Synthesis of Some Newer Analogues of 4-Hydroxyphenyl Acetic Acid as Potent Anti-inflammatory Agents

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A series of 1,3,4-oxadiazole and 1,2,4-triazole derivatives of 4-hydroxyphenyl acetic acid have been synthesized and evaluated for their anti-inflammatory activity by carrageenan induced rat paw edema method. The compounds, which showed good anti-inflammatory activity, were screened for their ulcerogenic and lipid peroxidation activities.

Keywords: 1,3,4-Oxadiazoles; 1,2,4-Triazoles; Anti-inflammatory; Ulcerogenic; Lipid peroxidation.

INTRODUCTION

The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme cyclooxygenases (COXs).[1,2] Recently it was discovered that COX exists in two isoforms, COX-1 and COX-2, which were regulated differently.[3-5] COX-1 provides cytoprotection in the gastrointestinal (GI) tract, whereas inducible COX-2 mediates inflammation.[6-8] Since most of the NSAIDs in the market show greater selectivity for COX-1 than COX-2,[9] chronic use of NSAIDs may elicit appreciable GI irritation, bleeding and ulceration.[10] The GI toxicity of acidic non-steroidal anti-inflammatory drugs is one of the most challenging problems in medicinal chemistry, since these side effects are usually related to the intrinsic mechanism responsible for the desired activity.[11,12] It is probably caused by a combination of local irritation produced by direct contact of free carboxylic group of NSAIDs and inhibition of enzyme COX-1 responsible for gastric mucosal protection.[1,13] A survey of the literature has also revealed that local generation of various reactive oxygen species (ROS) may be playing a significant role in the formation of gastric mucosal lesions associated with NSAID therapy.[14,15] This fact was further supported by the finding that indomethacin administration results in increased ROS production in the gastric mucosa,[16,17] followed by gastric ulceration. Thus the discovery of molecules which combine anti-inflammatory and antioxidant activities may lead to development of drugs with an improved therapeutic index.

It has been reported in the literature that derivatization of the carboxylate function of representative NSAIDs resulted in increased anti-inflammatory activity with reduced ulcerogenic effect.[18,19] It was also observed that certain 5-membered heterocyclic compounds bearing 1,3,4-oxadiazole and 1,2,4-triazole nuclei possess interesting anti-inflammatory activity with fewer GI side effects.[20-22] Moreover, phenolic compounds have also been reported to possess good anti-inflammatory and antioxidant activities.[23,24] In view of these observations and in continuation of our research programme on the synthesis of 5-membered heterocyclic compounds of acetic acid derivatives,[25,26] we herein report the synthesis of some newer, more potent analogues of 4-hydroxyphenyl acetic acid by replacing the carboxylic acid group with 1,3,4-oxadiazole and 1,2,4-triazole nuclei, which have been found to possess an interesting profile of anti-inflammatory activity with significant reduction in their ulcerogenic effect. The structure of the newly synthesized compounds was confirmed by elemental and spectral analysis.

RESULTS AND DISCUSSION

Chemistry

The acid hydrazide **1** was prepared by esterification of 4-hydroxyphenylacetic acid followed by treatment with hydrazine hydrate in absolute ethanol. The reaction of hydrazide **1** with carbon disulphide in alkaline medium af-

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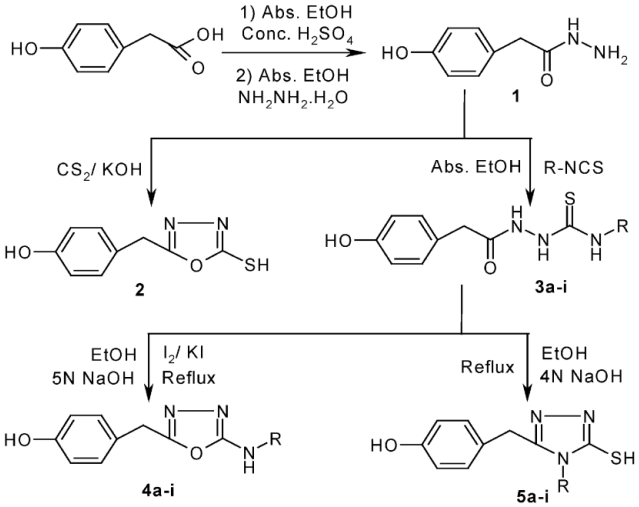
forded, after acidic treatment, 5-(4-hydroxyphenyl)methyl-2-mercapto-1,3,4-oxadiazole **2**. The IR spectra of compound **2** revealed the presence of absorption bands at 1165 cm^{-1} for the C=S group and at 1662 cm^{-1} due to C=N stretching vibrations. The ^1H NMR spectrum of compound **2** showed a broad singlet at δ 12.09 for the SH proton, confirming the formation of a mercaptotriazole ring. The mass spectra showed a molecular ion peak M^+ at *m/z* 208 corresponding to the molecular formula $C_9H_8N_3O_2S$. The hydrazide on treatment with various aryl/alkyl isothiocyanates gave N^1-[2-(4-hydroxyphenyl)acetyl] N^4-aryl/alkyl-3-thiosemicarbazides **3a-i**. The structure of thiosemicarbazide **3c** was confirmed by its IR spectrum, which displayed absorption bands at 3392 cm^{-1} for NH, 1696 cm^{-1} due to C=O and 1183 cm^{-1} corresponding to C=S stretching vibrations. The ^1H NMR spectrum showed a singlet at δ 9.27 for the NHAr-Cl proton. The CSNH and CONH protons were obtained as a broad singlet at δ 9.69 confirming the presence of a thiosemicarbazide group. The mass spectra showed a molecular ion peak M^+ at *m/z* 335 corresponding to the molecular formula $C_{15}H_{14}ClN_3O_2S$. The thiosemicarbazides were oxidatively cyclised to 5-(4-hydroxyphenyl)methyl-2-aryl/alkylamino-1,3,4-oxadiazoles **4a-i** by elimination of H_2S gas using iodine and potassium iodide in ethanolic sodium hydroxide. The IR spectrum of oxadiazole **4a** showed an absorption peak at 1664 cm^{-1} due to C=N stretching vibration. Its ^1H NMR spectrum showed a multiplet at δ 6.66-7.53 for 9 aromatic protons and a singlet for the NH proton at δ 9.02. In its ^{13}C NMR spectrum, CH_2 appeared at δ 31.0. The oxadiazole ring carbons C-5 and C-2 appeared at δ 152.1 and δ 168.2, respectively. The structure of compound **4a** was further supported by its mass spectra, which showed a molecular ion peak M^+ at *m/z* 267 corresponding to molecular formula $C_{15}H_{13}N_3O_2$. The thiosemicarbazides **3a-i** on heating with 4N NaOH in ethanol underwent smooth cyclisation through dehydration to afford 5-(4-hydroxyphenyl)methyl-4-aryl/alkyl-3-mercapto-1,2,4(*H*)-triazoles **5a-i** (Scheme I). The structure of triazole **5g** was confirmed by its IR spectrum, which displayed absorption bands at 1645 cm^{-1} for C=N and at 1162 cm^{-1} corresponding to C=S stretching vibrations. Its ^1H NMR spectrum showed two singlets at δ 2.36 and δ 3.70 for CH_3 and CH_2 protons, respectively. The singlet of the SH proton was obtained at δ 9.31. In its ^{13}C NMR spectrum, a CH_2 carbon appeared at δ 30.5, whereas a CH_3 carbon appeared at δ 20.7. The triazole ring carbons C-5 and C-3 were observed at δ 151.7 and δ 156.1, respectively. The structure of compound **5g**

Scheme I Protocol for synthesis of 4-hydroxyphenyl acetic acid derivatives



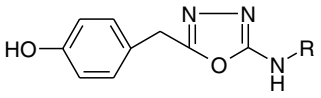
was further confirmed by its mass spectra, which showed a molecular ion peak M^+ at *m/z* 297 corresponding to the molecular formula $C_{16}H_{15}N_3OS$. The purity of the compounds was checked by TLC and elemental analysis.

Anti-inflammatory Activity

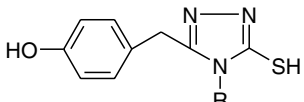
The anti-inflammatory activity of the synthesized compounds **2**, **4a-i & 5a-i** was evaluated by the carrageenan induced paw edema method of Winter et al., edema being measured after 3 and 4 hr of carrageenan treatment. Since % inhibition was found to be greater after 4 hr, this was made the basis of discussion. All compounds (**2**, **4a-i & 5a-i**) showed anti-inflammatory activity in this test at an equimolar oral dose relative to 70 mg/kg ibuprofen. The tested compounds showed anti-inflammatory activity ranging from 37.37 to 83.83% (Table 2), and the standard drug ibuprofen showed 86.36% inhibition after 4 hr. The oxadiazole derivative **4h** having a 4-methoxyphenyl amino group at the 2nd position of 1,3,4-oxadiazole nucleus, showed 77.77% activity, whereas the activity of **4a** void of the 4-methoxy from the C-2 substituent in **4h** was found to be minimal (37.37%). When the 4-methoxy group was replaced by 2-chloro (**4b**) and 4-methyl (**4g**), the compounds showed high activity, 65.55 and 62.62%, respectively. However, replacement of this 4-methoxy group by the halide, 4-chloro (**4c**), 4-bromo (**4d**), 4-fluoro (**4e**), or C-2 substituent by *n*-butyl amino group (**4i**) showed lower activity.

The 1,2,4-triazole derivatives showed anti-inflammatory activity ranging from 51.51 to 83.83% (Table 2) after 4

Table 1. Physical constants of synthesized compounds



4a-i



5a-i

Compound	R	Yield (%)	M.P. (°C)	Mol. Formula
2	--	45	166	$C_9H_8N_3O_2S$
4a	Phenyl	63	206	$C_{15}H_{13}N_3O_2$
4b	2-Chlorophenyl	56	198	$C_{15}H_{12}ClN_3O_2$
4c	4-Chlorophenyl	67	222	$C_{15}H_{12}ClN_3O_2$
4d	4-Bromophenyl	69	214	$C_{15}H_{12}BrN_3O_2$
4e	4-Fluorophenyl	62	242	$C_{15}H_{12}FN_3O_2$
4f	2-Methylphenyl	64	218	$C_{16}H_{15}N_3O_2$
4g	4-Methylphenyl	72	236	$C_{16}H_{15}N_3O_2$
4h	4-Methoxyphenyl	69	208	$C_{16}H_{15}N_3O_3$
4i	*n*-Butyl	73	124	$C_{13}H_{17}N_3O_2$
5a	Phenyl	68	192	$C_{15}H_{13}N_3OS$
5b	2-Chlorophenyl	67	206	$C_{15}H_{12}ClN_3OS$
5c	4-Chlorophenyl	76	236	$C_{15}H_{12}ClN_3OS$
5d	4-Bromophenyl	69	220	$C_{15}H_{12}BrN_3OS$
5e	4-Fluorophenyl	66	248	$C_{15}H_{12}FN_3OS$
5f	2-Methylphenyl	66	226	$C_{16}H_{15}N_3OS$
5g	4-Methylphenyl	81	250	$C_{16}H_{15}N_3OS$
5h	4-Methoxyphenyl	78	220	$C_{16}H_{15}N_3O_2S$
5i	*n*-Butyl	57	170	$C_{13}H_{17}N_3OS$

hr. The highest activity was found for compound **5i** having an *n*-butyl group at 4th position of the triazole ring. When this group was replaced by 2-methylphenyl group **5f**, the activity was found to be minimal (49.99%). The triazole derivative **5d** having a 4-bromophenyl group also showed high activity (65.15%). The rest of the compounds showed moderate activity.

Acute Ulcerogenicity

The compounds **4b-c**, **4g-i**, **5c-e & 5h-i** that showed anti-inflammatory activity higher than 55% were screened for their ulcerogenic activity. All the compounds were tested at an equimolar oral dose relative to 200 mg/kg ibuprofen. The maximum reduction in ulcerogenic activity (0.333 ± 0.10) was found in triazole derivatives **5c**, **5e & 5i** having 4-chlorophenyl, 4-fluorophenyl and *n*-butyl groups, respectively (Table 2). The other two triazole derivatives **5d & 5h** showed a moderate severity index. The oxadiazole derivative **4h** showing high anti-inflammatory activity also showed a reduction in severity index (0.417 ± 0.08). The rest of the oxadiazole derivatives showed moderate severity indexes. The standard drug ibuprofen showed a high severity index of 2.00 ± 0.12.

Lipid Peroxidation

All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation. Lipid peroxidation is measured as nmole of malondialdehyde (MDA)/100 mg of gastric mucosa tissue. Ibuprofen (standard drug) showed maximum lipid peroxidation (6.153 ± 0.18), whereas the control group showed 3.269 ± 0.05. It was found that all cyclised derivatives showing less ulcerogenic activity also showed a reduction in lipid peroxidation Table 2. Thus these studies showed that synthesized compounds have inhibited the induction of gastric mucosal lesions, and results further suggested that their protective effect is related to the inhibition of lipid peroxidation in the gastric mucosa.

CONCLUSION

In summary, various 1,3,4-oxadiazole and 1,2,4-triazole derivatives were prepared with the objective of developing better anti-inflammatory molecules with minimum ulcerogenic activity. It was interesting to note that five cyclised compounds **4b**, **4g**, **4h**, **5d** and **5i** were found to have more than 60% anti-inflammatory activity in com-

Table 2. Anti-inflammatory, ulcerogenic and lipid peroxidation activities of the synthesized compounds

Compound	Anti-inflammatory activity[#] (% inhibition ± SEM)		Ulcerogenic activity[#] (Severity Index ± SEM)	nmol MDA content ± SEM/100 mg tissue[#]
	After 3 hr	After 4 hr		
Control	0	0	0	3.269 ± 0.05
Ibuprofen	83.32 ± 2.79	86.36 ± 2.03	2.000 ± 0.12	6.153 ± 0.18
2	37.87 ± 2.53	46.96 ± 2.79[a]	-	-
4a	33.33 ± 1.96	37.37 ± 2.02[a]	-	-
4b	52.93 ± 2.15	65.65 ± 2.02[a]	0.667 ± 0.16[c]	4.518 ± 0.07[a]
4c	48.03 ± 1.80	55.55 ± 2.55[a]	0.500 ± 0.00[a]	4.193 ± 0.12[b]
4d	42.14 ± 1.80	48.48 ± 3.03[a]	-	-
4e	35.29 ± 2.14	44.44 ± 3.64[a]	-	-
4f	42.14 ± 2.35	54.54 ± 2.59[a]	-	-
4g	55.87 ± 2.52	62.62 ± 2.43[a]	0.500 ± 0.13[b]	4.241 ± 0.14[b]
4h	71.55 ± 2.35	77.77 ± 2.02[c]	0.417 ± 0.08[b]	4.177 ± 0.16[b]
4i	41.16 ± 2.14	57.57 ± 3.49[a]	0.500 ± 0.00[a]	4.257 ± 0.07[a]
5a	42.42 ± 3.03	51.51 ± 1.91[a]	-	-
5b	45.45 ± 3.31	51.51 ± 3.03[a]	-	-
5c	49.99 ± 2.03	59.08 ± 2.03[a]	0.333 ± 0.10[b]	4.122 ± 0.11[b]
5d	57.57 ± 3.03	65.15 ± 2.79[a]	0.417 ± 0.08[b]	4.160 ± 0.14[b]
5e	45.45 ± 2.34	56.78 ± 3.90[a]	0.333 ± 0.10[b]	4.131 ± 0.15[b]
5f	43.93 ± 2.79	49.99 ± 2.03[a]	-	-
5g	36.36 ± 2.34	45.45 ± 3.45[a]	-	-
5h	48.48 ± 1.91	59.08 ± 2.03[a]	0.417 ± 0.08[b]	4.134 ± 0.13[b]
5i	72.54 ± 1.96	83.83 ± 2.43	0.333 ± 0.10[b]	4.113 ± 0.13[b]

Data were analyzed by Student's *t* test for n = 6; [a] $p < 0.0001$, [b] $p < 0.001$, [c] $p < 0.01$.
[#] Relative to ibuprofen.

parison to the standard drug (ibuprofen 86.36%) against carrageenin induced paw edema in rats. The presence of a 4-methoxyphenylamino group at the second position of the oxadiazole ring (**4h**) showed a maximum anti-inflammatory activity of 77.77%. The compounds having a 2-chloro phenyl amino group **4b** and 4-methyl amino group **4g** also showed a high activity of 65.65 and 66.62%, respectively. It was further noted that the presence of the n-butyl group at the fourth position of the triazole ring (**5i**) showed the highest anti-inflammatory activity 83.83%, whereas the presence of an n-butyl amino group at the second position in an oxadiazole ring (**4i**) showed lower activity (57.57%). The presence of a 4-bromophenyl group at the fourth position of a triazole nucleus (**5d**) also showed high activity (65.15%).

These compounds were tested for ulcerogenic activity and showed a significant reduction in the severity index compared to the standard reference drug. It was noted that triazole derivatives showed maximum reduction in ulcerogenic activity followed by oxadiazole derivatives. From these studies, compound **5i**, a triazole derivative, has emerged as a lead compound, which showed maximum reduction in ulcerogenic activity and lipid peroxidation. Thus the basic objective of reducing ulcerogenic potential of synthesized compounds was achieved, and the series provided a new opportunity for possible modification of pharmacophoric requirements and future exploitations.

EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Nicolet, 5PC FTIR spectrometer (ν_{max} in cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker DRX-300 spectrometer using TMS as internal reference (chemical shift in δ ppm). Mass spectra were recorded on a Jeol SX-102 spectrometer. Chemicals were purchased from Merck Chemical Company, S. D. Fine (India) and Qualigens (India). Ethyl-2-(4-hydroxyphenyl) acetate was prepared by a procedure given in the

literature.[27]

4-Hydroxyphenyl acetic acid hydrazide (1)

To a mixture of ethyl-2-(4-hydroxyphenyl) acetate (0.01 mol) and hydrazine hydrate (0.20 mol), absolute ethanol (50 mL) was added, and it was refluxed for 24 hr on a water bath. The mixture was concentrated, cooled and poured into crushed ice. It was kept for 4-5 hr at room temperature, and the solid mass that separated out was filtered, dried and recrystallized from ethanol.

IR: (KBr) cm^{-1} 3596 (OH), 3406 (N-H), 2981 (C-H), 1690 (C=O), 1582 (C=C); ^1H NMR (DMSO-d_6): δ 3.20 (s, 2H, CH_2), 4.19 (s, 2H, NH_2), 6.65 (d, 2H, 2,6-ArH), 7.05 (d, 2H, 3,5-ArH), 9.13 (s, 1H, CONH), 9.22 (bs, 1H, OH).

5-(4-Hydroxyphenyl)methyl-2-mercapto-1,3,4-oxadiazole (2)

A mixture of 4-hydroxyphenyl acetic acid hydrazide **1** (0.005 mol), potassium hydroxide (0.005 mol) and carbon disulphide (5 mL) in ethanol (50 mL) was refluxed on a water bath for 10 hr. The solution was then concentrated, cooled and acidified with dilute hydrochloric acid. The solid that separated out was filtered and recrystallized from ethanol.

IR: (KBr) cm^{-1} 3611 (OH), 2987 (C-H), 1662 (C=N), 1586 (C=C), 1165 (C=S); ^1H NMR (DMSO-d_6): δ 3.70 (s, 2H, CH_2), 6.77 (d, 2H, 2,6-ArH), 7.19 (d, 2H, 3,5-ArH), 9.69 (bs, 1H, OH), 12.09 (bs, 1H, SH); MS (*m/z*) 208 (M^+); Anal. Calcd for $C_9H_8N_3O_2S$: C, 51.91; H, 3.87; N, 13.45; S, 15.80. Found: C, 51.93; H, 3.89; N, 13.44; S, 15.82.

General procedure for N^1-[2-(4-hydroxyphenyl)acetyl] N^4-alkyl/aryl-3-thiosemicarbazides (3a-i)

A mixture of 4-hydroxyphenyl acetic acid hydrazide **1** (0.10 mol), aryl/alkyl isothiocyanate (0.10 mol) and ethanol (50 mL) was refluxed on a water bath for 5-6 hr. It was then concentrated, cooled and kept overnight in refrigerator. The white solid that separated out was filtered, dried and recrystallised from a suitable solvent.

N^1-[2-(4-hydroxyphenyl)acetyl] N^4-(4-chlorophenyl)-3-thiosemicarbazide (3c)

IR: (KBr) cm^{-1} 3612 (OH), 3392 (N-H), 3028 (C-H), 1696 (C=O), 1589 (C=C), 1183 (C=S); ^1H NMR (DMSO-d_6): δ 3.50 (s, 2H, CH_2), 6.67 (d, 2H, 2,6-ArH), 7.08 (d, 2H, 3,5-ArH), 7.37 (d, 2H, 2′,6′-ArH-Cl), 7.46 (d, 2H, 3′,5′-ArH-Cl), 9.27 (s, 1H, NHAr-Cl), 9.69 (bs, 2H, CONH-NH-C=S), 10.09 (bs, 1H, OH); MS (*m/z*) 335 (M^+); Anal. Calcd for $C_{15}H_{14}ClN_3O_2S$: C, 53.65; H, 4.20; N, 12.51. Found: C, 53.53; H, 4.29; N, 12.45.

N^1-[2-(4-hydroxyphenyl)acetyl] N^4-(4-methoxyphenyl)-3-thiosemicarbazide (3h)

IR: (KBr) cm^{-1} 3571 (OH), 3237 (N-H), 2963 (C-H), 1660 (C=O), 1572 (C=C), 1146 (C=S); ^1H NMR (DMSO-d_6): δ 3.51 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 6.65 (d, 2H, 2,6-ArH), 7.01 (d, 2H, 3,5-ArH), 7.32 (d, 2H, 2′,6′-ArH-OCH_3), 7.45 (d, 2H, 3′,5′-ArH-OCH_3), 9.23 (s, 1H, NHAr-OCH_3), 9.58 (bs, 2H, CONH-NH-C=S), 10.02 (bs, 1H, OH); MS (*m/z*) 331 (M^+); Anal. Calcd for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68. Found: C, 57.80; H, 5.10; N, 12.72.

General procedure for 5-(4-hydroxyphenyl)methyl-2-aryl/alkylamino-1,3,4-oxadiazoles (4a-i)

A suspension of thiosemicarbazide (**3a-i**) (0.002 mol) in ethanol (50 mL) was dissolved in aqueous sodium hydroxide (5N, 1 mL) with cooling and stirring resulting in the formation of clear solution. To this, iodine in potassium iodide solution (5%) was added dropwise with stirring till the colour of iodine persisted at room temperature. The reaction mixture was refluxed for 3 hr on a water bath. It was then concentrated, kept overnight in the refrigerator, and the solid thus separated out was recrystallized from ethanol.

5-(4-Hydroxyphenyl)methyl-2-phenylamino-1,3,4-oxadiazole (4a)

IR: (KBr) cm^{-1} 3572 (OH), 3323 (N-H), 2978 (C-H), 1664 (C=N), 1591 (C=C); ^1H NMR (DMSO-d_6): δ 3.89 (s, 2H, CH_2), 6.66-7.53 (m, 9H, aromatic), 9.02 (s, 1H, NH), 10.48 (s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 31.0, 115.7, 124.9, 128.7, 129.6, 129.9, 134.0, 152.1, 156.5, 168.2; MS: *m/z* 267 (M^+); Anal. Calcd for $C_{15}H_{13}N_3O_2$: C, 67.41; H, 4.90; N, 15.72. Found: C, 67.39; H, 4.92; N, 15.71.

5-(4-Hydroxyphenyl)methyl-2-(2-chlorophenylamino)-1,3,4-oxadiazole (4b)

IR: (KBr) cm^{-1} 3577 (OH), 3359 (N-H), 2988 (C-H), 1652 (C=N), 1560 (C=C), 712 (C-Cl); ^1H NMR (DMSO-d_6): δ 3.90 (s, 2H, CH_2), 7.01-7.60 (m, 8H, aromatic), 8.99 (s, 1H, NH), 10.41 (s, 1H, OH); MS: *m/z* 301 (M^+); Anal. Calcd for $C_{15}H_{12}ClN_3O_2$: C, 59.71; H, 4.01; N, 13.93. Found: C, 59.70; H, 3.99; N, 13.96.

5-(4-Hydroxyphenyl)methyl-2-(4-chlorophenylamino)-1,3,4-oxadiazole (4c)

IR: (KBr) cm^{-1} 3575 (OH), 3314 (N-H), 2960 (C-H), 1633 (C=N), 1576 (C=C), 716 (C-Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.74 (s, 2H, CH_2), 6.56 (d, 2H, 2,6-ArH), 6.69 (d, 2H, 3,5-ArH), 7.23 (d, 2H, 2′,6′-ArH-Cl), 7.53 (d, 2H, 3′,5′-ArH-Cl), 9.33 (s, 1H, NH), 10.55 (s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 30.6, 115.1, 118.4, 124.3, 128.8, 129.2, 130.2, 134.0, 151.6, 156.1, 167.9; MS: *m/z* 301 (M^+); Anal. Calcd for $C_{15}H_{12}ClN_3O_2$: C, 59.71; H, 4.01; N, 13.93. Found: C, 59.73; H, 3.99; N, 13.95.

5-(4-Hydroxyphenyl)methyl-2-(4-bromophenylamino)-1,3,4-oxadiazole (4d)

IR: (KBr) cm^{-1} 3569 (OH), 3382 (N-H), 2988 (C-H), 1670 (C=N), 1591 (C=C), 596 (C-Br) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.69 (s, 2H, CH_2), 6.57 (d, 2H, 2,6-ArH), 6.70 (d, 2H, 3,5-ArH), 7.18 (d, 2H, 2′,6′-ArH-Br), 7.48 (d, 2H, 3′,5′-ArH-Br), 9.29 (s, 1H, NH), 10.41 (s, 1H, OH); MS: *m/z* 346 (M^+); Anal. Calcd for $C_{15}H_{12}BrN_3O_2$: C, 52.04; H, 3.49; N, 12.14. Found: C, 52.06; H, 3.53; N, 12.19.

5-(4-Hydroxyphenyl)methyl-2-(4-fluorophenylamino)-1,3,4-oxadiazole (4e)

IR: (KBr) cm^{-1} 3560 (OH), 3300 (N-H), 2980 (C-H), 1630 (C=N), 1573 (C=C), 1090 (C-F); ^1H NMR (DMSO-d_6): δ 3.77 (s, 2H, CH_2), 6.59 (d, 2H, 2,6-ArH), 6.71 (d, 2H, 3,5-ArH), 7.08 (d, 2H, 2′,6′-ArH-F), 7.53 (d, 2H, 3′,5′-ArH-F), 9.36 (s, 1H, NH), 10.47 (s, 1H, OH); MS: *m/z* 285 (M^+); Anal. Calcd for $C_{15}H_{12}FN_3O_2$: C, 63.15; H, 4.24; N, 14.73. Found: C, 63.16; H, 4.23; N, 14.79.

5-(4-Hydroxyphenyl)methyl-2-(2-methylphenylamino)-1,3,4-oxadiazole (4f)

IR: (KBr) cm^{-1} 3574 (OH), 3306 (N-H), 2960 (C-H), 1650 (C=N), 1571 (C=C); ^1H NMR (DMSO-d_6): δ 2.16 (s, 2H, CH_3), 3.70 (s, 2H, CH_2), 6.60-7.18 (m, 8H, aromatic), 8.81 (s, 1H, NH), 11.25 (bs, 1H, OH); MS: *m/z* 281 (M^+); Anal. Calcd for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94. Found: C, 68.32; H, 5.40; N, 14.90.

5-(4-Hydroxyphenyl)methyl-2-(4-methylphenylamino)-1,3,4-oxadiazole (4g)

IR: (KBr) cm^{-1} 3580 (OH), 3294 (N-H), 2975 (C-H), 1663 (C=N), 1575 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.36 (s, 2H, CH_3), 3.69 (s, 2H, CH_2), 6.57 (d, 2H, 2,6-ArH), 6.69 (d, 2H, 3,5-ArH), 7.06 (d, 2H, 2′,6′-ArH-CH_3), 7.26 (d, 2H, 3′,5′-ArH-CH_3), 9.26 (s, 1H, NH), 13.25 (bs, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 21.2, 31.0, 115.5, 117.2, 125.1, 128.4, 129.9, 131.5, 139.3, 152.1, 156.6, 168.3; MS: *m/z* 281 (M^+); Anal. Calcd for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94. Found: C, 68.40; H, 5.43; N, 14.91.

5-(4-Hydroxyphenyl)methyl-2-(4-methoxyphenylamino)-1,3,4-oxadiazole (4h)

IR: (KBr) cm^{-1} 3569 (OH), 3336 (N-H), 2976 (C-H), 1645 (C=N), 1574 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.70 (s, 2H, CH_2), 3.80 (s, 2H, OCH_3), 6.57 (d, 2H, 2,6-ArH), 6.69 (d, 2H, 3,5-ArH), 6.99 (d, 2H, 2′,6′-ArH-OCH_3), 7.10 (d, 2H, 3′,5′-ArH-OCH_3), 9.29 (s, 1H, NH), 13.79 (bs, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 30.0, 55.4, 114.4, 115.5, 118.3, 124.6, 126.1, 129.5, 152.0, 156.1, 159.6, 168.1; MS: *m/z* 297 (M^+); Anal. Calcd for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13. Found: C, 64.50; H, 5.01; N, 14.06.

5-(4-Hydroxyphenyl)methyl-2-*n*-butylamino-1,3,4-oxadiazole (4i)

IR: (KBr) cm^{-1} 3568 (OH), 3378 (N-H), 2966 (C-H), 1664 (C=N), 1573 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.76 (t, J = 6.7 Hz, 3H, CH_3), 1.16-1.22 (m, 4H, CH_2-CH_2), 3.18 (s, 2H, CH_2), 3.74 (t, J = 6.9 Hz, 2H, $NHCH_2$), 6.68 (d, 2H, 2,6-ArH), 7.03 (d, 2H, 3,5-ArH), 9.38 (s, 1H, NH), 13.53 (bs, 1H, OH); MS: *m/z* 247 (M^+); Anal. Calcd for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99. Found: C, 63.10; H, 6.99; N, 17.01.

General procedure for 5-(4-hydroxyphenyl)methyl-4-aryl/alkyl-3-mercapto-1,2,4(*H*)-triazoles (5a-i)

A suspension of thiosemicarbazide (**3a-i**) (0.001 mol) in ethanol (20 mL) was dissolved in aqueous sodium hydroxide solution (4N, 2 mL) and gently refluxed for 2 hr on a water bath. The resulting solution was concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid and kept aside for 1 hr. The solid thus separated out was filtered, washed with water, dried and recrystallized with ethanol.

5-(4-Hydroxyphenyl)methyl-4-phenyl-3-mercapto-1,2,4(*H*)-triazole (5a)

IR: (KBr) cm^{-1} 3574 (OH), 2974 (C-H), 1620 (C=N),

1578 (C=C), 1169 (C=S); ^{1}H NMR (DMSO-d_6): δ 3.80 (s, 2H, CH_2), 6.79-7.53 (m, 9H, aromatic), 10.09 (s, 1H, SH), 13.06 (s, 1H, OH); MS: *m/z* 283 (M^+); Anal. Calcd for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83. Found: C, 63.60; H, 4.66; N, 14.89.

5-(4-Hydroxyphenyl)methyl-4-(2-chlorophenyl)-3-mercapto-1,2,4(*H*)-triazole (5b)

IR: (KBr) cm^{-1} 3576 (OH), 2963 (C-H), 1648 (C=N), 1588 (C=C), 1181 (C=S), 769 (C-Cl); ^{1}H NMR (DMSO-d_6): δ 4.00 (s, 2H, CH_2), 6.71-7.53 (m, 8H, aromatic), 9.37 (s, 1H, SH), 13.83 (s, 1H, OH); MS: *m/z* 317 (M^+); Anal. Calcd for $C_{15}H_{12}ClN_3OS$: C, 56.69; H, 3.81; N, 13.22. Found: C, 56.63; H, 3.89; N, 13.29.

5-(4-Hydroxyphenyl)methyl-4-(4-chlorophenyl)-3-mercapto-1,2,4(*H*)-triazole (5c)

IR: (KBr) cm^{-1} 3566 (OH), 2992 (C-H), 1649 (C=N), 1586 (C=C), 1178 (C=S), 784 (C-Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.74 (s, 2H, CH_2), 6.56 (d, 2H, 2,6-ArH), 6.69 (d, 2H, 3,5-ArH), 7.23 (d, 2H, 2′,6′-ArH-Cl), 7.53 (d, 2H, 3′,5′-ArH-Cl), 9.32 (s, 1H, SH), 13.83 (s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 31.2, 115.5, 124.3, 129.5, 132.3, 135.3, 151.4, 156.5, 168.4; MS: *m/z* 317 (M^+); Anal. Calcd for $C_{15}H_{12}ClN_3OS$: C, 56.69; H, 3.81; N, 13.22. Found: C, 56.60; H, 3.78; N, 13.25.

5-(4-Hydroxyphenyl)methyl-4-(4-bromophenyl)-3-mercapto-1,2,4(*H*)-triazole (5d)

IR: (KBr) cm^{-1} 3555 (OH), 2981 (C-H), 1657 (C=N), 1586 (C=C), 1186 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.68 (s, 2H, CH_2), 6.54 (d, 2H, 2,6-ArH), 6.68 (d, 2H, 3,5-ArH), 7.21 (d, 2H, 2′,6′-ArH-Br), 7.50 (d, 2H, 3′,5′-ArH-Br), 9.29 (s, 1H, SH), 13.70 (bs, 1H, OH); MS: *m/z* 362 (M^+); Anal. Calcd for $C_{15}H_{12}BrN_3OS$: C, 49.74; H, 3.34; N, 11.60. Found: C, 49.70; H, 3.29; N, 11.50.

5-(4-Hydroxyphenyl)methyl-4-(4-fluorophenyl)-3-mercapto-1,2,4(*H*)-triazole (5e)

IR: (KBr) cm^{-1} 3545 (OH), 2986 (C-H), 1638 (C=N), 1596 (C=C), 1145 (C=S), 1090 (C-F); ^{1}H NMR (DMSO-d_6): δ 3.75 (s, 2H, CH_2), 6.66 (d, 2H, 2,6-ArH), 6.76 (d, 2H, 3,5-ArH), 7.08 (d, 2H, 2′,6′-ArH-F), 7.21 (d, 2H, 3′,5′-ArH-F), 9.99 (s, 1H, SH), 13.32 (bs, 1H, OH); MS: *m/z* 301 (M^+); Anal. Calcd for $C_{15}H_{12}FN_3OS$: C, 59.79; H, 4.01; N, 13.94. Found: C, 59.81; H, 4.00; N, 14.01.

5-(4-Hydroxyphenyl)methyl-4-(2-methylphenyl)-3-mercapto-1,2,4(*H*)-triazole (5f)

IR: (KBr) cm^{-1} 3561 (OH), 2971 (C-H), 1647 (C=N), 1586 (C=C), 1157 (C=S); ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 6.57-7.37 (m, 8H, aromatic), 9.42 (s, 1H, SH), 13.60 (bs, 1H, OH); MS: *m/z* 297 (M^+); Anal. Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13. Found: C, 64.65; H, 5.00; N, 14.15.

5-(4-Hydroxyphenyl)methyl-4-(4-methylphenyl)-3-mercapto-1,2,4(*H*)-triazole (5g)

IR: (KBr) cm^{-1} 3547 (OH), 2979 (C-H), 1645 (C=N), 1591 (C=C), 1162 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 6.56 (d, 2H, 2,6-ArH), 6.69 (d, 2H, 3,5-ArH), 7.07 (d, 2H, 2′,6′-ArH-CH_3), 7.27 (d, 2H, 3′,5′-ArH-CH_3), 9.31 (s, 1H, SH), 13.64 (bs, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 20.7, 30.5, 115.1, 124.6, 128.0, 129.5, 129.7, 131.0, 139.0, 151.7, 156.1, 167.9; MS: *m/z* 297 (M^+); Anal. Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13. Found: C, 64.56; H, 5.00; N, 14.09.

5-(4-Hydroxyphenyl)methyl-4-(4-methoxyphenyl)-3-mercapto-1,2,4(*H*)-triazole (5h)

IR: (KBr) cm^{-1} 3568 (OH), 2991 (C-H), 1631 (C=N), 1589 (C=C), 1163 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.69 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 6.57 (d, 2H, 2,6-ArH), 6.69 (d, 2H, 3,5-ArH), 6.99 (d, 2H, 2′,6′-ArH-OCH_3), 7.09 (d, 2H, 3′,5′-ArH-OCH_3), 9.29 (s, 1H, SH), 13.25 (bs, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 30.6, 55.4, 114.3, 115.2, 124.6, 126.2, 129.5, 151.9, 156.2, 159.6, 168.1; MS: *m/z* 313 (M^+); Anal. Calcd for $C_{16}H_{15}N_3O_2S$: C, 61.33; H, 4.82; N, 13.41. Found: C, 61.36; H, 4.89; N, 13.39.

5-(4-Hydroxyphenyl)methyl-4-*n*-butyl-3-mercapto-1,2,4(*H*)-triazole (5i)

IR: (KBr) cm^{-1} 3567 (OH), 2982 (C-H), 1629 (C=N), 1594 (C=C), 1174 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 0.76 (t, *J* = 6.7 Hz, 3H, CH_3), 1.16-1.27 (m, 4H, -CH_2-CH_2-), 3.20 (s, 2H, CH_2), 3.74 (t, *J* = 6.9 Hz, 2H, N-CH_2), 6.67 (d, 2H, 2,6-ArH), 7.02 (d, 2H, 3,5-ArH), 9.11 (s, 1H, SH), 13.55 (s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 13.4, 19.2, 29.2, 30.0, 42.9, 114.9, 126.3, 129.6, 151.5, 155.8, 166.6; MS: *m/z* 263 (M^+); Anal. Calcd for $C_{13}H_{17}N_3OS$: C, 59.29; H, 6.51; N, 15.96. Found: C, 59.20; H, 6.59; N, 16.00.

Biological Studies

Adult male Wistar strain rats of either sex, weighing

180-200 gm, were used. The animals were allowed food and water *ad libitum*. They were housed in a room at 25 ± 2 °C and 50 ± 5% relative humidity with a 12 hr light/dark cycle. The animals were randomly allocated into groups at the beginning of all the experiments. All the test compounds and the reference drugs were administered orally, suspended in 0.5% carboxymethyl cellulose (CMC) solution. The anti-inflammatory activity,[28] acute ulcerogenicity[29] and lipid peroxidation[30] activities were determined by procedures as reported in the literature.

Statistical Analysis

Data are expressed as mean ± SEM, the Student's *t*-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds.

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