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Preliminary communication

Novel terpene based 1,4,2-dioxazoles: Synthesis, characterization, molecular properties and screening against *Entamoeba histolytica*

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ABSTRACT

In present investigation a series of 20 dioxazole analogues (**1**–**20**) were synthesized, characterized and subjected to molecular properties prediction, anti-amoebic screening and cytotoxicity evaluation. Out of the twenty compounds *viz.* 3,5-substituted-1,4,2-dioxazoles, six compounds have shown IC_{50} values in the range (1.00–1.10 μM) lower than the standard drug metronidazole (IC_{50} = 1.45 μM). The toxicological studies of the active compounds on *H9c2 rat cardiac myoblasts* showed that all compounds were nontoxic. The *p*Ka, and log *P* values have also been predicted. Compound **8** showed the most promising results based on anti-amoebic evaluation, cytotoxicity studies and physico-chemical properties prediction.

1. Introduction

Amoebiasis, an infectious disease caused by *Entamoeba histolytica*, results in severe liver and brain abscess and causes high rate of morbidity and mortality in humans. Metronidazole, the 5-nitroimidazole drug has been the drug of choice for several decades in the treatment of amoebiasis [1]. However, there are concerns regarding its carcinogenicity [2] and recent studies have reported several toxic effects such as genotoxicity, gastric mucus irritation, and spermatozoid damage [3,4]. Furthermore, there are occasional reports of failure with metronidazole suggesting that this could probably be heralding the development of drug resistance clinically [5]. Recurrence of amoebic liver abscess even after treatment with metronidazole has been reported and parasites may survive in spite of adequate treatment [6]. Terpenoids are natural molecules with antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, antihyperglycemic, antiinflammatory, chemotherapeutic and immunomodulatory properties [7–18]. The versatile biological applications of azoles made it a targeted investigatory class of compound [19]. Counting the antiparasitic properties of terpenes [20–22] and anti-amoebic properties of 1,4,2-dioxazoles [23–25], we proposed the synthesis of terpene based 1,4,2-dioxazoles.

In the development of drugs intended for oral use, good drug absorption and appropriate drug delivery are very important [26]. About 30% of oral drugs fail in development due to poor pharmacokinetics [27]. Among the pharmacokinetic properties, a low and highly variable bioavailability is indeed the main reason for stopping further development of the drug [28]. Moreover, the knowledge of ionization constant is an important challenge for understanding various phenomena like biological uptake, pharmacological activity or in vivo studies. Hence, the discovery of new molecules require accurate determination of pKa and log *P* values. In the present research work some new terpene-based dioxazole derivatives **1**–**20** have been synthesized, screened for anti-amoebic activity and cell cytotoxicity. Their pKa and log *P* values have been determined in order to understand the chemical interactions between the compound of interest and its pharmacological target.

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2. Results and discussion

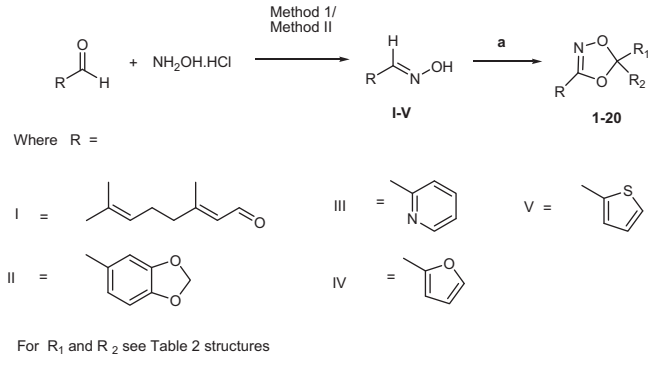
2.1. Chemistry

The outline synthesis of dioxazole derivatives (**1**–**20**) is given in Scheme 1. The reaction of corresponding oximes (**I**–**V**) with different aromatic aldehydes or ketones in ethylacetate using sodium hypochlorite and triethylamine yielded the respective dioxazole derivatives. The compounds in solid state showed sharp melting points and the elemental analysis was found in accordance with ±0.3%. The compounds were stable and were soluble in DMSO, methanol, and chloroform. These terpene based dioxazole derivatives **1**–**20** were recrystallized from appropriate solvents, and final yield of 25–60% was obtained. The dioxazole derivatives were characterized by electronic, IR, ^{1}H and ^{13}C NMR, and mass spectroscopy.

Characteristic IR bands provide significant indications for the formation of the oximes I–V and dioxazoles **1**–**20**. The absence of a band at/or around 2665 cm^{-1} due to aldehydic proton and the appearance of characteristic bands at 3245–3260 cm^{-1} and 1622–1635 cm^{-1} due to ν(NO–H) and ν(C=N) respectively, confirmed the formation of the oximes I–V. The absence of a band at 3245–3260 cm^{-1} in all the dioxazoles 1–20 confirmed the conversion of the corresponding oximes into their respective dioxazoles. The ν(C=N) band in dioxazoles was in the range of 1642–1660 cm^{-1} and a new band in the range of 1120–1185 cm^{-1} arose due to (C–O–C) group, however, in some cases this band split. In addition to these the representative bands due to carbon–carbon stretching of aliphatic and aromatic groups were also present.

The electronic spectra of the compounds **1**–**20** studied in the UV region, exhibited three absorption bands at 314–354 nm, 295–314 nm and 250–295 nm assigned to $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions respectively. The band at 314–354 nm was assigned to the transition involving the azomethine group (C=N). The other two absorption bands at 295–314 nm and 250–295 nm were due to $\pi \rightarrow \pi^*$ transition of dioxazole ring and $n \rightarrow \sigma^*$ transition of azomethine nitrogen respectively.

The structure of the oximes I–V was further confirmed by ^{1}H NMR spectra. A singlet at 6.90–7.90 ppm due to (C–H=N) proton showed condensation between carbonyl group of aldehydes and amino group of hydroxylamine hydrochloride. The signal at 9.43–11.13 ppm due to (N–OH) proton further confirmed the formation of the oximes. The structure of oximes I–V was further supported by ^{13}C NMR spectra. The absence of aldehydic carbon signal at 192 ppm and the presence of a signal at 146.0–149.80 ppm due to (C=N) confirmed the formation of oximes. The formation of dioxazoles was supported by the absence of a signal at 9.43–11.13 ppm and 6.90–7.80 due to (N–OH) and (N=CH) respectively in all the compounds **1**–**20**. The singlet at 5.65–6.96 ppm, which arose due to (C–H) group present at C-3 of the dioxazole ring, confirmed the condensation of oximes I–II with different hetero aromatic aldehydes/ketones and terpene moieties and oximes III–V with different terpene moieties. Methyl signals were obtained for compounds which contain methyl protons at C-3 of the dioxazole ring. For methyl group, a singlet at 1.90–2.54 ppm appeared in compounds 1, 3, 5, 8, 10 and 12. In addition, the signals for the different ring protons appeared in their respective range in all the compounds. ^{13}C NMR spectra further supported the confirmatory structures. The (C=N) signal around 155.1–169.6 ppm and (–OCO–) signal around 85.9–96.8 ppm clearly favoured the formation of dioxazole rings. The signals due to the different hetero aromatic and aliphatic carbons resonate at their usual positions and the values are given in the experimental section.



Scheme 1. General Method for the synthesis of Dioxazole derivatives via Oximes. Reagents and Conditions: Method I: Pyridine, C_2H_5OH, reflux 24 h. Method II: H_2O, $NaHCO_3$, Stirring, r.t. (a) aq. NaOCl, Et_3N, EtOAc, (R_1–CO–R_2), where (R_1–CO–R_2) represents the different aldehydes, ketones and terpene moieties as given in Table 2.

2.2. Pharmacology

All the dioxazole derivatives (**1**–**20**) were screened *in vitro* against *HM1:IMSS* strain of *E. histolytica* using microdilution method. All the experiments were carried out in triplicate at each concentration level and repeated thrice. Toxicity of active compounds was studied by MTT assay on H9c2 cardiac myoblasts. The results of biological activity and toxicity are summarized in Table 1 and Fig. 1. Besides the *in vitro* cytotoxic studies, their pKa and log *P* values have been determined, for understanding the chemical interactions, between the compound of interest and its pharmacological target.

2.2.1. Anti-amoebic activity

Preliminary experiments were carried out to determine the *in vitro* anti-amoebic activity of all the compounds **1**–**20** by microdilution method using *HM1:IMSS* strain of *E. histolytica*. The results are summarized in Table 1. The data are presented in terms of percent growth inhibition relative to untreated controls, and plotted as probit values as a function of drug concentration. The

Table 1
In vitro anti-amoebic activity of compounds (**1**–**20**) against *HM1:IMSS* strain of *E. histolytica* and toxicity profile. The compounds with bold font IC_{50} values are more active than metronidazole.

Compound	Anti-amoebic activity		Toxicity profile	
	IC_{50} (μM)	S.D.[a] (±)	IC_{50} (μM)	Safety index (SI)
1	2.25	0.62	N.D	N.C
2	2.98	0.13	N.D	N.C
3	2.02	0.46	N.D	N.C
4	2.42	0.27	N.D	N.C
5	3.01	0.29	N.D	N.C
6	2.90	0.16	N.D	N.C
7	1.48	0.18	N.D	N.C
8	**1.00**	0.25	**>200**	**>200**
9	**1.03**	0.28	40	38.83
10	**1.10**	0.14	>200	>181.81
11	**1.09**	0.13	>200	>183.48
12	2.68	0.20	N.D	N.C
13	3.50	0.36	N.D	N.C
14	1.39	0.39	N.D	N.C
15	2.22	0.22	N.D	N.C
16	**1.06**	0.43	85	>80.18
17	2.78	0.29	N.D	N.C
18	**1.05**	0.31	200	190.47
19	3.05	0.19	N.D	N.C
20	2.45	0.26	N.D	N.C
MNZ	1.45	0.33	>200	>137.90

N.D. Not done, N.C. Not Calculated, S.D. (±) Standard deviation.
[a] The value obtained in at least three separate assay done in triplicate.

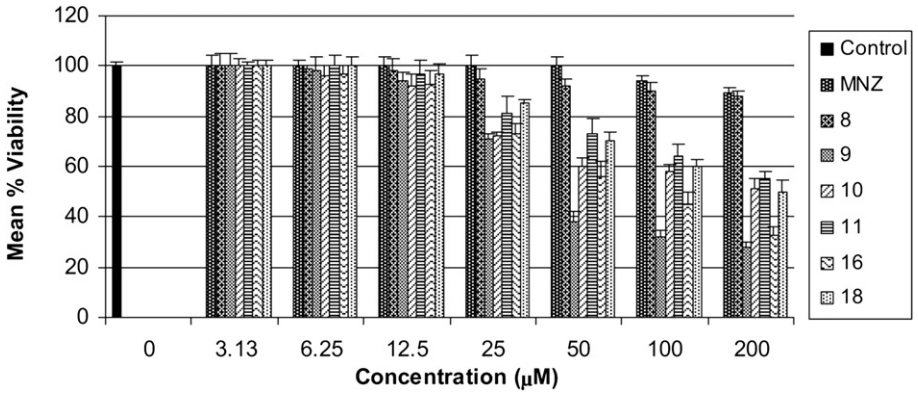


Fig. 1. Percentage of viable cells after 48 h pre-treatment of H9c2 myoblasts with Metronidazole, compounds **8**, **9**, **10**, **11**, **16** and **18**, evaluated by MTT assay.

anti-amoebic effect was compared with the most widely used anti-amoebic medication metronidazole with 50% inhibitory concentration (IC_{50}) of 1.45 μM in our experiments. Since the target compounds were deigned in three different ways based on the substituted molecules at position-3 and 5 of the dioxazole ring. The compounds (**1**–**7**), (**8**–**14**) and (**15**–**20**) showed activity in the range of IC_{50} = 1.40–3.01 μM, IC_{50} = 1.00–3.50 μM and IC_{50} = 1.05–3.05 μM respectively. The results showed that the compounds **8** (IC_{50} = 1.00 μM), **9** (IC_{50} = 1.03 μM), **10** (IC_{50} = 1.10 μM), **11** (IC_{50} = 1.09 μM), **16** (IC_{50} = 1.06 μM) and **18** (IC_{50} = 1.05 μM) exhibited better anti-amoebic activity than the standard drug metronidazole while compounds **7** & **14** were moderately active. Based on IC_{50} values, six compounds **8**, **9**, **10**, **11**, **16** & **18** were more active than the standard drug metronidazole. From the above results it can be inferred that most of the new compounds having piperonal ring skeleton in conjugation with pyridine ring or furan ring in the same compound showed significant anti-amoebic activity and the modification of functionality produced significant change of activity. Thus, in the dioxazole series, anti-amoebic activity can be positively modulated through the introduction of pyridine, furan and piperonal ring residue on the dioxazole ring. It is worthy to mention that the position of the CH_3 group at position 3 of the dioxazole ring has also a marked effect on the activity and toxicity of the synthesized compounds as depicted by compounds **8** and **9** besides the presence and position of pyridine ring has a noticeable effect on the anti-amoebic activity and cytotoxicity of the compounds. The results were also statistically evaluated by analysis of variance. The null hypothesis was tested using *t*-test. The significativity of the difference between the IC_{50} values of metronidazole and the compounds **8**, **9**, **10**, **11**, **16** and **18** was evaluated by *t*-test. The values of the calculated *T* were found higher than the Table value of *T* at 5% level, thus concluding that the character under study is said to be significantly influenced by the treatment.

2.2.2. MTT assay

To ensure the toxicity of the most active compounds **8**, **9**, **10**, **11**, **16** and **18**, they were tested against H9c2 cardiac myoblasts. A subconfluent population of H9c2 cells was treated with increasing concentrations of these compounds and the number of viable cells was measured after 48 h by MTT cell viability assay. The concentration range of all the compounds was 3.13–200 μM. Fig. 1 depicts that all the compounds including the reference compound metronidazole showed a viability of 100% at the concentration range of 3.13 μM. Fig. 1 depicts that the compounds **8**, **9**, **10**, **11**, **16** and **18** and metronidazole exhibited ≥82% viability at the concentration range of 3.13–25 μM. On increasing the concentration range up to 50, 100 and 200 μM the compounds showed moderate to high cytotoxicity against the H9c2 cardiac myoblasts. Compound **8** showed least cytotoxicity among all the compounds screened, with a remarkable viability of 88% at a concentration of 200 μM. The cytotoxicity IC_{50} values along with the standard deviation values of the compounds and metronidazole are given in Table 1. To further investigate the selectivity of the compounds, the "safety index" (SI), defined as the toxicity IC_{50}/protozoal IC_{50}, was calculated. This allows estimating the efficacy of compounds. The results are summarized in Table 1. Compound **8** showed higher safety index value. Compounds **10**, **11** and **18** show safety index values better than metronidazole. These results showed that further studies of these compounds can give a better lead in the drug field. The results of physico-chemical properties are also in good support with the above results.

2.3. Molecular properties prediction

A molecular property is a complex balance of various structural features which determine whether a particular molecule is similar to the known drugs. It generally means "molecules which contain functional groups and/or have physical properties consistent with most of the known drugs". These properties, mainly molecular size, flexibility, hydrophobicity and presence of various pharmacophoric features influence the behaviour of molecules in a living organism, including bioavailability. A good bioavailability can be achieved with an appropriate balance between solubility and partitioning properties. Thus in order to achieve good oral drugs we have subjected a series of 1,4,2-dioxazole derivatives (**1**–**20**) for the prediction of lipophilicity, solubility and Lipinski's "Rule of Five" [29].

The knowledge of acidity constant and lipophilicity is a key parameter for understanding the chemical interactions, between the compound of interest and its pharmacological target. Relationship between acidity constant, pKa and structure may prove useful in drug design studies and in explaining the biopharmaceutical properties of substances like benzoxa-, benzothia- or benzoselenazolinone [30,31]. Many biologically active molecules are fully or partially ionized at physiological pH, and it has often been shown that the presence of ionizable groups is necessary for biological activity and/or solubility. Moreover, the acid–base property of a drug molecule is the key parameter for drug development because it governs solubility, absorption, distribution, metabolism and elimination. Particularly for developing new API's, the pKa has become of great importance because the transport of drugs into cells and across other membranes is a function of physico-chemical properties, and of the pKa and log *P* of the drugs [32].

2.3.1. pKa and log P

Poor solubility and poor permeability are amongst the main causes for failure during drug development [33–35]. It is therefore,

important to determine the physico-chemical properties associated with a drug. In order to calculate pKa values of the compounds, Marvin was used that has been described as a method of choice in a published paper where comparison of predictive performance of five different pKa prediction tools was undertaken [36].

In cases where multiple tautomeric forms exist the one which is expected to be the most stable was considered for calculating pKa values. As shown in Fig. 2a, the pKa values can be correlated with the activity of the compounds under study. In particular, the most active compounds have pKa values of ~2 (Table 2), and at physiological pH values, their rings will be uncharged while those of the less active species will be charged (to a lesser or greater extent) [32]. The presence of certain substituents which result in resonance stabilization/charge delocalization have also a very major effect on the pKa of the compounds [37].

The octanol–water partition coefficient (*P*) (also referred to as Kow) is a measure of the propensity of a neutral compound to differentially dissolve in these immiscible phases. It is usually referred to as the logarithmic ratio, log *P*, and serves as a quantitative descriptor of lipophilicity. Compounds with log *P* values ≤5 (Lipinski's Rule of Five) mean that they can readily get past ester/phosphate groups in skin membranes. The log *P* values of the compounds are shown in Table 2. There also exists a good relationship between log *P* and activity and in this study we have found that compounds having lower log *P* and IC_{50} values are the most active. However the compounds having lower IC_{50} values and higher log *P* values are the least active and similarly the compounds having higher IC_{50} values and lower log *P* values are also least active. As shown in Fig. 2, the compounds with log *P* values close to the linear line and have lower IC_{50} values are the most active. Hansch et al. [38] have also shown the relationship of the type.

$$\text{Activity} = m \log P + K'(\text{Linear})$$

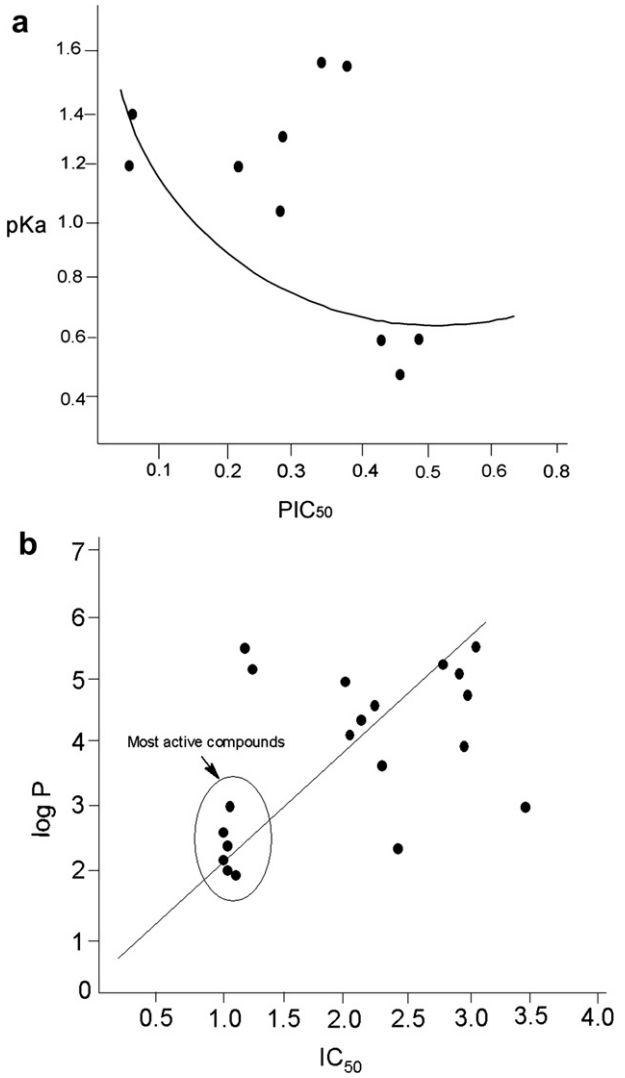


Fig. 2. a. Graph showing correlation between the PIC_{50} for *E. histolytica* growth inhibition by dioxazoles and the computed pka values of the parent bases. The line is to guide the eye. b. Graph showing correlation between log *P* and IC_{50} values. The compounds whose log *P* values are close to the linear line and have lower IC_{50} values are the most active. The most active compounds are encircled.

2.3.2. "Rule of Five" properties

High oral bioavailability is an important factor for the development of bioactive molecules as therapeutic agents. Good intestinal absorption, reduced molecular flexibility (measured by the number of rotatable bonds), low polar surface area or total hydrogen bond count (sum of donors and acceptors), are important predictors of good oral bioavailability [39,40]. Molecular properties such as membrane permeability and bioavailability are always associated with some basic molecular descriptors such as log *P* (partition coefficient), molecular weight (MW), or hydrogen bond acceptors and donors count in a molecule [41]. Lipinski [29] used these molecular properties in formulating his "Rule of Five". The rule states that most molecules with good membrane permeability have log $P \leq 5$, molecular weight ≤500, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors ≤5. This rule is widely used as a filter for drug-like properties. A compound that fulfils at least three out of the four criteria is said to adhere to Lipinski's "Rule of Five". A poor permeation or absorption is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors. The series (**1**–**20**) under investigation has most of the compounds possessing less number of hydrogen bond donors (<5) but do possess considerable number of acceptors (<10) as shown in Table 3. Lipinski's rule was used as a filter to choose the reasonable scaffolds as potential lead scaffolds. Tables 2 and 3 list the values of these properties for the new scaffolds and suggest that the active compounds are reasonable starting points for a drug discovery effort.

3. Conclusion

The combination of extended synthetic analogues of natural molecules leads to discovery of chemical entities which might be excellent anti-amoebic compounds as depicted in our results. 3,5-substituted 1,4,2-dioxazole derivatives **1**–**20**, bearing terpene moieties were synthesized and screened against *HM1:IMSS* strain of *E. histolytica*. Compound **8** (2-(3-(benzo[d][1,3]dioxol-5-yl)-5-methyl-1,4,2-dioxazol-5-yl)pyridine) showed the most promising results based on anti-amoebic screening, cytotoxicity studies and molecular properties prediction. Being highly anti-amoebic this compound can be explored in future as an option for decreasing pathogenic potential of infecting *E. histolytica* species. The compounds showing lower IC_{50} values than metronidazole also follow Lipinski's "Rule of Five".

4. Experimental protocol

Solvents and organic reagents were purchased from Sigma Aldrich, Merck (Germany) and Loba Chemie (India) and were used without further purification. Melting points (mp) were performed

Table 2
pKa and log *P* values of compounds **1**–**20**.

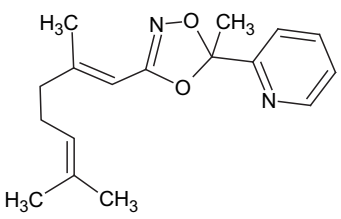
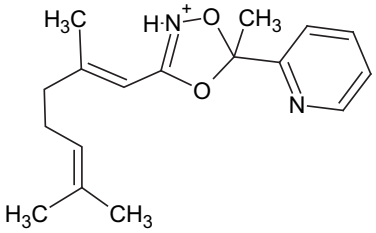
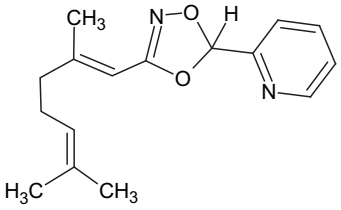
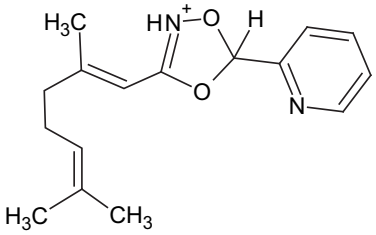
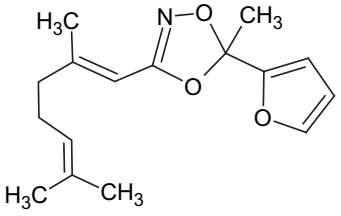
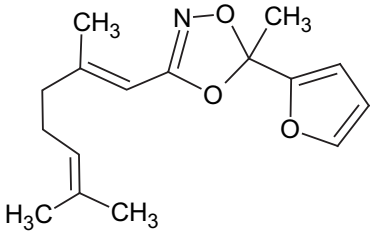
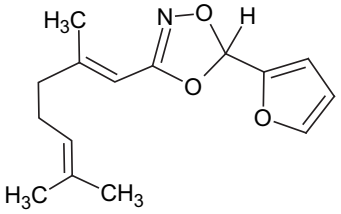
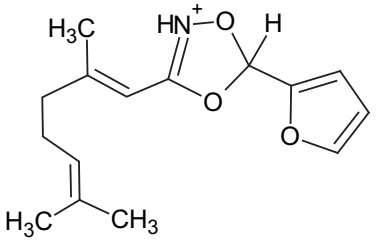
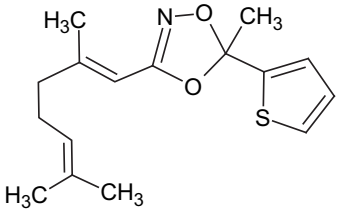
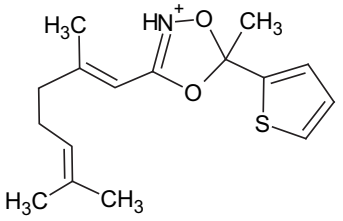
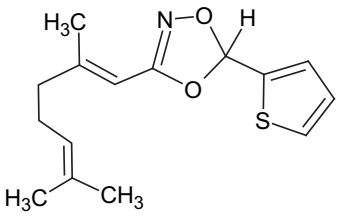
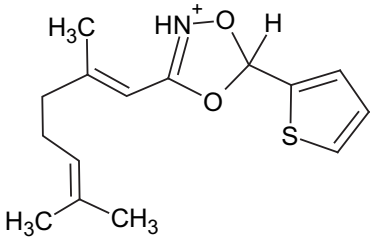
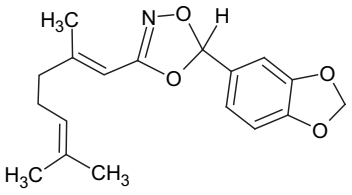
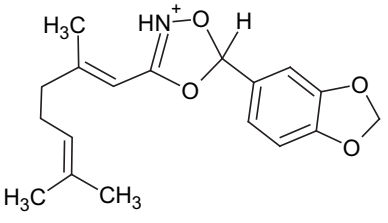
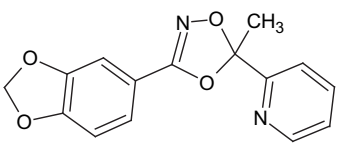
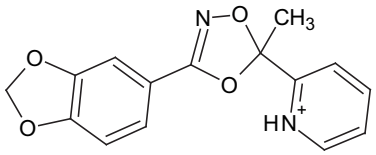
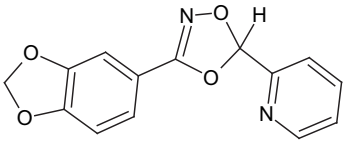
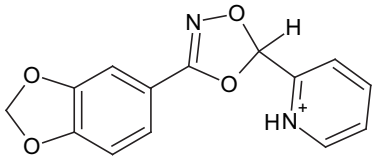
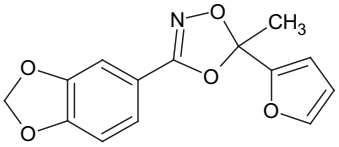
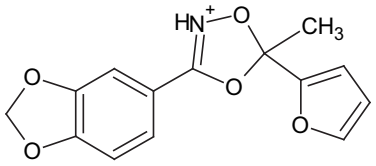
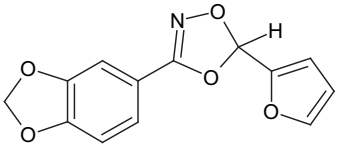
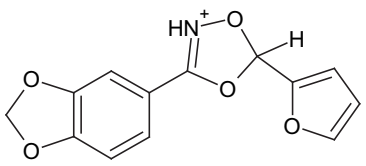
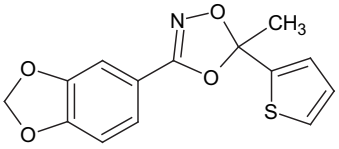
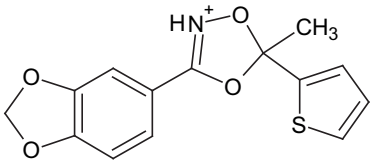
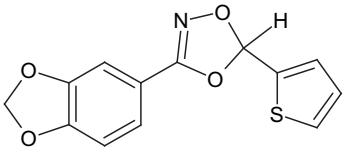
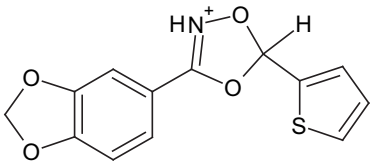
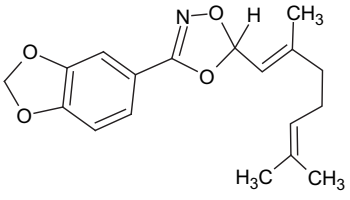
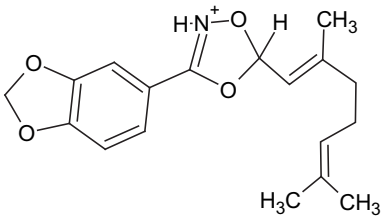
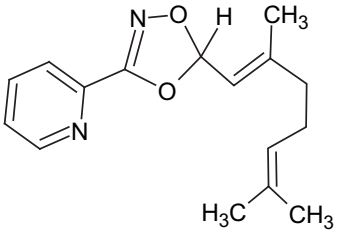
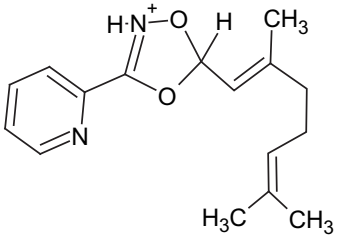
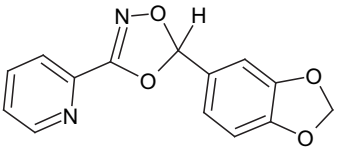
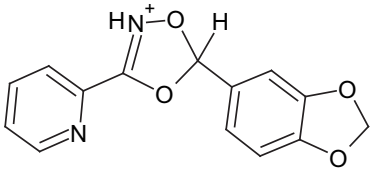
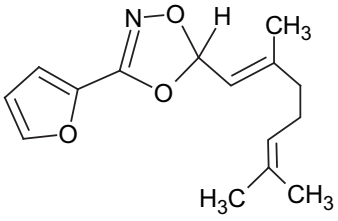
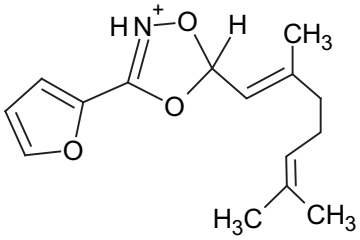
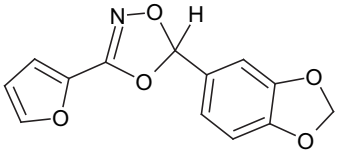
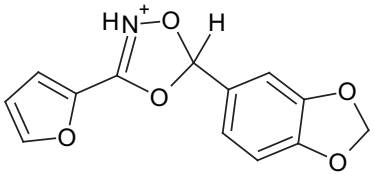
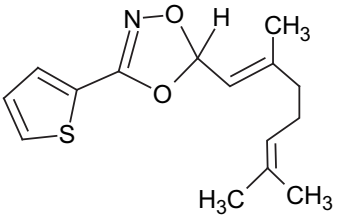
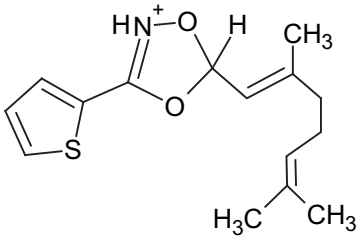
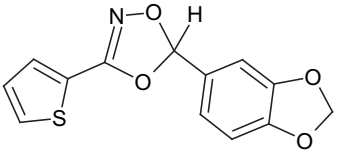
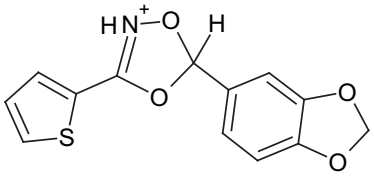
C. No.	% Yield	Neutral	Tautomer	LogP	pKa
1[a]	25			4.50 ± 0.66	Not Predicted
2[a]	30			3.96 ± 0.65	Not Predicted
3	25			5.15 ± 0.66	1.2–1.3
4	50			4.61 ± 0.66	1.2–1.3
5	40			5.67 ± 0.66	1.6
6	30			5.13 ± 0.66	1.6
7	40			5.31 ± 0.67	1.3–1.4

Table 2 (*continued*)

C. No.	% Yield	Neutral	Tautomer	LogP	pKa
8[a]	60			2.34 ± 0.91	0.30–0.40
9[a]	27			1.80 ± 0.90	0.20–0.30
10	29			2.99 ± 0.91	0.10–0.20
11	40			2.45 ± 0.90	0.10
12	50			3.51 ± 0.92	0.40–0.50
13	52			2.97 ± 0.91	0.40–0.50
14	58			5.44 ± 0.92	1.2–1.3
15[a]	60			4.08 ± 0.89	1.00

(*continued on next page*)

Table 2 (*continued*)

C. No.	% Yield	Neutral	Tautomer	LogP	pKa
16[a]	50			1.80 ± 0.90	Not Predicted
17	34			5.25 ± 0.90	0.50–0.60
18	32			2.97 ± 0.91	Not Predicted
19	60			4.74 ± 0.90	Not Predicted
20	50			2.45 ± 0.90	Not Predicted

[a] Have multiple tautomeric forms. The one which is the most stable has been mentioned.

using a Mel-temp instrument, and the results are uncorrected. Precoated aluminium sheets (silica gel 60 F_{254}, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. Elemental analyses were performed on Heraeus Vario EL III analyzer at Central Drug Research Institute, Lucknow, India. Electronic spectra were recorded on a Shimadzu UV 1601 PC UV–Visible spectrophotometer. IR spectra were recorded on Perkin–Elmer model 1600 FT-IR RX1 spectrophotometer as KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE 300 spectrometer using DMSO-d_6 as solvent with TMS as internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Chemical shift values are given in ppm. ESI-MS was recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer.

4.1. General procedure for the synthesis of oximes (I–V)

The oximes were synthesized by two methods (I & II). Although both methods resulted in good yields, however the method II is favoured, because of higher yield and less reaction time (Table 4).

4.1.1. Method I

An aldehyde (1 mmol) and hydroxylamine hydrochloride (1.25 mmol) in a solution of ethanol and pyridine (2:1) was refluxed with stirring for 24 h. After cooling, the mixture was concentrated and then poured into 600 ml of ice-cold water. The solid mass was collected, washed with water, and crystallized from methanol to give the corresponding oxime. However in case of citral oxime an oil layer was formed which was extracted with ether and separated. The organic layer was dried with $NaSO_4$ and concentrated under reduced pressure.

4.1.2. Method II

To a solution of aldehyde (1 eq) and hydroxylamine hydrochloride (1.25 eq) in water (5 ml), a solution of sodium bicarbonate (1.25 eq) in water (10 ml) was added gradually with stirring, and the mixture was stirred for further 2–5 h with the formation of a solid precipitate, which was filtered and dried. However in case of citral oxime (I), an organic layer was formed, which was separated and the aqueous layer was extracted with ether and added to the organic layer. The organic layer was dried with $NaSO_4$ and evaporated.

Table 3
The pharmacokinetic properties of compounds **1**–**20** according to Lipinski's rule.

Compound	Molecular weight	Log *P*	H-bond donors	H-bond acceptors	Rule of 5 criteria met
1	286.17	4.50 ± 0.66	0	4	Y
2	272.15	3.96 ± 0.65	0	4	Y
3	275.15	5.15 ± 0.66	0	4	N
4	261.14	4.61 ± 0.66	0	4	Y
5	291.13	5.67 ± 0.66	0	4	N
6	277.11	5.13 ± 0.66	0	4	N
7	315.15	5.31 ± 0.67	0	5	N
8	284.08	2.34 ± 0.91	0	6	Y
9	270.06	1.80 ± 0.90	0	6	Y
10	273.06	2.99 ± 0.91	0	6	Y
11	259.05	2.45 ± 0.90	0	6	Y
12	289.04	3.51 ± 0.92	0	6	Y
13	275.03	2.97 ± 0.91	0	6	Y
14	315.15	5.44 ± 0.92	0	5	N
15	272.15	4.08 ± 0.89	0	4	Y
16	270.06	1.80 ± 0.90	0	6	Y
17	261.14	5.25 ± 0.90	0	4	N
18	259.05	2.97 ± 0.91	0	6	Y
19	275.03	4.74 ± 0.90	0	6	Y
20	289.04	2.45 ± 0.90	0	6	Y

Y. Yes, N. No (Rule of 5 criteria met or not).

4.1.2.1. (1E,2E)-N-hydroxy-3,7-dimethylocta-2,6-dien-1-imine (I). Oil; Anal. Calc. For $C_{10}H_{17}NO$: C 71.81, H 10.25, N 8.37%; found: C 71.79, H 10.26, N 8.39%; IR ν_{max} cm^{-1}: 3250 (NO–H), 2864 (C–H), 1632 (C=N), 936 (N–O stretch); ^1H NMR (DMSO-d6) δ(ppm): 9.43 (broad s, 1H, N–OH), 7.32 (s, 1H, CH=N–OH), 5.90 (d, 1H,=CH–CH=N, J = 11.7 Hz), 5.08 (s, 1H,=CH–CH_2), 2.30–2.03 (m, 4H, CH_2–CH_2), 1.83 (s, 6H, CH_3), 1.68 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ(ppm): 154.58 (C=N), 151.2, 132.2, 125.6, 122.4, 43.5, 28.0, 26.2, 20.5, 18.9; ESI-MS m/z: [M^++1] 167.13.

4.1.2.2. (E)-1-(1,3-benzodioxol-5-yl)-N-hydroxymethanimine (II). - White; mp 100–105 °C; Anal. Calc. For $C_8H_7NO_3$: C 58.18, H 4.27, N 8.48%. found: C 58.34, H 4.36, N 8.49%; IR ν_{max} cm^{-1}: 3245 (NO–H), 2921 (C–H), 1635 (C=N), 937 (N–O stretch); ^1H NMR (DMSO-d6) δ(ppm): 10.85 (broad s, 1H, N–OH), 7.90 (s, 1H, CH=N–OH), 7.30–6.50 (m, 3H, Ar–H), 5.90 (s, 2H, –O–CH_2–O–); ^{13}C NMR (DMSO-d6) δ(ppm): 148.10 (C=N), 147.3, 145.0, 126.6, 122.5, 115.0, 112.8, 101.6 (–O–CH_2–O); ESI-MS m/z: [M^++1] 166.04.

4.1.2.3. (E)-N-hydroxy-1-(pyridin-2-yl)methanimine (III). Pink; mp 118–120 °C; Anal. Calc. For $C_6H_6N_2O$: C 59.01, H 4.95, N 22.94. found: C 58.95, H 4.96, N 23.09%; IR ν_{max} cm^{-1}: 3260 (NO–H), 2920 (C–H), 1634 (C=N), 1630 (C=N), 937 (N–O stretch); ^1H NMR (DMSO-d6) δ(ppm): 10.76 (broad s, 1H, N–OH), 8.60 (d, 1H, py-H, J = 7.4 Hz), 8.45 (d, 1H, py-H, J = 7.2 Hz), 8.03–8.11 (dd, 1H, py-H, J = 8.7 Hz), 7.90–8.02 (dd, 1H, py-H, J = 8.4 Hz), 6.97 (s, 1H, CH=N-OH); ^{13}C NMR (DMSO-d6) δ(ppm): 151.60 (C=N), 146.10 (C=N), 145.0, 136.9, 125.5, 101.6 (–O–CH_2–O); ESI-MS m/z: [M^++1] 122.04.

4.1.2.4. (E)-1-(furan-2-yl)-N-hydroxymethanimine (IV). White; mp 112–115 °C; Anal. Calc. For $C_5H_5NO_2$: C 47.23, H 3.96, N 11.01%. found: C 47.05, H 4.06, N 11.09%; IR ν_{max} cm^{-1}: 3254 (NO–H), 3132 (C–H), 1628 (C=N), 935 (N–O stretch); ^1H NMR (DMSO-d6) δ(ppm): 11.13 (broad s, 1H, N–OH), 6.90 (s, 1H, CH=N–OH), 7.20 (d, 1H, furan ring-H, J = 7.6 Hz) 6.01 (dd 2H furan ring-H, J = 8.5, 8.9 Hz); ^{13}C NMR (DMSO-d6) δ(ppm): 146.0 (C=N), 142.8, 134.7, 110.8, 109.5; ESI-MS m/z: [M^++1] 111.03.

Table 4
Synthesis of oximes I–V by methods I & II.

Oximes	Method I (Yield, %)	Reaction time (h)	Method II (Yield, %)	Reaction time (h)
I	76	24	92	2
II	80	24	90	3–5
III	70	24	83	2–3
IV	70	24	80	3
V	72	24	87	3–4

4.1.2.5. (E)-N-hydroxy-1-(thiophen-2-yl)methanimine (V). White; mp 98–101 °C; Anal. Calc. For C_5H_5NOS: C 47.23, H 3.96, N 11.01%. found: C 47.12, H 4.15, N 10.89%; IR ν_{max} cm^{-1}: 3256 (NO–H), 3064 (C–H), 1622 (C=N), 942 (N–O stretch); ^1H NMR (DMSO-d_6) δ(ppm): 11.05 (broad s, 1H, N–OH), 7.80 (s, 1H, CH=N–OH), 7.10–7.00 (dd, 2H, Thp-H, J = 7.8, 8.0 Hz), 6.80 (d, 1H, Thp-H, J = 5.4 Hz); ^{13}C NMR (DMSO-d_6) δ(ppm): 151.8 (C=N), 145.8, 129.5, 126.0, 124.9; ESI-MS m/z: [M^++1] 128.03.

*4.2. General procedure for the synthesis of dioxazoles (**1**–**20**)*

A 13% aqueous solution of NaOCl (1.6 equiv.) was added to a solution of aldehydes (1 equiv.) and triethylamine (0.1 equiv.) in ethylacetate under argon atmosphere. The oxime (1 equiv.) in ethylacetate was added dropwise (over a period of 1 h) at 0 °C to the above solution and stirred at room temperature for 12–15 h and refluxed for additional 10–12 h, depending upon the reaction time (monitored by TLC). The reaction mixture was cooled to room temperature and water was added to it. The organic layer was separated and the aqueous layer was extracted with ethylacetate. The combined organic layers were washed with water, brine, dried over $NaSO_4$, filtered and concentrated under vacuo. The compounds were crystallized using dichloromethane–hexane solution.

*4.2.1. 2-(5-methyl-3-((E)-2,6-dimethylhepta-1,5-dienyl)-1,4,2-dioxazole-5-yl) pyridine (**1**)*

Cream; mp 183–185 °C; Anal. Calc. For $C_{17}H_{22}N_2O_2$: C 71.30, H 7.74, N 9.78%; found: C 71.27, H 7.70, N 9.79%; UV λ_{max} nm: 352, 311, 250; IR ν_{max} cm^{-1}: 3054 (C–H stretch py), 1652 (C=N), 1645 (C=C stretch), 1615 (C=N, py); ^1H NMR (DMSO-d_6) δ(ppm): 8.60 (d, 1H, py-H, J = 7.4 Hz), 8.40 (d, 1H, py-H, J = 7.2 Hz), 8.12 (dd, 1H, py-H, J = 8.4, 8.5 Hz), 7.95 (dd, 1H, py-H, J = 7.8, 8.2 Hz), 5.86 (s,1H,=CH–C), 5.01 (s, 1H,=CH–CH_2), 2.57–2.61 (m, 4H, CH_2–CH_2), 2.17 (s, 3H, CH_3 dioxazole ring), 1.99 (s, 3H, CH_3), 1.68 (s, 6H, CH_3), ^{13}C NMR (DMSO-d_6) δ(ppm):162.4 (C=N), 160.1 (C=N), 154.2, 133.5, 123.7, 121.5, 40.5, 26.7, 25.1, 19.4, 18.1, (Aliphatic chain-C), 147.6, 134.4, 122.8, 120.6, (Ar–C), 95.1(O–C–O) 26.2 (C–CH_3 dioxazole ring); ESI-MS m/z: [M^++1] 287.17.

*4.2.2. 2-(3-((E)-2,6-dimethylhepta-1,5-dienyl)-1,4,2-dioxazole-5-yl) pyridine (**2**)*

Oil; Anal. Calc. For $C_{16}H_{20}N_2O_2$: C 70.56, H 7.40, N 10.29%; found: C 70.51, H 7.48, N 10.35%; UV λ_{max} nm: 321, 299, 267; IR ν_{max} cm^{-1}: 3064 (C–H stretch py), 1645 (C=N), 1660 (C=N)1642 (C=C stretch); ^1H NMR (DMSO-d_6) δ(ppm): 7.80 (dd, 1H, py-H), 7.71 (d, 1H, py-H, J = 7.4 Hz), 7.62 (d, 1H, py-H, J = 7.2 Hz),7.26 (dd, 1H, py-H), 6.91 (s, 1H, C–H (dioxazole ring)) 5.08 (s, 2H, –CH=C), 2.33–2.10 (m, 4H, CH_2–CH_2), 1.68 (s, 6H, CH_3), 1.55 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ(ppm): 155.1 (C=N), 158.5 (C=N py), 154.8, 134.8, 98.2, 38.5, 25.7, 24.3, 20.4, 18.7, 18.6, (Aliphatic-C) 149.3, 137.4, 123.9, 121.0, (Ar–C), 87.5(O–C–O); ESI-MS m/z: [M^++1] 273.15.

*4.2.3. 5-(furan-2-yl)-5-methyl-3-((E)-2,6-dimethylhepta-1,5-dienyl)-1,4,2-dioxazole (**3**)*

Oil; Anal. Calc. For $C_{16}H_{21}NO_3$: C 69.79, H 7.69, N 5.09%; found: C 69.76, H 7.70, N 5.11%; UV λ_{max} nm: 332, 298, 276; IR ν_{max} cm^{-1}: 3130 (C–H stretch furan ring), 1632 (C=N) 1641 (C=C stretch); ^1H NMR (DMSO-d_6) δ(ppm): 8.02 (d, 1H furan ring-H, J = 5.6 Hz), 6.52–6.45

(m 2H furan ring-H), 5.86 (s,1H,=CH–C), 5.07 (s, 1H,=CH–CH_2), 2.61–2.57 (m, 4H, CH_2–CH_2), 2.54 (s, 3H, CH_3 dioxazole ring), 1.68 (s, 3H, CH_3), 1.53 (s, 6H, CH_3), ^{13}C NMR (DMSO-d_6) δ(ppm): 159.6 (C=N), 152.2, 134.8, 124.3, 114.5, 41.2, 28.7, 26.6, 19.5, 18.3 (Aliphatic chain-C) 155.6, 140.2, 110.5, 106.4 (Ar–C) 96.8 (O–C–O), 25.5 (C–CH_3 dioxazole ring); ESI-MS m/z: [M^++1] 276.15.

4.2.4. 5-(furan-2-yl)-3-((E)-2, 6-dimethylhepta-1,5-dienyl)-1,4,2-dioxazole (**4**)

Oil; Anal. Calc. For $C_{15}H_{19}NO_3$: C 68.94, H 7.33, N 5.36%; found: C 69.14, H 7.28, N 5.37%; UV λ_{max} nm: 336, 312, 286; IR ν_{max} cm^{-1}: 3030 (C–H stretch furan ring), 1648 (C=N), 1643 (C=C stretch); ^{1}H NMR (DMSO-d_6) δ(ppm): 8.08 (d, 1H furan ring-H, J = 5.2 Hz), 6.52 (dd 1H furan ring-H, J = 5.6, 5.6 Hz), 6.16 (dd 1H furan ring-H, J = 5.8, 5.4 Hz), 5.90 (s, 1 H, C–H (dioxazole ring), 5.08 (s, 2H,=CH–CH_2), 2.25–2.05 (m, 4H, CH_2-CH_2), 1.83 (s, 3H, CH_3) 1.50 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6) δ(ppm): 168.4 (C=N), 162.4 (C=N) 122.6, 154.3, 137.8, 40.7, 28.1, 25.3, 20.5, 17.8 (Aliphatic chain-C) 151.6, 138.4, 111.7, 106.7, (Ar–C)), 90.7(O–C–O); ESI-MS m/z: [M^++1] 262.14.

4.2.5. 5-methyl-3-((E)-2, 6-dimethylhepta-1, 5-dienyl)-5-(thiophenyl-2-yl)-1,4,2-dioxazole (**5**)

Oil; Anal. Calc. For $C_{16}H_{21}NO_2S$: C 65.95, H 7.26, N 4.81%; found: C 65.84, H 7.28, N 4.87%; UV λ_{max} nm: 344, 304, 281; IR ν_{max} cm^{-1}: 3125 (C–H stretch thiophene ring), 1652 (C=N),1640 (C=C stretch); ^{1}H NMR (DMSO-d_6) δ(ppm): 7.83 (dd 1H Thp-H, J = 8.6, 8.4 Hz), 7.74 (d, 1H Thp-H, J = 7.6 Hz), 7.13 (d, 1H Thp-H, J = 7.6 Hz), 6.08 (s, 1H,=CH–C), 5.08 (s, 1H,=CH–CH_2), 2.54 (s, 3H, CH_3 dioxazole ring), 2.27–2.13 (m, 4H, CH_2–CH_2), 1.38 (s, 6H, CH_3), 1.35 (s, 3H, CH_3), ^{13}C NMR (DMSO-d_6) δ(ppm): 160.9 (C=N), 149.0, 133.0, 123.5, 36.5, 30.1, 21.6, 21.6, 16.9, (Aliphatic chain-C) 153.2, 135.5, 112.0, 105.6, 102.5, (Ar–C), 95.0 (O–C–O), 26.4 (C–CH_3 dioxazole ring); ESI-MS m/z: [M^++1] 292.13.

4.2.6. 3-((E)-2,6-dimethylhepta- 1,5-dienyl)- 5-(thiophen-2-yl)-1,4,2-dioxazole (**6**)

Oil; Anal. Calc. For $C_{15}H_{19}NO_2S$: C 64.95, H 6.90, N 5.05%; found: C 65.04, H 6.98, N 5.05%; UV λ_{max} nm: 319, 299, 261; IR ν_{max} cm^{-1}: 3095 (C–H stretch thiophene ring), 1660 (C=N), 1644 (C=C stretch), ^{1}H NMR (DMSO-d_6) δ(ppm): 7.74 (dd 1H Thp-H) 7.53 (d, 1H Thp-H, J = 5.6 Hz), 7.43 (d, 1H Thp-H, J = 5.6 Hz), 6.30 (s, 1H, C–H (dioxazole ring), 5.90 (s, 1H,=CH–C), 5.10 (s, 1H,=CH–CH_2), 2.25–2.13 (m, 4H, CH_2–CH_2), 1.38 (s, 6H, CH_3), 1.35 (s, 3H, CH_3), ^{13}C NMR (DMSO-d_6) δ(ppm): 166.5 (C=N), 153.0, 135.0, 121.2, 114.2, 34.5, 32.5, 25.6, 20.5, 17.0; (Aliphatic chain-C) 153.2, 135.5, 105.6, 102.5, (Ar–C), 89.5 (O–C–O); ESI-MS m/z: [M^++1] 278.17.

4.2.7. 5-(benzo[d][1,3]dioxol-5-yl)-3-((E)-2,6-dimethylhepta-1,5-dienyl)-1,4,2-dioxazole (**7**)

White; mp 123–125 °C; Anal. Calc. For $C_{18}H_{21}NO_4$: C 68.55, H 6.71, N 4.44%; found: C 68.54, H 6.78, N 4.40%; UV λ_{max} nm: 354, 314, 271; IR ν_{max} cm^{-1}: 1647 (C=N), 1645 (C=C stretch), ^{1}H NMR (DMSO-d_6) δ(ppm): 7.80–7.56 (m, 3H, Ar–H), 6.96 (s, 1H, C–H (dioxazole ring), 6.16 (s, 2H, –O–CH_2–O–), 5.43 (s, 1H,=CH–CH_2), 4.75 (s, 1H,=CH–C), 2.14–2.03 (m, 4H, CH_2–CH_2), 1.90 (s, 6H, CH_3), 1.38 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ(ppm): 162.4 (C=N), 150.5, 134.3, 120.5, 116.0, 34.9, 30.5, 23.5, 22.6, 17.5, (Aliphatic chain-C) 147.5, 145.6, 136.0, 120.5, 114.6, 110.7, 100.5, (Ar–C) 89.7(O–C–O); ESI-MS m/z: [M^++1] 316.18.

4.2.8. 2-(3-(benzo[d][1, 3]dioxol-5-yl)-5-methyl-1,4,2-dioxazol-5-yl) pyridine (**8**)

Cream; mp 140–145 °C; Anal. calc. for $C_{15}H_{12}N_2O_4$: C 63.38, H 4.25, N 9.85%; found: C 63.45, H 4.30, N 9.96%; UV λ_{max} nm: 340, 301, 265; IR ν_{max} cm^{-1}: 3054 (Ar–H), 3020 (C–H), 1660 (C=N), 1650 (C=N py); ^{1}H NMR (DMSO-d6) δ(ppm): 7.83 (d, 1H, py-H, J = 7.2 Hz), 7.71 (d, 1H, py-H, J = 7.2 Hz), 7.60 (dd, 1H, py-H, J = 7.8, 8.2 Hz), 7.34 (dd, 1H, py-H, J = 8.2, 8.6 Hz), 6.92–6.85 (m, 3H, Ar–H), 6.07 (s, 2H, –O–CH_2–O), 1.90 (s, 3H, CH_3 dioxazole ring); ^{13}C NMR (DMSO-d6) δ(ppm): 167.5 (C=N), 162.5 (C=N py), 150.6, 148.7, 146.2, 145.5, 123.4, 122.4, 121.2, 120.0, 115.7, 113.0, (Ar–C), 101.6 (–O–CH_2–O), 94.8 (O–C–O), 27.8 (C–CH_3 dioxazole ring); ESI-MS m/z: [M^++1] 285.10.

4.2.9. 2-(3-(benzo[d] [1,3]dioxol-5-yl)-1,4,2-dioxazol-5-yl) pyridine (**9**)

Cream; mp 130–134 °C; Anal. calc. for $C_{14}H_{10}N_2O_4$: C 62.22, H 3.73, N 10.37%; found: C 63.01, H 3.83, N 10.26%; UV λ_{max} nm: 346, 307, 255; IR ν_{max} cm^{-1}: 3065 (C–H), 3025 (Ar–H), 1656 (C=N), 1643 (C=N py); ^{1}H NMR (DMSO-d6) δ(ppm):): 8.81 (d, 1H, py-H, J = 7.6 Hz), 8.33 (d, 1H, py-H, J = 7.5 Hz), 8.02 (dd, 1H, py-H, J = 8.6, 8.4 Hz), 7.71 (dd, 1H, py-H, J = 8.2, 8.4 Hz), 6.91–7.59 (m, 3H, Ar–H), 6.52 (s, 1H, C–H (dioxazole ring), 5.94 (s, 2H, –O–CH_2–O–); ^{13}C NMR (DMSO-d6) δ(ppm): 162.4 (C=N), 159.8 (C=N py), 152.5, 150.2, 148.4, 146.5, 136.4, 124.5, 123.0, 122.1, 114.7, 112.5 (Ar–C), 103.5 (–O–CH_2–O), 87.5 (O–C–O); ESI-MS m/z: [M^++1] 271.08.

4.2.10. 3-(benzo[d][1,3]dioxol-5-yl)-5-(furan-2-yl)-5-methyl-1,4,2-dioxazole (**10**)

Cream; mp 150–155 °C; Anal. calc. for $C_{14}H_{11}NO_5$: C 61.54, H 4.06, N 5.13%; found: C 61.63, H 3.93, N 5.23%; UV λ_{max} nm: 338, 306, 275; IR ν_{max} cm^{-1}: 3072 (Ar–H), 3045 (C–H), 1650 (C=N); ^{1}H NMR (DMSO-d6) δ(ppm): 7.83 (d, 1H, furan ring-H, J = 5.6 Hz) 7.51–7.31 (m, 3H Ar-H), 7.25 (d, 1H, furan ring–H, J = 5.4 Hz), 7.13 (dd, 1H, furan ring-H, J = 7.6, 7.6 Hz), 5.86 (s, 2H, –O–CH_2–O–), 1.90 (s, 3H, dioxazole ring); ^{13}C NMR (DMSO-d6) δ(ppm): 162.4 (C=N), 157.2, 150.4, 148.1, 141.4, 128.5, 123.7, 121.6, 114.5, 110.5, 104.0, 101.5 (Ar–C), 91.5 (O–C–O), 25.6 (C–CH_3 dioxazole ring); ESI-MS m/z: [M^++1] 274.06.

4.2.11. 3-(benzo[d][1,3]dioxol-5-yl)-5-(furan-2-yl)-1,4,2-dioxazole (**11**)

Brown; mp 153–155 °C; Anal. calc. for $C_{13}H_9NO_5$: C 60.24, H 3.50, N 5.40%; found: C 60.58, H 3.42, N 5.33%; UV λ_{max} nm: 354, 312, 265; IR ν_{max} cm^{-1}: 3061 (Ar–H), 3027 (C–H), 1638 (C=N); ^{1}H NMR (DMSO-d6) δ(ppm): 7.80 (d, 1H furan ring-H, J = 5.7 Hz), 7.74–7.60 (m, 3H, Ar–H), 7.26 (dd 1H furan ring-H, J = 5.6, 5.6 Hz), 7.13 (d, 1H furan ring-H, J = 5.6 Hz), 6.14 (s, 2H, –O–CH_2–O–) 6.08 (s, 1H, C–H (dioxazole ring)); ^{13}C NMR (DMSO-d6) δ(ppm): 162.6 (C=N), 156.9, 150.4, 148.1, 141.4, 128.5, 123.7, 114.5, 110.5, 108.2, 104.0, 100.2 (Ar–C) 87.5 (O–C–O); ESI-MS m/z: [M^++1] 260.04.

4.2.12. 3-(benzo[d][1,3]dioxol-5-yl)-5-methyl-5-(thiophen-2-yl)-1,4,2-dioxazole (**12**)

Yellowish; mp 120–123 °C; Anal. calc. for $C_{14}H_{11}NO_4S$: C 58.12, H 3.83, N 4.84%; found: C 58.34, H 3.73, N 4.84%; UV λ_{max} nm: 330, 311, 291; IR ν_{max} cm^{-1}: 3100 (Ar–H), 3065 (C–H), 1656 (C=N); ^{1}H NMR (DMSO-d6) δ(ppm): 7.80 (dd, 1H, Thp-H, J = 5.8, 5.8 Hz), 7.71–7.56 (m, 3H, Ar-H), 7.26 (d, 1H Thp-H, J = 7.8 Hz), 6.91 (d, 1H Thp-H, J = 7.4 Hz), 6.09 (s, 2H, –O–CH_2–O–), 2.19 (s, 3H, dioxazole ring); ^{13}C NMR (DMSO-d6) δ(ppm): 164.4 (C=N), 154.6, 148.3, 147.5, 128.4, 126.8, 125.5, 123. 0, 115.4, 112.2, 108.5, 102.5 (Ar–C), 92.5 (O–C–O), 28.5 (C–CH_3 dioxazole ring); ESI-MS m/z: [M^++1] 290.04.

4.2.13. 3-(benzo[d][1,3]dioxol-5-yl)-5-(thiophen-2-yl)-1,4,2-dioxazole (**13**)

Oil; Anal. calc. for $C_{13}H_9NO_4S$: C 56.72, H 3.30, N 5.09%; found: C 56.80, H 3.40, N 5.23%; UV λ_{max} nm: 325, 297, 255; IR ν_{max} cm^{-1}: 3055 (C–H), 3034 (Ar–H), 1640 (C=N); ^{1}H NMR (DMSO-d6) δ(ppm): 7.83 (dd, 1H, Thp-H, J = 5.6, 5.6 Hz), 7.26–7.13 (m, 3H, Ar-

H), 6.92 (d, 1H, Thp-H, J = 7.2 Hz), 6.80 (d, 1H, Thp-H, J = 7.4 Hz), 6.04 (s, 1H, C–H (dioxazole ring)), 5.70 (s, 2H, –O–CH_2–O–); ^{13}C NMR (DMSO-d6) δ(ppm): 168.1 (C=N), 158.5, 152.6, 147.5, 128.2, 126.5, 124.5, 123.6, 121.5, 115.4, 113.5, 109.2, (Ar–C) 89.0 (O–C–O); ESI-MS m/z: [M^++1] 276.03.

4.2.14. 3-(benzo[d][1,3]dioxol-6-yl)-5-((E)-2,6-dimethylhepta-1,5-dienyl)-1,4,2-dioxazole (**14**)

Grey; mp 145–147 °C; Anal. calc. for $C_{18}H_{21}NO_4$: C 68.55, H 6.71, N 4.44%; found: C 68.32, H 6.73, N 4.33%; UV λ_{max} nm: 335, 298, 275; IR ν_{max} cm^{-1}: 3075 (C–H), 3065 (Ar–H), 1650 (C=N); ^{1}H NMR (DMSO-d6) δ(ppm): 7.53–7.13 (m, 3H, Ar-H), 6.04 (s, 2H, –O–CH_2–O–) 5.88 (s, 1H,=CH–C), 5.65 (s, 1H, C–H (dioxazole ring)), 5.10 (s, 1H,=CH–CH_2), 2.26–2.13 (m, 4H, CH_2–CH_2), 1.38 (s, 3H, CH_3), 1.35 (s, 6H, CH_3); ^{13}C NMR (DMSO-d6) δ(ppm): 162.4 (C=N), 135.0, 133.6, 124.5, 121.2, 32.5, 34.5, 25.6, 20.5, 17.0 (Aliphatic chain-C) 151.5, 146.2, 127.5, 123.5, 113.5, 109.2, 103.2 (Ar–C) 86.7 (O–C–O); ESI-MS m/z: [M^++1] 316.15.

4.2.15. 2-(5-((E)-2, 6-dimethylhepta-1,5-dienyl)-1,4,2-dioxazol-3-yl) pyridine (**15**)

Grey; mp 140–143 °C; Anal. calc. for $C_{16}H_{20}N_2O_2$: C 70.56, H 7.40, N 10.29%; found: C 70.64, H 7.33, N 10.24%; UV λ_{max} nm: 321, 303, 263; IR ν_{max} cm^{-1}: 1642 (C=C stretch), 3054 (C–H stretch py), 1650 (C=N); 1635 (C=N py); ^{1}H NMR (DMSO-d_6) δ(ppm): 8.08 (d, 1H, py-H, J = 5.2 Hz), 7.73 (d, 1H, py-H, J = 5.6 Hz), 7.56 (dd, 1H, py-H, J = 5.4, 5.4 Hz), 7.26 (dd, 1H, py-H, J = 5.6, 5.6 Hz), 6.07 (s, 1H, C–H (dioxazole ring), 5.90 (s, 1H,=CH–C), 5.08 (s, 1H,=CH–CH_2), 2.61–2.29 (m, 4H, CH_2–CH_2), 1.99 (s, 3H, CH_3), 1.68 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6) δ(ppm): 159.8 (C=N), 154.5 (C=N py), 138.8, 134.7, 122.8, 120.5, 36.9, 34.5, 24.6, 22.8, 17.5, (Aliphatic chain-C), 146.5, 136.9, 127.5, 123.5 (Ar-C) 87.5 (O–C–O); ESI-MS m/z: [M^++1] 273.15.

4.2.16. 2-(5-(benzo[d][1, 3] dioxol-5-yl)-1,4,2-dioxazol-3-yl) pyridine (**16**)

Yellowish; mp 135–137 °C; Anal. calc. for $C_{14}H_{10}N_2O_4$: C 62.22, H 3.73, N 10.37%; found: C 62.34, H 3.64, N 10.24%; UV λ_{max} nm: 350, 313, 265; IR ν_{max} cm^{-1}: 3075 (C–H), 3054 (Ar–H), 1659 (C=N); 1630 (C=N py); ^{1}H NMR (DMSO-d_6) δ(ppm): 8.50 (d, 1H, py-H, J = 7.6 Hz), 7.83 (d, 1H, py-H, J = 7.4 Hz), 7.74 (dd, 1H, py-H, J = 8.9, 8.6 Hz), 7.26 (dd, 1H, py-H, J = 8.4, 8.6 Hz), 6.92–6.85 (m, 3H, Ar–H), 6.04 (s, 1H, C–H (dioxazole ring)), 5.88 (s, 2H, –O–CH_2–O–); ^{13}C NMR (DMSO-d_6) δ(ppm): 160.5 (C=N), 156.9 (C=N), 150.4, 148.5, 147.2, 134.6, 128.5, 116.8, 126.6, 122.5, 112.9, 108.7, 102.5, (Ar–C), 93.7 (O–C–O); ESI-MS m/z: [M^++1] 271.06.

4.2.17. 3-(furan-2-yl)-5-((E)-2, 6-dimethylhepta-1, 5-dienyl)-1,4,2-dioxazole (**17**)

Oil; Anal. calc. for $C_{15}H_{19}NO_3$: C 68.94, H 7.33, N 5.36%; found: C 69.08, H 7.31, N 5.34%; UV λ_{max} nm: 322, 296, 273; IR ν_{max} cm^{-1}: 1642 (C=C stretch), 3125 (C–H), 1643 (C=N); ^{1}H NMR (DMSO-d6) δ(ppm): 8.81 (d, 1H furan ring-H, J = 5.6 Hz), 6.96 (dd, 2H furan ring –H, J = 5.8, 5.6 Hz) 5.86 (s, 1H, C–H (dioxazole ring)); 5.45 (s, 1H,=CH–C), 5.10 (s, 1H,=CH–CH_2), 2.61–2.54 (m, 4H, CH_2–CH_2), 1.99 (s, 3H, CH3), 1.60 (s, 6H, CH3), ^{13}C NMR (DMSO-d6) δ(ppm): 162.4 (C=N), 138.4, 134.7, 125.5, 120.5, 36.9, 34.5, 24.6, 22.8, 17.5 (Aliphatic chain-C), 140.5, 138.4, 109.1, 105.6, (Ar–C), 96.3 (O–C–O); ESI-MS m/z: [M^++1] 262.14.

4.2.18. 5-(benzo[d][1,3]dioxol-5-yl)-3-(furan-2-yl)-1,4,2-dioxazole (**18**)

Brown; mp 145–147 °C; Anal. calc. for $C_{13}H_9NO_5$: C 60.24, H 3.50, N 5.40%; found: C 60.14, H 3.51, N 5.44%; UV λ_{max} nm: 327, 303, 278; IR ν_{max} cm^{-1}: 3065 (Ar–H), 3030 (C-H), 1660 (C=N); ^{1}H NMR (DMSO-d_6) δ(ppm): 7.99 (d, 1H furan ring-H, J = 7.2 Hz), 7.26–6.98 (m, 3H, Ar–H), 6.55 (dd, 2H, furan ring-H, J = 7.8, 7.8 Hz), 5.94 (s, 2H, –O–CH_2–O–), 5.90 (s, 1H, C–H (dioxazole ring)); ^{13}C NMR (DMSO-d_6) δ(ppm): 167.0 (C=N), 152.0, 147.9, 142.6, 140.4, 135.6, 120.8, 117.4, 112.6, 109.5, 108.2, 98.5 (Ar–C), 96.3 (O–C–O); ESI-MS m/z: [M^++1] 260.05.

4.2.19. 5-((E)-2, 6-dimethylhepta-1,5-dienyl)-3-(thiophen-2-yl)-1,4,2-dioxazole (**19**)

Oil; Anal. calc. for $C_{15}H_{19}NO_2S$: C 64.95, H 6.90, N 5.05%; found: C 64.84, H 7.01, N 5.14%; UV λ_{max} nm: 348, 310, 291; IR ν_{max} cm^{-1}: 1642 (C=C stretch), 3135 (C–H stretch thiophene ring), 3054 (Ar–H), 1639 (C=N); ^{1}H NMR (DMSO-d_6) δ(ppm): 8.04 (dd, 2H, Thp-H, J = 5.6, 5.6 Hz) 7.73 (d, 1H Thp-H, J = 7.2 Hz), 6.04 (s, 1H, C–H (dioxazole ring)), 5.86 (s, 1H,=CH–C), 5.07 (s, 1H,=CH–CH_2), 2.14–2.03 (m, 4H, CH_2–CH_2), 1.90 (s, 3H, CH_3), 1.60 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6) δ(ppm): 164.5 (C=N), 134.9, 131.5, 126.8, 120.4, 40.5, 36.5, 27.4, 19.5, 17.8 (Aliphatic-C), 130.6, 127.5, 125.4, 123.2, (Ar–C), 87.3 (O–C–O); ESI-MS m/z: [M^++1] 278.11.

4.2.20. 5-(benzo[d][1,3]dioxol-5-yl)-3-(thiophen-2-yl)-1,4,2-dioxazole (**20**)

Oil; Anal. calc. for $C_{13}H_9NO_4S$: C 56.70, H 3.30, N 5.09%; found: C 56.84, H 3.47, N 5.14%; UV λ_{max} nm: 318, 295, 250; IR ν_{max} cm^{-1}: 3054 (Ar C–H), 3130 (C–H stretch thiophene ring) 1660 (C=N); ^{1}H NMR (DMSO-d_6) δ(ppm): 7.83 (dd, 2H, Thp-H, J = 5.6, 5.8 Hz), 7.13 (d, 1H. Thp-H, J = 7.2 Hz), 6.92–6.85 (m, 3H, Ar–H), 6.02 (s, 2H, –O–CH_2–O–), 5.86 (s, 1H, C–H (dioxazole ring)); ^{13}C NMR (DMSO-d_6) δ(ppm): 156.7 (C=N), 152.6, 149.9, 134.6, 130.6, 127.5, 125.6, 124.0, 122.0, 118.3, 114.6, 99.7, (Ar–C), 85.9 (O–C–O); ESI-MS m/z: [M^++1] 276.03.

4.3. In vitro anti-amoebic assay

All the compounds **1**–**20** were screened *in vitro* for anti-amoebic activity against *HM1:IMSS* strain of *E. histolytica* by microdilution method [42]. *E. histolytica* trophozoites were cultured in wells of 96-well microtiter plate by using Diamond TYIS-33 growth medium [43]. The test compounds (1 mg) were dissolved in DMSO (40 μl, level at which no inhibition of amoeba occurs) [44,45]. The stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/ml. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (costar). Each test includes metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) and a blank (culture medium only). All the experiments were carried out in triplicate at each concentration level and repeated thrice. The amoeba suspension was prepared from a confluent culture by pouring off the medium at 37 °C and adding 5 ml of fresh medium, chilling the culture tube on ice to detach the organisms from the side of the flask. The number of amoeba/ml was estimated with a haemocytometer, using trypan blue exclusion to confirm the viability. The suspension was diluted to 105 organism/ml by adding fresh medium and 170 μl of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 μl). An inoculum of 1.7 × 104 organisms/well was chosen so that confluent, but not excessive growth, took place in control wells. Plates were sealed and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h. After incubation, the growth of amoeba in the plate was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed with sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly and the plate was not allowed to cool in order to prevent the detachment of amoebae. The plate was allowed to dry at room temperature and the amoebae were fixed with methanol and when dried, stained with (0.5%) aqueous eosin for 15 min. The stained plate was washed

once with tap water, then twice with distilled water and allowed to dry. A 200 μl portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best fitting line from which the IC_{50} value was found. The IC_{50} values in μM are reported in Table 1.

4.4. Cytotoxicity studies (MTT assay)

H9c2 rat cardiac myoblasts were cultured and maintained as monolayer in Dulbecco's modified Eagle's medium (DMEM), high glucose, supplemented with 10% fetal bovine serum (heat inactivated), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin B, at 37 °C in humidified incubator with 5% CO_2 [46]. Only viable cells were used in the assay. Exponentially growing cells were plated at 1.2×10^4 cells per well into 96-well plates and incubated for 48 h before the addition of drugs to achieve the maximum confluency of the cells. Stock solutions were prepared by dissolving the compounds in 20% (v/v) DMSO and further diluted with fresh complete medium to achieve 1 M concentration. The growth-inhibitory effects of the compounds were measured using standard tetrazolium MTT assay. Cells were incubated with different concentrations of metronidazole and compounds **8**, **9**, **10**, **11**, **16** & **18** for 48 h at 37 °C in 5% CO_2 humidified incubator together with untreated control sample. At appropriate time points, cells were washed in PBS, treated with 50 μL MTT solution (5 mg/ml, tetrazolium salt) and incubated for 4 h at 37 °C. At the end of the incubation period, the medium was removed and pure DMSO 100 μL was added to each well. The metabolized MTT product dissolved in DMSO was quantified by measuring the absorbance at 570 nm on an ELISA plate reader (Labsystems Multiskan RC, Helsinki, Finland) with a reference wavelength of 655 nm. All assays were performed in triplicate and repeated thrice. Percent viability was defined as the relative absorbance of treated versus untreated control cells.

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References

[1] A. Lichtenstein, A.T. Kondo, G.S. Visvesvara, A. Fernandez, E.F. Paiva, T. Mauad, M. Dolhnikoff, M.A. Martins, Thorax 60 (2005) 350–352.
[2] P. Upcroft, J.A. Upcroft, Clin. Microbiol. Rev. 14 (2001) 150–164.
[3] F.A. el-Nahas, M.I. el-Ashmawy, Basic Clin. Pharmacol. Toxicol. 94 (2004) 226–231.
[4] V. Purohit, K.A. Basu, Chem. Res. Toxicol. 13 (2000) 673–692.
[5] M. Gomez, D.G. Perez, P. Ayala, J. Samuleson, E. Orozco, Arch. Med. Res. 27 (1996) 421–425.
[6] F.E. Pittman, J.C. Pittman, Am. J. Trop. Med. Hyg. 23 (1974) 146–150.
[7] R. Paduch, M. Kandefer-Szerszen, M. Trytek, J. Fiedurek, Arch. Immunol. Ther. Exp. 55 (2007) 315–327.
[8] E. Breitmaier, Terpenes: Flavors, Fragrances, Pharmaca, Pheromones. Wiley-VCH, Weinheim, Germany, 2006.
[9] K.A. Hammer, C.F. Carson, T.V. Riley, J. Appl. Microbiol. 95 (2003) 853–860.
[10] M. Friedman, P.R. Henika, R.E. Mandrell, J. Food Prot. 65 (2002) 1545–1560.
[11] N. Theis, M. Lerdau, Int. J. Plant Sci. 164 (2003) S93–S103.
[12] P.L. Crowell, R.R. Chang, Z.B. Ren, C.E. Elson, M.N. Gould, Biol. Chem. 266 (1991) 17679–17685.
[13] D.A. Wiseman, S.R. Werner, P.L. Crowell, J. Pharmacol. Exp. Ther. 320 (2007) 1163–1170.
[14] Y.D. Burke, A.S. Ayoubi, S.R. Werner, B.C. McFarland, D.K. Heilman, A. Ruggeri, P.L. Crowell, Anticancer. Res. 22 (2002) 3127–3134.
[15] T.J. Raphael, G. Kuttan, Immunopharmacol. Immunotoxicol. 25 (2003) 285–294.
[16] S.A. Look, W. Fenical, G.K. Matsumoto, J. Clardy, J. Org. Chem. 51 (1986) 5140–5145.
[17] A.T. Peana, P.S. D'Aquila, M.L. Chessa, M.D. Moretti, G. Serra, Eur. J. Pharmacol. 460 (2003) 37–41.
[18] A.T. Peana, P.S. D'Aquila, F. Panin, G. Serra, P. Pippia, M.D. Moretti, Phytomedicine 9 (2002) 721–726.
[19] N.H. Georgopapadakou, J.W. Thomas, Antimicrob. Agents Chemother. 40 (1996) 279–291.
[20] A.C.U. Uys, S.F. Malan, S. Dyk, R.L. Zyl, ChemInform 33 (2002) 180.
[21] H. Miyaoka, M. Shimomura, H. Kimura, Y. Yamada, H. Kim, Y. Wataya, Tetrahedron 54 (1998) 13467–13474.
[22] K.S. Moore, S. Wehrli, H. Roder, M. Rogers, J.N. Forrest, D. McCrimmon, M. Zasloff, Proc. Nat. Acad. Sci. U.S.A. 90 (1993) 1354–1358.
[23] A.R. Bhat, F. Athar, A. Azam, Eur. J. Med. Chem. 44 (2009) 926–936.
[24] P.F. Iqbal, H. Parveen, A.R. Bhat, F. Hayat, A. Azam, Eur. J. Med. Chem. 44 (2009) 4747–4751.
[25] I. Irfan, N. Sawangjaroen, A.R. Bhat, A. Azam, Eur. J. Med. Chem. 45 (2010) 1648–1653.
[26] T.J. Hou, X.J. Xu, Curr. Pharm. Des. 10 (2004) 1011–1033.
[27] H. De Waterbeemd, E. Gifford, Nat. Rev. Drug Discov. 2 (2003) 192–204.
[28] T. Hou, J. Wang, W. Zhang, X. Xu, J. Chem. Inf. Model. 47 (2007) 460–463.
[29] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 23 (1997) 3–25.
[30] J.H. Poupaert, P. Carato, E. Colacino, Curr. Med. Chem. 12 (2005) 877–885.
[31] C. Foulon, C. Danel, C. Vaccher, S. Yous, J.P. Bonte, J.F. Goossens, J. Chromatogr. A 1035 (2004) 131–136.
[32] M. Andrasi, P. Buglyo, L. Zekany, A. Gaspar, Pharm. Biomed. Anal. 44 (2007) 1040–1047..
[33] A. Avdeef, Curr. Top. Med. Chem. 1 (2001) 277–351.
[34] T.I. Oprea, Comput. J. Aided Mol. Des 16 (2002) 325–334.
[35] U. Norinder, A.S. Bergstr, Chem. Med. Chem. 1 (2006) 920–934.
[36] G.T. Balogh, B. Gyarmati, B. Nagy, L. Molnar, G.M. Keseru, Qsar Comb. Sci. 28 (2009) 1148–1155.
[37] G. Subhash, M.W. Julian, R.L. Christopher, A.M. Gary, C.L. Jared, S.T. Zev, M.F. Ryan, C.L. Timothy, B. Iris, K. Howard, L.C. Simon, G.K. Robert, K. Seiki, N. Tomoyoshi, O. Eric, J. Med. Chem. 47 (2004) 175–187.
[38] C. Hansch, T. Fujita, J. Am. Chem. Soc. 86 (1964) 1616–1625.
[39] D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kapple, J. Med. Chem. 45 (2002) 2615–2623.
[40] H.H.F. Refsgaard, B.F. Jensen, P.B. Brockhoff, S.B. Padkjaer, M. Guldbrandt, M.S. Christensen, J. Med. Chem. 48 (2005) 805–811.
[41] I. Muegge, Med. Res. Rev. 23 (2003) 302–321.
[42] C.W. Wright, M.J. O'Neill, J.D. Phillipson, D.C. Warhurst, Antimicrob. Agents Chemother. 32 (1988) 1725–1729.
[43] L.S. Diamond, D.R. Harlow, C. Cunnick, Trans. Soc. Trop. Med. Hyg. 72 (1978) 431–432.
[44] F.D. Gillin, D.S. Reiner, M. Suffness, Antimicrob. Agents Chemother. 22 (1982) 342–345.
[45] A.T. Keene, A. Harris, J.D. Phillipson, D.C. Warhurst, Planta. Med. 52 (1986) 278–285.
[46] M.K. Gupta, T.V. Neelakantan, M. Sanghamitra, R.K. Tyagi, A. Dinda, S. Mualik, C.K. Mukhopadhyay, S.K. Goswami, Antioxi. Redox Signal. 8 (2006) 1081–1093.