Synthesis of Acetylamino-Thiadiazoline Derivatives and In-Vitro screening of their antifungal activities

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ABSTRACT

Synthesis of Schiff’s base 1-[(2, 4-difluorophenyl)-2-(1H-1, 2, 4-triazol-1-yl)] ethanone thiosemicarbazone (1A) prepared by condensation of 1-(2,4-difluorophenyl)-2-[1(H)-1,2,4-triazol-1-yl] ethanone with thiosemicarbazide, followed by cyclization to 2-(2,4-difluorophenyl)-2-[(1H)-1,2,4-triazol-1-ylmethyl]-3-acetyl-5-acetylamino-thiadiazoline (2A) using acetic anhydride and finally hydrolysis of (2A) to form 2-(2,4-difluorophenyl)-2-[(1H)-1,2,4-triazol-1-ylmethyl]-3-acetyl-5-amino-thiadiazoline (3A) using hydrazine hydrate, all compounds having antifungal activities are reported.

Keywords: 1-[(2, 4-difluorophenyl)-2-(1H-1, 2, 4-triazol-1-yl)] ethanone thiosemicarbazone, 1-(2,4-difluorophenyl)-2-[1(H)-1,2,4-triazol-1-yl] ethanone, 2-(2,4-difluorophenyl)-2-[(1H)-1,2,4-triazol-1-ylmethyl]-3-acetyl-5-acetylamino-thiadiazoline, hydrolysis, 2-(2,4-difluorophenyl)-2-[(1H)-1,2,4-triazol-1-ylmethyl]-3-acetyl-5-amino-thiadiazoline.

INTRODUCTION

Antifungal agents are antibiotics, like microbial metabolites, are inhibitors of the growth of microorganisms. Most of the antibiotics act on microorganisms by inhibiting the biosynthesis of essential component of the cell of microorganisms [1]. Sometimes antibiotic resistance develops due to various reasons, so attempts have been made to synthesize second generation anti-fungal compounds having high potencies and efficacy.

Some group of heterocyclic compounds such as triazole derivatives and imidazole derivatives can have antifungal properties. From the many series of azoles that have been reported to have antifungal activity, several common structural features emerge: the presence of (a) an imidazole or triazole heme- coordinating group, (b) a halosubstituted aromatic ring separated from the azole moiety by two carbon atoms and (c) a side chain.
Because of development of resistance, further research works are to be continued as part of search for newer and more effective antifungal agents, through derivatization of functional groups present in Schiff’s base 1-(2, 4-difluorophenyl)-2-[1(H)-1, 2, 4-triazol-1-yl] ethanone which is shown in Figure 2 a triazole template previously used as an intermediate compound leading to formation of fluconazole (as triazole derivative), a known potent antifungal drugs.

Secondly, attempts have been made to modify the structure of the anti-fungal compounds and find out the potency in order to check the Structure-Activity-Relationship (SAR) of the newly synthetic drugs and their microbial activity is herein discussed.

**MATERIALS AND METHODS**

**Microorganism Materials**
The Schiff’s base was bought form Merck chemicals group, one of the world’s leading pharmaceutical, chemical and life science companies in Germany. Other chemicals like: thiosemicarbazide, acetic anhydride, hydrazine hydrate etc are purchased from Merck chemicals group along with Sigma-Aldrich; a life science and high technology company from United States.

**Experimental Procedure: Synthesis**
Melting points were recorded by a Digital automatic melting point apparatus, (Stuart SMP-10) which were uncorrected. The purity of the derivatives was checked by High Performance Liquid Chromatography (HPLC), SHIMADZU CLASS-VP10 using mobile phase (MeOH: Water, 50:50), column C-18 and spectrum was recorded under UV detector at 261 nm. Infra-red (IR) spectra were recorded on SHIMADZU IR-Prestige-21 spectrophotometer as a solid which was finely grounded in a small agate mortar in KBr disc. $^1$H & $^{13}$C NMR spectra were measured by Bruker DPX 400 MHz spectrometer using Dimethyl sulphoxide (DMSO-d$_6$) as solvent with (TMS) as internal standard. Mass spectra were recorded by LCT Premier TOF MS, KD-146 (Micromass) spectrometer.

**Synthesis of 1-[(2, 4-difluorophenyl)-2-(1H-1, 2, 4-triazol-1-yl)] ethanone thiosemicarbazon (1A) from 1-(2, 4-difluorophenyl)-2-[1(H)-1, 2, 4-triazol-1-yl] ethanone (1) with thiosemicarbamide**
To a boiling solution of compound-1 (2.23gm, 10 mmol) in methanol (10 mL) acidified with few drops of concentrated HCl (32%) in a three necked round bottom flask, the hot solution of thiosemicarbamide (0.914gm, 10 mmol) in methanol (50 mL) was added drop wise [2]. The reaction mixture was refluxed on water bath for 1.0 hour. The reaction was monitored by HPLC using (methanol: water 50: 50) as mobile phase. After completion of the reaction, the mixture was cooled to room temperature; then solvent was evaporated to dryness under vacuum at 40°C temperature. A solid mass was obtained which was washed successively with dichloromethane followed by distilled water. The crystalline material was dried and finally off-white crystalline powder of 2.6gm (87.8% yield) was obtained, melting point: 163° to 164°C, HPLC: retention time, 5.81 min.
Figure 2 Synthesis of 1-[(2, 4-difluorophenyl)-2-(1H-1, 2, 4-triazol-1-yl)] ethanone thiosemicarbazone (1A) from 1-(2, 4-difluorophenyl)-2-[1(H)-1, 2, 4-triazol-1-yl] ethanone (1) with thiosemicarbazide

The newly synthesized compound 1A had the following spectral data:

**IR (KBr) νmax (cm⁻¹) spectrum:** 3394, 3261 cm⁻¹ (NH₂), 3169 cm⁻¹ (NH), 3053 cm⁻¹ (C-H, aromatic), 1608 cm⁻¹ (C=N), 1589, 1506, 1460 cm⁻¹ (C=C aromatic), 1429 cm⁻¹ (C-F), 1273 cm⁻¹ (C-S), 1029 cm⁻¹ (N-N).

**¹H-NMR (DMSO-d6):** δ H (ppm): 10.18 (1H, br, s, NH₂); [8.42 (1H, d, J = 6.9 Hz, H-C-3), 7.89 (1H, d, J = 6.9 Hz, H-C-5) for triazole ring]; 7.31-7.02 (3H, m aromatic protons in the 2, 4-difluorophenyl ring); 5.57 (2H, s, NH₂); 5.3 (2H, s, H₂).

**¹³C-NMR (100 MHz, DMSO-d6):** δ C ppm: 179.5 (C=S), 158.22 (C=N); [152.01 (H-C-3), 145.27 (H-C-5) for triazole ring]; [120.5 (C-1), 162.14 (C-2), 105.4 (H-C-3), 164.6 (C-4), 112.14 (H-C-5), 131.5 (H-C-6), in the 2, 4-difluorophenyl ring].

**Mass Spectrum (TOF MS ES⁺):** (C₁₁H₁₀N₆F₂S): m/z (% of relative intensities): 297.025 [M+1]⁺ (100%), 280 (38%), 228 (9%).

**Synthesis of 2-(2, 4-difluorophenyl)-2-[(1H-1, 2, 4-triazol-1-yl)-3-acetyl-5-acetylamino thiazolizine (2A)] from 1-[(2, 4-difluorophenyl)-2-(1H-1, 2, 4-triazol-1-yl)] ethanone thiosemicarbazone (1A).**

Schiff base product (1A), (1.0 gm, 3.37 mmol) was treated with freshly distilled acetic anhydride (61 mL) and the mixture [3-6] was heated for 1.5 hrs on an oil bath at 90°C ~ 95°C. After completion of the reaction which was monitored by HPLC, mobile phase (methanol: water, 50:50), the solvent was removed from the reaction mixture under vacuum, giving a solid mass which was washed with methanol affording a white crystalline solid having a yield of 898 mg (70%), melting point: 235° to 238°C, HPLC: retention time, 11.747 min.
RESULTS AND DISCUSSION

Structure Elucidation

Compound 1A: This compound was prepared by refluxing starting compound 1 with thiosemicarbazide in methanol for 1.0 hour. HPLC chromatogram of the reaction mixture showed a single peak with retention time 5.81 which is different from starting compound.

IR spectrum of compound 1A showed a strong band at around 1608 cm⁻¹ showing the presence of C=N indicating Schiff base formation. In ¹H NMR spectrum the peak at δH 10.18 is safely assigned to NH group. The ¹³C-NMR spectrum shows signals for 11 carbons. The presence of a quaternary carbon signal at 179.5 ppm which can be assigned for >C=S and the absence of a >C=O carbon signal are indicative of the formation of the product 1A. In the mass spectrum, the molecular ion peak appearing at m/z 297.025 supports the formula C₁₁H₁₀N₆F₂S and thus formation of 1A.

Compound 2A: It was prepared by refluxing 1A with acetic anhydride. The HPLC chromatogram of the reaction product showed a single peak having retention time 11.74 which is different from that of compound 1A. The IR spectrum of compound 2A shows new single N-H band at 3101 cm⁻¹ showing the presence of C-N indicating Schiff base formation. In the ¹H NMR spectrum the peak at δH 10.18 is safely assigned to NH group. The ¹³C-NMR spectrum shows 15 signals indicating the presence of 15 carbons in the compound. The two signals at δC = 22.7 and 23.96 are due to the two CH₂ groups. The quaternary carbon signal at 677.70 ppm must be due to the C-2 of thiadiazoline ring in the Mass spectrum, the molecular ion peak appearing at m/z 381.094 indicates the formation of the diacetylated product 2A which corresponds to the molecular formula C₁₃H₁₂F₂N₆O₂S.
Compound 3A: Compound 3A was prepared by stirring 2A with hydrazine hydrate. From HPLC Chromatogram a single peak was observed with a different retention time (7.063). The IR spectrum of compound 3A showed the presence of -NH₂ group as was characterized by the presence of two strong bands at 3371 and 3297 cm⁻¹ (vNH stretching in primary amine). In ¹H NMR spectrum the peak at δH 6.32 can be safely assigned to presence of NH₂ group. The two doublets at δH 5.66 and δH 5.13 are due to two diastereotropic methylene protons. The methyl proton in NCOCH₃ appears at δH 2.07 as a singlet. The ¹³C spectrum of compound 3A shows the presence of 13 signals due to 13 carbons in the compound. In the mass spectrum, the molecular ion peak appearing at m/z 339.08 supports the formation of compound 3A which corresponds to molecular formula C₁₃H₁₂F₂N₆O₅S.

Antifungal Activity (In Vitro)
The compounds showed activity against different microorganisms at a concentration of 100µL. The compounds were dissolved in MeOH and diluted with phosphate buffer [6-10]. The standard agar plate diffusion technique was used to determine the activity of the tested compound. The antifungal activity was evaluated in Sabouraud Dextrose Agar media, methanol and phosphate buffer were used as solvent for the compound. Compound-1 (starting compound) was used as standard for comparison against the different fungal species. The results reveal that the newly synthesized compounds are more potent than the starting compound as well as the known antifungal compound Fluconazole. The MIC results are shown in the Table 1.

![Figure 3 The bar graph shows the comparative zone of inhibition of various compounds](image_url)

**Table 1: Antifungal activity of the new synthesized compounds**

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Conc. in µg/ml</th>
<th>Zone of inhibition (mm) of action of the compounds.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starting Material</td>
<td>Fluconazole</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>100</td>
<td>17.2</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>100</td>
<td>18.8</td>
</tr>
<tr>
<td>Colletotrichum spp.</td>
<td>100</td>
<td>19.4</td>
</tr>
<tr>
<td>Curvularia spp.</td>
<td>100</td>
<td>17.3</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>100</td>
<td>22.2</td>
</tr>
</tbody>
</table>
Figure 4 The bar graph shows the comparative zone of inhibition of various compounds.

Figure 5 The bar graph shows the comparative zone of inhibition of various compounds.

Figure 6 The bar graph shows the comparative zone of inhibition of various compounds.
DISCUSSION

Aim of our study was to examine whether molecular modification might result in detection of new potential antifungal drugs. A new series of compounds were prepared and assayed for their antifungal activity. The data reported in Table 1 shows that the effect of variation in chemical structure on activity was rather unpredictable. Compound 2A and 3A showed remarkable antifungal activity against Candida albicans and Aspergillus niger compared to that of standard Fluconazole. Compound 1A and 2A showed no antifungal activity against Fusarium spp. and compound 3A also had no activity against Curvularia spp.

CONCLUSION

The objective of the present study can be the hope of discovering new structural leads serving as antifungal agents. Our thiadiazoline derivatives have been prepared, and their physical properties were characterized. The antifungal activity of the compounds was evaluated by the agar diffusion method against Candida albicans, Aspergillus niger, Colletotrichum spp., Fusarium spp. and Curvularia spp. Our study in this article may be a helpful guide for the medicinal researchers.

REFERENCES