INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SCHIFF BASE DERIVATIVES OF 4-CHLORO-3-NITROBENZOIC ACID

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Article Received on: 24/10/12 Revised on: 21/11/12 Approved for publication: 10/12/12

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ABSTRACT

4-chloro-3-nitrobenzoic acid was selected as nucleus structure having good antibacterial property. It was thought worthwhile to synthesize Schiff base derivatives of 4-chloro-3-nitrobenzoic acid in search of better antibacterial agents. The compounds were synthesized by first reducing nitro group in presence of Sn/HCl to 3-amino-4-chlorobenzoic acid, which on treatment with substituted benzaldehyde afforded the synthesis of the envisaged compounds. The synthesized compounds were purified and chemically characterized. They were screened for their antibacterial activity and result suggested that SKY-1, SKY-5 and SKY-8 were highly active against both Gram (+) and Gram(-) bacteria like B.subtilis, S.aureus and E.coli whereas all are were found moderately to inactive against P.aeruginosa.

KEY WORDS: - Schiff base, 4-chloro-3-nitrobenzoic acid, 3-amino-4-chlorobenzoic acid, antibacterial activity.

INTRODUCTION

Benzoic acid is the simplest aromatic acid, which is being used in food and pharmaceutical preparation as preservatives owing to its antimicrobial activity. It is also one of the ingredients of Whitfield's ointment used in skin diseases like tinea, ring worm and Athlete's foot. Benzoic acid is an important precursor for the synthesis of many other organic substances. The salts and esters of benzoic acid are known as benzoates.[1, 2]

Schiff bases or *azomethine*, named after Hugo Schiff, is a class of substances that are achieved by condensing a primary amine with an aldehyde or ketone. The reaction of aromatic amines to obtain the corresponding imines occurs by acid catalysis. They have the general formula R1R2C = NR3. Hence, it is an imine which contain a characteristic C=N double bond. Schiff bases are the important compound owing to their wide range of biological activities and industrial application. They have been found to possess the pharmacological activities such as antimalarial, anticancer, antibacterial, antifungal, antitubercular, anti-inflammatory, antimicrobial and antiviral etc[5-11]. They also serve as a back bone for the synthesis of various heterocyclic compounds. The presence of azomethine and chloro functional group is responsible for antimicrobial activity, which can be altered depending upon the type of substituent present on the aromatic rings[12]. 4-chloro-3-nitrobenzoic acid was selected as nucleus structure having good antimicrobial property. It was thought worthwhile to synthesize Schiff base derivatives of 4-chloro-3-nitrobenzoic acid in search of better antimicrobial agents. The compounds were synthesized by first reducing nitro group in presence of Sn/HCl to 3-amino-4-chlorobenzoic acid, which on treatment with substituted benzaldehyde afforded the synthesis of the envisaged compounds. The synthesized compounds were purified and chemically characterized. They were screened for their antibacterial activity.

MATERIALS AND METHODS

All recorded melting points were determined on a laboratory melting point apparatus using the capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel-G on micro slide glass plates and spots were detected under iodine vapor. IR spectra were recorded in KBr disk on a Shimadzu FTIR-8400 spectrophotometer and 1HNMR spectra on a JEOL FT-NMR Spectrometer (300 MHz) using TMS as an internal standard. All chemical shift values were recorded as δ (ppm).

Synthesis of intermediates namely 4-chloro-3-aminobenzoic acid[13]

4-chloro-3-nitro benzoic acid (15 gm, 0.09 M) was placed in a 1-litre RBF fitted with a reflux condenser. Powdered tin (35gm, 0.295 M) and conc. HCl (75 ml) were introduced in to RBF. The mixture was heated gently to commence the reaction. The flame was removed and the reaction mixture was shaken and swirreled occasionally to take care that all insoluble acid adhering to the side of flask is transferred in to the reaction mixture. The mixture was kept warm during the reaction to get a clear solution. After about 30 minutes the solution was allowed to cool to room temperature and it was decanted to a 1-litre beaker. The residual in RBF was washed with water (15ml) and washing was added to beaker. Conc. ammonia solution was added to make the solution just alkaline to litmus. The suspension of precipitated hydrated tin oxide was digested on a steam bath for 20 minutes. It was then filtered at the pump and washed with water. The filter cake was transferred to a beaker, heated on water bath with 200 ml of water to ensure extraction of the product and refiltered. The filtrate was concentrated and washings were added to it and volume was reduced to 175-200 ml. The solid separated was filtered off. The liquid was acidified to litmus with glacial acetic acid and evaporated on a water bath until crystals commence to separate; cooled in ice, the crystals were filtered at the pump and dried in steam oven. The % yield 77%, m.p range 195-197^{O}C, the crystals were reddish brown needle shaped and soluble in DMSO. The purity of the compound was validated by single spot(Rf value 0.46) in Hexane: ethyl acetate :: 1:1solvent system.

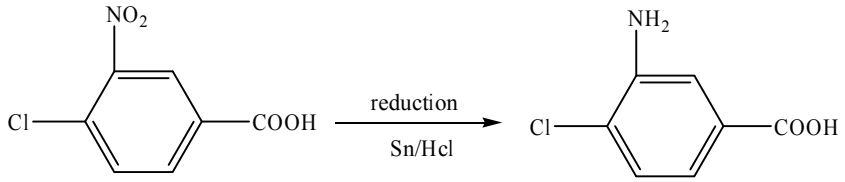
Synthesis of substituted Schiff base derivatives

All the derivatives were synthesized by following general procedure in which a mixture of 0.08575 (0.005 M) of 3-amino-4-chlorobenzoic acid and substituted benzaldehyde (0.005 M) in ethanol with catalytic amount of glacial acetic acid, was refluxed on water bath ,the progress of the reaction was monitored by thin layer chromatography. After

completion of reaction the content was cooled to get the product. The crude product was purified by recrystallization from appropriate solvents. The physical parameters of the synthesized compounds are depicted in the Table 1.

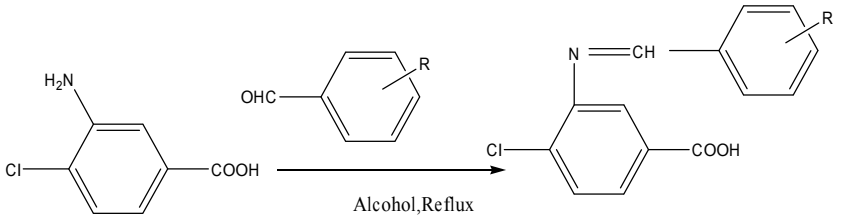
SCHEME 1

1. Synthesis of 3-amino-4-chlorobenzoic acid from 4-chloro-3-nitrobenzoic acid.



4-chloro-3-nitrobenzoic acid — 3-amino-4-chlorobenzoic acid

2. Synthesis of 3-[Substituted benzylidine amino]-4-chloro benzoic acid.



3-amino-4-chlorobenzoic acid — 3-(Substituted benzylidine amino)-4-chlorobenzoic acid as schiff base derivatives

R=4-Cl, 4-$OCH_2C_6H_5$, 2-Br, 3-NO_2, 2-CH_3, 2-Cl, 3, 4-$(OCH_3)_2$, 3-OCH_3

Antibacterial activiy[15, 16, 17]

The antibacterial activity was determined by the disc diffusion method at the concentration of 50 µg/disc. Each test compounds were dissolved in dimethylsulphoxide (DMSO) to get a concentration of 10 mg/mL. The discs (6 mm in diameter) were prepared and impregnated with 5 µL of each test solution, they were air dried and placed on the agar medium, previously seeded with 0.2 mL of broth culture of each organism for 18 hours. The plates were incubated at 37 °C for 24 hours and the inhibition zones measured in mm. Discs impregnated with DMSO were used as a control and ciprofloxacin discs as antibacterial reference standard.All the synthesized compounds were tested *in vitro* for their antibacterial activity against gram positive microorganisms such as *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli , Pseudomonas aeruginosa* (gram negative), using Ciprofloxacin as standard antibacterial.

Table 1: The physical parameters of the synthesized compounds

Compounds Code	R	% Yield	Recrystallization solvent	M.P range (^{0}C)	Solvent system	Rf value
SKY-1	4-Cl	56	Ethanol	210-211	Hexane: ethyl acetate :: 1:1	0.31
SKY-2	4-$OCH_2C_6H_5$	88	Ethanol	63-64	Hexane: ethyl acetate :: 1:1	0.25
SKY-3	2-Br	75	Ethanol	198-199	Hexane: ethyl acetate :: 1:1	0.24
SKY-4	3-NO_2	45	Ethanol	190-191	Hexane: ethyl acetate :: 1:1	0.39
SKY-5	2-CH_3	21	Ethanol	194-195	Hexane: ethyl acetate :: 1:1	0.30
SKY-6	2-Cl	95	Ethanol	234-235	Hexane: ethyl acetate :: 1:1	0.29
SKY-7	3,4-$(OCH_3)_2$	88	Ethanol	200	Hexane: ethyl acetate :: 1:1	0.75
SKY-8	3-OCH_3	17	Ethanol	144-145	Hexane: ethyl acetate :: 1:1	0.17

Table 2: The antibacterial activity of Schiff base derivatives of 4-chloro, 3-nitrobenzoic acid

COMPOUND 50 µg/disc	**Zone of inhibition in mm**			
	B.subtilis	***S.aureus***	***E. coli***	***P.aeruginosa***
SKY-1	+++	+++	+++	-
SKY-2	++	++	+++	++
SKY-3	+++	+++	-	++
SKY-4	-	++	+++	-
SKY-5	+++	++	+++	-
SKY-6	+	++	-	++
SKY-7	+	+++	+++	++
SKY-8	+++	+++	+++	++
Ciprofloxacin	+++	+++	+++	+++
Solvent control DMSO	-	-	-	-

Inactive (inhibition zone < 6 mm); slightly active = '+' (inhibition zone 7-9 mm); moderately Active = '++' (inhibition zone 10-13 mm); highly active = '+++' (inhibition zone > 14 mm).

RESULTS AND DISCUSSION

A survey of literature reveals that Schiff bases possess antibacterial activity which incentivised us to endeavour such a credible research work. Synthesis of 3-amino-4-chlorobenzoic acid as a starting material was carried out by following literature method of reduction.The IR spectral data of the δ value around showed the absorption band at around 1600-1630 cm^{-1} for the formation of -CH=N- group in all Schiff bases. The 1H NMR spectra also confirmed this synthesis by showing δ values for various hydrogens. The characteristic azomethine proton has the δ value around 8.0-8.8, for –COOH proton δ value around10.0-13.0 and for the

aromatic protons have the δ value around 7.0- 8.0. The mass spectra of the compounds have further confirmed the structure of the synthesized compounds.

Structure elucidation of the synthesized compounds

3-(4-chlorobenzylideneamino)-4-chlorobenzoicacid (SKY-1)

IR (KBr cm-1): 3090(-OH, Str -vibration of -COOH), 1682(-C=O Str -vibration of -COOH), 1630(-C=N-), 1053(Ar-Cl), ^{1}H NMR ($CDCl_3$, δ): 7.1490-8.1679(m, 7H, AR-H), 8.5973(s, 1H,-CH=N-), 12.947(s, 1H,-COOH)

3-[4-Benzyloxy benzylidene amino] 4-chloro benzoic acid (SKY-2)

IR (KBr cm-1): 2829(-OH, Str -vibration of -COOH), 1687(-C=O Str -vibration of -COOH), 1602(-C=N-), 1111(-C-O-C-),1032(Ar-Cl), ^{1}H NMR (DMSO, δ): 5.1579(s,2H,CH_2),7.0844-7.9241(m, 12H, AR-H), 8.4227(s, 1H,-CH=N-), 10.0000(s, 1H,-COOH),MS, 365(m+1 peak)

3-[2-Bromo benzylidene amino] 4-chloro benzoic acid (SKY-3)

IR (KBr cm-1): 2977(-OH, Str -vibration of -COOH), 1695(-C=O Str -vibration of -COOH), 1620(-C=N-), 1055(Ar-Cl),1027(Ar-Br), ^{1}H NMR ($CDCl_3$, δ): 7.3747-8.2423(m, 7H, AR-H), 8.7925(s, 1H,-CH=N-), 13.00(s, 1H,-COOH),MS:338(m+2 peak)

3-[3-Nitro benzylidene amino] 4-chloro benzoic acid(SKY-4)

IR (KBr cm-1): 2849(-OH, Str -vibration of -COOH), 1690(-C=O Str -vibration of -COOH), 1613(-C=N-),1351-1420(-NO_2), 1078(Ar-Cl), ^{1}H NMR ($CDCl_3$, δ): 7.2577-8.3675(m, 7H, AR-H), 8.5056(s, 1H,-CH=N-), 10.1627(s, 1H,-COOH)

3-[2-Methyl benzylidene amino] 4-chloro benzoic acid(SKY-5)

IR (KBr cm-1): 2978(CH str due to CH_3), 2819(-OH, Str -vibration of -COOH), 1681(-C=O Str -vibration of -COOH), 1609(-C=N-),1447(CH bending due to CH_3), 1039(Ar-Cl), ^{1}H NMR ($CDCl_3$, δ):2.5681-2.615(t,3H, CH_3), 7.1563-7.526(m, 7H, AR-H), 7.9737(s, 1H,-CH=N-), 10.2471(s, 1H,-COOH)

3-[2-Chlorobenzylidene amino] 4-chloro benzoic acid (SKY-6)

IR (KBr cm-1): 2990(-OH, Str -vibration of -COOH), 1692(-C=O Str -vibration of -COOH), 1621(-C=N-), 1054(Ar-Cl), ^{1}H NMR ($CDCl_3$, δ): 7.2329-8.2992(m, 7H, AR-H), 8.8690(s, 1H,-CH=N-), 10,4593(s, 1H,-COOH)

3-[3, 4-Dimethoxybenzylidene amino] 4-chloro benzoic acid (SKY-7)

IR (KBr cm-1): 2974 (CH str due to CH_3),2831(-OH, Str -vibration of -COOH), 1706(-C=O Str -vibration of -COOH), 1623(-C=N-), 1251(-C-O-C-),1054(Ar-Cl), ^{1}H NMR ($CDCl_3$, δ): 3.8289-3.8536(d,6H,$(CH3)_2$),7.0040-7.6650(m, 6H, AR-H), 7.7432(s, 1H,-CH=N-), 8.4295(s, 1H,-COOH)

3-[3-Methoxybenzylidene amino] 4-chloro benzoic acid (SKY-8)

IR (KBr cm-1): 3002 (CH str due to CH_3),2835(-OH, Str -vibration of -COOH), 1682(-C=O Str -vibration of -COOH), 1632(-C=N-), 1314(-C-O-C-),1039(Ar-Cl), ^{1}H NMR ($CDCl_3$, δ): 3.8277(s,3H,(CH_3),7.094-7.8112(m, 7H, AR-H), 8.521(s, 1H,-CH=N-), 9.9735(s, 1H,-COOH

Antibacterial Activity

The antibacterial activity was determined by the disc diffusion method at the concentration of 50 µg/disc. All the synthesized compounds were tested *in vitro* for their antibacterial activity against gram positive microorganisms such as *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli , Pseudomonas aeruginosa* (gram negative), using Ciprofloxacin as standard antibacterial, the results of activity are presented in the Table 2. The result suggested that SKY-1, SKY-5 and SKY-8 were especially highly active against both Gram (+) and Gram (-) bacteria where as all were found moderately to inactive against P. aerugenosa.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, College of Pharmacy and the Managing Director, I.F.T.M. Moradabad (India) for providing research facilities. We also extend our thanks to the Director, National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune (India) for providing the microbial strains.

REFERENCES

1. Indian pharmacopoeia,1966 edition, Ministry of Health, page 102
2. Remigton's Pharmaceutical Sciences, XVth edition Mack Publishing Company, USA, page 1164-1165
3. Karthikeyan MS, Prasad DJ, Poojary B, Subrahmanya Bhat K, Holl BS, Kumari NS, Synthesis and biological activity of Schiff and Mannich bases bearing 2, 4-dichloro-5-fluorophenyl moiety, Bioorg Med Chem 2006; 14:7482-9.
4. Singh K, Barwa MS, Tyagi P, Synthesis, characterization and biological studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with bidentate Schiff bases derived by heterocyclic ketone, Eur J Med Chem 2006; 41:147-53.
5. Panneerselvam P, Nair RR, Vijayalakshmi G, Subramanian EH, Sridhar SK,Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents, Eur J Med Chem 2005;40:225-9
6. Sridhar SK, Saravan M, Ramesh A. Synthesis and antibacterial screening of hydrazones, Schiff and Mannich bases of isatin derivatives, Eur J Med Chem 2001;36:615-25.
7. Pandeya SN, Sriram D, Nath G, Declercq E, Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4'-chlorophenyl)thiazol-2-yl] thiosemicarbazide, Eur J Pharm Sci 1999; 9:25-31.
8. Mladenova R, Ignatova M, Manolova N, Petrova T, Rashkov I, Preparation, characterization and biological activity of Schiff base compounds derived from 8-hydroxyquinoline-2-carboxaldehyde and Jeffamines ED, Eur Polym J 2002;38:989-99
9. Walsh OM, Meegan MJ, Prendergast RM, Nakib TA, Synthesis of 3-acetoxyazetidin-2-ones and 3-hydroxyazetidin-2-ones with antifugal and antifungal and antibacterial activity, Eur J Med Chem 1996; 31:989-1000
10.Sundberg RJ. Indoles. London: Academic Press; 1996
11.Katritzky AR, Pozharskii AF. Handbook of Heterocyclic Chemistry. Oxford: Pergamon Press; 2000
12.Kumar, S., Niranjan, M.S,,Chaluvaraju, K.C., Jamakhamdi, C.M & Kadadevar, D. Synthesis and antimicrobial study of some Schiff base of sulfonamides, Journal of Current Pharmaceutical Research., 2010,Vol.01,39-42
13.Vogel's Text book of practical organic chemistry,5th edition,Pearson education India, page 896.
14.Indian Pharmacopoeia., 1996, Vol-2nd Appendix 9.1; A 105-107
15.Pelzer M.J., Chan E.C.S., and Kriey N.R., (1993), Microbiology, Tata Mc Graw Hill publication company Ltd, New Delhi, 5th edition, 133-149
16.Collin, C.H. Microbiological Methods; Butterworths: London, UK, 1964; p. 92

Source of support: Nil, Conflict of interest: None Declared