Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 280–288
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Synthesis of new series of 5,6-dihydro-4*H*-1,2-oxazines via hetero Diels–Alder reaction and evaluation of antimicrobial activity

M.K. Manjula [a,*], K.M.L. Rai [a], S.L. Gaonkar [a], K.A. Raveesha [b], S. Satish [b]

[a] *Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore, Karnataka 570006, India*
[b] *Department of Studies in Botany and Microbiology, University of Mysore, Manasagangotri, Mysore, Karnataka 570006, India*

Received 23 October 2007; received in revised form 11 February 2008; accepted 14 February 2008
Available online 7 March 2008

Abstract

A new series of 5,6-dihydro-4*H*-1,2-oxazines were synthesized via hetero Diels–Alder reaction of α-nitrosoolefins with alkenes. α-Nitrosoolefins were generated from ketoximes by the action of chloramine-T. The newly synthesized compounds were characterized with IR, NMR, elemental analysis and screened for their antimicrobial activity; they exhibited excellent antimicrobial activity. The minimal inhibitory concentration of the compounds was in the range of 10–35 μg ml^{-1} for bacteria and 10–40 μg ml^{-1} for fungi.

Keywords: Synthesis; 1,2-Oxazines; Antimicrobial activity; Chloramine-T

1. Introduction

α-Nitrosoalkenes which are normally generated *in situ* by 1,4-elimination from α-monohaloketoximes [1–4] are of importance owing to their use as dienes for a (4 + 2) cycloaddition reaction. Of late, it has gained more importance since the cycloaddition leads to the formation of 5,6-dihydro-4*H*-1,2-oxazines which are of biological importance, viz: used as intermediates during the synthesis of glycosidase inhibitor analogues [5–7] and of functionalised pyrroles [8]. They have got considerable synthetic potential [9–11] and many bifunctional compounds are synthesized with them as intermediates [3,11–14]. Tetrahydro-4*H*-1,2-oxazines are the important structural constituents of many fungicides, herbicides [15] and broad spectrum bactericides [16]. Oxazines are used as key building blocks for natural products [17]. For instance, using oxazine as synthon one can synthesize pyrroles [18,19], pyrrolidine [20], pyridines [2] and γ-lactones [21] via the reductive cleavage of the C–O and N–O bonds and so on.

There are few methods [22,23] described in the literature for the preparation of 5,6-dihydro-4*H*-1,2-oxazine derivatives. Many syntheses of 1,2-oxazines rely on hetero Diels–Alder reaction of alkenes with α-nitrosoalkenes, derived from α-halo oximes [3,24,25] or on hetero Diels–Alder reaction of dienes with nitroso compounds [11,26,27]. Other methods rely on cyclizations of alkenyl-substituted oximes [28–31]. They are also obtained by the cyclization of oxime dianions with epibromohydrin [32]. Rai et al. obtained 5,6-dihydro-4*H*-1,2-oxazine derivatives in good yield by the mediation of chloramine-T [33].

In continuation to our previous work, we report here the synthesis and antimicrobial activity of a new series of 5,6-dihydro-4*H*-1,2-oxazine derivatives via hetero Diels–Alder reaction of nitrosoolefins derived from ketoximes with alkenes.

2. Chemistry

The required ketones were obtained from the corresponding aromatic hydrocarbons by Friedel–Crafts reaction. Later the ketones are converted to the corresponding ketoximes by routine method [34]. Chloramine-T was used as an effective

* Corresponding author. Tel.: +91 821 2340890.
E-mail address: mkmraichur@yahoo.co.in (M.K. Manjula).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.02.027

reagent for the generation of α-nitrosoolefins from the corresponding ketoximes **1**(**a**–**e**) which subsequently undergo hetero Diels–Alder reaction with an olefin **2**(**a**–**e**), to give 5,6-dihydro-4*H*-1,2-oxazine derivatives **3**(**a**–**y**) in absolute purity and good yield (Scheme 1) [33]; their structures are highlighted in Table 1.

3. Results and discussion

3.1. Chemistry

Formation of 5,6-dihydro-4*H*-1,2-oxazine derivatives (**3**) was confirmed on the basis of elemental analysis, IR and NMR.

The IR spectra of 1,2-oxazine derivative (**3a**) did not show absorption at 3420–3430 cm^{-1} and at 1620–1630 cm^{-1} for OH and C=N groups, respectively, but instead showed new peak at 1643 cm^{-1} owing to the ring nitrogen (C=N), at 2258 cm^{-1} due to (C≡N) and at 1598 cm^{-1} due to aromatic ring.

The ^1H NMR spectrum of **3a** showed a singlet at δ 2.38 due to the CH_3 protons. It showed two multiplets resonating at δ (1.86–2.07) and δ (2.57–2.79) due to the CH_2 protons of the oxazine ring.

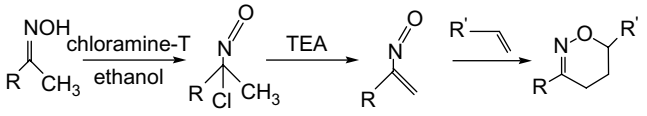
The four protons of the phenyl moiety resonated as two doublets at δ 7.16 and 7.59 with the coupling constants of $J = 6$ Hz and $J = 2$ Hz and CH proton resonated at δ 4.17 to give a triplet. In case of disubstituted benzene, instead of two doublets, three singlets are found.

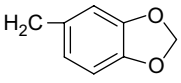
Further evidence for the formation of **3a** was given with ^{13}C NMR and elemental analysis.

3.2. Antimicrobial activity

3.2.1. Antibacterial activity

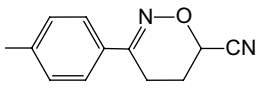
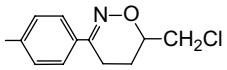
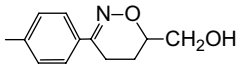
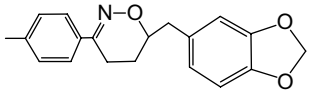
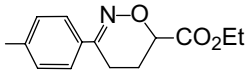
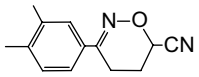
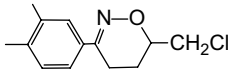
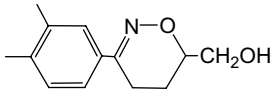
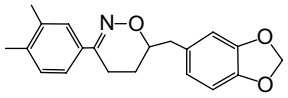
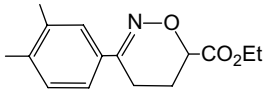
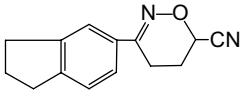
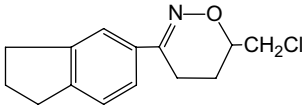
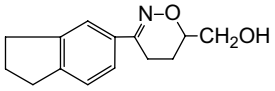
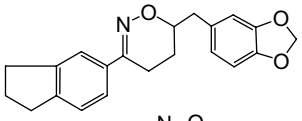
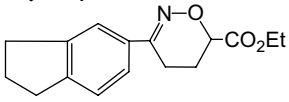
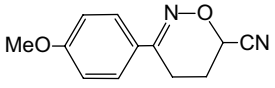
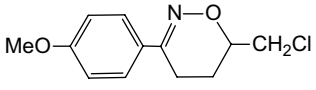
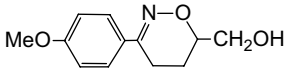
The newly synthesized oxazine derivatives excluding '**3e**' were screened for their antibacterial activity against four bacterial species, viz: *Escherichia coli*, *Klebsiella pneumoniae*,



1	R	2	R'
a	p-CH_3 . C_6H_4	a	CN
b	3,4-$(CH_3)_2$. C_6H_3	b	CH_2Cl
c	Indan-5-yl	c	CH_2OH
d	p-(OCH_3) . C_6H_4	d	
e	3,4-$(OCH_3)_2$. C_6H_3	e	CO_2Et

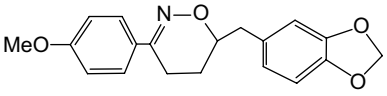
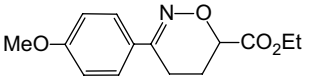
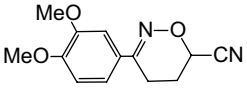
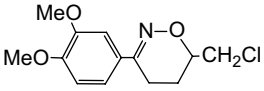
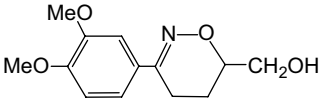
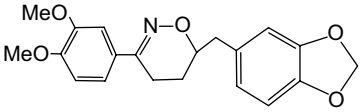
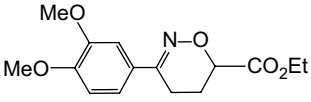
Scheme 1. Schematic representation of synthesis of 1,2-oxazine derivatives.

Table 1
Structural formulae of the synthesized compounds, **3(a–y)**

3	5,6-dihydro-4*H*-1,2-oxazines
a	
b	
c	
d	
e	
f	
g	
h	
i	
j	
k	
l	
m	
n	
o	
p	
q	
r	

(*continued on next page*)

Table 1 (*continued*)

3	5,6-dihydro-4*H*-1,2-oxazines
s	
t	
u	
v	
w	
x	
y	

Pseudomonas aeruginosa, *Staphylococcus aureus* by cup diffusion method [35]. Penicillin G and Gentamicin were used as positive reference to determine the sensitivity of each bacterial species tested. All compounds exhibited excellent *in vitro* antibacterial activity against Gram-positive and Gram-negative organisms. The results are summarized in Table 2. The results were subjected to statistical analysis and are summarized in Table 3.

The investigation of antibacterial screening data revealed that all the tested compounds showed considerable and varied activity against the four human pathogenic bacteria. Compound **3t** showed the maximum activity against the *E. coli*, which may be attributed to the aromatic ring, methoxy and carboxylate groups in *para* positions and in case of **3s**, the activity may be due to the methoxy and benzene dioxo group in *para* positions, the less activity may be due to the intervening methylene group. With *K. pneumoniae* **3l** showed the maximum activity, while **3k** and **3y** exhibited almost same level of activity with the said bacterium; with *P. aeruginosa* **3l** showed the maximum activity while **3k** and **3s** showed almost the same level of activity as **3l**. Against *S. aureus*, **3a** showed the maximum activity. The antibacterial activity may be due to the highly active chloro, nitrile, ester and methylene dioxyl groups. Most of the compounds exhibited better activity while some are even better than the standards.

3.2.2. Antifungal activity

Newly prepared compounds were screened for their antifungal activity against four fungal species namely *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme* and *Fusarium oxysporum* by cup diffusion method [35]. All compounds exhibited *in vitro* antifungal activity against all the tested fungal strains. The results are summarized in Table 4. Nystatin was used as positive reference to determine the sensitivity of each fungal species tested. The results were subjected to statistical analysis and are summarized in Table 5.

Table 4 clearly reveals that, most of the compounds are even more effective than the standard Nystatin. With all the four tested fungal strains, the maximum activity is exhibited by **3l** and the least by **3a**; **3o** and **3t** exhibited excellent activity almost on par with **3l**. The activity may be due to, the aromatic ring being common, the chloro (**3l**), ester (**3o**), methoxy and ester groups (**3t**).

4. Conclusion

In conclusion a new series of 5,6-dihydro-4*H*-1,2-oxazine derivatives were synthesized and their antimicrobial activities have been evaluated. The antibacterial and antifungal activities were observed in all the tested compounds. Majority of the compounds proved even better than the standard Gentamicin against the Gram-negative bacteria; with Gram-positive bacteria the activity is less than the control. The compounds exhibited excellent antifungal activity. Majority of the compounds exhibited better activity against the tested fungal strains than the standard Nystatin. Hence, it is concluded that there is ample scope for further developing this field.

5. Experimental protocols

Melting points were determined on Thomas Hoover melting point apparatus and are uncorrected. The IR spectra (in KBr pellets) were recorded on JASCO FT/IR − 460/113257 spectrometer (Japan) in the wave number range of 4000–400 cm^{-1}. ^{1}H NMR spectra were recorded on a Bruker AM 300 MHz spectrometer using $CDCl_3$ as solvent and tetramethylsilane as an internal standard. ^{13}C NMR spectra were measured on Jeol 400 (75 MHz) instrument. The chemical shifts are expressed in δ and the following abbreviations are used: s = singlet, d = doublet, t = triplet and m = multiplet. Elemental analyses were obtained on a Vario-EL instrument. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates using chloroform–acetone (8:2) as eluent.

5.1. General procedure for the synthesis of oxazine derivatives 3(a–y)

5.1.1. Synthesis of 3-p-tolyl-5,6-dihydro-4H-1,2-oxazine-6-carbonitrile 3a

Typical procedure. A mixture of **1a** (0.5 g, 3.35 mmol) and chloramine-T trihydrate (0.99 g, 3.52 mmol) in ethanol was taken in a 100 ml r.b. flask and refluxed for 2 h. The mixture was cooled to room temperature; triethylamine (1 ml) was added and stirred at room temperature for 15 min. To this, a solution of **2a** (1.8 g, 3.33 mmol) in ethanol (5 ml) was added

Table 2
Bacterial activity of the newly synthesized compounds and antibiotics against human pathogenic bacteria

Compound	*Escherichia coli* MTCC443	*Klebsiella pneumoniae* TCC109	*Pseudomonas aeruginosa* MTCC1688	*Staphylococcus aureus* MTCC 737
3a	11.50 ± 0.13	16.66 ± 0.06	12.66 ± 0.15	23.50 ± 0.25
3b	15.66 ± 0.15	16.50 ± 0.27	11.50 ± 0.25	16.66 ± 0.66
3c	08.83 ± 0.14	19.83 ± 0.20	10.75 ± 0.16	07.50 ± 0.19
3d	10.66 ± 0.11	19.66 ± 0.25	11.25 ± 0.25	12.83 ± 0.14
3f	15.50 ± 0.27	19.75 ± 0.16	15.25 ± 0.18	21.50 ± 0.25
3g	14.83 ± 0.20	15.83 ± 0.14	17.63 ± 0.25	20.66 ± 0.66
3h	13.66 ± 0.25	17.66 ± 0.15	12.75 ± 0.15	14.83 ± 0.20
3i	11.50 ± 0.12	15.50 ± 0.25	11.00 ± 0.18	21.33 ± 0.17
3j	13.50 ± 0.27	16.66 ± 0.66	12.83 ± 0.26	17.83 ± 0.20
3k	15.50 ± 0.25	23.83 ± 0.20	31.50 ± 0.27	17.66 ± 0.25
3l	11.83 ± 0.20	25.83 ± 0.20	34.50 ± 0.17	13.50 ± 0.17
3m	10.50 ± 0.12	17.66 ± 0.25	13.66 ± 0.15	10.66 ± 0.12
3n	11.50 ± 0.25	16.50 ± 0.17	21.50 ± 0.27	15.66 ± 0.11
3o	10.66 ± 0.06	13.50 ± 0.19	13.38 ± 0.18	18.66 ± 0.15
3p	10.16 ± 0.12	06.66 ± 0.12	12.16 ± 0.12	09.50 ± 0.13
3q	09.50 ± 0.12	17.66 ± 0.11	29.50 ± 0.12	14.66 ± 0.15
3r	10.66 ± 0.25	15.50 ± 0.13	16.66 ± 0.25	10.83 ± 0.14
3s	21.83 ± 0.14	19.66 ± 0.15	31.83 ± 0.14	21.66 ± 0.11
3t	24.66 ± 0.06	14.83 ± 0.14	23.66 ± 0.06	16.66 ± 0.06
3u	10.50 ± 0.25	07.75 ± 0.15	24.50 ± 0.25	15.50 ± 0.27
3v	13.83 ± 0.14	17.00 ± 0.18	09.83 ± 0.14	17.83 ± 0.20
3w	08.50 ± 0.13	14.50 ± 0.27	10.50 ± 0.13	07.66 ± 0.25
3x	08.12 ± 0.19	17.50 ± 0.17	14.12 ± 0.19	16.50 ± 0.12
3y	14.66 ± 0.11	20.66 ± 0.15	25.66 ± 0.11	16.50 ± 0.12
Penicillin G	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9.38 ± 0.18
Gentamicin	10.25 ± 0.25	17.50 ± 0.25	12.63 ± 0.18	24.63 ± 0.18

Zone of inhibition in mm (mean of six replicas ± standard error), $p < 0.05$.

and refluxed for an hour. It was then concentrated under reduced pressure and the residue was extracted with ether. The extract then washed with water (15 ml), with 1 N aq. NaOH and then with water and dried over anhydrous Na_2SO_4. Yellow solid, recrystallised from alcohol–*n*-hexane (0.36 g, 72%), m.p. 91 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.86–2.07 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.57–2.79 (m, 2H, CH_2), 4.17 (t, H, CH), 7.16 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.59 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.2 (q, CH_3), 16.8 (t, CH_2), 22.3 (t, CH_2), 72.1 (d, CH), 130.3 (d, CH), 117.2 (s, C), 133.0 (s, C), 141.2 (s, C), 150.3 (s, C). IR (KBr pellets cm^{-1}) ν 1598, 1643, 2258. Anal. CHN: calcd C 71.98, H 6.04, N 13.99, O 7.99, found C 71.95, H 6.04, N 13.98, O 7.99.

5.1.2. Synthesis of 6-chloromethyl-3-p-tolyl-5,6-dihydro-4H-1,2-oxazine ***3b***

Obtained from **1a** (0.5 g, 3.35 mmol), chloramine-T·3H_2O (0.99 g, 3.52 mmol) and **2b** (1.6 g, 2.09 mmol) as a red liquid (0.35 g, 70%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.88–2.09 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 2.55–2.75 (m, 2H, CH_2), 3.55–3.79 (m, 3H, CH_2–CH), 7.17 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.59 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.2 (q, CH_3), 11.5 (t, CH_2), 22.5 (t, CH_2), 47.9 (t, CH_2), 82.2 (d, CH), 130.1 (d, CH); 133.2 (s, C), 142.3 (s, C), 151.4 (s, C). IR (KBr pellets cm^{-1}) ν 782, 1595, 1637. Anal. CHN: calcd C 64.43, H 6.31, Cl 15.85, N 6.26, O 7.15, found C 64.42, H 6.31, Cl 15.83, N 6.25, O 7.15.

5.1.3. Synthesis of 6-hydroxymethyl-3-p-tolyl-5,6-dihydro-4H-1,2-oxazine ***3c***

Obtained from **1a** (0.5 g, 3.35 mmol), chloramine-T·3H_2O (0.99 g, 3.52 mmol) and **2c** (1.6 g, 2.56 mmol) as a colourless solid, recrystallised from alcohol–*n*-hexane (0.29 g, 58%), m.p. 80 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.08 (m, 2H, CH_2), 2.13 (br, 1H, OH), 2.37 (s, 3H, CH_3), 2.56–2.78 (m, 2H, CH_2), 3.74–3.93 (m, 3H, CH_2–CH), 7.18 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.59 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.2 (q, CH_3), 10.5 (t, CH_2), 23.3 (t, CH_2), 66.7 (t, CH_2), 82.4 (d, CH), 130.2 (d, CH), 132.5 (s, C), 141.9 (s, C), 151.9 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1647, 3340. Anal. CHN: calcd C 70.22, H 7.37, N 6.82, O 15.59, found C 70.22, H 7.37, N 6.80, O 15.60.

5.1.4. Synthesis of 6-(benzo[1,3]dioxol-5-yl)methyl-3-p-tolyl-5,6-dihydro-4H-1,2-oxazine ***3d***

Obtained from **1a** (0.5 g, 3.35 mmol), chloramine-T·3H_2O (0.99 g, 3.52 mmol) and **2d** (1.37 g, 0.85 mmol) as a red liquid (0.325 g, 65%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.09 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 2.55–2.77 (m, 2H, CH_2), 3.51–3.72 (m, 3H, CH_2–CH), 5.93 (s, 2H, O–CH_2–O), 6.51–6.62 (m, 3H, CH), 7.17 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.59 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.2 (q, CH_3), 14.6 (t, CH_2), 23.2 (t, CH_2), 41.9 (t, CH_2), 102.5 (t, CH_2), 81.9 (d, CH), 114.5 (d, CH), 116.3 (d, CH), 122.6 (d, CH), 130.1 (d, CH), 131.9 (s, C), 133.2 (s, C), 141.5 (s, C), 146.7 (s, C), 149.2 (s, C), 152.3 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1642. Anal. CHN: calcd C

Table 3
The minimal inhibitory concentration (MIC) in μg/mL of synthesized compounds against tested strains

Compound	*Escherichia coli* MTCC443	*Klebsiella pneumoniae* TCC109	*Pseudomonas aeruginosa* MTCC1688	*Staphylococcus aureus* MTCC 737
3a	25	20	20	15
3b	20	15	15	10
3c	35	30	25	25
3d	25	20	20	15
3f	25	15	15	10
3g	20	15	15	10
3h	25	20	25	15
3i	30	20	20	15
3j	20	15	20	10
3k	25	20	15	15
3l	20	15	15	10
3m	35	25	20	20
3n	25	15	15	10
3o	30	25	20	20
3p	25	15	15	10
3q	20	10	15	10
3r	35	25	20	20
3s	25	15	20	15
3t	35	25	20	20
3u	25	15	10	15
3v	20	10	15	10
3w	35	25	20	20
3x	25	15	20	10
3y	30	20	15	15
Penicillin G	–	–	–	–
Gentamicin	20	15	15	15

Table 4
Antifungal activity of the newly synthesized compounds and antifungal agent (Nystatin) against pathogenic fungi

Compound	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium moniliforme*	*Fusarium oxysporum*
3a	07.50 ± 0.25	06.83 ± 0.20	10.50 ± 0.12	13.66 ± 0.06
3b	14.50 ± 0.27	13.83 ± 0.20	17.66 ± 0.15	20.83 ± 0.14
3c	12.50 ± 0.12	11.50 ± 0.17	15.83 ± 0.14	19.83 ± 0.14
3d	17.83 ± 0.20	15.66 ± 0.25	19.50 ± 0.25	24.50 ± 0.25
3f	12.16 ± 0.12	11.66 ± 0.11	15.66 ± 0.15	21.50 ± 0.25
3g	09.50 ± 0.12	08.50 ± 0.13	11.50 ± 0.25	20.66 ± 0.66
3h	08.66 ± 0.25	10.66 ± 0.12	14.66 ± 0.66	19.83 ± 0.20
3i	18.83 ± 0.14	15.66 ± 0.11	22.83 ± 0.20	25.33 ± 0.17
3j	12.66 ± 0.06	11.50 ± 0.13	16.66 ± 0.25	19.83 ± 0.20
3k	09.50 ± 0.19	10.66 ± 0.15	13.50 ± 0.27	17.66 ± 0.25
3l	32.75 ± 0.16	28.83 ± 0.14	34.50 ± 0.17	38.50 ± 0.17
3m	09.83 ± 0.14	07.66 ± 0.11	13.66 ± 0.15	18.83 ± 0.14
3n	20.66 ± 0.15	18.66 ± 0.06	21.50 ± 0.27	26.66 ± 0.06
3o	28.50 ± 0.25	26.66 ± 0.12	32.83 ± 0.14	36.50 ± 0.13
3p	08.50 ± 0.25	09.50 ± 0.27	13.38 ± 0.18	18.50 ± 0.25
3q	21.66 ± 0.66	19.50 ± 0.17	25.25 ± 0.18	29.66 ± 0.66
3r	07.83 ± 0.20	08.66 ± 0.15	17.63 ± 0.25	19.50 ± 0.19
3s	09.66 ± 0.25	10.66 ± 0.15	12.75 ± 0.15	16.83 ± 0.14
3t	25.50 ± 0.12	24.16 ± 0.12	28.00 ± 0.18	30.66 ± 0.15
3u	10.50 ± 0.25	11.50 ± 0.12	12.83 ± 0.26	15.50 ± 0.27
3v	20.83 ± 0.14	18.66 ± 0.25	26.50 ± 0.25	28.66 ± 0.06
3w	08.50 ± 0.13	07.83 ± 0.14	10.75 ± 0.16	16.50 ± 0.27
3x	08.12 ± 0.19	07.75 ± 0.15	11.25 ± 0.25	15.83 ± 0.20
3y	09.66 ± 0.11	08.00 ± 0.18	11.66 ± 0.11	16.66 ± 0.25
Nystatin	13.25 ± 0.25	11.50 ± 0.25	15.63 ± 0.18	16.63 ± 0.18

Zone of inhibition in mm (mean of six replicas ± standard error), $p < 0.05$.

Table 5
The minimal inhibitory concentration (MIC) in μg/mL of synthesized compounds against fungal strains

Compound	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium moniliforme*	*Fusarium oxysporum*
3a	35	40	30	25
3b	15	15	10	15
3c	30	35	30	25
3d	15	20	20	10
3f	20	20	15	15
3g	15	15	20	10
3h	35	30	35	25
3i	10	15	20	10
3j	15	20	25	15
3k	40	35	40	35
3l	10	15	20	10
3m	30	30	35	25
3n	15	20	20	15
3o	10	15	20	10
3p	35	40	35	30
3q	15	20	15	10
3r	35	40	35	30
3s	40	35	35	35
3t	10	15	20	10
3u	35	35	30	30
3v	15	20	15	10
3w	40	40	35	30
3x	40	40	35	30
3y	35	35	30	35
Nystatin	15	10	15	20

73.77, H 6.19, N 4.53, O 15.52, found C 73.75, H 6.19, N 4.53, O 15.50.

*5.1.5. Synthesis of 3-p-tolyl-5,6-dihydro-4H-1,2-oxazine-6-carboxylic acid ethylester **3e***

Obtained from **1a** (0.5 g, 3.35 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2e** (1.63 g, 1.63 mmol) as a pale yellow solid recrystallised from ethanol–*n*-hexane (0.275 g, 55%), m.p.99 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.27 (t, 3H, CH_3), 2.28–2.52 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 2.56–2.78 (m, 2H, CH_2), 3.99–4.02 (m, 3H, CH_2–CH), 7.15 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.55 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.2 (q, CH_3), 25.1 (q, CH_3), 20.9 (t, CH_2), 22.0 (t, CH_2), 62.2 (t, CH_2), 86.3 (d, CH), 129.9 (d, CH), 132.0 (s, C), 141.5 (s, C), 152.2 (s, C), 171.5 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1648, 1778. Anal. CHN: calcd C 68.00, H 6.93, N 5.66, O 19.41, found C 68.00, H 6.93, N 5.66, O 19.40.

*5.1.6. Synthesis of 3-(3,4-dimethylphenyl)-5,6-dihydro-4H-1,2-oxazine-6-carbonitrile **3f***

Obtained from **1b** (0.5 g, 3 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2a** (1.8 g, 3.33 mmol) as a pale brown solid, recrystallised from ethanol–*n*-hexane (0.365 g, 73%), m.p. 121 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.85–2.08 (m, 2H, CH_2), 2.38 (s, 6H, CH_3), 2.56–2.78 (m, 2H, CH_2), 4.18 (t, H, CH), 7.05–7.34 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.5 (q, CH_3), 16.5 (t, CH_2), 21.9 (t, CH_2), 71.1 (d, CH), 126.9 (d, CH), 130.1 (d, CH), 119.3 (s, C), 131.5

(s, C), 137.9 (s, C), 140.8 (s, C), 151.6 (s, C). IR (KBr pellets cm^{-1}) ν 1598, 1645, 2260. Anal. CHN: calcd C 72.87, H 6.59, N 13.07, O 7.47, found C 72.85, H 6.58, N 13.07, O 7.47.

*5.1.7. Synthesis of 6-chloromethyl-3-(3,4-dimethylphenyl)-5,6-dihydro-4H-1,2-oxazine **3g***

Obtained from **1b** (0.5 g, 3 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2b** (1.6 g, 2.09 mmol) as a pale yellow solid, recrystallised from ethanol–*n*-hexane (0.375 g, 75%), m.p. 77 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.09 (m, 2H, CH_2), 2.37 (s, 6H, CH_3), 2.54–2.75 (m, 2H, CH_2), 3.54–3.79 (m, 3H, CH_2–CH), 7.06–7.35 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.6 (q, CH_3), 11.5 (t, CH_2), 22.4 (t, CH_2), 47.8 (t, CH_2), 82.3 (d, CH), 127.6 (d, CH), 130.5 (d, CH), 131.8 (s, C), 137.8 (s, C), 139.9 (s, C), 152.3 (s, C). IR (KBr pellets cm^{-1}) ν 779, 1600, 1640. Anal. CHN: calcd C 65.68, H 6.78, Cl 14.91, N 5.89, O 6.73, found C 65.66, H 6.75, Cl 14.91, N 5.89, O 6.73.

*5.1.8. Synthesis of [3-(3,4-dimethylphenyl)-5,6-dihydro-4H-1,2-oxazin-6-yl]-methanol **3h***

Obtained from **1b** (0.5 g, 3 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2c** (1.6 g, 2.56 mmol) as a colourless solid, recrystallised from ethanol–*n*-hexane (0.36 g, 72%), m.p. 91 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.08 (m, 2H, CH_2), 2.13 (br, 1H, OH), 2.36 (s, 6H, CH_3), 2.56–2.79 (m, 2H, CH_2), 3.73–3.92 (m, 3H, CH_2–CH), 7.03–7.32 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.7 (q, CH_3), 10.8 (t, CH_2), 23.2 (t, CH_2), 66.3 (t, CH_2), 82.6 (d, CH), 127.2 (d, CH), 130.5 (d, CH), 132.0 (s, C), 138.1 (s, C), 140.2 (s, C), 152.7 (s, C). IR (KBr pellets cm^{-1}) ν 1598, 1646, 3300. Anal. CHN: calcd C 71.21, H 7.81, N 6.39, O 14.59, found C 71.19, H 7.80, N 6.39, O 14.58.

*5.1.9. Synthesis of 6-(benzo[1,3]dioxol-5-yl)methyl-3-(3,4-dimethylphenyl)-5,6-dihydro-4H-1,2-oxazine **3i***

Obtained from **1b** (0.5 g, 3 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2d** (1.37 g, 0.85 mmol) as a red oil (0.35 g, 70%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.88–2.09 (m, 2H, CH_2), 2.37 (s, 6H, CH_3), 2.55–2.76 (m, 2H, CH_2), 3.51–3.73 (m, 3H, CH_2–CH), 5.92 (s, 2H, O–CH_2–O), 6.52–6.63 (m, 3H, CH), 7.06–7.33 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.8 (q, CH_3), 14.5 (t, CH_2), 23.4 (t, CH_2), 41.8 (t, CH_2), 102.5 (t, CH_2), 82.1 (d, CH), 114.3 (d, CH), 116.3 (d, CH), 122.7 (d, CH), 127.2 (d, CH), 130.5 (d, CH), 131.8 (s, C), 138.1 (s, C), 140.5 (s, C), 147.5 (s, C), 149.8 (s, C), 152.3 (s, C). IR (KBr pellets cm^{-1}) ν 1599, 1643. Anal. CHN: calcd C 74.28, H 6.55, N 4.33, O 14.84, found C 74.25, H 6.55, N 4.30, O 14.84.

*5.1.10. Synthesis of 3-(3,4-dimethylphenyl)-5,6-dihydro-4H-1,2-oxazine-6-carboxylic acid ethylester **3j***

Obtained from **1b** (0.5 g, 3 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2e** (1.63 g, 1.63 mmol) as a yellow crystalline solid, recrystallised from ethanol–*n*-hexane (0.29 g, 58%), m.p. 115 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.28 (t, 3H, CH_3), 2.28–2.53 (m, 2H, CH_2), 2.37 (s, 6H, CH_3), 2.55–2.78 (m, 2H, CH_2), 3.99–4.03 (m, 3H, CH_2–CH), 7.03–7.32 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.2 (q, CH_3), 18.9 (q, CH_3), 21.5 (t, CH_2), 22.5 (t, CH_2), 62.5 (t, CH_2), 86.6 (d, CH), 127.5 (d, CH), 130.3 (d, CH), 131.8 (s, C), 138.2 (s, C), 140.3 (s, C), 152.6 (s, C), 171.5 (s, C). IR (KBr pellets cm^{-1}) ν 1605, 1645, 1778. Anal. CHN: calcd C 68.94, H 7.33, N 5.36, O 18.37, found C 68.94, H 7.30, N 5.36, O 18.35.

*5.1.11. Synthesis of 3-indan-5-yl-5,6-dihydro-4H-1,2-oxazine-6-carbonitrile **3k***

Obtained from **1c** (0.5 g, 2.85 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2a** (1.8 g, 3.33 mmol) as a reddish brown oil (0.375 g, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.86–2.07 (m, 4H, CH_2), 2.57–2.78 (m, 2H, CH_2), 2.86 (t, 4H, CH_2), 4.19 (t, H, CH), 7.48–7.82 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6 (t, CH_2), 22.1 (t, CH_2), 26.5 (t, CH_2), 34.2 (t, CH_2), 71.4 (d, CH), 127.2 (d, CH), 129.2 (d, CH), 119.2 (s, C), 132.5 (s, C), 145.1 (s, C), 147.3 (s, C), 152.0 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1642, 2255. Anal. CHN: calcd C 74.31, H 6.24, N 12.38, O 7.07, found C 74.30, H 6.25, N 12.35, O 7.07.

*5.1.12. Synthesis of 6-chloromethyl-3-indan-5-yl-5,6-dihydro-4H-1,2-oxazine **3l***

Obtained from **1c** (0.5 g, 2.85 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2b** (1.6 g, 2.09 mmol) as a red oil (0.3 g, 60%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.88–2.08 (m, 4H, CH_2), 2.55–2.89 (m, 6H, CH_2), 3.55–3.78 (m, 3H, CH_2–CH), 7.45–7.82 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.6 (t, CH_2), 22.4 (t, CH_2), 26.4 (t, CH_2), 34.3 (t, CH_2), 47.1 (t, CH_2), 82.3 (d, CH), 127.3 (d, CH), 129.4 (d, CH), 132.3 (s, C), 145.1 (s, C), 147.3 (s, C), 152.3 (s, C). IR (KBr pellets cm^{-1}) ν 780, 1599, 1640. Anal. CHN: calcd C 67.33, H 6.46, Cl 14.20, N 5.61, O 6.41. found C 67.33, H 6.44, Cl 14.20, N 5.60, O 6.40.

*5.1.13. Synthesis of (3-indan-5-yl-5,6-dihydro-4H-1,2-oxazin-6-yl)-methanol **3m***

Obtained from **1c** (0.5 g, 2.85 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2c** (1.6 g, 2.56 mmol) as a red liquid (0.32 g, 64%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.09 (m, 4H, CH_2), 2.13 (br, 1H, OH), 2.57–2.90 (m, 6H, CH_2), 3.74–3.93 (m, 3H, CH_2–CH), 7.46–7.83 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.6 (t, CH_2), 22.9 (t, CH_2), 26.2 (t, CH_2), 33.9 (t, CH_2), 66.2 (t, CH_2), 82.3 (d, CH), 127.7 (d, CH), 129.3 (d, CH), 132.2 (s, C), 145.1 (s, C), 147.2 (s, C), 152.4 (s, C). IR (KBr pellets cm^{-1}) ν 1599, 1642, 3330. Anal. CHN: calcd C 72.70, H 7.41, N 6.06, O 13.83, found C 72.70, H 7.40, N 6.06, O 13.80.

*5.1.14. Synthesis of 6-(benzo[1,3]dioxol-5-yl)methyl-3-indan-5-yl-5,6-dihydro-4H-1,2-oxazine **3n***

Obtained from **1c** (0.5 g, 2.85 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2d** (1.37 g, 0.85 mmol) as a reddish liquid (0.325 g, 65%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.88–2.08 (m, 4H, CH_2), 2.56–2.88 (m, 6H, CH_2), 3.51–3.74 (m,

3H, CH_2–CH), 5.93 (s, 2H, O–CH_2–O), 6.53–6.62 (m, 3H, CH), 7.45–7.82 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 (t, CH_2), 23.0 (t, CH_2), 26.7 (t, CH_2), 34.1 (t, CH_2), 41.2 (t, CH_2), 102.3 (t, CH_2), 81.5 (d, CH), 114.2 (d, CH), 116.1 (d, CH), 122.2 (d, CH), 127.5 (d, CH), 129.5 (d, CH), 133.0 (s, C), 145.3 (s, C), 147.1 (s, C), 149.5 (s, C), 152.2 (s, C). IR (KBr pellets cm^{-1}) ν 1602, 1645. Anal. CHN: calcd C 75.20, H 6.31, N 4.18, O 14.31, found C 75.20, H 6.30, N 4.15, O 14.30.

*5.1.15. Synthesis of 3-indan-5-yl-5,6-dihydro-4H-1,2-oxazine-6-carboxylic acid ethylester **3o***

Obtained from **1c** (0.5 g, 2.85 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2e** (1.63 g, 1.63 mmol) as a reddish brown oil (0.3 g, 60%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.28 (t, 3H, CH_3), 1.98 (q, 2H, CH_2), 2.28–2.52 (m, 2H, CH_2), 2.56–2.87 (m, 6H, CH_2), 3.98–4.02 (m, 3H, CH_2–CH), 7.46–7.83 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.6 (q, CH_3), 21.4 (t, CH_2), 22.6 (t, CH_2), 26.4 (t, CH_2), 34.2 (t, CH_2), 62.8 (t, CH_2), 86.3 (d, CH), 127.4 (d, CH), 129.3 (d, CH), 132.3 (s, C), 145.1 (s, C), 147.1 (s, C), 152.6 (s, C), 171.5 (s, C). IR (KBr pellets cm^{-1}) ν 1598, 1643, 1774. Anal. CHN: calcd C 70.31, H 7.01, N 5.12, O 17.56, found C 70.31, H 7.01, N 5.10, O 17.55.

*5.1.16. Synthesis of 3-(4-methoxyphenyl)-5,6-dihydro-4H-1,2-oxazine-6-carbonitrile **3p***

Obtained from **1d** (0.5 g, 3.05 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2a** (1.8 g, 3.33 mmol) as a yellow solid, recrystallised from ethanol–*n*-hexane (0.38 g, 76%), m.p. 131 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.85–2.07 (m, 2H, CH_2), 2.55–2.79 (m, 2H, CH_2), 3.75 (s, 3H, OMe), 4.18 (t, H, CH), 6.88 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.57 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.9 (q, CH_3), 16.5 (t, CH_2), 22.0 (t, CH_2), 71.3 (d, CH), 115.6 (d, CH), 131.3 (d, CH), 119.2 (s, C), 127.1 (s, C), 152.0 (s, C), 164.2 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1642, 2258. Anal. CHN: calcd C 66.65, H 5.59, N 12.96, O 14.80, found C 66.65, H 5.55, N 12.93, O 14.80.

*5.1.17. Synthesis of 6-chloromethyl-3-(4-methoxyphenyl)-5,6-dihydro-4H-1,2-oxazine **3q***

Obtained from **1d** (0.5 g, 3.05 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2b** (1.6 g, 2.09 mmol) as a reddish brown oil (0.275 g, 55%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.86–2.08 (m, 2H, CH_2), 2.53–2.74 (m, 2H, CH_2), 3.54–3.73 (m, 3H, CH_2–CH), 3.75 (s, 3H, OMe), 6.89 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.58 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.8 (q, CH_3), 11.7 (t, CH_2), 21.9 (t, CH_2), 47.2 (t, CH_2), 82.3 (d, CH), 115.5 (d, CH), 131.4 (d, CH), 127.5 (s, C), 151.9 (s, C), 164.3 (s, C). IR (KBr pellets cm^{-1}) ν 780, 1600, 1645. Anal. CHN: calcd C 60.13, H 5.89, Cl 14.79, N 5.84, O 13.35, found C 60.13, H 5.87, Cl 14.79, N 5.82, O 13.35.

*5.1.18. Synthesis of [3-(4-methoxyphenyl)-5,6-dihydro-4H-1,2-oxazin-6-yl)-methanol **3r***

Obtained from **1d** (0.5 g, 3.05 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2c** (1.6 g, 2.56 mmol) as a yellow oil (0.32 g, 64%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.88–2.09 (m, 2H, CH_2), 2.13 (br, 1H, OH), 2.55–2.78 (m, 2H, CH_2), 3.72–3.93 (m, 3H, CH_2–CH), 3.76 (s, 3H, OMe), 6.87 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.58 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.8 (q, CH_3), 10.8 (t, CH_2), 23.0 (t, CH_2), 66.2 (t, CH_2), 82.2 (d, CH), 115.2 (d, CH), 131.3 (d, CH), 127.4 (s, C), 152.6 (s, C), 164.1 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1642, 3370. Anal. CHN: calcd C 65.14, H 6.83, N 6.33, O 21.69, found C 65.11, H 6.83, N 6.33, O 21.66.

*5.1.19. Synthesis of 6-(benzo[1,3]dioxol-5-yl)methyl-3-(4-methoxyphenyl)-5,6-dihydro-4H-1,2-oxazine **3s***

Obtained from **1d** (0.5 g, 3.05 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2d** (1.37 g, 0.85 mmol) as a red oil (0.375 g, 75%), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.08 (m, 2H, CH_2), 2.55–2.77 (m, 2H, CH_2), 3.51–3.73 (m, 3H, CH_2–CH), 3.75 (s, 3H, OMe), 5.92 (s, 2H, O–CH_2–O), 6.86 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.59 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.8 (q, CH_3), 14.1 (t, CH_2), 22.9 (t, CH_2), 41.8 (t, CH_2), 102.3 (t, CH_2), 81.5 (d, CH), 114.1 (d, CH), 115.1 (d, CH), 116.1 (d, CH), 122.4 (d, CH), 131.1 (d, CH), 127.2 (s, C), 133.0 (s, C), 147.1 (s, C), 149.5 (s, C), 152.2 (s, C), 164.0 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1640. Anal. CHN: calcd C 70.14, H 5.89, N 4.31, O 19.67, found C 70.14, H 5.89, N 4.30, O 19.65.

*5.1.20. Synthesis of 3-(4-methoxyphenyl)-5,6-dihydro-4H-1,2-oxazine-6-carboxylic acid ethylester **3t***

Obtained from **1d** (0.50 g, 3.05 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2e** (1.63 g, 1.63 mmol) as a red oil (0.34 g, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.28 (t, 3H, CH_3), 2.28–2.52 (m, 2H, CH_2), 2.57–2.79 (m, 2H, CH_2), 3.76 (s, 3H, OMe), 3.98–4.01 (m, 3H, CH_2–CH), 6.87 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH), 7.58 (dd, $J = 6$ Hz, $J = 2$ Hz, 2H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.2 (q, CH_3), 56.7 (q, CH_3), 21.6 (t, CH_2), 22.3 (t, CH_2), 62.3 (t, CH_2), 86.7 (d, CH), 115.5 (d, CH), 131.5 (d, CH), 127.5 (s, C), 152.5 (s, C), 164.3 (s, C), 171.8 (s, C). IR (KBr pellets cm^{-1}) ν 1598, 1645, 1775. Anal. CHN: calcd C 63.87, H 6.51, N 5.32, O 24.31, found C 63.82, H 6.51, N 5.32, O 24.30.

*5.1.21. Synthesis of 3-(3,4-dimethoxyphenyl)-5,6-dihydro-4H-1,2-oxazine-6-carbonitrile **3u***

Obtained from **1e** (0.5 g, 2.5 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2a** (1.8 g, 3.33 mmol) as a yellow solid, recrystallised from ethanol–*n*-hexane (0.4 g, 80%), m.p. 155 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.86–2.08 (m, 2H, CH_2), 2.56–2.78 (m, 2H, CH_2), 3.76 (s, 6H, OMe), 4.19 (t, H, CH), 6.77–7.19 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.3 (q, CH_3), 16.8 (t, CH_2), 22.2 (t, CH_2), 71.2 (d, CH), 115.5 (d, CH), 116.9 (d, CH), 123.8 (d, CH), 119.2

(s, C), 128.5 (s, C), 150.8 (s, C), 152.6 (s, C), 153.2 (s, C). IR (KBr pellets cm^{-1}) ν 1596, 1638, 2260. Anal. CHN: calcd C 63.40, H 5.73, N 11.38, O 19.49, found C 63.40, H 5.70, N 11.35, O 19.49.

*5.1.22. Synthesis of 6-chloromethyl-3-(3,4-dimethoxyphenyl)-5,6-dihydro-4H-1,2-oxazine **3v***

Obtained from **1e** (0.5 g, 2.5 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2b** (1.6 g, 2.09 mmol) as a reddish brown oil (0.35 g, 70%). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.09 (m, 2H, CH_2), 2.54–2.75 (m, 2H, CH_2), 3.54–3.73 (m, 3H, CH_2–CH), 3.76 (s, 6H, OMe), 6.78–7.19 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.2 (q, CH_3), 11.9 (t, CH_2), 22.5 (t, CH_2), 47.7 (t, CH_2), 82.5 (d, CH), 115.9 (d, CH), 116.8 (d, CH), 123.6 (d, CH), 128.4 (s, C), 150.7 (s, C), 152.3 (s, C), 153.1 (s, C). IR (KBr pellets cm^{-1}) ν 782, 1602, 1648. Anal. CHN: calcd C 57.89, H 5.98, Cl 13.14, N 5.19, O 17.80, found C 57.85, H 5.98, Cl 13.14, N 5.15, O 17.80.

*5.1.23. Synthesis of [3-(3,4-dimethoxyphenyl)-5,6-dihydro-4H-1,2-oxazin-6-yl]-methanol **3w***

Obtained from **1e** (0.5 g, 2.5 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2c** (1.6 g, 2.56 mmol) as a red oil (0.4 g, 80%). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.08 (m, 2H, CH_2), 2.13 (br, 1H, OH), 2.54–2.78 (m, 2H, CH_2), 3.72–3.93 (m, 3H, CH_2–CH), 3.75 (s, 6H, OMe), 6.77–7.18 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.3 (q, CH_3), 10.5 (t, CH_2), 23.0 (t, CH_2), 66.2 (t, CH_2), 82.6 (d, CH), 115.8 (d, CH), 116.7 (d, CH), 123.4 (d, CH), 128.5 (s, C), 150.8 (s, C), 152.6 (s, C), 153.1 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1640, 3360. Anal. CHN: calcd C 62.14, H 6.82, N 5.57, O 25.47, found C 62.14, H 6.82, N 5.55, O 25.45.

*5.1.24. Synthesis of 6-(benzo[1,3]dioxol-5-yl)methyl-3-(3,4-dimethoxyphenyl)-5,6-dihydro-4H-1,2- oxazine **3x***

Obtained from (0.5 g, 2.5 mmol) **1e**, chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2d** (1.37 g, 0.85 mmol) as a reddish brown oil (0.375 g, 75%). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.86–2.07 (m, 2H, CH_2), 2.56–2.77 (m, 2H, CH_2), 3.51–3.73 (m, 3H, CH_2–CH), 3.75 (s, 6H, OMe), 5.93 (s, 2H, O–CH_2–O), 6.75–7.18 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.2 (q, CH_3), 14.2 (t, CH_2), 23.1 (t, CH_2), 41.9 (t, CH_2), 102.2 (t, CH_2), 81.9 (d, CH), 114.3 (d, CH), 115.7 (d, CH), 116.9 (d, CH), 122.6 (d, CH), 123.5 (d, CH), 128.3 (s, C), 133.0 (s, C), 147.2 (s, C), 149.2 (s, C), 150.8 (s, C), 152.4 (s, C), 153.1 (s, C). IR (KBr pellets cm^{-1}) ν 1600, 1645. Anal. CHN: calcd C 67.59, H 5.96, N 3.94, O 22.51, found C 67.59, H 5.95, N 3.90, O 22.51.

*5.1.25. Synthesis of 3-(3,4-dimethoxyphenyl)-5,6-dihydro-4H-1,2-oxazine-6-carboxylic acid ethylester **3y***

Obtained from **1e** (0.5 g, 2.5 mmol), chloramine-T·$3H_2O$ (0.99 g, 3.52 mmol) and **2e** (1.63 g, 1.63 mmol) as a blood red viscous oil. (0.425 g, 85%). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (t, 3H, CH_3), 2.29–2.54 (m, 2H, CH_2), 2.56–2.79 (m, 2H, CH_2), 3.75 (s, 6H, OMe), 3.99–4.02 (m, 3H, CH_2–CH), 6.76–7.19 (m, 3H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.2 (q, CH_3), 57.2 (q, CH_3), 21.3 (t, CH_2), 22.6 (t, CH_2), 62.5 (t, CH_2), 86.6 (d, CH), 115.2 (d, CH), 116.3 (d, CH), 123.4 (d, CH), 128.3 (s, C), 150.5 (s, C), 152.6 (s, C) 153.0 (s, C), 171.8 (s, C). IR (KBr pellets cm^{-1}) ν 1605, 1641, 1776. Anal. CHN: calcd C 61.42, H 6.53, N 4.78, O 27.27, found C 61.42, H 6.50, N 4.75, O 27.27.

5.2. Biological activity

5.2.1. Antibacterial studies (materials and methods)

Four bacterial species, viz: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* were used as the antibacterial test strains. The *in vitro* antibacterial activity of the compounds was tested by cup diffusion method [35] by using the test bacteria maintained on the nutrient agar (NA) medium at 37 °C at 10^4 cells/mL and incubating in LB medium containing the indicated amount of the given compound. After overnight incubation at 37 °C, the optical density of the culture was determined. All compounds exhibited excellent *in vitro* antibacterial activity against Gram-positive and Gram-negative organisms.

The minimal inhibitory concentration (MIC) was determined by assaying the effect of each compound at concentrations of 0.1, 5, 10, 15, 20, 25, 30, 35 and 40 mg/mL. The sterile medium (20 ml) was poured onto 9 cm Petri plates. The medium was allowed to cool in a sterile condition and plates were then inculcated with 1×10^5 cfu cultures of test bacteria. The concentration of bacterial cells in the suspension was adjusted to a minimum of 1×10^5 cfu/ml in nutrient broth solution. Agar cups of 5 mm diameter were made in the plates. Each test sample was dissolved in dimethylformamide (DMF) and 50 µl of test solution containing 20 mg of the test compound was placed in each cup. The plates were left to stay for an hour in order to facilitate the diffusion of the drug solution. Negative controls were prepared using the same solvent (DMF) employed to dissolve the test compounds. Then the plates were incubated at 37 °C for 24 h. The zone of inhibition, if any, against the test bacteria was measured in mm. Penicillin G and Gentamicin were used as positive reference to determine the sensitivity of each bacterial species tested.

5.2.2. Antifungal studies (materials and methods)

Four fungal species namely *A. flavus*, *A. niger*, *F. moniliforme* and *F. oxysporum* were used as antifungal test strains to determine the antifungal activity of the synthesized compounds by cup diffusion method [35]. The filamentous fungi were maintained on potato dextrose agar (PDA) medium at 28 °C. The *in vitro* antifungal activity of the compounds was tested by using the test fungus at 10^6 spores/mL and was incubated in potato dextrose liquid medium containing the indicated amount of the given compound. After incubation for 48 h at 28 °C, the optical density of the culture was determined. All compounds exhibited *in vitro* antifungal activity against all the tested fungal strains.

MIC was determined by assaying the effect of each compound at concentrations of 0.1, 5, 10, 15, 20, 25, 30, 35 and

40 mg/mL. The sterile medium (20 ml) was poured onto 9 cm Petri plates. The medium was allowed to cool in a sterile condition and plates were then inculcated with 1×10^6 cfu cultures of test fungi. The concentration of spores in the suspension was adjusted to a minimum of 1×10^6 cfu/ml in potato dextrose broth solution. Agar cup of 5 mm diameter was made in the plates. Each test sample was dissolved in dimethylformamide (DMF), 50 μl of test solution containing 20 mg of the test compound was placed in the cup. The plates were left to stay for an hour in order to facilitate the diffusion of the drug solution. The same procedure was followed for the remaining compounds. Negative controls were prepared using the same solvent (DMF) employed to dissolve the test compounds. Then the plates were incubated at 28 °C for 48 h. The zone of inhibition, if any, against the test fungi were measured in mm. Nystatin was used as positive reference to determine the sensitivity of each fungal species tested. The results were subjected to statistical analysis.

Acknowledgements

One of the authors (M.K.M.) is grateful to the University Grants Commission for awarding the fellowship; the management of Taranath Shikshana Samsthe for permitting her to pursue Ph.D.; Dr. B.V. Suresh Kumar, Lecturer in Geology, for the IR analysis.

References

[1] J.H. Smith, J.H. Heidema, E.T. Kaiser, J.B. Wetherington, J.W. Moncrief, J. Am. Chem. Soc. 94 (1972) 9274–9276.
[2] R. Faragher, T.L. Gilchrist, J. Chem. Soc. Perkin Trans. 1 (1979) 258–262.
[3] T.L. Gilchrist, Chem. Soc. Rev. 12 (1983) 53–73 and references cited therein.
[4] T.L. Gilchrist, T.G. Roberts, J. Chem. Soc. Perkin Trans. 1 (1983) 1283–1291.
[5] A. Defoin, H. Sarazin, C. Strehler, J. Streith, Tetrahedron Lett. 35 (1994) 5653–5656.
[6] R. Henning, U. Learch, H. Urbach, Synthesis (1989) 265–268.
[7] P. Bach, M. Bols, Tetrahedron Lett. 40 (1999) 3461–3464.
[8] M. Miyashita, B.Z.E. Awen, A. Yoshikoshi, Tetrahedron 46 (1990) 7569–7586.
[9] O. Werbitzky, K. Klier, H. Felber, Liebigs Ann. Chem. (1990) 261–270.
[10] G.E. Keck, D.R. Romer, J. Org. Chem. 58 (1993) 6083–6089.
[11] M. Naruse, S. Aoyagi, C. Kibayashi, Tetrahedron Lett. 35 (1994) 595–598.
[12] P.G. Tsoungas, Heterocycles 57 (2002) 1149–1178.
[13] P.G. Tsoungas, Heterocycles 57 (2002) 915–953.
[14] G.W. Kirby, Chem. Soc. Rev. 6 (1977) 1–24.
[15] K.M. Patel, T.M. Stevenson, W.O. Patent 9 209 587, 1992.
[16] R. Zimmer, T. Arnold, K. Homann, H.-U. Reissig, Synthesis (1994) 1050–1056.
[17] R. Zimmer, M. Colias, M. Roth, H.-U. Reissig, Liebigs Ann. Chem. (1992) 709–714.
[18] W. Oppolzer, K. Battig, K. Hudlicky, Tetrahedron 37 (1981) 4359–4364.
[19] S. Nakanishi, Y. Shirai, K. Takahashi, Y. Otsuji, Chem. Lett. 10 (1981) 869–872.
[20] J. Angemann, K. Homann, H.-U. Reissig, R. Zimmer, Synlett 10 (1995) 1014–1016.
[21] T.L. Gilchrist, D.A. Lingham, T.G. Roberts, J. Chem. Soc. Chem. Commun. (1979) 1089–1090.
[22] H.A. Brandman, R.T. Conley, J. Org. Chem. 38 (1973) 2236–2238.
[23] R. Zimmer, H.U. Reissig, J. Org. Chem. 57 (1992) 339–347.
[24] T.L. Gilchrist, T.G. Roberts, J. Chem. Soc. Chem. Commun. (1978) 847–849.
[25] S.C. Yoon, K. Kim, Y.J. Park, J. Org. Chem. 66 (2001) 7334–7341.
[26] J. Streith, A. Defoin, Synlett 3 (1996) 189–193.
[27] A. Defoin, H. Sarazin, J. Streith, Tetrahedron 53 (1997) 13769–13782.
[28] W.R. Bowman, R.V. Davies, A.M.Z. Slavin, G.S. Sohal, R.B. Titman, D.J. Wilkins, J. Chem. Soc. Perkin Trans. 1 (1997) 155–158.
[29] H. Ali Dondas, R. Grigg, M. Hadjisoteriou, J. Markandu, P. Kennewell, M. Thornton-Pett, Tetrahedron 57 (2001) 1119–1128.
[30] M. Tiecco, L. Testaferri, M. Tingoli, L. Bagnoli, F.J. Marini, J. Chem. Soc. Perkin Trans. 1 (1993) 1989–1993.
[31] R. Grigg, M. Hadjisoteriou, P. Kennewell, J. Markandu, J. Chem. Soc. Chem. Commun. (1992) 1537–1538.
[32] U. Albrecht, K. Gerwien, P. Langer, J. Org. Chem. 71 (2006) 2293–2301.
[33] S.L. Gaonkar, K.M.L. Rai, J. Heterocyclic Chem. 42 (2005) 877–881.
[34] S.D. Larsen, C.H. Spilman, U.S. Patent 5 523 318, 1996.
[35] Anon, Pharmacopoeia of India, New Delhi, 1996.