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Der Chemica Sinica, 2012, 3(1):1-9

Der Chemica Sinica
Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Synthesis and characterization of 2-(4-fluoroPhenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H pyrrole-1- Cyclopropane and 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.

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INTRODUCTION

The cardiovascular system requires a delicate balance between excessive clotting causing obstructions, and leaks following a failure to clot. In most patients this balance is more or less correct, and controlled clinical trials have only supported the prophylactic use of anti-coagulants in two particular circumstances: (1) aspirin appears to give real protection against myocardial infarction, and (2) warfarin and heparin are of benefit in the management of deep vein thrombosis among those at risk. There is, however, a very clear benefit from speedy attempts to dissolve the clot blocking the coronary artery through the use of injected proteases during or immediately after a heart attack [1-3].

There are two routes for the activation of the clotting system. The intrinsic pathway is normally activated by contact with collagen from damaged blood vessels, but any negatively charged surface will suffice. Kaolin (clay) is used to artificially activate the pathway for the measurement of the activated partial thromboplastin time - a clinical test used to monitor the activity of this part of the clotting cascade. Clotting may alternatively be activated via the extrinsic pathway, which requires a tissue factor from the surface of extra vascular cells. The final stages of both pathways are common, and involve the proteolytic activation of thrombin which initiates the formation of a fibrin clot[4-6]. Existing cardiovascular drugs have a number of side effects some of them has been summarized as under:

- Hypotension: need to get the dose right, especially in combination with diuretics.
- Dry cough: probably due to bradykinin - use ATII receptor antagonists instead.
- Renal failure if the renal arteries are partially blocked.

- Hyperkalaemia: complication of renal failure.
- Rashes, taste disturbance, neutropenia, proteinuria - seen mainly at high doses, may result from the sulphydryl group.

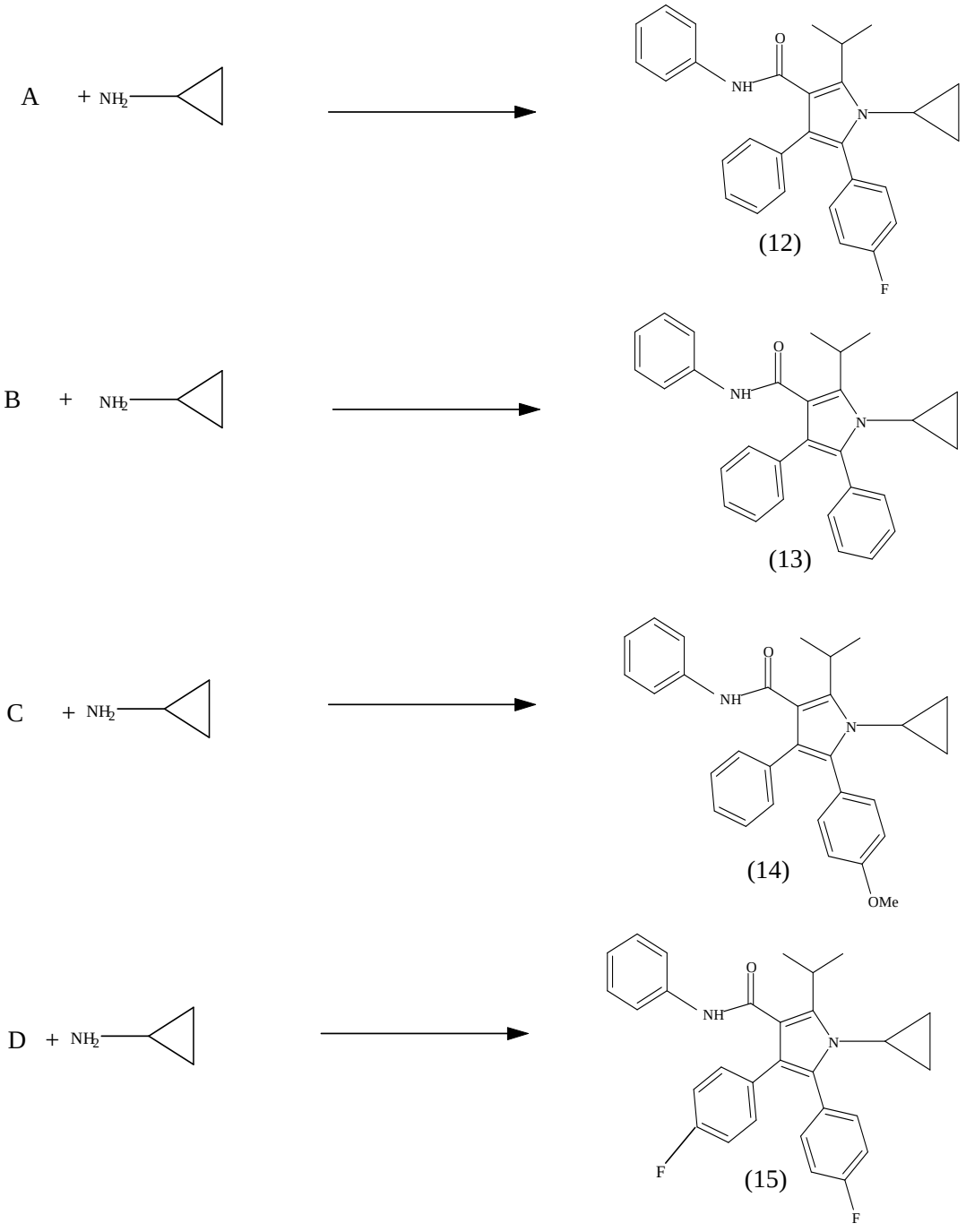
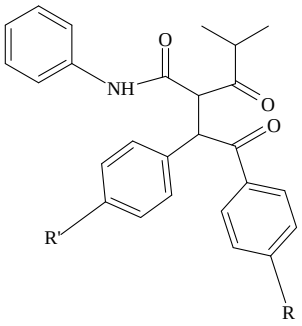


Figure 1: Structure of the compounds, synthesized

In view of above it is quite desirable to develop a cardiovascular: anti coagulating drug which should be in approach of general public and does not any significant side effect. In light of above the present paper deals with the synthesis

and characterization of derivatives of active precursor for an established cardiovascular moiety called Atorvastatin. The structure of the newly synthesized compounds has been shown in the figure 1.



A: R = F, R' = H
B: R = H, R' = H
C: R = OCH_3, R' = H
D: R = F, R' = F

MATERIALS AND METHODS

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 C-18 column of 250 mm length and 5 µm silica particles. Column temperature was maintained at 40 °C and samples were preserved at 8 °C.

RESULTS AND DISCUSSION

A. Synthesis:

a. Synthesis of 2-(4-fluoroPhenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H pyrrole-1-Cyclopropane (12):

A solution of 4-Fluoro-α-methyl-1-oxopropyl]γ-oxo-N-β-diphenylbenzenebutanemide (1 g, 0.0024 mol), cyclopropyl amine (0.0026 g, mol) and pivalic acid in 6ml cyclohexane were refluxed at 72 ºC (±3 °C). During refluxing water was removed azeotropically. Reaction was monitored by TLC (hexane: ethyl acetate 7:3). After completion of reaction 3 ml of 10 % sodium bicarbonate solution was added at 50-55 ºC and stirred well for 30 min. separated 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 light brown viscous oil. Degassed mass was dissolved in 5ml of isopropyl alcohol at 70-75 ºC. The solution was then gradually cooled down to 20-25 ºC. The resulting light yellow precipitate was filtered, washed with isopropyl alcohol and dried in *vacuo* to produce analytically pure product.

b. Synthesis of 5-(1-methylethyl)-2,3-biphenyl-4-[phenylamino)carbonyl] -1H pyrrole-1-Cyclopropane (13):

A solution of 4-Fluoro-α-methyl-1-oxopropyl]γ-oxo-N-β-diphenylbenzenebutanemide (1 g, 0.0024 mol), cyclopropyl amine (0.0026 g, mol) and pivalic acid in 6ml cyclohexane were refluxed at 83 ºC (±3 °C). During refluxing water was removed azeotropically. Reaction was monitored by TLC (hexane: ethyl acetate 7:3). After completion of reaction 3 ml of 10 % sodium bicarbonate solution was added at 50-55 ºC and stirred for 30 min. separated 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 yellowish viscous mass. Degassed mass was then dissolved in 5 ml of isopropyl alcohol at 70-75 ºC. The solution was then gradually

cooled down to 20-25ºC. The resulting pale yellow precipitate was filtered, washed with isopropyl alcohol and dried in *vacuo* to produce analytically pure product.

c. Synthesis of 2-(4-methoxyphenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H pyrrole-1-Cyclopropane (14):

A solution of 4-Fluoro-α-methyl-1-oxopropyl]γ-oxo-N-β-diphenylbenzenebutanemide (1 g, 0.0024 mol), cyclopropyl amine (0.0026 g, mol) and pivalic acid in 6ml cyclohexane were refluxed at 75 ºC (±3 °C). During refluxing water was removed azeotropically. Reaction was monitored by TLC (hexane: ethyl acetate 7:3). After completion of reaction 3 ml of 10 % sodium bicarbonate solution was added at 50-55 ºC and stirred well for 30 min. separated 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 oily mass. This oily mass was then degassed under vacuum. Degassed mass was dissolve in 5 ml of isopropyl alcohol at 70-75 ºC. The solution was then gradually cooled down to 20-25ºC. The resulting light yellow precipitate was filtered, washed with isopropyl alcohol and dried in *vacuo* to produce analytically pure product.

d. Synthesis of 2, 3-(4, 4-difluoro biphenyl)-5-(1-methylethyl)-4-[(phenyl amino) carbonyl]-1H pyrrole-1-Cyclopropane (15):

A solution of 4-Fluoro-α-methyl-1-oxopropyl]γ-oxo-N-β-diphenylbenzenebutanemide (1 g, 0.0024 mol), cyclopropyl amine (0.0026 g, mol) and pivalic acid in 6ml cyclohexane were refluxed at 78 ºC (±3 °C). During refluxing water was removed azeotropically. Reaction was monitored by TLC (hexane: ethyl acetate 7:3). After completion of reaction 3 ml of 10 % sodium bicarbonate solution was added at 50-55 ºC and stirred well for 30min. separated 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 off white floppy mass. Degassed mass was then dissolved in 5 ml of isopropyl alcohol at 70-75 ºC. The solution was then gradually cooled down to 20-25 ºC. The resulting light off white precipitate was filtered, washed with isopropyl alcohol and dried in *vacuo* to produce analytically pure product.

Table 1: Spectroscopic results of Compound # 12:

IR absorption band (ν cm^{-1})	**Group**
3381	N–$H_{str.}$
3081,3057	C–H_{str} (sp^2 Ar)
2965,2873	C-H_{str} (Methyl)
2928,2828	C-H_{str} (Methylene).
1660	C=$O_{str.}$ (Amide)
1602,1593	C=C_{str} (Ar)
1557,1525	C=C_{str} (Alkenes conjugated)
756,735+698	Phenyl (Mono subs. benzene)
^{1}H-NMR spectra (δ ppm)	**Protons**
0.52,0.75	2s, 4H (CH_2)
1.5	d, 6H (CH_3)
3.1	s, 1H (CH, isopropyl)
3.7	s, 1H (NH, amide)
6.7-7.2	m, 15H (Ar)
Mass: (M^+ m/z): 439 (M+1), 438(M^+, base peak), 346, 305	

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

B. Characterization:

All derivatives, synthesized and reported here, have been characterized by using modern spectroscopic techniques and physicochemical analysis tools[7-12]. Spectroscopic results support the proposed structure of the compounds [13-19]. The analytical data (IR and NMR) for compound # 12 to 15 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 98.83 to 99.32 % pure (Qualitatively) and the level of single highest most impurity was observed 0.16 to 0.47 %. Summarized results of HPLC analysis has been presented in table – 6.

Table 2: Spectroscopic results of Compound # 13:

IR absorption band (ν cm^{-1})	**Group**
3412	N–$H_{str.}$
3059,3003	C–H_{str} (sp^2 Ar)
2957,2867	C-H_{str} (Methyl)
2923	C-H_{str} (Methylene).
1667	C=$O_{str.}$ (Amide)
1652,1646	C=C_{str} (Ar)
1622,1615	C=C_{str} (Alkenes conjugated)
1257,1221	C-F_{str}
845,837	p-subst. benzene
756,735+698	Phenyl (Mono subs. benzene)
^{1}H-NMR spectra (δ ppm)	**Protons**
0.835,0.851	2s, 4H (CH_2)
1.5	d, 6H (CH_3)
3.2	s, 1H (CH, isopropyl)
3.8	s, 1H (NH, amide)
6.8-7.3	m, 14H (Ar)
Mass: (M^+ m/z): 4 22 (M+1), 421 (M^+, base peak), 260, 102	

Table 3: Spectroscopic results of Compound # 14

IR absorption band (ν cm^{-1})	**Group**
3393	N–$H_{str.}$
3074,3035	C–H_{str} (sp^2 Ar)
2960,2869	C-H_{str} (Methyl)
2925	C-H_{str} (Methylene).
1662	C=$O_{str.}$ (Amide)
1618,1601	C=C_{str} (Ar)
1245	C-O_{str}
857,840	p-subst. benzene
766,732+703,699	Phenyl (Mono subs. benzene)
^{1}H-NMR spectra (δ ppm)	**Protons**
0.65,0.83	2s, 4H (CH_2)
1.5	d, 6H (CH_3)
3.15	s, 1H (CH, isopropyl)
3.7	s, 1H (NH, amide)
3.8	s, 3H (C-O Methoxy)
6.8-7.3	m, 14H (Ar)
Mass: (M^+ m/z): 451 (M+1), 450 (M^+, base peak), 332, 102	

Table 4: Spectroscopic results of Compound # 15:

IR absorption band (ν cm^{-1})	**Group**
3387	N–$H_{str.}$
3066,3017	C–H_{str} (sp^2 Ar)
2963,2870	C-H_{str} (Methyl)
2928	C-H_{str} (Methylene)
1655	C=$O_{str.}$ (Amide)
1648,1603	C=C_{str} (Ar)
1250	C-O_{str}
847,834	p-subst. benzene
758,728+710,688	Phenyl (Mono subs. benzene)
^{1}H-NMR spectra (δ ppm)	**Protons**
0.63,0.78	2s, 4H (CH_2)
1.5	d, 6H (CH_3)
3.1	s, 1H (CH, isopropyl)
3.7	s, 1H (NH, amide)
6.7-7.4	m, 13H (Ar)
Mass: (M^+ m/z): 457 (M+1), 456(M^+, base peak), 350, 120	

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
$C_{29}H_{27}N_2OF$ (# 12)	438	DMF	Light Yellow	210	64	439	79.31 (79.45)	6.09 (6.16)	6.48 (6.39)
$C_{29}H_{28}N_2O$ (# 13)	420	DMF	Pale Yellow	203	69	422	80.74 (82.86)	6.77 (6.67)	6.52 (6.67)
$C_{30}H_{30}N_2O_2$ (# 14)	450	DMF	Light Yellow	218	88	451	80.09 (80.00)	6.75 (6.67)	6.10 (6.22)
$C_{29}H_{26}N_2OF_2$ (# 15)	456	DMF	Off White	197	72	457	76.20 (76.32)	5.61 (5.70)	6.19 (6.14)

Table 6: HPLC analysis data of the compounds

Sr. No. #	Compound	Purity by HPLC (%)	Single highest most impurity (%)	Total impurity (%)
1.	# 12	99.32	0.16	0.68
2.	# 13	99.04	0.31	0.96
3.	# 14	98.83	0.47	1.17
4.	# 15	98.92	0.28	1.08

Biological Screening

The entire compound has also been evaluated against several species of bacteria and different 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^{o}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 11-13, 10-12, 15-18 and 06-08 µg/ml for compound # 12 to 15, respectively, table 7. After drying it was placed on nutrient agar plates[23-24]. The inhibition areas were observed after 48h. 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 # 15 shows maximum inhibition capacity and the compound # 14 has minimum inhibition capacity among the group, reported here, Fig. 2.

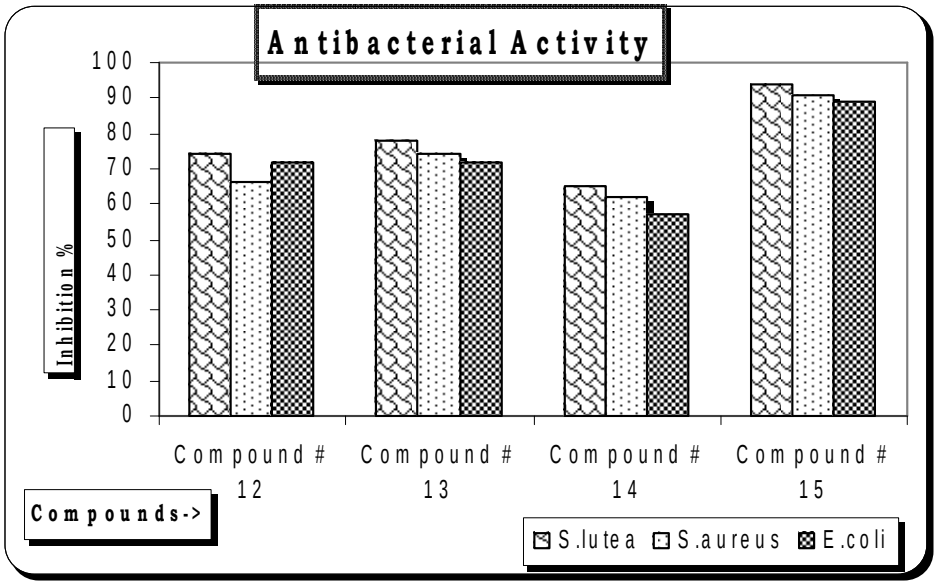


Fig. 2: Antibacterial action of the compounds

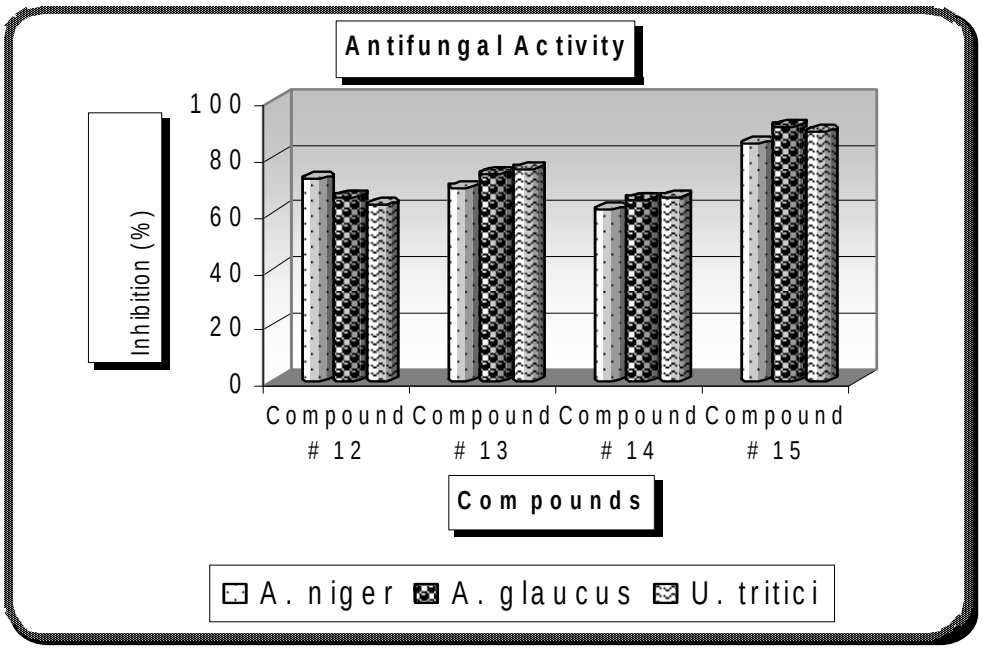


Fig. 3: Fungal growth inhibition capacity of the compounds

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 = 13-17, 14-15, 12-14 and 07-09 µg/ml for compound # 12 to 15, 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 48-72 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 # 14 and a maximum by compound # 15 for all the species, under study, Fig. 3.

Table 7: Antibacterial activity data of the compounds

Compound	Bacterial Inhibition % (MIC in µg/ml)		
	Sarcina lutea	*Staphylococcus aureus*	*Escherchia coli*
# 12	++++ (11)	++++ (13)	++++ (12)
# 13	++++ (10)	++++ (11)	++++ (12)
# 14	+++ (15)	+++ (16)	+++ (18)
# 15	++++ (06)	++++ (07)	++++ (08)

Table 8: Antifungal activity data of the compounds:

Compounds	Fungal Inhibition %(MIC in µg/ml)		
	Aspergillus niger	*Aspergillus glaucus*	*Ustilago tritici*
# 12	**** (13)	**** (15)	**** (17)
# 13	**** (15)	**** (14)	**** (14)
# 14	*** (14)	*** (12)	*** (12)
# 15	**** (09)	**** (07)	**** (08)

Acknowledgement
We are thankful to the Principal, Zakir Husain College for providing laboratory facilities and department of chemistry Jiwaji University Gwalior for providing instrumental support in characterization of compounds.

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