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

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Abstract:

Nitroimidazole scaffold developed a great interest in the last decade which ultimately led to the discovery of successful drug delamanid for multi-drug resistant tuberculosis (MDR-TB). Here, we report medicinal chemistry on 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 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** have shown good PK profile. In *in vivo* efficacy studies in intra-nasal model of acute infection, **1g** has shown 1.8 and 1 log CFU reduction with respect to the untreated and early control respectively. The lead compound **1g** had also shown 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 need further detail investigation.

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Introduction:

Tuberculosis (TB) is still one of the major health problem which has taken the lives of 1.3 million people worldwide in 2012.[1] Recent WHO report says globally 8.6 million people were infected with TB and among them 2.3 million were only in India.[2] The existing TB treatment is exceedingly lengthy therapy and emergence of multidrug resistant-TB (MDR-TB) and extensively drug resistant-TB (XDR-TB) has further complicated 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 situations necessitates the discovery and development of novel, potent, efficacious and less toxic anti-tuberculosis agents which in addition also should have capability of shortening the duration of therapy.

In the last decade, nitroimidazole scaffold has developed a great interest among the researchers of academic and industrial fields because of its promising potency against replicating and non-replicating phase of tuberculosis.[4] Anti-tubercular potential of 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 lead compound but unfortunately its mutagenic nature halted its clinical development.[5] Later, PathoGenesis Corporation and Otsuka pharmaceutical companies had overcome the mutagenicity and developed two drug candidates namely PA-824[6] (**B,** a nitroimidazopyran derivative, currently in Phase-II clinical trial) and OPC-67683[7] (**C**, delamanid, 6-nitro-2,3-dihydroimidazooxazole derivative, recently approved by European union for the treatment of MDR-TB[8]). Both the drug candidates are liphophilic in nature, which in fact might help in entry through the highly lipophilic cell wall of MTB and responsible for high potency.[9,10] Our literature survey revealed that

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several groups worked on the nitroimidazooxazine scaffold (PA-824) and synthesized their newer generation analogs,[11] however on the other hand, despite of having comparatively better anti-TB profile of delamanid, no further medicinal chemistry efforts were made on nitrodihydroimidazooxazole scaffold.

Keeping in view the importance of nitrodihydroimidazooxazole scaffold (particularly delamanid shown in Fig 1), we have initiated a medicinal chemistry program 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 anti-TB activity. Interestingly, the present work led to the discovery of new lead compound which has shown potent activity against sensitive MTB (*M. tuberculosis* $H_{37}Rv$), non-replicative MTB (*M. tuberculosis* 18b) and resistant-strain of MTB along with good safety index and *in vivo* efficacy.

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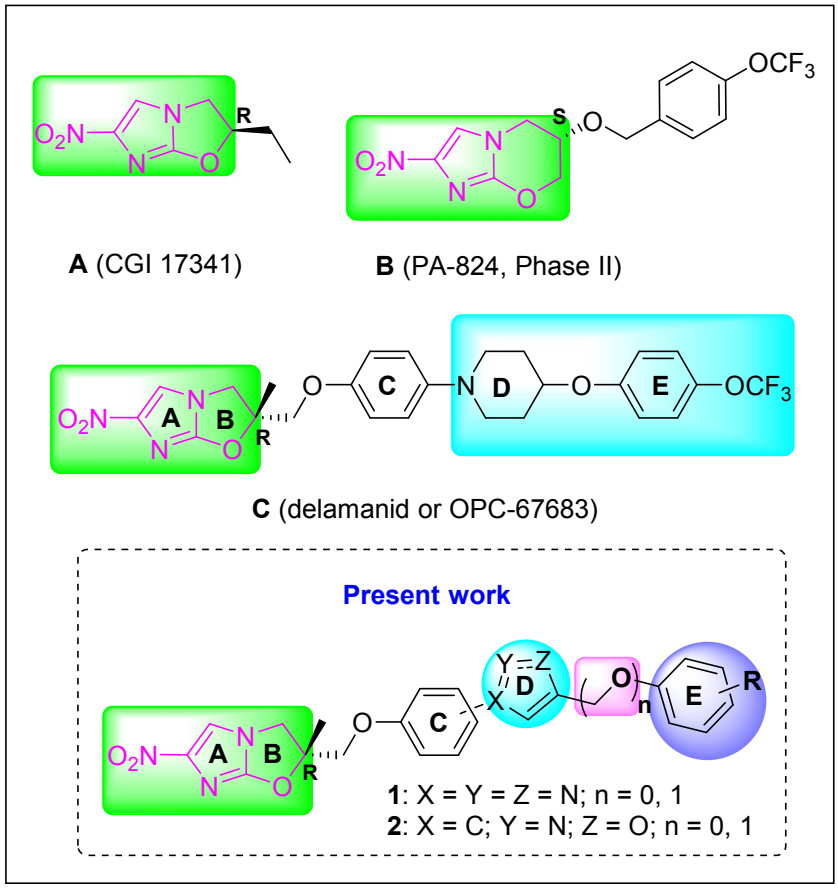

Fig 1: Structure of nitroimidazole based anti-TB agents

Chemistry:

The synthesis of target compounds *i.e.*, triazolyl and isoxazolyl containing 6-nitro-2,3-dihydroimidazooxazole (NHIO) compounds **1** and **2** requires two key intermediates, i) first (*R*)-2-chloro-1-{(2-methyloxiran-2-yl)methyl}-4-nitro-1*H*-imidazole **3** and ii) second is either of following such as 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 form commercially available starting material 4-nitroimidazole following reported method (details given in Supporting information).[7,12]

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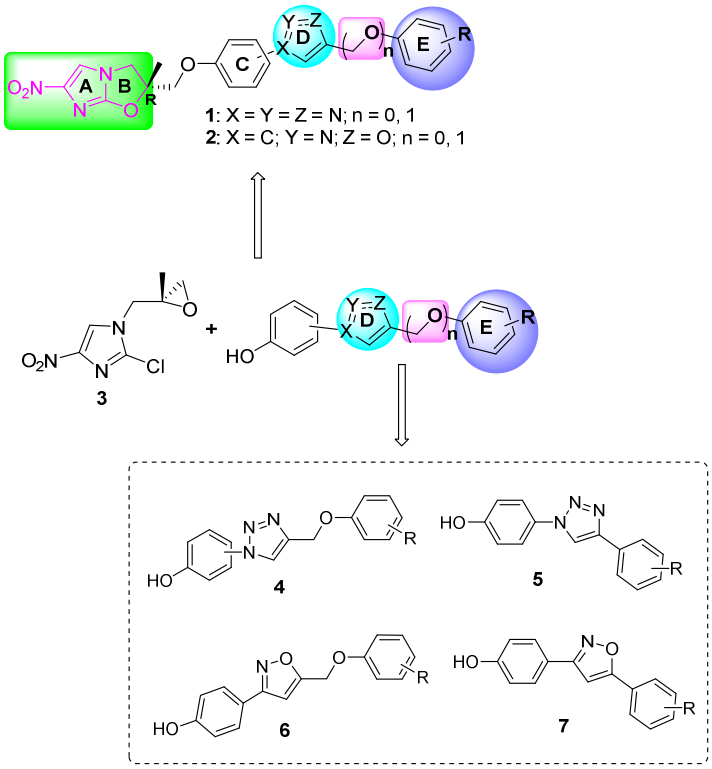

Fig 2: Strategy for the synthesis of target compounds

The other partners were synthesized by following the synthetic routes given in Scheme 1-4. Firstly, 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 second step underwent 2+3 cyclo-addition with azidophenols **12** (which in turn were synthesized from aminophenols **11**) under standard click condition afforded triazolyl containing key intermediates **4** in good yields (Scheme 1).[13] Similarly, the another triazolyl containing intermediates **5** were synthesized from azido phenols **12** on 2+3 cycloaddition with substituted phenylacetylenes **13** (Scheme 2). Finally, the triazolyl containing intermediates **4** and **5** were coupled with intermediate **3** in the presence of sodium hydride afforded 6-nitro-2,3-dihydroimidazooxazole (NHIO) compounds **1a-m** and **1n-s** respectively (Scheme 1 & 2).

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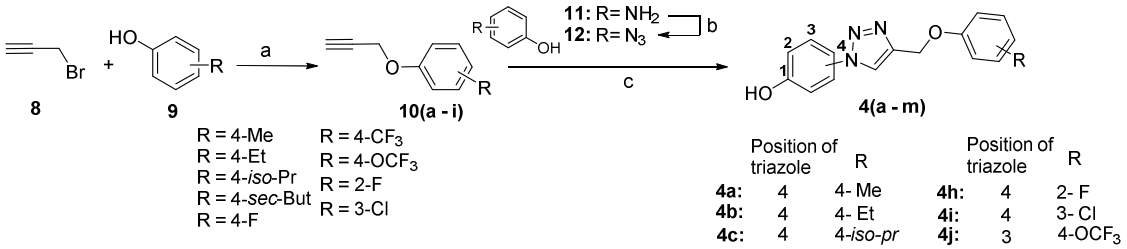
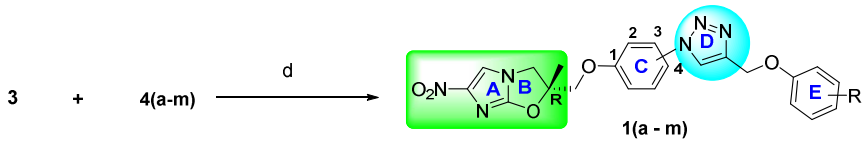
Scheme 1: Synthesis of compounds **1a-m**[a]



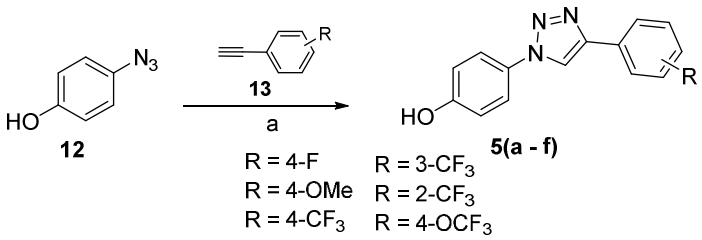
	Position of triazole	R		Position of triazole	R
4a:	4	4- Me	**4h:**	4	2- F
4b:	4	4- Et	**4i:**	4	3- Cl
4c:	4	4-*iso-pr*	**4j:**	3	4-OCF_3
4d:	4	4-*sec*-but	**4k:**	3	4-F
4e:	4	4-F	**4l:**	2	4-OCF_3
4f:	4	4-CF_3	**4m:**	2	4-F
4g:	4	4-OCF_3			

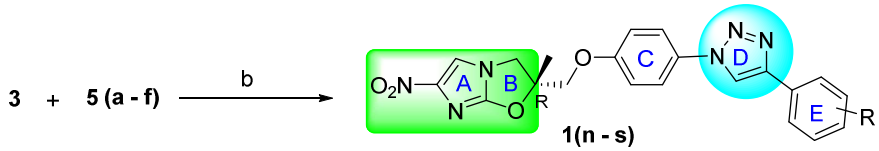


	Position of triazole	R		Position of triazole	R
1a:	4	4- Me	**1h:**	4	2- F
1b:	4	4- Et	**1i:**	4	3- Cl
1c:	4	4-*iso-pr*	**1j:**	3	4-OCF_3
1d:	4	4-*sec*-but	**1k:**	3	4-F
1e:	4	4-F	**1l:**	2	4-OCF_3
1f:	4	4-CF_3	**1m:**	2	4-F
1g:	4	4-OCF_3			

[a]**Reagents and conditions:** a) K_2CO_3, ACN, rt, 12 h, 85-90%; b) $NaNO_2$, HCl, 0-5 °C, 2h, NaN_3, H_2O, 2h, 85%; c) $CuSO_4$, ^{t}BuOH, 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**[a]





1n: R = 4-F **1q**: R = 3-CF_3
1o: R = 4-OMe **1r**: R = 2-CF_3
1p: R = 4-CF_3 **1s**: R = 4-OCF_3

[a]**Reagents and Conditions**: a) $CuSO_4$, ^{t}BuOH, H_2O, sodium ascorbate, rt, 12 h, 80-90%; b) NaH, DMF, 0 to 50 °C, 12 h, 20-40%.

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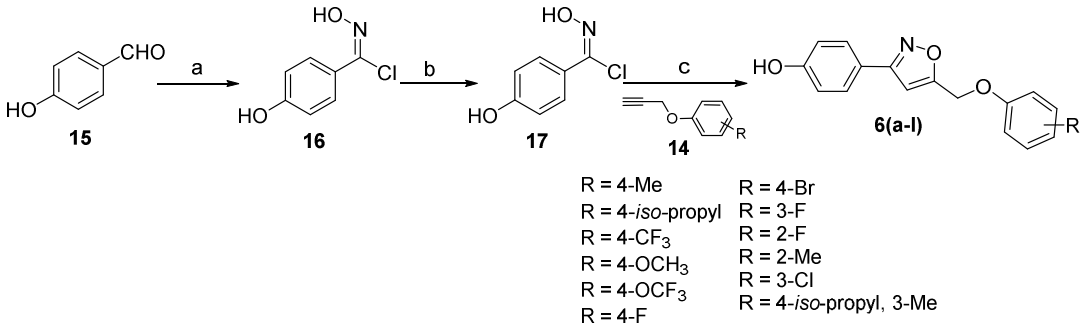
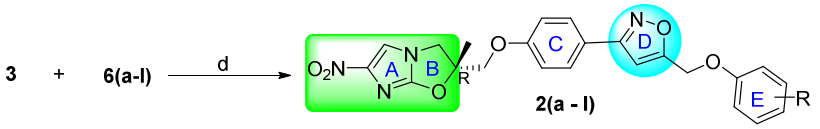
Next, the synthesis of isoxazolyl containing intermediates **6** were taken up which were synthesized from 4-hydroxybenzaldehyde **15** in three steps; i) first conversion of 4-hydroxybenzaldehyde **15** to aldoxime **16**; ii) then to chlorooxime **17** and iii) which further underwent dipolar cyclo-addition with substituted phenoxymethyl acetylenes **14** under standard condition afforded expected isoxazolyl containing intermediates **6** in good yields (Scheme 3).[14] Similarly, another isoxazole intermediates **7** were synthesized from chlorooxime **17** on dipolar cycloaddition with substituted phenyl acetylenes **18** (Scheme 4). Finally, the isoxazolyl containing intermediates **6** and **7** were coupled with intermediate **3** n the presence of sodium hydride which ultimately afforded isoxazolyl containing NHIO compounds **2a-l** and **2m-v** (Scheme 3 & 4).

Scheme 3: Synthesis of compounds **2a-l**[a]





2a: R = 4-Me
2b: R = 4-*iso*-propyl
2c: R = 4-CF_3
2d: R = 4-OCH_3
2e: R = 4-OCF_3
2f: R = 4-F
2g: R = 4-Br
2h: R = 3-F
2i: R = 2-F
2j: R = 2-Me
2k: R = 3-Cl
2l: R = 4-*iso*-propyl, 3-Me

[a]**Reagents and Conditions**: a) $NH_2OH.HCl$, 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 ^oC, 12 h, 20-40%.

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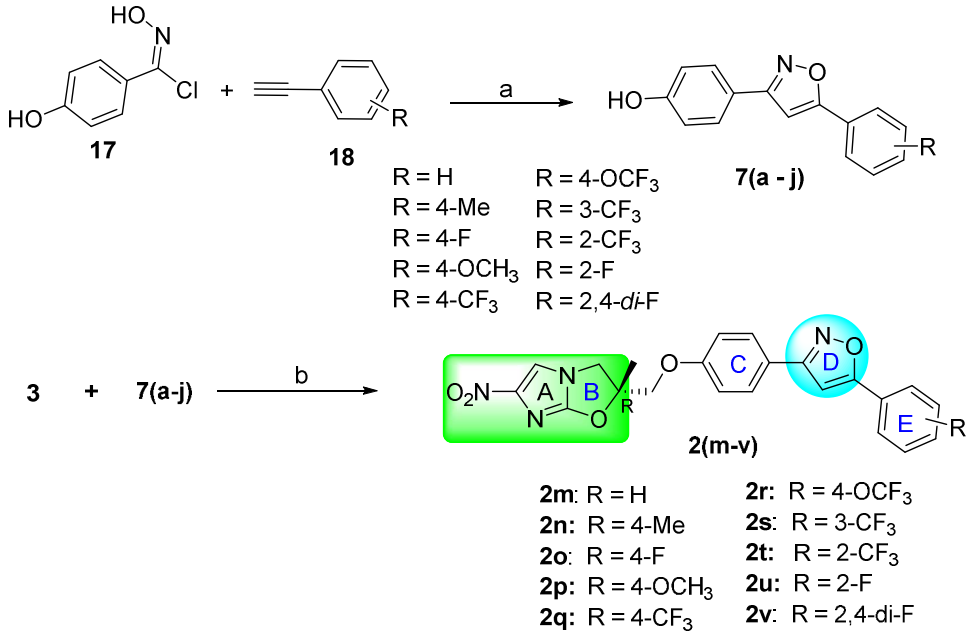
Scheme 4: Synthesis of compounds **2m-v**[a]



[a]**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%.

Biological evaluation and Discussion:

In total forty-one new triazolyl and isoxazolyl based NHIO compounds **1a-s** and **2a-v** respectively were synthesized having variations on the ring D and E and evaluated for *in vitro* activity against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294 strain) using micro-broth dilution method. The MIC of all the synthesized compounds is summarized in Table 1. Triazolyl based NHIO compounds **1a-i** wherein 4-[substitutedphenoxymethyl]triazolyl group present at 4th position of ring-C have shown MIC in the range of 1.02 to 0.23 µM. To evaluate the effect of position of triazolyl on activity, triazolyl based NHIO compounds **1j-k** having 4-[substitutedphenoxymethyl]triazolyl group at 3rd position of ring-C were also synthesized, which have shown MIC in the range of 1.07 to 0.23 µM. On the other hand, triazolyl based NHIO compound **1l-m** having 4-[substitutedphenoxymethyl]triazolyl group on the 2nd position of ring-C showed comparatively lower activity. Further, the role of methyleneoxy group between

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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 have been synthesized and among them, two compounds **1n** and **1o** showed the MIC of 0.57 & 0.56 μM. The nature and position of substituents on ring E also affect the potency wherein the *para*-substitution was more preferable and presence of -OCF_3 on ring E shown the most potent activity. In case of isoxazolyl based NHIO compounds **2a-l**, wherein 5-[substituted-phenoxymethyl]isoxazolyl group attached to 4^{th} position of ring-C have shown the MIC in the range of 1.0 to 0.13 μM except **2j** and **2k** which have shown MIC of 4.33 & 4.15 μM. However, isoxazolyl based NHIO compounds **2m-v,** wherein ring-E is directly attached to ring-D have shown MIC in the range of 2.31 to 0.14 μM. In case of isoxazolyl based NHIO compounds, the presence, nature and position of substituents on ring E greatly influence the potency. Among the isoxazolyl based NHIO series wherein rings D and E are attached through methyleneoxy group, the compounds having –F and –OCF_3 have shown most potent activity whereas isoxazolyl based NHIO series wherein rings D and E are directly attached, the un-substituted phenyl and –CF3 group bearing compounds have shown the most potent activity. Overall screening results suggested that the replacement of piperidyl ring i.e., ring D of delamanid with triazolyl and isoxazolyl is acceptable and further potency depends on the presence of substitution on ring-E. In most of the cases, the substitutions particularly -F, and -OCF_3 at ring-E were more favorable and have shown comparatively better activity (Fig 3).

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

Compound code	MIC ($H_{37}R_v$)[a] μM	Compound code	MIC ($H_{37}R_v$) μM	Compound code	MIC ($H_{37}R_v$) 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

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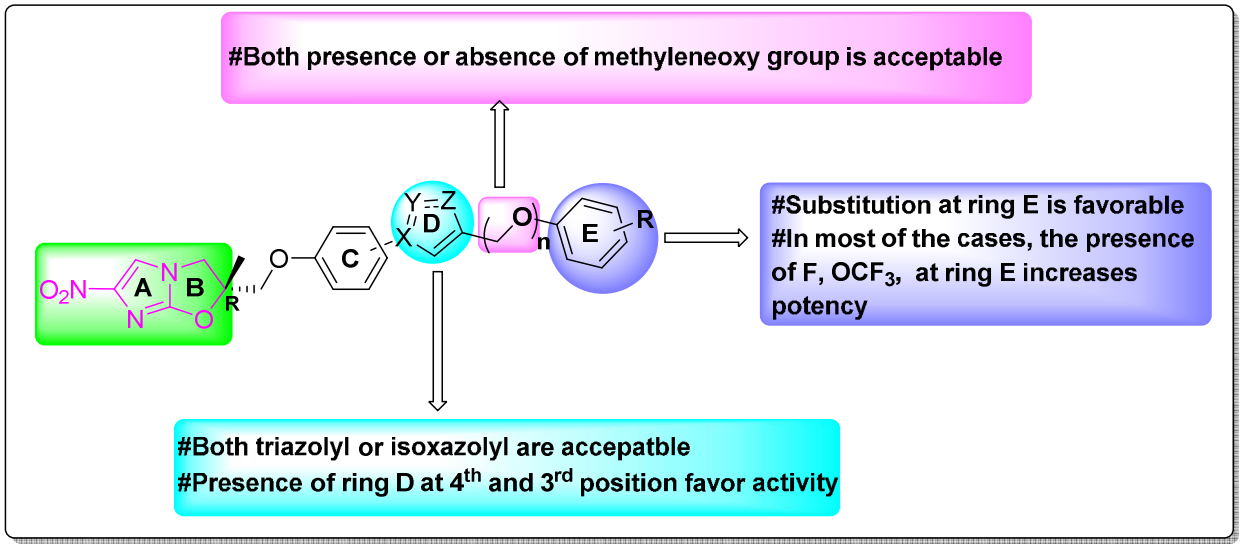

Fig 3: Structure-activity relationship of triazolyl/isoxazolyl NHIO compounds against $H_{37}Rv$ MTB

In the treatment of *M. tuberculosis*, the issue of dormant (non-replicating) as well as drug-resistant has further complicated the problem and the new chemical entities having efficacy against these will have advantages to addresses this unmet need. The use of streptomycin starved *M. tuberculosis* 18b as model for non-replicated cells has been validated and 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 compouds **2b, 2c, 2e, 2f, 2h, 2m** and **2s-u** having potent MIC in the range of 0.57 to 0.13 µM were selected and further screened against non-replicating as well as rifampicin (Rif^R) and multi-drug resistant (MDR) strain of MTB under *in vitro* condition. The screening results are summarized in Table 2. Among the twenty compounds, interestingly several compounds have shown single digit or less than single digit MIC against dormant strain of MTB. Against Rif^R and MDR strains of MTB, all the compounds have shown MIC in the range of 2.15 to 0.23 µM except **1o** (35.7 µM).

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

Compound	NRP[a] μM	MIC (Rif^R) μM	MIC (MDR) μM	CC_{50}[b] (μM)	SI[c] CC_{50}/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 reference 17, [e] reported in reference 7, [f] reported in reference 18, [g]Cytotoxicity against Vero epithelial cells using MTT assay reported in reference 19.

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The cytotoxic effect of potent compounds have also been determined on HepG2 cell line (Table 2), wherein none of the compounds had shown any cytotoxicity upto 40 µg/ml and have acceptable safety index. In order to evaluate the *in vivo* exposure of new 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 and compared with recently approved drug in this class delamanid. All the results are summarized in Table 3 and Fig 4. Among five tested compounds, two compounds **1g** and **2e** were shown good PK profile with C_{max} of 0.54 µg/ml and 1.34 µg/ml respectively and AUC_{0-t} of 7.42 µg/ml* h and 17.92 µg/ml* h respectively. As shown in Table 3, the compound **1g** has shown 1.5-times higher C_{max} and AUC_{0-t} and compound **2e** has shown 3.5- times higher C_{max} and AUC_{0-t} than delamanid (**C**).

Table 3: *In vivo* pharmacokinetic values in mice

Compound[a]	MIC (µg/ml)	Concentration (ng/ml)[b]								C_{max} (µg/ml)	AUC_{0-24} (µg/ml*h)	T_{max} (h)
		0.16 h	0.5 h	1 h	2 h	4 h	6 h	8 h	24 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, [b] each value represents mean ± SD (n=3) # Pharmacokinetic parameters were calculated by WINNONLIN software

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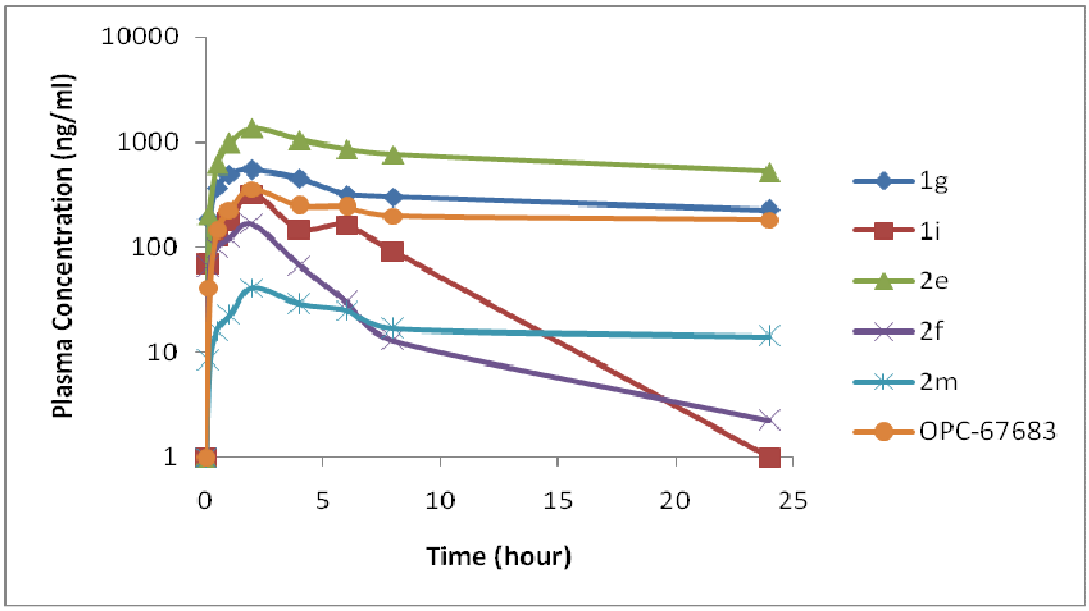

Fig 4: *In vivo* oral pharmacokinetic profile of selected compounds at 5 mg/kg.

Further, 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 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 once daily for 28 days. Compound **1g** has shown the 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 5). The group treated with compound **1g** at the dose of 100 mg/Kg daily for 28 days did not show any adverse effect and considered as safe. At the dose of 100 mg/Kg, compound **1g** has shown comparable *in vivo* activity with respect to delamanid as reported recently by Upton *et al.*[17] (activity has been performed in acute aerosol infection model). The compound **1g** has shown comparatively lesser activity than the rifampicin tested along in the regimen. However, the group treated with compound **2e** has shown mortality in

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some animals during the treatment and those survived also shown some toxic effect. Though this compound did not show cytotoxicity in the MTT assay (table 2), the apparent reason for mortality could be 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.

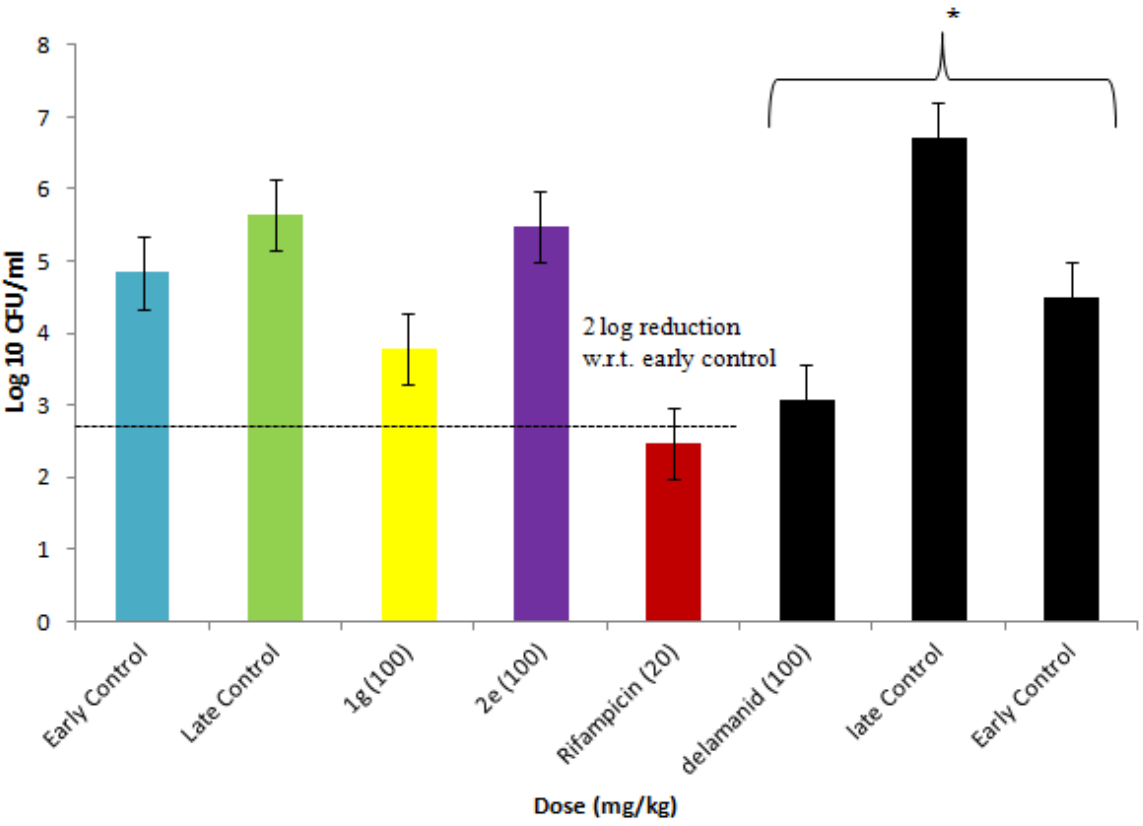

Fig 5: *In vivo* activity of **1g** and **2e** in intranasal model of acute infection Balb/c mice. Mice were orally dosed once daily for 28 days (n = 6) starting on the day after intravenous infection with 10^5 CFU of MTB; *the log10 CFU/ml value for delamanid taken from literature reported in 17.

In addition to this, *in vivo* efficacious lead **1g** was also studied for combination studies with three first line anti-TB drugs *viz.,* rifampicin (Rif), isoniazid (INH) and ethambutol (ETM) using checker board method (Table 4).The lead **1g** has shown additive effect with rifampicin, synergistic effect with isoniazid and additive effect with ethambutol. These results suggested that

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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 *viz*., 3A4, 2D6, 2C9, 1A2 and 2C19 at different concentrations using fluorescent based method (Table 5). The results indicated that the lead compound 1g did not show any CYP inhibition at all the tested concentrations.

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

Combinations	MIC μg/ml	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.

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

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CYP Isoforms	Test compound or Inhibitor	% of Inhibition		
		Concentration (μM)		
		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).

Conclusion:

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

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Experimental Section:

Chemistry: All chemicals for this study were purchased from Sigma-Aldrich, INDIA. ^{1}HNMR recorded on 200 MHz or 400 MHz or 500 MHz and ^{13}CNMR recorded on 101 MHz or 126 MHz Bruker-Avance DPX FT-NMR instruments. Chemical data for 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 in either MestReNova or Bruker software. Mass spectras were recorded with HRMS and LC-MS instrument. Melting points were recorded on digital melting point apparatus and are uncorrected. Purity of all final compounds (used for biological screening) was determined by using HPLC-Agilent Technologies 1260 infinity series system using one of the two methods. Method A: Coloumn RP 18e (Chromolith, 5µm, 4.6x100mm) and the gradient mixture of water /methanol was used as a Mobile phase over 50 minutes with a flow rate of 0.8 ml/min. Method B: Coloumn RP 18e (E-Merck, 5µm, 4.6x250mm) and the gradient mixture of water /methanol was used as a Mobile phase over 50 minutes with a flow rate of 0.6 ml/min. 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 ethylacetate twice, washed with brine solution and dried under *vaccuo*. This crude product was purified by silica gel column chromatography using a dichloromethane and ethyl acetate mixture as solvent to obtain the compounds **1a-s.**

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(*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 ^oC; ^1H 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 ^oC; ^1H 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 ^oC; ^1H 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,

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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),

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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%.

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(*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 ^oC; ^1H 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 ^oC; ^1H 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%.

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(*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 ^oC; ^1H 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^oC; ^1H 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-nitroimidazo[2,1-*b*]oxazole 1l (IIIM/MCD-52):

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TLC (EtOAc:DCM 1:9): R_f = 0.20; Light yellow solid; mp 160-162 ^oC; ^1H 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 ^oC; ^1H 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]phenoxymethyl}-2,3-dihydro-2-methyl-6-nitroimidazo[2,1-*b*]oxazole 1n (IIIM/MCD-65):

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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]phenoxymethyl}-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%.

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(*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 ^oC; ^1H 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 ^oC; ^1H 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):

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TLC (EtOAc:DCM 1:9): R_f = 0.25; Light yellow solid; mp 266-268 ^oC; ^1H 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:

Reaction of intermediate **3** and isoxazole intermediate **6 or 7** under 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]phenoxymethyl}-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 ^oC; ^1H 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%.

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(*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 ^oC; ^1H 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 ^oC; ^1H 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):

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TLC (EtOAc:DCM 1:9): R_f = 0.35; Light yellow solid; mp 198-200 ^oC; ^1H 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 ^oC; ^1H 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 ^oC; ^1H 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

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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,

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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* = 10.2Hz, 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,

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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%.

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(*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 ^oC; ^1H 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 ^oC; ^1H 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 ^oC; ^1H 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*

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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 ^oC; ^1H 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 ^oC; ^1H 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 ^oC; ^1H 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

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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%.

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(*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 ^oC; ^1H 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 ^oC; ^1H 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, Acetone); 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 clinical isolate *M. tuberculosis* MDR:

MIC determination: MIC was determined by 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 one of laboratory generated mutant *M. tuberculosis* $Rif^{R\ 15}$ (resistant to rifampicin) using micro-broth dilution method. The bacterial

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strains were grown for 10 to 15 days in Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) 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) under shaking conditions at 37 °C in 5% CO_2 to facilitate exponential-phase growth of the organism. Bacterial suspension was prepared by suspending *M. tuberculosis* growth in normal saline containing 0.5% tween 80 and turbidity was adjusted to 1 McFarland (McF) standard which is equivalent to 1.0 x 10^7 CFU/ml. 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 100 µl volume of this diluted inoculum was added to each well of the plate resulting in the final inoculum of 1.0 x 10^6 CFU/ml in the well and the final concentrations of compounds ranged from 0.015 to 32 µg/ml. The plates were incubated at 37 °C for seven days in 5% CO_2. For evaluation of results (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 plate including media and growth controls. After 48 h incubation, plates were read visually and the minimum concentration of the compound showing no change of colour was recorded as MIC.

***In vitro* activity against Non-Replicating MTB:**

Streptomycin starved *M tuberculosis* 18b (ss18b) in non replicating phase (NRP) of growth was grown according to method described earlier[16] using Middlebrook 7H9 media. In brief, ss18b was grown to mid-log phase in Middlebrook 7H9 supplemented with 10% ADC and 50µg/ml streptomycin. Streptomycin was removed from the media by washing the bacteria with phosphate buffer saline (pH 7.4) wash 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

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density of culture become nearly constant. A total of 0.2 O.D was used as inoculum for setting up MIC. Other conditions were same as used for MIC determination and evaluation by REMA method.

Evaluation of cytotoxicity in HepG2 cells:

Cytotoxicity of the compounds was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [20] Human HepG2 cell line was maintained in the *Dulbecco's Modified Eagle's medium* (Gibco Life Technology, NY). Cells were plated at a density of 10,000 cell /well in 96 microwell flat bottom plate and incubated for 24 h (37°C; 5% CO_2). Cells monolayer was exposed to the single concentration of 40µg/ml of the tested compounds and incubated for 24 h (37°C; 5% CO_2). MTT dye was added at concentration of 2.5mg/ml dissolved in phosphate buffer saline (PBS) and cell viability was determined by measuring the absorbance of the reduced formazan at 570 nm in a plate reader. The percent cytotoxicity achieved by the compounds was calculated according to standard methods using tamoxifen as a negative control and healthy cells as positive control. Cytotoxicity is reported as CC_{50}, the concentration that causes a 50% reduction in cell viability.

***In vivo* pharmacokinetics:**

Compounds were administered orally to female Balb/c mice (3 mice in each group) at a dose of 5 mg/kg as a suspension in 0.5% CMC and Tween 80. Samples 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, which were then analyzed by LC-MS/MS to generate the required pharmacokinetic parameters.

***In vivo* efficacy determination:**

For intranasal instillation purpose, actively growing culture of MTB $H_{37}Rv$ was used. In brief, freeze thawed vial was activated by incubating the culture in Middlebrook 7H9 Middle

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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 have average body weight of 20-22 g were taken for experiment. The animal were kept in cages in a Biosafetety laboratory for seven days prior to start of experiment for acclimation with levels 1g 100 mg/kg, 2e 100 mg/kg, Drug control (Rifampicin 20mg/kg) and placebo, no drug control. After sonicating *M. tuberculosis* suspension to make uniform suspension, 20 µl of it was given through nasal route to anesthetized mice.CFU determination was done after 48 h of postinfection to enumerate the bacterial load in lung of early control. Dosing was started after 48 h of infection (six days a week) and continued for 28 days. Bacterial load was determined in 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. Results were determined as CFU/ml.

Dose preparation and administration

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

Combination studies assay:

The efficacy of compound **1g** (conc range 0.25 µg/ml to 0.007 µg/ml) in combination with currently used anti-TB drugs such as rifampicin, isoniazid and ethambutol (each drug tested

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at conc range 4 µg/ml to 0.007 µg/ml), was determined *in vitro* using checkerboard method. The checkerboard procedure was performed based on the MIC values obtained from broth microdilution method. The checkerboard method was performed in 96 well U bottom microtitre plates. Hundred microliters of 4X of required concentration of drug was added to first column of the plate and fiflty microliters of plain media was added to remaining columns. Fifty microliters from first column was transferred to second column and was serially diluted in horizontal manner upto column 10 of the plate. Seven two fold serial dilutions of 4X of required concentration of compound were prepared in microcentrifuge tubes and fifty microliters of each concentration was added vertically starting from eleventh column of row eight to row second of the plate. Fiflty microliters of plain media was added to first row of the plate which served as drug control. Hundred microliters of 1:10 diluted of 1 Mc Farland inoculum was added to each well of the plate. Plates were then incubated at 37ºC for 14 days. MIC of drug alone and in 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. Results of 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 (Fluorescent based method):

Recombinant cytochrome P450 was aliquoted as per the total concentration required to conduct the study and stored in -80 ^{o}C until use. Total assay volume was adjusted to 200 µl and

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consists of three components: cofactors, inhibitor/vehicle, and enzyme substrate (ES) mix. Assay was conducted in black fluorogenic 96 well plates. Fifty microlitres of working concentration of 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 duplicates. Wells from 9,10 were control without inhibitor and 11 ,12 are blank wells. Enzyme substrate mix was prepared as per the required volume. Reaction plate with cofactor and test item and enzyme substrate mix prepared separately were pre incubated in 37 ^{o}C shaking incubator for 10 minutes. After the pre incubation, 100 µl of enzyme substrate mix was added to the required reaction wells in reaction plate and were incubation at 37 ^{o}C shaking incubator for 45 min. Incubation was terminated by dispensing 75µl of 100% acetonitrile after the incubation time. Fluorescence per well was measured using an excitation and emission wavelengths in Fluorescence Reader/Fluorescence Detector.[21]

Associated Content:

*S Supporting Information

Experimental procedures for intermediates along with characterization data, copies of NMR and Mass spectras of final compounds. This material is available free of charge via the Internet at

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