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Research Article

SYNTHESIS CHARECTERIZATION AND BIOLOGICAL EVALUATION OF A SERIES OF 4-CHLORO–*N*-(3-CHLORO-2-(ARYL)-4-OXOAZETIDIN-1-YL) BENZAMIDE DERIVATIVES

Atul H. Makwana*[1] and Hetal R. Makwana[2]

[1]*St.Xavier's College (Autonomous), (Affiliated to Gujarat University) Ahmedabad.*

[2]*Research Scholar, Gujarat University, St. Xavier's College (Autonomous), Ahmedabad.*

**Corresponding author: makwana_atul@yahoo.com*

ABSTRACT

Azetidinone is a four member heterocyclic moiety with one nitrogen atom and one carbonyl group.It is a also known as a β-lactam. It is named β-lactam because the nitrogen atom is attached to the β-carbon atom relative to the carbonyl group.It shows a wide spectrum of biological activities against gram positive and gram negative bacteria. Besides its antibacterial property azetidinones are found to exhibit anti-tubercular, anti-convulsant, anti-cancer, anti-inflammatory, anti-microbial, etc. activities. In the present work we have synthesized a series of 12 azetidinones derivatives. Structures of newly synthesized azetidinone derivatives were confirmed by analytical techniques like IR, ^{1}H-NMR and Mass spectrometry methods. Broth dilution method was used to determine anti-bacterial and anti-fungal activities of the synthesized compounds.

Keywords: Azetidinone derivatives, β-lactam, Schiff Base, Biological Activities, MIC

1. INTRODUCTION

In 1907 Hermann Staudinger was first to prepare azetidinone (β-lactam) derivatives. In the late 1990s, several groups reported novel methodologies for the synthesis of azetidinones.

The chemistry of β-lactam has taken an important place in organic chemistry since the discovery of Penicillin by Sir Alexander Fleming in 1928 and shortly thereafter Cephalosporin which were both used as successful antibiotics [1]. Even now the research in this area is stimulated because of development of bacterial resistance to widely used antibiotics of this type. The 2-azetitinone (β-lactams) ring is a common structural feature of a number of broad spectrum azetidinones (β-lactam)antibiotics including Penicillins I, Cephalosporins II, Carbapenems III, and monobactams which have been widely used as chemotherapeutic agents to treat bacterial infection and microbial diseases .

Azetidinone is a colorless solid, melting point 73-74°C. It is highly soluble in ethanol and chloroform. The physical state of other azetidinones (β-lactam) varies widely with the degree and nature of the substituents.

Azetidinones [2, 3] are very important class of compounds possessing wide range of biological activities such as antibacterial [4, 5], anti-inflammatory [6], anti-tubercular [7], anti-tumour [8], cytotoxic [9], enzyme inhibitors [10], elastase inhibitors [11], cholesterol absorption inhibitory activity [12], tryptase inhibitory [13], human leukocyte elastase inhibitory [14], anti-hyperglycemic [15] and vasopressin Vl antagonist activity [16].

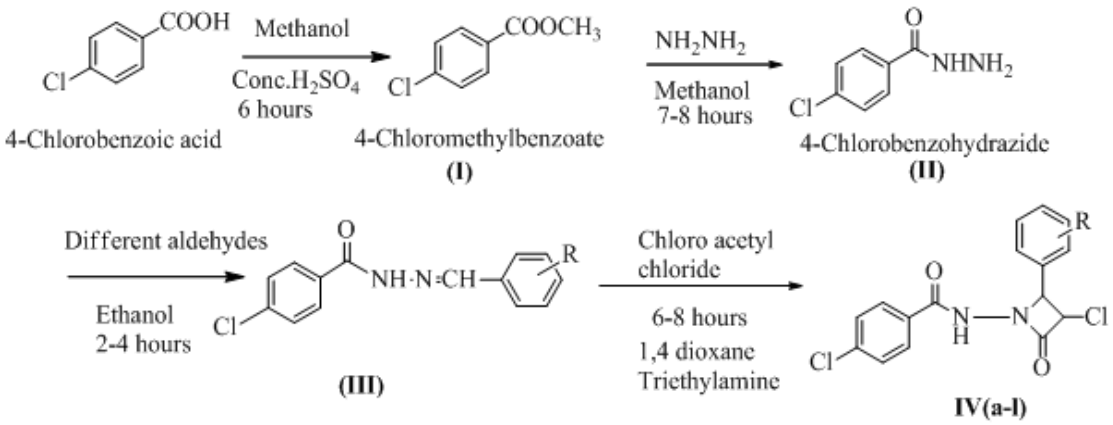
Looking to the biological importance to azetidinone derivatives we have prepared a series of azetidinones and evaluated them for their antimicrobial activities.

2. MATERIAL AND METHODS

2.1. General

All chemicals used in the synthesis of the titled compounds were of analytical grade. Melting points were reported by the open tube capillary method and are uncorrected. The reaction progress, completion and purity ofthe newly synthesized compounds were determined by Merck Kieselgel 60 F254TLC plates, using mobile phase ethyl acetate: chloroform (4:1). IR spectra were recorded on SHIMADZU FT-IR 8400 using potassium bromide pallets. The ^{1}H NMR spectra were recorded in DMSO d_6 solution in 5 mm tubes at room temperature, on a BRUKER 400 MHz FT-NMR, with TMS as internal standard.

Mass spectra were recorded on SHIMADZU QP-2010. The antimicrobial activity was carried out using broth dilution method to determine minimum inhibitory concentration (MIC).



Scheme 1: Reaction scheme for the synthesis of the compounds I (a-l).

2.2. Synthesis of 4-chloromethyl benzoate (I)

4-Chlorobenzoic acid (0.26 mol) was taken in a round bottom flask with 25 ml methanol and then sulfuric acid was added drop wise through a dropping funnel. The solution was heated to reflux for 6 hours. It was then cooled to room temperature and poured into ice of cold water to form product 4-chloro methyl benzoate. The product was re-crystallized from ethanol and the progress and completion of the reaction was confirmed by TLC.

2.3. Synthesis of 4-chlorobenzohydrazide (II)

A mixture of 4-chloro methyl benzoate (0.22mol) and hydrazine hydrate (0.22mol)in methanol was heated in a round bottom flask for7-8 hours. The reaction mixture was cooled to room temperature. A white precipitates obtained to form 4-chlorobenzohydrazide.

Finally the product was re-crystallized from ethanol and confirmed by TLC.

2.4. General procedure for synthesis of Schiff bases (III)

In a round bottom flask equimolar quantities of different aromatic aldehydes (0.1mol) and 4-chlorobenzo hydrazide (0.1mol) were dissolved in ethanol and refluxed for 2-4 hours and solid product obtained were respectively schiff base.

Finally the products were re-crystallized from ethanol. The progress and completion of the reaction were confirmed by TLC.

2.5. General Procedure for the preparation of IV(a-l)

In a round bottom flask a mixture of substituted schiff bases (III) (0.01mol), in 1,4-dioxane (25ml), and triethylamine (0.01mol) and chloro acetyl chloride (0.01mol) was taken. The reaction mixture was stirred for 30 min and then refluxed for 7-8 hours to form final products and completion of reaction was confirmed by TLC.

Finally the products were re-crystallized from ethanol or methanol.

3. RESULTS AND DISCUSSION

In the present work we have prepared a new series of azetidinone derivatives. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral analysis.

3.1. Spectral data of some of the synthesized compounds:

3.1.1. 4-chloro–N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)benzamide(IVa)

(IR cm^{-1}, KBr): 748 (C-Cl stretching), 1540 (N-H bending, CONH), 1644 (C=O); **^{1}H NMR:** (DMSO, 400MHz) (δ ppm)**:** 3.1 (1H, s), 4.3 (1H, s), 7.6 (2H, s), 7.7 (2H,t), 7.8(3H ,s), 7.9 (2H, d) , 8.5 (1H, s); **Mass (m/z):** 334.

3.1.2. 4-chloro–N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)benzamide(IVb)

(IR cm^{-1}, KBr): 847 (C-Cl stretching), 1540 (N-H bending, CONH), 1645 (C=O), 1528 (Ar-NO_2).; **^{1}H NMR:**(DMSO, 400MHz) (δ ppm)**:** 3.5 (1H, s), 4.4 (1H, s), 7.5 (2H, s), 7.7 (2H, t), 7.8 (2H, s), 7.9 (2H, d), 8.6 (1H, s); **Mass (m/z):** 380.

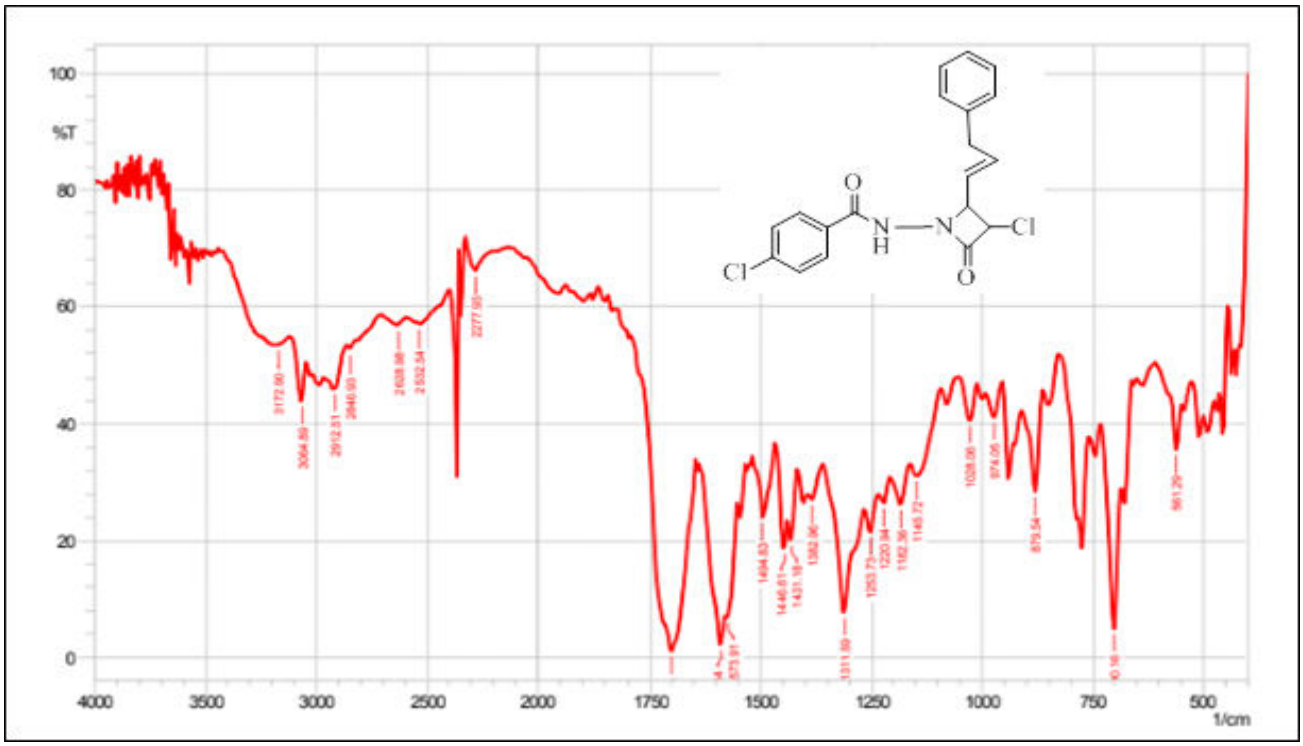


Fig. 1: IR Spectra of compound IVg

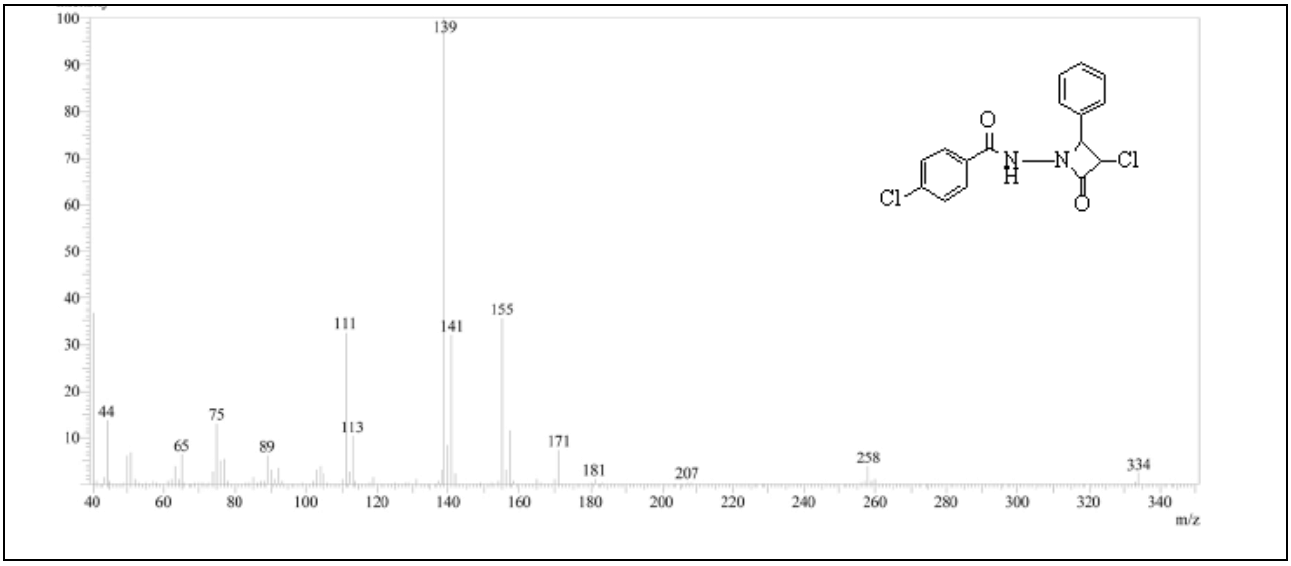


Fig. 2: Mass Spectra of compound IVa

3.1.3. 4-chloro–N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)benzamide (IVc)

(IR cm^{-1}, KBr): 750 (C-Cl stretching), 1521 (CONH, N-H bending), 1654 (C=O); **^{1}H NMR** (DMSO, 400MHz) (δ ppm)**:** 3.4 (1H, s), 4.2 (1H, s), 7.4 (2H, s), 7.7 (2H, t), 7.8 (2H, s), 7.9 (2H, d), 8.3 (1H, s); **Mass (m/z):** 370.

3.1.4. 4-chloro–N-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)benzamide (IVd)

(IR cm^{-1}, KBr): 773 (C-Cl stretching), 1572 (CONH, N-H bending), 1688 (C=O), 1254 (-OH)**; ^{1}H NMR:** (DMSO, 400MHz) (δ ppm)**:** 3.4 (1H, s), 4.3 (1H, s), 7.4 (2H, s), 7.5 (2H, t), 7.8 (3H, s), 7.9 (1H, d), 8.4 (1H, s), 11.2 (1H, s)**; Mass (m/z):** 351.

3.1.5.4-chloro–N-(3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)benzamide (IVe)

(IR cm^{-1}, KBr): 760 (C-Cl stretching), 1566(CONH, N-H bending), 1667 (C=O), 1259 (-OH); **^{1}H NMR** (DMSO, 400MHz) (δ ppm)**:** 4.2 (1H, s), 4.3 (1H, s), 7.3 (2H, d), 7.5 (2H, t), 7.8 (3H, s), 7.9 (1H, d), 8.4 (1H, s), 10.5 (1H, s); **Mass (m/z):** 351.

3.1.6.4-chloro–N-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl)benzamide(IVf)

(IR cm^{-1}, KBr): 849(C-Cl stretching),1525(CONH, N-H bending), 1644 (C=O), 2826(-OCH_3); **^{1}HNMR:**(DMSO, 400MHz) (δppm)**:** 3.4 (3H, s), 4.1 (1H, s), 4.2 (1H, s), 7.2 (2H, d), 7.5 (2H, t), 7.8 (3H, s), 7.9 (1H, d), 8.4 (1H, s); **Mass (m/z):**365.

3.1.7.4-chloro–N-(3-chloro-2-cinnamyl-4-oxoazetidin-1-yl)benzamide (IVg)

(IR cm^{-1}, KBr): 700 (C-Cl stretching), 1574(CONH, N-H bending), 1703(C=O), 1589(C=C); **^{1}H NMR:** (DMSO, 400MHz) (δ ppm)**:** 3.8 (2H, s), 3.5 (2H, s), 7.1 (2H, d), 7.5 (2H, t), 7.8 (3H, s), 7.9 (2H, d), 8.2 (1H, s); **Mass (m/z):**374.

3.1.8.4-chloro–N-(3-chloro-2-(4-dimethylamino -phenyl)-4-oxoazetidin-1-yl)benzamide(IVh)

(IR cm^{-1}, KBr): 847 (C-Cl stretching), 1520 (CONH, N-H bending), 1645 (C=O), 2808.36 (N-CH_3); **^{1}H NMR** (DMSO, 400MHz) (δ ppm)**:** 3.8 (6H, s), 4.2 (2H, s), 7.1 (2H, d), 7.5 (2H, t), 7.6 (1H, s), 7.8 (3H, s), 8.4 (1H, s); **Mass (m/z):**378.

3.1.9.4-chloro–N-(3-chloro-2-(3,4dimethoxyphenyl)-4-oxoazetidin-1-yl)benzamide (IVi)

(IR cm^{-1}, KBr): 748 (C-Cl stretching), 1520(CONH, N-H bending), 1648(C=O), 2830(-OCH_3); **^{1}H NMR:** (DMSO, 400MHz) (δ ppm)**:** 3.3 (6H, d), 4.2 (2H, s), 7.1 (2H, d), 7.3 (2H, t), 7.5 (1H, s) 7.8 (3H, s), 8.4 (1H, s); **Mass (m/z):**395.

3.1.10. 4-chloro –N-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)benzamide(IVj)

(IR cm^{-1}, KBr): 749 (C-Cl stretching), 1540 (CONH, N-H bending), 1645 (C=O); **^{1}H NMR:** (DMSO, 400 MHz) (δ ppm)**:** 3.2 (1H, s), 4.3 (1H, s), 7.5 (2H, s), 7.7 (2H, t), 7.8 (2H, s), 7.9 (2H, d), 8.3 (1H, s); **Mass (m/z):**370.

3.1.11. 4-chloro–N-(3-chloro-2-(2-nitrophenyl)-4-oxoazetidin-1-yl)benzamide (IVk)

(IR cm^{-1}, KBr): 745(C-Cl stretching),1568(CONH, N-H bending), 1641(C=O),1314(C-N),1520(Ar-NO_2); **^{1}H NMR:** (DMSO, 400MHz) (δ ppm)**:** 3.4 (1H, s), 4.3 (1H, s), 7.2 (2H, s), 7.7 (2H, t), 7.8 (2H, s), 7.9 (2H, d), 8.3 (1H, s); **Mass (m/z):**380.

3.1.12. 4-chloro–N-(3-chloro-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl)benzamide(IVl)

(IR cm^{-1}, KBr): 745 (C-Cl stretching),1570 (CONH, N-H bending), 1645 (C=O), 1312 (C-N), 2820 (-OCH_3), 1278(-OH); **^{1}H NMR:**(DMSO, 400MHz) (δ ppm)**:** 3.2 (3H, s), 4.1 (1H, s), 4.2 (1H, s), 7.2 (2H, d), 7.5 (2H, t), 7.8 (3H, s), 8.4 (1H, s), 10.2 (1H, s); **Mass (m/z):**381.

Table 1.Physical properties of the synthesized compounds

Sr. No.	Compound Id	R	Molecular Formula	M.W. gm/mole
1	IVa	-CHO	$C_{16}H_{12}Cl_2N_2O_2$	334
2	IVb	-3-NO_2	$C_{16}H_{11}Cl_2N_3O_4$	380
3	IVc	-4-Cl	$C_{16}H_{11}Cl_3N_2O_2$	370
4	IVd	-4- OH	$C_{16}H_{12}Cl_2N_2O_3$	351
5	IVe	-2-OH	$C_{16}H_{12}Cl_2N_2O_3$	351
6	IVf	-4- OCH_3	$C_{17}H_{14}Cl_2N_2O_3$	365
7	IVg	-4-C_8H_7	$C_{19}H_{14}Cl_2N_2O_2$	374
8	IVh	-N(CH_3)$_2$	$C_{18}H_{17}Cl_2N_3O_2$	378
9	IVi	-3,4,-(OCH_3)$_2$	$C_{18}H_{17}Cl_2N_2O_4$	395
10	IVj	-2-Cl	$C_{16}H_{11}Cl_3N_2O_2$	370
11	IVk	-2-NO_2	$C_{16}H_{11}Cl_2N_3O_4$	380
12	IVl	-3-OCH_3, 4 -OH	$C_{17}H_{14}Cl_2N_2O_4$	381

Compound **IVi** showed strong absorption at 1648 cm^{-1} due to carbonyl group. Compound **IVi** showed strong bending at 1520 cm^{-1}due to N-H group. Compound **IVi** showed 3.3 δ ppm to six protons of two methoxy group in NMR. The compound **Ive** showed singlet at 10.5 δ ppm due to one protons of –OH group and 4.2 δ ppm due to one protons of –CH group in azetidine ring.

Compound **IVl** showed singlet at 10.2 δ ppm of one proton of –OH group. The mass spectrum of compound **Iva** showed the molecular ion peak at m/z = 334 corresponding to the molecular formula $C_{16}H_{12}Cl_2N_2O_2$.

All the synthesized compounds were screened for antibacterial and antifungal activities by broth dilution method. The antimicrobial study was carried out using two gram positive bacteria (*Bacillus Cereus* and *S. aureus*) and two gram negative bacteria (*E. coli* and *P. Seudomonas*) and also one anti-fungal activity. Similarly From the study of the biological activity data in reference to Standard drug Ampicillin and Amphotericin B, we found that **IVb**, **IVc**, **IVh**, **IVk** exhibited moderate activity against *Bacillus Cereus*. Compounds **IVb**, **IVc** and **IVg** found to possess moderate activity against *S.aureus*. Compound **IVc** showed moderate activity against *E.coli* while compounds **IVa**, **IVh**, and **IVg** showed moderate activity against *P.Seudomonas*.

For anti-fungal activity compound **IVf** and **IVi** showed moderate activity against *Aspergillius niger*.

Table 2: Biological activities in terms of MIC of Synthesized Compounds

S.No.	Compound Id	Minimum inhibitory concentration (MIC) (µg/ml)				
		Anti-bacterial Activity				Anti-fungal activity
		Gram Positives bacteria		Gram Negative Bacteria		Fungus
		Bacillus Cereus MTCC 430	*S. aureus* MTCC 737	*E. coli* MTCC 1687	*P. Seudomonas* MTCC 1688	*Aspergillus niger* ATCC 16404
1	IVa	256	64	256	128	512
2	IVb	128	128	1024	512	256
3	IVc	256	128	128	256	1024
4	IVd	512	512	512	256	512
5	IVe	128	256	512	512	256
6	IVf	256	256	256	1024	128
7	IVg	512	128	128	512	256
8	IVh	128	1024	1024	128	256
9	IVi	256	1024	1024	256	128
10	IVj	1024	512	1024	128	1024
11	IVk	128	256	1024	512	256
12	IVl	256	1024	1024	256	256
	Ampicillin	10.0	12	2.0	8.0	-
	Amphotericin B	-	-	-	-	0.05

S.aureus: *Staphylococcusaureus*, *E.coli: Escherichiacoli, P.Seudomonas*: *Pseudomonas aeruginosa*

4. APPLICATIONS

In the present study azetidinone derivatives synthesized in the present work were screened for their anti-bacterial and anti-fungal activities. Some of the compounds are found to possess moderate to good biological activity. They are hopeful as active pharmacophore. Further work on these compounds may help for invention of lead molecule in future.

5. CONCLUSION

From the present study it was found that in the final structure of different substituted compounds affect the biological activities. Final compounds with hydroxy, chloro and nitro groups were found to possess moderate antibacterial activity. It was also observed that the synthesized compounds give moderate antifungal activity.

6. ACKNOWLEDGEMENT

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