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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL SCHIFF BASES OF CEPHALEXIN

N.T. Arun*[1] and B. Gowramma [2]

Anna University – BIT Campus [1], Trichy -24, Tamil Nadu, India
Department of Pharmaceutical Chemistry, JSS College of Pharmacy [2], Ooty, Tamil Nadu, India

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Correspondence to Author:

N.T. Arun

Anna University – Bit Campus, Tiruchirappalli, Tamil Nadu – 620 024, India

E-mail: arunyuvant@gmail.com

ABSTRACT: Cephalexin remain as one of the most versatile class of compounds against microbes and therefore, are useful substructures for further molecular exploration. A novel series of Schiff bases of cephalexin were synthesized. Thus condensation of 5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-[(amino phenyl acetyl) amino]-3-methyl-8-oxo, monohydrate with appropriate aldehydes afforded the Schiff bases. The purity of the compounds was determined by TLC. With elemental analysis and spectral data the structure of the synthesized compounds were elucidated. All the synthesized compounds were subjected for the screening of antimicrobial activity against 3 bacteria and two pathogenic fungi. Most of the synthesized compounds were found to possess moderate antibacterial and antifungal activity.

INTRODUCTION: The wide use of antibiotics in man and animals and their extensive use in areas other than the treatment and prophylaxis of disease have resulted in a serious problem of drug resistance.

More and more bacterial strains have become resistant to available drugs. Preparation of different synthetic derivatives of antibiotics based on structure-activity relationship has been one of the best approaches. A relation between the structure of the complexes and their anti-bacterial activity can be observed.

Cephalexin nucleus was reported to have significant antibacterial and antifungal activities.

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Cephalexin is employed in infections (caused by penicillin resistant Staphylococci, Klebisella species), respiratory tract infections (acute and chronic bronchitis & infected bronchiectasis) and ear, nose & throat infections (acute and chronic) and urinary tract infections resistant to penicillin and sulphonamides.

So it has been planned to synthesize some newer Schiff bases of Cephalexin. Since antimicrobial activities (antibacterial and antifungal) had not been reported so far to our titled compounds, these activities can be evaluated [1-9].

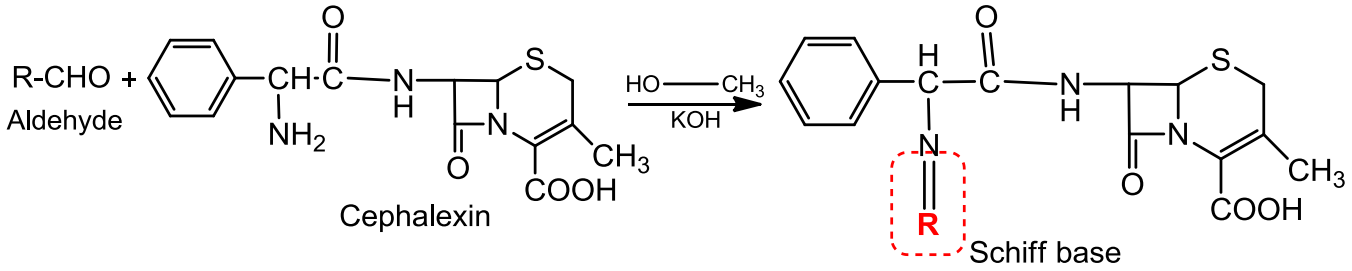
MATERIALS AND METHODS: Solvents and chemicals of laboratory and analytical were purchased from S.D. Fine Chemicals Ltd., India, reagents were purified and dried according to the procedure given in Vogel's text book of practical organic chemistry.

All the glassware were washed with distilled water and dried at 110^{o}C before commencing and during the reaction.

Chemistry: The reaction sequence for the different title compounds is outlined in the scheme. The Cephalexin (5 mmol) dissolved in methanol (50 ml) was mixed with the substituted aldehyde dissolved in methanol (50 ml). To this potassium hydroxide (0.1% in methanol) was added to adjust the pH of the solution between 7-8 and the mixture was refluxed for 30 minutes (approx.). A clear solution was obtained. The Schiff base was isolated by pouring the clear solution into crushed ice and the resulting solid was filtered and crystallized from DMF (60%) [10-11].

The crystalline product was dried under vacuum and kept in a desiccator till further use. The physical and analytical characteristics of the synthesized compounds were presented in **Table 1**. The purity of the compounds was determined by TLC and the structures of all the derivatives were confirmed by spectral data [12] and elemental analysis and they were in full agreement with the proposed structures.

SCHEME:



Synthetic pathway for the preparation of Schiff base (I-VIII)

Compound	R
I	C_7H_6
II	C_9H_8
III	C_5H_4O
IV	C_8H_8O
V	C_7H_5Cl
VI	C_7H_6O
VII	$C_8H_8O_2$
VIII	$C_9H_{11}N$

TABLE 1: PHYSICAL AND ANALYTICAL DATA OF SCHIFF BASES OF CEPHALEXIN

Compound	R	Molecular formula	Molecular weight	Melting point	Percentage yield	R_f value
I.	C_7H_6	$C_{23}H_{21}N_3O_4S$	435.50	156-161	65	0.68
II.	C_9H_8	$C_{25}H_{23}N_3O_4S$	461.53	125-130	96	0.50
III.	C_5H_4O	$C_{21}H_{19}N_3O_5S$	425.46	85-90	68	0.65
IV.	C_8H_8O	$C_{24}H_{23}N_3O_5S$	465.52	110-115	80	0.70
V.	C_7H_5Cl	$C_{23}H_{20}ClN_3O_4S$	469.94	125-130	85	0.64
VI.	C_7H_6O	$C_{23}H_{21}N_3O_5S$	451.49	120-125	78	0.71
VII.	$C_8H_8O_2$	$C_{24}H_{23}N_3O_6S$	481.52	170-185	65	0.73
VIII.	$C_9H_{11}N$	$C_{25}H_{26}N_4O_4S$	478.56	185-195	62	0.64

Biological Screening: The synthesized compounds were subjected to antimicrobial screening by cup plate method. The zone of inhibition was measured in millimeters [10].

The antibacterial activity was checked in nutrient agar medium against *Bacillus subtilis* (gram +ve), *Klebisella pneumonia (gram -ve)* and *Pseudomonas aeruginosa* (gram –ve) bacteria at the concentration of 25, 50 and 100 µg/well. The activity was determined after incubation for 24h at 37^{o}C by the comparison of inhibition of growth of bacteria by measured concentrations of the compounds (I-VIII) with that of the activity produced by known concentration of the standard drugs (gentamycin and tetracycline) using methanol as both the solvent and the control.

No inhibition zone was observed in control (i.e., for methanol).

The antifungal activity of the compounds (I-VIII) was assayed against *Candida albicans*, (NCIM - 3102), *Aspergillus niger* (NCIM - 1196), *Sachromyces cerviaceae* (NCIM - 3193) in Sabouraud's dextrose agar medium with an incubation of 48h at 28^{o}C.

The zone of inhibition was determined at three different concentrations 25, 50 and 100 µg/well of the synthesized compounds (I-VIII). Amphotericin-B was used ad reference standard and methanol was used both as a solvent and as a control. No inhibition zone was observed in control (i.e., for methanol).

RESULTS AND DISCUSSION: All the synthesized compounds (I-VIII) were first analyzed by performing thin layer chromatography and also the melting point was determined. The structure of the compounds was confirmed with the IR spectral analysis and the analytical data showed satisfactory results.

Then, the synthesized compounds were subjected to antimicrobial screening against three bacteria (*Bacillus subtilis, Klebisella pneumonia and Pseudomonas aeruginosa)* shown in **Fig. 1-3** & 3 and two fungi (*Candida albicans and Aspergillus niger*) shown in **Fig. 4-5**.

ANTIBACTERIAL SCREENING

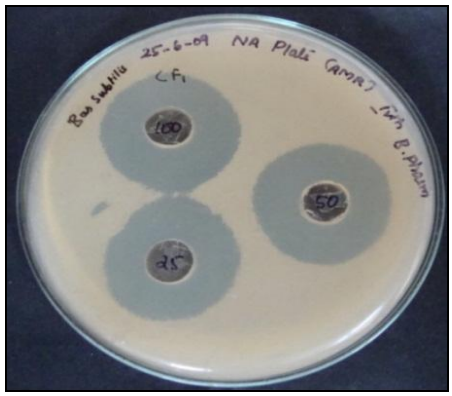


FIG. 1: ***BACILLUS SUBTILIS***

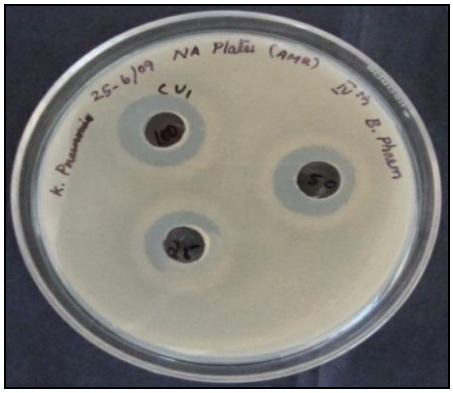


FIG. 2: ***KLEBISELLA PNEUMONIA***

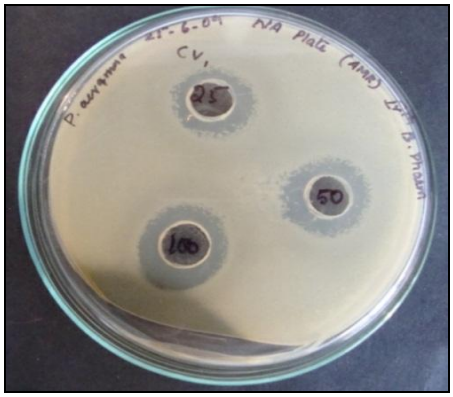


FIG. 3: ***PSEUDOMONAS AERUGINOSA***

ANTIFUNGAL SCREENING:

FIG. 4: ***SACHROMYCES CERVIACEAE***

FIG. 5: *CANDIDA ALBICANS*

Among the eight synthesized compounds, Compounds III**, IV, V** and **VII** showed good activity, whereas compounds **I, II, VI,** and **VIII** showed significant activity against *Bacillus subtilis.* Compounds **III, IV** and **V** were showed good activity, whereas compounds **I, II, VI, VII** and **VIII** showed significant activity against *Pseudomonas aeruginosa.* Compounds **III**, **IV** and **V** were showed good activity, whereas compounds **I**, **II**, **VI**, **VII** and **VIII** showed significant activity against *Klebisella pneumonia.*

The results of antibacterial activity screening of the tested compounds are depicted in **Table 2.** The results of antifungal activity of the tested compounds were found somewhat different from the antibacterial activity. In case of antifungal activity, compounds **I, III** and **VI** were showed mild activity against *Candida albicans*. All other titled compounds have not shown any activity against *Aspergillus niger*. The results of the antifungal of the tested compounds are summarized in **Table 3**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Compound	*Bacillus subtilis*			*Klebisella pneumonia*			*Pseudomonas aeruginosa*		
	Concentration (µg/well)			Concentration (µg/well)			Concentration (µg/well)		
	25	50	100	25	50	100	25	50	100
I	++	++	++	++	++	++	++	++	++
II	++	++	++	++	++	++	++	++	++
III	+	+	+	+	+	+	+	+	+
IV	+	+	+	+	+	+	+	+	+
V	+	+	+	+	+	+	+	+	+
VI	++	++	++	++	++	++	++	++	++
VII	+	+	+	++	++	++	++	++	++
VIII	++	++	++	++	++	++	++	++	++
Methanol	-	-	-	-	-	-	-	-	-
Tetracycline	++	++	++	++	++	++	++	++	++
Gentamycin	++	++	++	++	++	++	++	++	++

Symbols: + good activity, ++ significant activity, – no activity

TABLE 3: ANTIFUNGAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Compound	*Candida albicans*			*Aspergillus niger*			*Sachromyces cerviaceae*		
	Concentration (µg/well)			Concentration (µg/well)			Concentration (µg/well)		
	25	50	100	25	50	100	25	50	100
I	++	++	++	-	-	-	++	++	++
II	+	+	+	-	-	-	+	+	+
III	++	++	++	-	-	-	++	++	++
IV	+	+	+	-	-	-	+	+	+
V	+	+	+	-	-	-	+	+	+
VI	++	++	++	-	-	-	++	++	++
VII	+	+	+	-	-	-	+	+	+
VIII	+	+	+	-	-	-	+	+	+
Methanol	-	-	-	-	-	-	-	-	-
Amphotericin-B	+++	+++	+++	+++	+++	+++	+++	+++	+++

Symbols: + very mild activity, ++ mild activity, +++ significant activity, – no activity

CONCLUSION: Summarizing a series of novel Schiff bases of cephalexin have been synthesized successfully in appreciable yields and screened for their *in vitro antimicrobial activity* against bacterial strains (*Bacillus subtilis, Klebisella pneumonia & Pseudomonas aeruginosa*) and fungal strains (*Candida albicans and Aspergillus niger*).

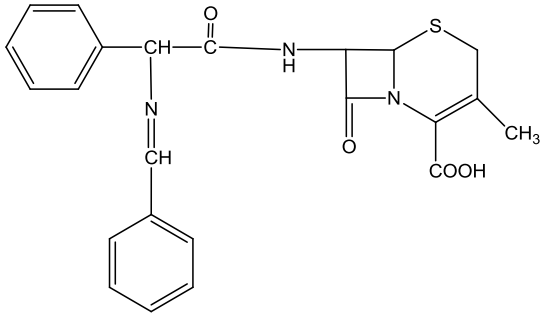
It is concluded that the synthesized compounds were found to possess moderate antibacterial and antifungal activity.

EXPERIMENTAL SECTION:

Physical measurements: The reactions and the purity of the products were monitored by TLC (Precoated- Merck G_{254}) using Methanol: Chloroform as mobile phase and UV chamber method and iodine as visualizing agents. Further the compounds were purified by recrystallization using suitable solvents. The melting point of the synthesized compounds were determined in open capillaries using Veego VMP-1 Apparatus and expressed in $^{\circ}C$ and are uncorrected.

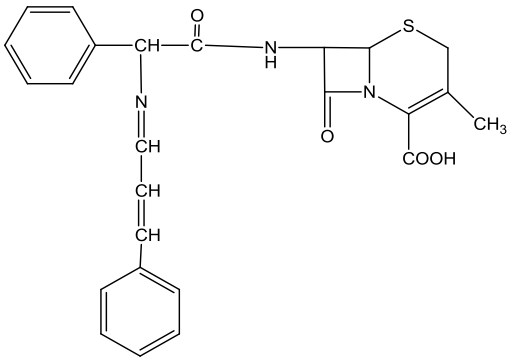
Spectral measurements: The IR spectra of the synthesized compounds were recorded on Shimadzu FT-IR Spectrophotometer using KBr pellets and are expressed in cm^{-1}. The Nuclear magnetic spectra (^{1}H-NMR) of the synthesized compounds were obtained from Bruker DRX-300 (300 MHz FT-NMR) Spectrophotometer using $CDCl_3$ as solvent with TMS as an internal standard.

Analytical data:



7-(2-(benzylideneamino)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid

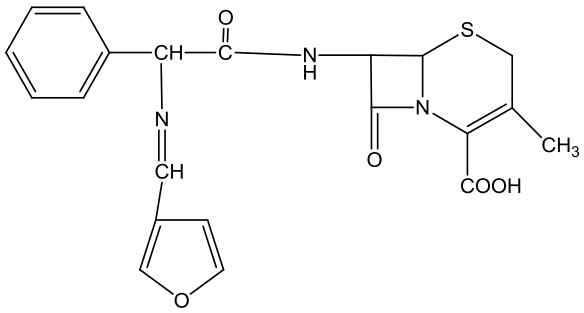
I*R* spectrum (KBr, in cm^{-1}) of the test compound **I** showed absorption band at: 1680(C=O), 1494(C=N), 1454 (C-H), 1340 (C=N), 698(Ar-CH), 3288 (OH), 2359 (C-S-C), 1205 (C=O).



3-methyl-8-oxo-7-(2-phenyl-2-(3-phenylallylideneamino)acetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

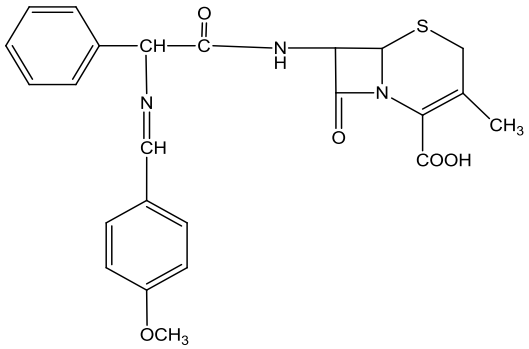
I*R* spectrum (KBr, in cm^{-1}) of the test compound **II** showed absorption band at: 3028 (C-H), 1674 (C-O), 1674 (C=N), 3236 (OH), 1450 (CH-Ar), 1494 (Ar hydrocarbon), 1282 (C-NH), 1124 (C=S), 698 (Ar-CH).

The ^{1}H-NMR spectrum (DMSO, in δ ppm) of the test compound **II** exhibited characteristic proton peaks at: 6.8 (10H, Ar, M), 8.8 (1H, NH, d), 3.9(1H, CH, q), 7.5(3H, CH-CH=CH, s), 2.7(3H, CH_3, s), 3.2 (2H,s), 9.7 (1H, COOH,s).



7-(2-(furan-3-ylmethyleneamino)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

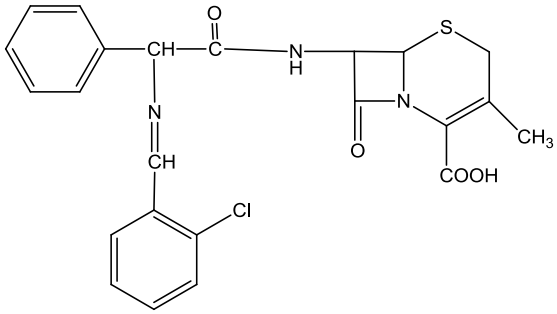
I*R* spectrum (KBr, in cm^{-1}) of the test compound **III** showed absorption band at: 3061(Furan CH-S), 1681(C=O), 1437 (Ar CH), 1012 (Alkane C-C), 744(Ar-CH), 698(Ar-CH).



7-(2-(4-methoxybenzylideneamino)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid

I*R* spectrum (KBr, in cm^{-1}) of the test compound **IV** showed absorption band at: 2931(Alkane CH), 1678(C=O), 1600 (C=C), 1510 (C-N), 1303(C-N), 1253 (C-O), 1028 (C-S), 835 (C-C), 700 (Ar-CH).

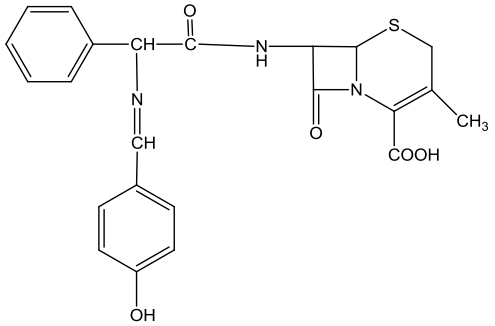
The ^{1}H-NMR spectrum (DMSO, in δ ppm) of the test compound **IV** exhibited characteristic proton peaks at: 6.7(8H, ArH M), 5.5(1H, CH s), 9.9(1H, COOH, s), 3.7(2H, CH_2 s), 3.5 (3H, OCH_3, s), 2(3H, CH_3 s), 6.7(1H, s), 8.7(1H, OH, d), 8.4(1H, CH, d), 4.9(1H, CH, q).



7-(2-(2-chlorobenzylideneamino)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

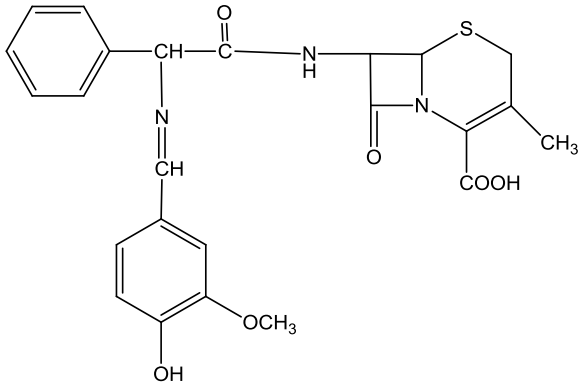
I*R* spectrum (KBr, in cm^{-1}) of the test compound **V** showed absorption band at: 1681(C=O), 1593(C=C), 1533(C-H), 1301(C-N), 1282 (C-NH), 1051(C-O-C), 698 (Ar-CH).

The ^{1}H-NMR spectrum (DMSO, in δ ppm) of the test compound **V** exhibited characteristic proton peaks at: 6.5(9H, ArH, M), 5.5(1H, CH, s), 8.8(1H, NH, d), 4.9(1H,CH, q), 8.4(1H, CH, d), 2.5 (3H, CH_3, s), 3.3 (2H, CH_2 ,s), 10.4 (1H, COOH, s),4(1H, CH, s).



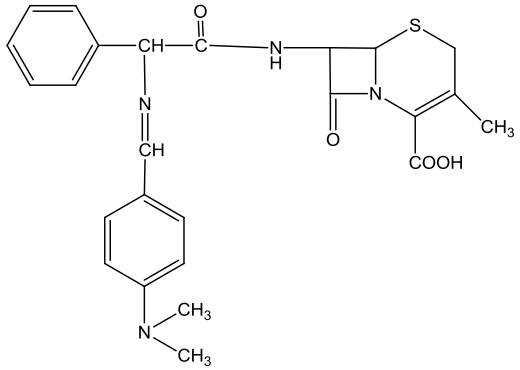
7-(2-(4-hydroxybenzylideneamino)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

I*R* spectrum (KBr, in cm^{-1}) of the test compound **VI** showed absorption band at: 3246 (NH), 1666 (C=O), 1600 (C-C), 1437(C-H), 1282(C-O), 1240(CH_3O), 1030(C-S), 839(C-C), 698(Ar-CH).



7-(2-(4-hydroxy-3-methoxybenzylideneamino)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

I*R* spectrum (KBr, in cm^{-1}) of the test compound **VII** showed absorption band at: 2359(C-S), 1670(C=O), 1591(C-C), 1512 (C-N), 1288 (C-N), 1028 (C-S), 698 (Ar-CH).



7-(2-(4-(dimethylamino)benzylideneamino)-2-phenylacetamido) -3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

I*R* spectrum (KBr, in cm^{-1}) of the test compound **VIII** showed absorption band at: 2914(C-H), 1602(N-H), 1550(C-N), 1535(C=N), 1446(C-H), 1064(C=S), 729 (Ar-CH).

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