Synthesis and antimicrobial evaluation of some novel thiazole derivatives

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ABSTRACT

Thiazole nucleus has been established as the potential entity in the largely growing chemical world of heterocyclic compounds possessing promising pharmacological characteristics. A series of pyrazoline thiazole derivatives were synthesized with an objective to develop novel and potent antimicrobial agents of synthetic origin. The required starting material 2-amino-4-aryl thiazole (1) was synthesized via a multicomponent condensation between thiourea, acetophenone and bromine. The Compound 1 was reacted with p-chloroacetophenone and various substituted aldehydes to synthesize the intermediates (3a-3d) which on cyclization with hydrazine hydrate yielded final products i.e. pyrazoline thiazole derivatives (4a-4d). Synthesized compounds were purified, characterized and evaluated for their antimicrobial activity. Most of the compounds exhibited moderate to significant activities.

Keywords: Thiazole, Pyrazole, Antibacterial Activity, Antifungal Activity.

INTRODUCTION

Substituted thiazoles and their biheterocycles have received considerable attention during last two decades as they are endowed with wide range of therapeutic properties. A number of thiazole derivatives have been reported to possess significant and diverse biological activities such as antimicrobial[1], analgesic[2], anti-inflammatory[3], antioxidant[4], anti-HIV[5] and antiallergic[6] activities.

In continuation to these efforts and with an objective to develop novel and potent therapeutic agents of synthetic origin, it was decided to synthesize certain pyrazoline thiazole derivatives and evaluate them for their antimicrobial potential.

MATERIALS AND METHODS

The melting points of synthesized compounds were determined in open capillary tubes using Kshitij Innovations melting point apparatus, expressed in ºC and are uncorrected. The IR spectra of compounds were recorded on Shimadzu Affinity-1 FTIR in KBr disc and absorption bands are expressed in cm\(^{-1}\). \(^1\)H NMR spectra were recorded on Bruker Advance 400.13 MHz NMR Spectrometer (Chemical shift if \(\delta\) ppm) using TMS as internal standard. The purity of the compounds was checked by TLC on silica gel G plates using ethyl acetate: benzene (1:1) solvent system and iodine vapors as a visualizing agent.

Synthesis of 2-amino 4-aryl thiazole (1)

Bromine (0.02 mol) was added drop wise to the reaction mixture containing acetophenone (0.01 mol) and thiourea (0.2 mol) with shaking. The reaction mixture was heated on water bath for 24 hours. Water was added to the
reaction mixture and was again heated until most of the solid had gone into solution. Reaction mixture was filtered when it was hot and the filtrate was cooled. It was made alkaline with concentrated ammonium hydroxide to separate 2-amino 4-aryl thiazole. The product was filtered, washed with water, dried over P₂O₅ and recrystallized from ethanol as colorless crystals.

**Synthesis of 1-[(4-phenyl-1,3-thiazol-2-yl)aminophenyl]ethanone (2)**

Equimolar quantities of Compound 1 (0.01 mol) and p-chloroacetophenone (0.01 mol) was dissolved in acetone (40 ml). The reaction mixture was refluxed for 6 hours. Periodically sodium carbonate solution was added to neutralize HCl evolved during the reaction. Finally the reaction mixture was cooled and poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

**Synthesis of (2E)-3-(2-substituted phenyl)-1-{4-[(4-phenyl-1,3-thiazol-2-yl)amino]phenyl} prop-2-en-1-one (3a-3d)**

Equimolar quantities of Compound 2 (0.01 mol) and substituted aromatic aldehyde (0.01 mol) was dissolved in minimum quantity of ethanol. To the above reaction mixture 3-4 drops of concentrated sodium hydroxide was added. The resultant mixture was stirred for a period of 2 hours. The reaction mixture was poured over crushed ice and was placed on ice chest over night. The precipitated product was filtered, dried and recrystallized from ethanol. Similar procedure was adopted for synthesizing other intermediates. Physical data of synthesized intermediates (3a-3d) is summarized in Table 1.

**Synthesis of pyrazoline thiazole derivatives (4a-4d)**

A mixture of Compound 3a (0.01 mol) in 25 ml dioxane and hydrazine hydrate (0.01 mol) was refluxed for 10 hours. Reaction was monitored via TLC and the reaction mixture was cooled and poured into crushed ice. The product separated out was filtered, washed with water and recrystallized from alcohol. Similar procedure was adopted for synthesizing other pyrazoline thiazole derivatives. Physical data of synthesized pyrazoline thiazole derivatives (4a-4d) is summarized in Table 2.

**Compound 4a:** IR (KBr, cm⁻¹): 3117 (Ar-CH str.), 710 (C-S str.), 1337 (C-N str.), 3433 (N-H str. coupled), 3260 (N-N str. of pyrazoline moiety), 2843 (C-H str.), 1248 (C-O-C str.), 1HNMR (CDCl₃, δ ppm): 1.9 (m, 2H, CH₂); 3.5 (s, 3H, OCH₃); 3.6 (m, 1H, CH); 4.505 (s, 1H, NH); 6.7 (s, 1H, S-CH); 6.8 (d, 1H, NH); 7.1 (m, 13H, Ar-H); Anal. Calcd. for C₂₅H₂₂N₄O: C (72.79), H (4.89), N (6.79), O (7.76); found: C (73.06), H (4.72), N (7.17), O (7.95), S (7.10); Mol. Wt.: 412.

**Compound 4b:** IR (KBr, cm⁻¹): 3116 (Ar-CH str.), 709 (C-S str.), 1338 (C-N str.), 3433 (N-H str. coupled), 2969 (C-H str.), 3332 (O-H str.); 1HNMR (CDCl₃, δ ppm): 1.8 (m, 2H, CH₂), 3.3 (m, 1H, CH), 3.9 (s, 1H, NH), 5.1 (s, 1H, OH), 6.7 (s, 1H, S-CH), 6.9 (d, 1H, NH), 7.1 (m, 13H, Ar-H); Anal. Calcd. for C₂₄H₂₀N₄O: C (72.34), H (4.55), N (7.03), O (8.03); found: C (72.56), H (4.81), N (6.69), O (7.56); Mol. Wt.: 398.

**Compound 4c:** IR (KBr, cm⁻¹): 3125 (Ar-CH str.), 3433 (N-H str. coupled), 707 (C-S str.), 3265 (N-N str. of pyrazoline moiety), 1339 (C-N str.), 2730 (C-H str.), 3335 (O-H str.); 1HNMR (CDCl₃, δ ppm): 1.8 (m, 2H, CH₂), 3.3 (m, 1H, CH), 3.9 (s, 1H, NH), 5.2 (s, 1H, OH), 6.2 (s, 1H, S-CH), 6.9 (d, 1H, NH), 7.2 (m, 13H, Ar-H); Anal. Calcd. for C₂₄H₂₀N₄O: C (72.34), H (4.55), N (7.03), O (8.03); found: C (72.56), H (4.81), N (6.69), O (7.56), S (8.41); Mol. Wt.: 398.

**Compound 4d:** IR (KBr, cm⁻¹): 3117 (Ar-CH str.), 3433 (N-H str. coupled), 711 (C-S str.), 1337 (C-N str.), 2818 (C-H str.), 3260 (O-H str.); 1HNMR (CDCl₃, δ ppm): 1.7 (m, 2H, CH₂), 3.6 (m, 1H, CH), 4.1 (s, 1H, NH), 5.1 (s, 2H, OH), 6.7 (s, 1H, S-CH), 6.8 (d, 1H, NH), 7.1 (m, 12H, Ar-H); Anal. Calcd. for C₂₄H₂₀N₂O₂S: C (69.55), H (4.38), N (6.76), O (11.58), S (7.74); found: C (70.15), H (4.91), N (6.69), O (7.65), S (8.39); Mol. Wt.: 414.

**Biological Screening of synthesized derivatives**

All the synthesized compounds were subjected to antimicrobial screening at a concentration of 100µg/ml involving two Gram-ve bacteria (Escherichia coli and Staphylococcus aureus); two Gram +ve (Bacillus subtilis and Streptococcus pneumoniae) and two fungal strains (P. aeruginosa and C. albicans) using Ampicillin as standard at the same concentration.
The work, in reference, was carried out by Agar disc diffusion method[7]. The response of organisms to the synthesized compounds were measured in terms of zone of inhibition and compared with that obtained with standard.

A) Preparation of Mueller Hinton Agar (MHA) Media
Mueller Hinton Agar Media was used for antimicrobial screening and its composition is as follows:

- Casein Acid Hydrolysate: 17.50gm
- Beef Heart Infusion: 2.00gm
- Starch, soluble: 1.50gm
- Agar: 17.00gm

For preparing Mueller Hinton Agar (MHA) Media, 38gm of Mueller Hinton Agar No. 2 was dissolved in 1000ml distilled water. It was mixed properly and heated to boil to dissolve the medium completely. It was autoclaved at 15lbs pressure for 15 minutes i.e. 121°C. It was then cooled and poured into sterilized plates. All the plates were kept for 4-5 hours in laminar airflow until the media got solidified. The plates were then kept in an incubator at 37°C.

B) Preparation of standard antibiotic solution
A solution (100µg/ml) of standard drug (Ampicillin) was prepared in sterile water.

C) Preparation of Test solution
10 mg of the synthesized compound(s) was dissolved in 10 ml of DMF. 1 ml of this solution was taken and diluted to 10 ml (with DMF) so that the concentration of the test solution became 100µg/ml.

D) Preparation of inoculum
For the preparation of inoculum, 5g of nutrient agar was dissolved in 100ml of distilled water and the pH was adjusted at 7.2 ± 0.2. It was poured in test-tubes as per requirement and then sterilized by autoclaving at 121°C. A 24 hour old culture was used for the preparation of bacterial suspension. Likewise suspensions of all the organisms were prepared as per standard procedure.

E) Preparation of discs
Discs of 6-7 mm in diameter were punched from No. 1 Whatmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C one hour. Standard and test solutions were added separately to these discs which were air dried later on.

F) Method of testing
Inoculums were added to the prepared media plates and allowed to solidify. The previously prepared discs were carefully kept on the solidified media by using sterilized forceps. These petridishes were kept for one-hour diffusion at room temperature and then for incubation at 37°C for 24 hours in an incubator. The zones of inhibition after 24 hours were measured in millimeters[8-10].

The results obtained are shown in Table 3.

RESULTS AND DISCUSSION

In a mixture of acetophenone and thiourea in ethanol, bromine was added drop wise with shaking. The mixture was heated on water bath for 24 hours afforded 2-amino-4-aryl-thiazole (1). Compound 1 on reaction with 4-chloroacetophenone give 1-{4-[(4-phenyl-1,3-thiazol-2-yl) amino] phenyl}ethanone (2) which on further stepwise reaction with various aromatic aldehydes and hydrazine hydrate afforded various pyrazoline thiazole derivatives respectively. The primary structural difference within the series involves the nature of various substituted aldehydes.

Synthesized compounds were found to be crystalline in nature and easily soluble in ethyl acetate, benzene, DMSO and DMF but insoluble in hexane and toluene.

With the help of analytical techniques such as melting point, IR and 1H-NMR, synthesized derivatives were characterized. These compounds showed a band at 3256-3265 cm⁻¹ for N-N stretching of pyrazoline ring. All the compounds showed NMR signals for different kinds of protons at their respective positions. All of them were found to be in full consignment with assigned structures.
All the synthesized compounds were screened for their antimicrobial activity. Compound 4c exhibited promising antibacterial activity while compound 4d exhibited promising antifungal activity. Therefore compound 4c and 4d can be recommended for further studies.

\[
\text{CH}_3\text{O} + \text{H}_2\text{N} = \text{S} \rightarrow \text{C}=\text{N} = \text{S} + \text{NH}_2 \\
(1)
\]

\[
\text{CH}_3\text{CO} \rightarrow \text{DMF} \rightarrow \text{C}=\text{N} = \text{S} + \text{NH} - \text{C} = \text{O} \rightarrow \text{R} \\
(2)
\]

\[
\text{R} = 4\text{-OCH}_3\text{C}_6\text{H}_4\text{CHO}; 2\text{-OHC}_6\text{H}_4\text{CHO}; 3\text{-OHC}_6\text{H}_4\text{CHO}; 2,4\text{-OHC}_6\text{H}_4\text{CHO}
\]

Synthetic Scheme
Table 1: Physical data of synthesized intermediates (3a-3d)

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<td>3a</td>
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Table 2: Physical data of pyrazoline thiazoles (4a-4d)

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Table 3: Antimicrobial Activity of synthesized pyrazoline thiazoles (4a-4d)

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<th>P. aeruginosa</th>
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CONCLUSION

The analytical and other informational data, available in literature so far, have rendered thiazole significantly important class of heterocyclic compounds and their applications in ever challenging chemotherapy of various ailments/ infections since last two decades immensely hiked interests of medicinal chemist and biochemist. This particular research study, in reference, would extend great deal of help to researchers in reckoning and determining the best and most productive, economical, suggestive and conclusive access to various thiazoles of clinical importance superseding other compounds of their class.

Further combinatorial libraries of these compounds can be generated which can be screened for optimal pharmacological activities by optimization techniques using 2D and 3D QSAR investigation.

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REFERENCES