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Short communication

2-Methyl-4/5-nitroimidazole derivatives potentiated against sexually transmitted *Trichomonas*: Design, synthesis, biology and 3D-QSAR study☆

Dhanaraju Mandalapu [a], Bhavana Kushwaha [b], Sonal Gupta [a, g], Nidhi Singh [c], Mahendra Shukla [d], Jitendra Kumar [e], Dilip K. Tanpula [f], Satya N. Sankhwar [h], Jagdamba P. Maikhuri [b], Mohammad I. Siddiqi [c], Jawahar Lal [d], Gopal Gupta [b, g], Vishnu L. Sharma [a, g, *]

[a] *Medicinal & Process Chemistry Division, CSIR-Central Drug Research Institute, Sitapur Road, Lucknow, 226031, India*
[b] *Endocrinology Division, CSIR-Central Drug Research Institute, Sitapur Road, Lucknow, 226031, India*
[c] *Molecular and Structural Biology Division, CSIR-Central Drug Research Institute, Sitapur Road, Lucknow, 226031, India*
[d] *Pharmacokinetic & Metabolism Division, CSIR-Central Drug Research Institute, Sitapur Road, Lucknow, 226031, India*
[e] *Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Raebareli, 229 010, India*
[f] *Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Raebareli, 229 010, India*
[g] *Academy of Scientific and Innovative Research (AcSIR), New Delhi, 110001, India*
[h] *Department of Urology, King George Medical University, Lucknow, 226003, India*

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ABSTRACT

Trichomoniasis is the most prevalent, non-viral sexually transmitted diseases (STD) caused by amitochondriate protozoan *Trichomonas vaginalis*. Increased resistance of *T. vaginalis* to the marketed drug Metronidazole necessitates the development of newer chemical entities. A library of sixty 2-methyl-4/5-nitroimidazole derivatives was synthesized via nucleophilic ring opening reaction of epoxide and the efficacies against drug-susceptible and -resistant *Trichomonas vaginalis* were evaluated. All the molecules except two were found to be active against both susceptible and resistant strains with MICs ranging 8.55–336.70 μM and 28.80–1445.08 μM, respectively. Most of the compounds were remarkably more effective than the standard Metronidazole. This study analyzes the *in vitro* and *in vivo* activities of the new 5-nitroimidazoles, which were found to be safe against human cervical *HeLa* cells with good selectivity index. The exploration of SAR by the synthesis of four different prototypes and 3D-QSAR study has shown the importance of prototype 1 over other prototypes.

1. Introduction

STDs are insidious, diabolic and have an intense impact on reproductive health of millions of men, women and infants worldwide, which can't be overlooked [1,2]. More than 30 infections have been identified to date that can be sexually transmitted. Over 358 million new cases of STDs occur every year, which include mostly the common curable STDs like gonorrhea, syphilis, chlamydia and trichomoniasis [3]. Trichomoniasis caused by *Trichomonas vaginalis* (TV) alone accounts for over half of the new cases detected in men and women of reproductive age [4] and thus is the most prevalent sexually transmitted protozoan pathogen, the highest among of all the curable and non-viral STDs

Abbreviations: STDs, sexually transmitted diseases; TV, *Trichomonas vaginalis*; DTC, dithiocarbamate; SAR, structure activity relationship; MTZ, Metronidazole; NI, nitroimidazoles; *HeLa*, epithelium cells; 3D-QSAR, 3D-quantitative structure–activity relationship; SAR, structure–activity relationship; MIC, minimum inhibitory concentration; IC_{50}, half maximal inhibitory concentration; CoMFA, comparative molecular field analysis; PLS, partial least squares; LDH, lactate dehydrogenase; DMEM, dulbecco's modified eagle's medium; TLC, thin layer chromatography.

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* Corresponding author. Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, Sector 10, Jankipuram ext., Lucknow, Uttar Pradesh, 226031, India.
E-mail addresses: vl_sharma@cdri.res.in, vlscdri@gmail.com (V.L. Sharma).

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in the world. Since 2005, the incidence of TV has increased by 11.5% and prevalence has increased by 22.2% [5]. TV, discovered by Alfred Donn in 1836, is an amitochondrial, microaerotolerant protozoan parasite of the human urogenital tract where it colonizes and causes the disease [6]. Trichomoniasis in women is frequently responsible for endometritis, vaginitis, pyosalpinx, adnexitis, bacterial vaginosis and increased risk of pelvic inflammatory disease, cervical cancer, HIV transmission and birth complications like infertility, preterm delivery, low birth weight [7–9]. Men are often asymptomatic carriers which results in persistent spread of over 50% of TV infections through heterosexual contacts. Complications associated with TV infection in men include urethral discharge, urethritis, dysuria, prostatitis, epididymitis, lower abdominal pain, pruritis, infertility and benign prostatic hyperplasia [10–12]. On the other hand, HIV-infected men with Trichomoniasis have nearly 6-fold-higher HIV concentrations in semen. Therefore, it would be quite rational to conclude that controlling trichomoniasis can also considerably reduce the incidence of new HIV infections [13].

The US-FDA approved Metronidazole (MTZ) serves as the primary drug for treatment of infections with TV. However, drug resistance against MTZ was reported as early as 1962 and the increased prevalence of resistant infections in the 21st century is a matter of concern, given that 2.5–10% of isolates are found resistant, which make the situation grimmer [14,15]. Since 1997, the incidence of resistance has increased by 17 fold and the treatment typically relies on increased doses of MTZ [6]. The mechanism of action of nitroimidazoles (NI) is assumed to be through the reduction of the nitro group for hydrogenosomal function, which is inhibited in refractory cases. Some other 5-NI class of drugs have been currently used in treatment but cross-resistance to anaerobic pathogen is a challenging problem with no universally successful treatment [16]. The emergence of drug resistance along with a declining pipeline of clinical agents warrants the development of novel anti-trichomonal agents. Now a days, dithiocarbamate (DTC) group has drawn a lot of attention due to its presence in various biologically active compounds [17,18] utilized as microbicidal spermicides [19–21], anesthetic [22], fungicidal [23,24], *anti*-HIV [25], mono glyceride lipase inhibitors [26], anti-tumor [27], anti-alcoholism [28] etc. Compounds having DTC groups along with other functional groups are of massive interest due to more than one pharmacophore [29–31].

The concept of hybridization is a rational approach of conquering the drug resistance by introducing a new pharmacophore in a biologically active molecule to get improved therapeutic potential [32–38]. Even though the 5-NIs have been in use since long yet the true potential of this important drug class is still not fully explored [16]. Numerous imidazole derivatives have been reported showing the importance of nitro group [16,38]. In a pursuit to develop novel hybrid molecules with enhanced *anti-Trichomonas* activity especially against the resistant strains it was found that the incorporation of the DTC group into an MTZ core generally resulted in improved biological activity where the modifications were carried out at terminal hydroxyl group of MTZ (Fig. 1) [38]. However, in the present study we tried to incorporate the DTC group while keeping the MTZ structure intact (Fig. 1).

In the present study several 4/5-nitroimidazole derived entities have been screened against MTZ-susceptible and -resistant *TV in vitro* and the most active structure was also tested *in vivo* against the former. Fluorescence labelling of biological targets, safety evaluation against cervical epithelium cells (*HeLa*) and 3D-QSAR study data have been reported.

2. Results and discussion

2.1. Chemistry

The general procedure for the synthesis of the designed prototypes has been illustrated in Schemes 1 and 2. In the first step, 2-methyl-4(5)-nitroimidazole (**1**) was reacted with epichlorohydrin in acidic conditions affording 1-chloro-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl)propan-2-ol (**2**) [39]. Whereas in basic conditions it was yielded 1-chloro-3-(2-methyl-4-nitro-1*H*-imidazol-1-yl) propan-2-ol (**51**) [40]. Further these open chain chlorohydrins compounds (**2** and **51**) were treated with aqueous NaOH to form oxirane intermediates 2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**) and 2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**) in good yields. Furthermore, nucleophilic ring opening of compounds **3** and **52** with different substituted-1-carbodithioates in water:acetone (10:1) at 0 °C for 2–4 h yields 2-hydroxy-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl)propyl substituted carbodithioate/carbamodithioate (prototype 1; **4**–**24**) and 2-hydroxy-3-(2-methyl-4-nitro-1*H*-imidazol-1-yl)propyl substituted carbodithioate/carbamodithioate (prototype 3; **53**–**61**) respectively in good yields [41]. Where on nucleophilic ring opening of oxirane intermediates **3** and **52** with different substituted amines/imidazoles/triazoles in acetonitrile at 80 °C for overnight to form 1-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-3-(substitutedamine)propan-2-ol (prototype 2; **25**–**50**) and 1-(2-methyl-4-nitro-1*H*-imidazol-1-yl)-3-(substitutedamine)propan-2-ol (prototype 4; **62**–**65**) respectively in good yields.

2.2. Biological evaluation

2.2.1. Anti-Trichomonas activity of synthesized compounds

The library of all the four prototypes (**4**–**50** and **53**–**65**, Table 1) was evaluated for *anti-Trichomonas* activity *in vitro* against drug-susceptible (ATCC 33592) and -resistant (ATCC 50143) strains of *T. vaginalis* and for safety towards cervical epithelium cells (HeLa). The *anti-Trichomonas* activity of the synthesized compounds (**4**–**50** and **53**–**65**, Table 1) demonstrated good to remarkable activity, which was mostly better than MTZ, against both susceptible as well as resistant strains of *T. vaginalis*. Interestingly, all the compounds tested were found to be active against MTZ-susceptible strain at MIC ranging from 8.55 to 336.70 μM and also against MTZ-resistant strain at MIC 28.80–1445.08 μM (except **55** and **63**). Among the compounds evaluated for *anti-Trichomonas* activity against MTZ–susceptible strain, fourteen compounds (**4**, **6**, **10**–**12**, **16**–**18**, **23**, **24**, **26**, **29**, **33** and **46**) were up to 2.4 fold more active (MIC range 8.55–18.86 μM) than the standard MTZ (MIC 21.05 μM) while the activity of nine compounds (**5**, **20**, **25**, **27**, **30**, **37**, **40**, **41** and **57**) was comparable to MTZ. Furthermore, forty six compounds (**4**–**20**, **22**–**41**, **43**–**50** and **57**) were up to 12.7 fold more active (MIC 28.80–344.35 μM) than the standard MTZ (MIC 365.50 μM). The most active compound (**6**) of this series demonstrated trichomonacidal activity at MIC 8.55 and 37.10 μM against MTZ-susceptible and -resistant strains of *Trichomonas,* respectively and was thus 2.4 and 9.8 fold more active than MTZ against these two strains.

2.2.2. Structure activity relationship (SAR) study

The *anti-Trichomonas* activity data of compounds (**4**–**50** and **53**–**65**) against MTZ-susceptible and -resistant strains, revealed that the amine substituents -NR^1R^2 and nitro group position both have been useful for the biological activity. To better understand the substitution requirements for desirable activity four prototypes (prototype 1, **4**–**24**; prototype 2, **25**–**50**; prototype 3, **53**–**61** and prototype 4, **62**–**65**) were synthesized.

Prototype 1 i.e., 2-hydroxy-3-(2-methyl-5-nitro-1*H*-imidazol-1-

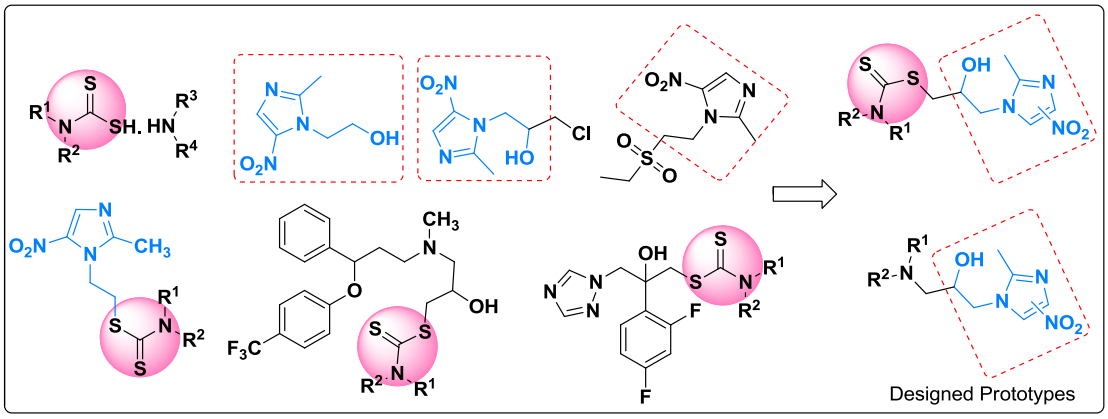
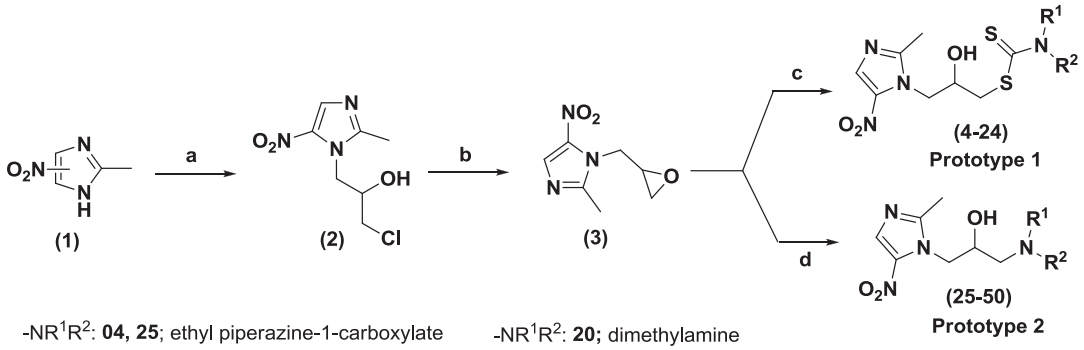


Fig. 1. Structures of 5-nitroimidazole drugs and dithiocarbamate hybrid molecules with enhanced activities.

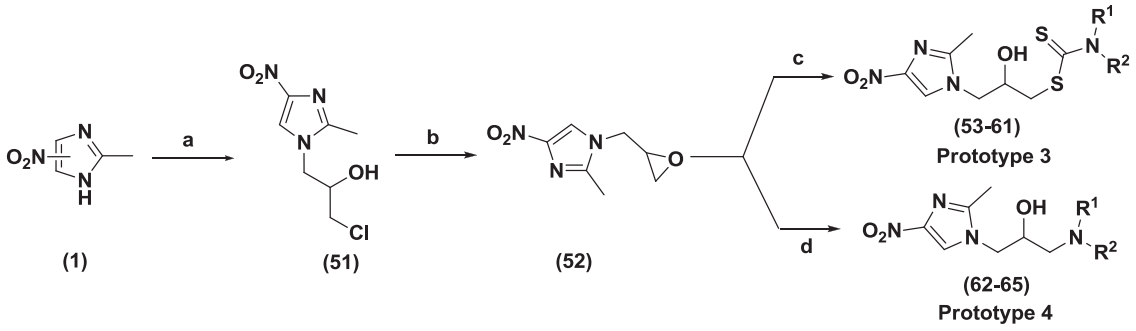


-NR^1R^2: **04, 25**; ethyl piperazine-1-carboxylate
05, 26; 1-ethylpiperazine
06, 27; 1-phenylpiperazine
07, 28; 1-(2-methoxyphenyl)piperazine
08, 29; 1-(pyridin-2-yl)piperazine
09, 30; 2-(piperazin-1-yl)pyrimidine
10, 31; 1-(2-fluorophenyl)piperazine
11, 32; 1-(3-chlorophenyl)piperazine
12, 33; 1-(2,3-dichlorophenyl)piperazine
13, 34; pyrrolidine
14. 35; morpholine
15, 36; piperidine
16, 37; 3-methylpiperidine
17, 38; 4-methylpiperidine
18, 39; 4-benzylpiperidine
19, 40; azepane

-NR^1R^2: **20;** dimethylamine
21; N-methylethanamine
22; diethylamine
23; 1-butylpiperazine
24; *tert*-butyl piperazine-1-carboxylate
41; 1-(furan-2-yl)piperazine
42; 2-methylpiperidine
43; 2,6-dimethylpiperidine
44; 4-nitro-1H-imidazole
45; 2-methyl-4-nitro-1H-imidazole
46; 1*H*-1,2,3-triazole
47; 1*H*-1,2,4-triazole
48; 2*H*-1,2,3-triazole
49; 1*H*-benzo[d]imidazole
50; 1*H*-benzo[d][1,2,3]triazole

Scheme 1:. Synthesis of compounds (**4**–**50**). ***Reagents and Reaction Conditions***: a) (±)-Epichlorohydrin, $AlCl_3$, EtOAc, 0 °C-rt, 24–26 h, 79% yield; b) Aq. NaOH, DCM, 2 h, 91% yield; c) Substituted-1-carbodithioate, Water:Acetone (10:1), 0 °C, 2–4 h, 68–91% yield; d) Substituted amines, Acetonitrile, 80 °C, overnight, 40–85% yield.

yl)propyl substituted carbodithioate/carbamodithioate (**4**–**24**) contained 2-methyl-5-nitro imidazole and DTC groups linked by a 2-hydroxy propyl chain. At -NR^1R^2 various amine substituents like acyclic amine (**20**–**22**), cyclic amine (**13**–**19**) and piperazine (**4**–**12**, **23** and **24**) were employed. Against MTZ-susceptible strain, in acyclic amines the activity decreased with the increasing the alkyl chain (**22** > **21** > **20**) while with cyclic amines the activity enhanced as the ring size increased (**34** < **35**<**40** < **37** = **38** < **39**) except in case of piperidine (**36**). In case of piperazines alkyl/carboxy substitutions at N^4 had activity better than MTZ where as in case of aryl/hetero aryl substitution the activity pattern followed was phenyl (**6**)>phenyl substituted with electron withdrawing groups (**10**–**12**)>electron donating groups (**7**) followed by hetero aryl substitution (**8** and **9**). the most desirable substituent at -NR^1R^2 was found to be N^4-phenylpiperazine (**6**). Against MTZ-resistant strain among acyclic amine dimethyl (**20**)/diethyl (**22**) substituents were more desirable than ethylmethyl substitution (**21**) and in cyclic amine substitution at -NR^1R^2 with increasing ring size from **13** < **15**<**19**. A further substitution on piperidine ring resulted in increased activity (**16**–**18**) whereas a hetero atom substitution decreased activity (**14**). These compounds (13–19) were 2.5–12.6 fold more active than MTZ. Alkyl/carboxy substitution at N^4 of piperazine had better activity than MTZ wherein aryl/hetero aryl substitution the order of activity was 2-pyrimidyl (**9**)>phenyl (**6**) >2-methoxyphenyl (**7**)>2-flurophenyl (**10**)>3-chlorophenyl (**11**) >2,3-dichlorophenyl (**12**)>2-pyridyl (**8**). The most desirable substituent was 2-pyrimidyl (**9**) as it was 12.3 fold more active than MTZ. In prototype 1 the essential substitution at -NR^1R^2 was aryl/heteroaryl-substituted piperazine against both susceptible and resistant strains. The SAR revealed that the loss of activity from



NR^1R^2: **53, 62**; 1-(2,3-dichlorophenyl)piperazine
54, 63; 1-ethylpiperazine
55; 1-butylpiperazine
56; ethyl piperazine-1-carboxylate
57; 1-phenylpiperazine
58; 1-(2-methoxyphenyl)piperazine

NR^1R^2: **59**; 1-(3-chlorophenyl)piperazine
60; 3-methylpiperidine
61; *tert*-butyl piperazine-1-carboxylate
62; 1-(2,3-dichlorophenyl)piperazine
63; 1-ethylpiperazine
64; 2-(piperazin-1-yl)pyrimidine
65; 1*H*-1,2,4-triazole

Scheme 2:. Synthesis of compounds (**53**–**65**). ***Reagents and Reaction Conditions***: a) (±)-Epichlorohydrin, K_2CO_3, 110 °C, 30 min, 60% yield; b) Aq. NaOH, DCM, 2 h, 86% yield; c) Substituted-1-carbodithioate, Water:Acetone (10:1), 0 °C, 2–4 h, 70–84% yield; d) Substituted amines, Acetonitrile, 80 °C, overnight, 55–72% yield.

susceptible to resistant strain in most of the compounds was much lesser (1–8 times) than MTZ (17 times). In order to explore the importance of DTC group, another prototype 2 i.e., 1-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-3-(substitutedamine)propan-2-ol (**25**–**50**) was devoid of DTC group from prototype 1. It was synthesized to observe the effect of DTC group. At -NR^1R^2 among cyclic amines (**34**–**40**, **42** and **43**) an increase in ring size (5-membered to 7-membered) enhanced the activity against susceptible strain while a further substitution in piperidine ring (**36**) decreased the activity (**38**, **39**, **42** and **43**) except 3-methyl substitution (**37**), which was comparable. In piperazines at N^4-position alkyl group (**26**) was more desirable over ethoxycarbonyl (**25**) or aryl/hetero aryl substitutions (**27**–**33**). Several azole groups (45–50) were also substituted at -NR^1R^2 which resulted in decreased activity as compared to MTZ except 1,2,3-triazole substitution (**46**). Against MTZ-resistant strain with cyclic amine substitution at -NR^1R^2 the activity pattern was to be similar as observed against susceptible strain of *T. vaginalis*. Further an N^4-heteroaryl/alkoxy carbonyl substitution in piperazine (**29**, **30**/**25**) was more favourable over alkyl/aryl substitutions (**26**, **27**, **28** and **31**–**33**). Among the various substituents attempted at -NR^1R^2 azoles were most promising (**44**–**50**) of which 1,2,3-benzotriazole (**50**) and 1,2,5-triazoles (**48**) found to be most desirable. In prototype 2 the required substitution at -NR^1R^2 was azoles against both susceptible and resistant strains. From the SAR study it was found that the loss of activity from susceptible to resistant strain in all of the compounds was 1.6–8 fold only as compared to 17 times in MTZ. From the activity data it was revealed that prototype 1 was more desirable for *anti-Trichomonas* activity in both susceptible and resistant strains. Against susceptible strain twelve compounds of prototype 1 and only four of prototype 2 were found to be more active than MTZ while against resistant strain four compounds of prototype 1 and only two of prototype 2 were more active at MIC <50 μM.

To further explore the effect of position of 5-nitro group in imidazole ring prototype 3 and 4 were synthesized with nitro group at 4-position along with and without DTC group respectively. The substituent at -NR^1R^2 were chosen according to the activity of their 5-nitro equivalents. In prototype 3 i.e., DTC derivatives (**53**–**61**) against susceptible strain the activity was lost by 1.6–8 fold and 4–20 fold against resistant strain as compared their 5-nitro analogues. While in prototype 4 i.e., without DTC group (**62**–**65**) against both the susceptible and resistant strains the activity was considerably lost by 3–28 fold as compared their 5-nitro analogues.

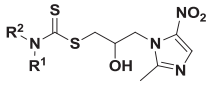
The SAR study suggested that prototype 1 was most desirable which comprised of both DTC and 5-nitro groups for the *anti-Trichomonas* activity better than MTZ against both susceptible and resistant strains. Compound **6** (2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl 4-phenylpiperazine-1-carbodithioate) was taken for the further biological studies based on its activity profile against both the strains tested. Safety data of all the synthesized compounds suggested that all compounds were safe against normal *HeLa* cells with higher SI values.

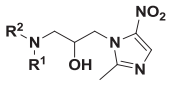
2.2.3. 3D-QSAR study

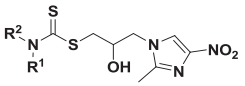
The reliability of CoMFA models is dependent on number of factors such as alignment rules, orientation of the aligned compounds, lattice shifting step size and probe atom type [42]. Molecular alignment was done through similarity based method considering compound **6** as template. The resulting alignment of whole dataset is shown in Fig. 2.

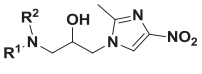
The leave-one-out partial least squares (PLS) analysis of the reported model yielded cross-validated q^2 value of 0.71 and non-cross validated correlation coefficient r^2 of 0.999. A significant predictive r^2 value of 0.654 was obtained for the test set molecules, indicating a reasonably good predictive power for the untested compounds. The experimental and predicted activity values for the compounds of training and test set is given in Supplementary Table 1. The results of CoMFA statistical analysis has been summarized in the Supplementary Table 2. The plot of experimental versus predicted values for CoMFA model is shown in Supplementary Fig. 1. The contour maps derived from the CoMFA-PLS model have permitted an understanding of the steric and electrostatic requirements for ligand binding. The total field contribution provided by electrostatic field is 49.7% and steric field is 50.3% for CoMFA model. Most active compound **6** was embedded in the CoMFA contour map (Fig. 3). The decrease in activity with increase in alkyl chain length is supported by prominent yellow contour near -NR^1R^2 substituent position as exemplified by compound **20** > **21** > **22**. The compounds **7** and **8** are also less active as compared to compound **23** and **24** and their substituent also fall in this yellow contour. The red contour in the vicinity of DTC group favors electronegative substitution and the compounds **6**, **10**, **18**, **26** and **46** are more active as compared to the compounds devoid of

Table 1
Anti-*Trichomonas* activity and cytotoxicity data of synthesized compounds (**4**–**50** and **53**–**65**)[a].


Prototype 1; (4-24)


Prototype 2; (25-50)


Prototype 3; (53-61)


Prototype 4; (62-65)

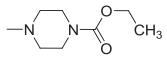
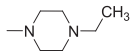
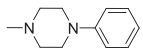
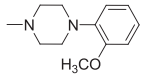
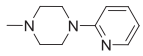
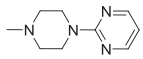
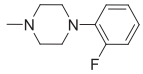
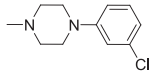
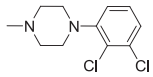
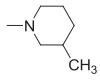
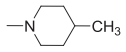
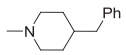
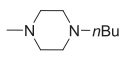
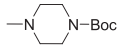
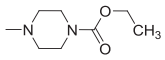
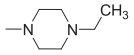
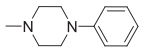
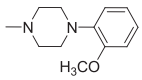
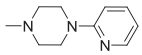
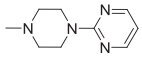
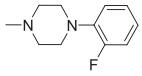
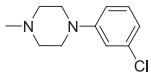
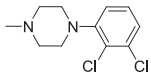
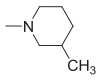
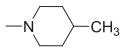
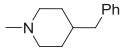
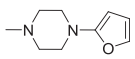
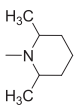
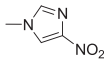
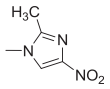
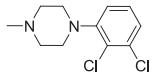
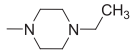
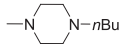
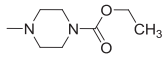
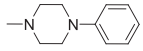
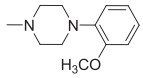
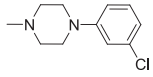
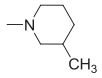
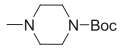
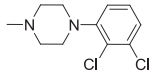
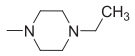
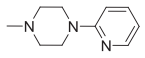
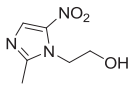
Compound	-NR^{1}R^2	MTZ susceptible strain[b] MIC[c] (μM)	MTZ resistant strain[d] MIC (μM)	*HeLa*[e] IC_{50}[f] (μM)	Selectivity index (IC_{50}/MIC)	
					MTZ susceptible strain	MTZ resistant strain
4		14.98	59.95	1609.11	107	26
5		20.93	83.78	>5000	>250	>60
6		8.55	37.10	>5000	>584	>135
7		27.71	55.43	>5000	>185	>90
8		29.62	148.10	2966.82	100	20
9		29.55	29.55	2044.91	69	69
10		14.23	56.94	3243.73	227	56
11		17.16	68.68	1076.92	62	15
12		15.97	127.81	1000.00	62	7
13		37.87	151.51	1342.42	35	9
14		36.12	144.50	4523.12	125	31
15		72.67	145.34	1404.06	19	9
16		17.45	69.83	608.93	35	8
17		17.45	69.83	1818.43	104	26
18		14.40	28.80	1315.66	91	45
19		34.91	69.83	410.61	11	6
20		20.55	82.23	>5000	>243	>61
21		24.55	393.08	4150.94	169	10
22		37.65	75.30	2457.83	65	32
23		15.58	31.17	1905.23	122	61
24		17.55	70.22	689	39	9

Table 1 (*continued*)

Compound	-NR[1]R[2]	MTZ susceptible strain[b] MIC[c] (μM)	MTZ resistant strain[d] MIC (μM)	*HeLa*[e] IC_{50}[f] (μM)	Selectivity index (IC_{50}/MIC)	
					MTZ susceptible strain	MTZ resistant strain
25		22.90	91.64	3671.55	160	40
26		12.12	105.21	4942.76	407	47
27		22.63	181.15	>5000	>220	>27
28		33.33	133.33	4389.33	131	33
29		18.06	72.25	1826.58	101	25
30		22.50	90.05	3927.95	174	43
31		43.03	344.35	>5000	>116	>14
32		131.92	329.81	1435.35	10	4
33		18.86	150.96	>5000	>265	>33
34		49.21	196.85	>5000	>102	>25
35		46.29	92.59	>5000	>108	>54
36		23.32	186.56	>5000	>217	>26
37		22.16	88.65	>5000	>227	>56
38		44.32	177.30	>5000	>113	>28
39		34.91	139.66	1430.16	40	10
40		22.16	44.32	>5000	>227	>113
41		21.51	172.17	3523.41	163	20
42		55.39	443.26	>5000	>90	>11
43		42.22	84.46	>5000	>119	>59
44		26.38	105.57	>5000	>192	>47
45		50.38	100.80	4354.83	86	43
46		12.38	99.20	>5000	>416	>50

(*continued on next page*)

Table 1 (*continued*)

Compound	-NR[1]R[2]	MTZ susceptible strain[b] MIC[c] (μM)	MTZ resistant strain[d] MIC (μM)	*HeLa*[e] IC_{50}[f] (μM)	Selectivity index (IC_{50}/MIC)	
					MTZ susceptible strain	MTZ resistant strain
47		30.99	99.20	4968.25	160	50
48		30.99	49.60	>5000	>161	>102
49		25.94	83.05	4528.23	181	54
50		25.86	41.39	4235.09	169	103
53		25.51	510.20	724.48	30	2
54		134.04	1340.48	>5000	>37	>3
55		124.68	Inactive	4331.67	34	Inactive
56		119.90	599.52	2359.71	19	4
57		22.69	296.91	>5000	>227	>16
58		221.72	1108.64	>5000	>22	>4
59		54.94	549.45	1736.26	31	3
60		34.91	1396.64	2695.53	105	2
61		28.08	561.79	>5000	>178	>9
62		120.77	1207.72	3623.18	30	3
63		336.70	Inactive	>5000	>14	Inactive
64		144.50	1445.08	>5000	>34	>3
65		99.20	992.06	>5000	>50	>5
MTZ		21.05	365.49	>5000	>238	>13

[a] All the experiments were carried out in triplicate.
[b] ATCC 33592 strain.
[c] Minimum inhibitory concentration.
[d] ATCC 50143 strain.
[e] Human cervical cell line.
[f] Inhibitory concentration killing 50% *HeLa* cells; MTZ = Metronidazole.

this group. The blue contour near -NR[1]R[2] substituent position favors the higher activity of compounds **18** and **46** than compounds **54** and **58** as observed. The large blue contour near nitroimidazole ring favors the electropositive substitution which is supported by the less activity of 4-nitro substituent at imidazole ring, lying in this contour than 5-nitro substituent, away from it (Fig. 3).

The 3D-QSAR study carried out in the present study shed light onto the regions of importance for steric and electrostatic contribution. The reported model helps in the understanding of observed variance in the activity and provides direction for structural

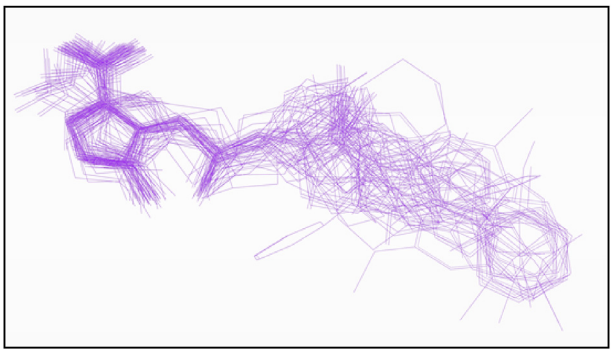


Fig. 2. Whole dataset aligned on the most active compound **6**.

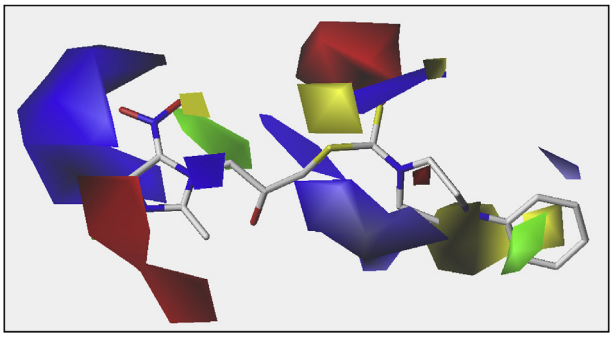


Fig. 3. CoMFA steric and electrostatic contours displayed with most potent compound **6**. The green areas in the figure indicate regions where steric bulks are well accomodated with an increase in activity, whereas yellow areas indicate regions where steric bulk is unfavourable. The red contours indicate regions where substitution with more electronegative substituent will increase the activity, whereas the blue contour show the reverse. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

requirement for compounds possessing better activity.

*2.2.4. Plausible mode of action of active compound **6***

T. vaginalis is an anaerobic parasite that contains hydrogenosomes (instead of mitochondria) to generate energy and produces molecular hydrogen, which maintains the redox balance. The metabolic pathway of hydrogenosome is different from that found in mitochondria and thus drugs intended for targeting hydrogenosomes generally do not affect the host cells. Metronidazole, FDA-approved drug for trichomoniasis management acts through the hydrogenosomal pathway. The nitro group is reduced in the hydrogenosome to produce cytotoxic nitro radicals liable for DNA destruction of parasite. The 2-methyl-4/5-nitroimidazole derivatives studied here also possibly act through the same pathway due to the presence of NO_2 group in the molecules [43,44].

Also the lack of glutathione makes *T. vaginalis* dependent on cysteine to overcome redox stress, making them very susceptible to sulfhydryl manipulating agents. The DTC compounds are known to be sulfhydryl binding agents [45–47]. Compound **6** was evaluated for its sulfhydryl binding ability due to the presence of DTC in its structure. Fig. 4 indicates a considerable inhibition in free thiol fluorescence of *Trichomonas* treated with compound **6**. In the control group the fluorescence intensities were visibly higher due to the higher number of free sulfhydryl groups than that in compound **6** treated. The diminished fluorescence indicates the interaction of compound **6** with the sulfhydryls present over *Trichomonas* further boosting its trichomonicidal mechanism. Even though non-DTC compounds of the series were also active but possible dual mechanism of this prototype with DTC group made prototype 1 more desirable scaffold for *anti-Trichomonas* activity than others.

*2.2.5. In vivo efficacy of compound **6***

The most promising compound **6** with high *in vitro* activity and absence of cytotoxicity against *HeLa* cells was screened for *in vivo* anti-trichomoniasis activity using the mouse abscess assay. Subcutaneous injections of live trichomonads resulted in a small pustule of ~60 mm^2 area on day-7 of injection (day-1 of treatment) in experimental and control animals that grew to ~120 mm^2 in area in controls but was reduced to ~1–5 mm^2 after 5-days of oral treatment with compound-6 or MTZ (Fig. 5). Thereafter the growth of abscess was exponential in controls and 7 days after the treatment it was >100 mm^2 in area in controls while it was further subdued to ~0.5–2 mm^2 in treated animals. On the day of autopsy (i.e. the day following seven days of treatment), the abscess was ~113 mm^2 in area in untreated controls, 1.57 mm^2 in 50 mg/kg of MTZ-treated group and 1.55 and 0.785 mm^2 in 50 mg and 100 mg/kg of compound-6 treated groups, respectively.

2.2.6. Pharmacokinetics study

A preliminary pharmacokinetic study involves monitoring drug substance in blood plasma carried out for compound **6**. The animals tolerated the treatment as no peculiarities in the animals' behaviour were observed. The mean peak serum concentration $C_{max} = 35.2 \pm 2.0$ ng/mL was achieved after 50 mg/kg oral dosing at 4.0 h. However, following oral administration, compound **6** could be monitored in serum up to 10 h post dose. The pharmacokinetic parameters were calculated using non compartmental approach and the calculated pharmacokinetic parameters are shown in Table 2. The volume of distribution (80.6 ± 7.2 L/kg) is larger than the total blood volume of rat (0.054 L/kg [48]) and systemic clearance (16.3 ± 1.8 L/h/kg) is higher than the total hepatic blood flow in rats (2.9 L/h/kg [48]) indicating extra vascular distribution along with the extra hepatic elimination of the compound.

3. Conclusion

A library of sixty 2-methyl-4/5-nitroimidazoles was designed which demonstrated improved trichomonicidal activities against MTZ–susceptible and –resistant strains *in vitro* than the marketed drug MTZ. Furthermore, these derivatives were also found to be safe in cytotoxicity study against *HeLa* cells with good selectivity index. The most active compound **6** demonstrated trichomonacidal activity at an MIC of 8.55 and 37.10 μM against MTZ-susceptible and -resistant strains of *Trichomonas* respectively, which was 2.4 and 9.8 fold better than MTZ. To further explore the importance of 5-nitro group the positional isomers 4-nitroimidazoles were also synthesized and evaluated. The SAR study revealed that change in the position of nitro group on imidazole ring from 5 to 4-position drastically reduced the activity against resistant *Trichomonas* which was a clear indication of the importance of the 5-nitro group. Some activity of 4-nitro molecules against MTZ–susceptible strain could also be due to the presence of DTC group. The finding have suggested that prototype 1 was more desirable over remaining prototypes plausibly due to the presence of both DTC and 5-nitro groups for better *anti-Trichomonas* activity against both the strains (inhibition of hydrogenosomal function by nitro group and sulfhydryl binding ability of DTC group, as also supported by sulfhydryl inhibition assay). The compound **6** was apparently nontoxic in animal infection models and was found to be active *in vivo* too. Preliminary pharmacokinetic study has shown good distribution and systemic clearance of compound **6** in rats and thus could serve as a novel lead molecule for the development of structurally

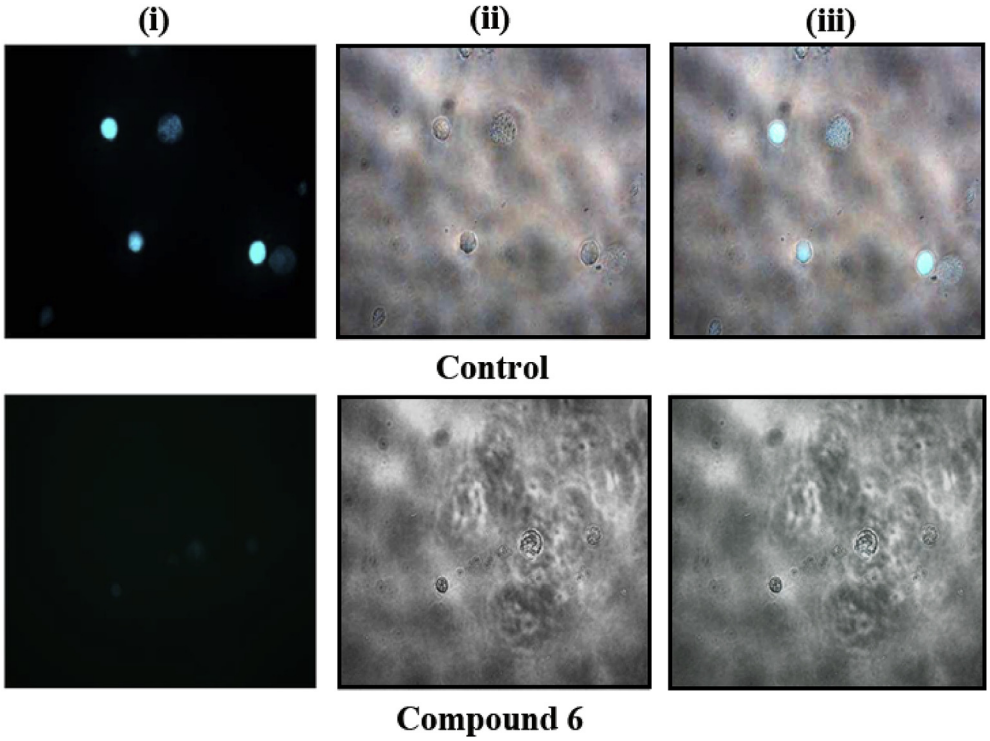


Fig. 4. Fluorescent labelling of *Trichomonas* sulfhydryls: (i) Labelled with fluorometric thiol detector specifically binds with free sulfhydryls, (ii) Phase contrast image, (iii) Merged image.

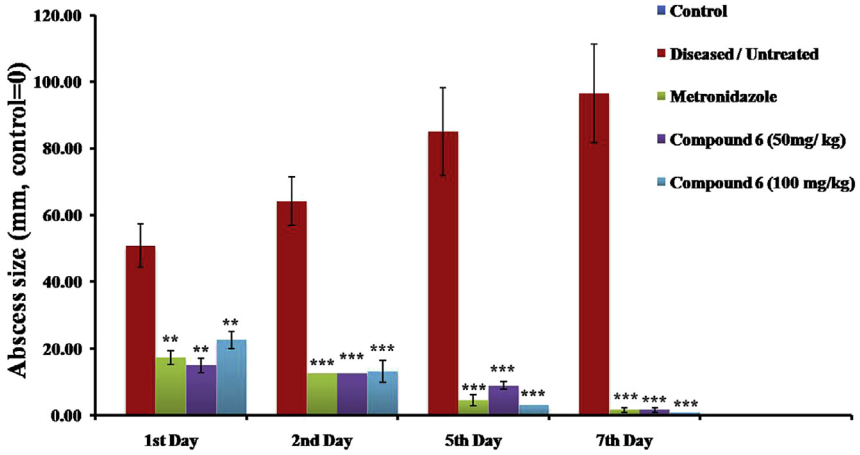


Fig. 5. Decrease of abscess size in subcutaneous abscess assay in mice model at days 1, 2, 5 and 7(autopsy day). Significant difference from infected-untreated animals is indicated as **P < 0.01; ***P < 0.001.

diverse, next-generation 5-nitroimidazoles.

4. Experimental section

4.1. Chemistry

In general, all reagents and solvents were of commercial quality and were used without further purification. Chromatography was carried on silica gel (100–200 and 60–120 mesh). All reactions were monitored by thin-layer chromatography (TLC) using F254 silica gel plates with fluorescence (Aldrich). Melting points were determined in open capillary tubes on an electrically heated block and are uncorrected. IR spectra (ν^{max} in cm^{-1}) of the compounds were recorded on Perkin Elmer FT-IR RX1 PC spectrophotometer. ^{1}H NMR spectra were recorded on Bruker Supercon Magnet Avance DPX-200/DRX-300 spectrometers (operating at 400 and 100 MHz, respectively, for ^{1}H and ^{13}C) in deuterated solvents with TMS as internal reference (chemical shifts δ in ppm, J in Hz.). Electrospray ionization mass spectra (ESI-MS) were recorded on Ion Ttrap LCQ Advantage Max-IT (Thermo Electron Corporation). High-resolution mass spectra (HRMS) were recorded on a 6520 Agilent Q Tof LC-MS/MS (accurate mass). Elemental analyses were performed on a Carlo Erba EA-1108 micro analyzer/Vario EL-III C, H, N analyzer. All compounds were analyzed of C, H, N and the results obtained were

Table 2
Pharmacokinetic parameters of compound **6** after single 50 mg/kg oral administration in male *Sprague Dawley* rats.[a]

Parameters	Compound 6
C_{max} (ng/mL)	35.2 ± 2.0
T_{max} (hr)	4.0 ± 0.0
AUC_{last} (ng*hr/mL)	149.1 ± 13.9
$t_{1/2}$ (hr)	5.0 ± 0.1[b]
Cl (L/hr/kg)	16.3 ± 1.8
V_{ss} (L/kg)	80.6 ± 7.2

Abbreviations: AUC_{last} = area under the concentration-time curve up to last observation, C_{max} = peak serum concentration, t_{max} = time to C_{max}, V_{ss} = steady-state volume of distribution, Cl = clearance, $T_{1/2}$ = elimination half-life.
[a] Each value represent the average of four rats.
[b] MRT(mean residence time).

within ±0.4% of calculated values. All final compounds were found to have >95% purity.

4.1.1. Synthesis of (±)–1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**2**)

A mixture of 2-methyl-4/5-nitro-1*H*-imidazole (**1**, 39.37 mmol) and anhydrous aluminium chloride (39.37 mmol) were dissolved in ethylacetate (50 mL) at 0 °C and stirred for 15 min then added (±)-epichlorohydrin (78.74 mmol) to the above reaction mixture and contents were further stirred for 15–20 h. After completion of reaction (as monitored by TLC) 30 mL of distilled water was added and further continued the stirring for 30 min and stopped the reaction. Then EtOAc (20 mL) and excess water (3 × 30 mL) was added to the reaction mixture and organic layer was separated, dried (anhyd. Na_2SO_4), concentrated under reduced pressure. The crude obtained was further purified by column chromatography over silica (60–120 mesh) with methanol/chloroform as eluent to get the title compounds **2** in 79% yield as white solid. mp: 84–86 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 1540, 1360, 1150, 771; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (s, 1H), 4.92 (s, 1H), 4.63–4.60 (m, 1H), 4.19–4.16 (m, 2H), 3.73–3.63 (m, 2H), 2.45 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 151.9, 138.3, 132.2, 69.8, 49.7, 46.9, 14.4; ESI-MS: *m/z* 220 ($M+H^+$).

4.1.2. Synthesis of (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1H-imidazole (**3**)

The title compound (**3**) was synthesized by dissolving compound **2** (29.68 mmol) in dichlorometane (10 mL) and added aqueous NaOH (29.68 mmol) at 0 °C. Stirred the contents for 40–45 min and after completion of reaction (as monitored by TLC) contents was extracted with DCM/water and organic layer was separated, dried (anhyd. Na_2SO_4), concentrated under reduced pressure. The crude obtained was recrystallized with EtOAc/hexane to get the final compound (**3**) in 91% yield as white solid. mp: 113–115 °C; IR (KBr) ν (cm^{-1}): 3405, 3110, 1540, 1362, 1151; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 1H), 4.85 (d/d, J = 2.3 & 15.0 Hz, 1H), 4.19 (d/d, J = 5.8 & 15.0 Hz, 1H), 3.37–3.33 (m, 1H), 2.85 (t, J = 4.2 Hz, 1H), 2.50 (d/d, J = 2.5 & 4.4 Hz, 1H), 2.47 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 151.4, 138.5, 132.8, 50.3, 47.5, 45.1, 14.3; ESI-MS: *m/z* 184 ($M+H^+$).

4.1.3. Synthesis of (±)–ethyl 4-((2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propylthio)carbonothioyl)piperazine-1-carboxylate (**4**)

To a solution of sodium 4-(ethoxycarbonyl)piperazine-1-carbodithioate (3.27 mmol) in water (20 mL) at 0 °C was added (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.64 mmol) in acetone (2 mL) and contents were stirred for 2.5 h. Reaction progress was monitored by TLC and after completion of the reaction EtOAc (20 mL), excess water (50 mL) was added and organic layer was separated, dried (anhyd. Na_2SO_4) and concentrated under reduced pressure. Crude product obtained was recrystallized from EtOAc/hexane to get the final compound (**4**) in 75% yield as white solid. mp: 88–90 °C; IR (KBr) ν (cm^{-1}): 3339, 3110, 1740, 1540, 1360, 1215, 1150; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 1H), 5.61–5.60 (m, 1H), 4.49 (d/d, J = 3.4 & 14.2 Hz, 1H), 4.25–3.98 (m, 8H), 3.58–3.51 (m, 5H), 3.39 (d/d, J = 7.3 & 13.6 Hz, 1H), 2.45 (s, 3H), 1.20 (t, J = 7.0 Hz, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.8, 155.0, 152.3, 138.9, 133.3, 68.6, 61.5, 50.9, 42.9, 41.3, 15.0, 14.8; ESI-MS: *m/z* 418 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{15}H_{23}N_5O_5S_2 + H^+$ ($M+H^+$): 418.1219. Found: 418.1219. Elemental analysis (%) for $C_{15}H_{23}N_5O_5S_2$: Calcd.: C, 43.15; H, 5.55; N, 16.77; Found, C, 43.40; H, 5.90; N, 16.69.

The compounds **5**–**24** were synthesized using a procedure similar to described for compound **4**.

4.1.4. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-ethylpiperazine-1-carbodithioate (**5**)

The title compound (**5**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.64 mmol) and sodium 4-ethylpiperazine-1-carbodithioate (3.27 mmol) in 70% yield as semi solid IR (KBr) ν (cm^{-1}): 3405, 3019, 1541, 1360, 1215, 1120; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (s, 1H), 4.66–4.63 (m, 1H), 4.35–4.06 (m, 9H), 3.73–3.69 (m, 1H), 3.63–3.60 (m, 5H), 2.51 (s, 3H), 1.30–1.26 (t, J = 7.1 Hz, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 196.8, 151.7, 138.4, 132.6, 69.9, 61.9, 50.8, 42.8, 40.7, 14.7, 14.6; ESI-MS: *m/z* 374 ($M+H^+$); Elemental analysis (%) for $C_{18}H_{23}ClN_5O_3S_2$: Calcd.: C, 47.41; H, 4.86; N, 15.36; Found, C, 47.31; H, 4.97; N, 15.39.

4.1.5. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-phenylpiperazine-1-carbodithioate (**6**)

The title compound (**6**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.18 mmol) and sodium 4-phenylpiperazine-1-carbodithioate (4.37 mmol) in 82% yield as light yellow solid. mp: 110–112 °C; IR (KBr) ν (cm^{-1}): 3400, 3112, 1560, 1545, 1361, 1217, 1140; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 1H), 7.26–7.22 (m, 2H), 6.96–6.94 (m, 2H), 6.81 (t, J = 7.2 Hz, 1H), 5.63–5.61 (m, 1H), 4.52 (d/d, J = 3.5 & 14.2 Hz, 1H), 4.38 (bs, 2H), 4.23 (d/d, J = 9.1 & 14.2 Hz, 1H), 4.11–4.00 (m, 3H), 3.57 (d/d, J = 4.8 & 13.6 Hz, 1H), 3.40 (d/d, J = 7.3 & 13.6 Hz, 1H), 3.34–3.28 (m, 4H), 2.46 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.3, 152.3, 150.5, 138.9, 133.3, 129.5, 119.7, 115.9, 68.6, 50.9, 49.6, 48.0, 41.3, 14.8; ESI-MS: *m/z* 422 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{18}H_{23}N_5O_3S_2 + H^+$ ($M+H^+$): 422.1321. Found: 422.1320. Elemental analysis (%) for $C_{18}H_{23}N_5O_3S_2$: Calcd.: C, 51.29; H, 5.50; N, 16.61; Found, C, 51.11; H, 5.74; N, 16.69.

4.1.6. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-(2-methoxyphenyl)piperazine-1-carbodithioate (**7**)

The title compound (**7**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.96 mmol) and sodium 4-(2-methoxyphenyl)piperazine-1-carbodithioate (3.93 mmol) in 80% yield as white solid. mp: 85–87 °C; IR (KBr) ν (cm^{-1}): 3400, 3120, 2995, 1562, 1550, 1361, 1216, 1145; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 1H), 7.00–6.97 (m, 2H), 6.92–6.88 (m, 2H), 4.53 (d/d, J = 3.5 & 14.2 Hz, 1H), 4.38–4.01 (m, 6H), 3.80 (s, 3H), 3.58 (d/d, J = 4.8 & 13.6 Hz, 1H), 3.41 (d/d, J = 7.3 & 13.6 Hz, 1H), 3.08–3.06 (m, 4H), 2.88–2.87 (m, 1H), 2.47 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.4, 152.4, 152.3, 140.5, 138.9, 133.3, 123.6, 121.2, 118.9, 112.3, 68.6, 55.8, 51.3, 50.9, 50.1, 41.3, 14.8; ESI-MS: *m/z* 452 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{19}H_{25}N_5O_4S_2 + H^+$ ($M+H^+$): 452.1426. Found: 452.1427. Elemental analysis (%) for $C_{19}H_{25}N_5O_4S_2$: Calcd.: C, 50.54; H, 5.58; N, 15.51; Found, C, 50.75; H,

5.89; N, 15.43.

*4.1.7. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-(pyridin-2-yl)piperazine-1-carbodithioate (**8**)*

The title compound (**8**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.24 mmol) and sodium 4-(pyridin-2-yl)piperazine-1-carbodithioate (4.48 mmol) in 77% yield as yellow solid. mp: 137–139 °C; IR (KBr) ν (cm^{-1}): 3399, 3115, 1560, 1545, 1356, 1215, 1146; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.14–8.13 (m, 1H), 8.02 (s, 1H), 7.59–7.55 (m, 1H), 6.82 (d, J = 8.6 Hz, 1H), 6.69–6.66 (m, 1H), 5.61 (d, J = 5.8 Hz, 1H), 4.52 (d/d, J = 3.5 & 14.2 Hz, 1H), 4.26–4.02 (m, 6H), 3.67 (bs, 4H), 3.58 (d/d, J = 4.7 & 13.6 Hz, 1H), 3.43–3.38 (m, 1H), 2.46 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.4, 158.6, 152.3, 148.0, 138.9, 138.1, 133.3, 113.7, 107.4, 68.6, 50.9, 49.6, 44.1, 41.3, 14.8; ESI-MS: *m/z* 440 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{17}H_{22}N_6O_3S_2$ + H$^+$ (M+H$^+$): 423.1273. Found: 423.1273. Elemental analysis (%) for $C_{17}H_{22}N_6O_3S_2$: Calcd.: C, 48.32; H, 5.25; N, 19.89; Found, C, 48.00; H, 5.54; N, 19.62.

*4.1.8. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-(pyrimidin-2-yl)piperazine-1-carbodithioate (**9**)*

The title compound (**9**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.18 mmol) and sodium 4-(pyrimidin-2-yl)piperazine-1-carbodithioate (4.37 mmol) in 85% yield as white solid. mp: 118–120 °C; IR (KBr) ν (cm^{-1}): 3406, 3111, 1562, 1540, 1356, 1215, 1151; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.39 (d, J = 4.7 Hz, 2H), 8.00 (s, 1H), 6.69 (t, J = 4.7 Hz, 1H), 5.67–5.66 (m, 1H), 4.52 (d/d, J = 3.4 & 14.2 Hz, 1H); 4.33–3.99 (m, 7H), 3.86 (bs, 4H), 3.39 (d/d, J = 7.4 & 13.7 Hz, 1H); 2.45 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.6, 161.2, 158.4, 152.4, 138.9, 133.2, 111.1, 68.6, 50.9, 49.6, 42.9, 41.2, 14.8; ESI-MS: *m/z* 424 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{16}H_{21}N_7O_3S_2$ + H$^+$ (M+H$^+$): 424.1226. Found: 424.1224. Elemental analysis (%) for $C_{16}H_{21}N_7O_3S_2$: Calcd.: C, 45.38; H, 5.00; N, 23.15; Found, C, 45.09; H, 5.32; N, 23.45.

*4.1.9. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-(2-fluorophenyl)piperazine-1-carbodithioate (**10**)*

The title compound (**10**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.85 mmol) and sodium 4-(2-fluorophenyl)piperazine-1-carbodithioate (3.71 mmol) in 72% yield as yellow solid. mp: 110–112 °C; IR (KBr) ν (cm^{-1}): 3400, 3109, 1562, 1540, 1361, 1215, 1143, 1010; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 1H), 7.19–7.00 (m, 4H), 5.62–5.61 (m, 1H), 4.52 (d/d, J = 3.5 & 14.2 Hz, 1H), 4.40 (bs, 2H), 4.23 (d/d, J = 9.1 & 14.2 Hz, 1H), 4.13–4.00 (m, 3H), 3.57 (d/d, J = 4.8 & 13.6 Hz, 1H), 3.43–3.34 (m, 4H), 3.13–2.99 (m, 1H), 2.46 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.6, 156.6, 154.1, 152.3, 139.3, 139.2, 138.9, 133.3, 125.3, 123.5, 123.4, 120.0, 116.6, 116.4, 68.6, 51.4, 50.9, 50.1, 41.4, 14.8; ESI-MS: *m/z* 440 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{18}H_{22}FN_5O_3S_2$ + H$^+$ (M+H$^+$): 440.1226. Found: 440.1221. Elemental analysis (%) for $C_{18}H_{22}FN_5O_3S_2$: Calcd.: C, 49.19; H, 5.05; N, 15.93; Found, C, 49.15; H, 5.42; N, 16.24.

*4.1.10. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-(3-chlorophenyl)piperazine-1-carbodithioate (**11**)*

The title compound (**11**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.40 mmol) and sodium 4-(3-chlorophenyl)piperazine-1-carbodithioate (4.80 mmol) in 76% yield as white solid. mp: 98–100 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 1565, 1536, 1361, 1215, 780; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (s, 1H), 7.23 (t, J = 8.1 Hz, 1H), 6.95–6.94 (m, 1H), 6.88 (d/d, J = 2.0 & 8.3 Hz, 1H), 6.81 (d/d, J = 1.4 & 7.8 Hz, 1H), 5.64–5.63 (m, 1H), 4.52 (d/d, J = 3.5 & 14.2 Hz, 1H), 4.26–4.00 (m, 6H), 3.57 (d/d, J = 4.8 & 13.7 Hz, 1H), 3.43–3.35 (m, 5H), 2.46 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.4, 152.4, 151.6, 138.9, 134.3, 133.3, 131.0, 118.7, 114.8, 113.8, 68.6, 50.9, 49.5, 47.2, 41.3, 14.8; ESI-MS: *m/z* 456 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{18}H_{22}ClN_5O_3S_2$ + H$^+$ (M+H$^+$): 456.0931. Found: 456.0949. Elemental analysis (%) for $C_{18}H_{22}ClN_5O_3S_2$: Calcd.: C, 47.41; H, 4.86; N, 15.36; Found, C, 47.31; H, 4.97; N, 15.39.

*4.1.11. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-(2,3-dichlorophenyl)piperazine-1-carbodithioate (**12**)*

The title compound (**12**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.53 mmol) and sodium 4-(3-chlorophenyl)piperazine-1-carbodithioate (3.06 mmol) in 85% yield as yellow solid. mp: 118–120 °C; IR (KBr) ν (cm^{-1}): 3400, 3119, 1590, 1542, 1370, 1216, 785; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 1H), 7.34–7.30 (m, 2H), 7.17 (d/d, J = 2.6 & 6.9 Hz, 1H), 5.64–5.63 (m, 1H), 4.53 (d/d, J = 3.5 & 14.2 Hz, 1H), 4.39 (bs, 2H), 4.23 (d/d, J = 9.1 & 14.2 Hz, 1H), 4.04–4.00 (m, 3H), 3.57 (d/d, J = 4.8 & 13.6 Hz, 1H), 3.12–3.02 (m, 5H), 2.46 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.9, 152.4, 150.5, 138.9, 133.3, 133.1, 128.9, 126.6, 125.4, 120.4, 68.6, 51.6, 50.9, 50.4, 45.0, 41.4, 14.8; ESI-MS: *m/z* 490 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{18}H_{21}Cl_2N_5O_3S_2$ + H$^+$ (M+H$^+$): 490.0541. Found: 490.0538. Elemental analysis (%) for $C_{18}H_{21}Cl_2N_5O_3S_2$: Calcd.: C, 44.08; H, 4.32; N, 14.28; Found, C, 44.47; H, 4.41; N, 14.01.

*4.1.12. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl pyrrolidine-1-carbodithioate (**13**)*

The title compound (**13**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.09 mmol) and sodium pyrrolidine-1-carbodithioate (2.18 mmol) in 91% yield as white solid. mp: 144–146 °C; IR (KBr) ν (cm^{-1}): 3400, 3119, 1542, 1362, 1215, 1134; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.00 (s, 1H), 5.63–5.61 (m, 1H), 4.49 (d/d, J = 3.5 & 14.2 Hz, 1H); 4.21 (q, J = 9.1 & 14.1 Hz, 1H); 3.95 (bs, 1H), 3.76 (t, J = 6.8 Hz, 2H); 3.63 (t, J = 6.8 Hz, 2H); 3.33 (q, J = 7.3 & 13.7 Hz, 1H); 2.44 (s, 3H), 2.05–1.98 (m, 2H), 1.94–1.87 (m, 2H); ^{13}C (100 MHz, DMSO-d_6): δ 191.0, 152.3, 138.9, 133.2, 68.8, 55.6, 51.0, 40.7, 26.0, 24.1, 14.8; ESI-MS: *m/z* 331 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{12}H_{18}N_4O_3S_2$ + H$^+$ (M+H$^+$): 331.0899. Found: 331.0897. Elemental analysis (%) for $C_{12}H_{18}N_4O_3S_2$: Calcd.: C, 43.62; H, 5.49; N, 16.96; Found, C, 43.79; H, 5.83; N, 16.75.

*4.1.13. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl morpholine-4-carbodithioate (**14**)*

The title compound (**14**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.63 mmol) and sodium morpholine-4-carbodithioate (3.27 mmol) in 84% yield as white solid. mp: 160–162 °C; IR (KBr) ν (cm^{-1}): 3400, 3122, 1550, 1352, 1217, 1094; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 1H), 5.61–5.60 (m, 1H), 4.52 (d/d, J = 3.4 & 14.2 Hz, 1H), 4.25–3.98 (m, 6H), 3.69–3.66 (m, 4H), 3.56 (d/d, J = 4.7 & 13.6 Hz, 1H), 3.39 (d/d, J = 7.3 & 13.7 Hz, 1H), 2.46 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.8, 152.3, 138.9, 133.3, 68.6, 66.0, 51.9, 50.9, 41.2, 14.8; ESI-MS: *m/z* 347 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{12}H_{18}N_4O_4S_2$ + H$^+$ (M+H$^+$): 347.0848. Found: 347.0846. Elemental analysis (%) for $C_{12}H_{18}N_4O_4S_2$: Calcd.: C, 41.60; H, 5.24; N, 16.17; Found, C, 41.77; H, 5.58; N, 16.39.

*4.1.14. (±)–2-Hydroxy-3-(5-methyl-2-nitro-1H-imidazol-1-yl) propyl piperidine-1-carbodithioate (**15**)*

The title compound (**15**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.80 mmol) and sodium piperidine-4-carbodithioate (3.60 mmol) in 78% yield as white solid. mp: 143–145 °C; IR (KBr) ν (cm^{-1}): 3400, 3125, 2995, 1543, 1362, 1215, 1135; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (s, 1H),

5.58–5.57 (m, 1H), 4.52 (d/d, J = 3.4 & 14.2 Hz, 1H); 4.25–3.96 (m, 6H), 3.52 (d/d, J = 5.0 & 13.6 Hz, 1H), 3.41–3.39 (m, 1H), 2.45 (s, 3H), 1.67–1.59 (m, 6H); ^{13}C (100 MHz, DMSO-d_6): δ 193.8, 152.3, 138.9, 133.3, 68.7, 53.0, 51.4, 50.9, 41.3, 26.2, 25.6, 24.0, 14.8; ESI-MS: *m/z* 345 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{13}H_{20}N_4O_3S_2$ + H$^+$ (M+H$^+$): 345.1055. Found: 345.1053. Elemental analysis (%) for $C_{13}H_{20}N_4O_3S_2$: Calcd.: C, 45.33; H, 5.85; N, 16.27; Found, C, 45.11; H, 6.09; N, 16.02.

4.1.15. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 3-methylpiperidine-1-carbodithioate (**16**)

The title compound (**16**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.24 mmol) and sodium 3-methylpiperidine-1-carbodithioate (4.48 mmol) in 77% yield as white solid. mp: 99–101 °C; IR (KBr) ν (cm^{-1}): 3400, 3113, 2990, 1535, 1362, 1215, 1144; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.78 (s, 1H), 5.28 (bs, 1H); 4.61–4.48 (m, 3H), 4.23–4.19 (m, 2H), 3.68–3.55 (m, 2H), 3.21 (bs, 1H), 2.97–2.79 (m, 1H), 2.46 (s, 3H), 1.93–1.58 (m, 4H), 1.27–1.20 (m, 1H), 0.95–0.93 (m, 3H); ^{13}C (100 MHz, CDCl$_3$): δ 194.7, 151.6, 138.4, 132.3, 69.9, 59.5, 57.6, 53.2, 50.8, 40.7, 32.7, 31.2, 25.3, 18.7, 14.6; ESI-MS: *m/z* 359 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{14}H_{22}N_4O_3S_2$ + H$^+$ (M+H$^+$): 359.1212. Found: 359.1200. Elemental analysis (%) for $C_{14}H_{22}N_4O_3S_2$: Calcd.: C, 46.91; H, 6.19; N, 15.63; Found, C, 46.70; H, 6.48; N, 15.31.

4.1.16. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-methylpiperidine-1-carbodithioate (**17**)

The title compound (**17**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.64 mmol) and sodium 4-methylpiperidine-1-carbodithioate (3.27 mmol) in 81% yield as white solid. mp: 125–127 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 2995, 1542, 1358, 1216, 1150; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (s, 1H), 5.58–5.56 (m, 1H), 5.27 (bs, 1H), 4.51 (d/d, J = 3.4 & 14.1 Hz, 2H), 4.21 (q, J = 9.2 & 14.1 Hz, 1H), 3.97 (bs, 1H), 3.51 (d/d, J = 5.0 & 13.6 Hz, 1H), 3.40–3.21 (m, 3H), 2.45 (s, 3H), 1.77–1.76 (m, 3H), 1.16–1.10 (m, 2H), 0.93–0.91 (m, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 194.0, 152.3, 138.9, 133.3, 68.7, 52.3, 50.9, 50.5, 41.4, 34.1, 33.7, 30.4, 21.5, 14.8; ESI-MS: *m/z* 359 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{14}H_{22}N_4O_3S_2$ + H$^+$ (M+H$^+$): 359.1212. Found: 359.1201. Elemental analysis (%) for $C_{14}H_{22}N_4O_3S_2$: Calcd.: C, 46.91; H, 6.19; N, 15.63; Found, C, 47.12; H, 6.47; N, 15.78.

4.1.17. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-benzylpiperidine-1-carbodithioate (**18**)

The title compound (**18**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.80 mmol) and sodium 4-benzylpiperidine-1-carbodithioate (3.60 mmol) in 71% yield as semi solid. IR (KBr) ν (cm^{-1}): 3400, 3112, 2990, 1540, 1361, 1215, 1150; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.93 (s, 1H), 7.33–7.29 (m, 2H), 7.25–7.23 (m, 1H), 7.15 (d, J = 6.8 Hz, 1H), 5.52–5.50 (m, 1H), 4.66–4.62 (m, 2H), 4.28–4.23 (m, 2H), 3.78–3.66 (m, 3H), 3.20–3.09 (m, 2H), 2.60–2.58 (m, 2H), 2.55 (s, 3H), 1.83–1.80 (m, 1H), 1.43–1.27 (m, 4H); ^{13}C (100 MHz, CDCl$_3$): δ 195.0, 151.7, 139.5, 138.5, 132.7, 129.0, 128.4, 126.2, 70.1, 50.9, 47.0, 42.4, 40.6, 37.9, 32.0, 31.5, 14.7; ESI-MS: *m/z* 435 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{20}H_{26}N_4O_3S_2$ + H$^+$ (M+H$^+$): 435.1525. Found: 435.1517. Elemental analysis (%) for $C_{20}H_{26}N_4O_3S_2$: Calcd.: C, 55.28; H, 6.03; N, 12.89; Found, C, 55.40; H, 6.40; N, 13.02.

4.1.18. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl azepane-1-carbodithioate (**19**)

The title compound (**19**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.18 mmol) and sodium azepane-1-carbodithioate (4.37 mmol) in 76% yield as white solid. mp: 114–116 °C; IR (KBr) ν (cm^{-1}): 3405, 3120, 2995, 1536, 1365, 1215, 1143; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.87 (s, 1H), 4.63–4.61 (m, 1H), 4.28–4.13 (m, 4H), 3.99–3.92 (m, 3H), 3.74–3.70 (m, 1H), 3.64–3.59 (m, 1H), 2.52 (s, 3H), 1.89–1.87 (m, 4H), 1.61–1.58 (m, 4H); ^{13}C (100 MHz, CDCl$_3$): δ 195.7, 151.7, 138.5, 132.7, 70.4, 56.4, 53.2, 50.9, 40.3, 27.2, 26.5, 26.4, 26.1, 14.7; ESI-MS: *m/z* 359 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{14}H_{22}N_4O_3S_2$ + H$^+$ (M+H$^+$): 359.1212. Found: 359.1212. Elemental analysis (%) for $C_{14}H_{22}N_4O_3S_2$: Calcd.: C, 46.91; H, 6.19; N, 15.63; Found, C, 46.99; H, 6.42; N, 15.59.

4.1.19. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl dimethylcarbamodithioate (**20**)

The title compound (**20**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.18 mmol) and sodium dimethylcarbamodithioate (4.37 mmol) in 68% yield as white solid. mp: 143–145 °C; IR (KBr) ν (cm^{-1}): 3401, 3111, 3010, 1540, 1365, 1215, 1135; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.88 (s, 1H), 4.64–4.61 (m, 1H), 4.27–4.10 (m, 2H), 3.73–3.68 (m, 1H), 3.60–3.43 (m, 6H), 3.26–3.06 (m, 1H), 2.52 (s, 3H); ^{13}C (100 MHz, CDCl$_3$): δ 196.6, 151.7, 138.5, 132.6, 70.2, 50.9, 45.9, 41.8, 41.0, 14.7; ESI-MS: *m/z* 305 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{10}H_{16}N_4O_3S_2$ + H$^+$ (M+H$^+$): 305.0742. Found: 305.0738. Elemental analysis (%) for $C_{10}H_{16}N_4O_3S_2$: Calcd.: C, 39.46; H, 5.30; N, 18.41; Found, C, 39.73; H, 5.69; N, 18.33.

4.1.20. 2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl ethyl(methyl)carbamodithioate (**21**)

The title compound (**21**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.42 mmol) and sodium dimethylcarbamodithioate (2.84 mmol) in 68% yield as white solid. mp: 80–82 °C; IR (KBr) ν (cm^{-1}): 3405, 3110, 3012, 1530, 1360, 1216, 1140; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.87 (s, 1H), 4.64–4.61 (m, 1H), 4.25–4.23 (m, 2H), 4.12–3.86 (m, 3H), 3.72–3.56 (m, 2H), 3.50 (s, 1H), 3.37 (s, 1H), 2.52 (s, 3H), 1.31–1.23 (m, 3H); ^{13}C (100 MHz, CDCl$_3$): δ 195.4, 151.7, 138.5, 132.6, 70.2, 52.7, 50.9, 49.7, 43.5, 40.8, 39.2, 14.7, 12.2, 11.1; ESI-MS: *m/z* 319 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{11}H_{18}N_4O_3S_2$ + H$^+$ (M+H$^+$): 319.0899. Found: 319.0892. Elemental analysis (%) for $C_{11}H_{18}N_4O_3S_2$: Calcd.: C, 41.49; H, 5.70; N, 17.60; Found, C, 41.63; H, 5.96; N, 17.31.

4.1.21. (±)–2-Hydroxy-3-(5-methyl-2-nitro-1H-imidazol-1-yl) propyl diethylcarbamodithioate (**22**)

The title compound (**22**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.82 mmol) and sodium diethylcarbamodithioate (3.64 mmol) in 71% yield as white solid. mp: 91–93 °C; IR (KBr) ν (cm^{-1}): 3400, 3105 2995, 1538, 1357, 1215, 1147; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (s, 1H), 5.58–5.57 (m, 1H), 4.51 (d/d, J = 3.4 & 14.2 Hz, 1H), 4.21 (q, J = 9.2 & 14.2 Hz, 1H), 3.99–3.93 (m, 3H), 3.78 (q, J = 7.0 & 14.2 Hz, 2H), 3.50 (d/d, J = 5.2 & 13.7 Hz, 1H), 3.38 (d/d, J = 7.0 & 13.7 Hz, 1H), 2.45 (s, 3H), 1.25 (t, J = 7.0 Hz, 3H), 1.17 (t, J = 7.0 Hz, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 194.0, 152.3, 138.9, 133.3, 68.6, 50.8, 49.7, 47.0, 41.2, 14.8, 12.8, 11.7; ESI-MS: *m/z* 333 (M+H$^+$); HRMS (ESI): *m/z* calculated for $C_{12}H_{20}N_4O_3S_2$ + H$^+$ (M+H$^+$): 333.1055. Found: 333.1044. Elemental analysis (%) for $C_{12}H_{20}N_4O_3S_2$: Calcd.: C, 43.35; H, 6.06; N, 16.85; Found, C, 43.59; H, 6.35; N, 16.59.

4.1.22. (±)–2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propyl 4-butylpiperazine-1-carbodithioate (**23**)

The title compound (**23**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.15 mmol) and sodium 4-butylpiperazine-1-carbodithioate (2.30 mmol) in 74% yield as white solid. mp: 73–75 °C; IR (KBr) ν (cm^{-1}): 3400, 3109 1545, 1361, 1215, 1149; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (s, 1H), 5.60–5.58 (m, 1H), 4.51 (d/d, J = 3.5 & 14.2 Hz, 1H), 4.25–4.19 (m,

3H), 3.98–3.94 (m, 3H), 3.53 (d/d, J = 4.8 & 13.7 Hz, 1H), 3.37 (q, J = 7.3 & 13.7 Hz, 1H), 2.45–2.43 (m, 7H), 2.30 (t, J = 7.2 Hz, 2H), 1.46–1.38 (m, 2H), 1.34–1.25 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.0, 152.3, 138.9, 133.3, 68.6, 57.3, 52.6, 51.7, 50.9, 50.1, 41.3, 28.8, 20.4, 14.8, 14.3; ESI-MS: m/z 402 (M+H$^+$); HRMS (ESI): m/z calculated for $C_{15}H_{23}N_5O_5S_2$ + H$^+$ (M+H$^+$): 402.1634. Found: 402.1625. Elemental analysis (%) for $C_{15}H_{23}N_5O_5S_2$: Calcd.: C, 47.86; H, 6.78; N, 17.44; Found, C, 48.11; H, 6.59; N, 17.12.

4.1.23. (±)–tert-Butyl 4-((2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propylthio)carbonothioyl)piperazine-1-carboxylate (**24**)

The title compound (**24**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.36 mmol) and sodium 4-(tert-butoxycarbonyl)piperazine-1-carbodithioate (2.73 mmol) in 85% yield as white solid. mp: 83–85 °C; IR (KBr) ν (cm^{-1}): 3410, 3106, 1740, 1538, 1357, 1215, 1145; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (s, 1H), 4.68–4.61 (m, 1H), 4.24–4.09 (m, 4H), 4.01–3.95 (m, 3H), 3.75–3.70 (m, 1H), 3.63–3.56 (m, 5H), 2.51 (s, 3H), 1.47 (s, 9H); ^{13}C (100 MHz, $CDCl_3$): δ 196.7, 154.4, 151.7, 138.4, 132.6, 80.7, 70.0, 51.5, 50.8, 49.9, 42.3, 40.6, 28.3, 14.7; ESI-MS: m/z 446 (M+H$^+$); HRMS (ESI): m/z calculated for $C_{17}H_{27}N_5O_5S_2$ + H$^+$ (M+H$^+$): 446.1532. Found: 446.1485. Elemental analysis (%) for $C_{17}H_{27}N_5O_5S_2$: Calcd.: C, 45.83; H, 6.11; N, 15.72; Found, C, 46.03; H, 6.44; N, 15.90.

4.1.24. (±)–Ethyl 4-(2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)piperazine-1-carboxylate (**25**)

To a solution of (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.36 mmol) in acetonitrile (20 mL) was added ethyl piperazine-1-carboxylate (1.50 mmol) and contents were stirred at 80 °C for overnight. Reaction progress was monitored by TLC and after completion of the reaction acetonitrile was evaporated. The crude obtained was extracted with EtOAc (20 mL), excess water (50 mL) and organic layer was separated, dried (anhyd. Na_2SO_4) and concentrated *in vacuo* to get the final compound (**25**) in 77% yield as semi solid. IR (KBr) ν (cm^{-1}): 3400, 3110, 1742, 1543, 1361, 1090; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (s, 1H), 4.60 (d/d, J = 6.4 & 18.12 Hz, 1H), 4.15–4.04 (m, 4H), 3.49–3.46 (m, 4H), 3.25 (bs, 1H), 2.62–2.53 (m, 6H), 2.43–2.40 (m, 3H), 1.26 (t, J = 7.1 Hz, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 155.4, 152.0, 138.4, 133.1, 66.8, 61.4, 61.1, 53.0, 50.2, 43.6, 14.7, 14.6; ESI-MS: m/z 341 (M+H$^+$); HRMS (ESI): m/z calculated for $C_{14}H_{23}N_5O_5$ + H$^+$ (M+H$^+$): 342.1777. Found: 342.1770. Elemental analysis (%) for $C_{14}H_{23}N_5O_5$: Calcd.: C, 49.26; H, 6.79; N, 20.52; Found, C, 49.41; H, 7.02; N, 20.42.

The compounds **26**–**50** were synthesized using a procedure similar to described for compound **25**.

4.1.25. (±)–1-(4-Ethylpiperazin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**26**)

The title compound (**26**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.18 mmol) and 1-ethylpiperazine (2.40 mmol) in 81% yield as semi solid. IR (KBr) ν (cm^{-1}): 3400, 3115, 1536, 1361, 1140; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.00 (s, 1H), 5.06–5.05 (m, 1H), 4.59 (d/d, J = 2.8 & 14.1 Hz, 1H), 4.05 (d/d, J = 9.3 & 14.1 Hz, 1H), 3.90 (bs, 1H), 2.50–2.26 (m, 15H), 0.98 (t, J = 7.1 Hz, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 152.6, 138.9, 133.3, 67.1, 62.7, 53.9, 52.8, 52.1, 51.0, 14.8, 12.4; ESI-MS: m/z 298 (M+H$^+$); HRMS (ESI): m/z calculated for $C_{13}H_{23}N_5O_3$ + H$^+$ (M+H$^+$): 298.1879. Found: 298.1898. Elemental analysis (%) for $C_{13}H_{23}N_5O_3$: Calcd.: C, 52.51; H, 7.80; N, 23.55; Found, C, 52.79; H, 8.11; N, 23.19.

4.1.26. (±)–1-(2-Methyl-5-nitro-1H-imidazol-1-yl)-3-(4-phenylpiperazin-1-yl)propan-2-ol (**27**)

The title compound (**27**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.42 mmol) and 1-phenylpiperazine (1.56 mmol) in 78% yield as white solid. mp: 78–80 °C; IR (KBr) ν (cm^{-1}): 3400, 3115, 1570, 1541, 1361, 1146; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H), 7.30–7.26 (m, 2H), 6.94–6.87 (m, 3H), 4.63–4.60 (m, 1H), 4.11–4.07 (m, 2H), 3.36 (bs, 1H), 3.22–3.18 (m, 4H), 2.85–2.80 (m, 2H), 2.66–2.60 (m, 3H), 2.58 (s, 3H), 2.49–2.44 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 151.0, 138.4, 133.1, 129.1, 120.0, 116.1, 66.7, 61.0, 53.3, 50.3, 49.1, 14.8; ESI-MS: m/z 345 (M+H$^+$); HRMS (ESI): m/z calculated for $C_{17}H_{23}N_5O_3$ + H$^+$ (M+H$^+$): 346.1879. Found: 346.1894. Elemental analysis (%) for $C_{17}H_{23}N_5O_3$: Calcd.: C, 59.12; H, 6.71; N, 20.28; Found, C, 59.41; H, 6.95; N, 20.00.

4.1.27. (±)–1-(4-(2-Methoxyphenyl)piperazin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**28**)

The title compound (**28**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.74 mmol) and 1-(2-methoxyphenyl)piperazine (1.91 mmol) in 74% yield as oil. IR (KBr) ν (cm^{-1}): 3400, 3120, 2995, 1562, 1546, 1360, 1153; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.02 (d/d, J = 4.5 & 7.9 Hz, 1H), 6.93 (d, J = 4.0 Hz, 2H), 6.88 (d, J = 7.9 Hz, 1H), 4.63–4.60 (m, 1H), 4.10–4.07 (m, 2H), 3.88 (s, 3H), 3.10–3.08 (m, 5H), 2.90–2.86 (m, 2H), 2.67–2.64 (m, 3H), 2.59 (s, 3H), 2.47–2.41 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): δ 152.1, 152.0, 140.9, 138.4, 133.1, 123.1, 120.9, 118.1, 111.2, 66.6, 61.0, 55.3, 53.4, 50.6, 50.4, 14.8; ESI-MS: m/z 376 (M+H$^+$); HRMS (ESI): m/z calculated for $C_{18}H_{25}N_5O_4$ + H$^+$ (M+H$^+$): 376.1985. Found: 376.1984. Elemental analysis (%) for $C_{18}H_{25}N_5O_4$: Calcd.: C, 57.59; H, 6.71; N, 18.65; Found, C, 57.70; H, 7.03; N, 18.53.

4.1.28. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(4-(pyridin-2-yl)piperazin-1-yl)propan-2-ol (**29**)

The title compound (**29**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.42 mmol) and 1-(pyridin-2-yl)piperazine (1.56 mmol) in 75% yield as white solid. mp: 130–132 °C; IR (KBr) ν (cm^{-1}): 3400, 3113, 1562, 1541, 1360, 1151; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21–8.19 (m, 1H), 7.99 (s, 1H), 7.52–7.48 (m, 1H), 6.67–6.65 (m, 2H), 4.63–4.61 (m, 1H), 4.11–4.09 (m, 2H), 3.60–3.51 (m, 5H), 2.81–2.76 (m, 2H), 2.59–2.58 (m, 6H), 2.45 (d/d, J = 9.6 & 12.1 Hz, 1H); ^{13}C (100 MHz, $CDCl_3$): δ 159.3, 152.1, 147.9, 138.3, 137.5, 133.1, 113.6, 107.2, 66.7, 61.1, 53.1, 50.3, 45.2, 14.8; ESI-MS: m/z 347 (M+H$^+$); HRMS (ESI): m/z calculated for $C_{16}H_{22}N_6O_3$ + H$^+$ (M+H$^+$): 347.1832. Found: 347.1824. Elemental analysis (%) for $C_{16}H_{22}N_6O_3$: Calcd.: C, 55.48; H, 6.40; N, 24.26; Found, C, 55.29; H, 6.78; N, 24.20.

4.1.29. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)propan-2-ol (**30**)

The title compound (**30**) was synthesized from **(±)**–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.91 mmol) and 2-(piperazin-1-yl)pyrimidine (2.10 mmol) in 79% yield as white solid. mp: 183–185 °C; IR (KBr) ν (cm^{-1}): 3401, 3110, 1565, 1550, 1355, 1150; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (d, J = 4.7 Hz, 2H), 7.97 (s, 1H), 6.51 (t, J = 4.7 Hz, 1H), 4.63–4.60 (m, 1H), 4.10–4.07 (m, 2H), 3.85–3.81 (m, 5H), 2.74–2.69 (m, 2H), 2.62–2.41 (m, 7H); ^{13}C (100 MHz, $CDCl_3$): δ 161.6, 157.7, 152.0, 138.4, 133.2, 110.1, 66.7, 61.0, 53.1, 50.2, 43.6, 14.8; ESI-MS: m/z 348 (M+H$^+$); HRMS (ESI): m/z calculated for $C_{15}H_{21}N_7O_3$ + H$^+$ (M+H$^+$): 348.1784. Found: 348.1784. Elemental analysis (%) for $C_{15}H_{21}N_7O_3$: Calcd.: C, 51.86; H, 6.09; N, 28.23; Found, C, 52.06; H, 5.89; N, 28.53.

*4.1.30. (±)–1-(4-(2-Fluorophenyl)piperazin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**31**)*

The title compound (**31**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.64 mmol) and 1-(2-fluorophenyl)piperazine (1.80 mmol) in 71% yield as light yellow solid. mp: 73–75 °C; IR (KBr) ν (cm^{-1}): 3400, 3111, 1563, 1551, 1362, 1150; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H), 7.08–7.02 (m, 2H), 6.97–6.92 (m, 2H), 4.64–4.59 (m, 1H), 4.11–4.05 (m, 2H), 3.13–3.05 (m, 5H), 2.86–2.81 (m, 2H), 2.67–2.62 (m, 3H), 2.58 (s, 3H), 2.48–2.42 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): δ 156.9, 154.4, 152.0, 139.8, 138.4, 133.1, 124.4, 122.7, 118.9, 116.2, 116.0, 66.7, 61.0, 53.3, 50.5, 46.1, 14.8; ESI-MS: *m/z* 364 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{17}H_{22}FN_5O_3 + H^+$ ($M+H^+$): 364.1785. Found: 364.1784. Elemental analysis (%) for $C_{17}H_{22}FN_5O_3$: Calcd.: C, 56.19; H, 6.10; N, 19.27; Found, C, 56.01; H, 6.41; N, 19.60.

*4.1.31. (±)–1-(4-(3-Chlorophenyl)piperazin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**32**)*

The title compound (**32**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.64 mmol) and 1-(3-chlorophenyl)piperazine (1.80 mmol) in 82% yield as oily. IR (KBr) ν (cm^{-1}): 3400, 3114, 1560, 1553, 1363, 1154, 780; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (s, 1H), 7.16 (t, J = 8.1 Hz, 1H), 6.87–6.76 (m, 3H), 4.63–4.60 (m, 1H), 4.08–4.05 (m, 2H), 3.20–3.14 (m, 4H), 3.03 (bs, 1H), 2.80–2.75 (m, 2H), 2.62–2.57 (m, 5H), 2.46–2.43 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): δ 152.6, 152.0, 138.4, 134.9, 133.1, 130.0, 119.5, 115.8, 113.9, 66.8, 61.0, 53.1, 50.3, 48.7, 14.7; ESI-MS: *m/z* 380 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{17}H_{22}ClN_5O_3 + H^+$ ($M+H^+$): 380.1489. Found: 380.1480. Elemental analysis (%) for $C_{17}H_{22}ClN_5O_3$: Calcd.: C, 53.75; H, 5.84; N, 18.44; Found, C, 53.51; H, 6.11; N, 18.63.

*4.1.32. (±)–1-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**33**)*

The title compound (**33**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.36 mmol) and 1-(2,3-dichlorophenyl)piperazine (1.50 mmol) in 79% yield as semi solid. IR (KBr) ν (cm^{-1}): 3400, 3120, 1594, 1546, 1366, 780; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.18–7.13 (m, 2H), 6.95–6.93 (m, 1H), 4.65–4.59 (m, 1H), 4.09–4.06 (m, 2H), 3.76–3.75 (m, 1H), 3.05–3.00 (m, 5H), 2.86–2.81 (m, 2H), 2.67–2.63 (m, 2H), 2.57 (s, 3H), 2.49–2.41 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 150.9, 138.4, 134.0, 133.1, 127.4, 124.7, 124.5, 118.5, 66.7, 61.0, 53.3, 51.3, 50.3, 14.8; ESI-MS: *m/z* 414 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{17}H_{21}Cl_2N_5O_3 + H^+$ ($M+H^+$): 414.1100. Found: 414.1082. Elemental analysis (%) for $C_{17}H_{21}Cl_2N_5O_3$: Calcd.: C, 49.29; H, 5.11; N, 16.90; Found, C, 49.47; H, 5.34; N, 16.66.

*4.1.33. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(pyrrolidin-1-yl)propan-2-ol (**34**)*

The title compound (**34**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.64 mmol) and pyrrolidine (1.80 mmol) in 83% yield as white solid. mp: 86–88 °C; IR (KBr) ν (cm^{-1}): 3400, 3124, 1546, 1361, 1130; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 4.58 (d/d, J = 2.2 & 14.0 Hz, 1H), 4.08 (d/d, J = 8.3 & 14.0 Hz, 1H), 4.01–3.96 (m, 1H), 2.69–2.65 (m, 3H), 2.58 (s, 3H), 2.54–2.50 (m, 3H), 1.80–1.77 (m, 4H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 138.4, 133.1, 68.4, 59.2, 54.1, 50.4, 23.5, 14.7; ESI-MS: *m/z* 255 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{11}H_{18}N_4O_3 + H^+$ ($M+H^+$): 255.1457. Found: 255.1444. Elemental analysis (%) for $C_{11}H_{18}N_4O_3$: Calcd.: C, 51.96; H, 7.13; N, 22.03; Found, C, 52.09; H, 7.46; N, 22.21.

*4.1.34. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-morpholinopropan-2-ol (**35**)*

The title compound (**35**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.36 mmol) and morpholine (1.50 mmol) in 76% yield as white solid. mp: 116–118 °C; IR (KBr) ν (cm^{-1}): 3400, 3120, 1550, 1350, 1104; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 4.62–4.59 (m, 1H), 4.09–4.05 (m, 2H), 3.73–3.71 (m, 4H), 3.50–3.41 (m, 1H), 2.68–2.63 (m, 2H), 2.58 (bs, 4H), 2.49–2.45 (m, 2H), 2.40–2.34 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 138.3, 133.0, 66.8, 66.6, 61.6, 53.7, 50.3, 14.7; ESI-MS: *m/z* 271 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{11}H_{18}N_4O_4 + H^+$ ($M+H^+$): 271.1406. Found: 271.1404. Elemental analysis (%) for $C_{11}H_{18}N_4O_4$: Calcd.: C, 48.88; H, 6.71; N, 20.73; Found, C, 48.97; H, 6.99; N, 20.79.

*4.1.35. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(piperidin-1-yl)propan-2-ol (**36**)*

The title compound (**36**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.91 mmol) and piperidine (2.10 mmol) in 80% yield as white solid. mp: 115–117 °C; IR (KBr) ν (cm^{-1}): 3400, 3124, 3011, 1540, 1365, 1132; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H), 4.56–4.53 (m, 1H), 4.05–3.98 (m, 2H), 2.55 (bs, 5H), 2.47 (d/d, J = 3.4 & 12.2 Hz, 1H), 2.33–2.25 (m, 3H), 1.55–1.41 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 138.4, 133.0, 66.5, 61.6, 54.6, 50.5, 25.9, 24.0, 14.7; ESI-MS: *m/z* 269 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{12}H_{20}N_4O_3 + H^+$ ($M+H^+$): 269.1614. Found: 269.1598. Elemental analysis (%) for $C_{12}H_{20}N_4O_3$: Calcd.: C, 53.72; H, 7.51; N, 20.88; Found, C, 53.44; H, 7.87; N, 20.98.

*4.1.36. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(3-methylpiperidin-1-yl)propan-2-ol (**37**)*

The title compound (**37**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.69 mmol) and 3-methylpiperidine (1.86 mmol) in 74% yield as oil. IR (KBr) ν (cm^{-1}): 3400, 3121, 2995, 1541, 1362, 1140; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (s, 1H), 4.58–4.55 (m, 1H), 4.06–4.00 (m, 2H), 2.86–2.79 (m, 1H), 2.69–2.66 (m, 1H), 2.58 (s, 3H), 2.52–2.47 (m, 1H), 2.31–2.25 (m, 2H), 1.96–1.92 (m, 1H), 1.68–1.57 (m, 5H), 0.93–0.85 (m, 4H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 138.4, 133.1, 66.6, 66.5, 61.4, 61.2, 60.5, 55.5, 50.4, 32.6, 31.1, 25.5, 19.4, 14.7; ESI-MS: *m/z* 283 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{13}H_{22}N_4O_3 + H^+$ ($M+H^+$): 283.1770. Found: 283.1757. Elemental analysis (%) for $C_{13}H_{22}N_4O_3$: Calcd.: C, 55.30; H, 7.85; N, 19.84; Found, C, 55.49; H, 8.14; N, 19.97.

*4.1.37. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(4-methylpiperidin-1-yl)propan-2-ol (**38**)*

The title compound (**38**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.91 mmol) and 4-methylpiperidine (2.10 mmol) in 73% yield as oil. IR (KBr) ν (cm^{-1}): 3400, 3110, 2990, 1544, 1365, 1150; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (s, 1H), 4.58–4.54 (m, 1H), 4.08–3.99 (m, 2H), 2.91–2.89 (m, 1H), 2.77–2.74 (m, 1H), 2.58 (s, 3H), 2.54–2.49 (m, 2H), 2.35–2.28 (m, 2H), 2.02–1.96 (m, 1H), 1.65–1.63 (m, 2H), 1.41–1.36 (m, 1H), 1.27–1.25 (m, 1H), 1.15–1.12 (m, 1H), 0.93 (t, J = 6.5 Hz, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 138.3, 133.1, 66.6, 61.3, 55.6, 52.6, 50.5, 34.1, 30.5, 21.7, 14.7; ESI-MS: *m/z* 283 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{13}H_{22}N_4O_3 + H^+$ ($M+H^+$): 283.1770. Found: 283.1762. Elemental analysis (%) for $C_{13}H_{22}N_4O_3$: Calcd.: C, 55.30; H, 7.85; N, 19.84; Found, C, 55.49; H, 8.13; N, 20.07.

*4.1.38. (±)–1-(4-Benzylpiperidin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**39**)*

The title compound (**39**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.42 mmol) and 4-

benzylpiperidine (1.56 mmol) in 68% yield as white solid. mp: 134–136 °C; IR (KBr) ν (cm^{-1}): 3400, 3115, 2998, 1545, 1360, 1150; ^1H NMR (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.31–7.27 (m, 2H), 7.22–7.19 (m, 1H), 7.16–7.14 (m, 2H), 4.56 (d/d, J = 1.6 & 13.5 Hz, 1H); 4.08–3.99 (m, 2H), 2.92–2.89 (m, 1H), 2.78–2.76 (m, 1H), 2.58–2.49 (m, 7H), 2.33–2.26 (m, 2H), 1.99–1.92 (m, 1H), 1.68–1.67 (m, 2H), 1.64–1.57 (m, 1H), 1.31–1.20 (m, 2H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 140.4, 138.4, 133.2, 129.0, 128.2, 125.8, 66.6, 61.1, 55.5, 52.4, 50.4, 43.0, 37.6, 32.3, 32.1, 14.8; ESI-MS: *m/z* 359 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{19}H_{26}N_4O_3 + H^+$ ($M+H^+$): 359.2083. Found: 359.2080. Elemental analysis (%) for $C_{19}H_{26}N_4O_3$: Calcd.: C, 63.67; H, 7.31; N, 15.63; Found, C, 63.81; H, 7.53; N, 15.50.

4.1.39. (±)–1-(Azepan-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**40**)

The title compound (**40**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.74 mmol) and azepane (1.92 mmol) in 80% yield as white solid. mp: 92–94 °C; IR (KBr) ν (cm^{-1}): 3400, 3122, 3011, 1542, 1364, 1147; ^1H NMR (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 4.56 (d/d, J = 2.1 & 14.0 Hz, 1H); 4.04 (d/d, J = 8.2 & 14.0 Hz, 1H); 3.93–3.88 (m, 1H), 2.82–2.73 (m, 3H), 2.66–2.61 (m, 2H), 2.59 (s, 3H), 2.35–2.30 (m, 1H), 1.70–1.61 (m, 8H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 138.3, 133.1, 67.3, 60.8, 55.7, 50.4, 28.4, 26.8, 14.8; ESI-MS: *m/z* 283 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{13}H_{22}N_4O_3 + H^+$ ($M+H^+$): 283.1770. Found: 283.1764. Elemental analysis (%) for $C_{13}H_{22}N_4O_3$: Calcd.: C, 55.30; H, 7.85; N, 19.84; Found, C, 55.04; H, 8.11; N, 19.55.

4.1.40. (±)–1-(4-(Furan-2-yl)piperazin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**41**)

The title compound (**41**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.36 mmol) and 1-(furan-2-yl)piperazine (1.50 mmol) in 82% yield as semi solid. IR (KBr) ν (cm^{-1}): 3400, 3121, 2995, 1550, 1360; ^1H NMR (400 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.47 (d, J = 0.8 Hz, 1H), 6.97 (d, J = 3.5 Hz, 1H), 6.48–6.46 (m, 1H), 4.65–4.62 (m, 1H), 4.11–4.00 (m, 2H), 3.78 (bs, 4H), 2.69–2.64 (m, 2H), 2.57–2.43 (m, 8H); ^{13}C (100 MHz, $CDCl_3$): δ 159.0, 152.0, 147.6, 143.8, 138.4, 133.0, 116.6, 111.3, 66.9, 61.2, 53.4, 50.4, 43.6, 14.7; ESI-MS: *m/z* 335 ($M+H^+$); Elemental analysis (%) for $C_{15}H_{21}N_5O_4$: Calcd.: C, 53.72; H, 6.31; N, 20.88; Found, C, 53.60; H, 6.00; N, 20.51.

4.1.41. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(2-methylpiperidin-1-yl)propan-2-ol (**42**)

The title compound (**42**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 2.18 mmol) and 2-methylpiperidine (2.39 mmol) in 60% yield as white solid. mp: 85–87 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 2995, 1544, 1364, 1150; ^1H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H), 4.58–4.52 (m, 1H), 4.12–3.94 (m, 2H), 2.81–2.77 (m, 1H), 2.68–2.63 (m, 1H), 2.57 (s, 3H), 2.32–2.20 (m, 2H), 2.17–2.14 (m, 1H), 1.65–1.26 (m, 7H), 1.04–1.03 (m, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 152.0, 138.4, 133.1, 68.0, 66.5, 57.7, 56.9, 55.5, 52.3, 50.7, 34.6, 33.1, 23.6, 21.6, 16.2, 14.7; ESI-MS: *m/z* 283 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{13}H_{22}N_4O_3 + H^+$ ($M+H^+$): 283.1770. Found: 283.1761. Elemental analysis (%) for $C_{13}H_{22}N_4O_3$: Calcd.: C, 55.30; H, 7.85; N, 19.84; Found, C, 55.65; H, 7.47; N, 20.04.

4.1.42. (±)–1-(2,6-Dimethylpiperidin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**43**)

The title compound (**43**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.80 mmol) and 2,6-dimethylpiperidine (1.98 mmol) in 40% yield as white solid. mp: 124–126 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 1541, 1362, 1150; ^1H NMR (400 MHz, $CDCl_3$): δ 7.97 (s, 1H), 4.54 (d/d, J = 2.0 & 14.0 Hz, 1H), 4.00–3.95 (m, 1H), 3.85–3.83 (m, 1H), 2.71 (d/d, J = 4.6 & 14.3 Hz, 1H), 2.58–2.50 (m, 5H), 2.48–2.46 (m, 1H), 1.69–1.27 (m, 6H), 1.17–1.06 (m, 7H); ESI-MS: *m/z* 297 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{14}H_{24}N_4O_3 + H^+$ ($M+H^+$): 297.1927. Found: 297.1915. Elemental analysis (%) for $C_{14}H_{24}N_4O_3$: Calcd.: C, 56.74; H, 8.16; N, 18.90; Found, C, 56.37; H, 8.45; N, 19.09.

4.1.43. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(4-nitro-1H-imidazol-1-yl)propan-2-ol (**44**)

The title compound (**44**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.64 mmol) and 4-nitro-1*H*-imidazole (1.80 mmol) in 42% yield as yellow solid. mp: 113–115 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 2996, 1542, 1362, 1150; ^1H NMR (400 MHz, $CDCl_3$+DMSO-d_6): δ 7.89 (s, 1H), 7.76 (s, 1H), 7.45 (s, 1H), 5.63–5.62 (m, 1H), 4.47–4.44 (m, 1H), 4.47–4.44 (m, 1H), 4.13–3.82 (m, 4H), 2.32 (s, 3H); ESI-MS: *m/z* 297 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{10}H_{12}N_6O_5 + H^+$ ($M+H^+$): 297.0947. Found: 297.0964. Elemental analysis (%) for $C_{10}H_{12}N_6O_5$: Calcd.: C, 40.54; H, 4.08; N, 28.37; Found, C, 40.62; H, 4.38; N, 28.17.

4.1.44. (±)–1-(2-methyl-4-nitro-1H-imidazol-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**45**)

The title compound (**45**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.91 mmol) and 2-methyl-4-nitro-1*H*-imidazole (2.10 mmol) in 52% yield as light yellow solid. mp: 190–192 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 2993, 1540, 1361, 1150; ^1H NMR (400 MHz, $CDCl_3$+DMSO-d_6): δ 8.19 (s, 1H), 7.92 (s, 1H), 5.61–5.59 (m, 1H), 4.48–4.44 (m, 1H), 4.19–4.10 (m, 2H), 4.01–3.96 (m, 2H), 2.46–2.41 (m, 3H), 2.35 (s, 3H); ^{13}C (100 MHz, $CDCl_3$+DMSO-d_6): δ 157.1, 150.6, 150.5, 143.6, 137.9, 127.7, 73.9, 55.1, 54.2, 19.5, 18.0; ESI-MS: *m/z* 311 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{11}H_{14}N_6O_5 + H^+$ ($M+H^+$): 311.1104. Found: 311.1095. Elemental analysis (%) for $C_{11}H_{14}N_6O_5$: Calcd.: C, 42.58; H, 4.55; N, 27.09; Found, C, 42.58; H, 4.55; N, 27.09.

4.1.45. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(1H-1,2,3-triazol-1-yl)propan-2-ol (**46**)

Into a solution of (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.91 mmol) in acetonitrile was added 1*H*-1,2,3-triazole (2.10 mmol) and stirred the contents for 14 h. After completion of the reaction, acetonitrile was evaporated and the crude obtained was separated through column chromatography using MeOH/$CHCl_3$ as eluent and the final compound (**46**) came out as first spot from column in 40% yield as white solid. mp: 127–129 °C; IR (KBr) ν (cm^{-1}): 3400, 3114, 2995, 1542, 1360, 1268, 1150; ^1H NMR (400 MHz, $CDCl_3$): δ 7.82 (s, 1H), 7.74 (s, 1H), 7.65 (s, 1H), 4.98 (s, 1H), 4.69–4.65 (m, 2H), 4.53–4.50 (m, 2H), 4.11–4.05 (m, 1H), 2.45 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 152.5, 138.9, 133.5, 133.3, 126.2, 68.8, 53.1, 49.6, 14.7; ESI-MS: *m/z* 253 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_9H_{12}N_6O_3 + H^+$ ($M+H^+$): 253.1049. Found: 253.1041. Elemental analysis (%) for $C_9H_{12}N_6O_3$: Calcd.: C, 42.86; H, 4.80; N, 33.32; Found, C, 42.72; H, 5.05; N, 33.11.

4.1.46. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**47**)

The title compound (**47**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.91 mmol) and 1*H*-1,2,4-triazole (2.10 mmol) in 53% yield as white solid. mp: 140–142 °C; IR (KBr) ν (cm^{-1}): 3400, 3120, 3011, 1545, 1360, 1268, 1150; ^1H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.88 (s, 1H), 7.84 (s, 1H), 5.12 (bs, 1H), 4.68 (d/d, J = 2.5 & 14.1 Hz, 1H), 4.57 (d/d, J = 3.0 & 13.6 Hz, 1H), 4.39–4.37 (m, 1H), 4.28 (Q, J = 7.1 & 13.6 Hz, 1H), 4.12 (d/d, J = 9.1 & 14.1 Hz, 1H), 2.46 (s, 3H); ^{13}C (100 MHz, $CDCl_3$+DMSO-d_6): δ 151.9, 151.4, 144.5, 138.3, 132.7, 68.2, 52.8, 49.4, 14.4; ESI-MS: *m/z* 253 ($M+H^+$); HRMS (ESI): *m/z* calculated for

$C_9H_{12}N_6O_3$ + H^+ ($M+H^+$): 253.1049. Found: 253.1044. Elemental analysis (%) for $C_9H_{12}N_6O_3$: Calcd.: C, 42.86; H, 4.80; N, 33.32; Found, C, 42.99; H, 5.18; N, 33.61.

4.1.47. (±)–1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-(2H-1,2,3-triazol-2-yl)propan-2-ol (**48**)

The title compound was obtained through a procedure described for the synthesis of compound (**46**) and the final compound **(48)** came out as second spot from column in 35% yield as white solid. mp: 113–115 °C; IR (KBr) ν (cm^{-1}): 3400, 3120, 2998, 1543, 1365, 1144; ^1H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H), 7.70 (s, 2H), 4.75 (d/d, J = 3.9 & 13.8 Hz, 1H), 4.68–4.59 (m, 2H), 4.51 (bs, 1H), 4.14 (d/d, J = 9.1 & 14.2 Hz, 1H), 3.90 (bs, 1H), 2.49 (s, 3H); ^{13}C (100 MHz, $CDCl_3$+DMSO-d_6): δ 156.8, 143.2, 139.2, 137.5, 73.8, 62.6, 54.6, 19.3; ESI-MS: m/z 253 ($M+H^+$); HRMS (ESI): m/z calculated for $C_9H_{12}N_6O_3$ + H^+ ($M+H^+$): 253.1049. Found: 253.1047. Elemental analysis (%) for $C_9H_{12}N_6O_3$: Calcd.: C, 42.86; H, 4.80; N, 33.32; Found, C, 42.99; H, 5.18; N, 33.61.

4.1.48. (±)–1-(1H-Benzo[d]imidazol-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**49**)

The title compound (**49**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.91 mmol) and 1*H*-benzo[*d*]imidazole (2.10 mmol) in 83% yield as white solid. mp: 198–200 °C; IR (KBr) ν (cm^{-1}): 3400, 3124, 1620, 1543, 1360, 1260, 1150; ^1H NMR (400 MHz, DMSO-d_6): δ 8.19 (s, 1H), 8.01 (s, 1H), 7.63 (d/d, J = 7.9 & 15.3 Hz, 2H), 7.30–7.19 (m, 2H), 5.59–5.58 (m, 1H), 4.56–4.46 (m, 2H), 4.32–4.16 (m, 3H), 2.43 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 152.5, 145.0, 143.7, 138.9, 134.5, 133.4, 122.7, 121.9, 119.8, 110.8, 68.8, 49.8, 48.4, 14.7; ESI-MS: m/z 302 ($M+H^+$); HRMS (ESI): m/z calculated for $C_{14}H_{15}N_5O_3$ + H^+ ($M+H^+$): 302.1253. Found: 302.1251. Elemental analysis (%) for $C_{14}H_{15}N_5O_3$: Calcd.: C, 55.81; H, 5.02; N, 23.24; Found, C, 55.56; H, 5.39; N, 23.09.

4.1.49. (±)–1-(1H-Benzo[d][1,2,3]triazol-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (**50**)

The title compound (**50**) was synthesized from (±)–2-methyl-5-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**3**, 1.91 mmol) and 1*H*-benzo[*d*][1,2,3]triazole (2.10 mmol) in 85% yield as white solid. mp: 173–175 °C; IR (KBr) ν (cm^{-1}): 3400, 3120, 1620, 1540, 1362, 1260, 1146; ^1H NMR (400 MHz, DMSO-d_6): δ 8.05–8.03 (m, 2H), 7.88–7.86 (m, 1H), 7.59–7.55 (m, 1H), 7.43–7.39 (m, 1H), 4.93 (d/d, J = 4.1 & 14.3 Hz, 1H), 4.84–4.79 (m, 1H), 4.62–4.57 (m, 1H), 4.34–4.25 (m, 2H), 2.44 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 152.5, 145.5, 138.8, 134.1, 133.1, 127.6, 124.3, 119.4, 111.4, 69.3, 51.6, 49.8, 14.7; ESI-MS: m/z 303 ($M+H^+$); HRMS (ESI): m/z calculated for $C_{13}H_{14}N_6O_3$ + H^+ ($M+H^+$): 303.1206. Found: 303.1194. Elemental analysis (%) for $C_{13}H_{14}N_6O_3$: Calcd.: C, 51.65; H, 4.67; N, 27.80; Found, C, 51.81; H, 4.85; N, 27.97.

4.1.50. (±)–1-Chloro-3-(2-methyl-4-nitro-1H-imidazol-1-yl) propan-2-ol (**51**)

Into a mixture of 2-methyl-(4/5-nitro-1*H*-imidazole (39.37 mmol) and potassium carbonate (3.93 mmol) was added excess (±)-epichlorohydrin (20 mL) at rt. The reaction mixture was refluxed at 110–115 °C with stirring for 15 min. Then the reaction mixture cooled to rt, poured over crushed ice in a beaker, and stirred vigorously. Solid precipitated out and water was decanted. Solid compound **51** was filtered off and recrystallized from ethanol in 60% yield as white solid. mp: 154–156 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 1542, 1360, 1150, 775; ^1H NMR (400 MHz, DMSO-d_6): δ 8.21 (s, 1H), 5.78 (s, 1H), 4.17–4.14 (m, 1H), 3.98–3.93 (m, 2H), 3.68–3.58 (m, 2H), 2.36 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 146.1, 145.6, 123.0, 69.6, 50.2, 47.3, 13.2; ESI-MS: m/z 220 ($M+H^+$).

4.1.51. (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1H-imidazole (**52**)

The title compound (**52**) was synthesized by dissolving compound **51** (27.39 mmol) in dichlorometane (10 mL) and added 27.39 mmol of aqueous NaOH at 0 °C. Stirred the contents for 45 min and after completion of reaction (as monitored by TLC) contents was extracted with DCM/water and organic layer was separated, dried (anhyd. Na_2SO_4), concentrated *in vacuo* to get the final compound (**52**) in 86% yield as white solid. mp: 94–96 °C; IR (KBr) ν (cm^{-1}): 3402, 3110, 1540, 1361, 1155; ^1H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H), 4.40 (d/d, J = 2.4 & 15.1 Hz, 1H), 3.89 (d/d, J = 6.2 & 15.1 Hz, 1H), 3.29–3.25 (m, 1H), 2.90 (t, J = 4.1 Hz, 1H), 2.53 (d/d, J = 2.5 & 4.4 Hz, 1H), 2.44 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 146.5, 145.2, 120.3, 49.9, 48.5, 44.9, 13.0; ESI-MS: m/z 184 ($M+H^+$).

The compounds **53**–**61** were synthesized using a procedure similar to described for compound **4**.

4.1.52. (±)–2-Hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl) propyl 4-(2,3-dichlorophenyl)piperazine-1-carbodithioate (**53**)

The title compound (**53**) was synthesized from **(±)**–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.36 mmol) and sodium 4-(2,3-dichlorophenyl)piperazine-1-carbodithioate (2.73 mmol) in 84% yield as white solid. mp: 180–182 °C; IR (KBr) ν (cm^{-1}): 3400, 3115, 1590, 1541, 1370, 1215, 780; ^1H NMR (400 MHz, DMSO-d_6): δ 8.24 (s, 1H), 7.36–7.31 (m, 2H), 7.18 (d/d, J = 2.4 & 7.1 Hz, 1H), 5.66–5.65 (m, 1H), 4.40–4.13 (m, 5H), 4.06–3.96 (m, 2H), 3.56 (d/d, J = 3.8 & 13.5 Hz, 1H), 3.36–3.34 (m, 1H), 3.12–3.09 (m, 4H), 2.37 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 196.0, 150.5, 145.9, 145.6, 133.1, 128.9, 126.6, 125.5, 123.3, 120.4, 68.6, 51.9, 50.9, 41.5, 13.3; ESI-MS: m/z 490 ($M+H^+$); HRMS (ESI): m/z calculated for $C_{18}H_{21}Cl_2N_5O_3S_2$ + H^+ ($M+H^+$): 490.0541. Found: 490.0555. Elemental analysis (%) for $C_{18}H_{21}Cl_2N_5O_3S_2$: Calcd.: C, 44.08; H, 4.32; N, 14.28; Found, C, 44.15; H, 4.21; N, 14.50.

4.1.53. (±)–2-Hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl) propyl 4-ethylpiperazine-1-carbodithioate (**54**)

The title compound (**54**) was synthesized from **(±)**–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.64 mmol) and sodium 4-ethylpiperazine-1-carbodithioate (3.27 mmol) in 72% yield as white solid. mp: 101–103 °C; IR (KBr) ν (cm^{-1}): 3400, 3020, 1544, 1360, 1215, 1125; ^1H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 1H), 5.64–5.63 (m, 1H), 4.23–4.16 (m, 3H), 3.99–3.94 (m, 4H), 3.55–3.51 (m, 1H), 3.33–3.28 (m, 1H), 2.46–2.43 (m, 4H), 2.37–2.36 (m, 5H), 1.01 (t, J = 7.1 Hz, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.1, 145.8, 145.6, 123.3, 68.6, 52.2, 51.9, 51.4, 50.2, 41.4, 13.3, 12.3; ESI-MS: m/z 374 ($M+H^+$); HRMS (ESI): m/z calculated for $C_{14}H_{23}N_5O_3S_2$ + H^+ ($M+H^+$): 374.1321. Found: 374.1317. Elemental analysis (%) for $C_{14}H_{23}N_5O_3S_2$: Calcd.: C, 45.02; H, 6.21; N, 18.75; Found, C, 44.69; H, 6.54; N, 18.48.

4.1.54. (±)–2-Hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl) propyl 4-butylpiperazine-1-carbodithioate (**55**)

The title compound (**55**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.64 mmol) and sodium 4-butylpiperazine-1-carbodithioate (3.27 mmol) in 82% yield as white solid. mp: 83–85 °C; IR (KBr) ν (cm^{-1}): 3400, 3100 1540, 1364, 1215, 1152; ^1H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 1H), 5.63–5.62 (m, 1H), 4.22–4.15 (m, 3H), 3.99–3.94 (m, 4H), 3.52 (d/d, J = 4.0 & 13.5 Hz, 1H), 3.32–3.27 (m, 1H), 2.45–2.42 (m, 4H), 2.36 (s, 3H), 2.32–2.28 (m, 2H), 1.45–1.38 (m, 2H), 1.34–1.25 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.1, 145.8, 145.6, 123.2, 68.6, 57.3, 52.6, 51.9, 50.2, 41.4, 28.8, 20.4, 14.3, 13.3; ESI-MS: m/z 402 ($M+H^+$); HRMS (ESI): m/z calculated for $C_{16}H_{27}N_5O_3S_2 + H^+$ ($M+H^+$): 402.1634. Found: 402.1634. Elemental analysis (%) for $C_{16}H_{27}N_5O_3S_2$: Calcd.: C, 47.86; H, 6.78; N, 17.44;

Found, C, 47.96; H, 6.99; N, 17.41.

*4.1.55. (±)–Ethyl 4-((2-hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl)propylthio)carbonothioyl)piperazine-1-carboxylate (**56**)*

The title compound (**56**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.64 mmol) and sodium 4-(ethoxycarbonyl)piperazine-1-carbodithioate (3.27 mmol) in 77% yield as white solid. mp: 92–94 °C; IR (KBr) ν (cm^{-1}): 3402, 3110, 1742, 1540, 1360, 1215, 1153; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (s, 1H), 5.66–5.64 (m, 1H), 4.32–3.94 (m, 10H), 3.52–3.51 (m, 5H), 2.36 (s, 3H), 1.20 (t, J = 7.0 Hz, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.9, 155.0, 145.9, 145.6, 123.2, 68.5, 61.5, 51.9, 51.0, 42.9, 41.4, 15.0, 13.3; ESI-MS: *m/z* 418 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{15}H_{23}N_5O_5S_2$ + H^+ ($M+H^+$): 418.1219. Found: 418.1216. Elemental analysis (%) for $C_{15}H_{23}N_5O_5S_2$: Calcd.: C, 43.15; H, 5.55; N, 16.77; Found, C, 43.25; H, 5.90; N, 16.32.

*4.1.56. (±)–2-Hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl) propyl 4-phenylpiperazine-1-carbodithioate (**57**)*

The title compound (**57**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.64 mmol) and sodium 4-phenylpiperazine-1-carbodithioate (3.27 mmol) in 82% yield as white solid. mp: 113–115 °C; IR (KBr) ν (cm^{-1}): 3400, 3115, 1562, 1540, 1360, 1215, 1140; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (s, 1H), 7.24 (d/d, J = 7.3 & 8.6 Hz, 2H), 6.95 (d, J = 7.9 Hz, 2H), 6.81 (d, J = 7.2 Hz, 1H), 5.66–5.64 (m, 1H), 4.38 (bs, 2H), 4.20–3.95 (m, 5H), 3.56 (d/d, J = 4.0 & 13.5 Hz, 1H), 3.36–3.28 (m, 5H), 2.37 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.4, 150.4, 145.9, 145.6, 129.5, 123.3, 119.7, 115.9, 68.6, 51.9, 51.2, 49.8, 48.0, 41.4, 13.3; ESI-MS: *m/z* 422 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{18}H_{23}N_5O_3S_2$ + H^+ ($M+H^+$): 422.1321. Found: 422.1316. Elemental analysis (%) for $C_{18}H_{23}N_5O_3S_2$: Calcd.: C, 51.29; H, 5.50; N, 16.61; Found, C, 51.60; H, 5.83; N, 16.33.

*4.1.57. (±)–2-Hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl) propyl 4-(2-methoxyphenyl)piperazine-1-carbodithioate (**58**)*

The title compound (**58**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.09 mmol) and sodium 4-(2-methoxyphenyl)piperazine-1-carbodithioate (2.18 mmol) in 80% yield as white solid. mp: 162–164 °C; IR (KBr) ν (cm^{-1}): 3400, 3120, 1560, 1552, 1355, 1215, 1147; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (s, 1H), 7.02–6.96 (m, 2H), 6.92–6.86 (m, 2H), 5.66–5.64 (m, 1H), 4.38–4.37 (m, 2H), 4.20–3.95 (m, 6H), 3.80 (s, 3H), 3.56 (d/d, J = 3.7 & 13.4 Hz, 1H), 3.08–3.05 (m, 4H), 2.37 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.5, 152.4, 145.8, 145.6, 140.4, 123.6, 123.3, 121.2, 118.9, 112.3, 68.6, 55.8, 51.9, 50.1, 41.4, 13.3; ESI-MS: *m/z* 452 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{19}H_{25}N_5O_4S_2 + H^+$ ($M+H^+$): 452.1426. Found: 452.1432. Elemental analysis (%) for $C_{19}H_{25}N_5O_4S_2$: Calcd.: C, 50.54; H, 5.58; N, 15.51; Found, C, 50.70; H, 5.90; N, 15.30.

*4.1.58. (±)–2-Hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl) propyl 4-(3-chlorophenyl)piperazine-1-carbodithioate (**59**)*

The title compound (**59**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.91 mmol) and sodium 4-(3-chlorophenyl)piperazine-1-carbodithioate (3.82 mmol) in 76% yield as white solid. mp: 118–120 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 1564, 1530, 1364, 1215, 790; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (s, 1H), 7.24 (t, J = 8.1 Hz, 1H), 6.96–6.95 (m, 1H), 6.89 (d/d, J = 2.0 & 8.3 Hz, 1H), 6.81 (d/d, J = 1.4 & 7.8 Hz, 1H), 5.67–5.65 (m, 1H), 4.36 (bs, 2H), 4.19–3.95 (m, 5H), 3.56 (d/d, J = 3.8 & 13.5 Hz, 1H), 3.38–3.36 (m, 5H), 2.36 (s, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 195.5, 151.6, 145.9, 145.6, 134.3, 130.9, 123.3, 118.7, 114.8, 113.8, 68.6, 51.9, 50.8, 47.1, 41.4, 13.3; ESI-MS: *m/z* 456 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{18}H_{22}ClN_5O_3S_2$ + H^+ ($M+H^+$): 456.0931. Found: 456.0932. Elemental analysis (%) for $C_{18}H_{22}ClN_5O_3S_2$: Calcd.: C, 47.41; H, 4.86; N, 15.36; Found, C, 47.70; H, 4.99; N, 15.65.

*4.1.59. (±)–2-Hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl) propyl 3-methylpiperidine-1-carbodithioate (**60**)*

The title compound (**60**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.09 mmol) and sodium 3-methylpiperidine-1-carbodithioate (2.18 mmol) in 76% yield as white solid. mp: 83–85 °C; IR (KBr) ν (cm^{-1}): 3400, 3110, 3011, 1540, 1358, 1215, 1150; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 1H), 5.62–5.60 (m, 1H), 5.09 (bs, 1H), 4.49–4.15 (m, 2H), 3.99–3.95 (m, 2H), 3.53–3.49 (m, 1H), 3.38 (d/d, J = 7.0 & 14.0 Hz, 1H), 3.14–2.95 (m, 1H), 2.36 (s, 3H), 1.79–1.63 (m, 3H), 1.47–1.40 (m, 1H), 1.27–1.24 (m, 1H), 0.91–0.90 (m, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 194.1, 145.8, 145.6, 123.3, 68.7, 58.7, 51.9, 41.5, 32.4, 25.1, 19.2, 18.9, 13.3; ESI-MS: *m/z* 359 ($M+H^+$). Elemental analysis (%) for $C_{14}H_{22}N_4O_3S_2$: Calcd.: C, 46.91; H, 6.19; N, 15.63; Found, C, 46.74; H, 6.50; N, 15.28.

*4.1.60. (±)–tert-Butyl 4-((2-hydroxy-3-(2-methyl-4-nitro-1H-imidazol-1-yl)propylthio)carbonothioyl)piperazine-1-carboxylate (**61**)*

The title compound (**61**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.36 mmol) and sodium 4-(*tert*-butoxycarbonyl)piperazine-1-carbodithioate (2.73 mmol) in 82% yield as white solid. mp: 144–146 °C; IR (KBr) ν (cm^{-1}): 3410, 3100, 1745, 1541, 1365, 1215, 1142; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 1H), 5.64–5.63 (m, 1H), 4.23–4.16 (m, 3H), 3.99–3.94 (m, 4H), 3.54 (d/d, J = 3.9 & 13.5 Hz, 1H), 3.48–3.45 (m, 4H), 2.36 (s, 3H), 1.42 (s, 9H); ^{13}C (100 MHz, DMSO-d_6): δ 195.8, 154.1, 145.9, 145.6, 123.3, 79.8, 68.6, 51.9, 49.8, 41.4, 28.4, 13.3; ESI-MS: *m/z* 446 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{17}H_{27}N_5O_5S_2 + H^+$ ($M+H^+$): 446.1532. Found: 446.1526. Elemental analysis (%) for $C_{17}H_{27}N_5O_5S_2$: Calcd.: C, 45.83; H, 6.11; N, 15.72; Found, C, 45.99; H, 6.42; N, 15.40.

The compounds **62**–**65** were synthesized using a procedure similar to described for compound **25**.

*4.1.61. (±)–1-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-(2-methyl-4-nitro-1H-imidazol-1-yl)propan-2-ol (**62**)*

The title compound (**62**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.91 mmol) and 1-(2,3-dichlorophenyl)piperazine (2.10 mmol) in 70% yield as white solid. mp: 164–166 °C; IR (KBr) ν (cm^{-1}): 3400, 3120, 1595, 1545, 1364, 785; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25 (s, 1H), 7.33–7.29 (m, 2H), 7.13 (d/d, J = 2.8 & 6.8 Hz, 1H), 5.12 (d, J = 5.1 Hz, 1H), 4.15 (d/d, J = 2.7 & 13.8 Hz, 1H), 4.01–3.98 (m, 1H), 3.91 (d, J = 7.9 & 13.8 Hz, 1H), 2.99 (s, 4H), 2.65–2.58 (m, 4H), 2.44–2.37 (m, 5H); ^{13}C (100 MHz, DMSO-d_6): δ 151.6, 146.1, 145.6, 133.1, 128.9, 126.4, 124.8, 123.3, 119.9, 67.2, 61.7, 53.8, 51.3, 13.3; ESI-MS: *m/z* 414 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{17}H_{21}Cl_2N_5O_3$ + H^+ ($M+H^+$): 414.1100. Found: 414.1101. Elemental analysis (%) for $C_{17}H_{21}Cl_2N_5O_3$: Calcd.: C, 49.29; H, 5.11; N, 16.90; Found, C, 49.02; H, 5.44; N, 16.63.

*4.1.62. (±)–1-(4-Ethylpiperazin-1-yl)-3-(2-methyl-4-nitro-1H-imidazol-1-yl)propan-2-ol (**63**)*

The title compound (**63**) was synthesized from (±)–2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.91 mmol) and 1-ethylpiperazine (2.10 mmol) in 66% yield as semi solid. IR (KBr) ν (cm^{-1}): 3400, 3105, 1535, 1361, 1140; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (s, 1H), 5.16 (bs, 1H), 4.10 (d/d, J = 2.4 & 13.5 Hz, 1H), 3.95–3.85 (m, 2H), 2.51–2.22 (m, 15H), 0.97 (t, J = 7.1 Hz, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 146.0, 145.6, 123.3, 67.1, 61.8, 53.8, 52.8,

52.1, 51.3, 13.3, 12.4; ESI-MS: *m/z* 298 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{13}H_{23}N_5O_3 + H^+$ ($M+H^+$): 298.1879. Found: 298.1885. Elemental analysis (%) for $C_{13}H_{23}N_5O_3$: Calcd.: C, 52.51; H, 7.80; N, 23.55; Found, C, 52.70; H, 8.09; N, 23.33.

4.1.63. (±)−1-(2-methyl-4-nitro-1H-imidazol-1-yl)-3-(4-(pyridin-2-yl)piperazin-1-yl)propan-2-ol (**64**)

The title compound (**64**) was synthesized from (±)−2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.91 mmol) and 1-(pyridin-2-yl)piperazine (2.10 mmol) in 72% yield as semi solid. IR (KBr) ν (cm^{-1}): 3400, 3110, 1560, 1542, 1360, 1155; ^1H NMR (400 MHz, $CDCl_3$): δ 8.18−8.16 (m, 1H), 7.86 (s, 1H), 7.51−7.46 (m, 1H), 6.65−6.62 (m, 2H), 4.12−4.07 (m, 2H), 3.88 (d/d, J = 7.6 & 14.8 Hz, 1H), 3.55−3.52 (m, 4H), 2.75−2.70 (m, 2H), 2.57−2.54 (m, 2H), 2.48−2.38 (m, 5H); ^{13}C (100 MHz, $CDCl_3$): δ 159.3, 147.9, 146.2, 145.4, 137.6, 120.9, 113.7, 107.2, 66.1, 60.6, 53.1, 50.6, 45.2, 13.2; ESI-MS: *m/z* 347 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_{16}H_{22}N_6O_3 + H^+$ ($M+H^+$): 347.1832. Found: 347.1822. Elemental analysis (%) for $C_{16}H_{22}N_6O_3$: Calcd.: C, 55.48; H, 6.40; N, 24.26; Found, C, 55.69; H, 6.72; N, 24.32.

4.1.64. (±)−1-(2-methyl-4-nitro-1H-imidazol-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**65**)

The title compound (**65**) was synthesized from (±)−2-methyl-4-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**52**, 1.91 mmol) and 1*H*-1,2,4-triazole (2.10 mmol) in 55% yield as white solid. mp: 148−150 °C; IR (KBr) ν (cm^{-1}): 3400, 3120, 2996, 1543, 1360, 12682, 1150; ^1H NMR (400 MHz, $CDCl_3$+DMSO-d_6): δ 8.02 (s, 1H), 7.77 (s, 1H), 7.70 (s, 1H), 5.50 (s, 1H), 4.13 (d/d, J = 4.1 & 13.5 Hz, 1H); 4.05−3.98 (m, 2H), 3.91−3.87 (m, 1H), 3.72−3.64 (m, 1H), 2.17 (s, 3H); ^{13}C (100 MHz, $CDCl_3$+DMSO-d_6): δ 151.6, 145.9, 145.4, 144.5, 121.3, 68.2, 52.3, 50.0, 13.0; ESI-MS: *m/z* 253 ($M+H^+$); HRMS (ESI): *m/z* calculated for $C_9H_{12}N_6O_3 + H^+$ ($M+H^+$): 253.1049. Found: 253.1034. Elemental analysis (%) for $C_9H_{12}N_6O_3$: Calcd.: C, 42.86; H, 4.80; N, 33.32; Found, C, 42.98; H, 5.12; N, 33.55.

4.2. Biological materials and methods

4.2.1. Anti-Trichomonas activity

Trichomonas vaginalis parasites to be used in assays (MTZ-susceptible ATCC-33592 and MTZ-resistant ATCC-50143 strains) were grown in TYM medium supplemented with 10% FCS, vitamin mixture and 100 U/mL penicillin/streptomycin, at 37 °C in 15 mL tubes for one day, followed by regular subculturing, and were in the log phase of growth. **[**20] The cultures routinely attained a concentration of 2×10^7 cells/mL in 48 h. Inoculums of 1×10^4 cells per tube were used for maintenance of the culture. *In vitro* drug susceptibility and resistant assays were carried out using the standard procedure. Stock solutions (100 μg/mL) of test compounds were prepared in DMSO. These stock solutions were serially diluted with TYM medium to obtain concentration up to 0.1 μg/mL in 48-well plates. DMSO/TYM was used as vehicle in control wells. Parasites (5×10^4 trophozoites/L) were added to these wells and incubated anaerobically at 37 °C. Cells were checked for viability at different time intervals from 3 to 48 h under the microscope at 40× magnification. The viability of the cells was determined by trypan blue exclusion assay. Minimum concentration of the test agent at which all cells were found dead in 48 h was considered as its MIC. The experiment was repeated three times to confirm the MIC (Table 1).

4.2.2. Cytotoxicity assay

The cytotoxic effect of test compounds was evaluated in an *in vitro* model of cervicovaginal epithelium (*HeLa*) cells line, using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay. Exponentially growing *HeLa* cells were seeded into 96-well tissue culture plates at a density of 2×104 cells per well (in triplicate) and were incubated in culture medium (DMEM with 10% fetal calf serum) for 24 h at 37 °C in a 5% CO_2−95% air atmosphere. After 24 h, the culture medium was replaced with fresh medium containing dilutions of test compounds in experimental wells and 0.05% DMSO in culture medium in control wells. After incubation for another 24 h, 5 μl of 5 mg/ml MTT solution in PBS [pH 7.4] was added to each well. The formazan crystals formed inside the viable cells were solubilized in DMSO, and the optical density at 540 nm (OD540) was recorded in a microplate reader (Microquant; BioTek) (Table 1).

4.2.3. 3D-QSAR study

QSAR model has been developed for the dataset of compounds, all calculations were performed using sybyl 2.1 [49]. The sketch module was used to draw the compounds and energy minimizationwas done using the Powell method. The compounds were assigned Tripos force field and Gasteiger-Huckel partial charges with termination criterion of 0.05 kcal/mol for 1000 iterations. The energy minimized conformation of most active compound **6** was used as a template for alignment and all other molecules were overlaid over it using surflex-Sim module [50]. Alignment of molecules is generated on the basis of morphological similarity function and fast poses generation technique. Similarity is function of molecular shape, hydrogen bonding and electrostatic properties. The activity values (in μM unit) were transformed into log unit and used as dependent variable in PLS analysis. The CoMFA descriptor was calculated and used as independent variable in partial least square regression analysis. CoMFA calculations were done using the Tripos force field with a distance-dependent dielectric constant at all interactions in a regularly spaced (2 Å) grid taking a sp^3 carbon atom as steric probe and a+1 charge as electrostatic probe, cut off was set to 30 kcal/mol [51]. The whole dataset was divided into two randomly generated sub datasets composed of 52 compounds in training set and 8 compounds in the test set. PLS method [42] was used to correlate the CoMFA fields to observed biological activity values. The cross-validation was performed using the leave-one-out (LOO) method in which one compound is removed from the dataset and its activity is predicted using the model derived from the rest of the molecules in the dataset. Non-cross-validation was performed to calculate conventional r^2 using the same number of components. It was used in the analysis of CoMFA result and the prediction of activity of compounds through the model. For evaluation of predictive ability of model, predictive r^2 for test set has been calculated.

4.2.4. Fluorescence labeling of sulfhydryl groups on Trichomonas vaginalis

The effect of test compound on *Trichomonas* sulfhydryl groups was examined and imaged by a method published earlier with slight modification [46] using a fluorescence microscope, after labelling with the fluorometric thiol detector using a thiol −detection assay kit (Cayman). Trichomonas vaginalis was treated with the vehicle or the test compound at MIC and incubated for 24 h at 37 °C. After incubation, trichomonads were pelleted at 700 × g for 10 min at 4 °C and washed 2−3 times with PBS. Thereafter 50 μL fluorometric thiol detector (pre-diluted 100× with dilution buffer), was added and incubated for 5 min in the dark. A drop of this sample was taken on a microscope slide, covered with a coverslip and imaged on a Nikon Eclipse 80i microscope equipped with epifluoresence illumination, using the UV-1A filter. Exposure times were the same for all samples.

4.2.5. In vivo anti-Trichomonas assay

All animal experiments were conducted in accordance and as per the approval of the Institutional Animal Ethics Committee. According to the modified method described by Escribano et al. [52] the mice were housed in polypropylene cages and kept in uniform husbandry conditions of temperature (25–26 °C), relative humidity (50–70%) and light/dark cycle (12/12 h). *T. vaginalis* were cultured under partial anaerobic condition in TYM medium and on attaining concentration of approximately 2×10^6 cells/mL (in ~48 h); trichomonads were harvested from the culture by centrifugation at 250 ×*g* for 10 min and then re-suspended in sterile saline. Six-week-old mice were inoculated subcutaneously with *T. vaginalis* (50 μL of 2×10^6 organisms per mL) into the left hind flank. Five groups were used for each experiment ($n = 3$). The abscess/lesion formation was determined by palpation 7 days after injection and measured daily thereafter. Fine needle biopsy specimens were taken from the lesion and examined microscopically to ensure infection. Infected animals were then treated with compounds in gum acacia solution (orally) with a dose of 50 mg and 100 mg per kg and control animals were given gum acacia solution only. Daily for 7 days, abscess size measured longitudinally and the area calculated as πr^2. Metronidazole was used as positive control. Controls did not result in abscess formation.

4.2.6. In vivo pharmacokinetic assay

The pharmacokinetic study of compound **6** was carried out in young and healthy male *Sprague Dawley* rats weighing 250 ± 25 g obtained from laboratory animal division, CSIR-CDRI, Lucknow. The animals were housed in plastic cages in standard laboratory conditions with a regular 12 h day-night cycle. Standard pelleted laboratory chow (Goldmohar Laboratory Animal Feed, Lipton India Ltd, Chandigarh, India) and water were allowed *ad libitum*. The rats were acclimatized to this environment for at least five days before conducting the experiment. The oral dose pharmacokinetic study was conducted in overnight fasted (12–16 h) rats (n = 4 per time point). All experiments, euthanasia and disposal of carcasses were carried out as per the guidelines of Local Ethics Committee for animal experimentation. Suspension formulations containing 12.5 mg/mL of compound was prepared separately by triturating the compound, gum acacia (1% w/v) and water (drop wise addition) in mortar and pestle. A single 50 mg/kg oral dose was given to conscious rats using rat feeding needle. Blood samples were withdrawn at various predefined times up to 24 h post dose. Serum samples were harvested and stored at −80 °C until analysis. A Shimadzu UFLC pump (LC-20AD) with online degasser (DGU-20A3), an auto-sampler (SIL-HTc) with a temperature-controlled peltier-tray and a triple quadrupole API 4000 mass spectrometer (Applied Biosystems, Toronto, Canada) was used for analysis. Chromatographic separation was made on a Discovery HS C-18 column (5 μm, 50 × 4.6 mm id) preceded with a guard column (5 μm, 20 × 4.0 mm, id) packed with the same material with mobile phase [acetonitrile: aqueous ammonium acetate buffer (0.01 M; pH, 4.5) (80:20, %v/v)] pumped at a flow rate of 0.6 mL/min under isocratic condition. The mobile phase was degassed by ultrasonication for 15 min before use. LC-MS/MS system was equilibrated for approximately 20 min before commencement of analysis. Total analysis time was 3 min per sample. The mass spectral analysis was performed in positive ionization mode at 5500 V using multiple reaction monitoring technique to monitor the transitions *m/z* 422.1 → *m/z* 295.0 and *m/z* 180.1 → *m/z* 138.1 for phenacetin (internal standard). Data acquisition and quantitation were performed using analyst software (version 1.4.2; AB Sciex, Toronto, Canada). The method utilizes 50 μL of serum. For sample clean up, liquid-liquid extraction was used. The method showed linearity over the range of 1–200 ng/mL with recovery of >50% and acceptable accuracy and precision [FDA, Guidance for Industry: Bioanalytical Method Validation [53].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.09.006.

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