NEWER CARBOSTYRIL DERIVATIVES FOR ANTIBACTERIAL ACTIVITY

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Summary

Compounds having carbostyril (2-quinolones) moiety are associated with interesting biological activities. In the present study, we synthesized Schiff bases of 7-amino-4-methyl carbostyrils and their antibacterial activity was evaluated by agar diffusion method. The 7-amino-4-methyl carbostyrils were prepared by reacting *m*-phenylene diamine and β-ketoester by the Conard-Limpach synthesis. Schiff bases of 7-amino-4-methyl carbostyrils (**1** to **10**) were prepared by reacting 7-amino-4-methyl carbostyril with substituted aromatic aldehydes. The final test compounds were purified and characterized by the UV, IR, ^{1}HNMR and Mass Spectral studies. They were evaluated for antibacterial activity. Four test compounds, such as **3**, **6**, **8** and **9** were active against *Bacillus subtilis* while Amoxicillin and Gentamycin were used as standards.

Key words: Carbostyrils; 2-Quinolones; Antibacterial; Schiff base

Introduction

The inhibition of bacterial DNA gyrase has been the target of a world wide research effort which began with the discovery of Nalidixic acid in the early 1960 [1]. Structure activity relationships (SAR) of compounds based on Nalidixic acid have led to a large group of synthetic antibacterial agents known collectively as the quinolones. The compounds prepared early in this effort such as Oxolinic acid, Rosoxacin and Pipemidic acid were most active against gram negative bacteria [2]. Introduction of a fluorine atom at the 6th position of the quinolone ring system led to Norfloxacin, which had a broad spectrum antibacterial activity [3]. Newer members of this family include Ciprofloxacin, Ofloxacin, Enoxacin, Lomefloxacin, Fluroxacin and Temafloxacin.

The ketone or hydroxy group in quinolone is completely in the carbonyl tautomeric form. For all practical purposes quinolone tautomeric form is suggested. The carbostyrils frame work is often found in quinoline alkaloids isolated from the Rutaceae family [4]. Other naturally occurring 2-quinolones such as Arboricine and Cinchonine alkaloids and their synthesis were reported. Hydrazones containing carbostyril and quinoline have been found to exhibit parasiticidal, tuberculostatic, anti-bacterial, antiviral, anticancer, anti-inflammatory, antifungal, antidiabetic, cardiotonic, diuretic and bronchodilator activsity [5-9]. 2-quinolones, the derivatives of carbostyrils are capable of inhibiting the bacterial DNA gyrase enzyme [10]. DNA gyrase enzyme controls the linking numbers of double stranded DNA molecule in Gram negative and Gram positive bacteria. Even though natural quinolones are highly potent, attempts have to be made to improve their stability, solubility, efficacy and kinetics.

Materials and methods

Chemicals

The chemicals used were of AR grade and LR grade, purchased from Loba Chemicals, Qualigens, NR Chemicals, Lancaster, Sigma, Reachem, S.D Fine Chemicals Ltd., Merck and Hi-Media.

Synthesis

Melting points were determined using melting point apparatus (Shital Scientific industries) and were uncorrected. The reactions were monitored on thin layer chromatography (TLC) and Rf value was determined by pre-coated aluminum plates (Merck Kieselghur). λ_{max}, ϵ_{max} for the synthesized compounds were established by UV- visible spectrometer (Shimadzu UV-Vis Spectrophotometer 1601). The log P value was carried out as it was explained in the literature [11-13]. The infrared (IR) spectra were recorded on FT-IR (Shimadzu FTIR 8700) spectrophotometer. ^{1}HNMR was recorded on 400 MHz spectrometer (AMX 400) and the mass spectra were recorded at 70eV on Sciex 3000 LC-MS-MS (Applied Biosystems, Canada), at Indian Institute of Science, Bangalore.

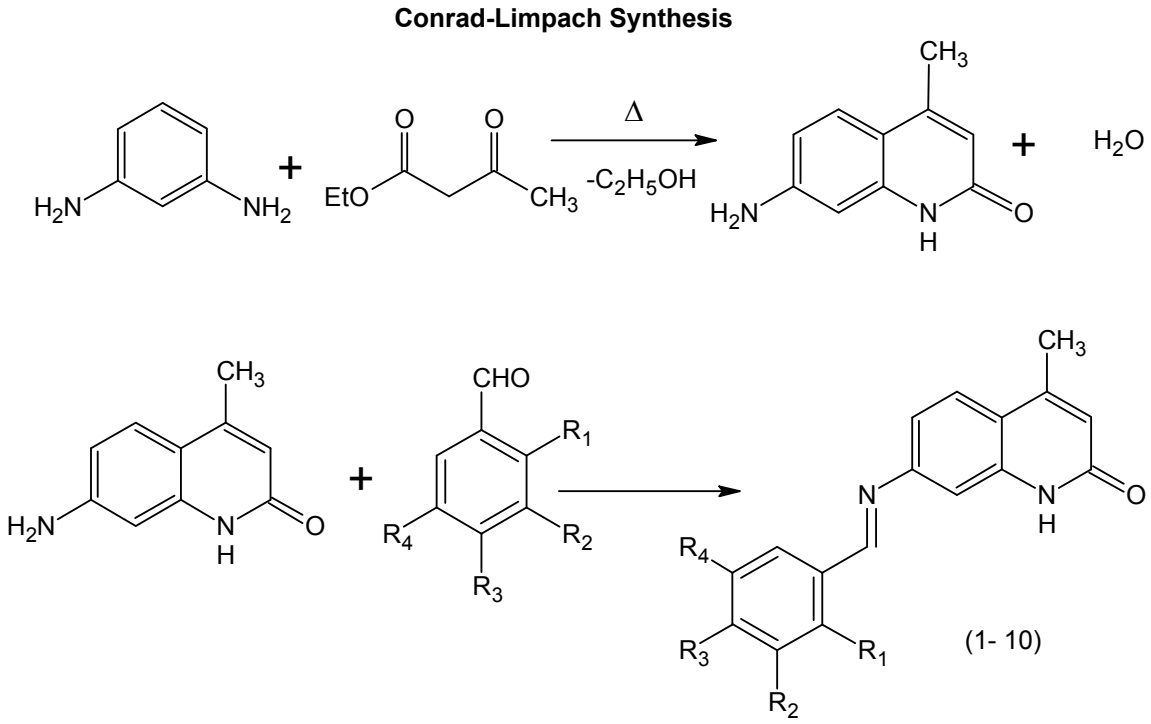
Preparation of starting material 7-amino-4-metyl carbostyril [14]

Equimolar concentration of *m*-phenylene diamine and ethylacetoacetate were mixed and heated together in a flask. The reaction was heated at 150°C for 18 h on an oil bath. At the end of reaction period, 100 to 200 ml of water was added to the flask. The contents were heated on a hot plate to the boiling temperature of the water. The mixture was then chilled, filtered and the precipitate dried in air. The compound was recrystallised from methanol.

Preparation of Schiff bases of 7-amino-4-methyl carbostyril

To a solution of 0.01 moles of 7-amino-4-methyl carbostyril in 30ml of ethanol and 0.01 mol of substituted benzaldehyde was added and refluxed for 3 h. The reaction mixture was allowed to cool to room temperature and then poured into 100 ml of ice cold water. The solid formed was filtered and washed twice with 30 ml of cold water, dried and recrystallised from aqueous ethanol.

Fig. 1: Scheme of synthesis



Compound	R_1	R_2	R_3	R_4
1	H	H	H	H
2	H	H	Cl	H
3	H	OCH_3	OCH_3	OCH_3
4	H	NO_2	H	H
5	H	H	CH_3	H
6	H	OCH_3	H	OCH_3
7	NO_2	H	H	H
8	H	H	NO_2	H
9	H	CH_3	H	H
10	Cl	H	H	H

Determination of antibacterial activity

All the ten synthesized test compounds were tested against four species of bacteria namely, *Bacillus subtilis* (gram positive) *Staphylococcus aureus* (gram positive) *Escherichia coli*, (gram negative) *Pseudomonas aeruginosa* (gram negative). Stock solutions of synthesized test compounds and standard drugs were prepared in 0.1% W/V of dimethylsulfoxide (DMSO). The test compounds were used at a concentration of 500µg/0.05ml. Amoxicillin and Gentamycin were used as standards at a concentration of 10µg /0.05ml and at a concentration of 5µg/0.05ml respectively. Mueller-Hinton agar medium was used for the agar diffusion method [15-16]. The inoculums were added to the medium and were poured into sterile petridishes for solidifying. Wells (bores) were made on the medium using sterile borer after solidification. 0.05 ml of the test and standard solutions were added to the respective bores. A control having only DMSO was maintained in each plate. The petridishes were kept at room temperature for 30 minutes for diffusion to take place and then incubated at 37°C for 24 hours. The zone of inhibition was measured using a scale (mm).

Results and discussion

Synthesis and the spectral studies

The starting material for the synthesis of 7-amino-4-methyl carbostyril *m*-phenylene diamine was treated with β-ketoester (ethyl-acetoacetate) at high temperature to get 7-amino-4-methyl carbostyril which was purified by recrystalisation from methanol, gave colorless needle shaped crystals and the purified test compound was checked by its melting point and thin layer chromatography. The structure was established by IR, ^{1}H-NMR and MS studies (yield = 53-54%).

7-amino-4-methyl carbostyril was refluxed with equal moles of different substituted benzaldehydes in presence of ethanol gave substituted 7- amino-4-methyl carbostyril. The crude test compounds were purified by recrystalisation from methanol. The purity of test compounds was checked by melting point and thin layer chromatography. The structures of test compounds were established by IR spectral studies (Yields 70-85%).

IR spectra of the 7-amino-4-methyl carbostyril showed characteristic band at 3423 and 3307 cm^{-1} due to NH_2 (primary amine), C=O stretching was observed at low frequency between 1658 cm^{-1} and this presumably due to carbonyl group tendency to conjugate with the double bond and inductive effect of nitrogen, indicating the formation of the 7-amino-4-methyl carbostyril. The absence of doublet of primary amine N-H stretching and presence of C=N stretching indicated completion of reaction and formation of Schiff bases of 7-amino-4-methyl carbostyril (**1** to **10**) conformed by IR spectra. C=N stretching observed at lower wave number due to presence of the electronegative groups like chloro, nitro and methoxy (**2, 3, 4, 5, 6, 7, 8, 9** and **10**). When these groups were present in *para* position of aryl molecule (**2** and **8**), it cause mesomeric effect on the double bond system of nitro group and the N=O stretching was observed at higher wave number (**8**). This effect was not shown in *meta* position (**4**), so it observed at lower wave number. In case of methoxy derivatives (**3**, **6** and **9**), C-O stretching was shown at 1128.32 to 1022.2cm^{-1} and changed in C=N stretching.

The spectral details of the parent compound 7-amino-4-methyl carbostyril: IR **(**KBr) cm^{-1}: NH_2 (str) 3423, N-H (str) 2^o amide 3307, N-H (b) amide 1637, C=O str 1658, C=C str 1421, 1475, 1552, C-H (str) aromatic methyl 1369. ^{1}H NMR (300 MHz, DMSO): 11.143 (s, 1H, NH (a)), 7.338 (d, 1H, Ar-H (e)), 6.353 (d, 1H, Ar-H (d)), 5.948(s, 1H, Ar-H, (b)), 5.75 (s, 2H, NH_2 (f)), 2.275 (d, 3H, CH_3(c)). GCMS: 174 $[M]^+$.

The λ_{max} and ϵ_{max} for 7-amino-4-methyl carbostyrils were found to be in the range of 408-380 nm and 2.4 x 10^3 to 3.3 x 10^3 respectively (Table 1). The partition coefficient values were found to be in the range of 0.821 –1.816 (table-1).

Antibacterial activity

All the ten test compounds synthesized, purified and characterized were screened for their qualitative antibacterial activity. They were tested against four species of bacteria namely, *Bacillus subtilis* (Gram-positive), *Escherichia coli* (Gram-negative), *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* (Gram-positive). The technique used was agar diffusion method using 10 µg/0.05ml of Amoxicillin and Gentamycin as standard. Stock solutions of the synthesized compounds were prepared in DMSO. Table 2 shows the antibacterial activity of Schiff bases of 7-amino-4-methyl carbostyril.

Table 1: λ_{max} , ϵ_{max} and Log P values of Schiff bases of 7-amino-4-methyl carbostyrils

Compound	Mol formula	Mol Weight	Yield (%)	Melting Point (^oC)	λ_{max}	ϵ_{max}	log P
1	$C_{17}H_{147}O_2N_2$	278	81	>300	398	2406.55	0.85
2	$C_{17}H_{14}N_2O_2Cl$	313	73	272	383	2785.92	1.81
3	$C_{20}H_{20}N_2O_4$	352	78	250	395	2664.20	0.92
4	$C_{17}H_{13}N_3O_3$	307	79	>300	390	3041.42	0.88
5	$C_{18}H_{16}N_2O$	276	79	266	396	2613.44	1.15
6	$C_{19}H_{18}O_5N_2$	322	79	266	402	3043.45	0.99
7	$C_{17}H_{18}N_3O_3$	307	85	> 300	395	3256.52	0.91
8	$C_{17}H_{13}N_3O_3$	307	85	> 300	380	3379.43	1.74
9	$C_{17}H_{13}N_3O_3$	292	69	270	399	2823.54	0.94
10	$C_{17}H_{13}N_2OCl$	296	79	274	392	2442.31	1.46

Table 2: Antibacterial Activity of Schiff Bases of 7-amino-4-methyl Carbostyrils

Compound	Zone of inhibition (mm)*			
	B.s	***P.a***	***E.c***	***S.a***
1	10	08	09	14
2	12	07	10	15
3	24	12	15	25
4	11	07	09	11
5	09	09	06	10
6	23	12	10	26
7	08	05	06	06
8	20	10	03	22
9	21	12	09	24
10	09	06	07	09
Amoxicillin	28	29	35	32
Gentamycin	26	31	29	33

*B.s: *Bacillus subtilis,* P.a: *Pseudomonas aeruginosa,* E.c: *Escherichia coli,* S.a: *Staphylococcus aureus*

Test compounds, such as **3**, **6**, **8** and **9** showed activity against gram positive micro-organisms greater than gram negative organisms and other test compounds such as **1**, **2**, **4**, **5**, **7** and **10** showed lower activity. The compounds **3**, **6**, **8** and **9** showed the zone of inhibition 25, 26, 22 and 24 mm respectively against *Staphylococcus aureus*. The same derivatives showed zones of inhibition at 15, 10, 13 and 9 mm against *Escherichia coli* and 24, 23, 20 and 21mm against *Bacillus subtilis* respectively. All the ten test compounds showed less activity against *Pseudomonas aeruginosa*.

Conclusion

The yields of all substituted carbostyrils were found to be in the range of 65 to 80%. Log P, λ_{max}, and ϵ_{max} were established for the newly synthesized substituted carbostyrils. However,

owing to solubility problems pKa for the test compounds were not established. Out of the ten compounds tested, four compounds showed good antibacterial activity against both gram positive and gram negative organisms and these were closer to the antibacterial activity of standard antibiotics Amoxicillin and Gentamycin. This study showed that the carbostyrils are the good candidates for future antibacterial lead molecules.

References

1. von Rosenstiel N, Adam D. Quinolone antibacterials. An update of their pharmacology and therapeutic use. *Drugs* 1994; 47(6):872-901, Erratum in: *Drugs* 1994; 48(2):326.
2. Cooper CS, Klock PL, Chu DT, Hardy DJ, Swanson RN, Plattner JJ. Preparation and in vitro and in vivo evaluation of quinolones with selective activity against gram-positive organisms. *J Med Chem* 1992; 35(8):1392-1398.
3. Higgins PG, Fluit AC, Schmitz FJ. Fluoroquinolones: structure and target sites. *Curr Drug Targets*. 2003; 4(2):181-190.
4. *da*-Silva MF, Soares MS, Fernandes JB, Vieria PC. Alkyl, aryl, alkylarylquinoline, and related alkaloids. *Alkaloids Chem Biol* 2007; 64:139-214.
5. Pan XS, Fisher LM. DNA Gyrase and Topoisomerase IV Are Dual Targets of Clinafloxacin Action in Streptococcus pneumonia. *Antimicrob Agents Chemother* 1998; 42(11): 2810–2816
6. Raitio KH, Savinainen JR, Vepsalainen J, Laitinen JT, Poso A, Jarvinen T, Nevalainen T. Synthesis and SAR studies of 2-oxoquinoline derivatives as CB2 receptor inverse agonists. *J Med Chem* 2006; 49(6): 2022-2027.
7. Milecki J, Baker SP, Standifer KM, Ishizu T, Chida Y, Kusiak JW. Carbostyril derivatives having potent β-adrenergic agonist properties. *J Med Chem* 1987; 30(9):1563-1566
8. Leclerc G, Marciniak G, Decker N, Schwartz J. Cardiotonic agents. 1. Synthesis and structure-activity relationships in a new class of 3,4 and 5-pyridyl-2(1H)-quinolone derivatives. *J Med Chem* 1986; 29(12): 2427-2432.
9. Joseph B, Darro F, Behard A, Frydman A, Lesur B, Collignon F. 3-aryl-2-quinolone derivatives: Synthesis and characterization of in vitro and in vivo anti-tumor effects with emphasis on a new therapeutical target connected with cell migration. *J Med Chem* 2002; 45(12):2543-2555
10. Schechner M, Scirockin F, Stote RH, Dejaegere AP. Functionality maps of the ATP-binding site of DNA gyrase-B: Generation of a consensus model of ligand binding. *J Med Chem* 2004; 47(18):4373-4390
11. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry, 4th ed part-II. CBS Publishers and Distributors, 2005: 275-278.
12. Taylor PJ. Hydrophobic properties of drug in comprehensive medicinal chemistry, Vol IV, 1st Indian edition, Pergamon Press Elsevier, 2005: 270-273.
13. Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR, Vogel's Textbook of Practical Organic Chemistry. 5th ed. Pearson Education, 2004: 1074.
14. Woods LL, Fooladi M. 5-aroyl-(or -acyl)-4-hydroxycoumarins. J Org Chem. 1968; 33(7):2966-2968.
15. Atta-ur-Rahman, Choudhary MI, Thomsen WJ. Bioassay techniques for drug development, Harward academic publishers, 2001: 14-26.
16. Cruikshank R, Duguid JP, Marmion BP, Swain RH. Medical Microbiology, 2, Churchill Livingstone, New York, 1975: 190.