1) of the crude mixture afforded **7** (61.0 mg, 83%). ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 1H), 7.21 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 7.6 Hz, 1H), 6.60 (d, J = 8.4 Hz, 2H), 5.35−5.20 (m, 1H), 3.90−3.50 (m, 2H), 2.80−2.55 (m, 2H), 2.65 (s, 3H), 2.20−2.00 (m, 1H), 2.00−1.80 (m, 3H), 1.23 (d, J = 6.4 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 156.4, 151.4, 147.8, 146.6, 139.2, 137.0, 128.9, 1224.8, 116.0, 112.6, 43.9, 42.6, 29.4, 22.9, 22.5, 19.6, 8.8; HRMS (ESI) calculated for $C_{20}H_{24}N_6O_2$ $[M + H]^+$ 381.2034, found 381.2037. HPLC purity: 96.1%.

***N*-[1-(4-Aminophenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-4-methylfurazan-3-carboxamide (8).** To a solution of **6u** (354.0 mg, 0.96 mmol) in EtOAc (20 mL) and DMF (10 mL) was added Pd/C (35.4 mg) in a thick-walled glass bottle. The bottle was degassed with N_2 and charged with H_2 (50 psi), and then the mixture was stirred at room temperature overnight. On completion of the reaction (TLC, eluent hexanes/EtOAc, 2:1), the solution was filtered and concentrated under reduced pressure. Silica gel chromatography (hexanes/EtOAc, 2:1) of the crude mixture afforded **8** (200.0 mg, 62%). ^{1}H NMR ($CDCl_3$) δ 7.51 (s, 1H), 7.40 (d, J = 7.2 Hz, 1H), 7.13 (d, J = 8.4 Hz, 2H), 6.70 (d, J = 8.4 Hz, 2H), 5.35−5.15 (m, 1H), 3.30−3.05 (br s, 2H), 2.75−2.50 (m, 2H), 2.60 (s, 3H), 2.20−1.98 (m, 1H), 1.98−1.75 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 157.0, 152.0, 150.0, 148.5, 139.2, 137.4, 128.9, 124.8, 117.7, 114.1, 42.9, 29.5, 22.6, 20.6, 9.0; ^{13}C NMR ($CDCl_3$) δ 156.6, 151.4, 147.8, 139.5, 138.4, 138.0, 132.0, 124.3, 120.6, 117.6, 42.3, 29.1, 22.7, 19.7, 8.7; HRMS (ESI) calculated for $C_{17}H_{18}N_6O_2$ $[M + H]^+$ 339.1564, found 339.1571. HPLC purity: 100.0%.

***N*-{1-[4-(2,2,2-Trifluoroacetylamino)phenyl]-1*H*-4,5,6,7-tetrahydroindazol-4-yl}-4-methylfurazan-3-carboxamide (9a). 9a** was synthesized by method C by using crude **8** and trifluoroacetic acid as starting materials. Yield 60%. ^{1}H NMR ($CDCl_3$) δ 9.03 (s, 1H), 7.66 (s, 1H), 7.56 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 8.8 Hz, 2H), 7.03 (d, J = 7.6 Hz, 1H), 5.40−5.25 (m, 1H), 2.85−2.60 (m, 2H), 2.67 (s, 3H), 2.25−2.05 (m, 1H), 2.00−1.85 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.9, 155.2 (q, J = 38 Hz), 151.8, 148.1, 140.2, 138.7, 137.0, 134.5, 124.3, 122.0, 117.8, 115.7 (q, J = 286 Hz), 42.7, 29.6, 22.9, 20.0, 9.1; HRMS (ESI) calculated for $C_{19}H_{17}F_3N_6O_3$ $[M + H]^+$ 435.1387, found 435.1388. HPLC purity: 98.0%.

***N*-{1-[4-(2,2-Dimethylpropionylamino)phenyl]-1*H*-4,5,6,7-tetrahydroindazol-4-yl}-4-methylfurazan-3-carboxamide (9b).** To a solution of **8** (44.7 mg, 0.13 mmol) in pyridine (2 mL) were added DMAP (1.6 mg, 0.013 mmol) and 2,2-dimethylpropionyl chloride (24.0 mg, 0.20 mmol). Then the resulting solution was stirred at room temperature overnight. On completion of the reaction (TLC, eluent hexanes/EtOAc, 2:1), the solution was concentrated under reduced pressure. The residue was diluted with EtOAc (10 mL) and washed thoroughly with 1 M aqueous HCl (5 mL), saturated aqueous $NaHCO_3$ (5 mL), and brine (5 mL), dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (hexanes/EtOAc, 2:1) of the crude mixture afforded **9b** (50.0 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 7.80−7.50 (m, 4H), 7.40 (d, J = 7.6 Hz, 2H), 7.06 (d, J = 6.4 Hz, 1H), 5.40−5.20 (m, 1H), 2.85−2.60 (m, 2H), 2.65 (s, 3H), 2.20−2.05 (m, 1H), 2.00−1.80 (m, 3H), 1.32 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 176.8, 156.8, 151.8, 148.2, 139.8, 138.3, 137.3, 135.4, 123.9, 120.6, 117.4, 42.8, 39.7, 29.6, 27.6, 23.0, 20.1, 9.1; HRMS (ESI) calculated for $C_{22}H_{26}N_6O_3$ $[M - H]^-$ 421.1994, found 421.2007. HPLC purity: 96.2%.

***N*-{1-[4-(4-Methylfurazan-3-carbonylamino)phenyl]-1*H*-4,5,6,7-tetrahydroindazol-4-yl}-4-methylfurazan-3-carboxamide (9c). 9c** was synthesized by method C by using crude **8** and 4-methylfurazan-3-carboxylic acid as starting materials. Yield 90%. ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.65 (s, 1H), 7.52 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 7.6 Hz, 1H), 5.40−5.25 (m, 1H), 2.90−2.65 (m, 2H), 2.69 (s, 3H), 2.67 (s, 3H), 2.25−2.05 (m, 1H), 2.00−1.85 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.5, 155.0, 151.6, 151.5, 14, 147.8, 139.4, 138.2, 136.2, 135.2, 123.7, 120.5, 117.3, 42.4, 29.2, 22.8, 19.7, 8.8; HRMS (ESI) calculated for $C_{21}H_{20}N_8O_4$ $[M + H]^+$ 449.1680, found 449.1686. HPLC purity: 96.2%.

1-(4-*tert*-Butylphenyl)-1,5,6,7-tetrahydrobenzotriazol-4-one (13). To a solution of 1-azido-4-*tert*-butylbenzene (409.0 mg, 2.3 mmol)

in xylene (20 mL) was added cyclohex-2-enone (224.4 mg, 2.3 mmol). Then the resulting solution was heated to 145 °C for 2 h. On completion of the reaction (TLC, eluent hexanes/EtOAc, 1:1), the solution was concentrated under reduced pressure. The residue was diluted with EtOAc (50 mL) and washed thoroughly with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (hexanes/EtOAc, 1:1) of the crude mixture afforded **13** (80.0 mg, 13%). ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 3.04 (d, J = 6.4 Hz, 2H), 2.66 (d, J = 6.4 Hz, 2H), 2.30−2.15 (m, 2H), 1.37 (s, 9H).

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydrobenzotriazol-4-yl]-4-methylfurazan-3-carboxamide. (15). 15** was synthesized by method C by using crude **14** and 4-methylfurazan-3-carboxylic acid as starting materials. Two-step overall yield 35%. ^{1}H NMR ($CDCl_3$) δ 7.54 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 6.8 Hz, 1H), 5.50−5.40 (m, 1H), 2.90−2.70 (m, 2H), 2.65 (s, 3H), 2.40−2.25 (m, 1H), 2.10−1.90 (m, 3H), 1.37 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 156.9, 152.1, 151.4, 147.8, 141.9, 133.8, 133.4, 126.2, 122.5, 43.5, 34.5, 30.9, 29.3, 21.1, 19.6, 8.7; HRMS (ESI) calculated for $C_{20}H_{24}N_6O_2$ $[M-H]^-$ 379.1888, found 379.1898. HPLC purity: 96.1%.

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-4,5,6,7-tetrahydroindazol-4-yl]-(4-methylfurazan-3-ylmethyl)amine (17).** To a solution of **5a** (45.0 mg, 0.17 mmol) in DMF (2 mL) were added 3-bromomethyl-4-methylfurazan (30.0 mg 0.17 mmol) and K_2CO_3 (70.0 mg, 0.50 mmol). Then the resulting solution was stirred at room temperature overnight. On completion of the reaction (TLC, eluent hexanes/EtOAc, 1:1), the solution was concentrated under reduced pressure. The residue was diluted with EtOAc (10 mL) and washed thoroughly with saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL), dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (hexanes/EtOAc, 1:1) of the crude mixture afforded **17** (7.0 mg, 12%). ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 1H), 7.52−7.43 (m, 2H), 7.43−7.35 (m, 2H), 4.10 (d, J = 1.2 Hz, 2H), 3.96−3.84 (m, 1H), 2.90−2.60 (m, 2H), 2.48 (s, 3H), 2.10−1.90 (m, 2H), 1.85−1.70 (m, 2H), 1.62 (br s, 1H), 1.36 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 153.0, 150.5, 149.8, 138.6, 137.7, 136.9, 125.6, 122.5, 119.4, 49.6, 39.3, 34.2, 30.9, 29.0, 23.0, 19.5, 8.1; HRMS (ESI) calculated for $C_{21}H_{27}N_5O$ $[M+H]^+$ 366.2288, found 366.2277. HPLC purity: 100.0%.

4-Nitro-1*H*-indazole (19). A solution of 2-methyl-3-nitroaniline **18** (1.0 g, 6.58 mmol) in glacial acetic acid (15 mL) was treated with a solution of sodium nitrite (454 mg, 6.58 mmol) in water (2 mL). The resultant solution was stirred for 15 min and allowed to stand at room temperature for 3 days. The acetic acid was evaporated. The residue was dissolved in EtOAc (50 mL), filtered through a plug of silica gel, and rinsed with EtOAc (5 mL × 2). The resulting solid was dried under reduced pressure to give **19** as a yellow powder (995.0 mg, 93%). ^{1}H NMR (DMSO-d_6) δ 13.93 (br s, 1H), 8.53 (s, 1H), 8.15 (d, J = 4.0 Hz, 1H), 8.09 (d, J = 3.6 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 141.8, 139.7, 132.3, 125.7, 118.7, 118.3, 115.3, 110.2, 130.7, 127.2, 122.5, 117.9, 117.7, 103.5, 101.2, 62.5, 55.9, 14.3.

1-(4-*tert*-Butylphenyl)-4-nitro-1*H*-indazole (20a). 20a was synthesized by method E by using **19** and 1-bromo-4-*tert*-butylbenzene as starting materials. Yield 47%. ^{1}H NMR ($CDCl_3$) δ 8.90 (s, 1H), 8.17 (d, J = 7.6 Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.60 (s, 4H), 7.52 (t, J = 8.0 Hz, 1H), 4.46 (br s, 2H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 151.4, 140.8, 140.6, 136.7, 134.5, 126.8, 126.2, 123.3, 118.9, 118.2, 117.7, 34.9, 31.7.

1-(3,5-Dimethylphenyl)-4-nitro-1*H*-indazole (20b). 20b was synthesized by method E by using **19** and 1-bromo-3,5-dimethylbenzene as starting materials. Yield 51%. ^{1}H NMR ($CDCl_3$) δ 8.62 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 8.0 Hz, 1H), 4.90−5.00 (m, 1H), 1.66 (t, J = 12.0 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 140.9, 140.4, 132.6, 125.1, 118.3, 117.2, 116.3, 51.3, 22.4.

1-(4-*tert*-Butylphenyl)-4-amino-1*H*-indazole (21a). 21a was synthesized by method F by using **20a** as a starting material. Yield 97%. ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1H), 7.55 (dd, J_1 = 16.0 Hz, J_2 = 4.0 Hz, 4H), 7.00−7.20 (m, 2H), 6.31 (d, J = 8.0 Hz, 1H), 4.46 (br s, 2H), 1.34 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 149.5, 140.7, 140.2, 137.9, 132.3, 128.6, 126.2, 122.3, 115.6, 104.4, 100.2, 34.6, 31.3.

1-(3,5-Dimethylphenyl)-4-amino-1*H*-indazole (21b). 21b was synthesized by method F by using **20b** as a starting material. Yield 99%. ^{1}H NMR ($CDCl_3$) δ 8.11 (s, 1H), 7.33 (s, 2H), 7.18 (t, J = 8.0 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.36 (d, J = 8.0 Hz, 1H), 4.25 (br s, 2H), 2.38 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 140.6, 140.4, 140.3, 139.2, 132.2, 128.6, 128.3, 120.6, 115.7, 104.5, 100.6, 21.4.

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-indazol-4-yl]-2,1,3-benzoxadiazole-5-carboxamide, 1-Oxide (22a). 22a** was synthesized by method G by using **21a** and 1-oxybenzo[1,2,5]oxadiazole-5-carboxylic acid as starting materials. Yield 21%. ^{1}H NMR (DMSO-d_6) δ 10.84 (s, 1H), 8.52 (s, 1H), 8.45 (br s, 1H), 7.93 (br s, 2H), 7.68 (d, J = 8.0 Hz, 2H), 7.62 (t, J = 8.6 Hz, 3H), 7.48 (t, J = 8.0 Hz, 1H), 1.35 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 163.7 (br), 152.8 (br), 149.3, 139.2, 137.2, 134.6, 132.8 (br), 131.0, 127.8, 126.4, 122.1, 118.9, 118.1 (br), 114.2, 107.2, 31.1, 34.4; HRMS (ESI) calculated for $C_{24}H_{21}N_5O_3$ $[M-H]^-$ 426.1572, found 426.1591. HPLC purity: 99.1%.

***N*-[1-(3,5-Dimethylphenyl)-1*H*-indazol-4-yl]-2,1,3-benzoxadiazole-5-carboxamide, 1-Oxide (22b). 22b** was synthesized by method G by using **21b** and 1-oxybenzo[1,2,5]oxadiazole-5-carboxylic acid as starting materials. Yield 33%. ^{1}H NMR (DMSO-d_6) δ 10.82 (s, 1H), 8.52 (s, 1H), 8.45 (br s, 1H), 7.91 (br s, 2H), 7.65 (d, J = 8.0 Hz, 2H), 7.48 (t, J = 8.0 Hz, 1H), 7.37 (s, 2H), 7.04 (s, 1H), 2.39 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 164.9 (br), 152.7 (br), 139.5, 139.1, 139.0, 134.6, 132.6 (br), 131.0, 128.1, 127.8, 120.0, 118.9, 117.7 (br), 104.0, 107.4, 20.9; HRMS (ESI) calculated for $C_{22}H_{17}N_5O_3$ $[M-H]^-$ 398.1259, found 398.1277. HPLC purity: 100.0%.

***N*-[1-(4-*tert*-Butylphenyl)-1*H*-indazol-4-yl]-4-methylfurazan-3-carboxamide (22c). 22c** was synthesized by method G by using **21a** and 4-methylfurazan-3-carboxylic acid as starting materials. Yield 37%. ^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1H), 8.25 (s, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.41 (t, J = 8.2 Hz, 1H), 7.29 (s, 2H), 7.02 (s, 1H), 2.70 (s, 3H), 2.40 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 155.6, 152.3, 148.5, 140.0, 139.7, 139.5, 131.9, 129.12, 129.08, 127.9, 121.0, 118.2, 113.2, 108.5, 21.5, 9.3; HRMS (ESI) calculated for $C_{21}H_{21}N_5O_2$ $[M-H]^-$ 374.1622, found 374.1639. HPLC purity: 98.4%.

***N*-[1-(3,5-Dimethylphenyl)-1*H*-indazol-4-yl]-4-methylfurazan-3-carboxamide (22d). 22d** was synthesized by method G by using **21b** and 4-methylfurazan-3-carboxylic acid as starting materials. Yield 33%. ^{1}H NMR ($CDCl_3$) δ 8.88 (s, 1H), 8.27 (s, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.50−7.70 (m, 5H), 7.43 (t, J = 8.0 Hz, 1H), 2.73 (s, 3H), 1.39 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 155.6, 152.3, 150.6, 148.6, 140.1, 137.4, 131.9, 129. 2, 127.9, 126.6, 123.0, 118.2, 108.5, 34.9, 31.6, 9.4; HRMS (ESI) calculated for $C_{19}H_{17}N_5O_2$ $[M-H]^-$ 346.1309, found 346.1322. HPLC purity: 97.0%.

Biology. The MICs were determined using MTB $H_{37}Rv$ ATCC 27294 in MABA[4] assays according to published procedures. Reported MICs are an average of two individual measurements.

Microplate Alamar Blue Assay (MABA). The compound MICs against R-TB were assessed by the MABA using **1** as a positive control. Briefly, compound stock solutions were prepared in DMSO at 12.8 mM, and the final test concentrations ranged from 128 to 0.5 μM. Two-fold dilutions of compounds were prepared in Middlebrook 7H12 medium (7H9 broth containing 0.1% w/v casitone, 5.6 μg/mL palmitic acid, 5 mg/mL bovine serum albumin, 4 mg/mL catalase, filter-sterilized) in a volume of 100 μL in 96-well microplates (black viewplates, manufacturer). MTB $H_{37}Rv$ (ATCC no. 27294) (100 μL inoculum of 1 × 105 cfu/mL) was added, yielding a final testing volume of 200 μL. The plates were incubated aerobically at

37 °C. On the seventh day of incubation, 12.5 μL of 20% Tween-80 and 20 μL of Alamar blue (Trek Diagnostic, Westlake, Ohio) were added to the test plate. After incubation at 37 °C for 16−24 h, fluorescence of the wells was measured (excitation at 530 nm, emission at 590 nm). The MICs were defined as the lowest concentration effecting a reduction in fluorescence of ≥90% relative to the mean of replicate bacteria-only controls.

Cytotoxicity Assay. Cytotoxicity was determined by exposing different concentrations of samples to Vero cells. Samples were dissolved at 12.8 mM in DMSO. Geometric 3-fold dilutions were performed in growth medium MEM (Gibco, Grand Island, NY), containing 10% fetal bovine serum (HyClone, Logan, UT), 25 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES, Gibco), 0.2% $NaHCO_3$ (Gibco), and 2 mM glutamine (Irvine Scientific, Santa Ana, CA). Final DMSO concentrations did not exceed 1% v/v. Drug dilutions were distributed in duplicate in 96-well tissue culture plates (Becton Dickinson Labware, Lincoln Park, NJ) at a volume of 50 μL per well. An equal volume containing either 5×10^5 log phase Vero cells (CCL-81; American Type Culture Collection, Rockville, MD) was added to each well, and the cultures were incubated at 37 °C in an atmosphere containing 5% of CO_2. After 72 h, cell viability was measured using the CellTiter 96 aqueous nonradioactive cell proliferation assay (Promega Corp., Madison, WI) according to the manufacturer's instructions. Absorbance at 490 nm was read in a Victor multilabel reader (PerkinElmer). The IC_{50} values were determined using a curve-fitting program.

Drug Metabolism and Pharmacokinetics. General Information. Reference compound **6d** was synthesized in house. Pooled mouse microsomes (protein concentration, 20 mg/mL) were purchased from XenoTech (Lenexa, KS). NADPH was purchased from Sigma Aldrich (St. Louis, MO). All solvents were HPLC grade and purchased from Fisher Scientific (Hanover Park, IL).

Microsomal Stability Assay. Compound stock solutions were prepared in DMSO. The final concentration of DMSO in the incubation media was 0.2% (v/v). The stability of compounds **6a** and **6m** in microsomes was determined in triplicate after their incubation at 1 μM with mouse liver microsomes (0.5 mg/mL) in 50 mM potassium phosphate buffer (pH 7.4) at 37 °C. The total incubation volume was 50 μL. The reaction mixture was prewarmed at 37 °C for 5 min before adding NADPH (1.0 mM). Reactions are quenched at 0, 5, 10, 15, 20, and 30 min by adding acetonitrile (150 μL) containing the internal standard **6d** at 0.01 μM. The samples were centrifuged at 4000*g* for 30 min before liquid chromatography/tandem mass spectrometry (LC−MS−MS) analysis of the parent compound. For control experiments, NADPH was omitted from these incubations.

LC−MS−MS Assay for 6a and 6m Quantitation. Qualitative assessments of compound metabolism were conducted by using an Agilent triple quadrapole mass spectrometer (Agilent, Santa Clara, CA). Reversed-phase HPLC separations during LC−MS−MS were carried out by using an XTerra MS C_{18} column (2.1 mm × 50 mm, 2.5 μm, Waters) connected to Agilent 1200 HPLC system.

Analysis Method. A binary gradient consisted of a mixture of a 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.5 mL/min. The LC gradient was programmed as follows: 50% to 70% B over 2.5 min, followed by an isocratic hold at 95% B over the next 2 min. The column was re-equilibrated for 5 min between injections. The column temperature was 40 °C, and the autosampler was maintained at 4 °C. Ionization was conducted in the positive ionization mode. The electrospray voltage was 4 kV, and the capillary temperature was 300 °C. Under these conditions, compounds **6a** and **6m** eluted at 2.076 and 1.447 min, respectively. The ion transitions with the collision energy, fragmentor, and retention times during selected reaction monitoring (SRM) for compounds **6a** and **6m** are listed in Table 5.

Table 5. SRMs and Retention Times of Analogues Detected by LC−MS−MS

compd	SRM ion pairs (*m*/*z*)	CE (eV)	fragmentor (eV)	t_R (min)
6a	380.1 → 323.2	30	160	2.076
6m	408.0 → 351.2	25	140	1.447

Dosing and in Vivo Sample Collection. Groups of 12 female BALB/c mice (20−22 g) were administered by a single oral 50 mg/kg dose of compounds **6a** and **6m**. The dose was formulated in 5% *N*-methylpyrrolidone (NMP) and 95% polyethylene glycol 400 (PEG400) with dosage of 50 mg/mL. Whole blood samples (250 μL) were collected aseptically from the retro orbital sinus at 0, 0.5, 1, 2, 4, 8, and 24 h postdose. Plasma was prepared from the whole blood by centrifugation at 2000*g* for 30 min. Three volumes of acetonitrile containing the internal standard (IS1, **6d**, 0.01 μM for compounds **6a** and **6m**) were added to each sample, and the samples were centrifuged at 4000*g* for 30 min before LC−MS−MS analysis.

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References

(1) World Health Organization. *Global Tuberculosis Control: Surveillance, Planning, and Financing*; WHO Report 2008; WHO Press: Geneva, Switzerland, 2008.
(2) (a) Spigelman, M. K. New tuberculosis therapeutics: a growing pipeline. *J. Infect. Dis.* **2007**, *196*, 28–34. (b) Janin, Y. L. Antituberculosis drugs: ten years of research. *Bioorg. Med. Chem.* **2007**, *15*, 2479–2513.
(3) Mao, J.; Wan, B.; Wang, Y.; Franzblau, S. G.; Kozikowski, A. P. HTS, chemical hybridization and drug design identify a chemically unique antituberculosis agent-coupling serendipity and rational approaches to drug discovery. *ChemMedChem* **2007**, *2*, 811–813.
(4) Collins, L.; Franzblau, S. G. Microplate Alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004–1009.
(5) Cantrell, C. L.; Lu, T.; Fronczek, F. R.; Fischer, N. H.; Adams, L. B.; Franzblau, S. G. Antimycobacterial cycloartanes from *Borrichia Frutescens*. *J. Nat. Prod.* **1996**, *59*, 1131–1136.
(6) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235–249.
(7) Baurin, N.; Baker, R.; Richardson, C.; Chen, I.; Foloppe, N.; Potter, A.; Jordan, A.; Roughley, S.; Parratt, M.; Greaney, P.; Morley, D; Hubbard, R. E. Drug-like annotation and duplicate analysis of a 23-supplier chemical database totalling 2.7 million compounds. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 643–651.
(8) Schenone, P.; Mosti, L.; Menozzi, G. Reaction of 2-dimethylaminomethylene-1,3-diones with dinucleophiles. I. Synthesis of 1,5-disubstituted 4-acylpyrazoles. *J. Heterocycl. Chem.* **1982**, *19*, 1355–1361.
(9) Souers, A. J.; Gao, J.; Brune, M.; Bush, E.; Wodka, D.; Vasudevan, A.; Judd, A. S.; Mulhern, Brodjian, M.; S.; Dayton, Shapiro, B.; R.; Hernandez, L. E.; Marsh, K. C.; Sham, H. L.; Collins, C. A.; Kym, P. R. Identification of 2-(4-benzyloxyphenyl)-*N*-[1-(2-pyrrolidin-1-yl-ethyl)-1*H*-indazol-6-yl]acetamide, an orally efficacious melanin-concentrating hormone receptor 1 antagonist for the treatment of obesity. *J. Med. Chem.* **2005**, *48*, 1318–1321.
(10) Anderson, K. W.; Tundel, R. E.; Ikawa, T.; Altman, R. A.; Buchwald, S. L. Monodentate phosphines provide highly active catalysts for Pd-catalyzed C−N bond-forming reactions of heteroaromatic halides/amines and (*H*) N-heterocycles. *Angew. Chem., Int. Ed.* **2006**, *45*, 6523–6527.
(11) Omura, T.; Sato, R. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J. Biol. Chem.* **1964**, *239*, 2379–2385.
(12) Houston, J. B. Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. *Biochem. Pharmacol.* **1994**, *47*, 1469–1479.
(13) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.