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Research Article

Synthesis, Characterisation and Pharmacological Evaluation of Substituted Thiazole Derivatives as Anti-Fungal Agents

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ABSTRACT

In this present communication, we have synthesized 2-amino-4-methyl-thiazole-5-carboxylic acid ethyl ester (A) by reacting with Thio urea and ethylacetoacetate in presence of N-bromosuccinimide and Benzoyl peroxide. Further compound (A) reacted with secondary amines, formaldehyde and ethanol to give newly synthesized compounds. All compounds are further characterized FTIR, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. These newly synthesized compounds are screened for in vitro anti-fungal activity against *Candida albicans*, *Trichophyton* and *Aspergillus Niger* by disc diffusion method. Compound A_3 [4-methyl-2- (Morpholine-4yl methyl)-amino-thiazole-carboxylic acid ethyl ester] have showed good anti-fungal activity against *Candida albicans* as compared to standard drug clotrimazole.

Keywords: Thiazole Synthesis, Anti-fungal activity, ^{1}H-NMR, ^{13}C-NMR, FTIR, Micro wave irradiation.

INTRODUCTION

The discovery and improvement of safe and effective drug has brought a progressive era in human health care system[1]. Thiazole is an important member of class of heterocyclic compound that contains a nitrogen and sulphur group and has a molecular formula C_3H_3NS[2]. It contains an electron donating group (-S-) and an electron withdrawing group (C=N)[3]. The thiazole ring is present in many synthetic and natural products such as vitamin B_1 (thiamine) helps in functioning of nervous system by playing a pivotal role in synthesis of acetylcholine[4]. The unique nature of thiazole is demonstrated by the fact that it is an important part of penicillin nucleus[5]. Substituted thiazole derivatives plays an important role in nature and have biologically diverse effect such as anti-microbial[6], anti-inflammatory[7], analgesic[8], anti-oxidant[9] and diuretic activities[10], anti-tubercular[11] and anti-cancer[12] agents. Thiazoles posses enhanced lipid solubility with hydrophilicity[13]. These are easily metabolized by regular biochemical reactions and are non-carcinogenic in nature[14]. The thiazole skeleton in variety of biologically active compounds, among those is several marketed drugs such as Meloxicam, Cefotaxime, Pramipexole, Bleomycin etc. Encouraged by these diverse biological activities of thiazole derivatives, we decided to prepare a new series of thiazole derivatives. Literature survey revealed that incorporation of different groups in thiazole ring enhanced anti-microbial activity.

MATERIALS AND METHOD

The chemicals used in the present research work were purchased from Loba, Merck and CDH manufacturer. The melting point of the synthesized compounds was determined in open capillary tube using Rexford digital melting point apparatus. TLC was performed on silica gel plates using methanol: chloroform (1:9) solvent system. The infrared spectra of the synthesized compounds were recorded using Perkin Elmer Version 10.03.05 with nujol oil and KBr (cm^{-1}) spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR data was recorded on Bruker Avance 500MHz using tetramethyl silane (TMS) as an internal standard. Microanalyses were obtained with an elemental analyses systemGmBh varioEL V300 ELEMENTAL analyzer. ^{1}H-NMR spectra were recorded with DMSO-d_6 as a solvent and the chemical shift data were expressed as δ values relative to TMS. The purity of these compounds were checked by pre coated $SiO_{2\ gel}$ plates using methanol and chloroform as mobile phase visualized in iodine chamber.

Experimental Procedure

Step-1: Synthesis of 2-amino-4-methyl thiazole-5-carboxylic acid ethyl ester

Equimolar quantities of Thio urea (0.1mol) and ethyl acetoacetate (0.1mol) were dissolved in presence of N-bromosuccinimide using a pinch of Benzoyl peroxide as catalyst. Few ml of benzene were also added. Also, a porcelain chip was added to avoid bumping. The entire reaction mixture was put inside a 300W microwave oven for 15 min. at 80°C. After the reaction was over, it was kept for cooling on a ice bath and then washed with water and recrystallized.

Step-2: Synthesis of substituted derivatives of 2-amino-4-methyl-thiazole-5-carboxylic acid ethyl ester (A_1-A_4)

Equimolar quantities of 2-amino-4-methyl-thiazole-5-carboxylic acid ethyl ester (0.02mol), ethanol and

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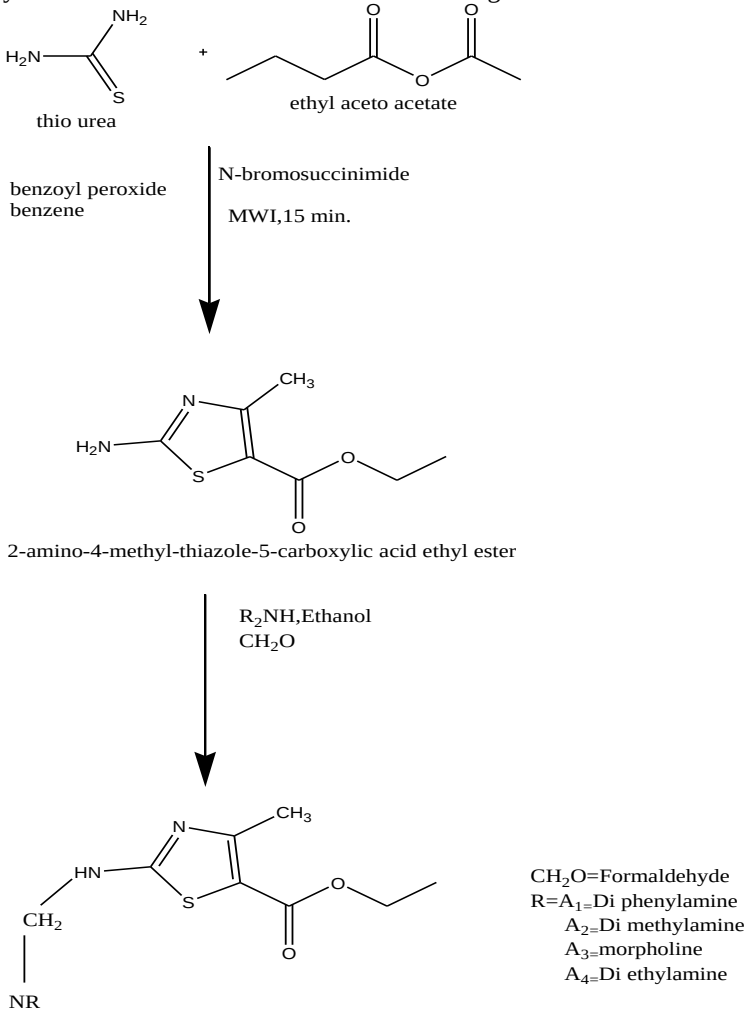
Scheme I: The scheme of synthesis of thiazole substituted derivatives is given below in scheme.



Substituted derivatives of 2- amino 4- methyl thiazole 5- carboxylic acid ethyl ester

Table 1: Physical data of substituted thiazole derivatives.

S.No	Comp. Code	Chemical Name	Mol. Formula	Mol. Weight (gm/mol)	M.P (°C)	% Yield	R_f Value
1.	A	2-amino-4-methyl-thiazole-5-carboxylic acid ethyl ester	$C_7H_{10}N_2O_2S$	186.23	158-159	62.72	0.22
2.	A_1	2-(diphenylamino)-4-methyl-thiazole-5-carboxylic acid ethyl ester	$C_{20}H_{21}N_3O_2S$	367.38	139-140	88.93	0.39
3.	A_2	2-(Dimethyl amino methyl-amino)-4-methyl thiazole -5-carboxylic acid ethyl ester	$C_{10}H_{17}N_3O_2S$	243.11	89	81.36	0.45
4.	A_3	4-methyl-2-(Morpholine-4yl methyl)-amino]-thiazole-carboxylic acid ethyl ester	$C_{12}H_{21}N_3O_3S$	287.13	100-101	50.43	0.32
5.	A_4	2-(di ethylamino methyl-amino)-4methyl-thiazole-5-carboxylic acid ethyl ester	$C_{12}H_{21}N_3O_2S$	271.23	76-77	75.69	0.20

formaldehyde were taken in a 250ml beaker. Then, secondary amine (0.02mol) was added in portion to the above solution under stirring at room temperature. After the addition was over, the entire solution was kept for stirring at room temperature for 3 hours. After the stirring was over, the entire reaction was kept inside a refrigerator 48 hours. The product was filtered and recrystallized. The physical data of newly synthesized thiazole substituted

Table 2: Characterization data of substituted thiazole derivatives.

Comp. code	IR (KBr/Nujol) cm^{-1}	^{1}H-NMR (DMSO-d_6) δ ppm	^{13}C-NMR (DMSO-d_6) δ ppm	Elemental Analysis (%)
A	(C-N)aromatic-1185,1025 (N-H)-str.3364, Bending. 1615 (C=O)-1704,1766 (C-S)-716,734	1.136-1.673(3H, triplet, CH_3), 2.669-2.846(3H, singlet, CH2), 3.309-3.323(2H, quartet, NH_2).	C-169.04(2 thiazole 1N), C-160.90(1-carboxyl), C-148.43(2-thiazole-1N), C-108.17(2-thiazole), C-61.84, C-30.02, C-14.5(aliphatic C)	Carbon-45.15 Hydrogen-5.41 Nitrogen-15.04 Oxygen- 17.18 Sulphir-17.22
A_1	(C-N)aromatic-1185,1025 (C=O)-1704,1766 (-CH_2)-Str.2904, bending.1455,1460,1376. (C-S)-721 (N-H)-bending 1615.	1.190-1.218(3H, triplet, CH_3), 2.44-2.89(3H, singlet, CH_3), 4.200-4.710(2H, quartet, CH_2), 5.048(2H, doublet, CH_2), 6.984-7.238(10H, multiplet, Benzene)	C-179.85(2-thiazole), C-168.6008(1-C=O), C-144.159,C-142.02[1-benzene(1-N)],C-117.45(1-benzene), C-119.62, C-119.84, C- 116.199,C-114.73, C- 128.953, CH-129.41(1-benzene),C-108(2 thiazole),CH_2-61.69, CH_3-56.54	Carbon-67.96 Hydrogen-5.99 Nitrogen-7.93 Oxygen-9.05 Sulphur-9.07
A_2	(C-N)aromatic-1273,1168,1153 (-CH_2) Aromatic-Str.2903,bending-1455,1376 (C=0)- 1703,1772 (N-H)-str,3166, bending 1608 (C-S)-722	2.8(3H, singlet, CH_3), 2.38-2.53(3H, triplet, CH_3), 1.03-1.87(3H, triplet, CH_3), 3.92-4.11(1H, triplet, C-NH), 4.28-4.63(2H, quartet, CH_2), 4.13-4.15(2H, doublet, CH_2).	C-172.14(2-thiazole),C-167.62(1-carboxyl),C-159.04(2-thiazole),C-109.37(3-thiazole),C-59.56(CH_2-aliphatic),C-14.64(CH_3 aliphatic),C-39.28,C-39.45CH_3	Carbon-49.36 Hydrogen-7.04 Nitrogen-17.27 Oxygen-13.15 Sulphur-13.18
A_3	(C-N)Aromatic-1304,1154 (C=O)- 1700, (N-H)-str.3169, Bending. 1609 (-CH_2)Aromatic-Str.2904.00, Bending-1456 (C-S)-722	2.8(3H, singlet, CH_3), 2.38-2.53(3H, triplet, CH_3), 1.03-1.87(3H, triplet, CH_3), 3.92-4.11(1H, triplet, C-NH), 4.28-4.63(2H, quartet, CH_2), 4.13-4.15(2H, doublet, CH_2).	C-172.14(2-thiazole),C-167.62(1-carboxyl), C-159.04(2-thiazole),C-109.37(3-thiazole), C-59.56(CH_2-aliphatic), C-14.64(CH_3 aliphatic),C-39.28,C-39.45(aliphatic).	Carbon-50.15 Hydrogen-7.37 Nitrogen-14.62 Oxygen-16.70 Sulphur-11.67
A_4	(C-N) Aromatic-1304,1154,1079 (-CH_2)Aromatic-Str.2905.00, bending. 1459,1376 (N-H)-Str.3168, bending.1611 (C-S)-721	4.49 (2H, quartet, CH_2), 4.32(2H, doublet, CH_2), 4.06(1H, triplet, C-NH), 2.60(3H, singlet, CH_3), 0.9(3H, triplet, CH_3), 1.11(3H, triplet, CH_3), 2.33(2H, quartet, CH_3).	C-171.80 (2-thiazole), C-168.82(1-carboxyl), C-108.76(3-thiazole),C-60.85(CH_2 aliphatic), C-41.98(CH_2-aliphatic), C-13.4,C-14.4,C-12.5(aliphatic CH_3)	Carbon-53.11 Hydrogen-7.80 Nitrogen-15.48 Oxygen-11.79 Sulphur-11.82

derivatives is shown in table 1 and the characterization data is shown in table 2.

RESULT AND DISCUSSION

The reaction employed for synthesis of titled compounds is shown in scheme 1. In this present work, the starting 2-amino-4-methyl-thiazole-5-carboxylic acid ethyl ester was prepared from thio urea and ethyl acetoacetate using N-bromosuccinimide and benzoyl peroxide. Further, compound (A) reacted with secondary amine, formaldehyde and ethanol to give newly synthesized compounds (A_1, A_2, A_3 and A_4). The progress of the reactions were monitored by precoated SiO_2 TLC plates using methanol and chloroform as mobile phase. The spots resolved were made visualized by using iodine chamber.

The stretching bands at (1780-1650) confirms the presence of C=O group with the assigned structure. The N-H stretching bands at (3500-3100) confirm the presence of N-H group. The CH_2 aliphatic stretch was observed bands at (2990-2850) cm^{-1}.

The presence of various bands around δ (1.190-1.218) showed the presence of CH_3 group and the presence of bands around δ (4.200-4.710) showed the presence of CH_2 group. The presence of bands at δ (3.92-4.11) confirms the presence of C-NH group with the assigned structure.

Table 3: Anti-Fungal activity of substituted thiazole derivatives.

Compound Name	Inhibition Zone Diameter(mm) Fungal Strains					
	Candida albicans		*Trichophyton*		*Aspergillus Niger*	
	100µg/ml	200µg/ml	100µg/ml	200µg/ml	100µg/ml	200µg/ml
A_1	-	-	-	-	-	-
A_2	-	+	+	+	-	-
A_3	++	++	-	-	-	-
A_4	+	+		-	-	-
Standard(Cl-otrimazole)	++	++	++	++	++	++
Control (DMSO)	-	-	-	-	-	-

(+) indicates absence of visible growth of fungi
(-) indicates presence of visible growth of fungi

The presence of various bands at C-172.14 confirms the presence of 2-thizole, C-167.62(1-carboxyl) confirms the presence of 1-carboxyl, C-59.56 confirms the presence of aliphatic CH_2 group and the bands C-14.64 confirms the presence of CH_3 aliphatic.

Anti-fungal Activity

The newly synthesized compounds (A_1, A2, A3 and A_4) were screened for anti-fungal activity against fungi *Candida albicans*, *Aspergillus Niger* and *Trichophyton* and were compared with standard anti-fungal drug clotrimazole. Stock solutions of 1000µg/ml of both (standard drug and newly synthesized drugs) were prepared in DMSO. The anti-fungal assay was carried out using commercial Potato dextrose agar liquid medium. Potato dextrose agar was dissolved in 100ml of distilled water while boiling. The pH was adjusted to 5.6±2. After adjusting the pH, the solution was autoclaved for 15min. at 121°C.

Evaluation of Anti-fungal Activity

The anti-fungal activity of newly synthesized derivatives was performed by disc diffusion method[15]. In this test, Whatmann filter paper sterile discs containing test solutions are placed on an agar plate where fungi have been placed, and the plate is left to be incubated. The Minimal Inhibitory Concentration (MIC) is the lowest concentration of an anti-fungal agent that prevents visible growth of fungal in agar diffusion test [16].

Procedure

PDA was poured in each sterile petri dish and allowed to solidify. A volume of 100µl of each culture was used as inoculums and distributed on Petri dish homogenously. Empty sterilized disc were impregnated with different concentration (100µg/ml and 200µg/ml) of all the compounds. Disc was placed on agar plates. The incubation was carried out in a fungal incubator at 25°C-28° C for 24 hours to 72 hours. The fungal zones of inhibition were measured in mm. The anti-fungal activity data is shown in table 3.

CONCLUSION

The in vitro anti fungal activity was carried out by disc diffusion method. Synthesized compound A_3 [4-methyl-2-(Morpholine-4yl methyl)-amino]-thiazole –carboxylic acid ethyl ester] showed significant zone of inhibition on fungi at 100µg/ml and 200µg/ml on *Candida albicans* as compared to standard drug clotrimazole. Synthesized Compound A_4 **[**2-(Diethyl amino methyl-amino)-thiazole -5-carboxylic acid ethyl ester**]** at 100µg/ml 200µg/ml showed moderate activity on fungi *Candida albicans* and *Trichophyton* and A_2 [2-(di methyl amino methyl-amino)-4methyl-thiazole-5-carboxylic acid ethyl ester] showed no significant activity at 100µg/ml and 200µg/ml on fungi *Candida albicans* and *Trichophyton* while Synthesized compound A_1 [2-(diphenylamino)-4-methyl-thiazole-5-carboxylic acid ethyl ester] didn't show any anti-fungal activity at any concentration.

The structure and biological activity relationship of titled compounds indicated that may be due to presence of basic nature of Morpholine in A_3[4-methyl-2- (Morpholine-4yl methyl)-amino]-thiazole –carboxylic acid ethyl ester] attached to thiazole ring were responsible for good anti-fungal activity and hence compound A_3 shows significant anti-fungal activity.

So, further research is required for their specific mode of their anti-fungal activity.

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