SYNTHESIS OF THIAZOLE SCAFFOLDS BY NOVEL METHOD AND THEIR IN VITRO ANTHELMINTIC ACTIVITY AGAINST INDIAN ADULT EARTHWORM

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ABSTRACT

A highly efficient and facile method has been described for the synthesis of substituted thiazoles and 2-aminothiazoles from phenacyl halide and thiourea in methanol using iodine as a catalyst at ambient temperature and product obtained in excellent yield within short time. The formation of these compounds was confirmed by IR, $^1$H NMR, $^{13}$C NMR and mass spectroscopy. The synthesized different substituted thiazoles (3a-3g) were taken for anthelmintic activity against Indian earthworm *Pheretima posthuma* at two concentration (50 mg/ml and 100 mg/ml) and results were expressed in terms of time for paralysis and time for death of worms. The albendazole (20 mg/ml) was used as reference standard. The products were evaluated for anthelmintic activity to develop anthelmintic agent. Some of the compounds showed good anthelmintic activity (3g) as compared to albendazole.

KEYWORDS: 2-Aminothiazole, Iodine, Methanol, phenacyl halide, Anthelmintic activity.

1. INTRODUCTION

Helminthes effecting human and animals: Helminthes are recognized as a major problem to livestock’s throughout tropics.[1] Helminth infections are one of the most prevalent diseases in developing and developed countries.[2] Helminthes infections, repeatedly entitled helminthiasis are among the most pervasive infection and a foremost degenerative disease distressing a large proportion of world’s population. In developing countries, they pose a large threat to public health and contribute to the of anemia, malnutrition, eosinophilia and pneumonia.[3] Most diseases caused by helminthes are of a chronic and debilitating in nature,
they probably cause more morbidity and greater economic and social deprivation among humans and animals than any other single group of parasites. The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms, which results in loss of appetite, weakness, reduced weight gain, decreased feed efficiency and decreased productivity. Helminths consume nutrients from their host, thereby causing or aggravating malnutrition which results in retarded growth and physical development. Consequently, symptoms like retarded cognitive development, iron-deficiency anaemia, abdominal pains and related health problems are characteristic features of most heavy helminth infections.[4-5]

![Figure1. General structure of thiazole derivatives](image)

The helminthes parasites mainly subsist in human body in internal tract, but they are also found in tissue, as their larve migrate towards them.[6] Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, development of resistance in helminthes.[7-8] against conventional anthelmintics is a foremost problem in treatment of helminthes diseases.[9-10] Therefore it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening synthetic compounds for their anthelmintic activity.

Helminth infections resulting to diseases such as ascariasis, hookworm infection and schistosomiasis constitute the bulk of the 13 diseases classified as neglected tropical diseases (NTDs) by the WHO.[11] These incapacitating diseases have continued to inflict severe disability and often deaths. It is more pronounced among the impoverished population living in marginalized areas of the world.[12] In the most developing countries, intestinal helminth infections are a major health concern because factors that pre-dispose humans to these infections abound in these areas.[13]
Thiazoles are one of the most intensively investigated classes of aromatic five membered heterocycles which exhibit pharmacological and biological activities. The thiazole ring system is a structural component of natural compounds such as vitamin B\(_1\) (thiamine), penicillin and carboxylase. The thiazoles and 2-aminothiazole ring system is a useful structural element in medicinal chemistry having application in drug development for the treatment of bacterial,\(^{[14]}\) schizophrenia,\(^{[15]}\) inflammation,\(^{[16]}\) allergies,\(^{[17]}\) hypertension,\(^{[18]}\) and HIV\(^{[19]}\) infections. The thiazolyl group has great importance as it appears frequently in the structure of various natural products and biologically active compounds, some antibiotic drugs like penicillin, micrococcin\(^{[20]}\) and many metabolic products of fungi and primitive marine animals etc.

These compounds attract particular attention in methodology design for its utility as a synthetic building block and widespread occurrence in target structures such as functional materials and biologically relevant compounds.\(^{[21-25]}\) Aminothiazoles are known to be ligands of estrogen receptors\(^{[26]}\) as well as a novel class of adenosine receptor antagonists\(^{[27]}\) whereas other analogues are used as fungicides, inhibiting in vivo growth of xanthomonas and as an ingredient of herbicides or as schistosomicidal and anthelmintic drugs.\(^{[28]}\) In view of the importance of 2-aminothiazole and its derivatives, several methods were reported in the literature. Solid supported synthesis has been used to generate small organic libraries\(^{[29]}\) and solution phase prepared in DMF\(^{[30]}\) as well as in dioxane.\(^{[31]}\)

Hantzch reaction of \(\alpha\)-halocarbonyl compounds with thioureas or thioamides provides a useful method for the synthesis of thiazoles.\(^{[32]}\) Recently many improved methods have been reported for the synthesis of thiazoles using catalyst such as, \(\beta\)-cyclodextrin in water,\(^{[33]}\) ammonium 12-molybdophosphate (AMP) in methanol,\(^{[34]}\) iodine,\(^{[35a-b]}\) silica chloride\(^{[36]}\) in organic solvents at elevated temperature and solvent such as 1-metyl -2 pyrrolidinone,\(^{[37]}\) by the use of microwave in ethanol\(^{[38]}\) and POCl\(_3\) catalyzed solvent free synthesis.\(^{[39]}\)

A different variety of compounds may serve as nucleophilic reagent in these reactions such as thioamide, thiourea, ammonium thiocarbamate, or dithiocarbamate and their derivatives.\(^{[40]}\) Therefore present work aim at the use of iodine catalyst in presence of polar protic solvent in cyclisation reactions\(^{[41]}\) prompted us to investigate a variety of cyclisation reactions using the substrate phenacyl halide with thiourea in methanol to get thiazoles and aminothiazoles respectively in high yield. To the best of our knowledge from the literature survey this is the first approach for the synthesis of thiazoles and 2-aminothiazole carried out in iodine catalyst.
with phenacyl halide and thiourea as substrates in methanol solvent at ambient temperature provide a homogeneous reaction medium. It can be observed that almost all reactions completed within 15-18 minutes.

Most diseases caused by helminthes are a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. Keeping in mind such outstanding properties exhibited by the aminothiazole, the present study was intended to investigate anthelmintic activity of 2-aminothiazole against Indian earthworm.

2. RESULTS AND DISCUSSION

2.1. Chemistry

An efficient synthesis of different substituted 2-aminothiazole carried out by reaction between Phenacyl halide with thiourea by using iodine as catalyst, the phenacyl halide forms complex with iodine then sulphur facilitates nucleophilic attack on electron deficient carbon in which halide (leaving group) is attached followed by attack of lone pair of nitrogen from imine species on carbonyl carbon, which on cyclization and condensation give thiazoles and 2-aminothiazole in excellent yield (92-95 %) in just 15-18 minutes (Scheme1 and Table 1).

The reaction proceeds smoothly without the formation of any rearranged products. The catalyst iodine was easily removed by simple washing of sodium thiosulphate solution (30 %). These reactions do take place with other catalysts and solvents however iodine in methanol used as better combination because iodine forms ion pair in methanol and behaves...
as a Lewis acid catalyst, which is useful for formation of complex with oxygen atom of phenacyl halide as soon as this complex is formed the reaction proceeds forwardly. Thus iodine binds the substrate and promotes the reaction in shorter times. Hydrogen bonding of chlorine with hydroxyl group of methanol may be facilitating the attack of the substrate nucleophile for the reaction to take place at room temperature. All the structures of compounds were assigned on the basis of IR, $^1$H NMR, $^{13}$C NMR, mass spectroscopy and elemental analysis, by comparison with authentic samples prepared by literature procedure. Several examples illustrating this simple and practical methodology summarized in Table 1. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

### Table 1: Physical data of various thiazole derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product code</th>
<th>Time (min)</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3a</td>
<td>16</td>
<td>94</td>
<td>129-131</td>
</tr>
<tr>
<td>2</td>
<td>3b</td>
<td>16</td>
<td>92</td>
<td>203-204</td>
</tr>
<tr>
<td>3</td>
<td>3c</td>
<td>17</td>
<td>95</td>
<td>180-182</td>
</tr>
<tr>
<td>4</td>
<td>3d</td>
<td>18</td>
<td>93</td>
<td>199-200</td>
</tr>
<tr>
<td>5</td>
<td>3e</td>
<td>16</td>
<td>92</td>
<td>186-188</td>
</tr>
<tr>
<td>6</td>
<td>3f</td>
<td>16</td>
<td>96</td>
<td>128-130</td>
</tr>
<tr>
<td>7</td>
<td>3g</td>
<td>15</td>
<td>97</td>
<td>122-124</td>
</tr>
</tbody>
</table>

#### 2.2. In vitro anthelmintic activity

The in vitro anthelmintic activity of seven compounds (3a-3g) were tested against Indian earthworm *Pheretima posthuma* by using two different concentration 50 mg/ml and 100 mg/ml. As shown in Table 2, out of the top seven compounds, 3g which had potent anthelmintic activity and 3a, 3b, 3e, 3c, 3d also showed good anthelmintic activity but 3f showed moderate activity. Compound 3g having paralysis time nearly equal to standard drug albendazole and having death time less than standard drug albendazole. All compounds showing death time less than standard for 100 mg/ml as shown in Table 2, structure–activity relationship in compounds 3a-3g demonstrated that compounds bearing electron donating group at ortho and para position of 4-substituted phenyl ring of thiazole showed better anthelmintic activity. Basically electron donating substituents showed high activity. In electron donating substituents chloro showed high activity as compared to methyl, methoxy and amino substituents. Compound (3e) having electron withdrawing substituent at its meta position (nitro) also show good activity as compared to standard. The above results indicated that thiazole derivatives might play an important role in the anthelmintic activity.
Table 2: Anthelmintic activity of thiazole derivatives.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paralysis time in Min</th>
<th>Death Time in Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>----</td>
<td>-----</td>
</tr>
<tr>
<td>Standard (Albendazole)</td>
<td>13 (20 mg/ml)</td>
<td>52 (20 mg/ml)</td>
</tr>
<tr>
<td>Thiazoles</td>
<td>50 mg/ml</td>
<td>100 mg/ml</td>
</tr>
<tr>
<td>3a</td>
<td>31.28333</td>
<td>61.73333</td>
</tr>
<tr>
<td>3b</td>
<td>31.18333</td>
<td>59.65</td>
</tr>
<tr>
<td>3c</td>
<td>34.5</td>
<td>63.36667</td>
</tr>
<tr>
<td>3d</td>
<td>36.83333</td>
<td>60.11667</td>
</tr>
<tr>
<td>3e</td>
<td>33.23333</td>
<td>70.35</td>
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<tr>
<td>3f</td>
<td>45.36667</td>
<td>72.23333</td>
</tr>
<tr>
<td>3g</td>
<td>19.56667</td>
<td>54.71667</td>
</tr>
</tbody>
</table>

CONCLUSIONS

In this paper, a series of thiazole derivatives (3a-3g) that may function as anthelmintic agent have been synthesized by new route. Some of them displayed potent anthelmintic activity (3g) and other have good to moderate activity. 2-Aminothiazole derivatives were efficiently prepared by our recently postulated synthetic methodology from phenacyl halide and thiourea. This reaction may also take place in aqueous and other organic solvent without catalyst but the iodine catalyzed reaction in methanol as a solvent is not only rapid but also economical, industrially scalable, environmentally friendly and follows various principles of green chemistry. The procedure offers several advantages including quantitative yield, mild conditions, no side reactions, simple experimental procedure, cleaner reactions and low cost which makes it a useful and attractive strategy in view of economic and environmental advantages. All compounds (3a-3g) showed prominent anthelmintic activity in Indian adult earthworm.
3. EXPERIMENTAL

3.1. Materials and measurements

$^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AV-400 spectrometer and Bruker AV-200 spectrometer in CDCl$_3$ using TMS as internal standard. Infrared spectra were recorded with ATI MATT-SON RS-1 FTIR spectrometer using KBr pellets. Elemental analyses were obtained using a flash EA 1112 thermofinnig as an instrument. TLC was performed on the glass backed silica gel sheets (Silica Gel-60 GF254) and visualized in UV light (254 nm). Melting points were recorded in open capillary on Buchi melting Point B-540 apparatus. All solvents and chemicals were of analytical grade and were used after freshly open as obtained from Merck and Aldrich.

3.2. Experimental worms

All the experiment was carried out in Indian earthworms (*Pheretima posthuma*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collect from moist soil and washed with water to remove all fecal matter.

3.3. General procedure for the synthesis of 2-aminothiazoles

A mixture of phenacyl halide (1mmol), thiourea (1.5 mmol) and iodine in catalytic amount was vigorously stirred for 15-18 minutes at room temperature in methanol. The progress of reaction was monitored by TLC. After completion of the reaction, reaction mixture was poured in ice cold water. The solid separated was filtered through Buckner funnel and washed with a solution of sodium thiosulphate (30%) to remove of excess iodine and finally with water. The resulting crude product was recrystallized from ethanol to obtain the title compound.

3.3.1. 5-(2,4-dichlorophenyl)thiazol-2-amine (3g)

Yellow solid; mp 122–124 °C; IR (KBr): 3458, 3279, 3117, 1633, 1585, 1556, 1540, 1466, 1371, 862, 836, 813 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz): δ = 5.16 (br s, 2H, NH$_2$), 7.08 (s, 1H, thiazole H), 7.29 (d, 1H, ArH), 7.461 (d, 1H, ArH), 7.814 (s, 1H, ArH); $^{13}$C NMR (CDCl$_3$, 400 MHz): δ = 108.5, 127.1, 130.1, 131.8, 131.9, 132.3, 133.5, 146.4, 166.4.

Anal. Calcd for C$_9$H$_6$N$_2$SCl$_2$: C, 44.08; H, 2.44; N, 11.42%. Found: C, 44.11; H, 2.50; N, 11.55%, MS: m/e 245
3.3.2. 4-Phenyl-thiazol-2-ylamine (3f)
Yellow solid; mp 128-130 °C; IR (KBr): 3400, 3300, 3100, 2950, 1635, 1605, 1510, 1450 cm⁻¹. ¹H NMR (CDCl₃, 400MHz): δ = 5.30 (s, 2H, NH₂), 6.74 (s, 1H, thiazole H), 7.28-7.80 (m, 5H). ¹³C NMR (CDCl₃, 400MHz); 102.29, 126.03, 127.74, 128.60, 134.72, 151.36, 167.42. Anal. Calcd for C₉H₈N₂S: C, 61.36%, H, 4.54%, N, 15.90%, S, 18.18%. Found: C, 61.38%, H, 4.57%, N, 4.55%, S, 18.21%, MS: m/e 177.

3.4 Method for evaluation of Anthelmintic activity[42]
All the experiments were carried out in Indian adult earthworms (Pheretima posthuma) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters.

Pheretima posthuma was placed in two petridish containing different concentrations (50 & 100 mg/ml) of compounds. Each petridish was placed with 2 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with Reference compound Albendazole (20 mg/ml) treated samples. Sodium Carboxy Methyl Cellulose (Sodium CMC) in 0.5% concentration was used as control. Sodium CMC was used to suspend the compounds in water as they are insoluble in water.

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