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Synthesis and Evaluation of some Thiazole derivatives as an Antifungal agent

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Abstract

The frequency of invasive and systemic fungal infections has increased dramatically in the population with altered immunity. Patients who become severely immune-compromised because of underlying diseases such as leukemia or recently acquired immunodeficiency syndrome or patients who undergo cancer chemotherapy or organ transplantation are particularly susceptible to opportunistic fungal infection. *Candida albicans* (*C. albicans*), and *Aspergillus fumigatus (A. fumigatus*) are the main causative fungi of the systemic mycosis. Unfortunately, none of the available antifungal are ideal in terms of efficacy, antifungal spectrum or safety. Although amphotericin B is efficacious against both Candidiasis and Aspergillosis, it shows severe renal toxicity. Azoles show drug–drug interactions with various drugs by inhibiting metabolic enzymes, the *CYP450* isoenzymes. To overcome the drawbacks of the current antifungal drugs and to obtain more efficacious drugs, there is a real perceived need for development of new antifungal agents having a biological therapeutic effect. *N*- myristoyltransferase attaches myristase to the proteins involved in a variety of signal transductions cascades and other critical cellular functions. The enzyme is essential for viability of *C.albicans*, which is a major cause of systemic fungal infection in immune compromised patient. Therefore it is worthwhile to develop new inhibitors against *NMT*.

Key-Words: Amphotericin B, Antifungal, Azole

Introduction

Invasive fungal infections now rank alongside bacterial infections as a major cause of morbidity and mortality in seriously debilitated and immunocompromised patients. [11, 12] Almost every fungus has the potential to cause invasive infections, but the most important pathogens in terms of incidence and mortality are *Candida spp*. and *Aspergillus spp.*, respectively. *Candida spp*. are the fourth most common cause of nosocomial (originating in hospitals) blood-stream infections in many hospitals and represent 8 to 11% of all blood-stream infections.[13, 14] The risk factors that are known to predispose patients to invasive candidiasis are intravascular cannulae, indwelling catheters, surgical procedures, prolonged use of broad-spectrum antibacterial antibiotics and immunocompromisation (e.g. AIDS, tissue transplant and cancer patients). Amphotericin B Deoxycholate (AmBD) has been the 'gold standard' in antifungal chemotherapy, despite its frequent toxicities. However, improved treatment options for invasive fungal infections (IFIs) have been developed during the last 15 years.

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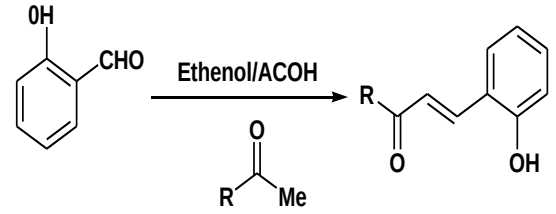
Newer antifungal agents, including less toxic lipid preparations of AmBD, triazole and the echinocandins, have been added to our armamentarium against IFIs. *N*-Myristoylation is catalyzed by myristoyl-CoA:protein*N*-myristoyltransferase (Nmt),[1] a member of the GNAT superfamily of proteins. Nineteen Nmts have been identified from 15 species. N-Myristoyltransferase represents an attractive therapeutic target given its requirement for the survival of several human pathogens. Different classes of Nmt inhibitors have been identified including analogs of myristate and myristoyl-CoA , myristoylpeptide derivatives, and histidine analogs. All studied Nmts exhibit a preference for myristoyl-CoA. However, they have divergent peptide substrate specificities. Therefore, peptide derivatives offer an opportunity to generate species-selective inhibitors. A large library of peptidomimetics has been developed through depeptidization of an octapeptide representing the N-terminal sequence of a known yeast Nmt substrate, Arf2.[28]The aim of pressent work is to synthesize and characterize heterocyclic compounds and to evaluate their anti fungal activity.

Material and Methods

Synthetic Scheme

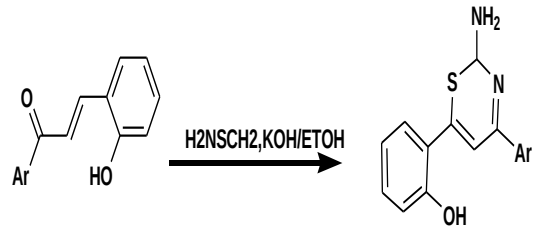
General synthetic scheme of Heterocyclic compound

Step-I: Synthesis of R1



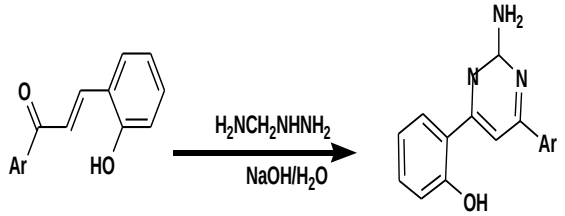
R1

Step-II : Synthesis of R2



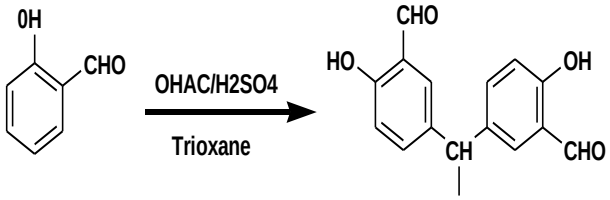
R2

Step-III: Synthesis of R3



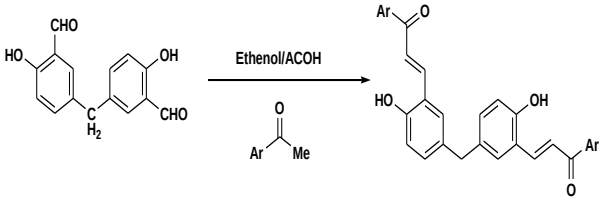
R3

Step-IV: Synthesis of R4



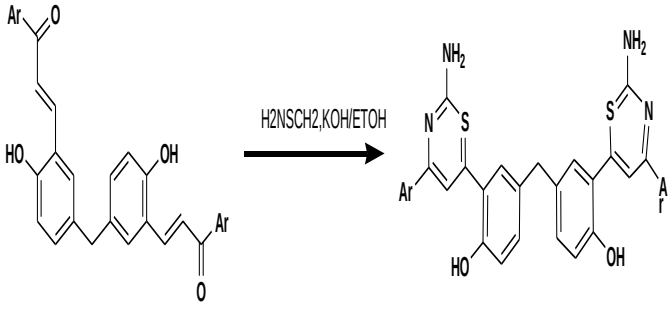
R4

Step-V: Synthesis of R5



R5

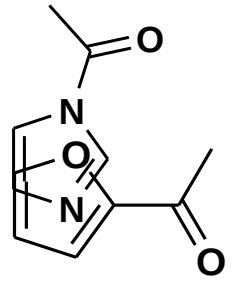
Step-VII: Synthesis of R6



Where **Ar**=

1.

2.



General method of synthesis

Synthesis of Substituted R1 (RG1 & RG2)

A solution of Salicyaldehyde(1 mmol) and substituted compound (1 mmol) in 5-7 mL of ethanol was treated with 3 mL of 60% KOH solution at 5-10 ºC. The reaction mixture was stirred at room temperature for 4 h. It was then diluted with water (10mL) and extracted with diethyl ether (3× 10 mL). The aqueous solution was acidified with dilute HCl. The solid obtained was filtered washed thoroughly with water and dried. The crude product was purified by crystallization from benzene: MeOH (3:2) gave R1 derivative

Synthesis of Substituted R2(RG1A & RG2A)

The compound R1(RG1 or RG2) (1mmol) and thiourea(1 mmol) in 5 mL ethanol was added 0.5 mL of alkali KOH (0.02 mol). The reaction mixture was refluxed. TLC (EtOAc: Pet-ether, 2:1) showed that the reaction was completed in 5 h.The reaction mixture was poured in 10 mL of 10% HCl solution (cold) and the precipitate was filtered. Filtered, washed with water until free from acid and recrystallized from benzene-ethanol gave compound R2

Synthesis of Substituted R3(RG3)

A solution of R1(RG1 or RG2) (0.001 mol) and guanidine hydrochloride (0.001mmol) in 5mL ethanol was added 0.5 mL of aqueous NaOH (0.02 mol). The reaction mixture was refluxed. TLC (EtOAc:Pet-ether, 2:1) showed that the reaction was complete after 6h. The reaction mixture was poured in 5 mL of 10% HCl

solution (cold) and the precipitate was filtered, washed with water until free from acid and recrystallized from benzene: ethenol gave compound R3

Synthesis of Substituted R4(RG4)

Salicyaldehyde (2mmol) and trioxane (3 mmol) in 6mL of glacial acetic acid was heated to a temperature of 90-95 ºC under nitrogen atmosphere. 1mL of mixture if concentrated sulfuric acid and glacial acetic acid(1:45) was added dropwise.The temperature was maintained for 22hr while stirring was continued during the entire period. Subsequently, the reaction mixture was poured into 200mL of ice-water and allowed to stand overnight. The precipated solid was filtered and extracted twice with n-hexane(2×5mL). The remaining solid was pulverised three times with diethylether (3×5mL) and the ether solution, with the tarry material, were decanted. Recrystalliza-tion from 7.5mL acetone gave the pure dialdehyde R4.

Synthesis of Substituted R5(RG5)

A solution of R4(1 mmol) and substituted compound (3 mmol) in 5-7 mL of ethanol was treated with 3 mL of 60% KOH solution at 5-10 ºC. The reaction mixture was stirred at room temperature for 4 h. It was then diluted with water (10mL) and extracted with diethyl ether (3× 10 mL). The aqueous solution was acidified with dilute HCl. The solid obtained was filtered washed thoroughly with water and dried. The crude product was purified by crystallization from benzene: MeOH (3:2) gave R5 derivative

Synthesis of Substituted R6(RG6)

A solution of R5 (0.01 mol) and thiourea (0.03 mol) in 10 mL ethanol was added 5 mL alcoholic KOH (0.02 mol). The reaction mixture was refluxed. TLC (EtOAc: Pet-ether, 2:1) showed that the reaction was completed in 5 h.The reaction mixture was poured in 10 mL of 10% HCl solution (cold) and the precipitate was filtered Filtered, washed with water until free from acid and recrystallized from benzene-ethanol gave compound R6.

Results and Discussion

The analytical data of synthesized compounds **RG1** is M.P. is 145-148ºC, Yield is 68.26%, Molecular formula is $C_{12}H_{10}N_2O_2$, Molecular weight is 214.22 and λ_{max} in methanol is 256.8. The synthesized compounds **RG1** is soluble in DMSO, ethyl acetate, methanol, acetone and R_f value is 0.71 in Pet. ether: Ethyl Acetate (2:3) solvent system. IR spectral data synthesized compounds RG1 of is 1706.1 for C=O, 1210 for C-O, 1221.8 for C-N,1610 for C=C(Ar),1312.6 for C=N, 3295.8 for OH. The MASS spectrum of compound RG1 showed molecular ion peak at *m/z* 215.5[M+1] in conformity with the molecular formula $C_{12}H_{10}N_2O_2$.

The analytical data of synthesized compounds **RG2** is M.P. is 182-185ºC, Yield is 56.3%, Molecular formula is $C_{13}H_{10}O_3$, Molecular weight is 214.22 and λ_{max} in methanol is 261.8. The synthesized compounds **RG2** is soluble in DMSO, ethyl acetate, methanol, acetone and R_f value is 0.63 in Pet. Ether: Ethyl Acetate (2:3) solvent system. IR spectral data synthesized compounds of **RG2** is 1679.3 for C=O, 1022 for C-O, 3261 for OH 1561.2 for C=C(Ar), 1251.1 for C=N. The MASS spectrum of compound **RG2** showed molecular ion peak at *m/z* 215.5[M+1] in conformity with the molecular formula $C_{13}H_{10}O_3$

.The analytical data of synthesized compounds **RG1A** is M.P. is 201-230ºC, Yield is 48.9%, Molecular formula is $C_{13}H_{12}N_4OS$, Molecular weight is 272.33 and λ_{max} in methanol is 279.0. The synthesized compounds **RG1A** is soluble in DMSO, ethyl acetate, methanol, acetone and R_f value is 0.28 in Pet.ether: Ethyl Acetate (2:3) solvent system. IR spectral data synthesized compounds cm^{-1} **RG1A** is 1164 for C-O, 1268.3 for C-N, 1591.5 &1456 for C=C(Ar.), 1201.8 for C=N, 3339.5 for OH, 3666.2 for NH_2. The MASS spectrum of compound **RG1A** showed molecular ion peak at *m/z* 273.2[M+1] in conformity with the molecular formula $C_{13}H_{12}N_4OS$.

The analytical data of synthesized compounds **RG2A** is M.P. is 113-116ºC, Yield is 49.9%, Molecular formula is $C_{14}H_{12}N_2O_2S$, Molecular weight is 272.32 and λ_{max} in methanol is 250.4. The synthesized compounds **RG2A** is soluble in DMSO, ethyl acetate, methanol, acetone and R_f value is 0.38 in Pet. ether: Ethyl Acetate (1.5:3.5) solvent system. IR spectral data synthesized compounds cm^{-1} **RG2A is** 1032.3 for C-O, 1231.5 for C-O, 1644.9&1455.9 for C=C(Ar.),1231.5 for C=N, 2949.8 for C-H(str), 3364.8 for NH_2 . The MASS spectrum of compound **RG2A** showed molecular ion peak at *m/z* 273.2[M+1] in conformity with the molecular formula $C_{14}H_{12}N_2O_2S$.

The analytical data of synthesized compounds **RG3** is M.P. is 150-153ºC, Yield is 67.8%, Molecular formula is $C_{13}H_{11}N_5O$, Molecular weight is 253.26 and λ_{max} in methanol is 281.3. The synthesized compounds **RG3** is soluble in DMSO, ethyl acetate, methanol, acetone and R_f value is 0.51 in Pet. Ether: Ethyl Acetate (2:3) solvent system. IR spectral data synthesized compounds **RG3** is 1032.3 for C-O, 1231.5 for C-N, 1644.9 & 1455.9 for C=C(Ar), 1231.5 for C=N, 2949.8 for C-H(stre), 3364.8 for NH_2. The MASS spectrum of compound **RG3** showed molecular ion peak at *m/z* 254.1[M+1] in conformity with the molecular formula $C_{13}H_{11}N_5O$.

The analytical data of synthesized compounds **RG5** is M.P. is 159-161°C, Yield is 58.3%, Molecular formula is $C_{25}H_{20}N_4O_4$, Molecular weight is 440.15 and λ_{max} in methanol is 278.4. The synthesized compounds **RG5** is soluble in DMSO, ethyl acetate, methanol, acetone and R_f value is 0.66 in Pet.ether: Ethyl Acetate (2:3) solvent system. IR spectral data synthesized compounds of **RG5** is 1094.8 for C-O, 1231.5 for C-N, 1634.6&1448.8 for C=C(Ar), 1363.9 for C=N, 1711.5 for C=O, 3333.1 for OH. The MASS spectrum of compound **RG5** showed molecular ion peak at *m/z* 441.2[M+1] in conformity with the molecular formula $C_{25}H_{20}N_4O_4$.

The analytical data of synthesized compounds **RG6** is M.P. is 172-173°C, Yield is 40.8%, Molecular formula is $C_{25}H_{20}N_8O_2S_2$, Molecular weight is 556.66 and λ_{max} in methanol is 291.2. The synthesized compounds **RG6** is soluble in DMSO, ethyl acetate, methanol, acetone and R_f value is 0.41 in Pet.ether: Ethyl Acetate (2:3) solvent system. IR spectral data synthesized compounds of RG6 is 1091.2 for C-O, 1224.3 for C-N, 1601.1 for C=C(Ar.) , 1191.3 for C=N, 3272.6 for OH, 3303.7 for NH_2.

Characteristics value of synthesized compounds as shown in table

Table 1: R_f Value, Melting Point, λ_{max} of Synthesized Compounds and % Yield

S/No.	Comp. Code	R_f Value	M.P °C	λ_{max}	% Yeild
1.	RG1	0.71	145-148	256.8	68.26
2.	RG2	0.63	182-185	261.8	56.3
3.	RG1A	0.28	201-203	279.0	48.9
4.	RG2A	0.38	113-116	250.4	49.9
5.	RG3	0.51	150-153	281.3	67.8
6.	RG5	0.66	159-161	278.4	58.3
7.	RG6	0.41	172-173	291.2	40.8

Biological evaluation

Tube dilution/turbidity method

This method is used to determine the susceptibility of an organism to antibiotics/antifungal as well as Minimal Inhibitory Concentration (MIC) of that antibiotic/antifungal. The MIC is the lowest concentration of an antimicrobial agent that inhibits the growth of the test microorganisms. A clinician to establish effective antimicrobial regimen for the treatment of bacterial/fungal infections may use quantitative data of this nature. This method is also used to compare the activity of an unknown crude antibiotic with the known antibiotic/antifungal. Lists of Fungal Strain as shown in table no. 2

Table 2: Lists of Fungal Strain

S/No.	Fungal strain
1.	*Candida albicans (3471)*
2.	*Aspergillus Niger* (545)

Table 3: MIC values of Synthesized Compounds against fungus

S/No.	Compound code	MIC value in µg/mL	
		A. niger	*C.albicans*
1.	R1(RG1)	64	256
2.	R1(RG2)	32	>256
3.	R2(RG1A)	128	32
4.	R2(RG2A)	8	8
5.	R3(RG3)	2	32
6.	R5(RG5)	64	128
7.	R6(RG6)	8	16
8.	Fluconazole	1	2

The synthesized compounds RG1, RG2, RG5, RG1A are equipotent as Fluconazole (standard antifungal drug) for *A. niger* with MIC values of 64, 32, 64, 128 µg/mL. The synthesized compounds RG2A , RG3 and RG6 shown the better activity against *A. niger* as compared to RG1, RG2, RG5, RG1A with MIC values of 8, 2, 8 µg/mL.

The synthesized compounds RG1A, RG2A, RG3, RG5, RG6 are equipotent as Fluconazole (standard antifungal drug) for *C. albicans* with MIC values of 32, 8, 32, 128, 16 µg/mL.

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