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Synthesis and Anti-inflammatory Evaluation of 1,2,4 Triazole Derivatives.

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Research Article

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ABSTRACT

A mixture of 4-hydroxyphenylacetic acid, absolute ethanol, few drops of concentrated sulphuric acid along with a small porcelain chip on reaction formed Ethyl-(4-hydroxyphenyl) acetate (1). On condensing mixture of (1), hydrazine hydrate and absolute ethanol formed 4-Hydroxyphenyl acetic acid hydrazide (2). Further mixture of 2, aryl/alkyl isothiocyanate and ethanol was refluxed and formed N^1-[2-(4-Hydroxyphenyl) acetyl] N^4-alkyl/aryl-3-thiosemicarbazide (3a-i). A suspension of (3a-3i) in ethanol, sodium hydroxide is refluxed for 2 hours on a water bath and formed 5-(Hydroxyphenyl) methyl-4-alkyl/aryl-2-mercpto-1,2,4(*H*)-triazoles (4a-i). The 1,2,4-triazole derivatives of 4-hydroxyphenyl acetic acid (4a-i) showed anti-inflammatory activity and Ibuprofen was taken as the standard drug.
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INTRODUCTION

Most of the present diseases are due to the invasion by the pathogenic organisms like bacteria, fungi, virus. To treat these diseases many potent and broad spectrum antibiotics were discovered eg: ampicillin, amoxicillin, Carbenicillin, Oflaxacin, Tetracyclines etc. Even though antibiotics are life saving drugs in therapeutics but they are potentially harmful. These effects include allergic and anaphylactic reactions, super-infection, development of resistance, destruction of normal non-pathogenic bacterial flora and selective toxicity like aplastic anemia, kidney damage etc. Infections often produce pain and inflammation. So there is need to search less toxic drugs than those based on natural sources resulted in the introduction of synthetic substances as drugs in the late 19th century and their widespread use in the 21th century. A considerable amount of research activities are directed towards potent, more specific and less toxic anti-inflammatory agents and it offers challenging task in the development of novel synthetic strategies; Nitrogen heterocycles; A five member ring containing three nitrogen is known as triazole such as 1.2.3 triazole, or 1.2.4 triazole. 1,2,4-triazoles, are among the various heterocycles that have received the most attention during the last two decaded as potential antimicrobial agents. The pharmacologically important heterocycles with nitrogen bridge derieved from 1,2,4-triazole played the way toward active research in trizole chemistry. 1,2,4-triazole and their derivatives are found to be associated with various biological activities such as antifungal [1,2], antimicrobial [3,4,5,6], insecticidal [7], cytotoxic [8], anti-tubercular [9], antiviral [10], anti-haemostatic activity [11], antitumor [12], antibacterial [13,14] and nematicidal [15].

In view of the versatile importance of the triazoles it is worthwhile to prepare and study some substituted 1,2,4-triazoles as a anti-inflammatory agent.

EXPERIMENTAL SECTION

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

The thiosemicarbazide **3a** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 2 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with

dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and re-crystallized with ethanol.

Purity of the compounds (3a-i) was checked by TLC on silica gel G plates using toluene: ethylacetate: formic acid (5:4:1) as solvent system and the spot was located by exposure to iodine vapours.

Yield: 68 %, m.p.: 192 ˚C, R_f: 0.61, Molecular formula: $C_{15}H_{13}N_3OS$, Molecular weight: 283.35. %N: Found: 15.06%; Calcd: 14.83 %.
IR (KBr): 3574 (OH), 2974 (C-H), 1620 (C=N), 1578 (C=C), 1169 (C=S).

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

The thiosemicarbazide **3b** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 3 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and recrystallized with ethanol.

Yield: %, m.p. 220˚C, R_f: 0.80, Molecular formula: $C_{15}H_{12}N_3OSBr$, Molecular weight: 362.24. %N: Found: 11.33%; Calcd: 11.60%.
IR (KBr): 3555 (OH), 2981 (C-H), 1657 (C=N), 1586 (C=C), 1186 (C=S).
^{1}HNMR (DMSO-d_6): 2.48 (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).
Mass (m/z): 362 (M^+), 329, 303, 133, 107.

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

The thiosemicarbazide **3c** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 2 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and recrystallized with ethanol.

Yield: 76 %, m.p.: 236 ˚C, R_f : 0.55, Molecular formula: $C_{15}H_{12}N_3OSCl$, Molecular weight: 317.79. %N: Found: 13.14%; Calcd: 13.22 %.
IR (KBr): 3566 (OH), 2992 (C-H), 1649 (C=N), 1586 (C=C), 1178 (C=S), 784 (C-Cl).
^{1}HNMR (DMSO-d_6): 2.50 (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).
Mass (m/z): 317 (M^+), 284, 258, 133, 107.

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

The thiosemicarbazide **3d** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 4 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and recrystallized with ethanol.

Yield: 67 %, m.p.: 206 ˚C, R_f : 0.62, Molecular formula: $C_{15}H_{12}N_3OSCl$, Molecular weight: 317.79. %N: Found: 12.96%; Calcd: 13.22 %.
IR (KBr): 3576 (OH), 2963 (C-H), 1648 (C=N), 1588 (C=C), 1181 (C=S), 769 (C-Cl).

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

The thiosemicarbazide **3e** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 3 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and recrystallized with ethanol.

Yield: 66 %, m.p. 248˚C, R_f: 0.66, Molecular formula: $C_{15}H_{12}N_3OSF$, Molecular weight: 301.34. %N: Found: 14.10%; Calcd: 13.94 %.

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IR (KBr): 3545 (OH), 2986 (C-H), 1638 (C=N), 1596 (C=C), 1145 (C=S), 1090 (C-F).

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

The thiosemicarbazide **3f** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 2 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and recrystallized with ethanol.

Yield: 81 %, m.p. 250 ˚C, R_f : 0.64, Molecular formula: $C_{16}H_{15}N_3OS$, Molecular weight: 297.37. %N: Found: 13.94%; Calcd: 14.13 %.
IR (KBr): 3547 (OH), 2979 (C-H), 1645 (C=N), 1591 (C=C), 1162 (C=S).
^{1}HNMR (DMSO-d_6): 2.36 (s, 2H, CH_3), 2.50 (s, 2H, CH_2), 6.56 (d, 2H, 2, 6-ArH-CH_3), 6.69 (d, 2H, 3, 5-ArH-CH_3), 7.07 (d, 2H, 2′, 6′-ArH), 7.27 (d, 2H, 3′, 5′-ArH), 9.31 (s, 1H, SH), 13.64 (bs, 1H, OH).
Mass (m/z): 297 (M^+), 298 (M+1), 264, 238, 133, 107.

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

The thiosemicarbazide **3g** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 4 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and recrystallized with ethanol.

Yield: 66 %, m.p.: 226 ˚C, R_f : 0.57, Molecular formula: $C_{16}H_{15}N_3OS$, Molecular weight: 297.37. %N: Found: 13.78%; Calcd: 14.13 %.
IR (KBr): 3561 (OH), 2971 (C-H), 1647 (C=N), 1586 (C=C), 1157 (C=S)

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

The thiosemicarbazide **3h** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 2 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and recrystallized with ethanol.

Yield: 78 %, m.p. 220˚C, R_f : 0.71, Molecular formula: $C_{16}H_{15}N_3O_2S$, Molecular weight: 313.37. %N: Found: 13.04%; Calcd: 13.41 %.
IR (KBr): 3568 (OH), 2991 (C-H), 1631 (C=N), 1589 (C=C), 1163 (C=S).
^{1}HNMR (DMSO-d_6): 2.50 (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).

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

The thiosemicarbazide **3i** (0.001 mole) was added to ethanol (20 ml) in a 100 ml round bottom flask. To it sodium hydroxide solution (4N, 2ml) was added, resulting in the formation of clear solution. The solution was refluxed for 3 hours on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted to 5-6 with dilute acetic acid. The reaction mixture was kept aside for 1 hour. Solid separated was filtered, washed with water, dried and recrystallized with ethanol.

Yield: 57 %, m.p. 170˚C, R_f : 0.73, Molecular formula: $C_{13}H_{17}N_3OS$, Molecular weight: 263.36. %N: Found: 15.72%; Calcd: 15.96 %.
IR (KBr): 3567 (OH), 2982 (C-H), 1629 (C=N), 1594 (C=C), 1174 (C=S).
^{1}HNMR(DMSO-d_6): 0.76-0.80 (t, 3H, CH_3), 1.16-1.27 (merged m, 4H, -CH-CH_2-), 2.50 (s, 2H, CH_2), 3.74-3.78 (t, 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).
Mass (m/z): 263 (M^+), 230, 204.

RESULT AND DISCUSSION

5-(Hydroxyphenyl) methyl-4-alkyl/aryl-2-mercpto-1,2,4(*H*)-triazoles (4a-i)

The purity of the compounds (4a-i) was checked by TLC and its characterization on the basis of IR, NMR and Mass spectral data.

The IR spectrum of the compounds (4a-i) showed peaks at 3576-3545 cm^{-1}, OH stretching; 2992-2963 cm^{-1}, CH stretching; 1657-1620 cm^{-1}, C=N stretching; 1596-1578 cm^{-1}, C=C stretching of aromatic rings and 1186-1145 cm^{-1}, C=S stretching vibrations.

The NMR spectrum of the compound 4b, 4c,4i showed a singlet respectively at δ 2.48, 2.50, at δ 2.50 indicating the presence of CH_2 protons.

4f, 4h showed two singlets respectively at δ 2.36 and δ 2.50 for CH_3 and CH_2 protons ,at δ 2.50 and δ 3.80 for CH_2 and OCH_3 protons.

4b, 4c, 4f, 4h, 4i showed a singlet and a broad singlet respectively at δ 9.29 at δ 13.70, at δ 9.32 and δ 13.83, at δ 9.31 and at δ 13.64, at δ 9.29 and at δ 13.25, at δ 9.11 and at δ 13.55 for SH and OH protons.

4b, 4c, 4f, 4h, 4i, showed two doublets in the aromatic region respectively centered at δ 6.54 and δ 6.68, at δ 6.56 and δ 6.69, at δ 6.56 and δ 6.69, at δ 6.57 and δ 6.69, at δ 6.57 and δ 6.69, indicating the presence of 2, 6- and 3, 5- phenolic protons respectively.

4b, 4c, 4f, 4h, showed four protons of p-bromophenyl ring as doublets respectively centered at δ 7.21 and δ 7.50, at δ 7.23 and δ 7.53, at δ 7.07 and δ 7.27, at δ 6.99 and δ 7.09 indicating the presence of 2′, 6′- and 3′, 5′- aromatic protons.

The NMR spectrum of the compound 4i showed a triplet at δ 0.76-0.80 indicating the presence of methyl protons of n-butyl group. The CH_3-CH_2CH_2 protons of n-butyl group were merged together and obtained as a multiplet at δ 1.16-1.27. The NH-CH_2 protons of n-butyl group was obtained as a triplet at δ 3.74-3.78.

The structure of the compounds 4b, 4c, 4f, 4i was further supported by their mass spectral data, which showed molecular ion peak M^+ respectively at m/z 362, at m/z 317, at m/z 297, at m/z 263and corresponding to the molecular formula respectively $C_{15}H_{12}N_3OSBr$, $C_{15}H_{12}N_3OSCl$, $C_{16}H_{15}N_3OS$, $C_{13}H_{17}N_3OS$ .Further peaks were observed respectively at m/z 329, 303,133 and107, at m/z 284, 258, 133 and 107, at m/z 264, 238, 133 and 107, at m/z 230 and 204.

Biological activity

The anti-inflammatory activity of 1,2,4-triazole derivatives was carried out by the method of Winter et al[180]. 4-Hydroxyphenyl acetic acid was used to synthesize triazole derivatives and were evaluated for anti-inflammatory activity. The 1,2,4-triazole derivatives of 4-hydroxyphenyl acetic acid (4a-i) showed anti-inflammatory activity ranging from 45.45% to 68.17% inhibition at 70 mg/Kg oral dose after 4 hours, whereas the standard drug Ibuprofen showed 86.35% inhibition at the same oral dose (Table-1). The triazole derivativbes having n-butyl group (4i) at the 4th position of the triazole nucleus showed maximum inhibition (68.17%). Replacement of n-butyl group with 4-bromophenyl (4b), 4-chlorophenyl (4c) and 4-methoxyphenyl (4h) results in slight decrease in the activity, but when these groups were replaced with 4-methylphenyl (4f) and 2-methylphenyl (4g) groups, a marked decrease in activity have been observed. Rest of the compounds showed moderate activity (Table-1).

Table 1: Anti-inflammatory Activity of 1,2,4-Triazole Derivatives

Compound No.	Mean Paw Volume ± SEM	% Inhibition ± SEM
4a	0.107 ± 0.0042	51.51 ± 1.916*
4b	0.077 ± 0.0061	65.15 ± 2.794*
4c	0.090 ± 0.0044	59.08 ± 2.033*
4d	0.106 ± 0.0066	51.51 ± 3.030*
4e	0.100 ± 0.0051	54.54 ± 2.347*
4f	0.120 ± 0.0051	45.45 ± 2.347*
4g	0.110 ± 0.0044	49.99 ± 2.033*
4h	0.090 ± 0.0044	59.08 ± 2.033*
4i	0.070 ± 0.0068	68.17 ± 3.105*

*P<0.0001, compared w.r.t. control. Data were analyzed by student's t-test for n= 6

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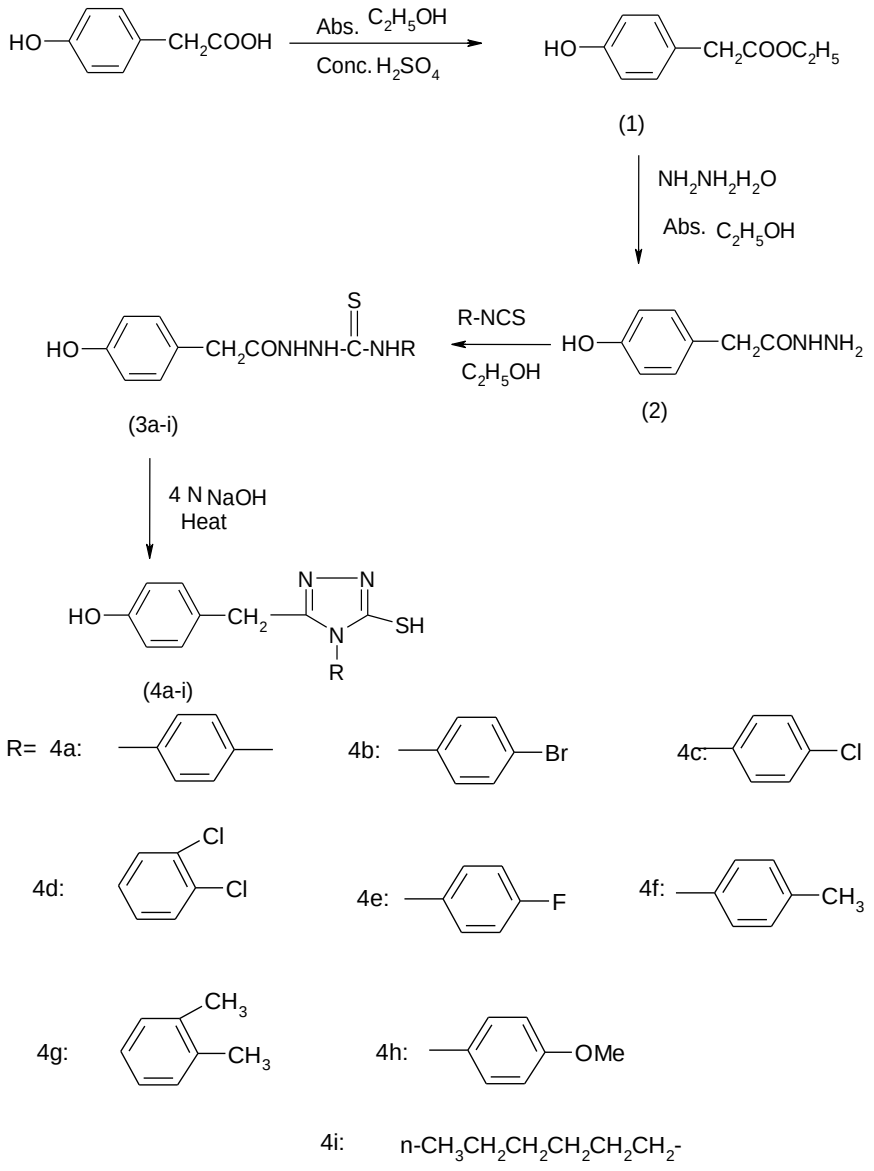

Scheme-1

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