ABSTRACT

Six substituted N-aryl,5-substituted phenyl 1,3,4-thiadiazole(7a-f) were synthesized by the reaction of different substituted benzaldehyde with different substituted 5-phenyl 1,3,4-thiadiazole 2-amino in the presence of sulphuric acid in refluxing methanol. The newly synthesized compounds were characterized by spectroscopic methods. Further, the synthesized compounds were screened for Toxicity study, Wound healing activity and Diuretic activity by standard method. Results of the activities reveal that some compounds exhibited less toxicity and moderate to good Wound healing and Diuretic activity.

KEYWORDS: Six substituted N-aryl,5-substituted phenyl 1,3,4-thiadiazole(7a-f)

INTRODUCTION

Medicinal chemistry is comprised of the ideas, knowledge and available tools that have advanced contemporary knowledge. Heterocyclic chemistry is a branch of organic chemistry. About half of the known compounds contain at least one hetero atom incorporated in their structure. Thiadiazole is the diunsaturated five membered ring structure having molecular formula C_2H_2N_2S. Thiadiazole is a versatile moiety having wide variety of pharmacological activities may be virtue of –N=C=S- (imene) grouping. Thiadiazole rings occurs in various types.
Various literature review shows that the thiadiazole nuclei have wide range of diversified biological activity e.g. antimicrobial\[4\], anti-inflammatory\[5\], anticancer\[6\], anticonvulsant\[7\], antidepressant\[8\], antioxidant\[9\], radio protective\[10\], anti-leishmanial\[11\], antiviral\[12\], diuretic\[13\], wound healing\[14\] and anti fungal activities.\[15\] The 1,3,4-thiadiazole is the most important heterocyclic nuclei which is a widespread and vital feature of a diversity of natural products and medicinal agents.

**EXPERIMENTAL**

Melting point of the synthesized compounds were determined using melting point apparatus i.e. Temp Star, Hindustan Scientific Linkers and uncorrected. The solvents and reagents were used as received or dried prior to use as needed. All reactions were monitored and purity of compound checked by TLC using silica gel G as a stationary phase. The spots resolved were visualized as brown colored spots by using UV 256 detector. The IR spectra of the synthesized compounds were recorded using KBr pellets in range of 4000-500 cm\(^{-1}\) on IR spectrometer, Simazdu. \(^1\)H-NMR (300 Mhz) spectra was recorded in DMSO-d\(_6\) in BRUKER DPX-300 NMR spectrophotometer using Tetramethylsilane as internal standard.

**Synthesis of Substituted Ethyl benzoate**

Ethyl benzoate was prepared by refluxing benzoic acid with absolute alcohol in presence of sulphuric acid for 10-12 hrs. The mixture was cooled and kept at room temperature overnight. Next day the excess solvent was distilled off and separated mass was poured into ice-water then separated solid mass was collected by filtration and dried. It was recrystalized from chloroform- ethanol. For compound 3a Melting point 155°C; yield-65%, R\(_f\) – 0.76 and for compound 3b Melting point 156°C, yield-69%, R\(_f\) – 0.75.
Synthesis of Substituted Benzohydrazide

Ethyl benzoate was refluxed with Hydrazine hydride in presence of methanol for 8-10 hrs. The mixture was cooled and kept at room temperature overnight. Next day the excess solvent was distilled off and separated mass was poured into ice-water then separated solid mass was collected by filtration and dried. It was recrystallized from chloroform- methanol. For compound 4a Melting point 167°C, yield-72%, Rf – 0.87 and for compound 4b Melting point 178°C, yield-68%, Rf – 0.64.

Synthesis of Substituted N-Carbamothioyl Benzamide

Benzohydrazide was refluxed with Potassium thiocyanide in presence of methanol for 5-6 hrs. The mixture was cooled and kept at room temperature overnight. Next day the excess solvent was distilled off and separated mass was poured into ice-water then separated solid mass was collected by filtration and dried. It was recrystallized from chloroform-methanol. For compound 5a Melting point 175°C; yield-55%, Rf – 0.47 and for compound 5b Melting point 189°C, yield-73%, Rf – 0.46.

Synthesis of Substituted 5-Phenyl-1,3,4- thiazole-2-amine

N-carbamothioylbenzamide and 5 ml of concentrated sulfuric acid was kept in room temperature for 5 -6 hrs in a closed glass container. Whole mass was poured into ice-water and the solid mass was collected by filtration and dried. Then obtained mass was recrystalized from rectified spirit-chloroform. For compound 6a Melting point 183°C; yield-59%, Rf – 0.56 and for compound 6b Melting point 200°C, yield-70%, Rf – 0.5. 6a IR (KBr) cm⁻¹: 3216 (NH), 2914(C-H), 1656 (C=N), 1567(C=C), 845(N-N), 688(C-S); 1HNMR (DMSO) δppm: 5.24(s,2H, NH), 7.16 - 7.95(m, 9H, Ar-H), 8.34(s, 1H, N=CH-); MS (m/z) : 177(M+).

Synthesis of Substituted (Z)-2-(((5-phenyl-1,3,4-thiazol-2-yl)imino)methyl) phenol

5-Phenyl-1,3,4- thiazole-2-amine was refluxed with salicyldehyde/p-Nitrobenzaldehyde/p-Chlorobenzaldehyde in presence of methanol for 10-12 hrs. The mixture was cooled and the solid mass was collected by filtration and dried to obtain the compounds 7a-f. It was recrystalized from chloroform-methanol.

For compound 7a Melting point 150-155°C, yield-68%, Rf – 0.51,IR (KBr)cm⁻¹ : 3600(OH), 2916C-H), 1657 (C=N), 1568(C=C), 847(N-N), 679(C-S); 1HNMR(DMSO)δppm; 9.54(s,1H, OH), 6.86-7.92(m, 9H, Ar-H), 8.26(S, 1H, N=CH-); MS (m/z): 281(M+).
For compound 7b Melting point 175-178°C, yield-62%, Rf – 0.38, IR (KBr)cm⁻¹: 2910(C-H), 1660 (=N), 1567(C=C), 1520(Ar-NO₂), 846(N-N), 690(C-S); 1HNMR(DMSO)δppm: 6.84-8.21(m, 9H, Ar-H), 8.24(S, 1H, N=CH-); MS (m/z): 310(M+).

For compound 7c Melting point 168-172°C, yield-66%, Rf – 0.64; IR (KBr)cm⁻¹ : 2915(C-H), 1658 (C=N), 1566(C=C), 843(N-N), 720(Ar-Cl), 688(C-S); 1HNMR(DMSO)δppm; 6.88-8.12(m, 9H, Ar-H), 8.17(S, 1H, N=CH-); MS (m/z) : 299(M+).

For compound 7d Melting point 212°C; yield-70%, Rf – 0.43, IR (KBr)cm⁻¹: 3559(Ar.OH), 3298(N-H), 2911(C-H), 1713(C=O),1652 (C=N), 1563(C=C), 1326(Ar. C-N), 844(N-N), 682(C-S); 1HNMR(DMSO)δppm: 9.74(s,1H, OH), 9.66(s,1H, NH) 6.72-7.8.08(m, 9H, Ar-H), 8.24(S, 1H, N=CH-); MS (m/z): 338(M+).

For compound 7e Melting point 208°C; yield-66%, Rf – 0.68.; IR (KBr)cm⁻¹: 3304(N-H), 2913(C-H), 1718(C=O), 1654 (C=N), 1324(Ar. C-N), 1565(C=C), 846(N-N), 689(C-S), 1510(Ar-NO₂); 1HNMR(DMSO)δppm; 10.62(s,1H, NH) 6.74-8.19(m, 9H, Ar-H), 8.62(S, 1H, N=CH-); MS (m/z): 367(M+).

For compound 7f Melting point 211°C; yield-53%, Rf – 0.54; IR (KBr)cm⁻¹: 2918(C-H), 1660 (=N), 3300(N-H),1328(Ar. C-N), 1715(C=O), 1562(C=C), 849(N-N), 720(Ar-Cl), 686(C-S); 1HNMR(DMSO)δppm: 9.76(s,1H, NH) 6.71-7.8.18(m, 9H, Ar-H), 8.27(S, 1H, N=CH-); MS (m/z): 356(M+).
Toxicity study\cite{16}

The median lethal dose (LD\textsubscript{50}) was determined to evaluate the toxicity of compounds according to Karber\cite{17}. Healthy albino mice of either sex weigh between 25-30 gm were selected. The mice were fasted overnight. Animal care and toxicity test procedure were carried out according to the method. The dosages of the compounds (100, 300, 500, 700, 1000 mg/kg body weight) suspended in 0.5% CMC were administered orally as a single dose. The mortality was observed at 700-1000 mg/kg body weight. So, for actual LD\textsubscript{50} determination the mortality levels were narrowed and 300, 400, 500, 600, 700 mg/kg body weight suspended in 0.5% CMC were administered. The normal control group was fed with 0.5% CMC orally (30 mg/kg body weight). Mice were housed in stainless cages in a room with controlled temperature (25°C) and humidity (40-60%) and 12 hr light / dark cycle. Animals were then kept under observation for 1 day to record toxicity and total mortality.

Wound Healing activity\cite{18}

Wound healing activity was performed by excision wound model according to references\cite{19} in albino rats. Healthy albino rats (150-250 g) of both gender and of approximately the same age group were used for the study. They were individually housed, maintained in clean cages and fed with commercially pellet diet (M/s Hindustan Lever Ltd., Mumbai) and water ad libitum. Eight groups, each containing six animals were used for excision wound model. The test compounds (7a-7f) were formulated as 10% w/w ointment using simple ointment IP as vehicle and were applied topically for each animal once daily (morning).

Animals were anesthetized prior to and during creation of wounds with 1ml of IV Ketamine Hcl (10 mg/kg). The dorsal fur of the animals was shaved and the anticipated area of the wound to be created was outlined with methylene blue. A full thickness of excision wound of circular area of 500mm\textsuperscript{2} and 2 mm depth was made along the markings using toothed forceps, scalpel and pointed scissors. Haemostasis was achieved by using normal saline and the wound was left open. All surgical procedures were performed under aseptic conditions. The group I animals were treated with the vehicle (simple ointment IP), the positive control (group II) was applied with 0.2% w/w in simple ointment IP. The wound closure rate was assessed by studying the wound on 1\textsuperscript{st}, 4\textsuperscript{th}, 8\textsuperscript{th}, 13\textsuperscript{rd}, and 16\textsuperscript{th} day.

Diuretic activity\cite{20}

The diuretic activity was evaluated on albino rats according to references\cite{21,22}. The wistar strain rats weighing 150-200 gm of either sex were divided into eight groups of three animals
each. They were placed in metabolic cages at $20^\circ \pm 0.5^\circ C$, which were provided with a wire mesh at the bottom and a funnel to collect urine. Sieves made up of stainless steel are placed in the funnel to retain feces. The rats were fed with standard diet and water *ad libitum*. Food and water were withdrawn 24 h prior to the experiment. For screening procedure six groups of animals were served with test compound and received 30 mg/kg body weight were administrated orally, which was suspended in 0.1% Tween-80. Another set of animals served standard and received 10 mg/kg body weight of Acetazolamide (aqueous solution) orally. The remaining group of animals served as control and received 0.1% Tween-80 solution. Sodium chloride solution (0.9%) at a dose of 5 ml/100 g body weight was given to all the animals by gavages before the experiment. Urine excretion was recorded after 5 h and the values were tabulated in [Table - 3].

**RESULT AND DISCUSSION**

The 2-amino-substituted 5-phenyl-1,3,4-Thiadiazole dericatives (7a-7f) were synthesized with good yields. The structures of the compound were confirmed on the basis of spectral data of IR, NMR and mass spectra. The median lethal dose ($LD_{50}$) data of 2-amino-substituted 5-phenyl-1,3,4-Thiadiazole derivatives towards mice are shown in Table No. 1. Among the toxicity study of compounds (7a-7f), the derivatives containing chlorine group in its structure i.e. 7c and 7f was found to be less toxic. The compound 7c displayed the lowest toxicity. This is may be due to chlorine group, which is an electron withdrawing group.

**Table No. 1: Toxicity study.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>7a</th>
<th>7b</th>
<th>7c</th>
<th>7d</th>
<th>7e</th>
<th>7f</th>
</tr>
</thead>
<tbody>
<tr>
<td>$LD_{50}$</td>
<td>4780</td>
<td>4595</td>
<td>4920</td>
<td>4699</td>
<td>4592</td>
<td>4808</td>
</tr>
</tbody>
</table>

$LD_{50}$ is the mean lethal dose in mg/kg.
Wound healing activity was performed by excision wound model in albino rats. For this, the compounds were formulated as 10% w/w ointment using Simple ointment IP as vehicle. The results of wound healing activity of compounds (7a-7f) showed significant promotion of wound healing activity in the excision wound models. In excision wound models, the mean percentage close of wound area was calculated on the 1st, 4th, 8th, 13th and 16th post wounding days as shown in Table 2. The animals treated with compound 7e showed faster epithelisation of wound than the animals treated with compounds (7b – 7f). The percentage of wound closure was 100.05 ± 0.00 in the case of standard drug Nitrofurazone on 16th day of treatment and the compound 7e demonstrated similar effects on 16th day, but the compound 7d and 7a showed moderate effect and the compound 7b, 7c and 7f did not reveal significant activity. The period of epithelisation was 17.23 ± 1.04 days for compound 7e treated group of animals as against 13.15 ± 1.47 for the standard drug treated group.

Table 2. Wound healing activity (Excision wound model).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Conc.</th>
<th>Percentage (%) Wound closure</th>
<th>Epithelization Period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4th Day</td>
<td>8th Day</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>22.42 ± 1.41</td>
<td>52.23 ± 1.17</td>
</tr>
<tr>
<td>II</td>
<td>Nitofurazone 0.2%</td>
<td></td>
<td>46.53 ± 2.92</td>
<td>84.6 ± 1.36</td>
</tr>
<tr>
<td>III</td>
<td>7a 10%</td>
<td></td>
<td>25.32 ± 1.22</td>
<td>77.31 ± 1.83</td>
</tr>
<tr>
<td>IV</td>
<td>7b 10%</td>
<td></td>
<td>22.46 ± 1.23</td>
<td>56.38 ± 3.32</td>
</tr>
<tr>
<td>V</td>
<td>7c 10%</td>
<td></td>
<td>24.75 ± 1.33</td>
<td>59.22 ± 2.37</td>
</tr>
<tr>
<td>VI</td>
<td>7d 10%</td>
<td></td>
<td>28.88 ± 1.37</td>
<td>80.21 ± 1.41</td>
</tr>
<tr>
<td>VII</td>
<td>7e 10%</td>
<td></td>
<td>30.18 ± 1.24</td>
<td>82.24 ± 1.31</td>
</tr>
<tr>
<td>VIII</td>
<td>7f 10%</td>
<td></td>
<td>22.47 ± 1.24</td>
<td>56.36 ± 3.21</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E (n= 6).

Diuretics are agents, which support the excretion of urine and also increase the rate of excretion of sodium and chlorine ions. In this study, the diuretic activity of the compounds (7a-7f) were investigated to rationalize its medicinal use as diuretic agents and are shown in the Table No. 3 and Figure No. 3. Compound 7a and 7d was found to show good diuretic activity. Among all the synthesized compounds 7a showed the best activity. The mechanism
of diuretic action is possibly due to the inhibition of the enzyme carbonic anhydrase which leads to the excretion of sodium, chloride ions along with water, a mechanism attributed to the standard drug used in this study.

Table 3. Diuretic Activity.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Vol. of urine collected after 5h</th>
<th>T/S (Lipschitz value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>10.5</td>
<td>1.00</td>
</tr>
<tr>
<td>7a</td>
<td>7.62</td>
<td>0.73</td>
</tr>
<tr>
<td>7b</td>
<td>4.15</td>
<td>0.4</td>
</tr>
<tr>
<td>7c</td>
<td>5.13</td>
<td>0.49</td>
</tr>
<tr>
<td>7d</td>
<td>7.18</td>
<td>0.68</td>
</tr>
<tr>
<td>7e</td>
<td>5.1</td>
<td>0.49</td>
</tr>
<tr>
<td>7f</td>
<td>2.97</td>
<td>0.28</td>
</tr>
<tr>
<td>Control</td>
<td>2.85</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Animals used were wistar rats and six animals per group were used. Standard drug used was Acetazolamide. T refers to urine collected for test compound and S refers to urine collected for standard drug. Control group received 0.1% Tween-80.

CONCLUSION

A series of 2-amino-substituted-5-phenyl-1,3,4-thiadiazole derivatives was synthesized with good yields and their structures were elucidated by spectral data. Compounds 7c and 7f were found to be less toxic which may be due to presence of an electron withdrawing group chlorine. Compound 7e showed the similar effect as compared to the standard drug Nitrofurazone used on 16th day. The period of epithelisation was 17.23 \( \pm \) 1.04 days for compound 7e against 13.15 \( \pm \) 1.47 for the standard drug treated group. Compound 7a and 7d was found to show good diuretic activity which may be due to the inhibition of the enzyme
carbonic anhydrase which leads to the excretion of sodium, chloride ions along with water, a mechanism attributed to the standard drug used in this study.

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