International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 1297-1302, Oct-Dec 2009

SYNTHESIS AND BIOLOGICAL EVALUATION OF SCHIFF BASES OF CINCHOPHEN AS ANTIMICROBIAL AGENTS

S. J. Wadher*, N. A. Karande, D. S. Borkar, and P. G. Yeole
Department of Pharmacectical Chemistry, Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha – 442 001,India.

Corres.author: sjwadher@rediffmail.com

ABSTRACT: Cinchophen (**I**) was synthesized from pyruvic acid, aniline and benzaldehyde by Doebner synthesis. Cinchophen acid chloride (**II**) was synthesised from cinchophen and oxalyl chloride by acylation reaction. The 2-phenylquinoline-4-carboxylic acid amide (**III**) was synthesized from acid chloride and ammonia by simple reaction. The 2-phenylquinoline-4-carboxylic acid amine (**IV**) was synthesized from cinchophen amide by the reduction reaction by using lithium alluminium hydride as reducing agent in the proper solvent like THF and ether. Then the compound **V** was prepared according to scheme **III** by the use of aldehyde The newly synthesized compound are characterized by spectral (I R, ^{1}H NMR and Mass) data. The purity of the compounds was confirmed by TLC. The physicochemical properties such as melting point and % yield were determined. A number of molecular docking experiments were carried out to identify potential inhibitor of AmpC enzyme of E. Coli HKY28. All these compounds were evaluated for their *in vitro* activity against several microbes. The results indicate that all the synthesized compound shown mild to good activity against the pathogenic bacteria and fungi and have been shown to be more potent than cinchophen with the reference standard ciprofloxacin and fluconazole.
Key Words : Cinchophen, Schiff base, physico-chemical properties and antimicrobial activity.

INTRODUCTION

Cinchophen (2-Phenylquinoline-4-carboxylic acid) has been proved to be a powerful antimicrobial agent.[1] Schiff bases are associated with antibacterial, antifungal activities and have diverse biological activities.[2] These compounds are of interest due to antibacterial and antifungal activities. Cinchophen derivatives occupy an important place in medicinal chemistry as they show a variety of microbiological activity. Therefore, an attempt was made to study the antibacterial and antifungal activity of Schiff base of cinchophen. In present investigation, cinchophen (**I**) was synthesised from pyruvic acid, aniline and benzaldehyde by Doebner synthesis. Cinchophen acid chloride (**II**) was synthesised from cinchophen and oxalyl chloride by acylation reaction. The 2-phenylquinoline-4-carboxylic acid amide (**III**) was synthesized from acid chloride and ammonia by simple reaction. The 2-phenylquinoline-4-carboxylic acid amine (**IV**) was synthesized from cinchophen amide by the reduction reaction by using lithium alluminium hydride as reducing agent in the proper solvent like THF and ether. The compounds **V** was prepared according to scheme **III** by the use of aldehyde. Employing the structure based CADD techniques, we have evaluated a series of virtual Schiff base of cinchophen using Ampc enzyme of E. Coli HKY28 enzyme. Based on these studies, we have taken up the compounds for synthesis and evaluated for antibacterial activity. The structural assignment of the products was based on their I R, NMR and Mass spectral data. The title compounds were screened for their antibacterial and antifungal activity.

MATERIALS AND METHOD

Melting points were obtained using DBK programmed melting point apparatus and are uncorrected. Mass spectra was recorded in a MICROMASS QUATTO II triple quadrapole mass spectrometer. The proton nuclear magnetic spectra [1H-NMR] were recorded on Bruker DRX-300, (300MHz FT NMR) using DMSO. Purity of the compounds was checked using TLC technique, spots were developed by exposure to iodine vapours. Ultra violet spectra were taken on U.V. 2401(PC) S 220V double beam U.V. spectrophotometer, Tokyo, Japan. Infrared spectra were recorded on FTIR spectrophotometer 8400 S, Shimadzu Corporation, Tokyo, Japan

MOLECULAR DOCKING ANALYSIS

A number of molecular docking experiments were carried out to identify potential of HKY28 E. coli AMPC. The structure was downloaded from PDB, active site characterization, energy minimization of molecules and docking (binding energy of molecules with enzymes express kcal/mol) has been done. The standard dock score is -96. The score –35 to –55 dock score means there is future scope to improve dock score. Binding energy improve by doing structure base drug design.

Commercial importance-

- It gives path to synthesize compound.

Methodology adopted by software is-

- Potential mean force[PMF]
- Genetic algorytham

Synthesis of 2-phenylquinoline-4-carboxylic acid (I) [Atophan or Cinchophen][3]

In a 500 ml RBF equipped with a reflux condenser, pyruvic acid (22 ml, 0.25 mol) in 200 ml of ethanol and benzaldehyde (24 ml, 0.236 mol) were placed. The mixture was heated to the boiling point on a water bath and slowly, with frequent shaking a solution of pure aniline (23 ml, 0.248 mol) in 100 ml of ethanol was added. The addition was done for 1 h. The mixture was refluxed for about 3 h and allowed to stand overnight. The crude compound was filtered off on pump and recrystallised using hot ethanol.

Method for synthesis of cinchophen acid chloride (II)[4]

2-Phenylquinoline-4-carboxylic acid, (2.49 g, 0.01 mole) was taken in a round bottomed flask, to it dichloromethane (6.5 ml) was added to form a suspension. To this suspension, oxalylchloride (0.01 mole) in 10 ml dichloromethane was added to form a clear solution. The solution was then stirred for 30 min at room temperature using magnetic stirrer. The solution was concentrated to give yellow solid of acid chloride of cinchophen, which was used for further reaction without purification.

Method for synthesis of cinchophen amide (III)[5]

(2-Phenylquinoline-4-carboxylic acid amide)

The acid chloride obtained was treated with solution of ammonia (20 % v/v) and stirred for 30 min. The addition of ammonia was made, part by part. The amide obtained was then dried and recrystalised from hot ethanol.

Method for reduction of cinchophen amide into cinchophen amine (IV)[6]

(2-Phenylquinoline-4-carboxylic acid amine)

A solution of 4.6 g of amide in 80 ml of tetrahydrofuran was cautiously added to a stirring suspension of 0.5 g. of lithium aluminum hydride in 60 ml of ether. The resulting mixture was refluxed for 2 h and allowed to stand at room temperature overnight. The reaction mixture was decomposed with aqueous sodium hydroxide, and the organic phase was separated, washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. After filtration, the solution was concentrated to provide the free base product, amine. The monohydrochloride salt was obtained by treating an ethereal solution of the free base with excess gaseous hydrogen chloride; after recrystallization from methanol.

General procedure for Schiff bases derived from aldehyde and cinchophen-amine (V)[7]

To a mixture of cinchophen amine (0.01M) and p-chlorobenzaldehyde (0.01M) dissolved in methanol, one drop of concentrated sulphuric acid was added. The reaction mixture was refluxed for 1 h. The reaction mixture was poured into crushed ice. Separeted solid was filtered, dried, and re-crystalised from ethanol.

Spectral data

Chemical name **:** 2-Phenylquinoline-4-carboxylic acid **(I)**

White crystals, m.p. 210 ^{0}C - 215 ^{0}C, yield 50 %, I R (KBr, cm^{-1}): 1704(C = O, COOH), 3034 (Ar-C-H str), 3360 (O-H str, COOH), ^{1}H NMR (DMSO) δ ppm : 7.526-7.504 (s, Ar-H), 7.603 (m, 1H, Ar-H), 7.796 -8.094 (s, Ar-H), 8.187 (s, Ar-H),8.496 (m, Ar-H), 9.598 (s,1H,COOH). MASS m/z: 249 calculated for $C_{16}H_{11}N_1O_2$, found 250.

Chemical name **:** 2-Phenylquinoline-4-carboxylic acid chloride **(II)**

Yellow crystals, m.p. 140 ^{0}C - 145 ^{0},yield 95 %, I R (KBr, cm^{-1}): 1716.53(CO-Cl), 3052.11 (Ar-C-H str), 1244, 765.59, 696.25.

Chemical name **:** 2-Phenylquinoline-4-carboxylic acid amide **(III)**

Yellow-whitish crystals, m.p. 192 ^{0}C - 196 ^{0}C, yield 85 %, I R (KBr, cm^{-1}): 1662.52 (C=O, Ar-CONH$_2$), 3056.96 (Ar-CH str.), 3353.98 (NH str.), ^{1}H NMR (DMSO) δ ppm : 3.296-3.335 (m, 1H, Ar-NH$_2$), 7.431-7.745 (s, Ar-H), 7.768- 7.746 (s, Ar-H), 8.192-8.366 (s, Ar-H), 8.526-8.544 (m,1H, Ar- H),). MASS m/z: 248 calculated for $C_{16}H_{12}N_2O$, found 249.

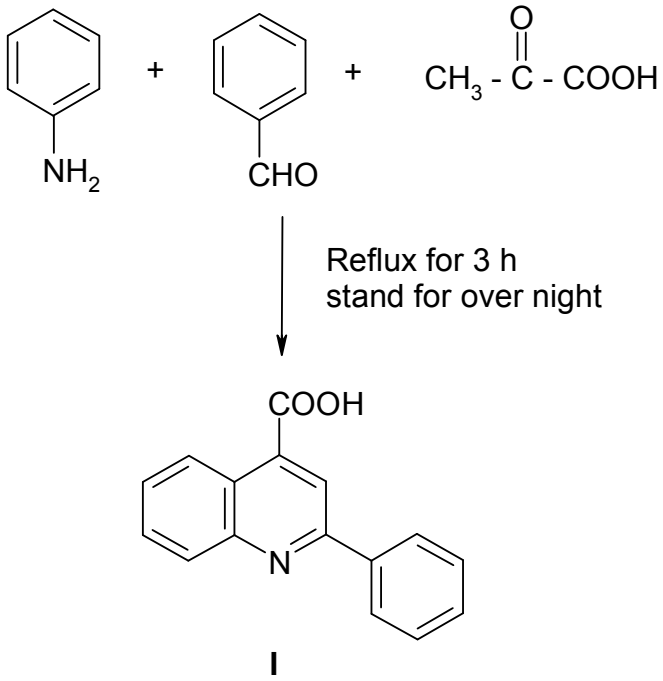
Chemical name : 2-Phenylquinoline-4-carboxylic acid amine **(IV)**

Yellow-brownish crystals, m.p. 205 ^{0}C - 208 ^{0}C, yield 55 %, I R (KBr, cm^{-1}): 3058.89 (Ar-CH str.), 3303.98 (NH str.), 1444.58.

Chemical name : N-(4-Chlorobenzylidene)-1-(2-phenylquinoline-4-yl) methanamines **(V)**

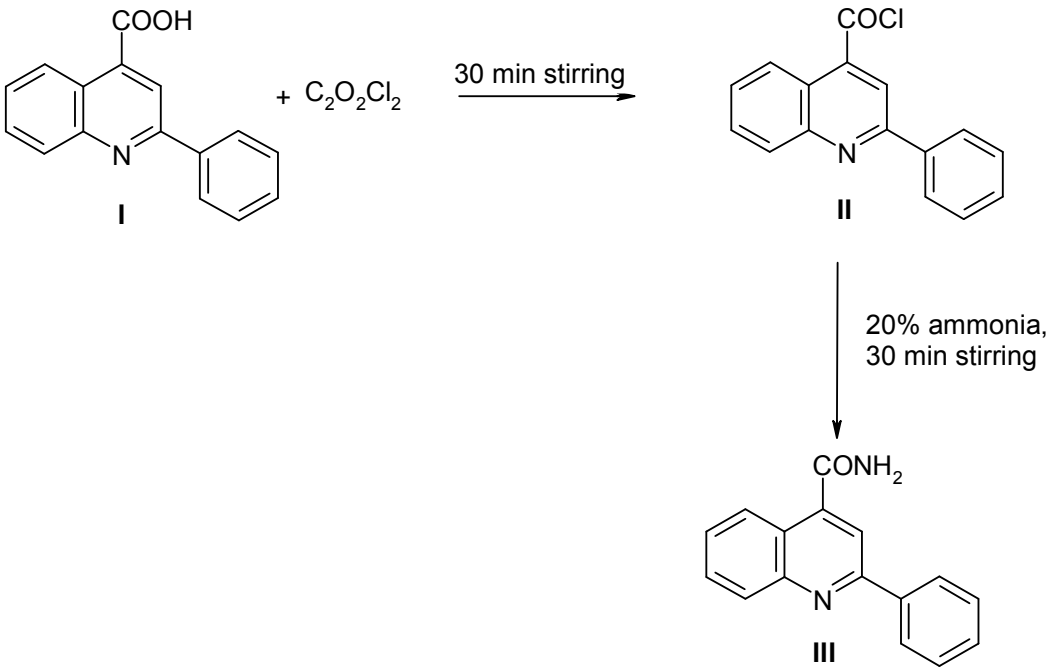
Yellow-whitish crystals, m.p. 185 ^{0}C - 190 ^{0}C, yield 72 %, I R (KBr, cm^{-1}) : 3052.16 (Ar- CH str), 1618 (C=N), 769, 698, ^{1}H NMR (DMSO) δ ppm: 4.866-4.906(s-CH$_2$),7.086-7.146 (s-Ar-H), 7.521 (s,1H-Ar-H),7.768-7.820 (m,1H-Ar-H), 7.942 (m,1H-Ar-H), 8.672 (s,1H-Ar-H), MASS m/z: 356 calculated for $C_{23}H_{17}ClN_2$, found 358

.



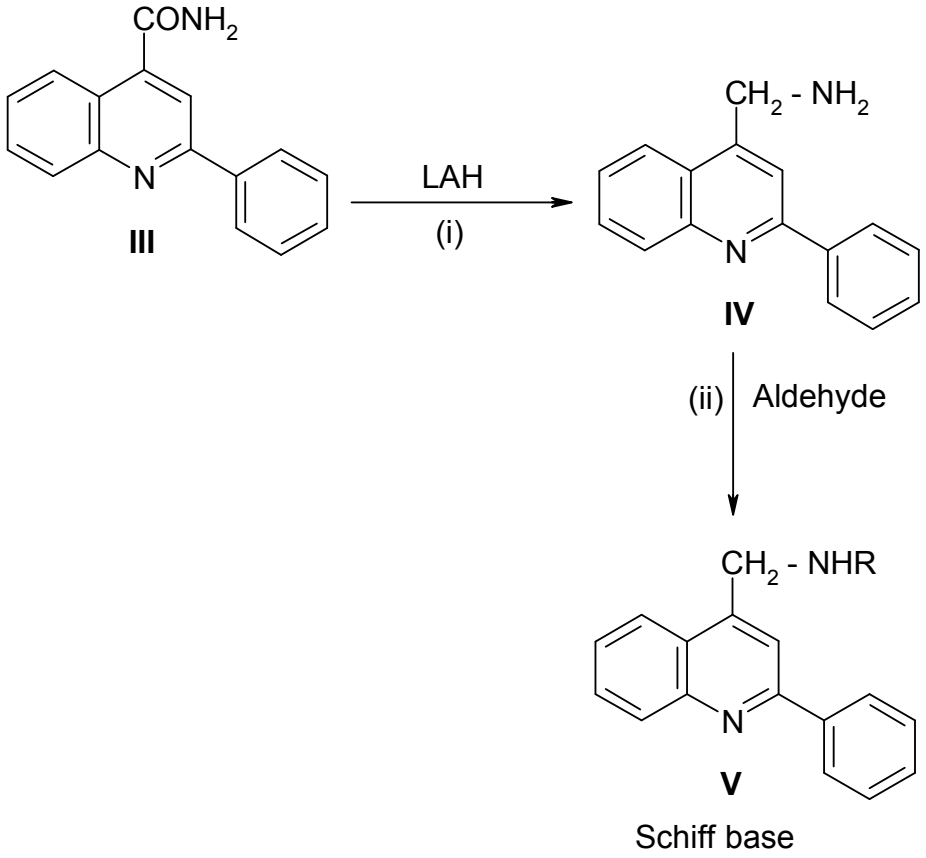
I

Scheme I : Synthesis of cinchophen.



I **II** **III**

Scheme II : Synthesis of acid chloride and amide of cinchophen.



Scheme III : Synthesis of Schiff base of cinchophen.

Reagents : LAH – Lithium Alluminium Hydride
Aldehyde – p-chloro benzaldehyde
Condition : i - Reflux 2 h.
ii - Reflux 1 h.

Table 1: Physico-chemical data of Cinchophen, acid chloride and amide of Cinchophen.

Compound	M.P. (^{0}C)	% Yield	Rf Value
I	210 - 215	50	0.79
II	140 - 145	95	0.76
III	192 - 196	85	0.70

Table 2: Physico-chemical data of synthesized Schiff base.

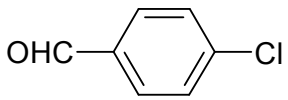
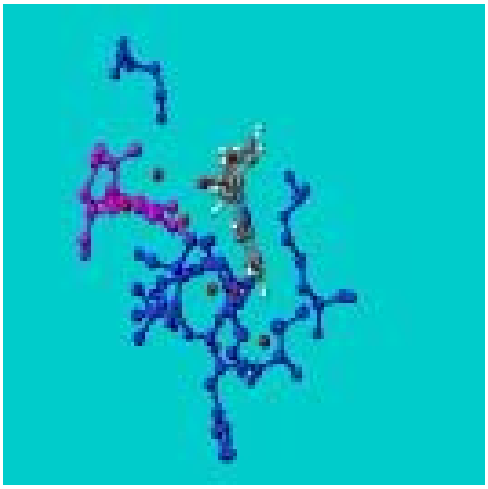
Compound	R	M.P. (^{0}C)	% Yield	Rf Value
V		185 – 190	72	0.72

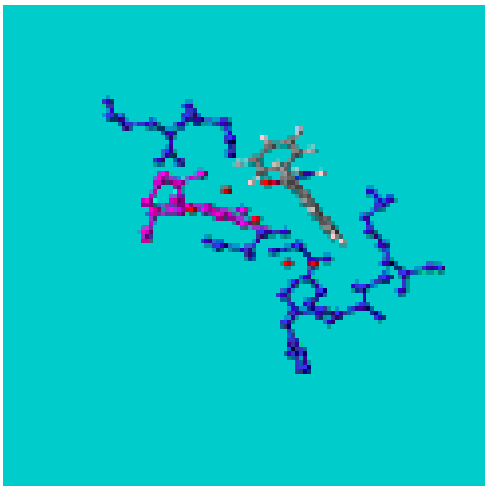
Table 3 : Antimicrobial activity data of synthesized compounds

Compound	Concentration (µg/ml)	Zone of inhibition in mm diameter against bacteria and fungi			
		S. aureus	*E. coli*	*C.albicans*	*A. niger*
I	100	16.2	17.2	16.8	17.2
III	100	18.6	18.2	16.4	16
V	100	17.4	17.8	24.2	23.8
Std 1	100	16.6	17	-	-
Std 2	100	-	-	18	18.4

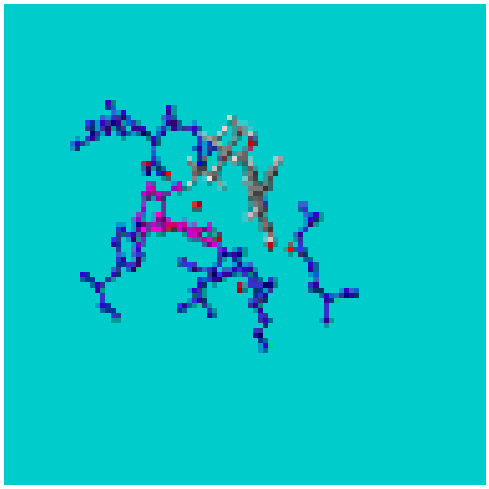
MOLECULAR DOCKING STUDY :



I
Dock score : -38.316



II
Dock score : -42.797



V
Dock score : -44.728

RESULT AND DISSCUSSION

The lead compound cinchophen **I**, was synthesised by the Doebnear synthesis using pyruvic acid, aniline and benzaldehyde. The acid chloride **II** and amide **III** was then prepared by reported method with good yield.

The reduction reaction for the synthesis of amine **IV** from amide by using LAH which is further use for the synthesis of Schiff base with appropriate yield.

The Schiff base of the amine of cinchophen **Va** was prepared by reaction with aldehyde in dried methanol by Schiff reaction with good yield and purity.

The structure of synthesised compounds were confirmed by spectral analysis (I.R., Mass and ^{1}H NMR).

Further synthesized compounds **(V)** were subjected for their anti-microbial activity. Employing the structure based CADD techniques, we have evaluated a series of virtual Schiff base of cinchophen using Ampc enzyme of E. Coli HKY28 enzyme. Based on these studies, we have taken up the compound **(V)** for synthesis and evaluated for antibacterial activity. Antibacterial screening of newly synthesized compound was carried out against *E. coli, S. aureus* and antifungal activity against *C. albicans and A. niger* according to cup-plate method.[8] The synthesized compound shown mild to good activity against the pathogenic bacteria and fungi. The synthesized compound, has been shown to be more potent than cinchophen.

The synthesized compound has found to be better antimicrobial activity than parent compounds.

REFERENCES

1) J. Stragacova, and Hueadcova, Some biological properties of new quinoline-4-carboxylic acid and quinoline-4-carboxamide derivatives, Folia Microbiol (Praha), **45** (4), 305 – 309 (2000).
2) B. Dash, M. Patra, Synthesis of some thiazole Schiff bases and their derivatives, Chem. Abst., published by the American Chemical Society, **105**, 208794f. (1985).
3) Daniel Lednicer, Lester A. Mischer, The Organic Chemistry of Drug Synthesis, **Vol-III**, Wiley Interscience Publication, New York, 183 (1984).
4) I. L. Finar, Organic Chemistry, The Fundamental Principle, **Vol-I**, ELBS with Longman Publication, Essex, England 5th Edn., 256, 448, 805 (1994).
5) Masaki Hosaka, Yoshikazu Asahina, Kazuhiko Iwase, Synthesis and antibacterial activity of 7-substituted, -4-quinolone-3-carboxylic acid derivatives, J. Med. Chem., **46**, 3194 - 4002 (2003).
6) James S. Kaltenbronn, Phenylbenzothiophene compounds, U. S. Patent number 3598839A (1971).
7) P. D. Mehata, and N. P. Sengar, Synthesis and biological activity studies of some thiazolidinones and azetidinone**,** Indian J. Pharm. Sci., **68**, 101-103 (2006).
8) O. A. Fathalla, and S. M. Awad, Synthesis of new 2-thiouracil-5-sulphonamide derivative with antibacterial and antifungal activity, Arch. Pharm. Res**., 28** (11), 1205 – 1212 (2005).

*****