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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 anti-fungal 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 halo-substituted aromatic ring separated from the azole moiety by two carbon
atoms and (c) a side chain.

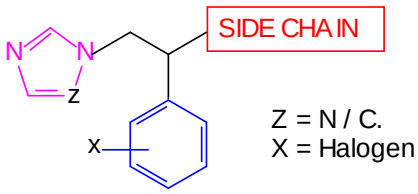

Figure 1 Variation of Side chain of Fluconazole

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 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 [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.

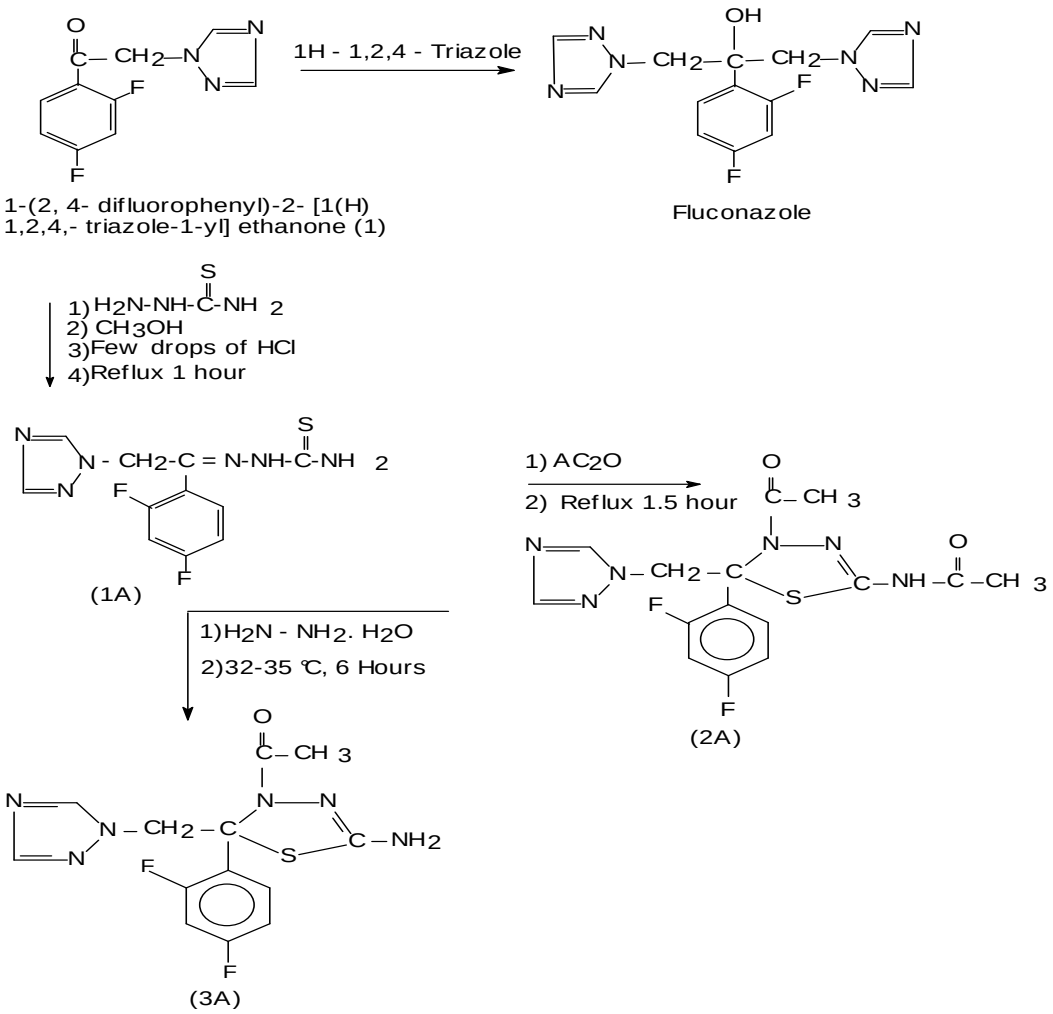

Figure 2 Synthesis of 1-[(2, 4-difluorophenyl)-2-(1H-1, 2, 4-triazol-1-yl)] ethanone thiosemicarbazone (1A) from 1-(2, 4-difluorophenyl)-2-[1(H)-1, 2, 4-triazol-1-yl] ethanone (1) with thiosemicarbazide

The newly synthesized compound **1A** had the following spectral data:
IR(KBr) ν_{max} (cm^{-1}) spectrum: 3394, 3261 cm^{-1} (NH_2), 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). **^{1}H-NMR (DMSO-d6): δ_H (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, NH_2); 5.3 (2H, s, H_2C).; **^{13}C-NMR (100 MHz, DMSO-d6):** δ^{13}C ppm: 179.5 (C = S), 158.22(C = N); 54.49 (CH_2); [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+):** ($C_{11}H_{10}N_6F_2S$): 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 [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.

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}H-NMR (DMSO-d6): δ_H (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 CH_2-] 2.12 (3H, s, NCOCH_3); 1.95 (3H, s, NHCOCH_3).; **^{13}C-NMR (100 MHz, DMSO-d6) :** δ^{13}C ppm: 169.46 (NHCOCH_3); 168.5 (NCOCH_3); 51.7 (-CH_2-); 23.96 (NCOCH_3); 22.7 (NHCOCH_3); [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-3), thiadiazoline ring].; **Mass Spectrum (TOF MS ES+):** ($C_{15}H_{14}F_2N_6O_2S$): 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 (205 mmol, 10 mL) at 32 ~ 35°C for 6 hours to form a precipitate [6-8]. 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: 3371cm^{-1}, 3297cm^{-1} (NH_2), 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}H-NMR (DMSO-d6): δ_H (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, NH_2); [5.66 (1H, d, J=14.4Hz), 5.13 (1H, d, J = 14.4Hz for -CH_2-] 2.07 (3H, s, NCOCH_3).; **13C-NMR (100 MHz, DMSO-d6) :** δ13C ppm :167.3 (NCOCH3); 51.7 (-CH2-); 24.49(NCOCH_3); [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-3) thiadiazoline ring].; **Mass Spectrum (TOF MS ES+):** ($C_{13}H_{12}F_2N_6OS$): m/z (% of relative intensities): 339.0891 $[M+1]^+$ (50%), 270.03 (5%).

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 ^{1}H NMR spectrum the peak at δ_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 >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 $C_{11}H_{10}N_6F_2S$ 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} accompanied by two C=O(amide) bands at 1689 cm^{-1} and 1664 cm^{-1} indicating the presence of a 3^o and a 2^o amide groups. In ^{1}H NMR spectrum the two protons of the methylene group appeared as two doublets at δ_H 5.60 ppm and δ_H 5.19 ppm indicating their diastereotropic nature as well as their linkage to the C-2 of the thiadiazoline rings. The two 3H singlets appearing at δ_H 2.12 and δ_H 1.95 can be assigned to -NCOCH_3 and NHCOCH_3 methyl protons. The ^{13}C spectrum of compound **2A** shows 15 signals indicating the presence of 15 carbons in the compound. The two signals at δ^{13}C = 22.7 and 23.96 are due to the two CH_3 groups. The quarternary carbon signal at δ77.70 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 $C_{15}H_{14}F_2N_6O_2S$.

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} (νNH stretching in primary amine). In ^1H NMR spectrum the peak at δ_H 6.32 can be safely assigned to presence of NH_2 group. The two doublets at δ_H 5.66 and δ_H 5.13 are due to two diastereotropic methylene protons. The methyl proton in $NCOCH_3$ appears at δ_H 2.07 as a singlet. The ^{13}C 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 $C_{13}H_{12}F_2N_6OS$.

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**.

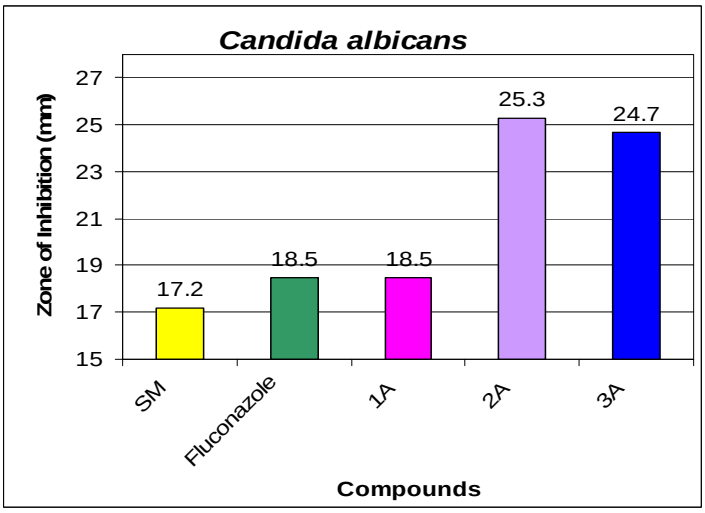

Figure 3 The bar graph shows the comparative zone of inhibition of various compounds
SM = Starting Material

Table 1: Antifungal activity of the new synthesized compounds

Test Organism	Conc. in µg/ml	Zone of inhibition (mm) of action of the compounds.				
		Starting Material	Fluconazole	1A	2A	3A
Candida albicans	100	17.2	18.5	18.5	25.3	24.7
Aspergillus niger	100	18.8	22.0	22.4	24.1	24.5
Colletotrichum spp.	100	19.4	22.3	21.9	22.4	22.1
Curvularia spp.	100	17.3	19.2	19.4	19.6	0
Fusarium spp.	100	22.2	22.9	0	0	24.3

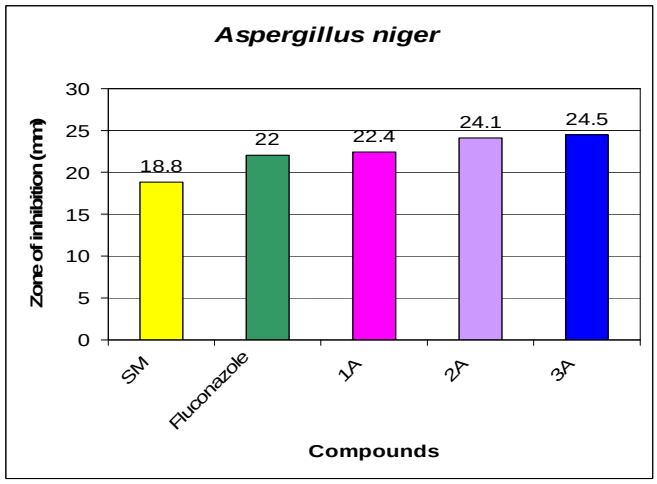

Figure 4 The bar graph shows the comparative zone of inhibition of various compounds

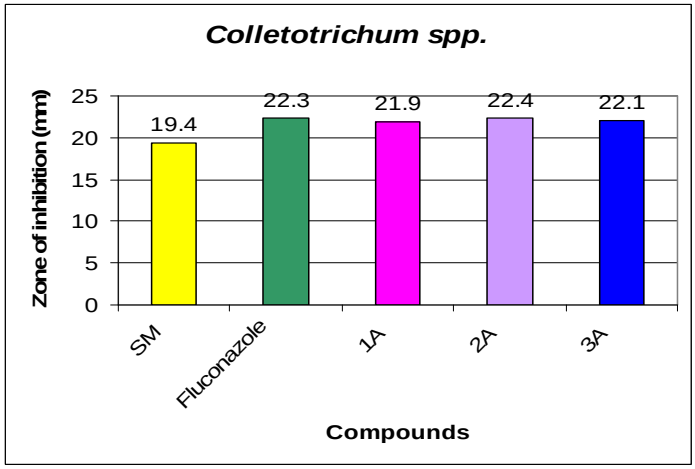

Figure 5 The bar graph shows the comparative zone of inhibition of various compounds

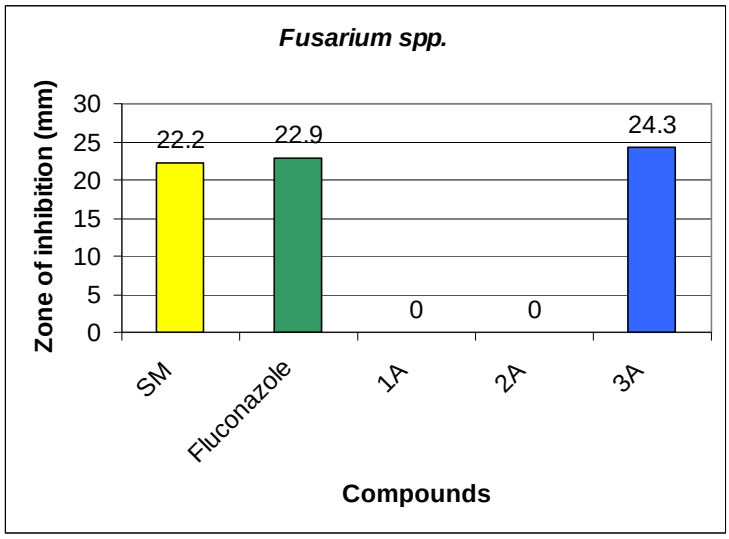

Figure 6 The bar graph shows the comparative zone of inhibition of various compounds

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.

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