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Research Article

SYNTHESIS & EVALUATION OF 2-CHLOROMETHYL-1H-BENZIMIDAZOLE DERIVATIVES AS ANTIFUNGAL AGENTS

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ABSTRACT

Objective: The objective of present research work is to synthesize & screen novel 2-chloromethyl-1H-benzimidazole derivatives for antifungal activity.

Methods: 2-chloromethyl-1H-benzimidazole derivatives were prepared by condensing 2-chloromethyl-1H-benzimidazole with different aromatic amines and heterocycle. The synthesized compounds were screened for their antifungal activity against *Candida albicans* by well plate method.

Results: Some of the syntheized compounds showed significant antifungal activity. VMKP 8 exhibited potent activity (MIC=12.5 µg/ml). The synthesized derivatives were characterized by means of TLC, IR, ^{1}HNMR spectral analysis for their structural confirmation.

Conclusion: The derivatives which showed better antifungal activity may serve as leads for further optimization.

Keywords: Antifungal agents, Benzimidazole, 2-chloromethyl-1H-Benzimidazole

INTRODUCTION

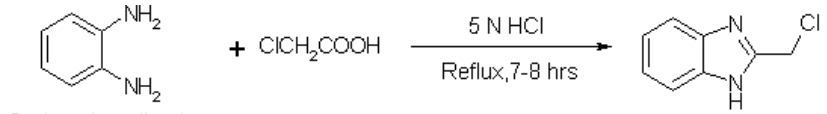
Invasive fungal infections present major diagnostic and therapeutic challenges for the clinician. Although many viral infections can now be prevented, superficial and systemic fungal infections remain important causes of morbidity and mortality in transplant recipients, even in view of the aggressive use of topical antifungal agents. In addition, antifungal drugs used in fungal infections have also become less effective owing to the development of fungal resistance.[1]In recent years, considerable attention has been given to the synthesis of benzimidazole/bis-benzimidazole derivatives because of their various pharmacological activities such as antitumour[2,3], anti-ulcer[4], antiinflammatory[5], antiviral[6,7], anthelmintic[8,9], antibacterial[10,11] and antifungal[12,13] properties. The objective of present study was to synthesize novel 2-chloromethyl-1 H-benzimidazole derivatives by using different aromatic amines and heterocycle and to evaluate them for antifungal activity.

MATERIALS AND METHODS

Chemistry

All the chemicals used were procured from Merck and purified prior to use. Melting points were determined on open capillary tube on Campbel Melting-point Apparatus and are uncorrected. The silica gel used for thin layer chromatography (TLC) was 'Silica Gel G' procured from Merck and was coated on laboratory glass slides. IR spectra were recorded using KBr disk on "JASCO 530V" and "SHIMADZU 3100", ^{1}H-NMR spectra were recorded in $CDCl_3$/DMSO solution on "FTNMR Varian-*Mercury 300*" using tetramethylsilane (TMS) as internal standard.

Synthesis of 2-Chloromethyl-1H-benzimidazole



O-phenylenediamine Chloroacetic acid 2-Chlormethyl-1Hbenzimidazole

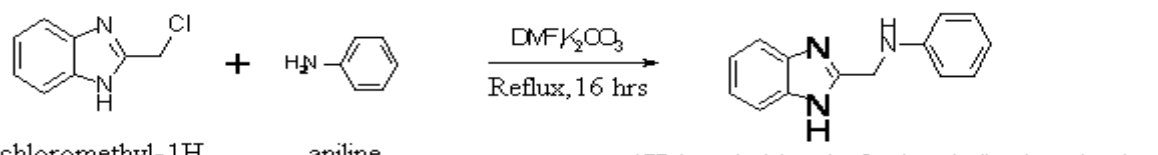
Procedure

In a 250 ml three necked flask a solution containing 7.5g (0.08 mole) of chloroacetic acid and 7.5g (0.07 moles) of 0-phenylenediamine dissolved in 60 ml of 5N HCl. The mixture was heated for 7.5 to 8 hrs with constant stirring. The reaction mixture is cooled to about 5^{0}C. It was neutralized with aq. ammonium hydroxide or dilute NaOH. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was re-crystallized from benzene: hexane[14,15].

Spectral Data:- Yield:60%; melting point:153-155^{0}C;TLC:Chloroform:Methanol(9:1);R_f: 0.68;IRv_{max}(KBr):3369(-NH)),3063(aromatic-CH),2939(alliphatic–CH),1345(-CN),780(C CL),1HNMR:δ-9.18(1H,s,-NH),7.16(4H,m.aromatic linkage),4.98(2H,s,CH_2linkage),2.6(1H,s,DMSO peak).

Synthesis of 2-Chloromethyl 1H-benzimidazole derivatives

VKMP-(1) Synthesis of (1H-Benzimidazole-2-ylmethyl)-phenyl amine



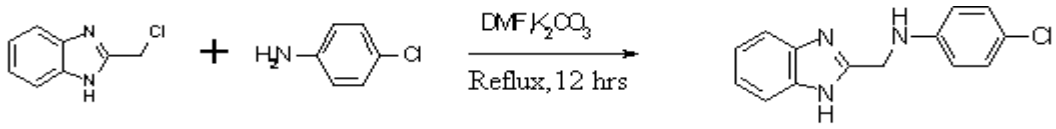
'2-chloromethyl-1H -benzimidazole aniline 1H-benzimidazole-2-ylmethyl)-phenylamine

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (0.01 mol,1.665gm) and K_2CO_3 (2.76gm,0.02mol) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added. After that aniline (0.095g, 0.01 mol) was added to reaction mixture which was refluxed for 16 hrs.until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate and concentrated to obtain crude product. The residue was recrystallized from diethyl ether to give pure compound.

Spectral Data:- Yield: 70%,melting point: 146-148^{0}C,TLC: Benzene: Ethyl acetate (4:1),R_f: 0.43,IRv_{max}(KBr):3352(-NH),3086(aromatic–CH),2905(aliphatic–CH),1310(-CN),^{1}HNMR:δ9.22(1H,s,,NHbenzimidazole),6.527.58(9H,m,aromatic protons),4.62(2H,s,CH_2linkage),3.1(1H,s,-NHof amine),1.4(1H,S,$CDCl_3$ peak).

VKMP-(2) Synthesis of (1H-Benzimidazole-2-ylmethyl)-(4-chloro-phenyl)-amine



2-chloromethyl-1H -benzimidazole

p-chloroaniline

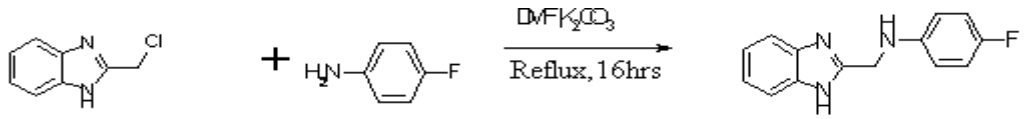
1H-benzimidazole-2-ylmethyl)-(4-chloro-phenyl)-amine

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (1.665, 0.01 mol) and K_2CO_3 (0.02mol, 2.76gm) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and *p*-chloroaniline (1.27g, 0.01 mol) was added. The reaction was refluxed for 12 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate and concentrated to obtain crude product. The residue was recrystallized from diethyl ether to give pure compound.

Spectral Data:- Yield: 58%,melting point.: 137-139^{0}C.TLC: Benzene: Ethyl acetate (2:4), R_f: 0.73,IRv_{max}KBr-3375(-NH),3082(aromatic–CH),1504(-CN),3011(aliphatic–CH),748(C-CL),^{1}HNMR-δ-9.19 (1H,s,-NH),6.46-7.58 (8H,m,aromatic protons),3.41 (1H,d,-NH of amine),4.42(2H,s,protons of CH_2 linkage),2.6(3H,s,DMSO peak).

VKMP-(3) Synthesis of (1H-Benzimidazole-2-ylmethyl)-(4-fluoro-phenyl)-amine



2-chloro-1H-benzimidazole

p-fluroaniline

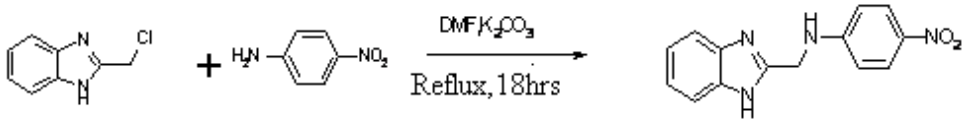
(1H-benzimidazole-2-ylmethyl)-4-fluro-phenyl)-amine

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (3.33g, 0.02 mol) and K_2CO_3 (0.02mol, 2.764g) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and *p*-fluoro aniline (2.22g, 0.02 mol) was added. The reaction was refluxed for 16 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate, and concentrated to obtain crude product. The residue was recrystallized from diethyl ether to give pure compound.

Spectral Data:- Yield: 53%,M.p.: 114-116^{0}C.TLC: Benzene: Ethyl acetate (2:4),R_f:0.70,IRv_{max}(KBr)-3269(-NH),3084(aromatic-CHstretching),2879(-C-N),1518(aliphatic –CH),1302(C-F).

VKMP-(4) Synthesis of (1H-Benzimidazole-2-ylmethyl)-(4-nitro-phenyl)-amine



2-chloromethyl-1H-benzimidazole

p-nitroaniline

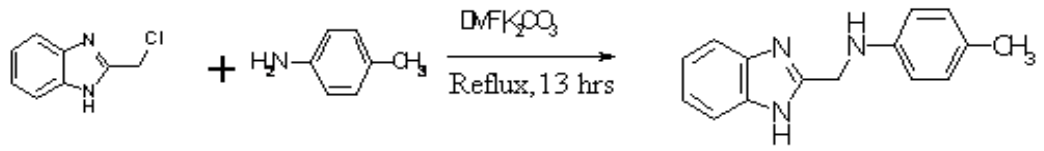
(1H-benzimidazole-2ylmethyl)-(4-nitro-phenyl) -amine

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (3.33g,, 0.02mol) and K_2CO_3 (0.02mol, 2.764) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and *p*-nitro aniline (2.76g, 0.02 mol) was added. The reaction was refluxed for 18 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate, and concentrated to obtain crude product.

Spectral Data:- Yield: 47%,M.p.: 118-120^{0}C.TLC: Benzene: Ethyl acetate (3:1.5), R_f:0.68, IRv_{max}(KBr)-3063(aromatic–CH), 2920 (aliphatic–CH) ,3395(-NH),1520(aromatic C-NO),1323(-C-N),1410(-NO_2).

VKMP-5) Synthesis of (1H-Benzimidazole-2-ylmethyl)-*p*-tolyl amine:

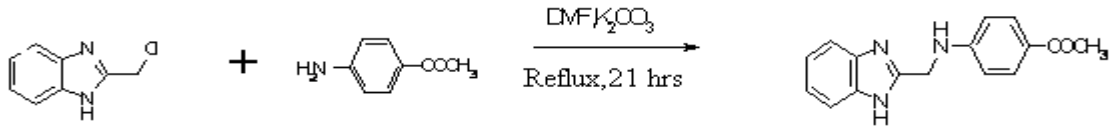


2-chloromethyl-1H-benzimidazole | p-toluidene | (1H-benzimidazole-2ylmethyl)-p-tolylamine

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (3.33g, 0.02mol) and K_2CO_3 (0.02mol, 2.764) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and *p*-toluidene (2.14g, 0.02 mol) was added reaction was refluxed for 13hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate, and concentrated to obtain crude product. Yield: 40%,melting point : 158-160^{0}C,TLC: Benzene: Ethyl acetate (3:1.5),R_f: 0.68,IRν_{max}(KBr)-3371(-NH),3053(-aromatic –CH),2953(aliphatic –CH),1305(-C-N),843(aromatic –C-H out of plane stretching).

VKMP-(6) Synthesis of 1-{4-[1H-Benzimidazole-2-ylmethyl)-amino]-phenyl}-ethanone



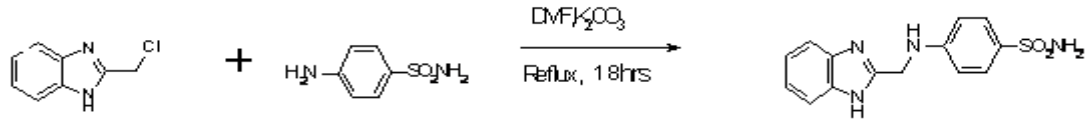
2-chloromethyl-1H-benzimidazole | p-aminoacetophenone | 1-{4-[(1H-benzimidazole-2-ylmethyl)-amino]-phenyl}-ethanone

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (3.33g, 0.02 mol) and K_2CO_3 (0.02mol, 2.764gm) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and *p*-amino acetophenone (2.70g, 0.02mol) was added reaction was refluxed for 21hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate, and concentrated to obtain crude product.

Spectral Data:- Yield: 48%,M.p.: 196-198^{0}C,TLC: Benzene: Ethyl acetate (3:1.5),R_f:0.63,IRν_{max}(KBr):3320(-NH),3055(aromatic–CH),2920(-CH,1655(C=O),1393(C-N).

VKMP-(7) Synthesis of 4[(1H-Benzimidazole-2-ylmethyl)-amino]-benzenesulfonamide



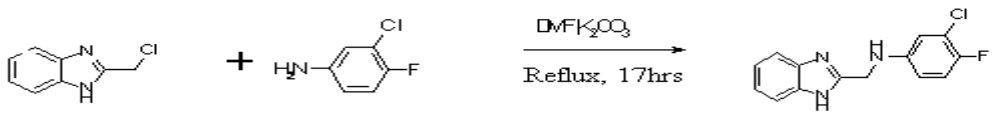
2-chloromethyl-1H-benzimidazole | sulphanilamide | [4-(1H-benzimidazole-2-ylmethyl)-amino]-benzenesulfonamide

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (1.665g, 0.01 mol) and K_2CO_3 (0.02mol, 2.764gm) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and sulphanilamide (1.72g, 0.01 mol) was added reaction was refluxed for 18 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate, and concentrated to obtain crude product.

Spectral Data:- Yield: 52%,M.p.: 212-214^{0}C,TLC: Benzene: Ethyl acetate (3:1.5),R_f: 0.64,IRν_{max}(KBr)-3425 (primary –NH_2),3398 (-NH),3261 (aromatic –CH),3063 (aliphatic –CH),1050 (S=O),1332 (sulfonamide),^{1}H-NMR:δ-9.21 (1H,s,-NH),6.71-7.57 (8H,m,protons of the aromatic ring),4.51 (2H,s,-CH_2),3.38 (1H,s,-NH of amine),2.5 (2H,s,-NH_2).

VKMP-(8) Synthesis of (1H-Benzimidazole -2-ylmethyl)-(3-chloro-4-fluoro-phenyl)-amine



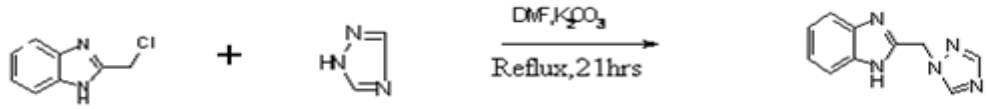
2-chloromethyl-1H benzimidazole | 3-chloro-4-fluroaniline | [(1H-benzimidazole-2-ylmethyl)-amino]-(3-chloro-4-fluro-phenyl)-amine]

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (3.33 gm, 0.02 mol) and K2CO3 (0.02mol, 2.764gm) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and 3-chloro4-fluro aniline (2.932g, 0.02mol) was added reaction was refluxed for 17 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate, and concentrated to obtain crude product.

Spectral Data:- Yield: 47%,M.p.: 132-134^{0}C,TLC: Benzene: Ethyl acetate (3:1.5),R_f: 0.67,IRv_{max} (KBr)-3415 (-NH),3035 (-aromatic –CH), 2987 (aliphatic –CH),1258 (C-N),1236 (C-F),889 (C-CL) .

VKMP-9) Synthesis of 2-[1, 2, 4] Triazol-1-ylmethyl-1H-benzimidazole



2-chloromethyl-1H-benzimidazole + 1,2,4-triazole → 2-[1,2,4]Triazol-1-ylmethyl-1H-benzimidazole

Procedure

In a 250 ml RBF, 2-Chloromethyl-1H-benzimidazole (4.99 gm, 0.03 mol) and K_2CO_3 (0.03mol, 4.14gm) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and 1,2,4 triazole (2.07g, 0.03mol) was added reaction was refluxed for 21 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate, and concentrated to obtain crude product.

Spectral Data:-Yield: 48%, M.p.: 143-145^{0}C,TLC: Benzene: Ethyl acetate (2:4), Rf: 0.62, IRvmax (KBr) -3053(aromatic –CH), 2897 (aliphatic –CH) 1587 (-N-N), 1273 (-C-N)

ANTIFUNGAL SCREENING[16,17,18,19]

Synthesized compounds were screened for their antifungal efficacy.

In vitro Antifungal screening strategies

Comprehensive antifungal screening strategies include the following primary and secondary evaluation methods:

Materials and Method for antifungal screening

a) Primary screening

Table 1: Detection of antifungal potential:

Method	**Agar diffusion quantitative bioassay (well plate method)**
Medium	Sabouraud'sDextrose agar (Hi Media).
Organismemployed	*Candida albicans* strain ATCC-3705 (NCIM 3471)
Inoculum	Growth from 3-4 old Sabouraud's Dextrose agar slope is uniformly suspended in 1 ml of sterile normal saline. Optical density of cell suspension is adjusted using Spectrophotometer at 640 nm to get absorbance 1.
Inoculum size	10^7 CFU/ml.
Stock solution	Prepared in dimetylsulfoxide for both synthesized compounds and standard drug.
Drug concentration	Ketoconazole at 6&12.5 μg/ml. Compounds at 6,12.5,25,50,100 μg/ml.
Incubation time	48-72hrs.
Incubation temp.	35-37^o C.
Interpretation	Any compound showing inhibition zone exceeding 9mm diameter and quality of zone better than or comparable to Ketoconazole is considered to be endowed with antifungal potential.

b) Secondary Screening

Table 2: Determination of Minimum Inhibitory Concentration (MIC μg/ml)

Method	**MIC by Agar diffusion technique (well plate method).**
Medium	Sabouraud'sDextrose agar (Hi Media).
Compounds conc.	12.5, 25, 50, 100 μg/ml.
Organismemployed	*Candida albicans* strain ATCC-3705 (NCIM 3471).
Inoculum preparation	Growth from 3-4 old Sabouraud's Dextrose agar slopes is uniformily suspended in 1 ml of sterile normal saline. Optical density of cell suspension is adjusted using Spectrophotometer at 640 nm to get absorbance 1.[4]
Incubation time & temperature	48-72hrs at 30^o C.
Endpoint definition	Concentration of compounds at which there is either complete disappearance or significant reduction in growth resulting in 5-10 colonies per spot is considered to be the MIC.
Interpretation	Synthesized compounds showing lower MIC than that shown by Ketoconazole would consider superior.

The synthesized compounds were evaluated for *in vitro* antifungal activity against *Candida albicans*(ATCC-3705). Ketoconazole was used as standard.

RESULT AND DISCUSSION

Nine derivatives of 2-Chloromethyl-1H-benzimidazole were synthesized using different aromatic amines and heterocycles as

substituents. Synthesized compounds were characterized by chromatographic methods, Infrared spectroscopy and Nuclear Magnetic Resonance spectroscopy for their structural confirmation.

These nine compounds were tested for antifungal activity by *in vitro* well plate method. Activity was presented in the form of zone of inhibition (mm) in agar culture plates and MIC.The zone of inhibition and MIC of the synthesized compounds are presented in table 4.

Table 3: Activity of Ketoconazole at different concentrations

Conc. (µg/ml)	Zone of inhibition (mm)
12.5	22
6	18

Compounds VMKP 1, 3, 4 and 5 (MIC = 100 µg/ml) exhibited moderate activity against *C. albicans*(ATCC 3705).

The activity of the compounds VMKP 2, 9 (MIC=50 µg/ml) 6, 7 (MIC=25 µg/ml) showed significant activity.

VMKP 8 exhibited maximum activity (MIC=12.5 µg/ml) with MIC equal to that of the Ketoconazole.

Table 4: In vitro antifungal activity of 2-Chloromethyl-1H-Benzimidazole) derivatives

Sr. No.	Molecular Code	Zone of inhibition (mm)	Conc. in µg/ml
1	VMKP-1	20	100
2	VMKP-2	24	50
3	VMKP-3	22	100
4	VMKP-4	23	100
5	VMKP-5	25	100
6	VMKP-6	22	25
7	VMKP-7	21	25
8	VMKP-8	20	12.5
9	VMKP-9	21	50

CONCLUSION

All the nine synthesized compounds screened for in vitro antifungal activity showed antifungal activity. VMKP 8 exhibited potent activity (MIC=12.5 µg/ml).In conclusion, these compounds provide preliminary insights into newer antifungal agents, which can help further modification to improve upon the activity profile.

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