European Journal of Medicinal Chemistry 44 (2009) 3898–3902

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and pharmacological evaluation of 1,3,4-oxadiazole bearing bis(heterocycle) derivatives as anti-inflammatory and analgesic agents

B. Jayashankar [a,*], K.M. Lokanath Rai [a], N. Baskaran [b], H.S. Sathish [b]

[a] Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570006, India
[b] Department of Biochemistry, Justice K.S. Hegde Medical Academy, Mangalore 574160, India

ARTICLE INFO

Article history:
Received 14 August 2008
Received in revised form
30 March 2009
Accepted 2 April 2009
Available online 14 April 2009

Keywords:
Bis(heterocycle)
Anti-inflammatory
Analgesic
1,3,4-Oxadiazole
Chloramine-T

ABSTRACT

A series of novel ether-linked bis(heterocycle)s have been synthesized via [3 + 2]-cycloaddition reaction of nitrile oxide with allyl alcohol followed by intramolecular 1,3-diploar cycloaddition reaction of nitrile imine with carbonyl group. All the newly synthesized compounds were screened for their anti-inflammatory and analgesic activities. Among the list of compounds (**7a**–**k**) studied, **7d**, **7g**, **7j**, and **7k** exhibited excellent activity comparable to ibuprofen and aspirin at the similar dosages.

1. Introduction

The wide occurrence of the heterocycles in bioactive natural products and pharmaceuticals has made them as important synthetic targets. Isoxazoline and 1,3,4-oxadiazoles represent a class of heterocyclic compounds of great importance in biological chemistry. For instance, compounds possessing 1,3,4-oxadiazole moiety show anticancer [1] and tyrosinase inhibitory activity [2]. Substituted 1,3,4-oxadiazoles have revealed antibacterial [3], antimycobacterial [4], antifungal [5], anti-inflammatory [6], analgesic [7], anticonvulsant [8], antihypoglycemic [9] and insecticidal properties [10]. Isoxazoline possesses biological activities like [11] (insecticidal, antibacterial, antibiotic, antitumour, antifungal, etc). In fact, Valdecoxib an isoxazoline derivative, is now widely used in the market as anti-inflammatory drug [12]. Literature studies reveal that 2,5-disubstituted-1,3,4-oxadiazoles have been synthesized either by microwave irradiation of the hydrazide and carboxylic acid mixture [13] or by thermal/acid catalyzed cyclization of 1,2-diacylhydrazines [14]. 2,5-Disubstituted-1,3,4-oxadiazoles have also been synthesized by oxidative cyclization of semicarbazone/hydrazone using chloramine-T as an oxidant [15].

1,3-Dipolar cycloaddition reactions are useful tools for constructing isoxazoline [16] and nitrile oxides serve as excellent 1,3-dipoles. Cycloaddition of nitrile oxide to olefinic compounds is of synthetic interest, since the product isoxazolines obtained are the versatile intermediates for the synthesis of bifuctional compounds [17]. Although various bis(heterocycle)s have been synthesized our attention was directed to the recent work of Padmavathi et al., who synthesized isoxazoline bearing bis(heterocycle) by the reaction of bischalcones [18] and bissulfones [19] as dipolarophiles with nitrile oxide as 1,3-dipole.

In our laboratory Rai and Hassner extensively used chloramine-T for the generation of nitrile oxide and nitrile imine from aldoxime and aldehyde hydrazone respectively [20]. For instance, we have recently reported the synthesis of ether-linked bis(isoxazoline) via 1,3-dipolar cycloaddition reactions of nitrile oxides with allyl alcohol and allyl ethers [21]. With this background, it is considered worthwhile to prepare bis(heterocycle) bearing both isoxazoline and 1,3,4-oxadiazole moieties starting from simple aromatic aldoxime and screen them for anti-inflammatory and analgesic activities. The present communication deals with the synthesis and pharmacological evaluation of hitherto unknown ether-linked bis(heterocycle) bearing 1,3,4-oxadiazole and an isoxazoline unit.

2. Results and discussion

2.1. Chemistry

The desired starting materials **1a**–**e** were prepared by the condensation of hydroxyl amine with corresponding aromatic

* Corresponding author. Tel.: +91 821 2419667.
E-mail address: bjshankar@yahoo.com (B. Jayashankar).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.04.006

aldehydes by employing well known method available in the literature [21]. Aromatic aldoximes **1a–e** were subjected to oxidative dehydrogenation using chloramine-T afforded expected nitrile oxides, which were intercept *in situ* by ethanolic solution of allyl alcohol. The reaction was carried under reflux conditions to obtain the pale yellow oils of (4,5-dihydro-3-arylisoxazol-5-yl)methanol **2a–e**.

The (4,5-dihydro-3-arylisoxazol-5-yl)methanol **2a–e** were further treated with ethyl chloroacetate in the presence of phase transfer catalyst such as tetrabutylammonium bromide and potassium hydroxide to get the etherialester of 4,5-dihydro-3-aryl-isoxazole **3a–e** as yellow oils. The esters **3a–e** were refluxed with hydrazine hydrate in ethanol to obtain the hydrazides **4a–e**, which were further condensed with aromatic aldehydes **5a–g** to yield the respective hydrazones **6a–k**. The oxidation of hydrazones **6a–k** with chloramine-T to generate nitrile imines followed by intramolecular cyclization with adjacent carbonyl group yielded the pale yellow solid, identified by NMR spectroscopy as 2-(((4,5-dihydro-3-aryl-isoxazol-5-yl)methoxy)methyl)-5-aryl-1,3,4-oxadiazole **7a–k** (Scheme 1).

^{1}H NMR spectra of compound **7a** showed multiplet at δ 3.88–3.98 ppm and doublet of doublet at 4.29 ppm ($J = 8.2$, 2.2 Hz) correspond to OCH_2 group attached to the isoxazoline and 1,3,4-oxadiazole respectively. Another multiplet (m) at δ 5.10 corresponds to the methine proton, doublet of doublet at δ 3.37 ppm ($J = 8.0$, 2.4 Hz) corresponds to the methylene proton of the isoxazoline ring. Aromatic protons and other substituents are at the expected region. Absence of amide NH frequency in the region 3400–3200 cm^{-1} confirms the formation of the product. The ^{13}C NMR and elemental analysis data further confirm the structures of **7a–k**.

2.2. Pharmacology

All the compounds were tested for anti-inflammatory activity in carrageenen-induced edema assay in rats at a dosage of 100 mg/kg po (Table 1). Four compounds (**7d**, **7g**, **7j**, and **7k**) showed significant activity. Among these compounds, the two dichlorophenyl derivatives, **7g** and **7j** revealed more than 50% activity. However at all of the doses they were less active than ibuprofen. Further, all of these compounds were tested for analgesic activity at 100 mg/kg in acetic acid-induced assay in mice (Table 2). Five compounds had significant activity and the compound **7g** exhibited the highest activity in the series. The compounds of statistical significance were also evaluated for their acute gastrolesivity in rats (at 200 mg/kg os dose): for all the compounds tested no acute gastrolesivity effect was detected in each of the eight treated rats (0/8), whereas indomethacin caused gastric ulcers in 7 of the 8 treated rats (7/8) at 10 mg/kg os dose [22].

3. Experimental section

3.1. Chemistry

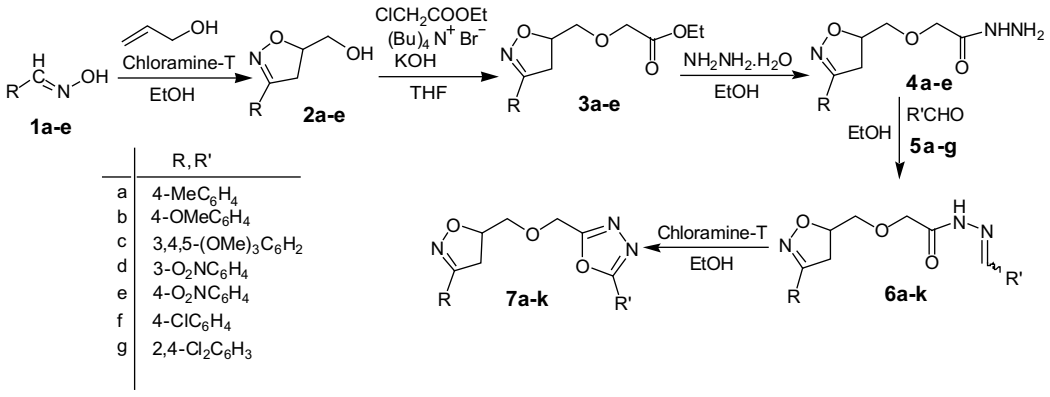
Melting points were determined on Thomas Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM 300 MHz spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard. ^{13}C NMR spectra were measured on Jeol 400 (100 MHz) instrument. The chemical shifts are expressed in δ and following abbreviations were used. s = singlet, d = doublet, t = triplet and m = multiplet. Infrared (IR) spectra were recorded on Shimadzu 8300 IR spectrometer. Elemental analyses were obtained on a Vario-EL instrument. Thin layer chromatography was carried out with BDH silica gel G on glass slides.

3.1.1. Procedure for the synthesis of (4,5-dihydro-3-arylisoxazol-5-yl) methanol 2(a–e) [21]

An equimolar mixture of **1**(**a–e**) (7.40 mmol) and chloramine-T trihydrate (7.41 mmol) in ethanol (20 mL) was stirred for 5 min at room temperature. To this mixture, allyl alcohol (7.41 mmol) in ethanol (5 mL) was added and warmed on a water bath for 3 h. After completion of the reaction (monitored by TLC), it was cooled to room temperature. Sodium chloride formed in the reaction mixture was filtered off and washed with ethanol (1 × 15 mL). The combined filtrate and washings were evaporated in vacuum. The residual part was extracted into ether (25 mL), washed successively with water (2 × 15 mL), 5% NaOH (2 × 15 mL) and saturated brine solution (1 × 10 mL). The organic layer was dried over anhydrous sodium sulphate. The solvent was evaporated and the remaining residue was subjected to the column chromatography using the chloroform–acetone (9:1) as eluent to get the **2**(**a–e**) as yellow oil. The same procedure was used for all.

3.1.2. Procedure for the synthesis of ethyl 2-((4,5-dihydro-3-phenylisoxazol-5-yl)methoxy)acetate 3(a–e)

A mixture of **2**(**a–e**) (5.23 mmol) and ethyl chloroacetate (5.24 mmol) was stirred overnight in the presence of tetrabutylammonium bromide (0.52 mmol) and powdered KOH (5.34 mmol) in THF (20 mL). After completion of the reaction, the mixture was diluted with (25 mL) water and product was extracted with the ethyl acetate (25 mL). The extract was washed with water (10 mL) and then dried (Na_2SO_4). The solvent was evaporated and the remaining pale



Scheme 1.

Table 1
Anti-inflammatory activity and acute gastrolesivity of compounds **7a–k**.

Compound	Edema volume[b] (mL) ± SD	Edema inhibition[c] (%)	Acute gastrolesivity in rat[g]
7a	0.27 ± 0.04[d]	42.5	–
7b	0.31 ± 0.07[e]	40.3	–
7c	0.32 ± 0.08[d]	32.0	–
7d	0.25 + 0.05[d]	46.8[a]	0/8
7e	0.36 ± 0.07[f]	37.9	–
7f	0.32 ± 0.05[e]	38.4	–
7g	0.22 ± 0.04[e]	53.1[a]	0/8
7h	0.27 ± 0.08[d]	42.5	0/8
7i	0.30 ± 0.05[d]	41.2	–
7j	0.25 ± 0.06[e]	51.9[a]	0/8
7k	0.27 ± 0.08[e]	48.0[a]	0/8
Ibuprofen	0.17 ± 0.06[f]	70.7[a]	–

[a] Statistically significant.
[b] At 100 mg/kg po, edema volume measured 3 h after carrageenen injection, and expressed as mean ± standard deviations (n = 4).
[c] Percentage of edema inhibition calculated by comparing with the vehicle-treated control animals.
[d] Control edema volume = 0.47 ± 0.04.
[e] Control edema volume = 0.52 ± 0.03.
[f] Control edema volume = 0.58 ± 0.05.
[g] Number of rats showing gastric lesions. For indomethacin gastrolesivity see Ref. [22].

yellow oily substance **3**(**a**–**e**) was purified by column chromatography (chloroform–acetone 9:1) and used for next step.

*3.1.3. Procedure for the synthesis of 2-((4,5-dihydro-3-phenylisoxazol-5-yl)methoxy)acetohydrazide **4**(**a**–**e**)*

The esters **3**(**a**–**e**) (3.61 mmol) were added drop wise to the 98% hydrazine hydrate (3 mL) with stirring and refluxed for 15 min. After that ethanol (15 mL) was added and refluxed for another 2 h. After completion of the reaction (TLC chloroform–acetone 9:1), the reaction mixture was cooled and extracted into ethyl acetate (25 mL), washed with water (2 × 15 mL) and dried (Na_2SO_4). Solvent was evaporated to yield **4**(**a**–**e**).

*3.1.4. Procedure for the synthesis 2-((4,5-dihydro-3-phenylisoxazol-5-yl)methoxy)-N′-benzylideneacetohydrazides **6**(**a**–**k**)*

An equimolar mixture of **4**(**a**–**e**) (3.80 mmol) and **5**(**a**–**g**) (3.82 mmol) was refluxed in ethanol for 3 h. The progress of the reaction was monitored by the TLC (chloroform–acetone 9.5:0.5).

Table 2
Analgesic activity of compounds **7a–k**.

Compound	No. of writhes in 15 min ± SD[a]	% Reduction from control[b]
7a	42 ± 11[c]	41.6
7b	43 ± 08[d]	36.8
7c	38 ± 07[d]	44.1
7d	31 + 12[e]	50.8[f]
7e	44 ± 06[c]	38.8
7f	38 ± 08[e]	39.7
7g	30 ± 14[e]	52.3[f]
7h	36 ± 12[d]	47.0[f]
7i	40 ± 07[c]	44.4
7j	35 ± 10[c]	51.4[f]
7k	40 ± 06[d]	48.5[f]
Aspirin	29 ± 06[d]	57.3[f]

[a] At 100 mg/kg po, number of writhes in 15 min beginning 5 min after acetic acid injection, expressed mean ± standard deviation (n = 6).
[b] Percentage of writhing inhibition calculated by comparing with vehicle-treated control animals.
[c] Control number of writhes = 72 ± 11.
[d] Control number of writhes = 68 ± 7.
[e] Control number of writhes = 63 ± 7.
[f] Statistically significant.

After completion of the reaction, the mass was cooled and the solid formed was filtered to give acetohydrazone **6**(**a**–**k**), which was used directly for next reaction.

*3.1.5. Synthesis of 2-(((4,5-dihydro-3-arylisoxazol-5-yl) methoxy)methyl)-5-aryl-1,3,4-oxadiazoles: **7**(**a**–**k**)*

*3.1.5.1. Synthesis of 2-(((4,5-dihydro-3-p-tolylisoxazol-5-yl)methoxy)methyl)-5-(3,4,5-trimethoxy phenyl)-1,3,4-oxadiazole: typical procedure (**7a**).* A mixture of **6a** (1.0 g, 2.26 mmol) and chloramine-T trihydrate (0.64 g, 2.27 mmol) in ethanol (20 mL) was refluxed under stirring for 3 h. It was then concentrated under reduced pressure and the residue was extracted with diethyl ether (25 mL). The extract was washed with water (15 mL), 1 N NaOH (2 × 10 mL) and then dried (Na_2SO_4). The solvent was evaporated and the remaining residue was subjected to the column chromatography using chloroform–acetone (9:1) as eluent. Compound **7a** obtained as yellow solid (0.82 g, 72%), m.p. 168–170 °C. The same procedure was used for all. ^{1}H NMR $CDCl_3$: 2.30 (s, 3H, CH_3), 3.37 (dd, 2H, J = 8 Hz, 2.4 Hz CH_2), 3.83 (s, 6H, OCH_3), 3.86 (s, 3H, OCH_3), 3.88–3.98 (m, 2H, OCH_2), 4.29 (dd, 2H, J = 8.2 Hz, 2.2 Hz, OCH_2), 5.10 (m, 1H, CH), 6.80 (s, 2H, ArH), 7.11–7.50 (m, 4H, ArH). ^{13}C NMR $CDCl_3$: 24.9 (C), 37.5 (C), 56.7 (2C), 57.1 (C), 71.1 (C), 75.6 (C), 78.2 (C), 104.7 (2C), 120.6 (C), 129.1 (2C), 129.3 (2C), 131.1 (C), 139.2 (C), 140.2 (C), 151.2 (2C), 156 (C), 160 (C), 164.5 (C). IR (KBr pellets cm^{-1}) ν 3104, 2944, 1688, 1672, 1668, 1390, 1288, 1265. Anal. Calcd for $C_{23}H_{25}N_3O_6$; C, 62.86; H, 5.73; N, 9.56; Found C, 62.95; H, 5.77; N, 9.53%.

*3.1.5.2. (((4,5-Dihydro-3-p-tolylisoxazol-5-yl)methoxy)methyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (**7b**).* Obtained from **6b** (1.0 g, 2.6 mmol) and chloramine-T trihydrate (0.73 g, 2.6 mmol) as yellow solid (0.69 g, 69%), m.p. 157–159 °C. ^{1}H NMR $CDCl_3$: 2.33 (s, 3H, CH_3), 3.37 (dd, 2H, J = 8.0 Hz, 2.4 Hz, CH_2), 3.86–3.96 (m, 2H, OCH_2), 4.32 (dd, 2H, J = 8.2 Hz, 2.4 Hz, OCH_2), 5.13 (m, 1H, CH), 7.13–7.52 (m, 8H, ArH), ^{13}C NMR $CDCl_3$: 24.2 (C), 37.4 (C), 70.4 (C), 75.4 (C), 78.1 (C), 124.6 (C), 128.9 (2C), 129.1 (2C), 129.3 (2C), 129.5 (2C), 131.1 (C), 134.5 (C), 140.9 (C), 156.6 (C), 161.1 (C), 164.5 (C). IR (KBr pellets cm^{-1}) ν 3071, 2924, 1681, 1666, 1648, 1382, 1283, 1260. Anal. Calcd for $C_{20}H_{18}ClN_3O_3$; C, 62.58; H, 4.73; N, 10.95; Found C, 62.63; H, 4.77; N, 10.92%.

*3.1.5.3. 2-(((4,5-Dihydro-3-p-tolylisoxazol-5-yl)methoxy)methyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (**7c**).* Obtained from **6c** (1.0 g, 2.52 mmol) and chloramine-T trihydrate (0.70 g, 2.52 mmol) as yellow solid (0.76 g, 76%), m.p. 163–165 °C. ^{1}H NMR $CDCl_3$: 2.36 (s, 3H, CH_3), 3.36 (dd, 2H, J = 8.0 Hz, 2.4 Hz, CH_2), 3.83–3.93 (m, 2H, OCH_2), 4.30 (dd, 2H, J = 8.2 Hz, 2.3 Hz, OCH_2), 5.05 (m, 1H, CH), 7.13 (d, 2H, ArH), 7.51 (d, 2H, ArH), 7.71 (d, 2H, ArH), 8.23 (d, 2H, ArH). ^{13}C NMR $CDCl_3$: 24.7 (C), 37.6 (C), 70.8 (C), 75.2 (C), 78.1 (C), 121.6 (2C), 128.5 (2C), 129.2 (2C), 129.4 (2C), 131.1 (C), 132.2 (C), 140.9 (C), 148.5 (C), 156.2 (C), 160.8 (C), 164.5 (C). IR (KBr pellets cm^{-1}) ν 3092, 2949, 1688, 1670, 1661, 1383, 1278, 1261. Anal. Calcd for $C_{20}H_{18}N_4O_5$; C, 60.91; H, 4.60; N, 14.21; Found C, 60.95; H, 4.67; N, 14.19%.

*3.1.5.4. 2-(((4,5-Dihydro-3-p-tolylisoxazol-5-yl)methoxy)methyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (**7d**).* Obtained from **6d** (1.0 g, 2.38 mmol) and chloramine-T trihydrate (0.68 g, 2.42 mmol) as yellow solid (0.66 g, 67%), m.p. 151–153 °C. ^{1}H NMR $CDCl_3$: 2.34 (s, 3H, CH_3), 3.39 (dd, 2H, J = 8.3 Hz, 2.2 Hz, CH_2), 3.86–3.96 (m, 2H, OCH_2), 4.30 (dd, 2H, J = 8.3 Hz, 2.2 Hz, OCH_2), 5.12 (m, 1H, CH), 7.12 (d, 2H, ArH), 7.51 (d, 2H, ArH), 7.28 (d, 2H, ArH), 7.63 (s, 1H, ArH). ^{13}C NMR $CDCl_3$: 24.5 (C), 37.3 (C), 70.7 (C), 75.3 (C), 78.0 (C), 127.6 (C), 129.0 (2C), 129.3 (2C), 130.3 (C), 130.9 (C), 131.0 (C), 133.9 (C), 135.2 (C), 135.9 (C), 140.6 (C), 156.5 (C), 161.1 (C), 164.5 (C). IR (KBr pellets cm^{-1}) ν 3101, 2962, 1683, 1677, 1670, 1392, 1282, 1262. Anal. Calcd

for $C_{20}H_{17}C_{12}N_3O_3$; C, 57.43; H, 4.10; N, 10.05; Found C, 57.45; H, 4.17; N, 10.02%.

*3.1.5.5. 2-(((4,5-Dihydro-3-(4-methoxyphenyl)isoxazol-5-yl)methoxy)methyl)-5-p-tolyl-1,3,4-oxadiazole (**7e**).* Obtained from **6e** (1.0 g, 2.67 mmol) and chloramine-T trihydrate (0.75 g, 2.67 mmol) as yellow oil (0.72 g, 73%). ^{1}H NMR $CDCl_3$: 2.33 (s, 3H, CH_3), 3.28 (dd, 2H, $J = 8.2$ Hz, 2.2 Hz, CH_2), 3.76 (s, 3H, OCH_3), 3.79–3.89 (m, 2H, OCH_2), 4.21 (dd, 2H, $J = 8.2$ Hz, 2.2 Hz, OCH_2), 4.98 (m, 1H, CH), 6.90 (d, 2H, ArH), 7.11 (d, 2H, ArH), 7.22 (s, 2H, ArH), 7.53 (d, 2H, ArH). ^{13}C NMR $CDCl_3$: 24.6 (C), 37.2 (C), 56.1 (C), 70.8 (C), 75.4 (C), 78.0 (C), 114.7 (2C), 123.4 (C), 126.4 (C), 127.1 (2C), 129.3 (2C), 130.5 (2C), 138.5 (C), 156.2 (C), 158.9 (C), 163.1 (C), 164.6 (C). IR (KBr pellets cm^{-1}) ν 3036, 2932, 1678, 1662, 1648, 1387, 1283, 1260. Anal. Calcd for $C_{21}H_{21}N_3O_4$; C, 66.48; H, 5.58; N, 11.08; Found C, 66.45; H, 5.57; N, 11.05%.

*3.1.5.6. 2-(((4,5-Dihydro-3-(4-methoxyphenyl)isoxazol-5-yl)methoxy)methyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (**7f**).* Obtained from **6f** (1.0 g, 2.43 mmol) and chloramine-T trihydrate (0.7 g, 2.49 mmol) as yellow oil (0.77 g, 78%). ^{1}H NMR $CDCl_3$: δ 3.27 (dd, 2H, $J = 8.2$ Hz, 2.2 Hz, CH_2), 3.76 (s, 3H, OCH_3), 3.83–3.93 (m, 2H, OCH_2), 4.28 (dd, 2H, $J = 8.4$ Hz, 2.5 Hz, OCH_2), 5.07 (m, 1H, CH), 6.88 (d, 2H, ArH), 7.33 (d, 2H, ArH), 7.75 (d, 2H), 8.26 (d, 2H). ^{13}C NMR $CDCl_3$: δ 37.5 (C), 56.2 (C), 70.8 (C), 75.5 (C), 78.3 (C), 114.7 (2C), 121.6 (2C), 126.4 (C), 128.3 (2C), 130.1 (2C), 132.2 (C), 148.2 (C), 156.7 (C), 160.0 (C), 163.1 (C), 164.5 (C). IR (KBr pellets cm^{-1}) ν 3087, 2964, 1694, 1682, 1667, 1391, 1281, 1270. Anal. Calcd for $C_{20}H_{18}N_4O_6$; C, 58.53; H, 4.42; N, 13.65; Found C, 58.55; H 4.47; N, 13.59%.

*3.1.5.7. 2-(((4,5-Dihydro-3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methoxy)methyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (**7g**).* Obtained from **6g** (1.0 g, 2.02 mmol) and chloramine-T trihydrate (0.58 g, 2.06 mmol) as yellow solid (0.65 g, 64%), m.p. 168–170 °C. ^{1}H NMR $CDCl_3$: 3.22 (dd, 2H, $J = 8.0$ Hz, 2.0 Hz, CH_2), 3.88 (s, 6H, OCH_3), 3.83 (s, 3H, OCH_3), 3.81–3.91 (m, 2H, OCH_2), 4.29 (dd, 2H, $J = 8.2$ Hz, 2.3 Hz, OCH_2), 5.01 (m, 1H, CH), 6.8 (s, 2H, ArH), 7.33 (s, 2H, ArH), 7.43 (s, 1H, ArH). ^{13}C NMR $CDCl_3$: 37.4 (C), 56.4 (2C), 56.8 (C), 70.8 (C), 75.3 (C), 78.1 (C), 106.7 (2C), 127.6 (C), 128.1 (C), 130.4 (C), 130.9 (C), 133.6 (C), 135.1 (C), 135.8 (C), 141.5 (C), 151.1 (2C), 156.5 (C), 159.8 (C), 164.7 (C). IR (KBr pellets cm^{-1}) ν 3058, 2934, 1678, 1665, 1656, 1380, 1278, 1256. Anal. Calcd for $C_{22}H_{21}Cl_2N_3O_6$: C, 53.45; H, 4.28; N, 8.50; Found C, 53.46; H, 4.27; N, 8.53%.

*3.1.5.8. 2-(((4,5-Dihydro-3-(3-nitrophenyl)isoxazol-5-yl)methoxy)methyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**7h**).* Obtained from **6h** (1.0 g, 2.11 mmol) and chloramine-T trihydrate (0.6 g, 2.13 mmol) as yellow solid (0.80 g, 79%), m.p. 177–179 °C. ^{1}H NMR $CDCl_3$: 3.34 (dd, 2H, $J = 8.3$ Hz, 2.0 Hz, CH_2), 3.86 (s, 6H, OCH_3), 3.80 (s, 3H, OCH_3), 3.79–3.89 (m, 2H, OCH_2), 4.26 (dd, 2H, $J = 8.2$ Hz, 2.0 Hz, OCH_2), 5.14 (m, 1H, CH), 6.48 (s, 2H, ArH), 7.89 (d, 2H, ArH), 8.26 (d, 2H, ArH), ^{13}C NMR $CDCl_3$: 37.7 (C), 56.3 (2C), 56.6 (C), 71.4 (C), 75.5 (C), 78.0 (C), 104.7 (2C), 120.7 (C), 123.3 (C), 124.2 (C), 130.2 (C), 134.9 (C), 135.3 (C), 139.2 (C), 148.2 (C), 151.4 (2C), 156.8 (C), 160.7 (C), 164.4 (C). IR (KBr pellets cm^{-1}) ν 3104, 2945, 1688, 1675, 1662, 1387, 1281, 1260. Anal. Calcd for $C_{22}H_{22}N_4O_8$; C, 56.17; H, 4.71; N, 11.91; Found C, 56.15; H, 4.74; N, 11.90%.

*3.1.5.9. 2-(((4,5-Dihydro-3-(3-nitrophenyl)isoxazol-5-yl)methoxy)methyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (**7i**).* Obtained from **6i** (1.0 g, 2.40 mmol) and chloramine-T trihydrate (0.68 g, 2.41 mmol) as yellow solid (0.80 g, 79%), m.p. 168–170 °C. ^{1}H NMR $CDCl_3$: 3.36 (dd, 2H, $J = 8.4$ Hz, 2.2 Hz, CH_2), 3.84–3.96 (m, 2H, OCH_2), 4.27 (dd, 2H, $J = 8.0$ Hz, 2.2 Hz, OCH_2), 5.12 (m, 1H, CH), 7.31 (d, 2H, ArH), 7.42 (d, 2H, ArH), 7.60 (d, 2H, ArH), 8.32 (d, 2H, ArH), ^{13}C NMR $CDCl_3$: 37.7 (C), 71.2 (C), 75.6 (C), 78.0 (C), 123.7 (C), 124.2 (C), 124.7 (C), 128.8 (2C), 129.8 (2C), 129.9 (C), 134.4 (C), 134.9 (C), 135.2 (C), 148.2 (C), 156.8 (C), 160.1 (C), 164.4 (C). IR (KBr pellets cm^{-1}) ν 3109, 2941, 1689, 1676, 1670, 1393, 1282, 1265. Anal. Calcd for $C_{19}H_{15}ClN_4O_5$; C, 55.02; H, 3.64; N, 13.51; Found C, 55.05; H, 3.62; N, 13.53%.

*3.1.5.10. 2-(((4,5-Dihydro-3-(4-nitrophenyl)isoxazol-5-yl)methoxy)methyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (**7j**).* Obtained from **6j** (1.0 g, 2.21 mmol) and chloramine-T trihydrate (0.63 g, 2.24 mmol) as yellow solid (0.67 g, 68%), m.p. 177–179 °C. ^{1}H NMR $CDCl_3$: 3.33 (dd, 2H, $J = 7.8$ Hz, 2.0 Hz, CH_2), 3.86–3.97 (m, 2H, OCH_2), 4.30 (dd, 2H, $J = 8.4$ Hz, 2.2 Hz, OCH_2), 5.13 (m, 1H, CH), 7.31 (d, 2H, ArH), 7.40 (s, 1H, ArH), 7.61 (d, 2H, ArH), 8.11 (d, 2H, ArH), ^{13}C NMR $CDCl_3$: 37.9 (C), 71.5 (C), 75.6 (C), 78.2 (C), 121.7 (2C), 127.2 (C), 130.0 (2C), 130.4 (C), 130.9 (C), 133.8 (C), 135.2 (C), 135.8 (C), 140.2 (C), 150.8 (C), 156.8 (C), 164.7 (C). IR (KBr pellets cm^{-1}) ν 3112, 2950, 1693, 1677, 1665, 1393, 1290, 1269. Anal. Calcd for $C_{19}H_{14}C_{12}N_4O_5$; C, 50.80; H, 3.14; N, 12.47; Found C, 50.82; H, 3.12; N, 12.49%.

*3.1.5.11. 2-(((4,5-Dihydro-3-(4-nitrophenyl)isoxazol-5-yl)methoxy)methyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**7k**).* Obtained from **6k** (1.0 g, 2.11 mmol) and chloramine-T trihydrate (0.6 g, 2.13 mmol) as yellow solid (0.81 g, 81%), m.p. 186–188 °C. ^{1}H NMR $CDCl_3$: 3.31 (dd, 2H, $J = 7.8$ Hz, 2.4 Hz, CH_2), 3.81 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 3.76–3.86 (m, 2H, OCH_2), 4.20–4.41 (dd, 2H, $J = 8.2$ Hz, 2.3 Hz, OCH_2), 5.14 (m, 1H, CH), 6.56 (s, 2H, ArH), 7.60 (d, 2H, ArH), 8.10 (d, 2H, ArH), ^{13}C NMR $CDCl_3$: 37.8 (C), 56.3 (2C), 56.6 (C), 71.6 (C), 75.7 (C), 78.3 (C), 104.8 (2C), 120.4 (C), 121.6 (2C), 130.2 (2C), 139.7 (C), 140.2 (C), 150.8 (C), 151.5 (2C), 156.7 (C), 161.1 (C), 164.5 (C). IR (KBr pellets cm^{-1}) ν 3114, 2948, 1691, 1677, 1670, 1389, 1290, 1267. Anal. Calcd for $C_{22}H_{22}N_4O_8$; C, 56.17; H, 4.71; N, 11.91; Found C, 56.18; H, 4.72; N, 11.89%.

3.2. Pharmacology

Albino rats of either sex (150–180 g) and albino mice of either sex (8–25 g) were used. The compounds were administered po using a feeding tube as homogenized suspensions in 0.5% sodium carboxymethyl cellulose; 0.5% sodium carboxymethyl cellulose was administered as the vehicle control.

3.2.1. Carrageenen-induced edema

Groups of four rats were dosed at 100 mg/kg po with the test compounds, 1 h before 0.05 mL of a 1% suspension of Type IV Lambda (Sigma) carrageenen was injected into the subplantar region at the right hind paw; additional groups of four rats were similarly pretreated with 100 mg/kg ibuprofen (positive control) or 10 mL/kg 0.5% sodium carboxymethyl cellulose (vehicle controls) [23]. Paw volumes were measured by water displacement in a plethysmograph immediately after carrageenen injection and again 3 h later. Edema volumes for test-compound-treated and positive-control rats were compared statistically with those for the vehicle-treated control rats; data are reported as percentage edema inhibition.

3.2.2. Gastrolesivity

The acute gastrolesivity of the test compounds was evaluated by examining the stomachs excised 5 h after oral administration of the drugs in rats. The stomachs fixed in 2% formalin, were opened and examined with a stereomicroscope by an observer unaware of the treatment the rats received. Acute gastrolesivity was expressed as the number of animals with gastric damage over the number of treated animals.

3.2.3. Analgesic activity

This method is based on acetic acid-induced writhings in mice [24]. Groups of six mice each were dosed with the test compounds or with aspirin at a dose of 100 mg/kg po, 1 h before the ip injection of 0.6% acetic acid (10 mL/kg). Mice were observed for 1.5 min beginning 5 min after the acetic acid injection and the total number of writhes recorded. The mean value of writhes for each group was calculated and compared statistically with that for the vehicle-treated control group ($n = 6$); data were reported as percent inhibition of the number of writhes. The test was repeated on additional groups of six mice, treated with compounds for which the reduction in writhes had been calculated to be $>10\%$; these results are shown in Table 2.

Acknowledgement

The authors thank Dr. V. Baskaran for his assistance in pharmacological studies.

References

[1] J.J. Bhatt, B.R. Shah, H.P. Shah, P.B. Trivedi, N.K. Undavia, N.C. Desai, Ind. J. Chem. 33B (1994) 189–192.
[2] M.T.H. Khan, M.I. Choudary, K.M. Khan, M. Rani, Atta-ur-Rahman, Bioorg. Med. Chem. 13 (2005) 3385–3395.
[3] B.S. Holla, R. Gonsalves, S. Shenoy, Eur. J. Med. Chem. 35 (2000) 267–271.
[4] F. Macaev, G. Rusu, S. Pogrebnoi, A. Gudima, E. Stingaci, L. Vlad, N. Shvets, F. Kandemirli, A. Dimogio, R. Reynolds, Bioorg. Med. Chem. 13 (2005) 4842–4850.
[5] X. Zou, Z. Zhang, G. Jin, J. Chem. Res. Synop. (2002) 228–230.
[6] E. Palaska, G. Sahin, P. Kelicen, N.T. Durlu, G. Altinok, Farmaco 57 (2002) 101–107.
[7] M. Amir, K. Shikha, Eur. J. Med. Chem. 39 (2004) 535–545.
[8] A. Zarghi, A. Sayyed, M. Tabatabai, A. Faizi, P. Ahadian, V. Navabi, A. Zanganeh, A. Shafiee, Bioorg. Med. Chem. Lett. 15 (2005) 1863–1865.
[9] M.I. Hussain, A. Kumar, R.C. Srivastava, Curr. Sci. 55 (1986) 644–646.
[10] X. Zheng, Z. Li, Y. Wang, W. Chen, O. Huang, C. Liu, C. Song, J. Fluorine Chem. 123 (2003) 163–169.
[11] R. Huisgen, Angew. Chem., Int. Ed. Engl. 2 (1963) 565–598.
[12] G. Dannahardt, W. Kiefer, G. Kramer, S. Maehrlein, U. Nowe, B. Fiebich, Eur. J. Med. Chem. 35 (2000) 499–510.
[13] K.M. Khan, Zia Ullah, M. Rani, S. Perveen, S.M. Haider, M.I. Choudary, Atta-ur-Rahman, W. Voelter, Lett. Org. Chem. 1 (2004) 50.
[14] S. Liras, M.P. Allen, B.E. Segelstein, Synth. Commun. 30 (2000) 437–443.
[15] S.L. Gaonkar, K.M.L. Rai, B. Prabhuswamy, Eur. J. Med. Chem. 41 (2004) 841–846.
[16] P. Caramella, P. Gruinanger, in: A. Padwa (Ed.), 1,3-Dipolar Cycloaddition Chemistry, vol. 1, John Wiley and Sons, New york, 1984, p. 337.
[17] K.B. Umesha, K. Ajay Kumar, K.M. Lokanath Rai, Synth. Commun. 32 (2002) 1841–1846.
[18] V. Padmavathi, B. Jagan Mohan Reddy, B. Chandra Obula, A. Padmaja, Tetrahedron 61 (2005) 2407–2411.
[19] V. Padmavathi, K. Reddy Venugopal, A. Padmaja, Bhaskar D. Reddy, Phosphorus, Sulfur, Silicon 178 (2003) 171–177.
[20] K.M. Lokanath Rai, A. Hassner, Ind. J. Chem. 36B (1997) 242–245.
[21] B. Jayashankar, K.M. Lokanath Rai ISSN: 1551-7012, Arkivoc (2008) 75–85.
[22] G. Grossi, M. Di Braccio, G. Roma, V. Ballabeni, M. Tognolini, E. Ballabeni, Eur. J. Med. Chem. 40 (2005) 155–165.
[23] C.A. Winter, E.A. Risley, G.W. Nuss, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
[24] R. Koster, M. Anderson, E.J. De Beer, Fed. Proc. 18 (1952) 412–421.