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Synthesis of new generation triazolyl- and isoxazolyl-containing 6-nitro-2,3-dihydroimidazooxazoles as anti-TB agents: *in vitro*, structure–activity relationship, pharmacokinetics and *in vivo* evaluation†

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The nitroimidazole scaffold has attracted great interest in the last decade, which ultimately led to the discovery of the successful drug Delamanid for multi-drug resistant tuberculosis (MDR-TB). Herein, we report medicinal chemistry on a 6-nitro-2,3-dihydroimidazooxazole (NHIO) scaffold with SAR on the novel series of triazolyl- and isoxazolyl-based NHIO compounds. In the present study, 41 novel triazolyl- and isoxazolyl-based NHIO compounds were synthesized and evaluated against *Mycobacterium tuberculosis* (MTB) $H_{37}Rv$. The active compounds with MIC of 0.57–0.13 μM were further screened against dormant, as well as against resistant strains of MTB. Based on the overall *in vitro* profile, five compounds were studied for *in vivo* oral pharmacokinetics, wherein two compounds: **1g** and **2e** showed a good PK profile. In *in vivo* efficacy studies in the intra-nasal model of acute infection, **1g** showed 1.8 and 1 log CFU reduction with respect to the untreated and early control, respectively. The lead compound **1g** also showed an additive to synergistic effect in combination studies with first line-TB drugs and no CYP inhibition. From the present studies, the compound **1g** represents another alternative lead candidate in this class and needs further detailed investigation.

Introduction

Tuberculosis (TB) is still one of the major global health problems, and claimed the lives of 1.3 million people worldwide in 2012.[1] A recent WHO report says that globally 8.6 million people were infected with TB, and among these 2.3 million were in India alone.[2] The existing TB treatment involves an exceedingly lengthy therapy, and the emergence of multidrug resistant-TB (MDR-TB) and extensively drug resistant-TB (XDR-TB) further complicates the world situation.[3] The WHO has estimated that if the present conditions remain unchanged, more than 30 million lives will be claimed by TB between 2000 and 2020.[2,3] Therefore, the current situation necessitates the discovery and development of novel, potent, efficacious, and less toxic anti-tuberculosis agents, which, in addition, should also have the capability of shortening the duration of therapy.

In the last decade, the nitroimidazole scaffold has attracted great interest among researchers in academic and industrial fields because of its promising potency against the replicating and non-replicating phases of tuberculosis.[4] The anti-tubercular potential of the nitroimidazole scaffold was initially identified by researchers of Ciba-Geigy India in 1989, wherein bicyclic nitroimidazooxazole compound, CGI-17341 (**A**, Fig. 1) was identified as the lead compound, but unfortunately its mutagenic nature halted its clinical development.[5] Later, PathoGenesis Corporation and Otsuka pharmaceutical companies overcame the mutagenicity and developed two drug candidates, namely PA-824[6] (**B**, a nitroimidazopyran derivative,

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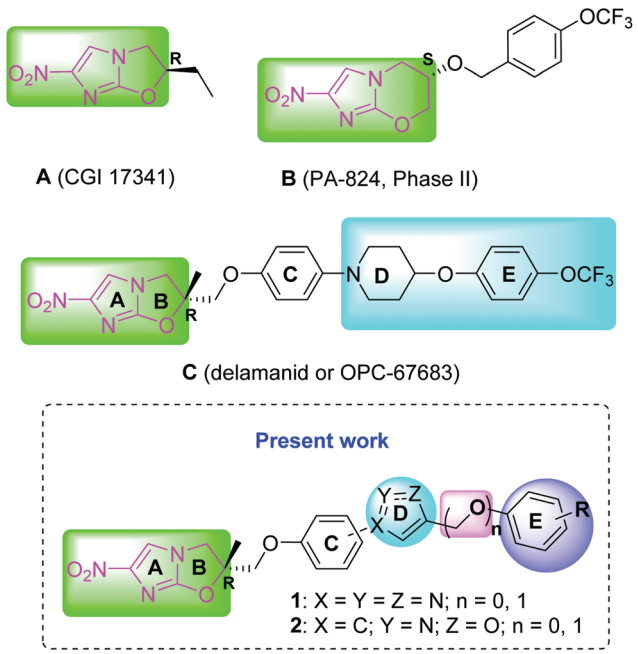


Fig. 1 Structure of nitroimidazole-based anti-TB agents.

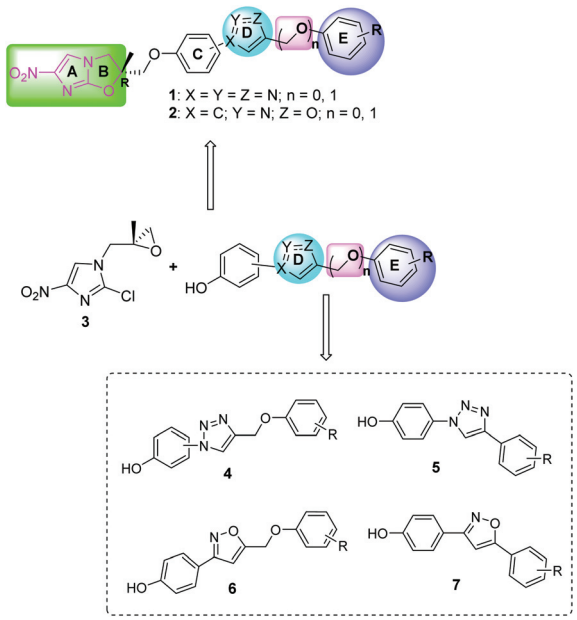


Fig. 2 Strategy for the synthesis of target compounds.

currently in Phase-II clinical trials) and OPC-67683[7] (C, delamanid, 6-nitro-2,3-dihydroimidazooxazole derivative, recently approved by the European Union for the treatment of MDR-TB[8]). Both the drug candidates are liphophilic in nature, which in fact might help entry through the highly lipophilic cell wall of MTB and be responsible for its high potency.[9,10] Our literature survey revealed that several groups worked on the nitroimidazooxazine scaffold (PA-824) and synthesized their newer generation analogs;[11] however, on the other hand, despite it having a comparatively better anti-TB profile than delamanid, no further medicinal chemistry efforts have been made on the nitrodihydroimidazooxazole scaffold.

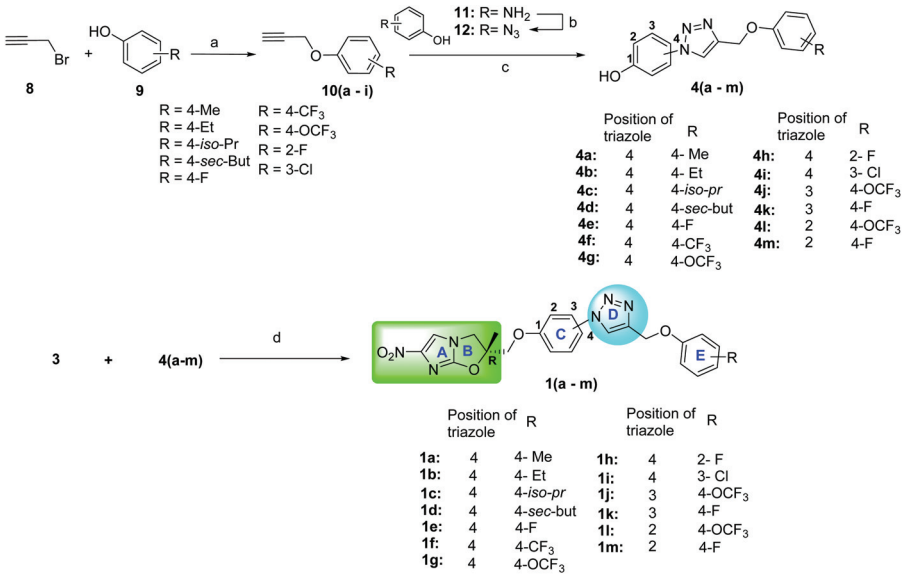
Keeping in view the importance of the nitrodihydroimidazooxazole scaffold (particularly delamanid, as shown in Fig. 1), we initiated a medicinal chemistry programme, wherein we replaced the substituted phenoxypiperidyl (ring D and E) of delamanid with heterocyclic moieties (triazole and isoxazole) and studied the effect of modifications on the anti-TB activity. Interestingly, the present work has led to the discovery of a new lead compound which has shown potent activity against sensitive MTB (*M. tuberculosis* $H_{37}Rv$), non-replicative MTB (*M. tuberculosis* 18b), and the resistant-strain of MTB, along with exhibiting a good safety index and *in vivo* efficacy.

Chemistry

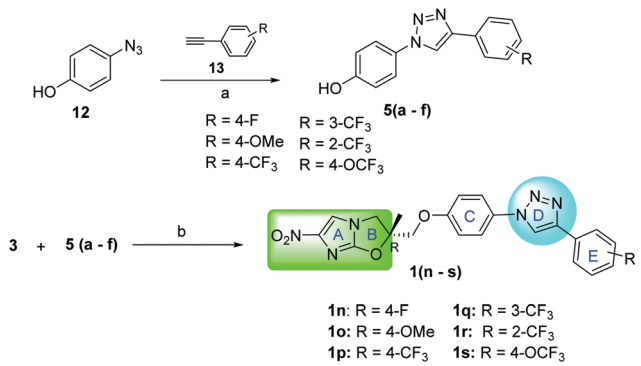
The synthesis of the target compounds, *i.e.*, triazolyl- and isoxazolyl-containing 6-nitro-2,3-dihydroimidazooxazole (NHIO) compounds **1** and **2**, required two key intermediates: (i) first (*R*)-2-chloro-1-{(2-methyloxiran-2-yl)methyl}-4-nitro-1*H*-imidazole **3**, and (ii) either of the following triazolyl- or isoxazolyl-containing phenols **4–7** (Fig. 2). The first key intermediate (*R*)-2-chloro-1-{(2-methyloxiran-2-yl)methyl}-4-nitro-1*H*-imidazole **3** was synthesized from commercially available starting material 4-nitroimidazole following the reported method (details given in ESI†).[7,12]

The other parts were synthesized by following the synthetic routes given in Schemes 1–4. First, the triazolyl containing intermediates **4** were taken up and synthesized in two steps from substituted phenol **9**. In the first step, substituted phenol **9** was converted into *O*-propargylated intermediate **10**, which in a second step underwent 2 + 3 cyclo-additions with azido-phenols **12** (which in turn were synthesized from aminophenols **11**) under standard click conditions to afford the triazolyl-containing key intermediates **4** in good yields (Scheme 1).[13] Similarly, the other triazolyl-containing intermediates **5** were synthesized from azido phenols **12** on 2 + 3 cycloadditions with the substituted phenylacetylenes **13** (Scheme 2). Finally, the triazolyl-containing intermediates **4** and **5** were coupled with intermediate **3** in the presence of sodium hydride to afford 6-nitro-2,3-dihydroimidazooxazole (NHIO) compounds **1a–m** and **1n–s**, respectively (Schemes 1 and 2).

Next, the synthesis of the isoxazolyl-containing intermediates **6** were taken up, which were synthesized from 4-hydroxybenzaldehyde **15** in three steps: (i) first, conversion of 4-hydroxybenzaldehyde **15** to aldoxime **16**; (ii) then to chlorooxime **17**, and (iii) then dipolar cyclo-addition with substituted phenoxymethyl acetylenes **14** under standard conditions to afford the expected isoxazolyl-containing intermediates **6** in good yields (Scheme 3).[14] Similarly, the other isoxazole intermediates **7** were synthesized from chlorooxime **17** on dipolar cycloaddition with substituted phenyl acetylenes **18** (Scheme 4). Finally, the isoxazolyl-containing intermediates **6**



Scheme 1 Synthesis of compounds **1a–m**. Reagents and conditions: (a) K_2CO_3, ACN, rt, 12 h, 85–90%; (b) $NaNO_2$, HCl, 0–5 °C, 2 h, NaN_3, H_2O, 2 h, 85%; (c) $CuSO_4$, ^tBuOH, H_2O, Sodium ascorbate, rt, 12 h, 80–90%; (d) NaH, DMF, 0 to 50 °C, 12 h, 20–40%.

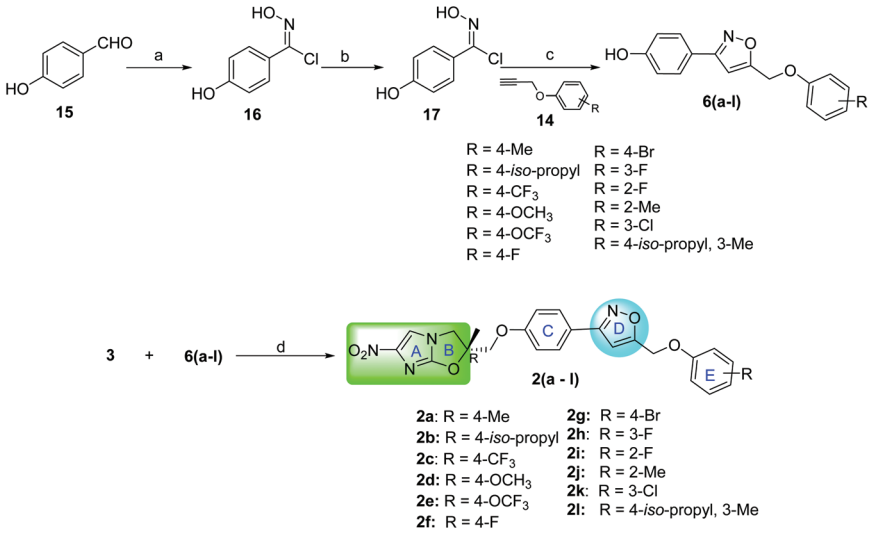


Scheme 2 Synthesis of compounds **1n–s**. Reagents and conditions: (a) $CuSO_4$, ^tBuOH, H_2O, sodium ascorbate, rt, 12 h, 80–90%; b) NaH, DMF, 0 to 50 °C, 12 h, 20–40%.

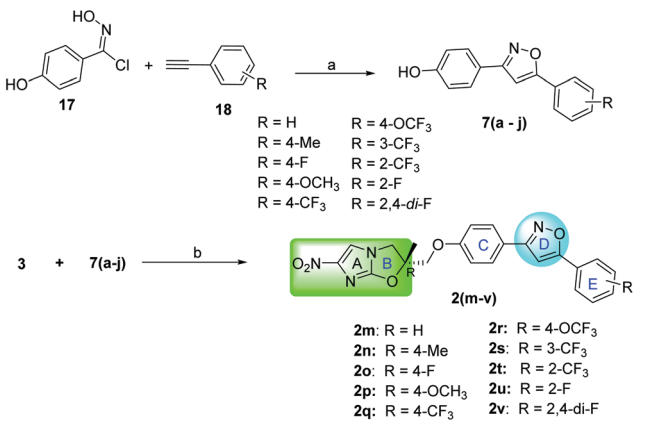
and 7 were coupled with intermediate **3** in the presence of sodium hydride, to ultimately afford isoxazolyl-containing NHIO compounds **2a–l** and **2m–v** (Schemes 3 and 4).

Biological evaluation and discussion

In total, forty-one new triazolyl- and isoxazolyl-based NHIO compounds **1a–s** and **2a–v**, respectively, were synthesized with variations on the ring D and E and were then evaluated for their *in vitro* activity against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294 strain) using the micro-broth dilution method. The MICs of all the synthesized compounds are summarized in Table 1. Triazolyl-based NHIO compounds **1a–i**, wherein the 4-[substituted-phenoxymethyl]triazolyl group is present at the 4th position of ring-C showed an MIC in the range of 1.02 μM to 0.23 μM. To evaluate the effect of the position of the triazolyl on activity, triazolyl-based NHIO compounds **1j–k** with the 4-[substituted-phenoxymethyl]triazolyl group at the 3rd position of ring-C were also synthesized, and showed an MIC in the range of 1.07 μM to 0.23 μM. On the other hand, triazolyl-based NHIO compound **1l–m** with the 4-[substitutedphenoxymethyl]triazolyl group at the 2nd position of ring-C showed comparatively lower activity. Furthermore, the role of the methyleneoxy group between ring-D and ring-E were studied by synthesizing NHIO analogs **1n–s**, wherein the ring-E was directly attached to ring-D. Six compounds were synthesized and among them, two compounds **1n** and **1o** showed MICs of 0.57 μM and 0.56 μM, respectively. The nature and position of the substituents on ring-E also affect the potency, wherein *para*-substitution was more preferable and the presence of -OCF_3 on ring-E showed the most potent activity. In the case of isoxazolyl-based NHIO compounds **2a–l**, where the 5-[substituted-phenoxymethyl]isoxazolyl group is attached to the 4th position of ring-C showed an MIC in the range of 1.0 μM to 0.13 μM, except for **2j** and **2k**, which showed MICs of 4.33 μM and 4.15 μM, respectively. However, isoxazolyl-based NHIO compounds **2m–v**, where ring-E is directly attached to ring-D showed MICs in the range of 2.31 μM to 0.14 μM. In the case of isoxazolyl-based NHIO compounds, the presence, nature, and position of the substituents on ring-E greatly influence the potency. Among the isoxazolyl-based NHIO series, wherein rings D and E are attached through a methyleneoxy group, the compounds with –F and –OCF_3 showed most potent activity, whereas isoxazolyl-based NHIO series, wherein rings D and E are directly attached, the un-substituted phenyl and –CF_3 group bearing compounds showed the most potent activity. Overall, the screening results suggested that the replacement of the piperidyl ring, *i.e.*, ring-D, of delamanid with triazolyl and isoxazolyl is acceptable and further potency depends on the presence of substitution on



Scheme 3 Synthesis of compounds **2a–l**. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, EtOH, NaOH soln, rt, 2 h, 80%; (b) NCS, DMF, rt, 2 h, 90%; c) $CuSO_4$, ^tBuOH, H_2O, sodium ascorbate, $KHCO_3$, rt, 12 h, 80–85%; d) NaH, DMF, 0 to 50 °C, 12 h, 20–40%.



Scheme 4 Synthesis of compounds **2m–v**. Reagents and conditions: (a) $CuSO_4$, ^tBuOH, H_2O, sodium ascorbate, $KHCO_3$, rt, 12 h, 80–85%; (b) NaH, DMF, 0 to 50 °C, 12 h, 20–40%.

ring-E. In most of the cases, the substitutions, particularly –F and $-OCF_3$ at ring-E, were more favorable and showed comparatively better activity (Fig. 3).

In the treatment of *M. tuberculosis*, the issue of dormancy (non-replicating), as well as drug-resistance, has further complicated the problem and new chemical entities with efficacy against these will have advantages to addresses this currently unmet need. The use of streptomycin-starved *M. tuberculosis* 18b as a model for non-replicated cells has been validated and is widely used to test the drugs that target latent tuberculosis[16] Among all the tested NHIO compounds, eleven triazolyl-based NHIO compounds: **1a**, **1b**, **1d–j**, **1n** and **1o**, and nine isoxazolyl-based NHIO compounds: **2b**, **2c**, **2e**, **2f**, **2h**, **2m** and **2s–u** with potent MICs in the range of 0.57 μM to 0.13 μM were selected and further screened against non-replicating, as well as rifampicin (Rif^R) and multi-drug resistant (MDR) strains of MTB under *in vitro* conditions. The screening results are sum-

Table 1 *In vitro* activity of **1a–s** and **2a–v** against MTB $H_{37}Rv$

Compound code	MIC ($H_{37}Rv$)[a]/μM	Compound code	MIC ($H_{37}Rv$)/μM	Compound code	MIC ($H_{37}Rv$)/μM
1a	0.54	**1p**	1.03	**2l**	0.99
1b	0.53	**1q**	8.23	**2m**	0.14
1c	1.02	**1r**	2.06	**2n**	2.31
1d	0.48	**1s**	1.00	**2o**	2.29
1e	0.54	**2a**	1.08	**2p**	1.12
1f	0.48	**2b**	0.51	**2q**	2.06
1g	0.23	**2c**	0.48	**2r**	1.03
1h	0.54	**2d**	1.05	**2s**	0.25
1i	0.52	**2e**	0.23	**2t**	0.51
1j	0.23	**2f**	0.13	**2u**	0.57
1k	1.07	**2g**	0.95	**2v**	1.1
1l	1.88	**2h**	0.26	**Delamanid (C)**	0.01
1m	8.58	**2i**	1.07	**Rifampicin**	0.07
1n	0.57	**2j**	4.33		
1o	0.56	**2k**	4.15		

[a] Minimum inhibitory concentration (MIC) against $H_{37}Rv$ MTB.

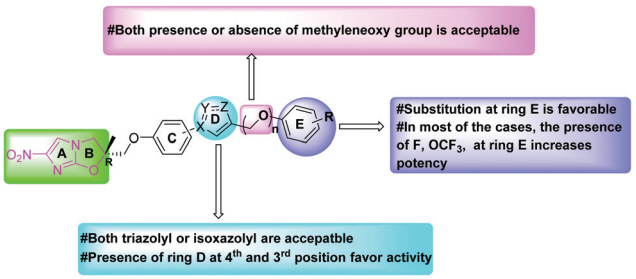


Fig. 3 Structure–activity relationship of triazolyl/isoxazolyl NHIO compounds against $H_{37}Rv$ MTB.

Table 2 *In vitro* activity against non-replicating and resistant strains of MTB, as well as cytotoxicity studies

Compound	NRP[a]/ μM	MIC (Rif^R)/μM	MIC (MDR)/μM	CC_{50}[b] (μM)	SI[c] CC50/ MIC
1a	8.66	0.54	0.54	nd	nd
1b	16.8	0.53	0.53	nd	nd
1d	>30.4	0.48	0.48	nd	nd
1e	8.6	2.15	2.15	nd	nd
1f	7.76	1.94	0.48	nd	nd
1g	7.52	0.23	0.11	>75	>326
1h	4.29	0.54	1.08	nd	nd
1i	8.3	0.52	0.52	nd	nd
1j	7.52	0.23	0.23	>75	>326
1n	4.59	0.57	1.14	nd	nd
1o	>35	35.7	8.925	nd	nd
2b	2.04	0.51	0.51	nd	nd
2c	7.75	0.48	0.96	nd	nd
2e	0.94	0.94	0.23	>75	>326
2f	2.15	0.26	0.26	>85	>600
2h	34.32	1.07	0.13	nd	nd
2m	9.57	1.2	0.14	>95	>600
2s	2.06	0.25	0.25	>82	>300
2t	>32	0.51	1.02	nd	nd
2u	>36	1.15	2.3	nd	nd
Delamanid (C)	0.7[d]	0.01[e]	0.05[f]	201.3[g]	10 710[g]
Rifampicin	2.43	311	155.5	—	—
GATI	2.66	2.66	1.33	—	—

[a] Non-replicating phase of *M. tb*. [b] Cytotoxicity (concentration causing death of 50% of cells; CC50) to HepG2 cells. [c] Selectivity index (CC_{50}/MIC). [d] NRP values using low oxygen recovery assay reported in ref. 17. [e] Reported in ref. 7. [f] Reported in ref. 18. [g] Cytotoxicity against Vero epithelial cells using MTT assay reported in ref. 19.

marized in Table 2. Among the twenty compounds, interestingly several compounds showed single digit or less than single digit MICs against the dormant strain of MTB. Against the Rif^R and MDR strains of MTB, all the compounds showed MICs in the range of 2.15 μM to 0.23 μM, except for **1o** (35.7 μM).

The cytotoxic effects of potent compounds were also determined on HepG2 cell lines (Table 2), wherein none of the compounds showed any cytotoxicity up to 40 μg ml^{-1} and all had an acceptable safety index. In order to evaluate the *in vivo* exposure of next generation triazolyl-/isoxazolyl-based NHIO compounds, five potent compounds: **1g**, **1j**, **2e**, **2f** and **2m** were taken up for oral *in vivo* pharmacokinetics studies in mice at the dose of 5 mg kg^{-1} and compared with the recently

Table 3 *In vivo* pharmacokinetic values in mice

Compound[a]	MIC (μg ml^{-1})	Concentration[b] (ng ml^{-1}) 0.16 h	0.5 h	1 h	2 h	4 h	6 h	8 h	24 h	*C*max (μg ml^{-1})	AUC_{0-24} (μg ml^{-1} h^{-1})	*T*max (h)
1g	0.12	183.03 ± 105.62	364.3 ± 142.53	491.57 ± 242.33	544.85 ± 153.81	451.55 ± 70.29	318.43 ± 67.11	298.92 ± 77.88	226.74 ± 118.41	0.54	7.428	2.00
1j	0.12	67.99 ± 17.21	126.0 ± 18.92	177.39 ± 28.62	316.55 ± 30.97	148.10 ± 62.01	162.25 ± 140.62	89.39 ± 73.30	0 ± 0	0.32	1.38	2.00
2e	0.12	202.53 ± 72.90	614.5 ± 158.06	981.92 ± 248.97	1348.29 ± 297.61	1044.88 ± 134.41	857.08 ± 55.48	765.45 ± 138.98	520.08 ± 144.33	1.34	17.92	2.00
2f	0.06	64.77 ± 10.58	98.13 ± 7.50	123.05 ± 9.06	164.38 ± 14.74	67.55 ± 17.62	30.13 ± 4.88	12.79 ± 3.37	2.23 ± 2.01	0.16	0.724	2.00
2m	0.06	8.32 ± 2.72	16.00 ± 4.47	22.32 ± 4.39	40.80 ± 6.93	28.57 ± 4.18	24.76 ± 7.53	16.68 ± 1.71	13.99 ± 3.31	0.04	0.45	2.00
C (**delamanid**)	0.007	40.82 ± 11.85	141.4 ± 33.79	221.86 ± 63.81	356.83 ± 96.01	246.62 ± 97.42	240.1 ± 76.61	198.35 ± 44.91	181.21 ± 88.33	0.36	4.97	2.00

[a] p.o. at 5 mg kg^{-1}. [b] Each value represents mean ± SD (n = 3) # Pharmacokinetic parameters were calculated by WINNONLIN software.

approved drug in this class—delamanid. All the results are summarized in Table 3 and Fig. 4. Among the five tested compounds, two compounds **1g** and **2e** were shown to have a good PK profile with a C_{max} of 0.54 μg ml^{-1} and 1.34 μg ml^{-1}, respectively, and an $AUC_{0\text{-}t}$ of 7.42 μg ml^{-1} h^{-1} and 17.92 μg ml^{-1} h^{-1}, respectively. As shown in Table 3, the compound **1g** showed a 1.5-times higher C_{max} and $AUC_{0\text{-}t}$ and compound **2e** showed a 3.5-times higher C_{max} and $AUC_{0\text{-}t}$ than delamanid (**C**).

Furthermore, based on the *in vitro* activity and *in vivo* pharmacokinetic profile, both the compounds **1g** and **2e** were evaluated for their *in vivo* efficacy in an intranasal mice model of acute infection in Balb/c mice. After one week of post MTB infection, the compounds were orally administered at 100 mg kg^{-1} once daily for 28 days. Compound **1g** showed a significant 1.8 log CFU (colony forming unit) reduction compared to the untreated control (late control group run parallel without drug treatment) and 1 log CFU reductions compared to the early control (group at the start of treatment). On the other hand, **2e** did not show any efficacy with respect to early and late control

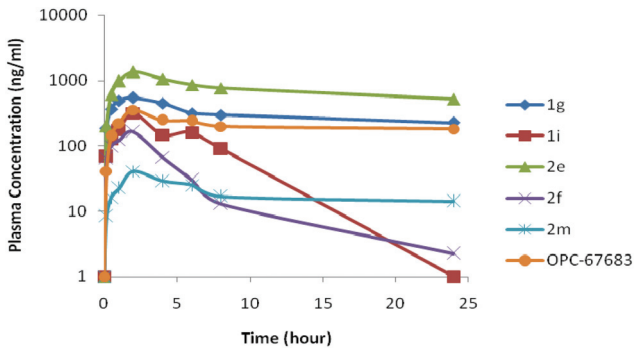


Fig. 4 *In vivo* oral pharmacokinetic profile of selected compounds at 5 mg kg^{-1}.

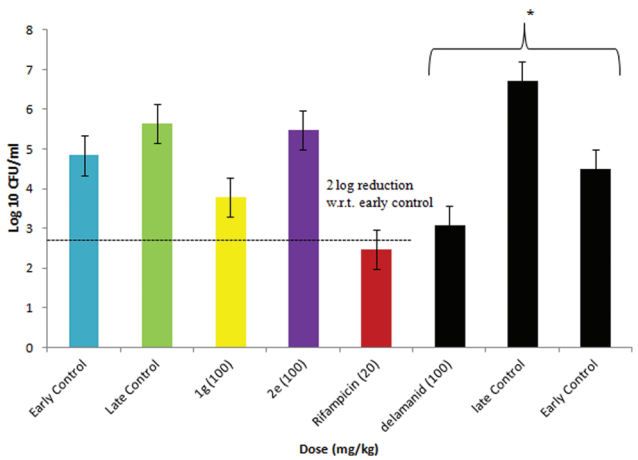


Fig. 5 *In vivo* activity of **1g** and **2e** in an intranasal model of acute infection Balb/c mice. The mice were orally dosed once daily for 28 days (n = 6), starting on the day after intravenous infection with 10^5 CFU of MTB; *the log 10 CFU ml^{-1} value for delamanid is taken from a previous study, reported in ref. 17.

Table 4 *In vitro* combination studies of lead **1g** with first line drugs

Combinations	MIC μg ml^{-1}	FIC[a]	FIC index[b]	Observation
Rif	0.12	FIC *A* = 0.25	0.75	Additive
Rif-**1g**	0.03			
1g	0.12	FIC *B* = 0.5		
1g-Rif	0.06			
INH	0.25	FIC *A* = 0.25	0.50	Synergistic
INH-**1g**	0.06			
1g	0.12	FIC *B* = 0.25		
1g-INH	0.03			
ETM	1	FIC *A* = 0.25	0.75	Additive
ETM-**1g**	0.25			
1g	0.12	FIC *B* = 0.5		
1g-ETM	0.06			

[a] MIC of combination/MIC of alone. [b] FIC A + FIC B; FIC index; ≤0.5 is synergistic, 0.75 to 1 is additive, 1 to 4 is indifference and >4.0 is antagonism.

(Fig. 5). The group treated with compound **1g** at the dose of 100 mg kg^{-1} daily for 28 days did not show any adverse effect and was considered as safe. At the dose of 100 mg kg^{-1}, compound **1g** showed comparable *in vivo* activity with respect to delamanid, as reported recently by Upton *et al.*[17] (activity was performed in the acute aerosol infection model). Compound **1g** showed comparatively lesser activity than the rifampicin tested along with it in the same regimen. However, the group treated with compound **2e** showed mortality in some animals during the treatment, while those that survived also showed some toxic effects. Though this compound did not show cytotoxicity in the MTT assay (Table 2), the apparent reason for the mortality could be a high accumulation of compound in the gut due to its poor solubility. Based on the *in vivo* efficacy results, compound **1g** was taken up for further studies.

In addition to this, *in vivo* efficacious of the lead **1g** was also studied in combination studies with three first line anti-TB drugs, namely rifampicin (Rif), isoniazid (INH), and ethambutol (ETM) using the checkerboard method (Table 4).

Table 5 Effect of lead **1g** on Cyp isoforms

		% of inhibition		
		Concentration (μM)		
CYP Isoforms	Test compound or inhibitor	100	30	10
CYP 1A2	**1g**	14.74	6.95	0.29
	Alpha Napthaflavone			95.25
CYP 2C9	**1g**	0.70	3.56	4.42
	Sulphaphenazole			66.78
CYP 2C19	**1g**	14.74	6.95	0.29
	Ticlopidine			71.09
CYP 2D6	**1g**	0.69	1.24	6.62
	Paraxotine			63.37
CYP 3A4	**1g**	3.39	10.36	5.27
	Ketoconazole			95.86

The substrate concentration used for each assay were; 5 μM 3-cyano-7-ethoxycoumarin (CYP1A2), 25 μM 3-cyano-7-ethoxycoumarin (CYP2C9 & 2C19), 50 μM 3-cyano-7-ethoxycoumarin (CYP2D6), 50 μM 7-benzyloxy-4-(trifluoromethyl)-coumarin (CYP3A4).

The lead **1g** showed an additive effect with rifampicin, a synergistic effect with isoniazid, and an additive effect with ethambutol. These results suggested that the newly generated lead **1g** is suitable for combination with first-line anti-TB drugs. The lead **1g** was also investigated for CYP inhibition by testing for their effect on five major CYP isoforms, namely 3A4, 2D6, 2C9, 1A2, and 2C19 at different concentrations using a fluorescence-based method (Table 5). The results indicated that the lead compound **1g** did not show any CYP inhibition at any of the tested concentrations.

Conclusion

In summary, forty-one new triazolyl- and isoxazolyl-based NHIO compounds were synthesized and evaluated for their detailed anti-TB potential, and a preliminary SAR for this scaffold was also established. The present study suggested that the replacement of rings D and E of delamanid with other heteroaryl and aryl groups is acceptable. The potential compounds were evaluated for oral *in vivo* pharmacokinetics studies, wherein two compounds **1g** and **2e** showed comparable PK profiles to the best in class drug: delamanid. Furthermore, compound **1g** showed an *in vivo* efficacy in an intranasal mice model of acute infection. The lead compound **1g** also showed synergistic to additive effects with current first-line anti-tubercular drugs without any CYP liabilities. The interesting and promising profile of compound **1g** represents another alternative lead candidate in this class. Moreover, detailed evaluation of the lead compound **1g**, as well as the *in vivo* combination studies with first-line anti-TB drugs, is presently undergoing in our laboratory and the results will be published in due course.

Experimental section

Chemistry

All the chemicals for this study were purchased from Sigma-Aldrich, INDIA. ^{1}HNMR were recorded on 200 MHz or 400 MHz or 500 MHz and ^{13}CNMR on 101 MHz or 126 MHz Bruker-Avance DPX FT-NMR instruments. Chemical data for the protons are reported in parts per million (ppm, scale) downfield from tetramethylsilane and are referenced to the residual proton in the NMR solvent ($CDCl_3$: δ 7.26, acetone-d_6: δ 2.1, DMSO-d_6: δ 2.5, or other solvents as mentioned). All the NMR spectra were processed with either MestReNova or Bruker software. Mass spectra were recorded with an HRMS and LC-MS instrument. Melting points were recorded on a digital melting point apparatus and are uncorrected. The purity of all the final compounds (*i.e.*, used for the biological screening) were determined using an HPLC-Agilent Technologies 1260 infinity series system using one of the two methods: Method A: column RP 18e (Chromolith, 5 μm, 4.6 mm × 100 mm) and a gradient mixture of water–methanol as the mobile phase over 50 minutes with a flow rate of 0.8 ml min^{-1}; Method B: column RP 18e (E-Merck, 5 μm, 4.6 mm × 250 mm) and a gradient mixture of water–methanol as the mobile phase over 50 minutes with a flow rate of 0.6 ml min^{-1}. UV recorded at 254 nm.

General procedure for the preparation of triazolyl-based NHIO compounds 1a–s

To a mixture of **3** (0.586 mmol) and **4** or **5** (0.468 mmol) in *N*,*N*-dimethylformamide (3 ml) was added 60% sodium hydride (0.936 mmol) at 0 °C portion wise. After the mixture was stirred at 50 °C for 12 h under a nitrogen atmosphere, the reaction mixture was cooled in an ice bath and quenched with ethyl acetate (2.3 ml) and ice water (0.5 mL). The thus-obtained mixture was poured into water (30 ml) and extracted with ethyl acetate twice, washed with brine solution, and then dried under *vaccuo*. This crude product was purified by silica gel column chromatography using a dichloromethane and ethyl acetate mixture as the solvent to obtain the compounds **1a–s**.

(*R*)-2-{4-[4-(4-Methylphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1a (IIIM/MCD-023). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 200–202 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.65 (d, J = 8.9 Hz, 2H), 7.58 (s, 1H), 7.11 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 8.9 Hz, 2H), 6.92 (d, J = 8.5 Hz, 2H), 5.26 (s, 2H), 4.52 (d, J = 10.3 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.15 (d, J = 10.1 Hz, 1H), 4.08 (d, J = 10.2 Hz, 1H), 2.29 (s, 3H), 1.82 (s, 3H); $[\alpha]_D$ −9.04° (*c* 0.42, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{22}N_6O_5$ $[M + Na]^+$ 485.1550, found 485.1551; HPLC-purity (Method A) 98.8%.

(*R*)-2-{4-[4-(4-Ethylphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 1b (IIIM/MCD-24). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 181–183 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.65 (d, J = 9.0 Hz, 2H), 7.58 (s, 1H), 7.14 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 9.0 Hz, 2H), 6.94 (d, J = 8.6 Hz, 2H), 5.27 (s, 2H), 4.53 (d, J = 10.3 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.15 (d, J = 10.1 Hz, 1H), 4.09 (d, J = 10.3 Hz, 1H), 2.60 (q, J = 7.6 Hz, 2H), 1.82 (s, 3H), 1.21 (t, J = 7.6 Hz, 3H); $[\alpha]_D$ −8.4° (*c* 0.41, acetone); HRMS (ESI-TOF) calcd for $C_{24}H_{24}N_6O_5$ $[M + H]^+$ 477.1886, found 477.1889; HPLC-purity (Method A) 99.05%.

(*R*)-2-{4-[4-(4-Iso-propylylphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1c (IIIM/MCD-26). TLC (EtOAc–DCM 1 : 9): R_f = 0.28; light yellow solid; mp 183–185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.65 (d, J = 8.8 Hz, 2H), 7.58 (s, 1H), 7.17 (d, J = 8.6 Hz, 2H), 6.99–6.94 (m, 4H), 5.27 (s, 2H), 4.53 (d, J = 10.2 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.15 (d, J = 10.1 Hz, 1H), 4.08 (d, J = 10.3 Hz, 1H), 2.90–2.83 (m, 1H), 1.82 (s, 3H), 1.23 (d, J = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, acetone-d_6) δ 159.29, 157.65, 157.20, 156.90, 145.51, 142.13, 132.31, 128.07, 122.82, 122.74, 116.62, 115.51, 114.99, 94.41, 73.11, 62.40, 52.09, 34.01, 24.51, 22.67; $[\alpha]_D$ −10.21° (*c* 0.46, acetone); HRMS (ESI-TOF) calcd for $C_{25}H_{26}N_6O_5$ $[M + Na]^+$ 513.1863, found 513.1871; HPLC-purity (Method A) 97.8%.

(*R*)-2-{4-[4-(4-*sec*-Butylphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1d (IIIM/MCD-27). TLC (EtOAc–DCM 1 : 9): R_f = 0.30; light yellow solid; mp 180–182 °C; ^{1}H NMR (400 MHz, $CDCl_3$)

δ 7.98 (s, 1H), 7.65 (d, J = 8.7 Hz, 2H), 7.58 (s, 1H), 7.12 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 5.27 (s, 2H), 4.53 (d, J = 10.3 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.16 (d, J = 10.1 Hz, 1H), 4.09 (d, J = 10.3 Hz, 1H), 2.61–2.50 (m, 1H), 1.82 (s, 3H), 1.58–1.51 (m, 3H), 1.21 (d, J = 6.9 Hz, 2H), 0.81 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 159.29, 157.71, 156.91, 146.50, 145.51, 140.80, 132.30, 128.71, 122.83, 122.77, 116.62, 115.46, 115.01, 94.42, 73.10, 62.37, 52.09, 41.60, 31.94, 22.68, 22.44, 12.52; $[\alpha]_D$ −10.9° (c 0.33, acetone); HRMS (ESI-TOF) calcd for $C_{26}H_{28}N_6O_5$ $[M + H]^+$ 527.2019, found 527.2027; HPLC-purity (Method A) 99.4%.

(*R*)-2-{4-[4-(4-Fluorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1e (IIIM/MCD-28). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 187–189 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.65 (d, J = 8.8 Hz, 2H), 7.59 (s, 1H), 7.02–6.94 (m, 6H), 5.25 (s, 2H), 4.53 (d, J = 10.3 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.16 (d, J = 10.1 Hz, 1H), 4.09 (d, J = 10.2 Hz, 1H), 1.82 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 159.33, 158.25 (d, J = 236.7 Hz), 156.90, 155.78 (d, J = 1.9 Hz), 145.13, 144.74, 132.27, 122.90, 122.84, 117.01 (d, J = 8.1 Hz), 116.63, 116.57 (d, J = 23.2 Hz), 114.99, 94.42, 73.12, 62.96, 52.09, 22.67; $[\alpha]_D$ −9.58° (c 0.48, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{19}FN_6O_5$ $[M + Na]^+$ 489.1299, found 489.1312; HPLC-purity (Method A) 95.05%.

(*R*)-2-{4-[4-(4-Trifluoromethylphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]phenoxy methyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 1f (IIIM/MCD-25). TLC (EtOAc–DCM 1 : 9): R_f = 0.30; light yellow solid; mp 192–194 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.66 (d, J = 9.0 Hz, 2H), 7.58–7.56 (m, 3H), 7.10 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 9.0 Hz, 2H), 5.33 (s, 2H), 4.52 (d, J = 10.2 Hz, 1H), 4.31 (d, J = 10.2 Hz, 1H), 4.16 (d, J = 10.2 Hz, 1H), 4.09 (d, J = 10.2 Hz, 1H), 1.82 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.22, 159.37, 156.91, 147.76, 144.59, 132.22, 127.80 (q, J = 3.8 Hz), 126.97, 123.16, 122.89, 116.65, 116.10, 115.00, 94.43, 73.13, 62.61, 52.10, 22.67;$[\alpha]_D$ −10° (c 0.4, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{19}F_3N_6O_5$ $[M + Na]^+$ 539.1267, found 539.1277; HPLC-purity (Method A) 99.7%.

(*R*)-2-{4-[4-(4-Trifluoromethoxyphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]phenoxy methyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 1g (IIIM/MCD-19). TLC (EtOAc–DCM 1 : 9): R_f = 0.35; light yellow solid; mp 188–190 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.60 (s, 1H), 7.91 (s, 1H), 7.82 (d, J = 9.0 Hz, 2H), 7.29 (d, J = 8.6 Hz, 2H), 7.23–7.12 (m, 4H), 5.30 (s, 2H), 4.67 (d, J = 10.8 Hz, 1H), 4.50 (d, J = 10.6 Hz, 1H), 4.46 (d, J = 10.7 Hz, 1H), 4.37 (d, J = 10.8 Hz, 1H), 1.85 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 159.35, 158.30, 156.91, 147.76, 144.86, 143.64, 132.24, 123.36, 123.05, 122.87, 116.86, 116.64, 115.02, 94.45, 73.13, 62.81, 52.10, 22.67. $[\alpha]_D$ −9.6° (c 0.5, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{19}F_3N_6O_6$ $[M + Na]^+$ 555.1216, found 555.1232; HPLC-purity (Method A) 99.7%.

(*R*)-2-{4-[4-(2-Fluorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1h (IIIM/MCD-31). TLC (EtOAc–DCM 1 : 9): R_f = 0.3; light yellow solid; mp 180–182 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.59 (s, 1H), 7.16 (t, J = 7.9 Hz, 1H), 7.13–7.06 (m, 2H), 6.99 (d, J = 9.0 Hz, 2H), 6.96–6.92 (m, 1H), 5.36 (s, 2H), 4.53 (d, J = 10.3 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.16 (d, J = 11.1 Hz, 1H), 4.09 (d, J = 11.4 Hz, 1H), 1.82 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 159.32, 156.91, 154.47, 153.49 (d, J = 244.4 Hz), 147.37 (d, J = 10.5 Hz), 144.76, 132.21, 125.48 (d, J = 4.1 Hz), 123.18, 122.87, 122.45 (d, J = 5.1 Hz), 116.89 (d, J = 18.3 Hz), 116.60, 116.53, 115.07 (d, J = 5.3 Hz), 94.44, 73.08, 63.32, 52.22, 22.67; $[\alpha]_D$ −7.39° (c 0.46, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{19}FN_6O_5$ $[M + Na]^+$ 489.1299, found 489.1298; HPLC-purity (Method B) 99.6%.

(*R*)-2-{4-[4-(3-Chlorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1i (IIIM/MCD-30). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 167–169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.66 (d, J = 8.9 Hz, 2H), 7.58 (s, 1H), 7.23 (t, J = 8.2 Hz, 1H), 7.08–6.86 (m, 5H), 5.27 (s, 2H), 4.53 (d, J = 10.3 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.16 (d, J = 10.2 Hz, 1H), 4.09 (dd, J = 10.2 Hz, 1H), 1.82 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 160.38, 159.33, 156.91, 147.73, 144.78, 135.29, 132.24, 131.60, 123.09, 122.86, 121.88, 116.63, 115.95, 115.02, 114.51, 94.42, 73.11, 62.61, 52.09, 22.67; $[\alpha]_D$ −10.68° (c 0.44, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{19}ClN_6O_5$ $[M + Na]^+$ 505.1003, found 505.0997; HPLC-purity (Method B) 95.6%.

(*R*)-2-{3-[4-(4-Trifluoromethoxyphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]phenoxy methyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 1j (IIIM/MCD-47). TLC (EtOAc–DCM 1 : 9): R_f = 0.35; light yellow solid; mp 156–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.57 (s, 1H), 7.44 (t, J = 8.2 Hz, 1H), 7.34 (dd, J = 17.3, 5.4 Hz, 2H), 7.17 (d, J = 8.9 Hz, 2H), 7.02 (d, J = 9.1 Hz, 2H), 6.93 (dd, J = 8.2, 2.1 Hz, 1H), 5.28 (s, 2H), 4.51 (d, J = 10.2 Hz, 1H), 4.34 (d, J = 10.3 Hz, 1H), 4.18 (d, J = 10.2 Hz, 1H), 4.08 (d, J = 10.2 Hz, 1H), 1.81 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 160.19, 158.25, 156.88, 147.70, 145.03, 143.60, 139.09, 131.80, 123.43, 123.17, 116.82, 116.09, 115.14, 113.99, 107.66, 94.45, 73.04, 62.71, 52.07, 22.70; $[\alpha]_D$ −8.75° (c 0.4, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{19}F_3N_6O_6$ $[M + H]^+$ 533.13963, found 533.1391; HPLC-purity (Method A) 95.22%.

(*R*)-2-{3-[4-(4-Fluorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]-phenoxy methyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1k (IIIM/MCD-48). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 154–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.57 (s, 1H), 7.43 (t, J = 8.2 Hz, 1H), 7.33 (dd, J = 17.9, 5.0 Hz, 2H), 7.03–6.90 (m, 5H), 5.25 (s, 2H), 4.51 (d, J = 10.3 Hz, 1H), 4.34 (d, J = 10.2 Hz, 1H), 4.18 (d, J = 10.2 Hz, 1H), 4.08 (d, J = 10.2 Hz, 1H), 1.81 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 160.18, 158.25 (d, J = 236.7 Hz), 156.88, 155.71 (d, J = 2.0 Hz), 147.70, 145.30, 139.10, 131.80, 123.04, 116.99 (d, J = 8.0 Hz), 116.61 (d, J = 23.2 Hz), 116.10, 115.15, 113.96, 107.65, 94.46, 73.04, 62.86, 52.07, 22.71; $[\alpha]_D$ −9.52° (c 0.42, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{19}FN_6O_5$ $[M + H]^+$ 467.1479, found 467.1471; HPLC-purity (Method A) 95.4%.

(*R*)-2-{2-[4-(4-Trifluoromethoxyphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]phenoxy methyl}-2,3-dihydro-2-methyl-6-nitroimi-

dazo[2,1-*b*]oxazole 1l (IIIM/MCD-52). TLC (EtOAc–DCM 1 : 9): R_f = 0.20; light yellow solid; mp 160–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.51–7.47 (m, 2H), 7.45 (s, 1H), 7.20–7.16 (t, J = 7.8 Hz, 3H), 7.09 (d, J = 8.0 Hz, 1H), 7.03 (d, J = 9.2 Hz, 2H), 5.16 (q, J = 12.1 Hz, 2H), 4.38 (d, J = 10.3 Hz, 1H), 4.34 (d, J = 10.2 Hz, 1H), 4.09 (d, J = 10.3 Hz, 1H), 3.87 (d, J = 10.2 Hz, 1H), 1.67 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 158.32, 156.61, 151.58, 147.67, 143.69, 143.59, 131.58, 127.50, 127.14, 126.33, 123.34, 122.82, 116.77, 115.15, 114.85, 94.16, 73.35, 62.49, 51.82, 22.91; $[\alpha]_D$ −4.7° (*c* 0.34, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{19}F_3N_6O_6$ $[M + H]^+$ 533.13963, found 533.1387; HPLC-purity (Method A) 96.4%.

(*R*)-2-{2-[4-(4-Fluorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl]-phenoxy methyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1m (IIIM/MCD-63). TLC (EtOAc–DCM 1 : 9): R_f = 0.20; light yellow solid; mp 162–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.50–7.46 (m, 2H), 7.44 (s, 1H), 7.17 (t, J = 7.7 Hz, 1H), 7.09 (d, J = 8.2 Hz, 1H), 7.03–6.95 (m, 4H), 5.13 (q, J = 12.1 Hz, 2H), 4.37 (d, J = 10.3 Hz, 1H), 4.33 (d, J = 10.3 Hz, 1H), 4.09 (d, J = 10.3 Hz, 1H), 3.87 (d, J = 10.3 Hz, 1H), 1.67 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 158.20 (d, J = 236.5 Hz), 156.61, 155.76 (d, J = 2.0 Hz), 151.56, 147.56, 143.91, 131.63, 127.42, 127.16, 126.28, 122.80, 116.87 (d, J = 8.0 Hz), 116.57 (d, J = 23.1 Hz), 115.29, 114.79, 94.23, 73.32, 62.50, 51.79, 22.93; $[\alpha]_D$ −3.15° (*c* 0.38, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{19}FN_6O_5$ $[M + H]^+$ 467.1479, found 467.1479; HPLC-purity (Method B) 96.7%.

(*R*)-2-{4-[4-(4-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl]phenoxy-methyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 1n (IIIM/MCD-65). TLC (EtOAc–DCM 1 : 9): R_f = 0.20; light yellow solid; mp 254–256 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 8.90 (s, 1H), 8.03 (dd, J = 6.1, 2.7 Hz, 3H), 7.87 (d, J = 9.0 Hz, 2H), 7.26 (t, J = 8.9 Hz, 2H), 7.19 (d, J = 9.1 Hz, 2H), 4.68 (d, J = 10.8 Hz, 1H), 4.52 (d, J = 10.6 Hz, 2H), 4.48 (d, J = 10.6 Hz, 2H), 4.38 (d, J = 10.8 Hz, 1H), 1.86 (s, 3H); $[\alpha]_D$ −9.4° (*c* 0.43, acetone); HRMS (ESI-TOF) calcd for $C_{21}H_{17}FN_6O_4$ $[M + Na]^+$ 459.1193, found 459.1193; HPLC-purity (Method B) 95.5%.

(*R*)-2-{4-[4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenoxy-methyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 1o (IIIM/MCD-66). TLC (EtOAc–DCM 1 : 9): R_f = 0.15; light yellow solid; mp 277–279 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.77 (s, 1H), 8.02 (s, 1H), 7.90 (d, J = 10.0 Hz, 2H), 7.86 (d, J = 9.1 Hz, 2H), 7.18 (d, J = 9.1 Hz, 2H), 7.04 (d, J = 8.9 Hz, 2H), 4.68 (d, J = 10.8 Hz, 1H), 4.51 (d, J = 10.7 Hz, 1H), 4.47 (d, J = 10.6 Hz, 1H), 4.38 (d, J = 10.8 Hz, 1H), 3.85 (s, 3H), 1.85 (s, 3H); $[\alpha]_D$ −9.5° (*c* 0.45, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{20}N_6O_5$ $[M + H]^+$ 449.1573, found 449.1567; HPLC-purity (Method B) 98.9%.

(*R*)-2-{4-[4-(4-Trifluoromethylphenyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1p (IIIM/MCD-67). TLC (EtOAc–DCM 1 : 9): R_f = 0.20; light yellow solid; mp 258–260 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 9.08 (s, 1H), 8.21 (d, J = 7.9 Hz, 2H), 8.02 (s, 1H), 7.93–7.86 (m, 2H), 7.83 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 9.0 Hz, 2H), 4.69 (d, J = 10.8 Hz, 1H), 4.52 (d, J = 10.6 Hz, 1H), 4.48 (d, J = 10.6 Hz, 1H), 4.39 (d, J = 10.8 Hz, 1H), 1.86 (s, 3H); $[\alpha]_D$ −9.5° (*c* 0.41, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{17}F_3N_6O_4$ $[M + H]^+$ 487.1341, found 487.1340; HPLC-purity (Method B) 95.9%.

(*R*)-2-{4-[4-(3-Trifluoromethylphenyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1q (IIIM/MCD-178). TLC (EtOAc–DCM 1 : 9): R_f = 0.30; light yellow solid; mp 219–220 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 9.11 (s, 1H), 8.29 (d, J = 8.0 Hz, 2H), 7.93 (s, 1H), 7.96–7.87 (m, 3H), 7.73 (d, J = 6.2 Hz, 2H), 7.20 (d, J = 9.0 Hz, 2H), 4.69 (d, J = 10.8 Hz, 1H), 4.52 (d, J = 10.6 Hz, 1H), 4.48 (d, J = 10.7 Hz, 1H), 4.39 (d, J = 10.8 Hz, 1H), 1.86 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 158.55, 156.02, 146.86, 146.29, 132.08, 131.29, 130.73 (q, J = 32.1 Hz), 129.93, 129.05, 124.52 (q, J = 3.7 Hz), 121.95 (q, J = 3.6 Hz), 121.87, 119.71, 115.81, 114.16, 93.56, 72.25, 51.21, 21.79; $[\alpha]_D$ −8.03° (*c* 0.51, acetone); LC-MS (ESI+): *m*/*z* 509.18 [M + Na]; HPLC-purity (Method B) 96.15%.

(*R*)-2-{4-[4-(2-Trifluoromethylphenyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1r (IIIM/MCD-51). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 217–219 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.05 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 9.1 Hz, 2H), 7.70–7.65 (m, 1H), 7.59 (s, 1H), 7.53 (t, J = 8.2 Hz, 1H), 7.02 (d, J = 9.1 Hz, 2H), 4.54 (d, J = 10.3 Hz, 1H), 4.33 (d, J = 10.1 Hz, 1H), 4.17 (d, J = 10.1 Hz, 1H), 4.09 (d, J = 10.3 Hz, 1H), 1.83 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 159.44, 156.91, 145.71, 133.26, 132.98, 132.10, 130.68, 129.73, 127.19, 127.14, 126.57, 122.97, 122.33, 116.69, 115.02, 94.43, 73.11, 52.10, 22.68; $[\alpha]_D$ −11.52° (*c* 0.46, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{17}F_3N_6O_4$ $[M + H]^+$ 487.1341, found 487.1334; HPLC-purity (Method A) 96.22%.

(*R*)-2-{4-[4-(4-Trifluoromethoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 1s (IIIM/MCD-68). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 266–268 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.96 (s, 1H), 8.11 (d, J = 8.9 Hz, 2H), 8.02 (s, 1H), 7.92–7.86 (m, 2H), 7.45 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 9.1 Hz, 2H), 4.68 (d, J = 10.8 Hz, 1H), 4.52 (d, J = 10.7 Hz, 1H), 4.48 (d, J = 10.7 Hz, 1H), 4.38 (d, J = 10.8 Hz, 1H), 1.85 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 159.37, 156.91, 149.56, 147.67, 147.31, 132.19, 131.12, 128.08, 122.74, 122.47, 120.19, 116.66, 115.15, 94.50, 73.10, 52.08, 22.68; $[\alpha]_D$ −9.63° (*c* 0.41, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{17}F_3N_6O_5$ $[M + H]^+$ 503.1291, found 503.1283; HPLC-purity (Method B) 97.7%.

General procedure for the preparation of isoxazolyl-based NHIO compounds 2a–v

The reaction of intermediate **3** and isoxazole intermediate **6** or **7** was carried out using the same procedure as mentioned for triazole-NHIO compounds, followed by silica gel column chromatography using a dichloromethane and ethyl acetate mixture as eluents to obtain the isoxazole-NHIO compounds **2a–v.**

(*R*)-2-{4-[5-(4-Methylphenoxymethyl)isoxazol-3-yl]phenoxy-methyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2a (IIIM/MCD-118). TLC (EtOAc–DCM 1 : 9): R_f = 0.3; light yellow

solid; 185–187 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.8 Hz, 2H), 7.59 (s, 1H), 7.13 (d, J = 7.8 Hz, 2H), 6.95–6.89 (m, 4H), 6.60 (s, 1H), 5.19 (s, 2H), 4.53 (d, J = 10.3 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.15 (d, J = 10.1 Hz, 1H), 4.08 (d, J = 10.3 Hz, 1H), 2.32 (s, 3H), 1.83 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 169.78, 162.59, 160.67, 156.97, 156.91, 131.52, 130.83, 130.50, 129.10, 123.29, 116.10, 115.60, 115.01, 102.29, 94.43, 72.79, 61.79, 52.09, 22.69, 20.46; $[\alpha]_D$ −8.56° (c 0.52, acetone); HRMS (ESI-TOF) calcd for $C_{24}H_{22}N_4O_6$ $[M + Na]^+$ 485.1437, found 485.1389; HPLC-purity (Method B) 95.594%.

(*R*)-2-{4-[5-(4-Iso-Propylphenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2b (IIIM/MCD-116). TLC (EtOAc–DCM 1 : 9): R_f = 0.45; light yellow solid; mp 191–193 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.7 Hz, 2H), 7.58 (s, 1H), 7.19 (d, J = 8.0 Hz, 2H), 6.93 (d, J = 8.1 Hz, 4H), 6.61 (s, 1H), 5.18 (s, 2H), 4.53 (d, J = 10.3 Hz, 1H), 4.30 (d, J = 10.0 Hz, 1H), 4.15 (d, J = 10.1 Hz, 1H), 4.09 (d, J = 10.3 Hz, 1H), 2.93–2.86 (m, 1H), 1.82 (s, 3H), 1.25 (d, J = 6.9 Hz, 6H); ^{13}C NMR (126 MHz, acetone-d_6) δ 169.80, 162.61, 160.66, 157.15, 156.89, 147.68, 142.73, 129.11, 128.21, 123.24, 116.08, 115.52, 115.12, 102.28, 94.47, 72.77, 61.72, 52.08, 34.02, 24.50, 22.69; $[\alpha]_D$ −7.77° (c 0.36, acetone); HRMS (ESI-TOF) calcd for $C_{26}H_{26}N_4O_6$ $[M + H]^+$ 491.193, found 491.194; HPLC-purity (Method B) 95.807%.

(*R*)-2-{4-[5-(4-Trifluoromethylphenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2c (IIIM/MCD-115). TLC (EtOAc–DCM 1 : 9): R_f = 0.35; light yellow solid; mp 200–202 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.9 Hz, 2H), 7.59 (d, J = 8.6 Hz, 2H), 7.57 (s, 1H), 7.06 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.9 Hz, 2H), 6.61 (s, 1H), 5.24 (s, 2H), 4.51 (d, J = 10.2 Hz, 1H), 4.29 (d, J = 10.1 Hz, 1H), 4.14 (d, J = 10.1 Hz, 1H), 4.06 (d, J = 10.2 Hz, 1H), 1.81 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 168.81, 162.71, 161.72, 160.73, 156.92, 147.73, 129.13, 127.94 (q, J = 3.9 Hz), 124.03, 123.13, 116.11, 116.10, 115.05, 102.78, 94.39, 72.79, 61.81, 52.09, 22.69; $[\alpha]_D$ −9.05° (c 0.53, acetone); HRMS (ESI-TOF) calcd for $C_{24}H_{19}F_3N_4O_6$ $[M + H]^+$ 517.1335, found 517.1333; HPLC-purity (Method B) 97.278%.

(*R*)-2-{4-[5-(4-Methoxyphenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2d (IIIM/MCD-128). TLC (EtOAc–DCM 1 : 9): R_f = 0.35; light yellow solid; mp 198–200 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.8 Hz, 2H), 7.57 (s, 1H), 6.94–6.91 (m, 4H), 6.85 (d, J = 9.2 Hz, 2H), 6.57 (s, 1H), 5.14 (s, 2H), 4.51 (d, J = 10.2 Hz, 1H), 4.29 (d, J = 10.1 Hz, 1H), 4.14 (d, J = 10.1 Hz, 1H), 4.06 (d, J = 10.2 Hz, 1H), 3.78 (s, 3H), 1.81 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 169.87, 162.60, 160.67, 156.92, 155.57, 153.01, 130.51, 129.11, 123.24, 116.91, 116.11, 115.52, 115.14, 102.32, 94.52, 72.79, 62.41, 55.87, 52.09, 22.67; $[\alpha]_D$ −10.6° (c 0.33, acetone); HRMS (ESI-TOF) calcd for $C_{24}H_{22}N_4O_7$ $[M + H]^+$ 479.1566, found 479.1559; HPLC-purity (Method B) 95.891%.

(*R*)-2-{4-[5-(4-Trifluoromethoxyphenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2e (IIIM/MCD-69). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 195–197 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 8.9 Hz, 2H), 7.56 (s, 1H), 7.18 (d, J = 9.0 Hz, 2H), 6.98 (d, J = 9.2 Hz, 2H), 6.92 (d, J = 8.9 Hz, 2H), 6.60 (s, 1H), 5.18 (s, 2H), 4.51 (d, J = 10.2 Hz, 1H), 4.29 (d, J = 10.1 Hz, 1H), 4.14 (d, J = 10.1 Hz, 1H), 4.06 (d, J = 10.2 Hz, 1H), 1.81 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 169.10, 162.67, 160.72, 157.81, 156.91, 144.02, 144.01, 129.11, 123.48, 123.20, 116.94, 116.12, 114.99, 102.61, 94.42, 72.80, 62.12, 52.10, 22.69; $[\alpha]_D$ −7.77° (c 0.54, acetone); HRMS (ESI-TOF) calcd for $C_{24}H_{19}F_3N_4O_6$ $[M + H]^+$ 533.1284, found 533.1277; HPLC-purity (Method B) 99.75%.

(*R*)-2-{4-[5-(4-Fluorophenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2f (IIIM/MCD-114). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 175–177 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 8.8 Hz, 2H), 7.57 (s, 1H), 7.03–6.99 (m, 2H), 6.95–6.90 (m, 4H), 6.58 (s, 1H), 5.16 (s, 2H), 4.51 (d, J = 10.2 Hz, 1H), 4.29 (d, J = 10.1 Hz, 1H), 4.14 (d, J = 10.1 Hz, 1H), 4.06 (d, J = 10.2 Hz, 1H), 1.81 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 169.40, 162.63, 160.71, 158.8 (d, J = 237.4 Hz), 156.91, 155.32 (d, J = 2.0 Hz), 147.78, 129.10, 123.27, 117.22 (d, J = 8.1 Hz), 116.71 (d, J = 23.4 Hz), 116.12, 114.94, 102.44, 94.40, 72.82, 62.38, 52.10, 22.69; $[\alpha]_D$ −7.0° (c 0.1, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{19}FN_4O_6$ $[M + H]^+$ 467.1367, found 467.1364; HPLC-purity (Method A) 97.964%.

(*R*)-2-{4-[5-(4-Bromophenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2g (IIIM/MCD-127). TLC (EtOAc–DCM 1 : 9): R_f = 0.45; light yellow solid; mp 208–210 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.92 (s, 1H), 7.85 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 9.0 Hz, 2H), 7.09–7.06 (m, 4H), 7.00 (s, 1H), 5.35 (s, 2H), 4.67 (d, J = 10.8 Hz, 1H), 4.49 (d, J = 10.7 Hz, 1H), 4.44 (d, J = 10.7 Hz, 1H), 4.38 (d, J = 10.8 Hz, 1H), 1.85 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 169.12, 162.65, 160.71, 158.31, 156.91, 147.73, 133.28, 129.12, 123.17, 117.89, 116.10, 115.05, 114.15, 102.61, 94.43, 72.78, 61.88, 52.09, 22.69; $[\alpha]_D$ −8.68° (c 0.38, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{19}BrN_4O_6$ $[M + H]^+$ 527.0566, found 527.0559; HPLC-purity (Method B) 95.24%.

(*R*)-2-{4-[5-(3-Fluorophenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole 2h (IIIM/MCD-175). TLC (EtOAc–DCM 1 : 9): R_f = 0.3; light yellow solid; mp 164–166 °C;^{1}H NMR (400 MHz, acetone-d_6) δ 7.92 (s, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.36 (dd, J = 15.3, 8.0 Hz, 1H), 7.07 (d, J = 8.8 Hz, 2H), 7.01 (s, 1H), 6.94–6.88 (m, 2H), 6.80–6.76 (m, 1H), 5.35 (s, 2H), 4.66 (d, J = 10.8 Hz, 1H), 4.47 (d, J = 10.6 Hz, 2H), 4.42 (d, J = 10.6 Hz, 2H), 4.36 (d, J = 10.8 Hz, 1H), 1.84 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 169.04, 164.45 (d, J = 243.8 Hz), 162.67, 160.43 (d, J = 10.9 Hz), 160.38, 156.91, 147.74, 131.66 (d, J = 10.1 Hz), 129.12, 123.19, 116.11, 115.03, 111.79 (d, J = 2.9 Hz), 108.98 (d, J = 21.4 Hz), 103.32 (d, J = 25.3 Hz), 102.64, 94.44, 72.80, 61.92, 52.10, 22.69; $[\alpha]_D$ −9.42° (c 0.35, acetone); LC-MS (ESI+): m/z 467.14 [M + H]; HPLC-purity (Method B) 95.52%.

(*R*)-2-{4-[5-(2-Fluorophenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2i (IIIM/MCD-117). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 174–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.8 Hz, 2H), 7.57 (s, 1H), 7.15–7.10 (m, 1H), 7.09–7.02 (m, 2H),

7.01–6.96 (m, 1H), 6.92 (d, J = 8.8 Hz, 2H), 6.63 (s, 1H), 5.26 (s, 2H), 4.51 (d, J = 10.2 Hz, 1H), 4.29 (d, J = 10.1 Hz, 1H), 4.14 (d, J = 10.1 Hz, 1H), 4.06 (d, J = 10.2 Hz, 1H), 1.81 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 169.04, 162.67, 160.71, 156.91, 152.39, 146.88, 130.57, 129.12, 125.57 (d, J = 3.8 Hz), 123.28, 123.20 (d, J = 2.7 Hz), 117.14 (d, J = 18.2 Hz), 116.90, 116.10, 115.02, 102.75, 94.42, 72.79, 62.78, 52.09, 22; $[\alpha]_D$ −8.0° (c 0.4, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{19}FN_4O_6$ $[M + H]^+$ 467.1367, found 467.1367; HPLC-purity (Method B) 98.817%.

(*R*)-2-{4-[5-(2-Methylphenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2j (IIIM/MCD-177). TLC (EtOAc–DCM 1 : 9): R_f = 0.32; light yellow solid; mp 187–188 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.92 (s, 1H), 7.85 (d, J = 8.9 Hz, 2H), 7.18 (m, 2H), 7.08 (m, 3H), 6.99 (s, 1H), 6.90 (t, J = 7.4 Hz, 1H), 5.32 (s, 2H), 4.66 (d, J = 10.8 Hz, 1H), 4.47 (d, J = 10.6 Hz, 1H), 4.43 (d, J = 10.6 Hz, 1H), 4.37 (d, J = 10.8 Hz, 1H), 2.23 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 169.87, 162.61, 160.67, 157.08, 156.91, 131.64, 129.12, 127.85, 127.56, 123.25, 122.18, 116.07, 116.01, 115.09, 112.60, 102.18, 94.45, 72.76, 61.93, 52.08, 22.69, 16.34; $[\alpha]_D$ −9.51° (c 0.41, acetone); LC-MS (ESI+): m/z 463.10 [M + H]; HPLC-purity (Method B) 98.31%.

(*R*)-2-{4-[5-(3-Chlorophenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2k (IIIM/MCD-138). TLC (EtOAc–DCM 1 : 9): R_f = 0.3; light yellow solid; mp 162–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.8 Hz, 2H), 7.58 (s, 1H), 7.23 (d, J = 7.9 Hz, 1H), 7.00 (t, J = 4.2 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.90–6.85 (m, 1H), 6.60 (s, 1H), 5.18 (s, 2H), 4.52 (d, J = 10.3 Hz, 1H), 4.29 (d, J = 10.1 Hz, 1H), 4.14 (d, J = 10.1 Hz, 1H), 4.07 (d, J = 10.3 Hz, 1H), 1.81 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 169.02, 162.68, 160.71, 159.88, 156.92, 147.73, 135.39, 131.73, 130.50, 129.13, 123.16, 122.46, 116.11, 115.07, 114.50, 102.66, 94.45, 72.78, 61.90, 52.09, 22.70; $[\alpha]_D$ −8.37° (c 0.43, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{19}ClN_4O_6$ $[M + H]^+$ 483.1071, found 483.1072; HPLC-purity (Method B) 96.93%.

(*R*)-2-{4-[5-(3-Methyl,4-iso-propylphenoxymethyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]-oxazole 2l (IIIM/MCD-139). TLC (EtOAc–DCM 1 : 9): R_f = 0.5; light yellow solid; mp 176–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.8 Hz, 2H), 7.57 (s, 1H), 7.17 (d, J = 8.3 Hz, 1H), 6.92 (d, J = 8.9 Hz, 2H), 6.82–6.75 (m, 2H), 6.59 (s, 1H), 5.16 (s, 2H), 4.51 (d, J = 10.3 Hz, 1H), 4.29 (d, J = 10.1 Hz, 1H), 4.13 (d, J = 10.1 Hz, 1H), 4.07 (d, J = 10.3 Hz, 1H), 3.11–3.04 (m, 1H), 2.32 (s, 3H), 1.81 (s, 3H), 1.20 (d, J = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, acetone-d_6) δ 169.92, 162.59, 160.67, 156.91, 156.78, 147.75, 140.75, 137.17, 129.10, 126.54, 123.31, 117.48, 116.10, 114.96, 113.10, 102.20, 94.43, 72.80, 61.68, 52.10, 29.27, 23.70, 22.69, 19.50; $[\alpha]_D$ −10.93° (c 0.58, acetone); HRMS (ESI-TOF) calcd for $C_{27}H_{28}N_4O_6$ $[M + H]^+$ 505.2087, found 505.2101; HPLC-purity (Method B) 98.4614%.

(*R*)-2-{4-[5-Phenylisoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6nitroimidazo [2,1-*b*]oxazole 2m (IIIM/MCD-50). TLC (EtOAc–DCM 1 : 9): R_f = 0.35; light yellow solid; mp 233–235 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85–7.81 (m, 4H), 7.58 (s, 1H), 7.51–7.46 (m, 3H), 6.95 (d, J = 8.8 Hz, 2H), 6.78 (s, 1H), 4.52 (d, J = 10.2 Hz, 1H), 4.31 (d, J = 10.0 Hz, 1H), 4.16 (d, J = 10.0 Hz, 1H), 4.07 (d, J = 10.2 Hz, 1H), 1.82 (s, 3H); ^{13}C NMR (126 MHz, Pyridine-d_5) δ 169.33, 161.75, 158.73, 155.27, 146.54, 129.39, 128.31, 127.61, 126.77, 124.93, 114.52, 113.71, 113.62, 97.29, 92.59, 70.93, 50.23, 21.30; $[\alpha]_D$ −4.49° (c 0.51, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{18}N_4O_5$ $[M + H]^+$ 419.1355, found 419.1348; HPLC-purity (Method A) 97.42%.

(*R*)-2-{4-[5-(4-Methylphenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2n (IIIM/MCD-72). TLC (EtOAc–DCM 1 : 9): R_f = 0.5; light yellow solid; mp 256–258 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.88 (d, J = 8.9 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.5 Hz, 2H), 7.24 (s, 1H), 7.09 (d, J = 8.9 Hz, 2H), 4.66 (d, J = 10.8 Hz, 1H), 4.48 (d, J = 10.6 Hz, 1H), 4.44 (d, J = 10.6 Hz, 1H), 4.36 (d, J = 10.8 Hz, 1H), 2.40 (s, 3H), 1.84 (s, 3H); $[\alpha]_D$ −3.59° (c 0.30, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{20}N_4O_5$ $[M + H]^+$ 433.1521, found 433.1513; HPLC-purity (Method B) 98.77%.

(*R*)-2-{4-[5-(4-Fluorophenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2o (IIIM/MCD-119). TLC (EtOAc–DCM 1 : 9): R_f = 0.4; light yellow solid; mp 248–250 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.99 (dd, J = 9.0, 5.3 Hz, 2H), 7.91 (s, 1H), 7.89 (d, J = 8.9 Hz, 2H), 7.34 (t, J = 8.9 Hz, 2H), 7.30 (s, 1H), 7.10 (d, J = 8.9 Hz, 2H), 4.67 (d, J = 10.8 Hz, 1H), 4.49 (d, J = 10.6 Hz, 1H), 4.44 (d, J = 10.6 Hz, 1H), 4.37 (d, J = 10.8 Hz, 1H), 1.85 (s, 3H); $[\alpha]_D$ −2.66° (c 0.30, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{17}FN_4O_5$ $[M + H]^+$ 437.1261, found 437.1258; HPLC-purity (Method B) 98.86%.

(*R*)-2-{4-[5-(4-Methoxyphenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2p (IIIM/MCD-71). TLC (EtOAc–DCM 1 : 9): R_f = 0.35; light yellow solid; mp 252–254 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.92 (s, 1H), 7.89–7.84 (m, 4H), 7.17 (s, 1H), 7.11–7.08 (m, 4H), 4.67 (d, J = 10.8 Hz, 1H), 4.49 (d, J = 10.6 Hz, 1H), 4.44 (d, J = 10.6 Hz, 1H) 4.37 (d, J = 10.8 Hz, 1H), 3.89 (s, 3H), 1.85 (s, 3H); $[\alpha]_D$ −2.5° (c 0.32, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{20}N_4O_6$ $[M + H]^+$ 449.1461, found 449.1463; HPLC-purity (Method B) 99.69%.

(*R*)-2-{4-[5-(4-Trifluoromethylphenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2q (IIIM/MCD-73). TLC (EtOAc–DCM 1 : 9): R_f = 0.15; light yellow solid; mp 258–260 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.16 (d, J = 8.1 Hz, 2H), 8.02 (s, 1H), 7.93–7.90 (m, 4H), 7.53 (s, 1H), 7.11 (d, J = 8.9 Hz, 2H), 4.67 (d, J = 10.8 Hz, 1H), 4.50 (d, J = 10.6 Hz, 1H), 4.45 (d, J = 10.7 Hz, 1H), 4.37 (d, J = 10.8 Hz, 1H), 1.85 (s, 3H); $[\alpha]_D$ −3.45° (c 0.33, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{17}F_3N_4O_5$ $[M + H]^+$ 487.1229, found 487.1229; HPLC-purity (Method B) 98.76%.

(*R*)-2-{4-[5-(4-Trifluoromethoxyphenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2r (IIIM/MCD-74). TLC (EtOAc–DCM 1 : 9): R_f = 0.3; light yellow solid; mp 254–256 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.07 (d, J = 8.9 Hz, 2H), 7.93–7.86 (m, 3H), 7.54 (d, J = 8.2 Hz, 2H), 7.40 (s, 1H), 7.10 (d, J = 8.9 Hz, 2H), 4.67 (d, J = 10.8 Hz, 1H), 4.49 (d, J = 10.6 Hz, 1H), 4.45 (d, J = 10.6 Hz, 1H), 4.37 (d, J = 10.8 Hz, 1H), 1.85 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 169.51, 163.43, 160.75, 156.92, 150.98, 129.10, 128.58, 127.53, 123.23, 122.62, 120.32, 116.13, 115.14, 99.42, 94.49, 72.84,

52.13, 22.73; $[\alpha]_D$ −5.8° (*c* 0.5, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{17}F_3N_4O_6$ $[M + H]^+$ 503.1178, found 503.1175; HPLC-purity (Method B) 95.94%.

(*R*)-2-{4-[5-(3-Trifluoromethylphenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2s (IIIM/MCD-125). TLC (EtOAc–DCM 1 : 9): R_f = 0.3; light yellow solid; mp 233–235 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.25 (s, 1H), 8.23 (m, 1H), 7.99–7.78 (m, 5H), 7.57 (s, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 4.69 (d, *J* = 10.8 Hz, 1H), 4.51 (d, *J* = 10.6 Hz, 1H), 4.47 (d, *J* = 10.6 Hz, 1H), 4.39 (d, *J* = 10.8 Hz, 1H), 1.86 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6) δ 169.24, 163.50, 160.80, 156.93, 132.04, 131.78, 131.28, 130.11, 129.37, 129.12, 127.58 (q, *J* = 7.4 Hz), 123.17 (q, *J* = 3.8 Hz), 116.77, 116.15, 115.08, 100.06, 94.46, 72.81, 52.10, 22.70; $[\alpha]_D$ −5.63° (*c* 0.55, acetone); HRMS (ESI-TOF) calcd for $C_{23}H_{17}F_3N_4O_5$ $[M + H]^+$ 487.1229, found 487.1223; HPLC-purity (Method B) 99.8446%.

(*R*)-2-{4-[5-(2-Trifluoromethylphenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2t (IIIM/MCD-176). TLC (EtOAc–DCM 2 : 8): R_f = 0.20; light yellow solid; mp 221–223 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.98 (d, *J* = 7.8 Hz, 1H), 7.95–7.78 (m, 6H), 7.16 (s, 1H), 7.11 (d, *J* = 8.7 Hz, 2H), 4.67 (d, *J* = 10.8 Hz, 1H), 4.50 (d, *J* = 10.7 Hz, 1H), 4.45 (d, *J* = 10.7 Hz, 1H), 4.38 (d, *J* = 10.8 Hz, 1H), 1.85 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 168.76, 162.93, 160.78, 156.92, 133.61, 132.38, 131.77, 129.17, 128.60 (q, *J* = 31.4 Hz), 127.73 (q, *J* = 5.5 Hz), 127.17 (q, *J* = 4.1 Hz), 126.08, 123.14, 116.17, 115.04, 103.14 (q, *J* = 2.2 Hz), 94.45, 72.81, 52.11, 22.70; $[\alpha]_D$ −10.25° (*c* 0.40, acetone); LC-MS (ESI+): *m*/*z* 487.0 $[M + H]^+$; HPLC-purity (Method B) 96.76%.

(*R*)-2-{4-[5-(2-Fluorophenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2u (IIIM/MCD-126). TLC (EtOAc–DCM 1 : 9): R_f = 0.20; light yellow solid; mp 235–237 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.03–7.99 (m, 1H), 7.94 (d, *J* = 8.9 Hz, 2H), 7.91 (s, 1H), 7.64–7.57 (m, 1H), 7.46–7.35 (m, 2H), 7.25 (d, *J* = 3.3 Hz, 1H), 7.10 (d, *J* = 8.9 Hz, 2H), 4.67 (d, *J* = 10.8 Hz, 1H), 4.50 (d, *J* = 10.6 Hz, 1H), 4.45 (d, *J* = 10.6 Hz, 1H), 4.37 (d, *J* = 10.8 Hz, 1H), 1.85 (s, 3H); $[\alpha]_D$ −3.44° (*c* 0.44, acetone); HRMS (ESI-TOF) calcd for $C_{22}H_{17}FN_4O_5$ $[M + H]^+$ 437.1261, found 437.1262; HPLC-purity (Method B) 95.7503%.

(*R*)-2-{4-[5-(2,4-Difluorophenyl)isoxazol-3-yl]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 2v (IIIM/MCD-70). TLC (EtOAc–DCM 1 : 9): R_f = 0.25; light yellow solid; mp 269–271 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.99 (m, 1H), 7.88 (d, *J* = 8.9 Hz, 2H), 7.86 (s, 1H), 7.29–7.18 (m, 2H), 7.17 (d, *J* = 4.3 Hz, 1H), 7.05 (d, *J* = 8.9 Hz, 2H), 4.62 (d, *J* = 10.8 Hz, 1H), 4.45 (d, *J* = 10.6 Hz, 1H), 4.40 (d, *J* = 10.6 Hz, 1H), 4.32 (d, *J* = 10.8 Hz, 1H), 1.80 (s, 3H); $[\alpha]_D$ −2.47 (*c* 0.42, cetone); HRMS (ESI-TOF) calcd for $C_{22}H_{16}F_2N_4O_5$ $[M + H]^+$ 455.1167, found 455.1166; HPLC-purity (Method B) 99.436%.

Biological evaluation

***In vitro* activity against *M. tuberculosis* H_{37}Rv and against the clinical isolate *M. tuberculosis* MDR.** MIC determination: MIC was determined by the broth dilution method against *M. tuberculosis* H_{37}Rv (ATCC 27294; American Type Culture Collection, Manassas, VA, USA), *M. tuberculosis* MDR (resistant to isoniazid and rifampicin), and against one laboratory-generated mutant: *M. tuberculosis* Rif^R (ref. 15) (resistant to rifampicin) using the micro-broth dilution method. The bacterial strains were grown for 10 to 15 days in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI, USA) supplemented with 0.5% (v/v) glycerol, 0.25% (v/v) Tween 80 (Himedia, Mumbai, India), and 10% ADC (albumin dextrose catalase, Becton Dickinson, Sparks, MD, USA) under shaking conditions at 37 °C in 5% CO_2 to facilitate exponential-phase growth of the organism. A bacterial suspension was prepared by suspending the *M. tuberculosis* growth in normal saline containing 0.5% Tween 80 and the turbidity was adjusted to 1 McFarland (McF) standard, which is equivalent to 1.0×10^7 CFU ml^{-1}. The 2-fold serial dilutions of compounds were prepared in Middle brook 7H9 (Difco laboratories) for *M. tuberculosis* in 100 μl per well in 96-well U bottom microtitre plates (Tarson, Mumbai, India). The above-mentioned bacterial suspension was further diluted 1 : 10 in the growth media and a 100 μl volume of this diluted inoculum was added to each well of the plate, resulting in a final inoculum of 1.0×10^6 CFU ml^{-1} in the well, and the final concentrations of compounds ranging from 0.015 μg ml^{-1} to 32 μg ml^{-1}. The plates were incubated at 37 °C for seven days in 5% CO_2. For evaluation of the results, the Resaurin Microtitre Assay (REMA) method was used. After incubation, 15 μl of 0.04% resazurin and 12.5 μl of 20% Tween 80 was added in each well of the plate, including in the media and growth controls. After 48 h incubation, the plates were read visually and the minimum concentration of the compound showing no change of colour was recorded as the MIC.

***In vitro* activity against non-replicating MTB.** Streptomycin-starved *M. tuberculosis* 18b (ss18b) in a non-replicating phase (NRP) of growth was grown according to the method described earlier[16] using Middlebrook 7H9 media. In brief, ss18b was grown to the mid-log phase in Middlebrook 7H9 supplemented with 10% ADC and 50 μg ml^{-1} streptomycin. Streptomycin was then removed from the media by washing the bacteria with phosphate buffer saline (pH 7.4) three times and then incubated at 37 °C in 5% CO_2 in streptomycin-free 7H9 media for a period for 14–16 days until it stopped growing and the optical density of the culture become nearly constant. A total of 0.2 O.D was used as the inoculum for setting up the MIC. All other conditions were the same as those used for MIC determination and for evaluation by the REMA method.

Evaluation of cytotoxicity in HepG2 cells

The cytotoxicity of the compounds was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[20] The Human HepG2 cell line was maintained in *Dulbecco's Modified Eagle's medium* (Gibco Life Technology, NY). The cells were plated at a density of 10 000 cell per well in a 96-microwell flat-bottom plate and incubated for 24 h (37 °C; 5% CO_2). The cells monolayer was exposed to the single concentration of 40 μg ml^{-1} of the tested compounds and incubated for 24 h (37 °C; 5% CO_2). MTT dye was added at a concentration of 2.5 mg ml^{-1} dissolved in phosphate buffer

saline (PBS) and the cell viability was determined by measuring the absorbance of the reduced formazan at 570 nm in a plate reader. The per cent cytotoxicity achieved by the compounds was calculated according to standard methods using tamoxifen as a negative control and healthy cells as a positive control. The cytotoxicity is reported as CC_{50} – the concentration that causes a 50% reduction in cell viability.

In vivo pharmacokinetics

The compounds were administered orally to female Balb/c mice (three mice in each group) at a dose of 5 mg kg^{-1} as a suspension in 0.5% CMC and Tween 80. The samples were derived from plasma at different time points: 0.16 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h, and were then analyzed by LC-MS/MS to generate the required pharmacokinetic parameters.

In vivo efficacy determination

For intranasal instillation purposes, an active growing culture of MTB $H_{37}Rv$ was used. In brief, a freeze-thawed vial was activated by incubating the culture in Middlebrook 7H9 Middle brook broth containing 10% OADC for 48 h under shaking at 37 °C, keeping the final density of cells to 1 McF. A total of five groups of Balb/c female mice containing six numbers and with an average body weight of 20–22 g were taken for experiment. The animals were kept in cages in a Biosafety laboratory for seven days prior to the start of the experiment for acclimation with levels of **1g** 100 mg kg^{-1}, **2e** 100 mg kg^{-1}, drug control (Rifampicin 20 mg kg^{-1}) and placebo, and no drug control. After sonicating the *M. tuberculosis* suspension to make a uniform suspension, 20 μl of it was given through the nasal route to the anesthetized mice. CFU determination was done after 48 h post-infection to enumerate the bacterial load in the lungs for early control. Dosing was started after 48 h of infection (six days a week) and continued for 28 days. The bacterial load was determined in the left lung of each infected mice (six in each group). Serial tenfold dilutions made from homogenized lungs in normal saline solution were applied on Middlebrook 7H10 Agar supplemented with 10% OADC plates. The results were determined as CFU ml^{-1}.

Dose preparation and administration

For preparing the dose, the compound or drug was dissolved in a minimum amount of DMSO and then mixed in alcohol, so as to make final volume up to 5% ethanol and 95% PEG 400 (v/v) mixed. The compounds were dissolved to make a final concentration of 100 mg kg^{-1}, while Rifampicin was prepared at a concentration of 20 mg kg^{-1}. A total of 200 μl volume of the respective dose was administered orally (oral gavage) in a biosafety cabinet to each group. The same volume of mixture, *i.e.*, 5% ethanol and 95% PEG 400 (v/v), was given to the placebo group. A group of mice was kept without dosing to serve as the control.

Combination studies assay

The efficacy of compound **1g** (conc. range 0.25 μg ml^{-1} to 0.007 μg ml^{-1}) in combination with currently used anti-TB drugs, such as rifampicin, isoniazid, and ethambutol (each drug tested at conc. range of 4 μg ml^{-1} to 0.007 μg ml^{-1}), was determined *in vitro* using the checkerboard method. The checkerboard procedure was performed based on the MIC values obtained from the broth microdilution method. The checkerboard method was performed in 96-well U bottom microtitre plates. 100 μl of 4× of the required concentration of drug was added to the first column of the plate and 50 μl of plain media was added to the remaining columns. 50 μl from the first column was then transferred to the second column and was serially diluted in a horizontal manner up to column 10 of the plate. Seven twofold serial dilutions of 4× of the required concentration of compound were prepared in microcentrifuge tubes and 50 μl of each concentration was added vertically, starting from eleventh column of row eight to the second row of the plate. 50 μl of plain media was added to the first row of the plate, to serve as a drug control. 100 μl of 1 : 10 diluted 1 Mc Farland inoculum was added to each well of the plate. The plates were then incubated at 37 °C for 14 days. The MIC of the drug alone and in the presence of compound and *vice versa* was observed visually. The level of synergy was determined by calculating the fractional inhibitory concentration (FIC) index based on the following formula: FIC of drug A = MIC of drug A in combination/MIC of drug A alone; FIC of drug B = MIC of drug B in combination/MIC of drug B alone; and FIC index = FIC of drug A + FIC of drug B. The results of the FIC index were interpreted as follows: ≤0.5: synergy, >0.5 to 0.75: partial synergy, >0.75 to 1.0: additive effect, >1.0 to 4.0: indifference, and >4.0: antagonism. We calculated the FIC index value for each concentration of two-drug combination and the minimum value was adopted.

CYP enzymes assay (fluorescence-based method)

Recombinant cytochrome P450 was aliquoted as per the total concentration required to conduct the study and stored at −80 °C until further use. The total assay volume was adjusted to 200 μl and consisted of three components: cofactors, inhibitor/vehicle, and enzyme substrate (ES) mix. The assay was conducted in black fluorogenic 96-well plates. 50 μl of working concentration of the cofactor was dispensed to all the specified wells, followed by the addition of 50 μl of the diluted working concentrations of **1g** to the specified wells from 1 to 8 in duplicate. Wells from 9 and 10 were the controls without inhibitor, and 11 and 12 were blank wells. Enzyme substrate mix was prepared as per the required volume. The reaction plate with cofactor and the test item and enzyme substrate mix were prepared separately and were pre-incubated at 37 °C in a shaking incubator for 10 minutes. After the pre-incubation, 100 μl of the enzyme substrate mix was added to the required reaction wells in the reaction plate and were incubated at 37 °C in a shaking incubator for 45 min. Incubation was terminated by dispensing 75 μl of 100% acetonitrile after the incubation

time. The fluorescence per well was measured using excitation and emission wavelengths in a Fluorescence Reader/ Fluorescence Detector.[21]

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