Journal of Enzyme Inhibition and Medicinal Chemistry, 2011; 26(1): 141–148

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756366.2010.481622

informa
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RESEARCH ARTICLE

Design, synthesis and pharmacological evaluation of novel azole derivatives of aryl acetic acid as anti-inflammatory and analgesic agents

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Abstract

A series of substituted azole derivatives (**3a**–**e**, **4a**–**e** and **5a**–**e**) were synthesised by the cyclisation of N^1(diphenylethanoyl)-N^4-substituted phenyl thiosemicarbazides under various reaction conditions. These compounds were tested *in vivo* for their anti-inflammatory activity. The compounds which showed activity comparable to the standard drug ibuprofen, were screened for their analgesic, ulcerogenic and lipid peroxidation activities. The compounds 5-(diphenylmethyl)-N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (**3b**) and 5-(diphenylmethyl)-N-(3-chloro-4-fluorophenyl)-1,3,4-oxadiazol-2-amine (**3c**) emerged as the most active compounds of the series, and were moderately more potent than the standard drug, ibuprofen. (This abstract was published in Inflammation Research, Supplement 2, Volume 56, page A101, 2008.)

Keywords: Diphenyl acetic acids, anti-inflammatory, analgesic, acute ulcerogenicity, lipid peroxidation

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, are among the most widely prescribed drugs worldwide. Through their anti-inflammatory, antipyretic and analgesic activities, they represent a choice treatment in various inflammatory diseases, so as to relieve the aches and pains of every day life. Chronic NSAID therapy effectively reduces the symptoms of many painful arthritic syndromes, but there can be adverse gastrointestinal (GI) complications ranging from stomach irritation to life threatening GI ulceration and bleeding. Long term NSAID therapy makes NSAID induced GI toxicity a substantial patient risk, and a costly healthcare and societal burden in terms of the associated hospitalisation and morbidity [1]. NSAIDs exert their anti-inflammatory effect mainly through inhibition of the cyclooxygenases (COXs), as the key enzymes in prostaglandin (PG) biosynthesis from arachidonic acid [2–4]. There are at least two mammalian COX isoforms, COX-1 and COX-2 [5]. COX-1 is the constitutive isoform, which performs a housekeeping function to synthesise prostaglandins, which regulate normal cell activity. The second isoform COX-2, in contrast, is induced in inflammatory cells in response to pro-inflammatory stimuli such as cytokines, tumour promoting agents, and bacterial endotoxins. The prostaglandins (PGs) produced by COX-2 play a major role in inflammatory reactions and are responsible for the characteristic inflammatory symptoms [6]. These findings stimulated the development of selective COX-2 inhibitors (coxibs) as a new generation of NSAIDs, free from GI toxicity [7,8]. Unfortunately careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effects [9]. Therefore the development of novel compounds having anti-inflammatory and analgesic activity with an improved safety profile is still a necessity. Studies have shown that derivatisation of the carboxylate function of arylalkonic acids resulted in a retained anti-inflammatory activity and reduced ulcerogenic potential [10–13]. Furthermore it has been reported in the literature that the substituted azole derivatives such as 1,3,4-oxadiazole/thiadiazole [14,15] and 1,2,4 triazole [16] possess significant anti-inflammatory activity.

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(Received 20 August 2009; revised 28 October 2009; accepted 10 November 2009)

In view of these observations and in continuation of our research programme on the synthesis of five membered heterocyclic compounds [17–19], we report here the synthesis of some new azole derivatives of diphenyl acetic acid. The synthesised compounds have been found to possess an interesting profile of anti-inflammatory and analgesic activity, with a significant reduction in their ulcerogenic effect (Scheme 1).

Materials and methods

Chemistry

All chemicals for synthesis were supplied by Merck (Darmstadt, Germany) and S.D. Fine Chemicals (Delhi, India). The melting points of the newly synthesised compounds were determined in open glass capillary and are uncorrected. IR (KBr) spectra were recorded on a Nicolet 5PC (MA, USA) FTIR spectrophotometer (υ_{max} in cm^{-1}) and ^1H NMR spectra were recorded on a Brucker Model - 400 NMR (Fallanden, Switzerland) spectrometer in $CDCl_3$ and DMSO-d_6 using tetramethysilane (TMS) as the internal reference (chemical shifts in δ ppm). ^{13}C NMR spectra were measured on Bruker - 400 instrument (100 MHz) with complete proton decoupling. Chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). Mass spectra were recorded on a Jeol SX- 102/DA-6000 (Tokyo, Japan) spectrometer. The purity of the compounds was checked on silica gel G plates using iodine vapours as the visualising agent.

Synthesis of diphenyl acetic acid hydrazide (1)

Compound **1** was prepared by the procedure given in the literature. [14]

Synthesis of N^1(diphenylethanoyl)-N^4-aryl thiosemicarbazides (2a–e)

A mixture of diphenyl acetic acid hydrazide (0.1 M), aryl isothiocyanate (0.1 M) and ethanol (50 mL) was placed in a 100 mL round bottom flask and refluxed for 3–4 h on a water bath. It was then concentrated, cooled and kept overnight in a refrigerator. The solid was separated out, then filtered, dried and recrystallised from a suitable solvent.

N^1(Diphenylethanoyl)-N^4-(4-chlorophenyl)thiosemicarbazide (2a)

Yield: 70%; mp: 148°C; IR (KBr, cm^{-1}): 3280 (NH), 1676 (C=O), 1079 (C=S); ^1H NMR ($CDCl_3$) δ (ppm): 5.09 (s, 1H, CH), 6.9–7.35 (m, 14H, ArH), 8.83 (bs, 1H, NH), 9.9 (bs 1H, CSNH), 10.83 (bs, 1H, CONH); MS: *m/z* 380 (M^+), 382 (M^++2); Anal. Calcd for $C_{21}H_{17}ClN_2OS$: C, 66.22; H, 4.5; N, 7.35; Found: C, 66.08; H, 4.32; N, 7.12%.

N^1(Diphenylethanoyl)-N^4-(4-flourophenyl) thiosemicarbazide(2b)

Yield: 65%; mp: 162°C; IR (KBr, cm^{-1}): 3289 (NH), 1682 (C=O), 1091 (C=S); ^1H NMR ($CDCl_3$) δ (ppm): 5.13 (s, 1H, CH), 6.97–7.48 (m, 14H, ArH), 8.87 (bs, 1H, NH), 9.93 (bs, 1H, CSNH), 10.89 (bs, 1H, CONH); MS: *m/z* 364 (M^+); Anal. Calcd for $C_{21}H_{17}FN_2OS$: C, 69.21; H, 4.7; N, 7.69; Found: C, 69.03; H, 4.52; N, 7.58%.

N^1(Diphenylethanoyl)-N^4-(3-chloro-4-flourophenyl) thiosemicarbazide (2c)

Yield: 60%; mp: 165°C; IR (KBr, cm^{-1}): 3307 (NH), 1686 (C=O), 1083 (C=S); ^1H NMR ($CDCl_3$) δ (ppm): 5.21 (s, 1H, CH), 7.12–7.63 (m, 14H, ArH), 8.94 (bs, 1H, NH), 9.98 (bs, 1H, CSNH), 10.96 (bs, 1H, CONH); MS: *m/z* 398 (M^+), 400 (M^++2); Anal. Calcd for $C_{21}H_{17}ClFN_2OS$: C, 63.23; H, 4.04; N, 7.02; Found: C, 63.12; H, 3.89; N, 6.89%.

N^1(Diphenylethanoyl)-N^4-(4-methylphenyl)thiosemicarbazide (2d)

Yield: 80%; mp: 185°C; IR (KBr, cm^{-1}): 3274 (NH), 1654 (C=O), 1079 (C=S); ^1H NMR ($CDCl_3$) δ (ppm): 5.86 (s, 1H, CH), 6.3–7.41 (m, 14H, ArH), 8.86 (bs. 1H, NH), 9.87 (bs 1H, CSNH), 10.75 (bs, 1H, CONH); MS: *m/z* 398 (M^+); Anal. Calcd for $C_{22}H_{20}N_2OS$: C, 73.30, H, 5.59, N, 7.77; Found: C, 73.21; H, 5.42; N, 7.68%.

N^1(Diphenylethanoyl)-N^4-(4-methoxyphenyl) thiosemicarbazide (2e)

Yield: 78%; mp: 190°C; IR (KBr, cm^{-1}): 3265 (NH), 1674 (C=O), 1091 (C=S); ^1H NMR ($CDCl_3$) δ (ppm): 3.68 (s, 3H, OCH_3), 5.8 (s, 1H, CH), 6.87–7.43 (m, 14H, ArH), 8.81 (bs, 1H, NH), 9.83 (bs, 1H, CSNH), 10.21 (bs, 1H, CONH); MS: *m/z* 376 (M^+); Anal. Calcd for $C_{22}H_{20}N_2O_2S$: C, 70.19; H, 5.35; N, 7.44; Found: C, 70.03; H, 5.19; N, 7.31%.

General method for synthesis of 5-(diphenylmethyl)-2-(4-substituted phenyl)amino-1,3,4-oxadiazoles (3a–e)

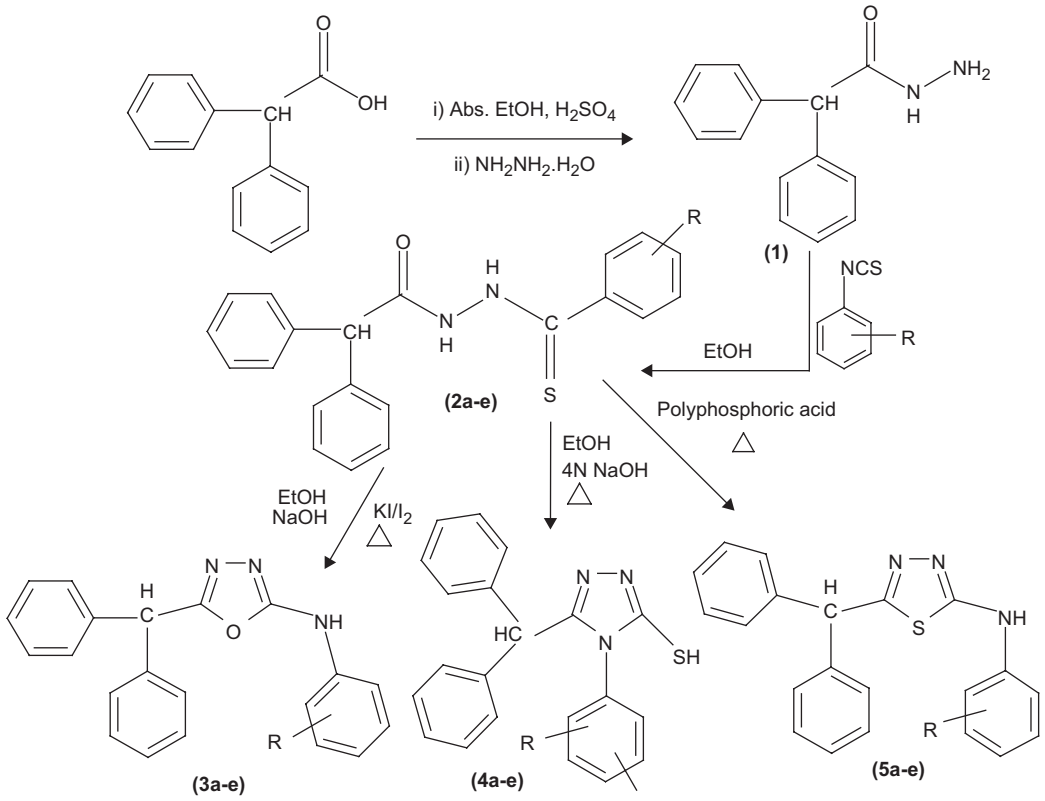
A suspension of thiosemicarbazide (0.002 moles) in ethanol (50 mL) was dissolved in sodium hydroxide (5N, 1 mL) with cooling and stirring resulting in the formation of a clear solution. To this iodine, in potassium iodide solution (5%, 10 mL), was added drop wise with stirring till the colour of the iodine persisted at room temperature. The reaction mixture was then refluxed for 3 h on a water bath. It was then concentrated, cooled, the solid was separated out, filtered, dried and recrystallised with ethanol.

5-(Diphenylmethyl)-2-(4-chlorophenyl)amino-1,3,4-oxadiazole (3a)

Yield: 71%, mp: 142°C; IR (KBr, cm^{-1}): 3330 (NH), 1615 (C=N), 1226 (C-O-C); ^1H NMR ($CDCl_3$) δ (ppm): 5.61 (s, 1H, CH), 6.93–7.31 (m, 14H, ArH), 7.91 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 45.32 (CH), 121.13 ($2CH_{arom}$), 127.38 ($4CH_{arom}$), 127.71 ($4CH_{arom}$), 128.76 ($2CH_{arom}$), 130.17 ($2CH_{arom}$), 132.47 (CH_{arom}), 134.31 (CH_{arom}), 137.17 ($2CH_{arom}$), 158.37 ($C_5/_{arom}$), 159.13 ($C_2/_{arom}$); MS: *m/z* 362 (M^+), 364 (M^++2); Anal. Calcd for $C_{21}H_{16}ClN_3O$: C, 69.71; H, 4.46; N, 11.61; Found: C, 69.86; H, 4.32; N, 11.73%.

5-(Diphenylmethyl)-2-(4-fluorophenyl)amino-1,3,4-oxadiazole (3b)

Yield: 65.8%, mp: 145°C; IR (KBr, cm^{-1}): 3342 (NH), 1610 (C=N), 1221 (C-O-C); ^1H NMR ($CDCl_3$) δ (ppm): 5.64 (s,



R = a: 4-Cl; b: 4-F; c: 3-Cl-4-F; d: 4-CH_3 ; e: 4-OCH_3

Scheme 1. Synthesis of azole derivatives **3**, **4** and **5**.

1H, CH), 6.99–7.37 (m, 14H, ArH), 7.95 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 47.24 (CH), 117.16 ($2CH_{arom}$), 118.27 ($2CH_{arom}$), 128.83 ($2CH_{arom}$), 130.29 ($4CH_{arom}$), 130.52 ($4CH_{arom}$), 137.59 (CH_{arom}), 140.31 ($2CH_{arom}$), 155.38 (CH_{arom}), 159.87 ($C_5/_{arom}$), 160.59 ($C_2/_{arom}$);MS: *m/z* 345 (M^+); Anal. Calcd for $C_{21}H_{16}FN_3O$: C, 73.03; H, 4.67; N, 12.17; Found: C, 73.21; H, 4.53; N, 11.98%.

5-(Diphenylmethyl)-2-(3-chloro-4-fluorophenyl)amino-1,3,4-oxadiazole (3c)

Yield: 66.9%; mp: 170°C; IR (KBr, cm^{-1}): 3314 (NH), 1625 (C=N), 1246 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 5.68 (s, 1H, CH), 7.16–7.47 (m, 14H, ArH), 8.1 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 43.52 (CH), 111.53 (CH_{arom}), 111.75 (CH_{arom}), 114.07 (CH_{arom}), 115.66 (CH_{arom}), 122.66 ($2CH_{arom}$), 123.65 ($4CH_{arom}$), 123.9 ($4CH_{arom}$), 130.77 (CH_{arom}), 133.66 ($2CH_{arom}$), 149.43 (CH_{arom}), 155.48 ($C_5/_{arom}$), 155.65 ($C_2/_{arom}$); MS: *m/z* 379 (M^+), 381 (M^++2); Anal. Calcd for $C_{21}H_{15}ClFN_3O$: C, 66.41; H, 3.98; N, 11.06; Found: C, 66.73; H, 3.87; N, 10.93%.

5-(Diphenylmethyl)-2-(4-methylphenyl)amino-1,3,4-oxadiazole (3d)

Yield: 75%, mp: 180°C; IR (KBr, cm^{-1}): 3310 (NH), 1612 (C=N), 1228 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.3 (s, 3H, CH_3), 5.63 (s, 1H, CH), 7.1–7.36 (m, 14H, ArH), 7.57 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 20.75 (CH_3), 48.51 (CH), 117.55 ($2CH_{arom}$), 127.59 ($2CH_{arom}$), 128.62 ($4CH_{arom}$), 128.81 ($4CH_{arom}$), 129.86 ($2CH_{arom}$), 132.65 (CH_{arom}), 135.02 (CH_{arom}), 138.52 ($2CH_{arom}$), 160.49 (C_5/arom), 160.79 (C_2/arom); MS: *m/z* 341 (M^+); Anal. Calcd for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31; Found: C, 77.29; H, 5.53; N, 12.24%.

5-(Diphenylmethyl)-2-(4-methoxyphenyl)amino-1,3,4-oxadiazole (3e)

Yield: 80%, mp: 175°C; IR (KBr, cm^{-1}): 3318 (NH), 1620 (C=N), 1231 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 5.59 (s, 1H, CH), 6.98–7.33 (m, 14H, ArH), 7.52 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 47.84 (CH), 55.12 (OCH_3), 113.36 ($2CH_{arom}$), 125.17 (CH_{arom}), 126.26 ($2CH_{arom}$), 127.91 ($4CH_{arom}$), 128.13 ($4CH_{arom}$), 129.27 ($2CH_{arom}$), 137.48 ($2CH_{arom}$), 152.71 ($C_5/_{arom}$), 159.39 ($C_2/_{arom}$), 167.58 (CH_{arom}); MS: *m/z* 357 (M^+); Anal. Calcd for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76; Found: C, 73.78; H, 5.14; N, 11.83%.

General method for synthesis of 5-(diphenylmethyl)-4-(4-substituted phenyl)-3-mercapto (4H)-1,2,4-triazoles (4a–e)

In a 100 ml round bottom flask, thiosemicarbazide (0.001 M) and ethanol (20 mL) was placed. To it sodium hydroxide solution (4N, 2 mL) was added, resulting in the formation of a clear solution. The reaction mixture was refluxed for 6 h on a water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to between pH 5–6 with acetic acid and kept aside for 1–2 h. The solid

obtained was separated, then filtered, washed with water, dried and recrystallised from ethanol.

5-(Diphenylmethyl)-4-(4-chlorophenyl)-3-mercapto(4H)-1,2,4-triazole (4a)

Yield: 74%; mp 225°C; IR (KBr, cm^{-1}): 2550 (SH), 1610 (C=N); ^1H NMR ($CDCl_3$) δ (ppm): 5.03 (s, 1H, CH), 6.94–7.39 (m, 14H, ArH), 11.49 (bs, 1H, SH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.5 (CH), 127.4 ($2CH_{arom}$), 128.51 ($4CH_{arom}$), 128.73 ($4CH_{arom}$), 129.58 ($2CH_{arom}$), 129.65 ($2CH_{arom}$), 131.92 (CH_{arom}), 135.61 (CH_{arom}), 138.15 ($2CH_{arom}$), 152.87 ($C_5/_{arom}$), 168.46 ($C_3/_{arom}$); MS: *m/z* 378 (M^+), 380 (M^++2); Anal. Calcd for $C_{21}H_{16}ClN_3S$: C, 66.75; H, 4.27; N, 11.12; Found: C, 66.89; H, 4.16; N, 10.98%.

5-(Diphenylmethyl)-4-(4-fluorophenyl)-3-mercapto (4H)-1,2,4-triazole (4b)

Yield: 70%; mp: 245°C; IR (KBr, cm^{-1}): 2680 (SH), 1588 (C=N); ^1H NMR ($CDCl_3$) δ (ppm): 5.12 (s, 1H, CH), 6.97–7.45 (m, 14H, ArH), 11.53 (bs, 1H, SH); ^{13}C NMR ($CDCl_3$) δ (ppm): 47.89 (CH), 118.37 ($2CH_{arom}$), 119.69 ($2CH_{arom}$), 128.73 ($2CH_{arom}$), 131.25 ($4CH_{arom}$), 131.86 ($4CH_{arom}$), 136.67 (CH_{arom}), 140.17 ($2CH_{arom}$), 147.36 (CH_{arom}), 152.63 ($C_5/_{arom}$), 168.25 ($C_3/_{arom}$); MS: *m/z* 361 (M^+); Anal. Calcd for $C_{21}H_{16}FN_3S$: C, 69.78; H, 4.27; N, 11.63; Found: C, 69.64; H, 4.16; N, 11.51%.

5-(Diphenylmethyl)-4-(3-chloro-4-flurophenyl)-3-mercapto (4H)-1,2,4 triazole (4c)

Yield: 62%; mp: 252°C; IR (KBr, cm^{-1}): 2650 (SH), 1610 (C=N); ^1H NMR (CDCl3) δ (ppm): 5.19 (s, 1H, CH), 7.09–7.52 (m, 14H, ArH), 11.63 (bs, 1H, SH); ^{13}C NMR ($CDCl_3$) δ (ppm): 45.47 (CH), 111.64 (CH_{arom}), 111.95 (CH_{arom}), 114.35 (CH_{arom}), 115.98 (CH_{arom}), 122.84 ($2CH_{arom}$), 123.93 ($4CH_{arom}$), 124.37 ($4CH_{arom}$), 131.27 (CH_{arom}), 133.98 ($2CH_{arom}$), 149.76 (CH_{arom}), 151.13 ($C5/_{arom}$), 163.41 ($C2/_{arom}$); MS: *m/z* 396 (M^+), 398 (M^{++2}); Anal. Calcd for $C_{21}H_{15}ClFN_3S$: C, 63.71; H, 3.82; N, 10.61; Found: C, 63.62; H, 3.7; N, 10.52%.

5-(Diphenylmethyl)-4-(4-methylphenyl)-3-mercapto(4H)-1,2,4-triazole (4d)

Yield: 80%, mp: 254°C; IR (KBr, cm^{-1}): 2564 (SH), 1623 (C=N); ^1H NMR ($CDCl_3$) δ (ppm): 2.39 (s, 3H, CH_3), 5.05 (s, 1H, CH), 6.88–7.31 (m, 14H, ArH), 11.35 (bs, 1H, SH); ^{13}C NMR ($CDCl_3$) δ (ppm): 20.63 (CH_3), 48.42 (CH), 127.64 ($2CH_{arom}$), 128.71 ($4CH_{arom}$), 128.93 ($4CH_{arom}$), 129.87 ($2CH_{arom}$), 130.16 ($2CH_{arom}$), 132.17 (CH_{arom}), 135.84 (CH_{arom}), 138.34 ($2CH_{arom}$), 152.96($C_5/_{arom}$), 168.75 ($C_3/_{arom}$); MS: *m/z* 357 (M^+); Anal. Calcd for $C_{22}H_{19}N_3S$: C, 73.92; H, 5.36; N, 11.75; Found: C, 73.78; H, 5.24; N, 11.87%.

5-(Diphenylmethyl)-4-(4-methoxyphenyl)-3-mercapto(4H)-1,2,4-triazole (4e)

Yield: 81%, mp: 232°C; IR (KBr, cm^{-1}): 2557 (SH), 1608 (C=N); ^1H NMR ($CDCl_3$) δ (ppm): 3.83 (s, 3H, OCH_3), 5.05 (s, 1H, CH), 6.88–7.32 (m, 14H, ArH), 10.78 (bs, 1H, SH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.49 (CH), 55.57 (OCH_3), 114.65 ($2CH_{arom}$), 126.01 (CH_{arom}), 127.44 ($2CH_{arom}$), 128.63 ($4CH_{arom}$), 128.76 ($4CH_{arom}$), 129.58 ($2CH_{arom}$), 138.8 ($2CH_{arom}$), 153.56 ($C_5/_{arom}$), 160.16 ($C_3/_{arom}$), 168.7 (CH_{arom}); MS: *m/z* 373 (M^+); Anal. Calcd for $C_{22}H_{19}N_3OS$: C, 70.75; H, 5.13; N, 11.25; Found: C, 70.59; H, 5.27; N, 11.13%.

General method for synthesis of 5-(diphenylmethyl)-2-(4-substituted phenyl)amino-1,3,4-thiadiazoles (5a–e)

The thiosemicarbazide (0.001 M) was added gradually with stirring to polyphosphoric acid (12–15 mL) at 120°C for 20 min. The reaction mixture was heated with stirring for another 3 h and poured over crushed ice. The precipitated mass was filtered, washed with water, dried and recrystallised from methanol.

5-(Diphenylmethyl)-2-(4-chlorophenyl)amino-1,3,4-thiadiazole (5a)

Yield: 75%; mp: 115°C; IR (KBr, cm^{-1}): 3388 (NH), 1636 (C=N); ^1H NMR ($CDCl_3$) δ (ppm): 5.61 (s, 1H, CH), 7.25–7.87 (m, 14H, ArH), 7.97 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 51.47 (CH), 116.46 ($2CH_{arom}$), 116.78 ($2CH_{arom}$), 127.92 ($2CH_{arom}$), 129.35 ($4CH_{arom}$), 129.57 ($4CH_{arom}$), 137.76 (CH_{arom}), 142.35 ($2CH_{arom}$), 156.68 (CH_{arom}), 162.47 ($C_5/_{arom}$), 165.37 ($C_2/_{arom}$); MS: *m/z* 378 (M^+), 380 (M^++2); Anal. Calcd for $C_{21}H_{16}ClN_3S$: C, 66.75; H, 4.27; N, 11.12; Found: C, 66.58; H, 4.12; N, 11.29%.

5-(Diphenylmethyl)-2-(4-fluorophenyl)amino-1,3,4-thiadiazole (5b)

Yield: 65.5%; mp: 132°C; IR (KBr, cm^{-1}): 3346 (NH), 1632 (C=N); ^1H NMR ($CDCl_3$) δ (ppm): 5.67 (s, 1H, CH), 7.34–7.91 (m, 14H, ArH), 8.03 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm) 51.28 (CH), 115.97 ($2CH_{arom}$), 116.19 ($2CH_{arom}$), 127.59 ($2CH_{arom}$), 129.03 ($4CH_{arom}$), 129.11 ($4CH_{arom}$), 137.59 (CH_{arom}), 141.99 ($2CH_{arom}$), 156.46 (CH_{arom}), 162.22 ($C_5/_{arom}$), 165.13 ($C_2/_{arom}$); MS: *m/z* 361 (M^+); Anal. Calcd. for $C_{21}H_{16}FN_3S$: C, 69.78; H, 4.46; N, 11.63; Found: C, 69.59; H, 4.37; N, 11.52%.

5-(Diphenylmethyl)-2-(3-chloro-4-fluorophenyl)amino-1,3,4-thiadiazole (5c)

Yield: 58%; mp: 113°C; IR (KBr, cm^{-1}): 3343 (NH), 1612 (C=N); ^1H NMR ($CDCl_3$), δ (ppm): 5.72 (s, 1H, CH), 7.41–7.97 (m, 14H, ArH) 8.14 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.12 (CH), 112.35 (CH_{arom}), 112.68 (CH_{arom}), 115.63 (CH_{arom}), 116.12 (CH_{arom}), 123.59 ($2CH_{arom}$), 124.54 ($4CH_{arom}$), 124.97 ($4CH_{arom}$), 132.32 (CH_{arom}), 134.84 ($2CH_{arom}$), 152.43 (CH_{arom}), 161.57 ($C_5/_{arom}$), 165.21 ($C_2/_{arom}$); MS: *m/z* 396 (M^+), 398 (M^++2); Anal. Calcd for $C_{21}H_{15}ClFN_3S$: C, 63.71; H, 3.82; N, 10.61; Found: C, 63.56; H, 3.71; N, 10.49%.

5-(Diphenylmethyl)-2-(4-methylphenyl)amino-1,3,4-thiadiazole (5d)

Yield: 75.6%, mp: 130°C; IR (KBr, cm^{-1}): 3337 (NH), 1623 (C=N); ^1H NMR (DMSO d_6) δ (ppm): 2.29 (s, 3H, CH_3), 5.62 (s, 1H, CH), 7.09–7.34 (m, 14H, ArH), 7.78 (s, 1H, NH); ^{13}C NMR (DMSO d_6) δ (ppm): 25.54 (CH_3), 56.96 (CH),

122.88 ($2CH_{arom}$), 132.05 ($2CH_{arom}$), 133.46 ($4CH_{arom}$), 133.5 ($4CH_{arom}$), 134.3 ($2CH_{arom}$), 136.43 (CH_{arom}), 143.16 (CH_{arom}), 146.07 ($2CH_{arom}$), 166.28 ($C_5/_{arom}$), 170.32 ($C_2/_{arom}$); MS: *m/z* 357 (M^+); Anal. Calcd for $C_{22}H_{19}N_3S$: C, 73.92; H, 5.36; N, 11.75; Found: C, 73.74, H, 5.27; N, 11.59%.

5-(Diphenylmethyl)-2-(4-methoxyphenyl)amino-1,3,4-thiadiazole (5e)

Yield: 80%; mp: 110°C; IR (KBr, cm^{-1}): 3358 (NH), 1624 (C=N); ^1H NMR (DMSO d_6) δ (ppm): 3.81 (s, 3H, OCH_3), 5.65 (s, 1H, CH), 7.14–7.43 (m, 14, 14ArH), 7.91 (s, 1H, NH); ^{13}C NMR (DMSO d_6) δ (ppm): 54.27 (CH), 58.69 (OCH_3), 118.68 ($2CH_{arom}$), 129.65 (CH_{arom}), 132.53 ($2CH_{arom}$), 134.27 ($4CH_{arom}$), 134.82 ($4CH_{arom}$), 135.69 ($2CH_{arom}$), 140.13 ($2CH_{arom}$), 163.37 ($C_5/_{arom}$), 165.19 ($C_2/_{arom}$), 169.24 (CH_{arom}); MS: *m/z* 373 (M^+); Anal. Calcd for $C_{22}H_{19}N_3OS$: C, 70.75; H, 5.13; N, 11.25; Found: C, 70.63, H, 5.02; N, 11.13%.

Pharmacology

The synthesised compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and lipid-peroxidation activities. The Wister rats and albino mice used in the present study were housed and kept in accordance with the Hamdard University Animal Care Unit, which applies the guidelines and rules laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. All the test compounds and standard drug were administered in the form of solution (0.5% w/v carboxymethyl cellulose as a vehicle) by an oral route. Each group consisted of six animals. All the animals were procured from the CPCSEA and maintained in colony cages at 25 ± 2°C, relative humidity of 45–55%, under a 12h light and dark cycle and were fed a standard animal feed. All the animals were acclimatised for a week before use. The anti-inflammatory activity of the test compounds were compared with the control. The analgesic, ulcerogenic and lipid peroxidation activities were compared with the standard drug ibuprofen. Data were analysed by student's t test for n=6.

Anti-inflammatory activity

Anti-inflammatory activity was carried out by the carrageenan induced paw oedema test in the Wister albino rat according to the method of Winter et al. [20]. The standard drug, ibuprofen and test compounds were given orally (70mg/kg body weight) as a suspension using 0.5% w/v carboxymethyl cellulose as a vehicle. After one hour, foot paw oedema was induced by injecting 0.1 ml of 1% carrageenan subcutaneously into the planter portion of the right hind paw of each rat. The initial paw volume was measured immediately using a mercury plethysmometer. The paw volume was again measured after the time intervals of 3 and 4 h. The percentage inhibition of inflammation was calculated for the standard drugs and other test compounds and a comparison was made. The percentage inhibition of inflammation was calculated according to the formula: % anti-inflammatory activity = 100 × (1-Vt/Vc) where, Vt and Vc are the volume of oedema for the test and control groups respectively.

Analgesic activity

Analgesic activity was evaluated by the tail immersion method [21] using Swiss albino mice (25–30 g) of either sex selected by a random sampling technique. The standard drug, ibuprofen and test compounds and standard drugs were administered orally (70mg/kg body weight) as a suspension using 0.5% w/v carboxymethyl cellulose as a vehicle. The lower 5 cm portion of the tail was gently immersed into thermostatically controlled water at 55 ± 0.5°C. The time for tail withdrawal from the water (in seconds) was taken as the reaction time with a cut of time of immersion, set at 10 seconds for both control and treatment groups. The reaction time was measured before and after a 4 h interval after the administration of the test compounds and standard drugs.

Acute ulcerogenicity

An acute ulcerogenesis test was performed according to Cioli et al. [22] using Wistar rats (180–200 gm) of either sex. The animals were divided into groups, with each group consisting of 6 rats. All the rats were fasted for 24 h with free access to water. The control groups of animals were administered a 0.5% CMC solution intraperitoneally. One group was administered with the standard drug ibuprofen orally in a dose of 210 mg/kg once a day for three days. The remaining group of animals was administered with the test compounds through the same route. The animals were immediately fed and kept for 17 hrs after the dose administration. After 17 h they were killed and dissected for the estimation of ulcerogenic activity. The stomach was dissected out and washed with running water, opened along the greater curvature and carefully observed with a magnifying glass. The mucosal damage for each stomach was assessed according to the following scoring system:

0.5: redness, 1: spot ulcers, 1.5: haemorrhagic streak, 2.0: ulcers >3 but ≤5, 3.0: ulcers >5. The mean score of each treated group minus the mean score of the control group was regarded as the severity index of gastric mucosal damage.

Lipid peroxidation

The lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al [23]. After the evaluation of each stomach for ulcers, the gastric mucosa of the glandular portion was scrapped, weighed (100 mg), homogenised in a pestle and mortar and the homogenate was prepared in 1.8 ml of ice cold 1.15 % KCl solution. The homogenate was supplemented with 0.2 ml of 8.1 % sodium dodecyl sulfate (SDS), 1.5 ml of acetate buffer and 1.5 ml of 0.8 % thiobarbituric acid (TBA). The mixture was incubated at 95°C for 60 minutes on a boiling water bath then extracted with a mixture of n-butanol: pyridine (15:1, v/v; 5 mL) by shaking vigorously for 1 minute and then kept in ice for 2 minutes. The organic

layer of reaction mixture was centrifuged at 3000 rpm for 10 minutes and the absorbance was measured at 532 nm on a UV spectrophotometer. The results were expressed as nmol malondialdehyde /100 mg tissue.

Results and discussion

Chemistry

The key intermediate N^1(diphenylethanoyl)-N^4-aryl thiosemicarbazides (**2a**–**e**) were synthesised by refluxing a mixture of diphenyl acetic acid hydrazide and the substituted phenyl isothiocyanate in ethanol. The structure of thiosemicarbazide **2d** was confirmed by its IR spectrum which displayed IR peaks at 3274 cm^{-1} for NH, 1654 cm^{-1} for C=O and 1079 cm^{-1} corresponding to C=S stretching vibrations. The ^{1}H NMR spectrum showed a multiplet at δ 6.30–7.41 for 14 aromatic protons. The CSNH and CONH protons were observed at δ 9.87 and 10.75 respectively confirming the formation of the semicarbazide. The mass spectrum of the compound **2d** showed a molecular ion peak M^+ at an *m/z* of 398 corresponding to the molecular formula $C_{22}H_{20}N_2OS$. The thiosemicarbazide (**2a**–**e**) were oxidatively cyclised to 5-(diphenylmethyl)-2-(4-substituted phenyl) amino-1,3,4-oxadiazoles (**3a**–**e**) by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. The IR spectrum of the oxadiazole **3d** showed an absorption peak at 1612 cm^{-1} corresponding to C=N stretching vibrations. The structure was further supported by its ^{1}H NMR spectrum, which showed a multiplet at δ 7.10–7.36 for fourteen aromatic protons. The disappearance of both CSNH and CONH singlet signals of thiosemicarbazide and the appearance of an NH signal at δ 8.57 confirmed the formation of an oxadiazole ring. The mass spectrum of compound **3d** showed a molecular ion peak M^+ at an *m/z* of 323 corresponding to the molecular formula $C_{22}H_{19}N_3O$. On heating with 4N-NaOH in ethanol the thiosemicarbazide (**2a**–**e**) underwent smooth cyclisation through dehydration to afford the 5-(diphenylmethyl)-4-(4-substituted phenyl)-3-mercapto (4H)-1,2,4-triazole (**4a**–**e**). The structure of triazole **4d** was confirmed by its IR spectrum, which displayed an absorption peak at 1623 cm^{-1} due to C=N and at 2564 cm^{-1} corresponding to an SH group. The formation of the triazole ring was further supported by its ^{1}H NMR spectrum which showed a broad singlet of the SH proton at δ 11.35. The mass spectrum of the compound showed a molecular ion peak M^+ at an *m/z* of 357 corresponding to the molecular formula $C_{27}H_{19}N_3S$. The 5-(diphenylmethyl)-2-(4-substiued phenyl)amino-1,3,4-thiadiazole (**5a**–**e**) were obtained by cyclisation of **2a**–**e** with polyphosphoric acid. The IR spectrum of the compound **5d** showed absorption peak at 1623 cm^{-1} due to the C=N stretching vibrations. In the ^{1}H NMR spectra of the compound the singlet of CSNH and CONH of the semicarbazide disappeared. The NH proton at the second position of the thiadiazole ring appeared as a singlet at δ 7.91 confirming the structure. The mass spectra of the compound showed a molecular ion peak M^+ at an *m/z* of 357 corresponding to the molecular formula $C_{22}H_{19}N_3S$. All the compounds were synthesised in a good yield of between 62–85.4%. TLC and elemental analysis were used to check the purity of the compounds. Both analytical and spectral data (IR and ^{1}H NMR) confirmed the structure of the compounds.

Pharmacology

Anti-inflammatory activity

The anti-inflammatory activity of the synthesised compounds **3a**–**e**, **4a**–**e** and **5a**–**e** were evaluated by carrageenan induced paw edema method of Winter et al. The compounds were tested at an equimolar oral dose relative to 70 mg/kg of ibuprofen. The percentage inhibition was calculated after 3 and 4 h, and since it was found to be more after 4h, this was made to be the basis of this discussion. The tested compounds showed anti-inflammatory activity ranging from 50.82% to 82.25% (Table 1). Whereas the standard drug ibuprofen showed 75.12% inhibition after 4h. The anti-inflammatory activity of 1,3,4 oxadiazole derivatives **3a**–**e** were in the range of 50.82 to 82.25%. The oxadiazole derivative **3b** having a 4-fluoro phenyl amino group showed the highest activity (82.25%) and this was more than the standard drug ibuprofen (75.12%). When the group was replaced by a 3Cl-4F-phenyl amino group (**3c**) there was a slight decrease in activity (81.43%). It was observed that oxadiazole derivatives having 4Cl-phenyl amino (**3a**), 4-methoxy phenyl amino (**3e**) and 4-methyl phenyl amino (**3d**) groups at the second position showed a decreasing

Table 1. Anti-inflammatory activity of compounds (**3a**–**e**, **4a**–**e** and **5a**–**e**).

	Anti-inflammatory activity % inhibition ± SEM#			Anti-inflammatory activity % inhibition ± SEM#	
Compound	After 3h	After 4h	Compound	After 3h	After 4h
Ibuprofen	72.67 ± 3.42	75.12 ± 3.95	**4c**	58.95 ± 4.06	70.95 ± 3.2[c]
3a	58.33 ± 3.6	62.87 ± 3.79[a]	**4d**	53.24 ± 4.56	65.52 ± 4.54[c]
3b	81.68 ± 1.73	82.25 ± 1.55[c]	**4e**	64.38 ± 4.77	72.58 ± 2.99[c]
3c	77.24 ± 4	81.43 ± 2.17[c]	**5a**	52.56 ± 2.56	55.29 ± 2.47[a]
3d	50.81 ± 5.64	50.82 ± 4.24[a]	**5b**	58.18 ± 3.13	63.26 ± 3.53[b]
3e	50.16 ± 3.35	51.24 ± 4.72[a]	**5c**	62.3 ± 3.6	67.92 ± 1.52[c]
4a	56.81 ± 1.94	59.85 ± 2.73[a]	**5d**	59.68 ± 3.78	64.95 ± 3.93[c]
4b	56.38 ± 3.34	57.95 ± 1.87[a]	**5e**	62.41 ± 3.58	65.11 ± 2.24[c]

#Relative to standard and data were analyzed by student's t test for n=6

[a]p <0.01; [b]p <0.05; [c]p <0.5

Table 2. Analgesic, ulcerogenic and lipid peroxidation activities of selected compounds.

Compound	Analgesic Activity[#] Pre-treatment/ normal 0 h (s)	Post-treatment /after 4 h (s)	% Inhibition	UlcerogenicActivity (Severityindex ± SEM) [#]	nmol MDA content ± SEM / 100 mg tissue[#]
3b	1.59 ± 0.021	2.95 ± 0.018	85.53 ± 2.09[b]	0.333 ± 0.1[e]	4.27 ± 0.59[c]
3c	1.28 ± 0.013	2.21 ± 0.010	73.8 ± 1.24	0.166 ± 0.1[c]	3.41 ± 0.03[a]
4c	1.27 ± 0.013	2.11 ± 0.010	66.14 ± 1.9[c]	0.5 ± 0.18	5.25 ± 0.33[c]
4d	1.86 ± 0.021	2.50 ± 0.016	34.4 ± 1.27[a]	—	—
4e	1.37 ± 0.025	2.14 ± 0.015	68.5 ± 1.42[b]	0.333 ± 0.1[e]	4.21 ± 0.17[a]
5c	1.36 ± 0.011	2.17 ± 0.013	59.55 ± 0.84[a]	—	—
5e	1.26 ± 0.014	2.10 ± 0.016	67.46 ± 1.67[e]	0.333 ± 0.1[e]	5.19 ± 0.26[b]
Control	—	—	—	0	3.25 ± 0.05
Ibuprofen	1.36 ± 0.009	2.37 ± 0.012	74.26 ± 1.25	0.5 ± 0	6.55 ± 0.03

#Relative to standard and data where analysed by student t test for n=6
[a]p <0.0001; [b]p <0.001; [c]p <0.01; [d]p <0.05; [e]p <0.5

order of anti-inflammatory activity of 62.87%, 51.24% and 50.81% respectively.

The anti-inflammatory activity of the 1,2,4-triazole derivatives **4a**–**e** were found to be between 59.75% and 72.58%. The maximum activity was found in the triazole derivatives **4e**, having a 4-methoxy phenyl group at position 4. When this group was replaced by a 3Cl, 4F-phenyl group (**4c**), the activity was slightly decreased. The triazole derivatives showed moderate to weak anti-inflammatory activity for this test. The 1,3,4-thiadiazole derivatives **5a**–**e** showed anti-inflammatory activity between 55.29% to 67.92%. It was observed that thiadiazole derivatives having 4F-phenyl amino (**5b**), 3Cl,4F-phenyl amino (**5c**), 4-methyl phenyl amino (**5d**) and 4-methoxy phenyl amino (**5e**) group showed good activity of 63.26%, 67.92%, 64.95% and 65.11% respectively. Compound **5a** having a 4-chloro phenyl amino group showed weak anti-inflammatory activity. The compounds **3b**–**e**, **4c**–**e**, **5b** and **5e** showed 65% anti-inflammatory activity, and were further tested for their analgesic activity at an equimolar oral dose relative to 70 mg/kg ibuprofen (Table 2). The compounds showed analgesic activity ranging from 34.4% to 85.53% whereas the standard drug ibuprofen showed 74.26% inhibition. It was found that compound **3b**, an oxadiazole derivative having the highest anti-inflammatory activity, also showed the highest analgesic activity (85.53%). The compound (**3c**) having a 3Cl-4F-phenyl amino group also showed significant analgesic activity (73.5%). The other oxadiazole, triazole and thiadiazole derivatives showed moderate to good analgesic activity except compound (**4d**), a triazole derivative which showed minimum analgesic activity (34.4%).

Acute ulcerogenic activity

The compounds **3b**, **3c**, **4c**, **4e** and **5e** showing high analgesic activity were further screened for their acute ulcerogenic activity. The compounds were tested at an equimolar oral dose relative to 210 mg/kg ibuprofen. The tested compound showed a significant reduction in ulcerogenic activity ranging from 0.5 ± 0 to 0.166 ± 0.105 whereas the standard drug, ibuprofen showed a severity index of 0.5 ± 0. The maximum reduction in ulcerogenic activity (0.166 ± 0.105) was found for compound (**3c**) having a 3Cl-4F-phenylamino group at the second position of the oxadiazole ring. Rest of the compound also showed an improved GI safety profile compared to ibuprofen, as illustrated in Table 2. Thus the results showed that substitution of the carboxylic group by a 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole, has resulted in significant anti-inflammatory and analgesic activities along with reduced ulcerogenic potential. As the anti-inflammatory, analgesic and ulcerogenic activity were performed only on a single dose, the dose related or graded pharmacological activity has not been explored.

Lipid peroxidation

It has been reported that compounds with a lower ulcerogenic activity also had a reduced malondialdehyde (MDA) content, a by product of lipid peroxidation [24]. Therefore, an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were also analysed for their effect on lipid peroxidation. The lipid peroxidation was measured as nanomoles of malondialdehyde (MDA)/100 mg of gastric mucosa tissue. Ibuprofen exhibited maximum tissue lipid peroxidation 6.55 ± 0.026, whereas the control group showed a level of 3.25 ± 0.05. It was found that all the cyclise derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (range of 3.41–5.25, Table 2). Thus these studies showed that the synthesised compounds have inhibited the induction of gastric mucosal lesions and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa.

Conclusion

Various derivatives of diphenyl acetic acid containing 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole nucleus were successfully synthesised and screened for anti-inflammatory, analgesic, ulcerogenic activities and lipid peroxidation studies. Several of these compounds have

been evaluated as potential anti-inflammatory-analgesic agents with minimum ulcerogenic potential and lipid peroxidation. The tested compounds were found to be less irritating to the gastric mucosa as indicated by the severity index. The lipid peroxidation values of the compounds tested were also reduced. Among the synthesised compounds, **3b** and **3c** possessed the most prominent and consistent activity with the maximum reduction in gastrointestinal toxicity and minimum lipid peroxidation. Further studies to acquire more information about the structure-activity relationships are in progress in our laboratories.

Acknowledgements

The authors would like to thank the Central Drug Research Institute (CDRI) and the Central Instrumentation Facility (CIF), Hamdard University for the spectral analysis of the compounds. The authors would also like to thank Shaukat Shah, (in-charge of the animal house) and Jamia Hamdard for providing the Wistar rats.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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