

SYNTHESIS OF SCHIFF BASE HAVING A HETEROCYCLIC MOIETY ALONG WITH ITS CYCLIZED DERIVATIVES AND STUDY OF THEIR ANTIFUNGAL ACTIVITIES

RABEYA KHATUN, A. S. M ANSARUL ISLAM AND M. A. HAI*

Supreme Pharmaceuticals Ltd., Savar, Dhaka-1344, Bangladesh.

Department of Chemistry, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

Abstract

Syntheses of Schiff's base¹ 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 cyclization² to 2-(2,4-difluorophenyl)-2-[(1H)-1,2,4-triazol-1-ylmethyl]-3-acetyl-5-acetylamino- thiadiazoline (**2A**), using acetic anhydride, and finally hydrolysis³ 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 anti-fungal activities, are reported.

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 their cells. Sometimes antibiotic resistance develops due to various reasons. So, attempts have been made to synthesize second generation anti-fungal compounds having higher potencies and efficacy.

Some group of heterocyclic compounds such as triazole derivatives and imidazole derivatives can have antifungal properties. Among 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 halo-substituted aromatic ring separated from the azole moiety by two carbon atoms and (c) a sidechain⁴ (Fig.1).

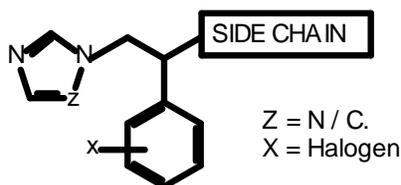


Fig.1

Because of development of resistance, further research-work are to be continued as part of search for newer and more effective antifungal agents through derivatization of functional groups present in 1-(2,4-difluorophenyl)-2-[1 (H)-1,2,4-triazol-1-yl]ethanone

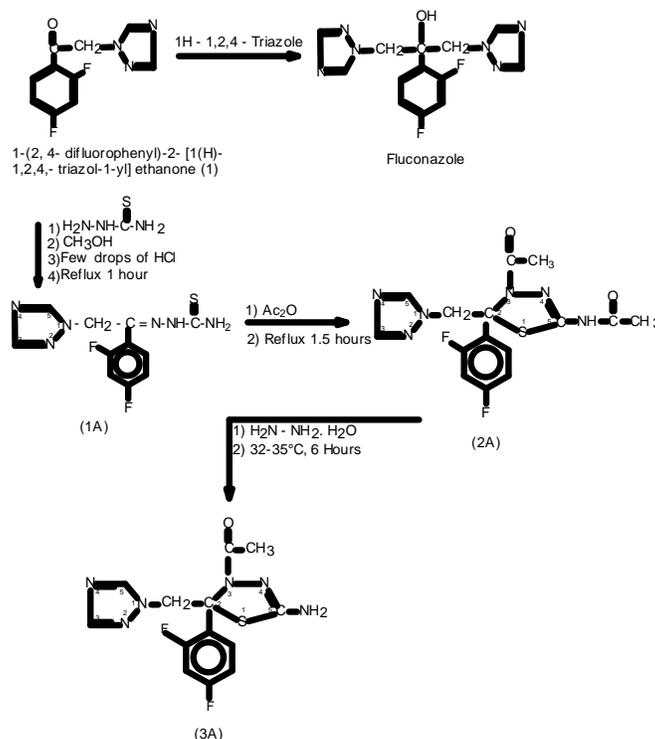
*Author for Correspondence; e-mail: mahai123@yahoo.com

(Scheme-1), a triazole template previously used as an intermediate compound leading to formation of fluconazole (a triazole derivative), a known potent antifungal drug.

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 here in discussed.

Experimental

Melting points were recorded by a Digital automatic melting point apparatus, (Stuart SMP-10) which are uncorrected. The purity of the derivatives were checked by High Performance Liquid Chromatography (HPLC), SHIMADZU CLASS-VP10 using mobile phase (MeOH: Water, 50:50), column C18 and spectrum was recorded under UV detector at 261 nm. Infra-red (IR) spectra were recorded on SHIMADZU FTIR (IRPrestige-21) spectrophotometer as a solid which was finely grounded in a small agate mortar in KBr disc. ^1H & ^{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.



Scheme -1

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.

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 thiosemicarbazide (0.914gm, 10 mmol) in methanol (50 mL) was added drop wise. 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 2.6 gm (87.8% yield) was obtained, melting point: 163° to 164°C, HPLC: retention time, 5.81 min.

The newly synthesized compound (**1A**) had the following spectral data.

IR (KBr) ν_{\max} (cm⁻¹) spectrum: 3394, 3261 cm⁻¹ (NH₂), 3169cm⁻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-N), 1300cm⁻¹ (C=S), 1273cm⁻¹ (C-F), 1029cm⁻¹ (N-N).

¹H-NMR (DMSO-d₆): δ (ppm): 10.18 (1H, br, s, NH); [8.42 (1H, d, J = 6.9 Hz, HC-3), 7.89 (1H, d, J = 6.9 Hz, HC-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, HC).

¹³C-NMR (100 MHz, DMSO-d₆): ¹³C ppm: 179.5 (C = S), 158.22(C = N); 54.49 (CH₂); [152.01 (HC-3), 145.27 (HC-5) for triazole ring]; [120.5 (C-1), 162.14(C-2), 105.4 (HC-3), 164.6 (C-4), 112.14 (HC-5), 131.5 (HC-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 methyl]-3-acetyl-5-acetylamino thiadiazoline (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 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.

The newly synthesized Compound (**2A**) had the following spectral data:

IR (KBr) ν_{\max} (cm^{-1}) spectrum : 3101 cm^{-1} (>NH), 3086 cm^{-1} (C-H aromatic), 1689, 1664 cm^{-1} (C=O), 1625 cm^{-1} (C=N), 1600, 1506 and 1425 cm^{-1} (C=C aromatic), 1398 cm^{-1} (C-N), 1246 cm^{-1} (C-F), 1139 cm^{-1} (C-S), 1022 cm^{-1} (N-N).

$^1\text{H-NMR}$ (DMSO- d_6): δ (ppm): 11.45 (1H, s, NH); [8.55 (1H, s, HC-3); 8.0 (1H, s, HC-5) for triazole ring]; 7.54-7.13 (3H, m, aromatic protons in 2, 4-difluorophenyl ring); [5.60 (1H, d, J=14.3Hz), 5.19 (1H, d, J = 14.3Hz) for CH₂-] 2.12 (3H, s, NCOCH₃); 1.95 (3H, s, NHC₂H₅).

$^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6) : ^{13}C ppm: 169.46 (NHC₂H₅); 168.5 (NCOCH₃); 51.7 (-CH₂-); 23.96 (NCOCH₃); 22.7 (NHC₂H₅); [152.2 (HC-3); 146.5 (HC-5), triazole ring]; [124.15 (C-1), 160.59 (C-2), 105.61 (HC-3); 163.21 (C-4), 111.65 (HC-5), 128.84 (HC-6) for 2, 4-difluorophenyl ring]; [77.7 (C-2) and 158.1(C-5), thiadiazoline ring].

Mass Spectrum (TOF MS ES+):

(C₁₅H₁₄F₂N₆O₂S): m/z (% of relative intensities): 381.094 [M+1]⁺ (100%), 339.08 (10%).

Synthesis of 2-(2, 4-difluorophenyl)-2-[(1H)-1, 2, 4-triazol-1-yl methyl]-3-acetyl-5-amino thiadiazoline (3A) from

2-(2, 4-difluorophenyl)-2-[(1H)-1, 2, 4-triazol-1-yl methyl]-3-acetyl-5-acetylamino thiadiazoline (2A).

Compound **2A**, (200mg) was stirred with hydrazine hydrate (10 mL) at 32 ~ 35°C for 6 hours to form a precipitate. The resulting precipitate was collected by filtration and dried well to obtain a bright white fine powder, melting point: 171° to 173°C and yield: 130mg (73%), HPLC: retention time, 7.063 min.

The newly synthesized compound (**3A**) had the following spectral data:

IR (KBr) ν_{\max} (cm^{-1}) spectrum: 3371 cm^{-1} , 3297 cm^{-1} (NH₂), 3178 cm^{-1} (N-H), 3106 cm^{-1} (C=C aromatic), 1643 cm^{-1} (C=O), 1618 cm^{-1} (C=N), 1597, 1579 and 1504 cm^{-1} (C=C aromatic), 1366 cm^{-1} (C-N), 1278 cm^{-1} (C-F), 1142 cm^{-1} (C-S).

$^1\text{H-NMR}$ (DMSO- d_6): δ (ppm): [8.50 (1H, s, HC-3), 7.99 (1H, s, HC-5) for triazole ring]; 7.48-7.12 (3H, m, aromatic protons 2, 4-difluorophenyl ring); 6.32 (2H, s, NH₂); [5.66 (1H, d, J=14.4Hz), 5.13 (1H, d, J = 14.4Hz for -CH₂-] 2.07 (3H, s, NCOCH₃).

$^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6) : ^{13}C ppm : 167.3 (NCOCH₃); 51.7 (-CH₂-); 24.49 (NCOCH₃); [151.9 (HC-3), 146.31 (HC-5) for triazole ring]; [124.1 (C-1), 160.2 (C-2), 105.55 (HC-3), 163.16 (C-4), 111.6 (HC-5) and 129.12 (HC-6), for 2, 4-difluorophenyl ring]; [81.08 (C-2) and 160.82 (C-5) thiadiazoline ring].

Mass Spectrum (TOF MS ES+): (C₁₃H₁₂F₂N₆O₂S): m/z (% of relative intensities): 339.089 [M+1]⁺ (50%), 270.03 (5%).

Results and discussion

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 new peak at $\delta_{\text{H}} 10.18$ is safely assigned to NH group.

The ^{13}C -NMR spectrum shows signals for 11 carbons. The presence of a quaternary carbon signal at 179.5 ppm which can be assigned for $>\text{C}=\text{S}$ and the absence of a $>\text{C}=\text{O}$ carbon signal are indicating the formation of the compound **1A**.

In the mass spectrum, the molecular ion peak appearing at m/z 297.025 supports the formula $\text{C}_{11}\text{H}_{10}\text{N}_6\text{F}_2\text{S}$ 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 11.747 which is different from that of compound **1A**.

The IR spectrum of compound **2A** shows new single N-H band at 3101 cm^{-1} accompanied by two C=O (amide) bands at 1689 cm^{-1} and 1664 cm^{-1} indicating the presence of a 3° and a 2° amide groups.

In ^1H NMR spectrum the two protons of the methylene group appeared as two doublets at $\delta_{\text{H}} 5.60$ ppm and $\delta_{\text{H}} 5.19$ ppm indicating their diastereotropic natures as well as their linkage to the C-2 of the thiadiazoline ring. The two singlets appearing at $\delta_{\text{H}} 2.12$ and $\delta_{\text{H}} 1.95$ can be assigned to $-\text{NCOCH}_3$ and NHCOCH_3 methyl protons.

The ^{13}C NMR spectrum of compound **2A** shows 15 signals indicating the presence of 15 carbons in the compound. The two signals at $\delta^{13}\text{C} = 22.7$ and 23.96 are due to the two CH_3 groups. The quaternary carbon signal at $\delta 77.70$ 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 $\text{C}_{15}\text{H}_{14}\text{F}_2\text{N}_6\text{O}_2\text{S}$.

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 -NH_2 group as was characterized by the presence of two strong bands at 3371 and 3297 cm^{-1} (ν_{NH} stretching in primary amine).

In ^1H NMR spectrum the peak at $\delta_{\text{H}} 6.32$ can be assigned to NH_2 group. The two doublets at $\delta_{\text{H}} 5.66$ and $\delta_{\text{H}} 5.13$ are due to two diastereotropic methylene protons. The methyl proton in NCOCH_3 appears at $\delta_{\text{H}} 2.07$ as a singlet.

The ^{13}C NMR 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 $m/z 339.089$ supports the formation of compound **3A** which corresponds to molecular formula $\text{C}_{13}\text{H}_{12}\text{F}_2\text{N}_6\text{OS}$.

Antifungal Activity (In vitro)

The compounds showed activity against different microorganisms at a concentration of $100\mu\text{g/L}$. The compounds were dissolved in MeOH and diluted with phosphate buffer. The standard agar plate diffusion technique⁵⁻⁶ was used to determine the activity of the tested compounds. The antifungal activity was evaluated in Sabouraud Dextrose Agar media, methanol and phosphate buffer were used as solvent for the compound. Compound-1 (Starting Material) 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 given below.

Table: Antifungal activity of the new synthesized compounds.

Test Organism	Conc. in $\mu\text{g/mL}$	Zone of inhibition (mm) of action of the compounds.				
		Starting Material	Fluconazole	1A	2A	3A
<i>Candida albicans</i>	100	17.2	18.5	18.5	25.3	24.7
<i>Aspergillus niger</i>	100	18.8	22.0	22.4	24.1	24.5
<i>Colletotrichum spp.</i>	100	19.4	22.3	21.9	22.4	22.1
<i>Curvularia spp.</i>	100	17.3	19.2	19.4	19.6	0
<i>Fusarium spp.</i>	100	22.2	22.9	0	0	24.3

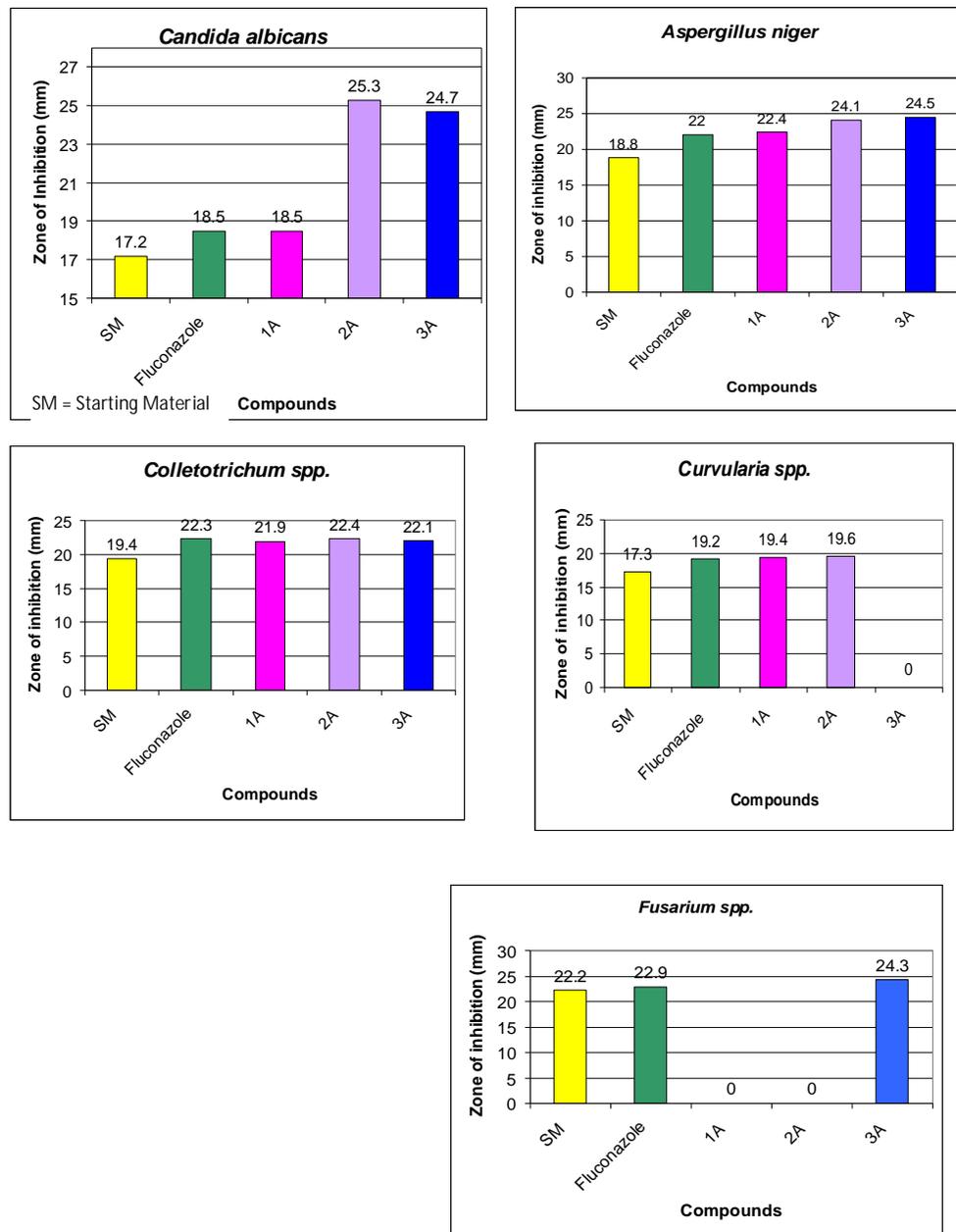


Fig. 2: The bar graph shows the comparative zone of inhibition of various compounds.

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