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Short communication

New derivatives of 3,5-substituted-1,4,2-dioxazoles: Synthesis and activity against *Entamoeba histolytica*

Abdul R. Bhat, Fareeda Athar[1], Amir Azam*

Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India

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Abstract

Dioxazole derivatives (**1**–**33**) were synthesized in two steps via their corresponding oximes (**I**–**III**). All the compounds were characterized using various spectroscopic techniques. A comparative study of *in vitro* antiamoebic activity of these heterocyclic compounds, *viz*. 3-*o*-chloro (**1**–**11**), 3-*m*-chloro (**12**–**22**) and 3-*p*-chloro (**23**–**33**) dioxazoles having same substituents at 5-position of dioxazole ring, was performed against HM1:IMSS strain of *Entamoeba histolytica*. The results showed a regular pattern in the activity and out of 33 compounds assayed 15 compounds showed better antiamoebic activity than metronidazole with IC_{50} values in the range 0.41–1.73 μM and 1.80 μM. Dioxazoles having *o*-chloro, *m*-chloro, *p*-chloro, dichloro and pyridine substituents at 5-position were more active than the standard drug metronidazole. The toxicity studies against human kidney epithelial cell line showed that all the compounds were non-toxic. 3,5-Bis-[2-chlorophenyl]-1,4,2-dioxazole (**10**) was most active and least toxic among all the compounds.

Keywords: Dioxazole; Antiamoebic activity; *Entamoeba histolytica*; MTT assay

1. Introduction

Amoebiasis, causative protozoan parasite *Entamoeba histolytica*, is the second leading cause of death from parasitic disease worldwide [1]. *E. histolytica* causes approximately 50 million cases and approximately 100,000 deaths annually [2,3]. Liver abscesses and brain abscesses are dreadful complications of this disease [4]. The treatment for amoebiasis remains the nitroimidazole derivatives (metronidazole, tinidazole, ornidazole, emitine). However, there are reports of failure of treatment with metronidazole [1,5]. Although most antiamoebic drugs have shown to be relatively efficient for the treatment of clinical cases, the long-term use of medications produces undesirable side effects in patients. The side effects of metronidazole are metallic taste, nausea, vomiting, diarrhea, sensory neuropathies and toxicity with ataxia, vertigo, seizures and encephalopathy [6,7]. Despite these side effects, there is lack of ideal drug, and immunity acquired to already available drugs and the side effects is a major hurdle in eradicating these diseases [8,9].

Azoles have long been targeted for synthetic investigation because of their known biological properties like cognitive-enhancing and anxiolytic-like activities [10–12]. The antifungal agents of azoles are useful drugs and are widely used for the treatment of topic or inner mycoses, in particular AIDS-related mycotic pathologies [13–16]. Oxazoles, an important member of the azole family also contains a number of biologically active molecules, which play an important role in the drug chemistry. A number of compounds had been screened for antituberculosis agents including dihydrophinazines [17], indoles and ureas [18]. Oxybenzylglycine, possessing an oxazoline group is currently in clinical development for the treatment of type 2 diabetes and dyslipidemia [19]. Considering the facts that nearly all the classes of the azole family are biologically active in one way or the another, we tried to

* Corresponding author. Tel.: +91 11 26981717/3250; fax: +91 11 26981232.
E-mail address: amir_sumbul@yahoo.co.in (A. Azam).
[1] Present address: Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India.

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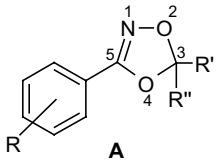
develop a new series of dioxazoles and screened it against the HM1:IMSS strain of *E. histolytica* to develop an effective drug that can be better than metronidazole, a member of azole family and the commercially available drug for amoebiasis.

2. Results and discussion

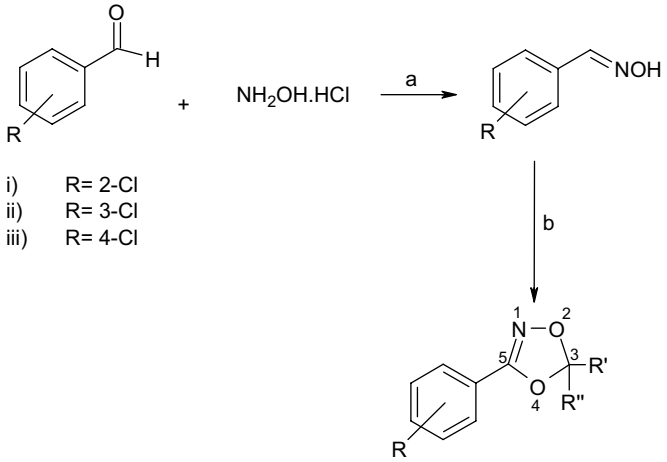
The dioxazoles were prepared by treating aldo-oximes with different aldehydes and ketones as described elsewhere [20] (Scheme 1). The oximes (**I–III**) and dioxazoles (**1–33**) were characterized by different spectroscopic techniques and the purity was confirmed by the elemental analysis and melting points. All the compounds showed sharp melting points and the elemental analysis was found in accordance with ±0.3%. The oximes (**I–III**) and dioxazoles (**1–33**) were confirmed by IR spectroscopy. The characteristic bands of ν(NO–H) and ν(C=N) in oximes showed at 3267–3250 cm^{-1} and 1634–1623 cm^{-1}, respectively. Besides the common aromatic bands, the band ν(C=C) at 1611–1434 cm^{-1} and the bands for *ortho*-substituted benzene in oxime **I** at 773 cm^{-1}, *meta*-substituted benzene in oxime **II** at 748 cm^{-1} and 805 cm^{-1}, and for *para*-substituted benzene in oxime **III** at 811 cm^{-1} were also found. The ν(C=N) band in dioxazoles was in the range 1611–1673 cm^{-1} and a new band in the range from 1193 cm^{-1} to 1125 cm^{-1} arises due to C–O–C group, which was splitted in some cases. In addition to C=C of aromatic region, the respective bands of substituted benzene were also present. The structure was further confirmed by ^{1}H NMR. In the oximes (**I–III**), a singlet at 6.74–6.94 ppm represents the CH=N proton which shows the occurrence of condensation between the CH=O and H_2–N–OH groups. The signal at 11.16–11.37 ppm due to N–OH proton further supports the formation of the oximes (**I–III**).

The electronic spectra of the cyclised dioxazole analogues studied in the UV region in chloroform exhibited three absorption bands at 356–317 nm, 318–299 nm and 290–250 nm assigned to n → π*, π → π* and n → σ* transition, respectively. The band at 356–317 nm was assigned to the transition involving the azomethine group (C=N). Two other absorption bands at 318–299 nm and 290–250 nm were due to π → π* transition of dioxazole ring and n → π* transition of azomethine nitrogen, respectively.



A

The formation of dioxazoles was supported by the absence of a signal at 11.16–11.37 ppm due to N–OH in all the compounds (**1–33**). The singlet at 5.86–6.28 ppm arises due to CH group present at C-3 of the dioxazole ring (**A**) in compounds **1–4**, **10–15**, **21–26**, **32** and **33**. Methyl and ethyl signals were found in compounds where methyl and ethyl groups are attached at C-3 of the dioxazole ring. For methyl group, a singlet at 2.10–2.53 ppm was found in compounds **5**, **7**, **9**, **16**, **18**, **20**, **27**, **29** and **31**, while for ethyl group, a multiplet at 1.87–2.34 ppm for CH_2 protons and a triplet at 1.11–1.54 ppm for CH_3 protons were found in compounds **6**, **9**, **17**, **19**, **28** and **30**. Additional signals for aromatic region were found in 6.9–7.8 ppm with least chemical shifting in all the compounds. The compounds **9**, **20** and **31** containing pyridine showed two double doublets at 7.89–8.03 ppm and 8.06–8.17 ppm and two doublets at 8.21–8.51 ppm and 8.34–8.68 ppm. The structure of the compounds **1**, **2**, **9**, **12**, **13**, **20**, **23**, **24** and **31** were further supported by ^{13}C NMR. The C=N signal was found in the range of 157.1–164.2 ppm and the characteristic signal for –OCO– was found in the range 111.1–115.8 ppm which clearly favors the formation of dioxazole ring. The signals due to the aromatic aliphatic carbons resonate at their usual positions and are shown in the data given in Section 3.



Scheme 1. General method for the synthesis of dioxazole derivatives. (a) Pyridine, C_2H_5OH, reflux 24 h; (b) aq. NaOCl, Et_3N, CH_2Cl_2, (R′–CO–R″), where (R′–CO–R″) represents the different aldehydes and ketones as given in Table 1.

2.1. Antiamoebic activities

Preliminary experiments were carried out to determine the *in vitro* antiamoebic activities by using HM1:IMSS strain of *E. histolytica* cultured in TYIS-33 growth medium. The results are summarized in Tables 1–3. Out of 33 compounds, nine showed best activity with 2.09–4.39 times more active than metronidazole. The IC_{50} values for these compounds were 0.41 μM (**10**), 0.48 μM (**32**), 0.51 μM (**13**), 0.53 μM (**24**), 0.62 μM (**21**), 0.71 μM (**2**), 0.72 μM (**11**), 0.81 μM (**1**), and 0.86 μM (**33**) in comparison to metronidazole (IC_{50} 1.8 μM). Six compounds were found to be moderately active with 1.04–1.97 times better than metronidazole. The IC_{50} values of these compounds were 0.91 μM (**22**), 1.13 μM (**23**), 1.21 μM (**12**), 1.58 μM (**31**), 1.62 μM (**9**), and 1.73 μM (**20**). The results were statistically evaluated by analyses of variance. The null hypothesis was tested using *t*-test and the significance of the differences between the IC_{50} value(s) of metronidazole vs. **1**, **2**, **10**, **11**, **13**, **21**, **24**, **32** and **33** was evaluated. The calculated *t*-values were higher than the table values at the 4% level. Hence, the character under study was significantly influenced by the treatment [21]. The activity of the dioxazole derivatives was found structure dependent. It was found that 4-chloro and 2-chloro phenyl groups present at 5-position of the dioxazole ring were more active than the compounds with 3-chloro phenyl groups. The activity further depends upon the nature of the substituents at carbon-3 of the dioxazole ring. Compounds with only phenyl groups attached at carbon-3 were found more active than those with additional methyl or ethyl groups present at the same carbon. SAR further reveals that the phenyl rings with electron-withdrawing groups present at carbon-3 of the dioxazole ring were more active than those with electron-donating groups. The phenyl rings substituted with Br atom were not found active that may be due to the presence of the methyl and ethyl groups at carbon-3 atom of the dioxazole ring.

2.2. Toxicity profile

Compounds **1–33** were tested to find the toxic effects on human kidney epithelial cell line. No one inhibited cell growth at a concentration of 100 μM. To investigate the selectivity of the compounds, the ''safety index'' (SI) was calculated and defined as toxicity IC_{50}/protozoal IC_{50}, where toxicity IC_{50} is defined as the concentration of compound that kills 50% of the human (kidney epithelial) cell line and protozoal IC_{50} is the concentration that kills 50% of amoeba protozoa. This allows to estimate which compound might be efficacious or toxic against human cells and potentially in vivo. The numerical results for each compound are given in Tables 1–3.

3. Experimental

All the chemicals were purchased from Aldrich Chemical Company (USA). Analytical thin-layer chromatography was performed on precoated silica gel 60 F_{254} plates and flash column chromatography was accomplished using silica gel, 60 Å (200–400 mesh). Elemental analyses were performed by Central Drug Research Institute, Lucknow, using Heraeus Vario EL III analyzer and the results were within ±0.3 of the theoretical values. Electronic spectra were recorded in methanol on a Shimadzu UV-1601 PC UV–visible spectrophotometer. IR spectra on KBr disks were recorded on a Perkin Elmer model 1620 FT-IR spectrophotometer. All the ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at ambient temperature using a Bruker Spectrospin DPX-300 MHz spectrophotometer with TMS as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values are given in parts per million. The FAB mass spectra of all the compounds were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer/Data System using argon/xenon (6 kV, 10 mA) as the FAB gas and *m*-nitro benzyl alcohol (NBA) was used as the matrix.

3.1. Preparation of oxime *I*

A solution of 2-chlorobenzaldehyde (1 equiv) and hydroxylamine hydrochloride (1.08 equiv) 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 precipitated solid was collected and recrystallized from methanol to give the corresponding oxime.

Yield 70%, m.p. 73 °C; IR ν_{max} (cm^{-1}): 3250 (N–OH), 3011 (ArC–H), 2885 (CH), 1634 (C–N), 773 (benzene); ^1H NMR ($CDCl_3$) δ/ppm: 7.20–7.34 (m, 4H, Ar–H), 6.94 (s, 1H, CH=N), 11.37 (s, 1H, N–OH); FAB MS: *m/z* ($C_7H_6NOCl + H$, $M^+ + 1$) 156.7, calcd 155.5.

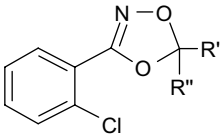
3.2. General procedure for the preparation of 3,5-substituted 1,4,2-dioxazoles (*1–11*)

The 4% aqueous solution of NaOCl (1.6 equiv) was added to a solution of dipolarophile (respective aldehyde and ketone, 1 equiv) and triethylamine (0.1 equiv) in dichloromethane under argon atmosphere. The oxime **I** (1 equiv) in dichloromethane was added dropwise (over a period of 1 h) at 0 °C to the above solution. After being stirred at room temperature for 24 h and refluxed for additional 24 h, water was added to the reaction mixture and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water and brine, dried ($MgSO_4$), filtered and concentrated under vacuum. The compounds were recrystallized using dichloromethane hexane solution.

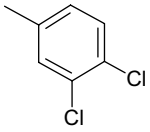
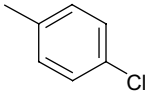
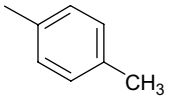
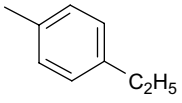
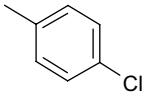
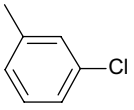
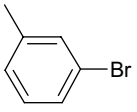
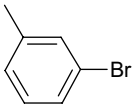
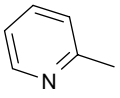
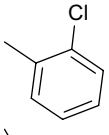
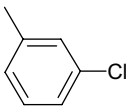
3.2.1. 3-[3,4-Dichlorophenyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (*1*)

Yield 34%, m.p. 130 °C. Anal. calcd for $C_{14}H_8NO_2Cl_3$: C 51.13, H 2.45, N 4.26%; found: C 51.19, H 2.41, N 4.30%; UV/vis λ (nm): 317, 311, 256, 221; IR ν_{max} (cm^{-1}): 3036 (ArC–H), 2851 (CH), 1621 (C=N), 773, 825 (benzene); ^1H NMR ($CDCl_3$) δ/ppm: 5.93 (s, 1H, CH), 7.05 (d, 1H, 7.3 Hz, ArC–H), 7.12 (s, 1H, ArC–H), 7.14 (d, 1H, 7.3 Hz, ArC–H), 7.31–7.37 (m, 4H, Ar–H); ^{13}C NMR ($CDCl_3$)

Table 1
Dioxazoles (**1**–**11**), their antiamoebic activity against HM1:IMSS strain of *E. histolytica* and toxicity profile

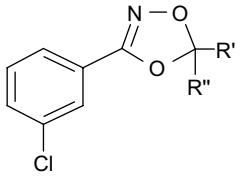


(I)

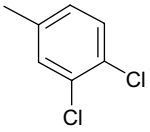
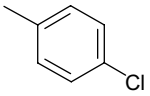
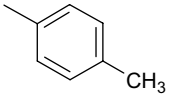
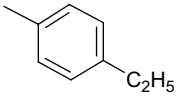
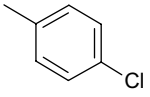
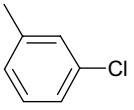
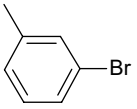
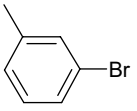
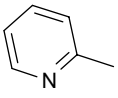
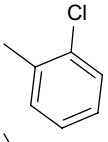
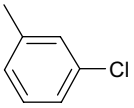
Compound	R′	R″	Antiamoebic activity		Toxicity profile	
			IC_{50} (μM)	S.D.[a] (±)	IC_{50} (μM)	Safety index (SI)
1	H		**0.81**	0.55	>100	>123.5
2	H		**0.51**	0.38	>100	>196.1
3	H		>1.80	1.32	>100	>55.55
4	H		>1.80	1.26	>100	>55.55
5	CH_3		>1.80	1.09	>100	>55.55
6	C_2H_5		>1.80	0.71	>100	>55.55
7	CH_3		>1.80	0.51	>100	>55.55
8	C_2H_5		>1.80	0.76	>100	>55.55
9	CH_3		**1.62**	0.61	>100	>61.72
10	H		**0.41**	0.32	>100	>243.9
11	H		**0.72**	0.26	>100	>138.8

[a] Standard deviation. The compounds with bold font IC_{50} values are more active than metronidazole.

Table 2
Dioxazoles (**12**–**22**), their antiamoebic activity against HM1:IMSS strain of *E. histolytica* and toxicity profile

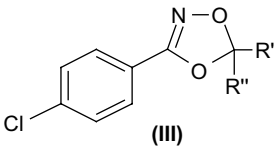


(II)

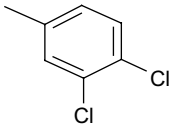
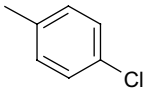
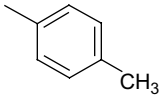
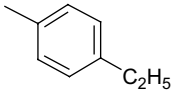
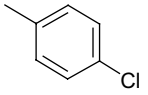
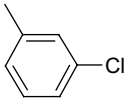
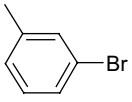
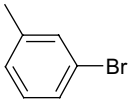
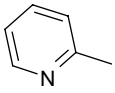
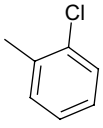
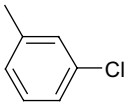
Compound	R′	R″	Antiamoebic activity		Toxicity profile	
			IC_{50} (μM)	S.D.[a] (±)	IC_{50} (μM)	Safety index (SI)
12	H		**1.21**	0.65	>100	>82.6
13	H		**0.71**	0.53	>100	>140.8
14	H		>1.80	1.03	>100	>55.55
15	H		>1.80	0.98	>100	>55.55
16	CH_3		>1.80	1.14	>100	>55.55
17	C_2H_5		>1.80	0.96	>100	>55.55
18	CH_3		>1.80	0.91	>100	>55.55
19	C_2H_5		>1.80	0.53	>100	>55.55
20	CH_3		**1.73**	0.39	>100	>57.8
21	H		**0.62**	0.23	>100	>161.3
22	H		**0.91**	0.34	>100	>109.89

[a] Standard deviation. The compounds with bold font IC_{50} values are more active than metronidazole.

Table 3
Dioxazoles (**23**–**33**), their antiamoebic activity against HM1:IMSS strain of *E. histolytica* and toxicity profile



(III)

Compound	R′	R″	Antiamoebic activity		Toxicity profile	
			IC_{50} (μM)	S.D.[a] (±)	IC_{50} (μM)	Safety index (SI)
23	H		**1.13**	0.86	>100	>88.5
24	H		**0.53**	0.29	>100	>188.7
25	H		>1.80	0.71	>100	>55.55
26	H		>1.80	0.36	>100	>55.55
27	CH_3		>1.80	0.71	>100	>55.55
28	C_2H_5		>1.80	0.45	>100	>55.55
29	CH_3		>1.80	0.61	>100	>55.55
30	C_2H_5		>1.80	0.53	>100	>55.55
31	CH_3		**1.58**	0.68	>100	>63.3
32	H		**0.48**	0.28	>100	>208.3
33	H		**0.86**	0.54	>100	>116.27
		Metronidazole	1.80	0.32	>100	>55.55

[a] Standard deviation. The compounds with bold font IC_{50} values are more active than metronidazole.

δ/ppm: 157.1 (C=N), 139.0, 137.1, 135.8, 134.6, 130.9, 130.2, 128.2, 121.9 (Ar–C), 111.1 (–O–C–O–); FAB MS: *m/z* ($M^+ + 1$) 329.73, calcd 328.56.

3.2.2. 3-[4-Chlorophenyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**2**)

Yield 34%, m.p. 235 °C. Anal. calcd for $C_{14}H_8NO_2Cl_3$: C 57.12, H 3.08, N 4.76%; found: C 57.15, H 3.12, N 4.72%; UV/vis λ (nm): 250, 299, 318; IR ν_{max} (cm^{-1}): 3045 (ArC–H), 2872 (CH), 1611 (C=N), 757, 813 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.2 (s, 1H, CH), 7.13 (d, 2H, 7.5 Hz, Ar-C–H), 7.16 (d, 2H, 7.5 Hz, ArC–H), 7.21–7.28 (m, 4H, Ar–H); ^{13}C NMR ($CDCl_3$) δ/ppm: 158.2 (C=N), 140.3, 137.8, 136.6, 135.1, 131.4, 128.6, 122.3 (Ar–C), 112.8 (–O–C–O–); FAB MS: *m/z* ($M^+ + 1$) 294.36, calcd 294.07.

3.2.3. 3-[4-Methylphenyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**3**)

Yield 29%, m.p. 138 °C. Anal. calcd for $C_{15}H_{12}NO_2Cl_2$: C 58.23, H 3.91, N 4.51%; found: C 58.28, H 3.87, N 4.53%; UV/vis λ (nm): 255, 318, 331; IR ν_{max} (cm^{-1}): 3026 (ArC–H), 2857 (CH), 1614 (C=N), 776, 819 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.31 (s, 3H, CH_3), 5.86 (s, 1H, CH), 6.97 (d, 1H, 7.6 Hz, ArC–H), 7.02 (d, 2H, 7.6 Hz, ArC–H), 7.11–7.16 (m, 4H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 310.29, calcd 309.09.

3.2.4. 3-[4-Ethylphenyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**4**)

Yield 37%, m.p. 125 °C. Anal. calcd for $C_{16}H_{14}NO_2Cl$: C 67.07, H 4.95, N 4.91%; found: C 67.13, H 5.01, N 5.89%; UV/vis λ (nm): 271, 309, 334; IR ν_{max} (cm^{-1}): 3043 (ArC–H), 2915 (CH_3), 2895 (CH_2), 1645 (C=N), 743, 756 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.53 (t, 3H, CH_3), 2.14 (m, 2H, CH_2), 6.13 (s, 1H, CH), 6.93 (d, 2H, 7.7 Hz, ArC–H), 6.97 (d, 2H, 7.7 Hz, ArC–H), 7.16–7.23 (m, 4H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 285.46, calcd 284.61.

3.2.5. 3-[4-Chlorophenyl],3-[methyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**5**)

Yield 19%, m.p. 129 °C. Anal. calcd for $C_{15}H_{11}NO_2Cl$: C 58.42, H 3.59, N 4.54%; found: C 58.47, H 3.53, N 4.58%; UV/vis λ (nm): 255, 317, 334; IR ν_{max} (cm^{-1}): 3053 (ArC–H), 2946 (CH_3), 1657 (C=N), 746, 793 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.53 (s, 3H, CH_3), 7.16 (d, 2H, 7.2 Hz, ArC–H), 7.19 (d, 2H, 7.2 Hz, ArC–H), 7.32–7.39 (m, 4H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 309.35, calcd 308.08.

3.2.6. 3-[3-Chlorophenyl],3-[ethyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**6**)

Yield 21%, m.p. 137 °C. Anal. calcd for $C_{16}H_{13}NO_2Cl_2$: C 59.60, H 4.06, N 4.34%; found: C 59.65, H 4.13, N 4.38%; UV/vis λ (nm): 290, 318, 331; IR ν_{max} (cm^{-1}): 3051 (ArC–H), 2927 (CH_3), 2892 (CH_2), 1661 (C=N), 720, 748 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.34 (t, 3H, CH_3), 2.34 (m, 2H, CH_2), 7.14 (s, 1H, Ar–H), 7.16 (d, 1H, 7.2 Hz, ArC–H), 7.19 (d, 1H, 7.2 Hz, ArC–H), 7.23 (dd, 1H, Ar–H), 7.32–7.39 (m, 4H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 322.37, calcd 322.10.

3.2.7. 3-[3-Bromophenyl],3-[methyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**7**)

Yield 37%, m.p. 147 °C. Anal. calcd for $C_{15}H_{11}NO_2BrCl$: C 51.07, H 3.14, N 3.97%; found: C 51.16, H 3.19, N 3.92%; UV/vis λ (nm): 290, 305, 334; IR ν_{max} (cm^{-1}): 3041 (ArC–H), 2963 (CH_3), 1637 (C=N), 753, 813 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.23 (s, 3H, CH_3), 7.15 (s, 1H, Ar–H), 7.26 (dd, 1H, ArC–H), 7.34–7.41 (m, 5H, Ar–H), 7.77–7.80 (d, 1H, 7.6 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 353.76, calcd 352.40.

3.2.8. 3-[3-Bromophenyl],3-[ethyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**8**)

Yield 34%, m.p. 163 °C. Anal. calcd for $C_{16}H_{13}NO_2BrCl$: C 52.38, H 3.57, N 3.87%; found: C 52.41, H 3.82, N 3.92%; UV/vis λ (nm): 290, 304, 342; IR ν_{max} (cm^{-1}): 3058 (ArC–H), 2925 (CH_3), 2871 (CH_2), 1664 (C=N), 758, 829 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.19 (t, 3H, CH_3), 1.94 (m, 2H, CH_2), 7.17 (s, 1H, Ar–H), 7.24 (d, 1H, 7.8 Hz, Ar–H), 7.31–7.39 (m, 5H, Ar–H), 7.41–7.44 (d, 1H, 7.8 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 367.68, calcd 366.50.

3.2.9. 3-[2-Pyridyl],3-[methyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**9**)

Yield 34%, m.p. 134 °C. Anal. calcd for $C_{14}H_{11}N_2O_2Cl$: C 61.20, H 4.03, N 5.08%; found: C 61.25, H 4.09, N 5.13%; UV/vis λ (nm): 290, 317, 341; IR ν_{max} (cm^{-1}): 3035 (ArC–H), 2948 (CH_3), 1664 (C=N), 758 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.32 (s, 3H, CH_3), 7.53–7.77 (m, 4H, Ar–H), 7.93–8.01 (dd, 1H, py–H), 8.09–8.16 (dd, 1H, py–H), 8.47–8.50 (d, 1H, py–H), 8.62–8.65 (d, 1H, py–H); ^{13}C NMR ($CDCl_3$) δ/ppm: 158.4 (C=N), 154.4 (C=N), 140.2, 139.8, 139.1, 135.4, 134.1, 130.8, 130.2, 128.2 (Ar–C), 115.1 (–O–C–O–), 26.2 (CH_3); FAB MS: *m/z* ($M^+ + 1$) 275.76, calcd 274.50.

3.2.10. 3,5-Bis-[2-chlorophenyl]-1,4,2-dioxazole (**10**)

Yield 34%, m.p. 142 °C. Anal. calcd for $C_{14}H_9NO_2Cl_2$: C 57.12, H 3.08, N 4.76%; found: C 57.19, H 3.14, N 4.71%; UV/vis λ (nm): 256, 305, 346; IR ν_{max} (cm^{-1}): 3023 (ArC–H), 2856 (CH), 1625 (C=N), 758 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.12 (s, 1H, CH), 7.53–7.64 (m, 8H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 295.38, calcd 294.07.

3.2.11. 3-[3-Chlorophenyl]-5-[2-chlorophenyl]-1,4,2-dioxazole (**11**)

Yield 34%, m.p. 156 °C. Anal. calcd for $C_{14}H_9NO_2Cl_2$: C 57.12, H 3.08, N 4.76%; found: C 57.15, H 2.98, N 4.71%; UV/vis λ (nm): 275, 321, 342; IR ν_{max} (cm^{-1}): 3029 (ArC–H), 2869 (CH), 1639 (C=N), 751, 816 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.09 (s, 1H, CH), 7.16 (s, 1H, Ar–H), 7.18 (d, 1H, 7.2 Hz, ArC–H), 7.22 (d, 1H, 7.2 Hz, ArC–H), 7.26 (dd, 1H, Ar–H), 7.29–7.36 (m, 4H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 295.28, calcd 294.07.

3.3. Preparation of oxime **II**

A solution of 3-chlorobenzaldehyde (1 equiv) and hydroxylamine hydrochloride (1.08 equiv) 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 water. The precipitated solid was collected and then recrystallized from methanol to give the corresponding oxime.

Yield 57%, m.p. 84 °C; IR ν_{max} (cm^{-1}): 3267 (N–OH), 3035 (ArC–H), 2849 (CH), 1630 (C–N), 805, 748 (*m*-substituted benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.74 (s, 1H, CH=N), 7.13 (s, 1H, Ar–H), 7.17 (d, 1H, 7.4 Hz, ArC–H), 7.23 (d, 1H, 7.4 Hz, ArC–H), 7.27 (dd, 1H, Ar–H), 11.56 (s, 1H, N–OH); FAB MS: *m/z* ($M^+ + 1$) 156.43, calcd 155.5.

3.4. General procedure for the preparation of 3,5-substituted 1,4,2-dioxazoles (**12–22**)

The 4% aqueous solution of NaOCl (1.6 equiv) was added to a solution of dipolarophile (respective aldehyde and ketone, 1 equiv) and triethylamine (0.1 equiv) in dichloromethane under argon atmosphere. The oxime **II** (1 equiv) in dichloromethane was added dropwise (over a period of 1 h) at 0 °C to the above solution. After being stirred at room temperature for 24 h and refluxed for additional 24 h, water was added to the reaction mixture and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water and brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The compounds were recrystallized using dichloromethane hexane solution.

3.4.1. 3-[3,4-Dichlorophenyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**12**)

Yield 32%, m.p. 152 °C. Anal. calcd for $C_{14}H_8NO_2Cl_3$: C 51.13, H 2.45, N 4.26%; found: C 51.07, H 2.39, N 4.21%; UV/vis λ (nm): 252, 308, 354; IR ν_{max} (cm^{-1}): 3074 (Ar-C–H), 2843 (CH), 1616 (C=N), 790, 885 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.17 (s, 1H, CH), 7.08 (d, 1H, 7.6 Hz, Ar–H), 7.14 (s, 1H, Ar–H), 7.16 (d, 1H, 7.6 Hz, ArC–H), 7.21 (dd, 1H, Ar–H), 7.25 (s, 1H, Ar–H), 7.28 (d, 1H, 7.3 Hz, ArC–H), 7.31 (d, 1H, 7.3 Hz, Ar–H); ^{13}C NMR ($CDCl_3$) δ/ppm: 157.2 (C=N), 138.9, 138.1, 137.6, 136.8, 135.1, 134.3, 130.4, 130.1, 128.5 (Ar–C), 114.1 (–O–C–O–); FAB MS: *m/z* ($M^+ + 1$) 329.7, calcd 328.56.

3.4.2. 3-[4-Chlorophenyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**13**)

Yield 31%, m.p. 163 °C. Anal. calcd for $C_{14}H_8NO_2Cl_3$: C 57.12, H 3.08, N 4.76%; found: C 57.17, H 3.16, N 4.78; UV/vis λ (nm): 257, 300, 329; IR ν_{max} (cm^{-1}): 3074 (Ar–H), 2846 (CH), 1617 (C=N), 787, 868 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.21 (s, 1H, CH), 7.07 (d, 1H, 7.3 Hz, Ar–H), 7.11 (s, 1H, Ar–H), 7.14 (d, 1H, 7.3 Hz, Ar–H), 7.18 (dd, 1H, Ar–H), 7.25 (d, 2H, 7.6 Hz, Ar–H), 7.29 (d, 2H, 7.6 Hz, Ar–H); ^{13}C NMR ($CDCl_3$) δ/ppm: 160.8 (C=N), 136.4, 135.9, 135.2, 134.8, 132.9, 131.6, 130.1, 128.9, 128.6, 126.2 (Ar–C), 110.9 (–O–C–O–); FAB MS: *m/z* ($M^+ + 1$) 295.07, calcd 294.07.

3.4.3. 3-[4-Methylphenyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**14**)

Yield 29%, m.p. 135 °C. Anal. calcd for $C_{15}H_{12}NO_2Cl_2$: C 58.23, H 3.91, N 4.51%; found: C 58.28, H 4.03, N 4.56%; UV/vis λ (nm): 251, 322, 346; IR ν_{max} (cm^{-1}): 3078 (ArC–H), 2864 (CH), 1658 (C=N), 790, 889 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.43 (s, 3H, CH_3), 6.08 (s, 1H, CH), 6.95 (d, 2H, 7.2 Hz, Ar–H), 6.98 (d, 2H, 7.2 Hz, Ar–H), 7.04 (d, 1H, 7.3 Hz, Ar–H), 7.09 (s, 1H, Ar–H), 7.12 (d, 1H, 7.3 Hz, Ar–H), 7.16 (dd, 1H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 310.41, calcd 309.09.

3.4.4. 3-[4-Ethylphenyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**15**)

Yield 29%, m.p. 145 °C. Anal. calcd for $C_{16}H_{14}NO_2Cl$: C 67.07, H 4.95, N 4.91%; found: C 67.16, H 5.03, N 4.86%; UV/vis λ (nm): 253, 317, 346; IR ν_{max} (cm^{-1}): 3062 (Ar-C–H), 2918 (CH_3), 2891 (CH_2), 1629 (C=N), 791, 869 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.58 (t, 3H, CH_3), 2.19 (m, 2H, CH_2), 5.96 (s, 1H, CH), 6.95 (d, 2H, 7.8 Hz, ArC–H), 6.99 (d, 2H, 7.8 Hz, ArC–H), 7.11 (d, 1H, 7.4 Hz, Ar–H), 7.14 (s, 1H, Ar–H), 7.16 (dd, 1H, Ar–H), 7.20 (d, 1H, 7.3 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 288.39, calcd 287.61.

3.4.5. 3-[4-Chlorophenyl],3-[methyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**16**)

Yield 22%, m.p. 149 °C. Anal. calcd for $C_{15}H_{11}NO_2Cl$: C 58.42, H 3.59, N 4.54%; found: C 58.47, H 3.65, N 4.59%; UV/vis λ (nm): 283, 313, 354; IR ν_{max} (cm^{-1}): 3048 (ArC–H), 2925 (CH_3), 1637 (C=N), 789, 886 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.52 (s, 3H, CH_3), 7.01 (d, 2H, 7.6 Hz, Ar–H), 7.21 (d, 1H, 7.3 Hz, Ar–H), 7.24 (s, 1H, Ar–H), 7.26 (d, 2H, 7.3 Hz, Ar–H), 7.29 (d, 2H, 7.3 Hz, Ar–H), 7.31 (dd, 1H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 309.42, calcd 308.08.

3.4.6. 3,5-Bis[3-chlorophenyl]-3-[ethyl]-1,4,2-dioxazole (**17**)

Yield 31%, m.p. 129 °C. Anal. calcd for $C_{16}H_{13}NO_2Cl_2$: C 59.60, H 4.06, N 4.34%; found: C 59.64, H 4.05, N 4.39%; UV/vis λ (nm): 285, 321, 334; IR ν_{max} (cm^{-1}): 3075 (ArC–H), 2852 (CH_2), 2935 (CH_3), 1673 (C=N), 783, 846 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.13 (t, 3H, CH_3), 2.19 (m, 2H, CH_2), 7.10 (s, 1H, Ar–H), 7.11 (d, 1H, 7.3 Hz, Ar–H), 7.13 (d, 1H, 7.3 Hz, Ar–H), 7.17 (dd, 1H, Ar–H), 7.21 (s, 1H, Ar–H), 7.24 (d, 2H, 7.2 Hz, Ar–H), 7.26 (d, 2H, 7.2 Hz, Ar–H), 7.28 (dd, 1H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 323.05, calcd 322.04.

3.4.7. 3-[3-Bromophenyl],3-[methyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**18**)

Yield 43%, m.p. 175 °C. Anal. calcd for $C_{15}H_{11}NO_2BrCl$: C 51.07, H 3.14, N 3.97%; found: C 50.95, H 3.18, N 4.04%; UV/vis λ (nm): 290, 318, 346; IR ν_{max} (cm^{-1}): 3035

(ArC–H), 2981 (CH_3), 1652 (C=N), 790, 864 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.29 (s, 3H, CH_3), 7.11 (s, 1H, Ar–H), 7.13 (d, 2H, 7.2 Hz, Ar–H), 7.15 (dd, 1H, Ar–H), 7.26 (s, 1H, Ar–H), 7.28 (d, 2H, 7.5 Hz, Ar–H), 7.33 (d, 2H, 7.5 Hz, Ar–H), 7.42 (dd, 1H, Ar–H), 7.81–7.89 (d, 1H, 7.4 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 353.6, calcd 352.4.

*3.4.8. 3-[3-Bromophenyl],3-[ethyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**19**)*

Yield 39%, m.p. 165 °C. Anal. calcd for $C_{16}H_{13}NO_2BrCl$: C 52.38, H 3.57, N 3.87%; found: C 52.39, H 3.52, N 3.91%; UV/vis λ (nm): 256, 300, 346; IR ν_{max} (cm^{-1}): 3065 (ArC–H), 2913 (CH_3), 2865 (CH_2), 1651 (C=N), 732, 861 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.23 (t, 3H, CH_3), 1.87 (m, 2H, CH_2), 7.18 (s, 1H, Ar–H), 7.21 (d, 1H, 7.8 Hz, Ar–H), 7.24 (d, 1H, 7.8 Hz, Ar–H), 7.28 (dd, 1H, Ar–H), 7.33 (s, 1H, Ar–H), 7.34 (d, 1H, 7.4 Hz, Ar–H), 7.37 (d, 1H, 7.4 Hz, Ar–H), 7.43 (dd, 1H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 367.23, calcd 366.50.

*3.4.9. 3-[2-Pyridyl],3-[methyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**20**)*

Yield 44%, m.p. 154 °C. Anal. calcd for $C_{14}H_{11}N_2O_2Cl$: C 61.20, H 4.03, N 5.08%; found: C 61.24, H 4.08, N 5.13%; UV/vis λ (nm): 278, 321, 354; IR ν_{max} (cm^{-1}): 3021 (ArC–H), 2976 (CH_3), 1658 (C=N), 790, 893 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.48 (s, 3H, CH_3), 7.23 (s, 1H, Ar–H), 7.25 (d, 1H, 7.4 Hz, Ar–H), 7.28 (d, 1H, 7.4 Hz, Ar–H), 7.31 (dd, 1H, Ar–H), 8.03–8.15 (dd, 1H, py–H), 8.17–8.25 (dd, 1H, py–H), 8.51–8.53 (d, 1H, py–H), 8.68–8.71 (d, 1H, py–H); ^{13}C NMR ($CDCl_3$) δ/ppm: 164.2 (C=N), 160.2 (C=N), 139.2, 138.4, 136.2, 132.9, 130.6, 130.1, 128.9, 128.2 (Ar–C), 113.8 (–O–C–O–), 26.5 (CH_3); FAB MS: *m/z* ($M^+ + 1$) 275.41, calcd 274.50.

*3.4.10. 3-[2-Chlorophenyl]-5-[3-chlorophenyl]-1,4,2-dioxazole (**21**)*

Yield 26%, m.p. 134 °C. Anal. calcd for $C_{14}H_9NO_2Cl_2$: C 57.12, H 3.08, N 4.76%; found: C 57.08, H 3.13, N 4.69%; UV/vis λ (nm): 281, 309, 348; IR ν_{max} (cm^{-1}): 3041 (ArC–H), 2823 (CH), 1648 (C=N), 754, 887 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.28 (s, 1H, CH), 7.15 (s, 1H, Ar–H), 7.22 (d, 1H, 7.5 Hz, Ar–H), 7.25 (d, 1H, 7.5 Hz, Ar–H), 7.28 (dd, 1H, Ar–H), 7.48–7.59 (m, 8H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 295.09, calcd 294.00.

*3.4.11. 3,5-Bis-[3-chlorophenyl]-1,4,2-dioxazole (**22**)*

Yield 29%, m.p. 152 °C. Anal. calcd for $C_{14}H_9NO_2Cl_2$: C 57.12, H 3.08, N 4.76%; found: C 57.18, H 3.14, N 4.79%; UV/vis λ (nm): 251, 300, 353; IR ν_{max} (cm^{-1}): 3030 (ArC–H), 2861 (CH), 1655 (C=N), 762, 873 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 5.99 (s, 1H, CH), 7.17 (d, 1H, 7.4 Hz, Ar–H), 7.21 (d, 1H, 7.6 Hz, Ar–H), 7.24 (d, 1H, 7.6 Hz, Ar–H), 7.26–7.41 (m, 4H, Ar–H), 7.43 (d, 1H, 7.4 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 295.01, calcd 294.00.

*3.5. Preparation of oxime **III***

A solution of 4-chlorobenzaldehyde (1 equiv) and hydroxylamine hydrochloride (1.08 equiv) 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 water. The precipitated solid was collected and then recrystallized from methanol to give the corresponding oxime.

Yield 79%, m.p. 67 °C; IR ν_{max} (cm^{-1}): 3261 (N–OH), 3034 (ArC–H), 2879 (CH), 1623 (C=N), 781 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.89 (s, 1H, CH=N), 7.29 (d, 2H, 7.6 Hz, Ar–H), 7.32 (d, 2H, 7.6 Hz, Ar–H), 11.16 (s, 1H, N–OH); FAB MS: *m/z* 156.6 ($M^+ + 1$), calcd 155.5.

*3.6. General procedure for the preparation of 3,5-substituted 1,4,2-dioxazoles (**23–33**)*

The 4% aqueous solution of NaOCl (1.6 equiv) was added to a solution of dipolarophile (respective aldehyde and ketone, 1 equiv) and triethylamine (0.1 equiv) in dichloromethane under argon atmosphere. The oxime **III** (1 equiv) in dichloromethane was added dropwise (over a period of 1 h) at 0 °C to the above solution. After being stirred at room temperature for 24 h and refluxed for additional 24 h, water was added to the reaction mixture and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water and brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The compounds were recrystallized using dichloromethane hexane solution.

*3.6.1. 3-[3,4-Dichlorophenyl]-5-[4-chlorophenyl]-1,4,2-dioxazole (**23**)*

Yield 38%, m.p. 172 °C. Anal. calcd for $C_{14}H_8NO_2Cl_3$: C 51.13, H 2.45, N 4.26%; found: C 51.19, H 2.43, N 4.31%; UV/vis λ (nm): 259, 302, 334; IR ν_{max} (cm^{-1}): 3024 (ArC–H), 2813 (CH), 1611 (C=N), 728, 830 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.26 (s, 1H, CH), 7.21 (s, 1H, ArC–H), 7.23 (d, 1H, 7.5 Hz, ArC–H), 7.28 (d, 1H, 7.5 Hz, Ar–H), 7.35 (d, 2H, 7.4 Hz, Ar–H), 7.39 (d, 2H, 7.4 Hz, Ar–H); ^{13}C NMR ($CDCl_3$) δ/ppm: 160.5 (C=N), 142.0, 139.8, 136.1, 135.6, 131.2, 128.6, 121.8 (Ar–C), 112.2 (–O–C–O–); FAB MS: *m/z* ($M^+ + 1$) 329.65, calcd 328.61.

*3.6.2. 3,5-Bis-[4-chlorophenyl]-1,4,2-dioxazole (**24**)*

Yield 31%, m.p. 162 °C. Anal. calcd for $C_{14}H_8NO_2Cl_3$: C 57.12, H 3.08, N 4.76%; found: C 57.19, H 3.16, N 4.79%; UV/vis λ (nm): 254, 300, 347; IR ν_{max} (cm^{-1}): 2998 (Ar–H), 2821 (CH), 1659 (C=N), 756, 838 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.11 (s, 1H, CH), 7.19 (d, 2H, 7.6 Hz, Ar–H), 7.24 (d, 2H, 7.7 Hz, Ar–H), 7.33 (d, 2H, 7.6 Hz, Ar–H), 7.38 (d, 2H, 7.7 Hz, Ar–H); ^{13}C NMR ($CDCl_3$) δ/ppm: 158.5 (C=N), 141.2, 138.6, 138.2, 136.2, 135.8, 130.9, 129.1, 122.5 (Ar–C), 115.5 (–O–C–O–); FAB MS: *m/z* ($M^+ + 1$) 295.31, calcd 294.07.

*3.6.3. 3-[4-Methylphenyl]-5-[4-chlorophenyl]-1, 4,2-dioxazole (**25**)*

Yield 29%, m.p. 139 °C. Anal. calcd for $C_{15}H_{12}NO_2Cl_2$: C 58.23, H 3.91, N 4.51%; found: C 58.27, H 3.95, N 4.56%; UV/vis λ (nm): 255, 309, 354; IR ν_{max} (cm^{-1}): 3068 (ArC–H), 2852 (CH), 1658 (C=N), 775, 834 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.51 (s, 3H, CH_3), 5.99 (s, 1H, CH), 6.98 (d, 2H, 7.3 Hz, Ar–H), 7.04 (d, 2H, 7.3 Hz, Ar–H), 7.26 (d, 2H, 7.5 Hz, Ar–H), 7.31 (d, 2H, 7.5 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 310.29, calcd 309.09.

*3.6.4. 3-[4-Ethylphenyl]-5-[4-chlorophenyl]-1, 4,2-dioxazole (**26**)*

Yield 41%, m.p. 158 °C. Anal. calcd for $C_{16}H_{14}NO_2Cl$: C 67.07, H 4.95, N 4.91%; found: C 67.13, H 5.01, N 4.96%; UV/vis λ (nm): 257, 313, 346; IR ν_{max} (cm^{-1}): 3075 (ArC–H), 2947 (CH_3), 2876 (CH_2), 1656 (C=N), 791, 834 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.42 (t, 3H, CH_3), 2.24 (m, 2H, CH_2), 6.02 (s, 1H, CH), 6.92 (d, 2H, 7.5 Hz, Ar–H), 6.97 (d, 2H, 7.5 Hz, Ar–H), 7.22 (d, 2H, 7.6 Hz, Ar–H), 7.26 (d, 2H, 7.6 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 288.84, calcd 287.61.

*3.6.5. 3,5-Bis-[4-chlorophenyl]-3-[methyl]1, 4,2-dioxazole (**27**)*

Yield 27%, m.p. 152 °C. Anal. calcd for $C_{15}H_{11}NO_2Cl$: C 58.42, H 3.59, N 4.54%; found: C 58.47, H 3.56, N 4.59%; UV/vis λ (nm): 282, 311, 356; IR ν_{max} (cm^{-1}): 3034 (ArC–H), 2941 (CH_3), 1657 (C=N), 753, 828 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.10 (3H, CH_3), 7.12 (d, 2H, 7.4 Hz, Ar–H), 7.17 (d, 1H, 7.4 Hz, Ar–H), 7.29 (d, 2H, 7.6 Hz, Ar–H), 7.34 (d, 2H, 7.6 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 309.2, calcd 308.08.

*3.6.6. 3-[3-Chlorophenyl],3-[ethyl]-5-[4-chlorophenyl]-1, 4,2-dioxazole (**28**)*

Yield 31%, m.p. 146 °C. Anal. calcd for $C_{16}H_{13}NO_2Cl_2$: C 59.60, H 4.06, N 4.34%; found: C 59.64, H 4.03, N 4.39%; UV/vis λ (nm): 256, 300, 336; IR ν_{max} (cm^{-1}): 3033 (ArC–H), 2941 (CH_3), 2896 (CH_2), 1656 (C=N), 756, 837 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.54 (t, 3H, CH_3), 2.14 (m, 2H, CH_2), 7.09 (d, 1H, 7.4 Hz, Ar–H), 7.13 (s, 1H, Ar–H), 7.15 (d, 1H, 7.4 Hz, Ar–H), 7.19 (dd, 1H, Ar–H), 7.25 (d, 2H, 7.7 Hz, Ar–H), 7.29 (d, 2H, 7.7 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 323.14, calcd 322.10.

*3.6.7. 3-[3-Bromophenyl],3-[methyl]-5-[4-chlorophenyl]-1, 4,2-dioxazole (**29**)*

Yield 46%, m.p. 151 °C. Anal. calcd for $C_{15}H_{11}NO_2BrCl$: C 51.07, H 3.14, N 3.97%; found: C 51.13, H 3.16, N 3.93%; UV/vis λ (nm): 253, 317, 343; IR ν_{max} (cm^{-1}): 3046 (ArC–H), 2972 (CH_3), 1657 (C=N), 789, 843 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.46 (s, 3H, CH_3), 7.06–7.18 (m, 4H, Ar–H), 7.23 (d, 2H, 7.3 Hz, Ar–H), 7.29 (d, 2H, 7.3 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 353.32, calcd 352.4.

*3.6.8. 3-[3-Bromophenyl],3-[ethyl]-5-[4-chlorophenyl]-1, 4,2-dioxazole (**30**)*

Yield 37%, m.p. 136 °C. Anal. calcd for $C_{16}H_{13}NO_2BrCl$: C 52.38, H 3.57, N 3.87%; found: C 52.43, H 3.62, N 3.79%; UV/vis λ (nm): 259, 306, 356; IR ν_{max} (cm^{-1}): 3065 (ArC–H), 2937 (CH_3), 2897 (CH_2), 1657 (C=N), 754, 830 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.11 (t, 3H, CH_3), 1.98 (m, 2H, CH_2), 7.04 (d, 2H, 7.2 Hz, Ar–H), 7.07 (d, 2H, 7.2 Hz, Ar–H), 7.17–7.31 (m, 4H, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 367.63, calcd 366.50.

*3.6.9. 3-[2-Pyridyl],3-[methyl]-5-[4-chlorophenyl]-1, 4,2-dioxazole (**31**)*

Yield 40%, m.p. 129 °C. Anal. calcd for $C_{14}H_{11}N_2O_2Cl$: C 61.20, H 4.03, N 5.08%; found: C 61.23, H 4.12, N 5.12%; UV/vis λ (nm): 254, 313, 346; IR ν_{max} (cm^{-1}): 3045 (ArC–H), 2948 (CH_3), 1658 (C=N), 755, 830 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.39 (s, 3H, CH_3), 7.11 (d, 2H, 7.6 Hz, Ar–H), 7.15 (d, 2H, 7.6 Hz, Ar–H), 7.89–7.95 (dd, 1H, py–H), 8.06–8.17 (dd, 1H, py–H), 8.21–8.24 (d, 1H, py–H), 8.34–8.38 (d, 1H, py–H); ^{13}C NMR ($CDCl_3$) δ/ppm: 160.4 (C=N), 153.2 (C=N), 141.3, 138.8, 136.2, 134.1, 130.6, 130.2, 128.9, 128.3 (Ar–C), 116.8 (–O–C–O–), 25.9 (CH_3); FAB MS: *m/z* ($M^+ + 1$) 275.32, calcd 274.50.

*3.6.10. 3-[2-Chlorophenyl]-5-[4-chlorophenyl]-1, 4,2-dioxazole (**32**)*

Yield 35%, m.p. 154 °C. Anal. calcd for $C_{14}H_9NO_2Cl_2$: C 57.12, H 3.08, N 4.76%; found: C 57.16, H 3.11, N 4.81%; UV/vis λ (nm): 256, 309, 346; IR ν_{max} (cm^{-1}): 3051 (ArC–H), 1654 (C=N), 753, 830 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.16 (s, 1H, CH), 7.09–7.24 (m, 4H, Ar–H), 7.32 (d, 2H, 7.7 Hz, Ar–H), 7.38 (d, 2H, 7.7 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 295.24, calcd 294.00.

*3.6.11. 3-[3-Chlorophenyl]-5-[4-chlorophenyl]-1, 4,2-dioxazole (**33**)*

Yield 29%, m.p. 165 °C. Anal. calcd for $C_{14}H_9NO_2Cl_2$: C 57.12, H 3.08, N 4.76%; found: C 57.16, H 3.13, N 4.72%; UV/vis λ (nm): 260, 316, 336; IR ν_{max} (cm^{-1}): 3038 (ArC–H), 2861 (CH), 1659 (C=N), 754, 830 (benzene); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.18 (s, 1H, CH), 7.08 (d, 1H, 7.4 Hz, Ar–H), 7.12 (d, 1H, 7.4 Hz, Ar–H), 7.16 (s, 1H, Ar–H), 7.18 (dd, 1H, Ar–H), 7.27 (d, 2H, 7.6 Hz, Ar–H), 7.32 (d, 2H, 7.6 Hz, Ar–H); FAB MS: *m/z* ($M^+ + 1$) 295.32, calcd 294.0.

3.7. Antiamoebic activity

Compounds **1–33** were screened *in vitro* for antiamoebic activity against the HM1:IMSS strain of *E. histolytica* by the microdilution method [22]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously in 96-well microtiter plates (Costar) [23]. The test compounds were dissolved in DMSO (40 μl), at which level no inhibition of the amoeba occurs [24,25]. Then culture medium was added to obtain a concentration of 1 mg/ml. Twofold serial

dilutions were then made. Each test includes metronidazole as a standard amoebic drug, control wells (culture medium plus amoeba), and a blank (culture medium only). The number of amoeba per millimeter was estimated with a haemocytometer and trypan blue exclusion was used to confirm viability. The cell suspension used was diluted to 10^5 organisms/ml by adding fresh medium, and 170 μl of this suspension was added to the test and control wells in the plate. An inoculum of 1.7×10^4 organisms/well was chosen, so that confluent, but not excessive, growth took place. The plates were sealed and gassed for 10 min with N_2, and then incubated at 37 °C for 72 h. After incubation the growth of the amoeba was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. The plates were immediately washed with 0.9% aq. NaCl solution at 37 °C. This procedure was performed quickly, and the plate was not allowed to cool to prevent the detachment of amoebae. The plate was allowed to dry at room temperature, and the amoebae were fixed with chilled methanol by keeping it in an ice bath for 15 min, dried, and stained with 0.5% aq. eosin for 15 min. The stained plate was washed once with tap H_2O and then twice with distilled H_2O, and allowed to dry. Then 0.1 N aq. NaOH solution (200 μl) was added to each well to dissolve the protein and to release the dye (eosin). The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The inhibition (in %) 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 straight line from which IC_{50} values were determined.

3.8. MTT toxicity assay

For the toxicity assay, transformed human kidney epithelial (Graham) cells were continuously maintained in culture at 37 °C in 5% CO_2. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, USB) cellular viability assay was used to determine the toxicity profile of the compounds [26]. The trypsinized cell suspension was adjusted to 0.5 million cells/ml and plated out with the various compounds. After 44 h of incubation, 2 mM MTT was added to the plates and incubated for a further 4 h. DMSO was then added to stop the reaction and dissolve the formazan crystals. The absorbance was taken at 540 nm and reference wavelength of 690 nm and the percentage cellular viability calculated with appropriate controls taken into account. The means ± S.D. values of IC_{50} values in Tables 1–3 are from three independent experiments.

4. Conclusion

This work examined the biological activities of the 3,5-substituted-1,4,2-dioxazoles prepared from the oximes. Out of the 33 compounds prepared, nine were found with 2–4 times better activity than the reference drug metronidazole. The MTT assay revealed that all the compounds are non-toxic to the human kidney epithelial (Graham) cells. The in vivo studies of these nine compounds are currently in progress.

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