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SYNTHESIS CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 2-(4-FLUOROPHENYL)-5-(1-METHYLETHYL)-3-PHENYL-4-[(PHENYL AMINO) CARBONYL]-1*H* PYRROLE-1-ETHANOL AND ITS DERIVATIVES

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ABSTRACT

The synthesis of derivative precursors for pharmaceutically established cardiovascular drug substance that is Atorvastatin Calcium has been carried out with eco-friendly and conventional method. The synthesis process has been designed in such a way that can be easily commercialized or scaled up in any pharmaceutical industry. The ultimate goal of research is to make available the technology for the cheap and effective production of cardiovascular pharmaceutical ingredients.

In tune on above four compounds has been synthesized, characterized and reported in this paper. The quality of the products has been established by using modern spectroscopic and physicochemical tools for analysis that includes: Mass, IR, NMR, HPLC, Melting point CHN analysis etc.

Keywords: *Synthesis, Cardiovascular, Characterization, Antibacterial and Antifungal screening.*

Introduction

The science of contemporary drug development is a tremendously complex and costly process but it has successfully advanced our understanding of modern diseases and has improved public health significantly by providing society with many valuable drug treatments. A critical step in the drug development process is the submission of non-clinical and clinical data and information in a new drug application (NDA) to the Food and Drug Administration (FDA) by a sponsor seeking marketing authorization. A typical new molecular entity (NME) that is the subject of a NDA has most likely been studied pre-clinically for 5-7 years and has been in clinical trials for 6-7 years. The average cost of bringing an NME to market is somewhere 500 to 800 million US Dollars including the cost of lost opportunities and lead compound failures. With this investment of time and money many scientists involved in drug development as efficient, and yet informative, as possible. Despite its successes, the drug development process, including regulatory decision making based on benefit/risk assessment, can be improved in three areas:

1. By providing a greater understanding of human health and the causes of diseases at a genomic and molecular level. It would address the well known heterogeneity of diseases states that underlies the wide inter-individual variation in efficacy observed with many common treatments.
2. Improve the safety of medicines. Adverse Drug Reactions (ADRs) have had a major impact on morbidity, mortality and health economics. It can be achieved by improving the drug quality and

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controlling/identifying the impurity profile in the drug substance.

3. Optimize drug doses and dosing schedules. Approximately 70% of drug related adverse events are due to extended pharmacological actions.

The work presented in this paper is inline of focusing point # 2, to develop novel drugs (Analogous to the known cardiovascular drugs) by novel routes. As the pollution and the green house effects are the burning issue now a day. It is a great challenge in front of all scientists, around the globe, to reduce the pollution coming out from different sources, major from the industries and to care of our environment. The scheme, designed for the synthesis of drug substances, mentioned above, has been prepared keeping in mind these aspects. The structure of the newly synthesized compounds has been shown in the figure 1.

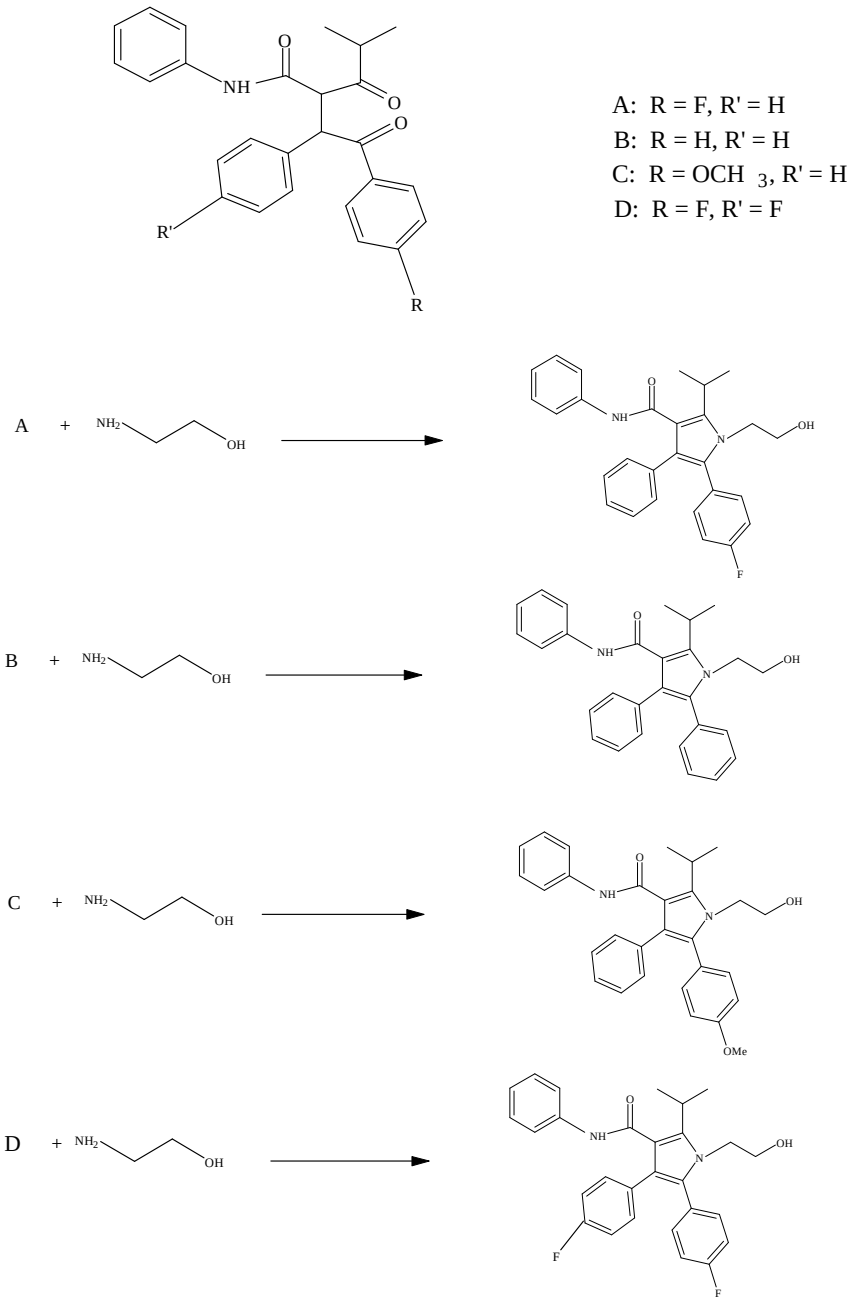

Figure 1: Structure of the compounds, synthesized

RESULTS AND DISCUSSION

A. Synthesis:

a. Synthesis of 2-(4-FluoroPhenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H pyrrole-1-ethanol (4):

A solution of 4-fluoro-α-methyl-1-oxopropyl]γ-oxo-N-β-diphenylbenzenebutanamide (1 g, 0.0024 mol), ethanol amine (0.0026 g, mol) and pivalic acid in 6 ml of cyclohexane were taken and refluxed at 78ºC (±3 °C). During refluxing water was removed azeotropically. Reaction progress has been monitored by TLC (hexane: ethyl acetate:: 7:3). On completion of the reaction, 3 ml of 10 % sodium bicarbonate solution was added at 50-55 ºC (±3 °C) and stirred well for about 30 minutes. Separated out the layer and washed the organic layer with 10 % sodium bicarbonate solution. Organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give yellow viscous oil. Residual mass was then dissolved in 5 ml of isopropyl alcohol at 70-75 ºC (±3 °C). The resultant solution was then gradually cooled down to 20-25ºC (±3 °C). Obtained light yellow precipitate was filtered, washed with isopropyl alcohol and dried in vacuum to produce analytically pure product.

b. Synthesis of 5-(1-methylethyl)-2,3-biphenyl-4-[phenyl amino)carbonyl] 1H pyrrole-1-ethanol (5):

A solution of α-methyl-1-oxopropyl]-γ-oxo-N-β-diphenylbenzenebutanamide (1 g, 0.0024 mol), ethanol amine (0.0026 g, mol) and pivalic acid in 6 ml cyclohexane were taken and refluxed at 78ºC (±3 °C). During refluxing water was removed azeotropically. Reaction progress has been monitored by TLC (hexane: ethyl acetate:: 7:3). On completion of the reaction, 3 ml of 10 % sodium bicarbonate solution was added at 50-55 ºC (±3 °C) and stirred well for about 30 minutes. Separated out the layer and washed the organic layer with 10 % sodium bicarbonate solution. Organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give brownish viscous oil. Residual mass was then dissolved in 5 ml of isopropyl alcohol at 70-75 ºC (±3 °C). The resultant solution was then gradually cooled down to 20-25ºC (±3 °C). Obtained light brown precipitate was filtered, washed with isopropyl alcohol and dried in vacuum to produce analytically pure product.

c. Synthesis of 2-(4-methoxyphenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H pyrrole-1-ethanol (6):

A solution of 4-methoxy-[-methyl-1-oxopropyl]γ-oxo-N-β-diphenylbenzenebutanamide (1 g,

0.0024 mol), ethanol amine (0.0026 g, mol) and pivalic acid in 6ml cyclohexane were taken and refluxed at 78ºC (±3 °C). During refluxing water was removed azeotropically. Reaction progress has been monitored by TLC (hexane: ethyl acetate:: 7:3). On completion of the reaction, 3 ml of 10 % sodium bicarbonate solution was added at 50-55 ºC (±3 °C) and stirred well for about 30 minutes. Separated out the layer and washed the organic layer with 10 % sodium bicarbonate solution. Organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give clear semi viscous oily mass. Residual mass was then dissolved in 5 ml of isopropyl alcohol at 70-75 ºC (±3 °C). The resultant solution was then gradually cooled down to 20-25ºC (±3 °C). Obtained cream colored precipitate was filtered, washed with isopropyl alcohol and dried in vacuum to produce analytically pure product.

d. Synthesis of 2,3-(4,4-difluoro biphenyl)-5-(1-methylethyl)-4-[(phenyl amino) carbonyl]-1H pyrrole-1-ethanol (7):

A solution of 4-fluoro-α- [methyl-1-oxopropyl]γ-oxo-N-phenyl β-4-fluoro benzenebutanamide (1 g, 0.0024 mol), ethanol amine (0.0026 g, mol) and pivalic acid in 6ml cyclohexane were taken and refluxed at 78ºC (±3 °C). During refluxing water was removed azeotropically. Reaction progress has been monitored by TLC (hexane: ethyl acetate:: 7:3). On completion of

the reaction, 3 ml of 10 % sodium bicarbonate solution was added at 50-55 ºC (±3 °C) and stirred well for about 30 minutes. Separated out the layer and washed the organic layer with 10 % sodium bicarbonate solution. Organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give creamish hazy mass. Residual mass was then dissolved in 5 ml of isopropyl alcohol at 70-75 ºC (±3 °C). The resultant solution was then gradually cooled down to 20-25ºC (±3 °C). Obtained off white precipitate was filtered, washed with isopropyl alcohol and dried in vacuum to produce analytically pure product.

B. Characterization:

All derivatives, synthesized and reported in this paper, have been characterized by using modern spectroscopic techniques and physicochemical analytical tools [7-12]. Spectroscopic results support the proposed structure of the compounds [13-19]. The analytical data (IR and NMR) for compound # 4 to 7 has been tabulated and reported in table 1 to 4, respectively. The observed values of CHN analysis are in tune of calculated values. Yield, color, melting point, mass and CHN results has been summarized in table – 5. The HPLC analysis of all compounds gives an idea about the purity of compounds [20-22]. Compounds have been observed to be 98.48 to 99.12 % pure (Qualitatively) and the level of single highest most impurity was observed 0.16 to 0.39 %. Summarized results of HPLC analysis has been presented in table – 6.

Table 1: Spectroscopic results of Compound # 4:

IR absorption band (ν cm^{-1})	**Group**
3304	O-H (Alcohols)
3297	N–$H_{str.}$(Amide)
3178,3134	C–H_{str} (Aromatic)
3121,3038	C-H_{str} (Alkenes)
2964	C-H_{str} (Methylene).
2352,2327	C-N (Amides)
1651	C=$O_{str.}$ (Amide)
1598,1580	C=C_{str} (Alkenes)
1310,1230	C-O (Alcohols)
1266,1218	C-F_{str}
766	p-substituted benzene
H^1NMR spectra (δ ppm)	**Protons**
1.5	d, 6H (2×CH_3 isopropyl)
1.8	s, 1H (OH, Alcohols,D_2O exchanged)
3.45	s, 1H (CH, isopropyl)
3.65,4.05	2×t, 4H (CH_2)
6.85	s, 1H (NH, amide)
6.9-7.3	m, 14H (Ar)
Mass (M^+ m/z): 443 (M+1), 442(M^+), 351, 350 (base peak), 252.	

Note: s (singlet), d (doublet), m (multiplet), Ar (Aromatic)

Table 2: Spectroscopic results of Compound # 5:

IR absorption band (ν cm^{-1})	Group
3558	O-H (Alcohols)
3282	N–$H_{str.}$(Amide)
3054	C–H_{str} (Aromatic)
2965,2921	C-H_{str} (Methylene)
1648	C=$O_{str.}$ (Amide)
1596	C=C_{str} (Alkenes)
1594,1517	C=C_{str} (Alkenes conjugated)
1315,1236	C-O (Alcohols)
766+759,743+723	Phenyl (Mono subs. benzene)
H^1NMR spectra (δ ppm)	**Protons**
1.4	d, 6H (2×CH_3 isopropyl)
1.5	s, 1H (OH, Alcohols,D_2O exchanged)
	s, 1H (CH, isopropyl)
3.4	2×t, 4H (CH_2)
3.5,4.0	s, 1H (NH, amide)
6.8	m, 15H (Ar)
6.9-7.25	
Mass (M^+ m/z): 426 (M+1), 425(M^+, base peak), 306, 236, 102.	

Table 3: Spectroscopic results of Compound # 6:

IR absorption band (ν cm^{-1})	Group
3511	O-H (Alcohols)
3290	N–$H_{str.}$
3180,3150	C–H_{str} (Aromatic)
2965	C-H_{str} (Alkanes)
1677	C=$O_{str.}$ (Amide)
1596,1541	C=C_{str}
1316,1261	C-O (Alcohols)
1108,1024	C-O_{str} (Ethers)
799	p-subst. benzene
H^1NMR spectra (δ ppm)	**Protons**
1.5	d, 6H (2×CH_3 isopropyl)
1.4	s, 1H (OH, Alcohols,D_2O exchanged)
	s, 1H (CH, isopropyl)
3.5	2×t, 4H (CH_2)
3.6,4.0	s, 3H (OCH_3)
3.8	s, 1H (NH, amide)
6.8	m, 14H (Ar)
6.9-7.25	
Mass (M^+ m/z): 456 (M+1), 455(M^+, base peak), 336,102.	

Table 4: Spectroscopic results of Compound # 7

IR absorption band (ν cm^{-1})	Group
3541	O-H (Alcohols)
3285	$N–H_{str.}$
3136, 3125	$C–H_{str}$ (Ar)
3092,3003	C-H $_{str}$ (Alkenes)
2968, 2945	C-H $_{str}$ (Alkanes)
2365,2340	C-N $_{str}$ (Amides)
1671	$C=O_{str.}$ (Amide)
1590,1577	$C=C_{str}$
1265,1220	$C-F_{str}$
845,833	p-subst. benzene
750,739+708,681	Phenyl (Mono subs. benzene)
H^1NMR spectra (δ ppm)	**Protons**
1.4	s, 1H (OH, Alcohols,D_2O exchanged)
1.5	d, 6H (2×CH_3 isopropyl)
3.5	s, 1H (CH, isopropyl)
3.6,4.0	2×t, 4H (CH_2)
6.8	s, 1H (NH, amide)
6.9-7.25	m, 13H (Ar)
Mass (M^+ m/z): 461 (M+1), 640(M^+, base peak), 336, 102.	

Table 5: Physical parameters and elemental analysis data of the compounds:

Compound #	MW	Solubility	Colour	M.P. °C	Yield %	Elemental analysis data Found (Calculated)%			
						Observed mass (m/z: M^+)	C	H	N
$C_{28}H_{27}N_2O_2F$ (# 4)	442	DMF	Light Yellow	241	69	443	76.19 (76.02)	6.01 (6.11)	6.27 (6.33)
$C_{28}H_{28}N_2O_2$ (# 5)	424	DMF	Light Yellow	256	73	426	79.04 (79.25)	6.68 (6.60)	6.65 (6.60)
$C_{29}H_{30}N_2O_3$ (# 6)	454	DMF	Cream	219	77	456	76.73 (76.65)	6.69 (6.61)	6.26 (6.17)
$C_{28}H_{26}N_2O_2F_2$ (# 7)	460	DMF	Off White	238	68	461	72.91 (73.04)	5.68 (5.65)	6.00 (6.09)

Table 6: HPLC analysis data of the compounds:

Sr. No. #	Compound	Purity by HPLC (%)	Single highest most impurity (%)	Total impurity (%)
1.	# 4	99.05	0.23	0.95
2.	# 5	99.12	0.16	0.88
3.	# 6	98.48	0.39	1.52
4.	# 7	98.83	0.28	1.17

BIOLOGICAL SCREENING

The newly synthesized compounds have also been screened against several species of bacteria and different plant pathogenic fungi.

Antibacterial screening

The antibacterial action of the compounds has been evaluated by the disc diffusion technique [21-22]. This was done on Sarcina lutea (gram-positive), Staphylococcus aureus and Escherchia coli (gram-negative) bacteria at 35°C. The disc of Whatmann no. 4 filter paper having the diameter 6.00 mm were soaked

in the solution of compounds in DMF [Minimum Inhibitory Concentrations (MICs) were in the range 12-13, 14-15, 12-14 and 16-18 µg/ml for compound # 4 to 7, respectively, table 7. After drying it was placed on nutrient agar plates [23-24]. The inhibition areas were observed after 52h. DMF was used as a control and Gentamycin as a standard drug.

100% growth of bacteria which is represented as +, 50% growth by- ++, less then 50% growth by-+++ and noble inhibition by-++++. Compound # 4 shows maximum inhibition capacity and the compound # 7 has minimum inhibition capacity among the group, reported here, Fig. 2.

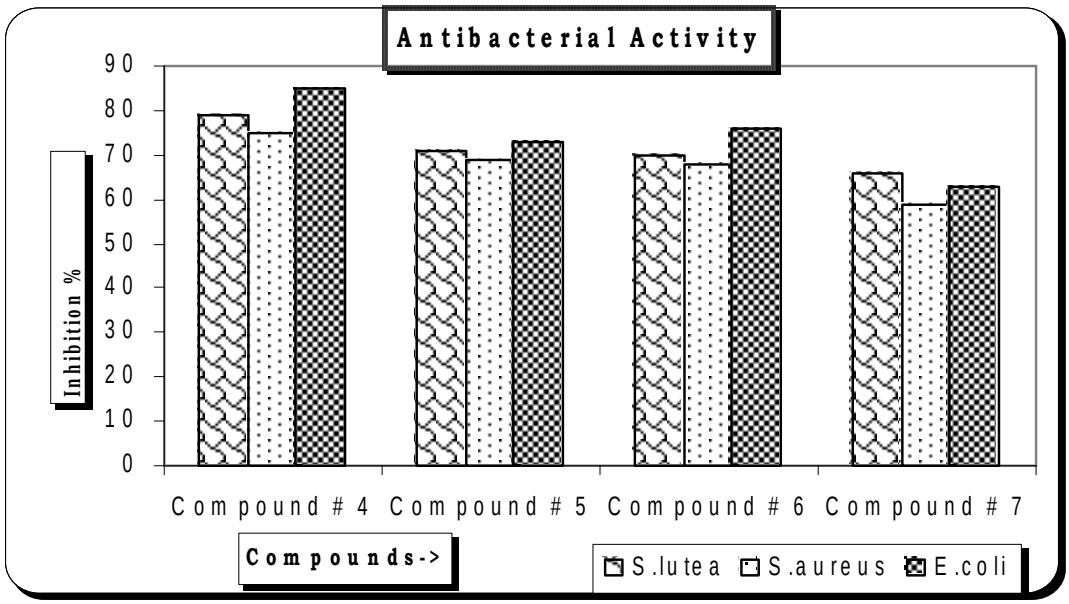

Fig. 2: Antibacterial action of the compounds

Table 7: Antibacterial activity data of the compounds:

Compound	Bacterial Inhibition % (MIC in µg/ml)		
	Sarcina lutea	Staphylococcus aureus	Escherchia coli
# 4	++++ (13)	++++ (13)	++++ (12)
#5	++++ (14)	+++ (15)	++++ (14)
# 6	++++ (14)	++++ (14)	++++ (12)
# 7	+++ (16)	+++ (18)	+++ (17)

Antifungal screening

The Antifungal activity of all the compounds has been screened by the agar plate technique [25] for the Aspergillus-niger, Aspergillus-glaucus and Ustilago tritici fungi. The compounds were directly mixed to the medium in different concentrations [MICs = 11-14, 14-16, 17-19, 18-21 µg/ml for compound # 16 to 19, respectively] (Table-8). The fungus was placed on the medium with the help of the inoculum needle. The pettridishes were wrapped in polythene sheets, containing some drops of EtOH and put in incubator at 32 ± 1°C for 52-72 h. The growth of fungus was measured by the recording the diameter of fungal colony. The following relation calculated the fungal growth inhibition [26-27]:

Fungal growth inhibition % = (A-B) x 100/A

Where: A= diameter of fungal colony in control plate.
B= diameter of fungal colony in test plate.

100% growth of fungus which is represented as *, 50% growth by- **, less then 50% growth by-*** and noble inhibition by-****.

The observed results are in accordance of antibacterial activity as the minimum inhibition has been shown by compound # 7 and a maximum by compound # 4 for all the species, under study, Fig. 3.

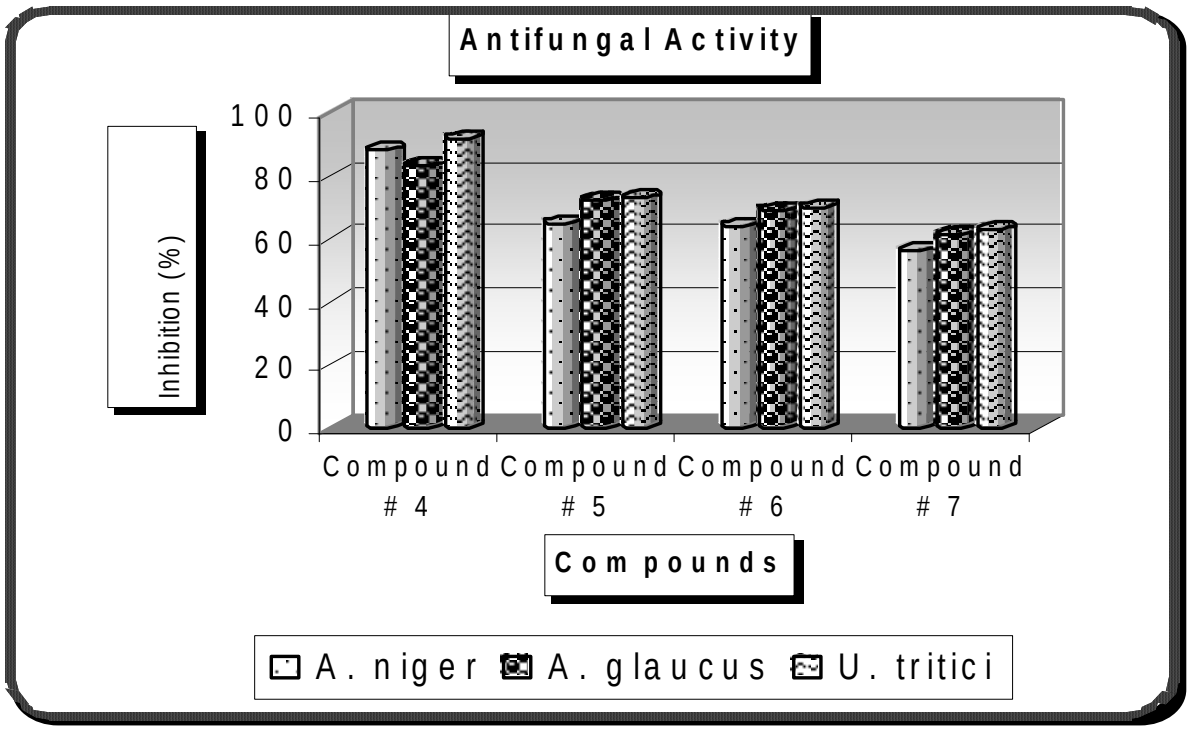

Fig. 3: Fungal growth inhibition of the compounds

Table 8: Antifungal activity data of the compounds:

Compounds	Fungal Inhibition %(MIC in µg/ml)		
	Aspergillus niger	Aspergillus glaucus	Ustilago tritici
# 16	**** (14)	**** (13)	**** (11)
# 17	*** (16)	**** (14)	*** (15)
# 18	*** (18)	*** (19)	*** (17)
# 19	*** (18)	** (20)	** (21)

EXPERIMENTAL

All the chemicals used were of AnalaR grade, and procured from Sigma Aldrich and Fluka. Metal salts were purchased from Glaxo/Spectrochem/Merck and were used as received. All solvents used were odf spectroscopic grade.

Physical measurements

The C, H and N were analysed on a Carlo-Erba 1106 elemental analyzer. MS spectra were recorded on JEOL, JMS, DX-303 mass spectrometer. ^{1}HNMR spectra were recorded on Hitachi FT-NMR, model R-600 spectrometer using DMF as solvent. The chemical shifts are given in ppm relative to tetramethylsilane. IR spectra (KBr) were recorded on a FTIR Spectrum BX-II Perkin Elmer spectrophotometer. The electronic spectra were recorded in DMF on Shimadzu UV 2400

double beam spectrophotometer. The purity of compounds has been established on reverse phase Agilent 1200 series High Performance Liquid Chromatography (HPLC) by using Waters Sunfire C-18 column of 250 mm length and 3.5 µm silica particles. Column temperature was maintained at 38 °C and samples were preserved at 6 °C.

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REFERENCES

1) *A.E.F. Nassar, J.F. Rusling, J. Am. Chem. Soc., 1996, 118, 3043; M.G. Bhowan, J. Laulloo, M. Dowlut, S. Curpen and V. Vijayalaxmi, Trans. Met. Chem., 2005, 30, 35.*
2) *M.J. Abalson and W.J. Stubbe, Biochemistry, 1995, 34, 2076; D.R. McMillin and K.M. McNett, Chem. Rev., 1998, 98, 1201.*
3) *D.S. Sigman, A. Mazumder and D.M. Perrin, Chem. Rev., 1993, 93, 2295; A. Sigel and H. Sigel, Metal Ions in Biological Systems, Marcel Dekker, New York 1996, 33, 324.*
4) *K.E. Erkkila, D.T. Odom and J.K. Barton, Chem. Rev., 1999, 99, 2777; C. Orvig and M.J. Abrams, Chem. Rev., 1999, 99, 2201; D.S. Sigman, Acc. Chem. Res., 1986, 19, 180.*
5) *D.S. Sigman, A. Mazumder and D.M. Perrin, Chem. Rev., 1993, 93, 2295.*
6) *D.L. Carlson, D.H. Huchital, E.J. Mantilla, R.V. Sheardy and W.L. Murphy Jr, J. Am. Chem. Soc., 1993, 115, 6424.*
7) *R.R. Fenton, R. Gauci, P.C. Junc, L.F. Lindoy, R.C. Luckay, G.V. Meehan, J.R. Price, P. Turner and G. Wei, J. Chem. Soc. Dalton Trans., 2002, 2185.*
8) *Y. Baran and I. Yilmaz, Trans. Metal Chem., 2001, 26, 36.*
9) *B. Ramdhanie, L.N. Zakhrov, A.L. Rheingold and D.P. Goldberg, Inorg.Chem., 2002, 41, 4105.*
10) *M. Formica, V. Fusi, M. Micheloni, R. Pontellini and P. Romani, Polyhedron, 2000, 19, 2501.*
11) *Y. Jenkins, A.E. Friedman, N.J. Turro and J.K. Barton, Biochemistry, 1992, 31, 10809.*
12. *P.S. Kalsi, Spectroscopy of organic compounds, 4th Edn., New Age International (P) Ltd., New Delhi, 1999.*
13 *P. Kopel, Z. Travnicek, J. Kvitek, M. Biler, M. Pavlicek and Z. Sindelar, Trans.Metal.Chem., 2001, 26, 282.*
13. *R.J. Dudley and B.J. Hathaway, J. Chem. Soc., (1970) 1725.; B.J. Hathaway and*
12) *D.E. Billing , Cord. Chem. Rev., 5, (1970) 143.*
13) *K. Nakamoto, Infrared Spectra of Inorganic and Coordination Compounds, Wiley Interscience, New York. 1970.*
14) *S. Chandra and K. Gupta, Synth.React.Inorg.Met.Org.Chem., 2001, 31(7), 1205.*
15) *A.B.P. Lever, Inorganic Electronic Spectroscopy, Elsevier, Amsterdam, 1984.*
16) *F.A. Cotton Wilkinson, Advanced Inorganic Chemistry 5th Ed., Wiley, New York, 1988.*
17) *A.B.P. Lever, Crystal Field Spectra. Inorganic Electronic Spectroscopy; Ist Ed; Elsevier; Amsterdam, 249-360, 1968.*
18) *B.N. Figgis and lewis Lewis, J., Inorg. Chem., 1964, 37, 239.*
19) *N.K. Singh and S.B. Singh, Trans.Metal.Chem., 2001, 26, 487.*
20) *A. Ali, G.E. Taylor, K. Ellsworth, G. Harris, R. Painter, L.L. Silver and K.J. Young,*
21) *J. Med. Chem., 2003, 46, 1824.*
22) *W.G. Hanna and M.M. Maowad, Trans.Metal.Chem., 2001, 26, 644.*
23) *N.K. Singh and S.B. Singh., Trans. Met. Chem., 2001, 26, 487.*
24) *N. Raman, A. Kulandaswamy, A. Shanmugasundaram and K. Jeyasubramanian,*
25) *Trans. Met. Chem., 2001, 26, 131.*
26) *R.S. Srivastava, Inorg. Chim. Acta., 1981, 56, 65.*
27) *M. Robert, R.J. Motekaits and A.E. Martell, Inorg. Chim. Acta., 1985, 103, 73.*

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