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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Design, synthesis, antimicrobial, anti-inflammatory, and analgesic activity of novel dihydrobenzo furo[3,2-*e*]isoxazolo[4,5-*b*] azepin-5(5*aH*)-ones

**E. Rajanarendar · K. Govardhan Reddy · S. Rama Krishna · B. Shireesha · Y. N. Reddy · M. V. Rajam**

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**Abstract** Novel series of dihydro benzofuro[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones **6** have been synthesized from 3,5-dimethyl-4-nitroisoxazole **1**. Compound **1** on treatment with salicyl aldehydes afforded the corresponding nitrostyrylisoxazoles **3**, which upon reaction with ethyl bromo acetate followed by cyclization with triethylamine furnished ethyl 2,3-dihydro-3-[(3-methyl-4-nitro-5-isoxazolyl)methyl] benzofuran-2-carboxylates **5**. Reductive cyclization of compounds **5** was effected with $SnCl_2$–MeOH to give the title compounds **6**. Compounds **4**–**6** were characterized by IR, $^1$H NMR, $^{13}$C NMR and Mass spectral data. The title compounds **6a**–**g** were evaluated for their antimicrobial, anti-inflammatory, LOX-5 inhibitory, and analgesic activity. Compounds **6b** and **6c** exhibited significant antimicrobial activity, potent anti-inflammatory and analgesic activities as that of standard drugs.

**Keywords** Dihydrobenzofuro[3,2-*e*]isoxazolo[4,5-*b*] azepin-5(5*aH*)-ones · Reductive cyclization · Antimicrobial activity · Anti-inflammatory activity · Analgesic activity

E. Rajanarendar (✉) · K. Govardhan Reddy · S. Rama Krishna
Department of Chemistry, Kakatiya University, Warangal 506 009, Andhra Pradesh, India
e-mail: rajanarendareligeti@gmail.com

B. Shireesha · Y. N. Reddy
Department of Pharmacology and Toxicology, Kakatiya University, Warangal 506 009, Andhra Pradesh, India

M. V. Rajam
Department of Genetics, University of Delhi, South Campus, New Delhi 110 02, India

## Introduction

2,3-Disubstituted dihydrobenzofuran moiety is ubiquitous in nature and display remarkably diverse biological properties such as antimicrobial (Pauletti *et al.,* 2000), antimitotic (Pieters *et al.,* 1999), antiangiogenic (Apers *et al.,* 2003), and HIV integrase inhibition activity [Abd-Elazem *et al.,* 2002). Remarkable examples of this class are (+)-conocarpan (**1**) which exhibits insecticidal, antifungal, and antitrypanosomal activity (De Campos *et al.,* 2005), as well as anti-tumor neo-lignane (2*R*, 3*S*)-3′,4-di-*o*-methylcedrusin (**2**) (Pieters *et al.,* 1999). Recently benzofuran-fused azepinone system was identified as potent and selective protein kinase D (PKD) inhibitor (LaValle *et al.,* 2010).

Azepine derivatives such as isoxazolo[4,5-*c*] azepine (**3**) are found to contain many pharmacological applications (Krogsgaard-Larsen *et al.,* 1988), whereas isoxazolo[4,5-*b*] azepine (**4**) and isoxazolo[5,4-*b*] azepine (**5**) (Fig. 1) are active against Gram-positive and Gram-negative bacteria and exhibit cytotoxicity (Rao *et al.,* 1981a, b). Inspired by the biological activity of 2,3-dihydrobenzofuran and isoxazolo azepines, we embarked on the synthesis of title compounds having isoxazolo azepine and 2,3-dihydro benzofuran moieties embedded in a fused molecular framework to improve specificity and efficacy of these scaffolds against microorganisms, and these compounds promise to offer fascinating scaffolds of fundamental interest to both synthetic and medicinal chemists. As a part of our ongoing research in development of new biologically active fused isoxazole derivatives (Rajanarendar *et al.,* 2012a, b, c, d) from readily available starting materials, we envisaged a novel modular synthesis leading to dihydrobenzo furo[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-one derivatives. Herein, we report the discovery of furo[3,2-*e*] isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones from 3,5-dimethyl-4-

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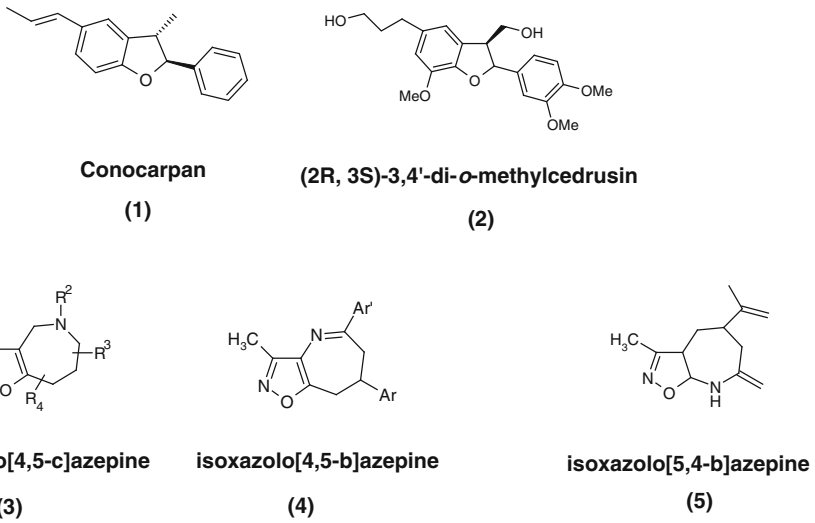



**Fig. 1** Biologically active dihydrobenzofurans and isoxazolo azepines

nitroisoxazole synthon and their antimicrobial, anti-inflammatory, and analgesic activities.

## Experimental

### Synthesis

*General procedure for the synthesis of (E)-2-(2-(3-methyl-4-nitroisoxazole-5-yl)vinyl)phenols (**3a–g**)*

A mixture of 3,5-dimethyl-4-nitroisoxazole **1** (1 mmol) and 2-hydroxy benzaldehyde (1 mmol) in ethanol (10 mL) were heated at 70 °C in the presence of piperidine (0.1 mmol) for 2 h. After completion of reaction (monitored by TLC), the reaction mixture was cooled, and the solid separated was filtered and recrystallized from ethanol to afford the pure product.

Spectral data of each compound are given below

*2-((E)-2-(3-Methyl-4-nitroisoxazol-5-yl)vinyl)phenol (**3a**)* Yield 79 %, m.p. 160–162 °C. IR (KBr) $cm^{-1}$: 3450. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.30 (s, 3H, isoxazole–$CH_3$), 6.62 (d, 1H, CH=CH, $J = 12$ Hz), 6.71 (d, 1H, CH=CH, $J = 12$ Hz), 7.14–7.58 (m, 4H, ArH), 8.52 (s, 1H, OH, $D_2O$ exchangeable). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.39, 102.53, 113.29, 115.28, 120.38, 123.54, 126.74, 128.75, 132.81, 149.65, 157.89, 159.89. ESI-MS (70 eV) *m/z*: 247 $[M+H]^+$. Anal. Calcd for $C_{12}H_{10}N_2O_4$: C, 58.54; H, 4.09; N, 11.38. Found: C, 58.48; H, 4.08; N, 11.41 %.

*4-Methyl-2-((E)-2-(3-methyl-4-nitroisoxazol-5-yl)vinyl)phenol (**3b**)* Yield 82 %, m.p. 157–159 °C. IR (KBr) $cm^{-1}$: 3445. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.28 (s, 3H, isoxazole–$CH_3$), 2.82 (s, 3H, Ar–$CH_3$), 6.64 (d, 1H, CH=CH, $J = 12$ Hz), 6.78 (d, 1H, CH=CH, $J = 12$ Hz), 7.10–7.68 (m, 3H, ArH), 8.60 (s, 1H, OH, $D_2O$ exchangeable). $^{13}C$NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.32, 41.53, 102.49, 113.29, 115.48, 120.29, 123.64, 126.81, 128.68, 132.89, 149.58, 158.03, 159.11. ESI-MS (70 eV) *m/z*: 261 $[M+H]^+$. Anal. Calcd for $C_{13}H_{12}N_2O_4$: C, 60.00; H, 4.65; N, 10.76. Found: C, 60.04; H, 4.69; N, 10.79 %.

*4-Methoxy-2-((E)-2-(3-methyl-4-nitroisoxazol-5-yl)vinyl) phenol (**3c**)* Yield 85 %, m.p. 152–154 °C. IR (KBr) $cm^{-1}$: 3448. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.25 (s, 3H, isoxazole–$CH_3$), 3.68 (s, 3H, Ar $OCH_3$), 6.69 (d, 1H, CH=CH, $J = 12$ Hz), 6.81 (d, 1H, CH=CH, $J = 12$ Hz), 7.10–7.68 (m, 3H, ArH), 8.64 (s, 1H, OH, $D_2O$ exchangeable). $^{13}C$NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.31, 60.97, 101.83, 113.52, 115.39, 120.41, 123.69, 126.79, 128.59, 132.93, 149.61, 158.09, 159.28. ESI-MS (70 eV) *m/z*: 277 $[M+H]^+$. Anal. Calcd for $C_{13}H_{12}N_2O_5$: C, 56.52; H, 4.38; N, 10.14. Found: C, 56.49; H, 4.41; N, 10.10 %.

*4-Chloro-2-((E)-2-(3-methyl-4-nitroisoxazol-5-yl)vinyl) phenol (**3d**)* Yield 87 %, m.p. 168–170 °C. IR (KBr) $cm^{-1}$: 3457. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.28 (s, 3H, isoxazole–$CH_3$), 6.67 (d, 1H, CH=CH, $J = 12$ Hz), 6.73 (d, 1H, CH=CH, $J = 12$ Hz), 7.08–7.68 (m, 3H, ArH), 8.73 (s, 1H, OH, $D_2O$ exchangeable). $^{13}C$NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.29, 102.38, 113.53, 115.42, 120.32, 123.69, 126.92, 128.73, 132.69, 149.72, 158.19, 159.28. ESI-MS (70 eV) *m/z*: 281 $[M+H]^+$. Anal. Calcd for $C_{12}H_9ClN_2O_4$: C, 51.35; H, 3.23; N, 9.98. Found: C, 51.39; H, 3.19; Cl, 12.58; N, 9.95 %.

*2,4-Dichloro-6-((E)-2-(3-methyl-4-nitroisoxazol-5-yl)vinyl) phenol (**3e**)* Yield 81 %, m.p. 163–165 °C. IR (KBr) $cm^{-1}$: 3437. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.25 (s, 3H, isoxazole–$CH_3$), 6.62 (d, 1H, CH=CH, $J = 12$ Hz),

6.73 (d, 1H, CH=CH, $J = 12$ Hz), 7.09–7.98 (m, 2H, ArH), 8.71 (s, 1H, OH, $D_2O$ exchangeable). $^{13}$CNMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.32, 101.83, 113.74, 114.98, 121.09, 123.75, 127.13, 129.03, 132.58, 150.11, 158.32, 159.35. ESI-MS (70 eV) *m/z*: 315 $[M+H]^+$. Anal. Calcd for $C_{12}H_8Cl_2N_2O_4$: C, 45.74; H, 2.56; N, 8.89. Found: C, 45.71; H, 2.60; N, 8.92 %.

*4-Bromo-2-((E)-2-(3-methyl-4-nitroisoxazol-5-yl)vinyl)phenol* ***(3f)*** Yield 83 %, m.p. 170–172 °C. IR (KBr) $cm^{-1}$: 3440. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.24 (s, 3H, isoxazole–$CH_3$), 6.70 (d, 1H, CH=CH, $J = 12$ Hz), 6.79 (d, 1H, CH=CH, $J = 12$ Hz), 7.11–7.69 (m, 3H, ArH), 8.81 (s, 1H, OH, $D_2O$ exchangeable). $^{13}$CNMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.35, 102.23, 113.69, 115.23, 120.97, 123.69, 127.29, 128.75, 133.08, 150.24, 159.32, 160.09. ESI-MS (70 eV) *m/z*: 325 $[M+H]^+$. Anal. Calcd for $C_{12}H_9BrN_2O_4$: C, 44.33; H, 2.79; N, 8.62. Found: C, 44.29; H, 2.82; N, 8.59 %.

*2,4-Dibromo-6-((E)-2-(3-methyl-4-nitroisoxazol-5-yl)vinyl) phenol* ***(3g)*** Yield 88 %, m.p. 173–175 °C. IR (KBr) $cm^{-1}$: 3451. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.27 (s, 3H, isoxazole–$CH_3$), 6.68 (d, 1H, CH=CH, $J = 12$ Hz), 6.81 (d, 1H, CH=CH, $J = 12$ Hz), 7.13–7.75 (m, 3H, ArH), 8.69 (s, 1H, OH, $D_2O$ exchangeable). $^{13}$CNMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.37, 101.79, 113.73, 114.98, 121.24, 123.76, 127.35, 129.17, 132.79, 150.16, 159.52, 160.62. ESI-MS (70 eV) *m/z*: 403 $[M+H]^+$. Anal. Calcd for $C_{12}H_8Br_2N_2O_4$: C, 35.67; H, 2.00; N, 6.93. Found: C, 35.71; H, 2.04; N, 6.89 %.

*General procedure for the synthesis of ethyl 2-[2-[(E)-2-(3-methyl-4-nitroisoxazole-5-yl)vinyl] phenoxy]acetates* ***(4a–g)***

To a solution of **3** (1 mmol), in dry acetone (10 mL), ethyl bromoacetate (1 mmol) and anhydrous $K_2CO_3$ (2 mmol) was added, and the contents were refluxed for 2 h. After the completion of the reaction as indicated by TLC the reaction mixture is filtered and the acetone is evaporated. The crude product is purified by passing through silica gel column by eluting with ethyl acetate:hexane (25:75).

Spectral data of each compound are given below

*(E)-Ethyl 2-[2-[2-(3-methyl-4-nitro-5-isoxazolyl)vinyl] phenoxy]acetate* ***(4a)*** Yield 73 %, m.p. 137–139 °C. IR (KBr) $cm^{-1}$: 1760, 1540, 1326. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.87 (t, 3H, $OCH_2CH_3$), 2.32 (s, 3H, isoxazole–$CH_3$), 4.10 (q, 2H, $OCH_2CH_3$), 5.21 (s, 2H, $OCH_2CO$), 6.62 (d, 1H, CH=CH, $J = 12$ Hz), 6.71 (d, 1H, CH=CH, $J = 12$ Hz), 7.14–7.58 (m, 4H, ArH). .$^{13}$CNMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.29, 14.23, 60.82, 64.69, 99.78, 114.23, 120.75, 122.07, 123.21, 126.51, 128.87, 134.08, 149.76, 157.39, 157.93, 170.78. ESI-MS (70 eV) *m/z*: 333 $[M+H]^+$. Anal. Calcd for $C_{16}H_{16}N_2O_6$: C, 57.83; H, 4.85; N, 8.43. Found: C, 57.88; H, 4.81; N, 8.40 %.

*(E)-Ethyl 2-[4-methyl-2-[2-(3-methyl-4-nitro-5-isoxazolyl) vinyl]phenoxy]acetate* ***(4b)*** Yield 73 %, m.p. 167–169 °C. IR (KBr) $cm^{-1}$: 1763, 1525, 1335. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.90 (t, 3H, $OCH_2CH_3$), 2.27 (s, 3H, isoxazole–$CH_3$), 2.47 (s, 3H, Ar–$CH_3$), 4.21 (q, 2H, $OCH_2CH_3$), 5.18 (s, 2H, $OCH_2CO$), 6.60 (d, 1H, CH=CH, $J = 12$ Hz), 6.75 (d, 1H, CH=CH, $J = 12$ Hz), 7.00–7.62 (m, 3H, ArH). $^{13}$CNMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.34, 14.31, 42.23, 61.07, 64.73, 100.20, 114.37, 120.81, 122.19, 122.98, 127.06, 128.91, 134.15, 150.18, 157.43, 158.11, 169.79. ESI-MS (70 eV) *m/z*: 347 $[M+H]^+$. Anal. Calcd for $C_{17}H_{18}N_2O_6$: C, 58.96; H, 5.24; N, 8.09. Found: C, 58.93; H, 5.29; N, 8.14 %.

*(E)-Ethyl 2-[4-methoxy-2-[2-(3-methyl-4-nitro-5-isoxazolyl) vinyl]phenoxy]acetate* ***(4c)*** Yield 77 %, m.p. 162–164 °C. IR (KBr) $cm^{-1}$: 1757, 1521, 1325. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.91 (t, 3H, $OCH_2CH_3$), 2.30 (s, 3H, isoxazole–$CH_3$), 3.69 (s, 3H, $OCH_3$), 4.27 (q, 2H, $OCH_2CH_3$), 5.28 (s, 2H, $OCH_2CO$), 6.67 (d, 1H, CH=CH, $J = 12$ Hz), 6.71 (d, 1H, CH=CH, $J = 12$ Hz), 7.09–7.57 (m, 3H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.39, 14.42, 61.23, 61.31, 65.03, 101.31, 114.43, 121.09, 122.29, 123.07, 127.12, 129.09, 134.23, 150.21, 156.98, 158.23, 170.11.ESI-MS (70 eV) *m/z*: 363 $[M+H]^+$. Anal. Calcd for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.01; N, 7.73. Found: C, 56.30; H, 5.07; N, 7.67 %.

*(E)-Ethyl 2-[2-chloro-6-[2-(3-methyl-4-nitro-5-isoxazolyl) vinyl]phenoxy]acetate* ***(4d)*** Yield 77 %, m.p. 148–150 °C. IR (KBr) $cm^{-1}$: 1775, 1535, 1310. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.93 (t, 3H, $OCH_2CH_3$), 2.29 (s, 3H, isoxazole–$CH_3$), 4.12 (q, 2H, $OCH_2CH_3$), 5.25 (s, 2H, $OCH_2CO$), 6.58 (d, 1H, CH=CH, $J = 12$ Hz), 6.61 (d, 1H, CH=CH, $J = 12$ Hz), 7.00–7.62 (m, 3H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.42, 14.51, 60.98, 102.09, 113.89, 121.11, 122.31, 122.97, 127.29, 128.87, 134.38, 149.76, 150.53, 156.99, 158.32, 169.79.EI-MS (70 eV) *m/z*: 367 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}ClN_2O_6$: C, 52.40; H, 4.12; N, 7.64. Found: C, 52.40; H, 4.12; N, 7.64 %.

*(E)-Ethyl 2-[2,4-dichloro-6-[2-(3-methyl-4-nitro-5-isoxazolyl) vinyl]phenoxy]acetate* ***(4e)*** Yield 74 %, m.p. 141–143 °C. IR (KBr) $cm^{-1}$: 1765, 1540, 1315. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.86 (t, 3H, $OCH_2CH_3$), 2.31 (s, 3H, isoxazole–$CH_3$), 4.00 (q, 2H, $OCH_2CH_3$), 5.20 (s, 2H, $OCH_2CO$), 6.66 (d, 1H, CH=CH, $J = 12$ Hz), 6.74 (d, 1H, CH=CH, $J = 12$ Hz), 7.45 (s, 1H, ArH), 7.57 (2, 1H,

ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.45, 14.39, 61.07, 102.21, 114.11, 121.39, 122.42, 123.01, 127.37, 129.11, 134.43, 151.03, 157.11, 158.48, 170.09.ESI-MS (70 eV) *m/z*: 401 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_6$: C, 47.90; H, 3.52; N, 6.98. Found: C, 47.86; H, 3.48; N, 7.02 %.

*(E)-Ethyl 2-[4-bromo-2-[2-(3-methyl-4-nitro-5-isoxazolyl) vinyl]phenoxy]acetate* ***(4f)*** Yield 71 %, m.p. 145–147 °C. IR (KBr) $cm^{-1}$: 1761, 1532, 1311. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.91 (t, 3H, $OCH_2CH_3$), 2.33 (s, 3H, isoxazole–$CH_3$), 4.21 (q, 2H, $OCH_2CH_3$), 5.28 (s, 2H, $OCH_2CO$), 6.59 (d, 1H, CH=CH, $J = 12$ Hz), 6.68 (d, 1H, CH=CH, $J = 12$ Hz), 7.11–7.69 (m, 3H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.32, 14.46, 59.78, 65.12, 99.51, 113.97, 121.35, 121.94, 123.62, 127.01, 129.11, 133.72, 149.51, 158.16, 158.19, 170.38. ESI-MS (70 eV) *m/z*: 411 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}BrN_2O_6$: C, 46.73; H, 3.68; N, 6.81. Found: C, 46.79; H, 3.70; N, 6.77 %.

*(E)-Ethyl 2-[2,4-dibromo-6-[2-(3-methyl-4-nitro-5-isoxazolyl) vinyl]phenoxy]acetate* ***(4g)*** Yield 75 %, m.p. 153–155 °C. IR (KBr) $cm^{-1}$: 1755, 1530, 1315. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.95 (t, 3H, $OCH_2CH_3$), 2.30 (s, 3H, isoxazole–$CH_3$), 4.22 (q, 2H, $OCH_2CH_3$), 5.11 (s, 2H, $OCH_2CO$), 6.61 (d, 1H, CH=CH, $J = 12$ Hz), 6.70 (d, 1H, CH=CH, $J = 12$ Hz), 7.39 (s, 1H, ArH), 7.48 (s, 1H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.49, 14.47, 61.29, 102.21, 113.97, 12.53, 121.98, 122.79, 127.48, 129.18, 133.97, 151.03, 157.11, 158.49, 170.09.ESI-MS (70 eV) *m/z*: 489 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}Br_2N_2O_6$: C, 39.21; H, 2.88; N, 5.72. Found: C, 39.24; H, 2.82; N, 5.67 %.

*General procedure for the preparation of ethyl 2,3-dihydro-3-[(3-methyl-4-nitroisoxazole-5-yl)methyl]benzofuran-2-carboxylates* ***(5a–g)***

To a solution of **4** (1 mmol) in ethanol, $Et_3N$ (0.5 mmol) was added and the contents are heated at 100 °C for 8 h. After completion of the reaction as indicated by TLC, ethanol is evaporated and the crude product is purified by passing through a column of silica gel with ethylacetate:hexane (20:80) as elute.

Spectral data of each compound are given below

*Ethyl-3-[(3-methyl-4-nitro-5-isoxazolyl)methyl]-2,3-dihydrobenzofuran-2-carboxylate* ***(5a)*** Yield 70 %, m.p. 170–172 °C. IR (KBr) $cm^{-1}$: 1746, 1520, 1315. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.98 (t, 3H, $OCH_2CH_3$), 2.32 (s, 3H, isoxazole–$CH_3$), 3.48 (dd, 1H, $J = 4.5$, 18 Hz, $CH_2$), 3.65 (dd, 1H, $J = 5.5$, 18 Hz, $CH_2$), 4.03 (m, 1H, H-3), 4.27 (q, 2H, $OCH_2CH_3$), 5.48 (m, 1H, H-2), 7.02–7.69 (m, 4H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.42, 14.29, 26.73, 41.28, 60.79, 81.59, 100.73, 113.98, 120.23, 125.78, 126.11, 130.78, 149.69, 158.23, 158.35, 172.24. EI-MS (70 eV) *m/z*: 333 $[M+H]^+$. Anal. Calcd for $C_{16}H_{16}N_2O_6$: C, 57.83; H, 4.85; N, 8.43. Found: C, 57.89; H, 4.88; N, 8.38 %.

*Ethyl-3-[(3-methyl-4-nitro-5-isoxazolyl)methyl]-2,3-dihydrobenzofuran-2-carboxylate* ***(5b)*** Yield 70 %, m.p. 191–193 °C. IR (KBr) $cm^{-1}$: 1751, 1532, 1321. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.97 (t, 3H, $OCH_2CH_3$), 2.32 (s, 3H, isoxazole–$CH_3$), 2.65 (s, 3H, $ArCH_3$), 3.39 (dd, 1H, $J = 4.5$, 18 Hz, $CH_2$), 3.66 (dd, 1H, $J = 5.5$, 18 Hz, $CH_2$), 4.06 (m, 1H, H-3), 4.31 (q, 2H, $OCH_2CH_3$), 5.42 (m, 1H, H-2), 7.13–7.67 (m, 3H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.48, 14.31, 41.39, 27.09, 40.89, 61.09, 81.63, 100.81, 114.02, 120.53, 126.09, 131.11, 150.07, 158.35, 158.44, 172.38. ESI-MS (70 eV) *m/z*: 347 $[M+H]^+$. Anal. Calcd for $C_{17}H_{18}N_2O_6$: C, 39.21; H, 2.88; N, 5.72. Found: C, 39.25; H, 2.84; N, 5.76 %.

*Ethyl-5-methoxy-3-[(3-methyl-4-nitro-5-isoxazolyl)methyl]-2,3-dihydrobenzofuran-2-carboxylate* ***(5c)*** Yield 70 %, m.p. 191–193 °C. IR (KBr) $cm^{-1}$: 1752, 1537, 1333. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.90 (t, 3H, $OCH_2CH_3$), 2.28 (s, 3H, isoxazole–$CH_3$), 3.40 (dd, 1H, $J = 4.5$, 18 Hz, $CH_2$), 3.64 (dd, 1H, $J = 5.5$, 18 Hz, $CH_2$), 3.68 (s, 3H, $OCH_3$), 4.03 (m, 1H, H-3), 4.25 (q, 2H, $OCH_2CH_3$), 5.44 (m, 1H, H-2), 7.00–7.64 (m, 3H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.40, 15.09, 26.98, 40.91, 60.78, 61.23, 82.09, 101.1, 114.23, 121.13, 125.97, 126.29, 131.28, 149.78, 158.43, 158.71, 173.08. ESI-MS (70 eV) *m/z*: 363 $[M+H]^+$. Anal. Calcd for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.01; N, 7.73. Found: C, 56.41; H, 4.98; N, 7.76 %.

*Ethyl-7-chloro-3-[(3-methyl-4-nitro-5-isoxazolyl)methyl]-2,3-dihydrobenzofuran-2-carboxylate* ***(5d)*** Yield 74 %, m.p. 178–180 °C. IR (KBr) $cm^{-1}$: 1755, 1528, 1310. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.90 (t, 3H, $OCH_2CH_3$), 2.26 (s, 3H, isoxazole–$CH_3$), 3.42 (dd, 1H, $J = 4.5$, 18 Hz, $CH_2$), 3.67 (dd, 1H, $J = 5.5$, 18 Hz, $CH_2$), 4.10 (m, 1H, H-3), 4.26 (q, 2H, $OCH_2CH_3$), 5.40 (m, 1H, H-2), 7.11–7.73 (m, 3H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.43, 16.21, 27.03, 41.12, 60.83, 100.79, 114.38, 121.73, 126.15, 131.42, 150.04, 158.59, 158.83, 173.08. ESI-MS (70 eV) *m/z*: 367 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}ClN_2O_6$: C, 52.40; H, 4.12; N, 7.64. Found: C, 52.45; H, 4.18; N, 7.60 %.

*Ethyl-5,7-dichloro-3-[(3-methyl-4-nitro-5-isoxazolyl)methyl]-2,3-dihydrobenzofuran-2-carboxylate* ***(5e)*** Yield 73 %, m.p. 181–183 °C. IR (KBr) $cm^{-1}$: 1742, 1521, 1322. $^1$H

NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.96 (t, 3H, $OCH_2CH_3$), 2.30 (s, 3H, isoxazole–$CH_3$), 3.42 (dd, 1H, $J = 4.5$, 18 Hz, $CH_2$), 3.64 (dd, 1H, $J = 5.5$, 18 Hz, $CH_2$), 4.00 (m, 1H, H-3), 4.29 (q, 2H, $OCH_2CH_3$), 5.44 (m, 1H, H-2), 7.31 (s, 1H, ArH), 7.43 (s, 1H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.49, 15.78, 27.19, 41.37, 61.12, 82.31, 101.03, 144.53, 121.95, 126.53, 127.08, 131.59, 149.78, 158.35, 159.13, 172.81. ESI-MS (70 eV) *m/z*: 401 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_6$: C, 47.90; H, 3.52; N, 6.98. Found: C, 47.84; H, 3.48; N, 7.01 %.

*Ethyl-7-bromo-3-[(3-methyl-4-nitro-5-isoxazolyl)methyl]-2,3-dihydrobenzofuran-2-carboxylate (**5f**)* Yield 70 %, m.p. 175–177 °C. IR (KBr) $cm^{-1}$: 1740, 1525, 1315. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.91 (t, 3H, $OCH_2CH_3$), 2.30 (s, 3H, isoxazole–$CH_3$), 3.39 (dd, 1H, $J = 4.5$, 18 Hz, $CH_2$), 3.64 (dd, 1H, $J = 5.5$, 18 Hz, $CH_2$), 4.05 (m, 1H, H-3), 4.30 (q, 2H, $OCH_2CH_3$), 5.45 (m, 1H, H-2), 7.11–7.70 (m, 3H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.39, 27.83, 42.11, 60.79, 82.48, 101.28, 114.75, 122.16, 127.13, 132.09, 150.11, 159.11, 159.27, 173.08. ESI-MS (70 eV) *m/z*: 412 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}BrN_2O_6$: C, 46.73; H, 3.68; N, 6.81. Found: C, 46.77; H, 3.62; N, 6.87 %.

*Ethyl-5,7-dibromo-3-[(3-methyl-4-nitro-5-isoxazolyl)methyl]-2,3-dihydrobenzofuran-2-carboxylate (**5g**)* Yield 77 %, m.p. 184–186 °C. IR (KBr) $cm^{-1}$: 1745, 1535, 1320. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 0.93 (t, 3H, $OCH_2CH_3$), 2.26 (s, 3H, isoxazole–$CH_3$), 3.43 (dd, 1H, $J = 4.5$, 18 Hz, $CH_2$), 3.65 (dd, 1H, $J = 5.5$, 18 Hz, $CH_2$), 4.04 (m, 1H, H-3), 4.30 (q, 2H, $OCH_2CH_3$), 5.41 (m, 1H, H-2), 7.33 (s, 1H, ArH), 7.47 (s, 1H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.45, 28.11, 61.27, 82.42, 100.98, 114.79, 122.09, 127.12, 127.29, 131.75, 150.09, 158.73, 160.15, 172.57. ESI-MS (70 eV) *m/z*: 491 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}Br_2N_2O_6$: C, 39.21; H, 2.88; N, 5.72. Found: C, 39.17; H, 2.93; N, 5.68 %.

*General procedure for the synthesis of 3-methyl-10b,11-dihydro-4H-benzofuro[3,2-e] isoxazole[4,5-b]azepin-5(5aH)-ones (**6a–g**)*

Spectral data of each compound are given below

Compound **5** (1 mmol) and $SnCl_2{\cdot}2H_2O$ (3 mmol) were dissolved in 20 mL of methanol and refluxed for 4 h. After completion of the reaction (monitored by TLC), solvent was removed in vacuum. The solid mass was decomposed with cold water and the reaction solution was carefully adjusted to pH 8 with 10 % $NaHCO_3$ solution and then extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried over $Na_2SO_4$ and evaporated under vacuum and purified by recrystallization from ethanol to give pure product **6**.

*3-Methyl-10b,11-dihydro-4H-benzofuro[3,2-e]isoxazolo[4,5-b]azepin-5(5aH)-one (**6a**)* Yield 73 %, m.p. 198–200 °C. IR (KBr) $cm^{-1}$: 3200, 1710. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.22 (s, 3H, isoxazole–$CH_3$), 3.50 (dd, 1H, $J = 4.6$, 18 Hz, 11-$CH_2$), 3.66 (dd, 1H, $J = 6.6$, 18 Hz, 11-$CH_2$), 4.05 (m, 1H, H-10b), 5.48 (d, 1H, $J = 4$ Hz, H-5a), 7.01–7.83 (m, 4H, ArH), 8.32 (bs, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.51, 26.21, 40.79, 85.63, 114.21, 115.76, 119.58, 126.78, 126.91, 150.08, 156.54, 160.21, 174.79. ESI-MS (70 eV) *m/z*: 257 $[M+H]^+$. Anal. Calcd for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.66; H, 4.78; N, 10.89 %.

*3,9-Dimethyl-10b,11-dihydro-4H-benzofuro[3,2-e]isoxazolo[4,5-b]azepin-5(5aH)-one (**6b**)* Yield 73 %, m.p. 222–225 °C. IR (KBr) $cm^{-1}$: 3210, 1725. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.25 (s, 3H, isoxazole–$CH_3$), 2.48 (s, 3H, $ArCH_3$), 3.52 (dd, 1H, $J = 4.6$, 18 Hz, 11-$CH_2$), 3.64 (dd, 1H, $J = 6.6$, 18 Hz, 11-$CH_2$), 4.13 (m, 1H, H-10b), 5.52 (d, 1H, $J = 4$ Hz, H-5a), 7.10–7.64 (m, 3H, ArH), 8.11 (bs, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.48, 25.79, 41.13, 41.79, 86.08, 113.79, 115.53, 120.12, 127.29, 127.58, 131.96, 149.79, 156.63, 160.48, 175.04. ESI-MS (70 eV) *m/z*: 271 $[M+H]^+$. Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.70; H, 5.28; N, 10.33 %.

*9-Methoxy-3-methyl-10b,11-dihydro-4H-benzofuro[3,2-e]isoxazolo[4,5-b]azepin-5(5aH)-one (**6c**)* Yield 75 %, m.p. 219–221 °C. IR (KBr) $cm^{-1}$: 3218, 1725. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.20 (s, 3H, isoxazole–$CH_3$), 3.54 (dd, 1H, $J = 4.6$, 18 Hz, 11-$CH_2$), 3.58 (s, 3H, $OCH_3$), 3.63 (dd, 1H, $J = 6.6$, 18 Hz, 11-$CH_2$), 4.00 (m, 1H, H-10b), 5.40 (d, 1H, $J = 4$ Hz, H-5a), 7.13–7.83 (m, 3H, ArH), 8.33 (bs, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.53, 26.13, 41.78, 62.21, 85.97, 114.09, 115.74, 119.78, 127.81, 128.11, 131.23, 149.78, 156.63, 160.49, 175.04. ESI-MS (70 eV) *m/z*: 287 $[M+H]^+$. Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.89; H, 4.90; N, 9.74 %.

*9-Chloro-3-methyl-10b,11-dihydro-4H-benzofuro[3,2-e]isoxazolo[4,5-b]azepin-5(5aH)-one (**6d**)* Yield 77 %, m.p. 211–213 °C. IR (KBr) $cm^{-1}$: 3215, 1723. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.28 (s, 3H, isoxazole–$CH_3$), 3.48 (dd, 1H, $J = 4.6$, 18 Hz, 11-$CH_2$), 3.64 (dd, 1H, $J = 6.6$, 18 Hz, 11-$CH_2$), 4.00 (m, 1H, H-10b), 5.42 (d, 1H, $J = 4$ Hz, H-5a), 7.00–7.81 (m, 3H, ArH), 8.21 (bs,

1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.41, 26.73, 42.11, 86.24, 113.89, 116.31, 120.12, 128.09, 128.29, 131.83, 150.12, 157.13, 161.11, 174.79. ESI-MS (70 eV) *m/z*: 291 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}ClN_2O_3$: C, 57.84; H, 3.81; N, 9.64. Found: C, 57.80; H, 3.86; N, 9.62 %.

*7,9-Dichloro-3-methyl-10b,11-dihydro-4H-benzofuro[3,2-e]isoxazolo[4,5-b]azepin-5(5aH)-one* ***(6e)*** Yield 72 %, m.p. 218–220 °C. IR (KBr) $cm^{-1}$: 3210, 1720. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.31 (s, 3H, isoxazole–$CH_3$), 3.50 (dd, 1H, $J = 4.6$, 18 Hz, 11-$CH_2$), 3.65 (dd, 1H, $J = 6.6$, 18 Hz, 11-$CH_2$), 4.13 (m, 1H, H-10b), 5.44 (d, 1H, $J = 4$ Hz, H-5a), 7.21 (s, 1H, ArH), 7.35 (s, 1H, ArH), 8.28 (bs, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.49, 27.19, 42.08, 86.11, 113.89, 116.31, 120.09, 128.09, 128.29, 132.19, 149.78, 157.13, 161.11, 174.79. ESI-MS (70 eV) *m/z*: 325 $[M+H]^+$. Anal. Calcd for $C_{14}H_{10}Cl_2N_2O_3$: C, 51.72; H, 3.10; N, 8.62. Found: C, 51.68; H, 3.13; N, 8.60 %.

*9-Bromo-3-methyl-10b,11-dihydro-4H-benzofuro[3,2-e]isoxazolo[4,5-b]azepin-5(5aH)-one* ***(6f)*** Yield 70 %, m.p. 205–207 °C. IR (KBr) $cm^{-1}$: 3216, 1720. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.30 (s, 3H, isoxazole–$CH_3$), 3.53 (dd, 1H, $J = 4.6$, 18 Hz, 11-$CH_2$), 3.61 (dd, 1H, $J = 6.6$, 18 Hz, 11-$CH_2$), 4.11 (m, 1H, H-10b), 5.39 (d, 1H, $J = 4$ Hz, H-5a), 7.11–7.61 (m, 3H, ArH), 8.11 (bs, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.44, 28.03, 41.79, 84.55, 114.23, 117.11, 120.42,127.89, 128.53, 133.03, 150.11, 157.91, 163.11, 174.79. ESI-MS (70 eV) *m/z*: 335 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}BrN_2O_3$: C, 50.17; H, 3.31; N, 8.36. Found: C, 50.12; H, 3.35; N, 8.31 %.

*7,9-Dibromo-3-methyl-10b,11-dihydro-4H-benzofuro[3,2-e]isoxazolo[4,5-b]azepin-5(5aH)-one* ***(6g)*** Yield 76 %, m.p. 215–217 °C. IR (KBr) $cm^{-1}$: 3225, 1716. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 2.28 (s, 3H, isoxazole–$CH_3$), 3.48 (dd, 1H, $J = 4.6$, 18 Hz, 11-$CH_2$), 3.67 (dd, 1H, $J = 6.6$, 18 Hz, 11-$CH_2$), 4.00 (m, 1H, H-10b), 5.43 (d, 1H, $J = 4$ Hz, H-5a), 7.25 (s, 1H, ArH), 7.37 (s, 1H, ArH), 8.18 (bs, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 12.79, 28.45, 41.94, 85.38, 114.58, 117.51, 121.34,128.42, 129.31, 133.59, 149.79, 158.13, 163.42, 175.05. EI-MS (70 eV) *m/z*: 413 $[M+H]^+$. Anal. Calcd for $C_{14}H_{10}Br_2N_2O_3$: C, 40.61; H, 2.43; N, 6.77. Found: C, 40.57; H, 2.40; N, 6.80 %.

Seven new derivatives for each class of compounds (**4**, **5**, and **6**) are herein reported. The structures of all newly synthesized compounds were confirmed by analytical and spectral data (IR, $^1H$ NMR, and MS).

## Pharmacological screening

### *Antibacterial assay*

The ready-made nutrient broth medium (Himedia, 24 g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and test tubes were autoclaved at a pressure of 15 lb/inc$^2$ for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound was dissolved in acetone and a concentration of 100 µg/mL of the test compound was added in the first test tube, which was serially diluted. A fixed volume of 0.5 mL of overnight culture was added in all the test tubes which were incubated at 37 °C for 24 h. After 24 h these tubes were measured for turbidity.

### *Antifungal assay*

For the antifungal assay, the ready-made potato dextrose agar medium (Himedia, 39 g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and glass petri dishes were autoclaved at a pressure of 15 lb/in C$^2$ for 20 min. The medium was poured into sterile petri dishes under aseptic conditions in a Laminar flow chamber. When the medium in the plates solidified, 0.5 mL of the culture (one-week-old) of fungal spore suspension was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving test compound in acetone. After inoculation, cups were scooped out with 6-mm sterile cork borer and the lids of the dishes were replaced. To each cup, different concentrations of test solution were added. Controls were maintained with acetone and fluconazole. The treated and the controls were kept at room temperature for 72–96 h. The minimum inhibitory concentration (MIC) was recorded in µg/mL. Three to four replicates were maintained for each treatment.

### *Anti-inflammatory analysis*

Anti-inflammatory activity was determined by carrageenan-induced paw edema method (Winter *et al.,* 1990). Wistar rats of either sex weighing 150–200 g were divided into 6 groups ($n = 6$) and they were fasted 18 h before the experiment with water ad libitum. Group-I received 1 % sodium CMC (negative control), Group-II received ibuprofen at a dose of 100 mg/kg (positive control), and Group-III–VI were given the compounds **6a**–**g** (100 mg/kg). All the compounds **6a**–**g** were given in oral route. After 30 min, 0.1 mL of 1 % carrageenan suspension in normal saline was injected into the subplantar region of the

left hind paw of each rat to induce edema. The edema volumes of the injected paw were measured with the help of plethysmograph at the interval of 0, 1, 2, 4, and 6 h. The difference between the paw volumes of treated animals were compared with that of the control group and the mean edema volume was calculated. Percentage inhibition was calculated as per the formula, %inhibition $= [V_o - V_t)/V_o] \times 100$, where $V_o =$ volume of the paw control at time $t$, $V_t =$ volume of the paw of drug treated at time $t$.

### *5-Lox inhibitory activity*

The in vitro anti-inflammatory activity (5-LOX inhibition assay) was determined using UV–Vis spectrophotometer (Reddenna *et al.,* 1990; Ulusu *et al.,* 2002). The assay mixture contained 80 mM linoleic acid and sufficient amount of potato 5-lipoxygenase enzyme in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by the enzyme buffer mix to substrate (linoleic acid) and the enzyme activity was monitored as an increase in absorbance at 234 nm. The reaction was monitored for 10 s. using UV Kinetic mode on Varian Cary-50-UV–Vis spectrophotometer. In the inhibition studies, the activities were measured by incubating various concentrations of test substance with enzyme buffer mix for 2 min before addition of the substrate. The assay was performed in triplicate and mean values were used for the calculation. The activity of 5-Lipoxygenase was compared with the standard positive control LI01020. Percentage inhibition was calculated as per the formula, % inhibition $= 1 -$ [O.D. in sample well)/(O.D. in control well)] $\times$ 100.

### *Analgesic analysis*

The analgesic activity was determined by acetic acid-induced writhing method (Syamsudin and Rahayn, 2010). Swiss albino mice ($n = 6$) of either sex selected by random sampling technique were used for the study and divided into 6 groups. The animals were fasted 18 h before the experiment with water ad libitum. Diclofenac (50 mg/kg) was administered as standard drug for comparison. The test compounds **6a–g** (50 mg/kg) were administered orally 30 min after the administration of compounds. All the mice were given 1.0 % v/v solution of acetic acid, i.p. the dose being 1 mL/100 g of the mice and the writhings produced in these animals were counted for 20 min. The number of writhings produced in the treated groups was compared with those in the control group. Percentage inhibition was calculated as per the formula, % inhibition = [(Average writhes in control − Average writhes in test)/Average writhes in control] × 100.

## Results and discussion

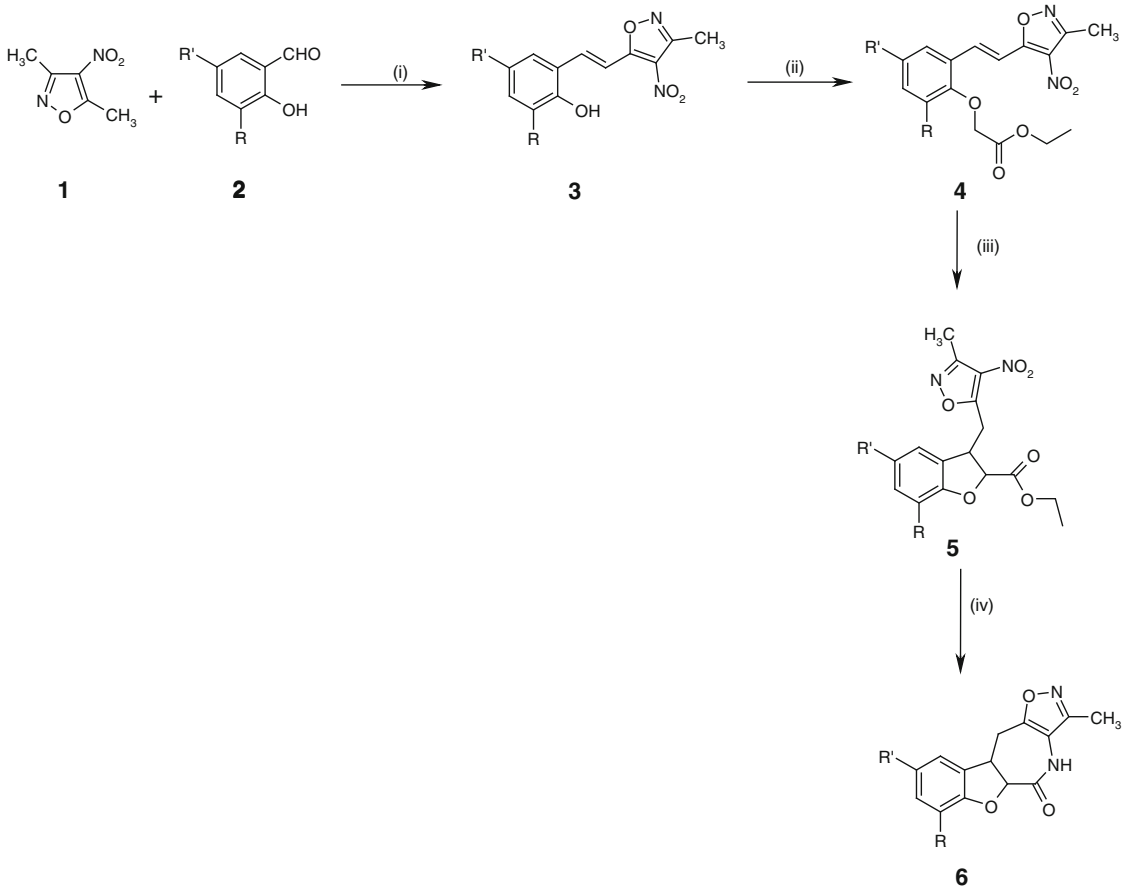
### Synthesis

The synthesis of title compounds was accomplished by synthetic sequence shown in Scheme 1. Knoevenagel condensation of 3,5-dimethyl-4-nitroisoxazole (**1**) (purchased from Sigma-Aldrich) with salicyl aldehydes (**2**) in refluxing ethanol in the presence of piperidine affords nitrostyrylisoxazoles (**3**) in good yields (Martinez, 1980; Chimichi, 1989; Malla Reddy *et al.,* 1981). Compound **3** on treatment with ethyl bromoacetate in dry acetone in the presence of anhydrous $K_2CO_3$ under refluxing condition led to the formation of ethyl 2-[2-[(*E*)-2-(3-methyl-4-nitro-5-isoxazolyl)vinyl]phenoxy]acetates (**4**). Compounds **4** underwent cyclization to afford ethyl 2,3-dihydro-3-((3-methyl-4-nitro-5-isoxazolyl)methyl)benzofuran-2-carboxylates (**5**) on treatment with triethyl amine in refluxing alcohol. These compounds (**5**) were further converted into 3-methyl-10*b*,11-dihydro-4*H*-benzofuro[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones (**6**) by reductive cyclization on treatment with $SnCl_2$–MeOH (Scheme 1).

## Biological screening

### Antimicrobial activity

The newly synthesized title compounds **6a–g** were evaluated for their in vitro antibacterial activity against Gram-positive bacteria viz., *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511), and *Staphylococcus aureus* (MTCC 96) and Gram-negative bacteria viz., *Pseudomonas aeruginosa* (MTCC 741), *Klobsinella aerogenes* (MTCC 39), and *Chromobacterium violaceum* (MTCC 2656) at 100 µg/mL concentration. The in vitro antibacterial activity of the tested compounds was assessed by MIC using broth dilution method (National committee for clinical laboratory standards (NCCLS) 1982). Ciprofloxacin was used as standard drug for comparison. The results of antibacterial screening (Table 1) reveal that the compounds **6a–g** displayed a better activity and were more active than the standard Ciprofloxacin. Compounds **6b** and **6c** carrying methyl and methoxy substituents on the benzene ring showed best activity and more active than the standard Ciprofloxacin. Compounds **6d**, **6e**, **6f**, and **6g** carrying chloro and bromo substitutions on benzene ring did not exhibit much activity. Compound **6a** showed least activity because it has no substituent on the benzene ring. However, the degree of inhibition varied both with the test compound and with the bacteria used in the present investigation. In conclusion, compounds **6b** and **6c** showed




**Scheme 1** Synthesis of novel dihydrobenzo furo[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones (**6a**–**g**). **3, 4, 5,** and **6**: (a) R=H, R′=H; (b) R=H, R′=$CH_3$; (c) R=H, R′=$OCH_3$; (d) R=H, R′=Cl; (e) R=Cl, R′=Cl; (f) R=H, R′=Br; (g) R=Br, R′=Br. Reagents and conditions: (*i*) ethanol, piperidine, Δ; (*ii*) $BrCH_2COOEt$, $K_2CO_3$, acetone, reflux, Δ; (*iii*) $Et_3N$, ethanol, 100 °C, 8 h; (*iv*) $SnCl_2{\cdot}2H_2O$/MeOH, 90 °C, 24 h

maximum activity by inhibiting the growth of all the bacteria under investigation compared to standard Ciprofloxacin, hence can be exploited for the formulation of bacteriocides after further studies.

The title compounds **6a**–**g** were also evaluated for their antifungal activity against *Fusarium oxysporum*, *Verticillium dahliae*, *Alternaria solani*, *Rhizoctonia solani*, *Colletotrichum capsici*, and *Pythium aphanidermatum* in acetone by agar cup bioassay method (Margery Linday, 1962), using fluconazole as the standard drug. The antifungal activity data (Table 2) reveal that compounds **6a**–**g** are highly toxic toward all the fungi under investigation. Compounds **6b** and **6c** exhibited high antifungal activity by inhibiting the growth of fungi to a remarkable extent, when compared to standard drug fluconazole which may be due to the presence of methyl and methoxy substituents on the benzene ring. Compound **6a** showed good activity. Compounds **6d**, **6e**, **6f**, and **6g** are moderately active. However, the degree of spore germination inhibition varied with the test compound as well as with the fungi under investigation. It is noteworthy that compounds **6b** and **6c** showed better activity, when compared with the standard drug fluconazole, hence, may be exploited for control of wilt diseases of different crops as fungicides after further studies.

## Anti-inflammatory activity

The anti-inflammatory activity of the title compounds, **6a**–**g** were evaluated by carrageenan-induced paw edema method in rats (Winter *et al.,* 1990) at a dose of 100 mg/kg body weight using ibuprofen as a reference drug. Results were expressed as a mean ± SE. The anti-inflammatory properties were recorded at successive intervals of 0, 1, 2, 4, and 6 h and compared with that of standard ibuprofen. The anti-inflammatory activity data (Table 3) indicated that all the compounds **6a**–**g** exhibited significant activity by decreasing the paw volume that was produced by carrageenan. Among all the compounds tested, it is interesting to note that the compounds **6b** and **6c** showed better anti-inflammatory activity, may be due to the presence of methyl and methoxy substituents on the benzene ring, besides benzofuran-fused isoxazoloazepine moiety. Compounds **6b** and **6c** exhibited maximum activity at the

**Table 1** Antibacterial activity of dihydrobenzo furo[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones (**6a**–**g**)

| Compound | Minimum inhibitory concentration in μg/mL (MIC) | | | | | |
|---|---|---|---|---|---|---|
| | Gram +ve bacteria | | | Gram –ve bacteria | | |
| | B. subtilis | *B. sphaericus* | *S. aureus* | *P. aeruginosa* | *K. aerogenes* | *C. violaceum* |
| **6a** | 18 | 20 | 20 | 22 | 20 | 20 |
| **6b** | 8 | 10 | 9 | 8 | 7 | 6 |
| **6c** | 10 | 10 | 8 | 10 | 8 | 10 |
| **6d** | 20 | 18 | 22 | 21 | 19 | 16 |
| **6e** | 18 | 22 | 21 | 24 | 18 | 18 |
| **6f** | 16 | 12 | 14 | 16 | 15 | 16 |
| **6g** | 18 | 15 | 16 | 16 | 18 | 18 |
| Ciprofloxacin | 20 | 22 | 26 | 25 | 20 | 22 |

Negative control (acetone)—No activity

Values are indicated in μg/mL

**Table 2** Antifungal activity of dihydrobenzo furo[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones (**6a**–**g**)

| Compound | Minimum inhibitory concentration in μg/mL (MIC) | | | | | |
|---|---|---|---|---|---|---|
| | F. oxysporum | *V. dahale* | *A. solani* | *R. solani* | *C. capsici* | *P. aphanidermatum* |
| **6a** | 16 | 14 | 12 | 12 | 15 | 18 |
| **6b** | 8 | 8 | 10 | 8 | 9 | 9 |
| **6c** | 8 | 10 | 11 | 10 | 10 | 8 |
| **6d** | 16 | 16 | 14 | 16 | 16 | 15 |
| **6e** | 14 | 14 | 15 | 14 | 18 | 20 |
| **6f** | 14 | 17 | 18 | 14 | 17 | 17 |
| **6g** | 16 | 16 | 20 | 12 | 18 | 20 |
| Flucanozole | 18 | 16 | 20 | 16 | 18 | 22 |

Negative control (acetone)—No activity

interval of 6 h. The presence of electron withdrawing chloro and bromo groups on benzene ring (**6d**, **6e**, **6f**, and **6g**) did not influence the activity much.

### 5-Lox inhibitory activity

The 5-Lox inhibitory assay of compounds **6a**–**g** were evaluated using UV–Vis spectrophotometer (Reddenna *et a.,* 1990; Ulusu *et al.,* 2002). All the compounds **6a**–**g** showed dose-dependant inhibition of 5-lipoxygenase enzyme. The compounds **6b** and **6c** showed more significant 5-Lox inhibitory activity compared to other compounds. Rest of the compounds are moderately active. The results are illustrated in Table 4.

### Analgesic activity

The analgesic activity of the newly synthesized compounds **6a**–**g** were determined in vivo by acetic acid-induced writhing method (Syamsudin and Rahayn, 2010) in mice at a dose of 50 mg/Kg body weight. All the compounds **6a**–**g** produced significant activity when compared to standard diclofenac. Compounds **6b** and **6c** showed more significant activity compared to other test compounds (Table 5). These compounds **6b** and **6c** decreased the number of writhings produced in mice to a remarkable extent. Rest of the compounds (**6a**, **6d**, **6e**, **6f**, and **6g**) are moderately active.

## Conclusion

In conclusion, we report the synthesis of novel dihydro benzofuro[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones using inexpensive and commercially available materials with potential medicinal properties. The newly synthesized title compounds **6a**–**g** were evaluated for their antimicrobial, anti-inflammatory, and analgesic activities. It has been found that compounds **6a**–**g** exhibited good antimicrobial, anti-inflammatory, and analgesic activity compared to that

**Table 3** Anti-inflammatory activity of dihydrobenzo furo[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones (**6a–g**)

| Paw volume (mL of Hg)[a] | | | | | |
|---|---|---|---|---|---|
| Group[b] | 0 h | 1 h | 2 h | 4 h | 6 h |
| **6a** | 0.36 ± 0.03 | 0.76 ± 0.03 | 0.86 ± 0.08$^{ns}$ | 0.76 ± 0.06$^{**}$ | 0.63 ± 0.03$^{**}$ |
| **6b** | 0.26 ± 0.06 | 0.53 ± 0.8 | 0.70 ± 0.05$^{ns}$ | 0.66 ± 0.03$^{**}$ | 0.36 ± 0.01$^{***}$ |
| **6c** | 0.22 ± 0.02 | 0.56 ± 0.06 | 0.71 ± 0.04 | 0.58 ± 0.02$^{**}$ | 0.40 ± 0.03$^{***}$ |
| **6d** | 0.30 ± 0.05 | 0.70 ± 0.05 | 0.83 ± 0.03$^{ns}$ | 0.83 ± 0.03$^{**}$ | 0.55 ± 0.05$^{**}$ |
| **6e** | 0.26 ± 0.08 | 0.80 ± 0.05 | 0.90 ± 0.05$^{ns}$ | 0.83 ± 0.03$^{**}$ | 0.60 ± 0.01$^{**}$ |
| **6f** | 0.31 ± 0.04 | 0.78 ± 0.02 | 0.84 ± 0.03 | 0.81 ± 0.03$^{*}$ | 0.53 ± 0.02$^{**}$ |
| **6g** | 0.34 ± 0.03 | 0.82 ± 0.04 | 0.80 ± 0.03 | 0.82 ± 0.04$^{*}$ | 0.54 ± 0.02$^{**}$ |
| Control | 0.36 ± 0.3 | 0.90 ± 0.05 | 1.07 ± 0.08 | 1.2 ± 0.05 | 0.96 ± 0.03 |
| Ibuprofen | 0.30 ± 0.5 | 0.66 ± 0.03$^{*}$ | 0.70 ± 0.05$^{***}$ | 0.60 ± 0.05$^{***}$ | 0.40 ± 0.05$^{***}$ |

$n = 6$, number of animals used in each group

[ns] Nonsignificant, compared to control

Statistically significant compound to respective control value $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$, $^{***}$ $P < 0.001$

[a] Values are expressed as mean ± SE

[b] Dose levels: test compound (100 mg/kg b.wt) Ibuprofen (100 mg/kg b.wt)

**Table 4** 5-LOX inhibitory activity (in vitro anti-inflammatory activity) of dihydrobenzo furo[3,2-*e*] isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones (**6a–g**)

| Compound[a] | $IC_{50}$ (μg/mL)[b] |
|---|---|
| **6a** | 19.90 ± 1.0 |
| **6b** | 13.31 ± 0.2 |
| **6c** | 14.62 ± 0.3 |
| **6d** | 28.29 ± 1.5 |
| **6e** | 20.99 ± 1.2 |
| **6f** | 25.21 ± 0.3 |
| **6g** | 24.25 ± 0.4 |
| LI0I020 | 4.33 ± 0.2 |

[a] Values are expressed as mean ± SEM

[b] $IC_{50}$ conentration corresponding to 50 % inhibition

**Table 5** Analgesic activity of dihydrobenzo furo[3,2-*e*]isoxazolo[4,5-*b*]azepin-5(5*aH*)-ones (**6a–g**)

| Animal group[a] | No. of writhings[b] | %inhibition |
|---|---|---|
| **6a** | 26 ± 1.18$^{**}$ | 36.58 ± 1.40 |
| **6b** | 22.6 ± 1.52$^{***}$ | 44.87 ± 2.05 |
| **6c** | 20.3 ± 1.60$^{***}$ | 50.48 ± 1.62 |
| **6d** | 33 ± 4.35$^{*}$ | 19.51 ± 1.82 |
| **6e** | 34. ± 2.51$^{*}$ | 16.34 ± 1.31 |
| **6f** | 33.61 ± 2.40$^{*}$ | 19.42 ± 2.10 |
| **6g** | 34.60 ± 2.80$^{*}$ | 16.52 ± 1.50 |
| Control | 41 ± 1.25 | – |
| Diclofenac | 19 ± 1.23$^{***}$ | 53.65 ± 1.92 |

Statistically significant compound to respective control value $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$, $^{***}$ $P < 0.001$

[a] Dose levels: test compound (50 mg/kg b.wt) Diclofenac (50 mg/kg)

[b] Values are expressed as mean ± SE

of reference drugs. Among all the test compounds, it is interesting to note that compounds **6b** and **6c** showed better antimicrobial, anti-inflammatory, and analgesic activity.

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