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A Study on Synthesis and Antimicrobial Activity of Substituted (4-Aminosulfonyl) Phenyl-N'(Phenylimino) Benzamidine

NIRANJAN MS, SANTOSH KUMAR, CHOUGALA SS, RAKESH D AND CHALUVARAJU KC*

Department of Pharmaceutical Chemistry, Government College of Pharmacy. Bangalore 560 027.

ABSTRACT

The present study involves the synthesis of nine substituted (4-aminosulfonyl) phenyl-N'(phenylimino) benzamidine (2a to 2i) from respective Schiff's bases benzylidine-4-sulfonamide benzenamines (1a to 1i) by reacting these with benzene diazonium chloride in pyridine at 0-5^{0}C. These synthesized benzamidines were characterized using physical data (m.p, R_f), spectral data (IR, ^{1}H NMR) and studied for their potentiality of antimicrobial activity using pathogenic strains of bacteria- *B.subtilis, S.aureus, E.coli, S.typhi* and fungi- *A.niger, C.albicans* by Agar diffusion method against the standard drugs Ciprofloxacin and ketoconazole respectively. The compound **2i** was found to be the most active antimicrobial compound amongst others in the series.

Key words: Benzimidines, Sulfonamides, Schiff's bases, antibacterial, antifungal.

Introduction

Schiff's bases are widely used oral antibacterial, extensively indicated for infections caused by many bacterial pathogens in most tissues and body fluids with good potency[1]. Next to atomic and space research, development of antimicrobial drugs at the present time is gaining importance due to the development of drug resistance in microorganisms. Increased indiscriminate prescribing has led to the recent occasional emergence of Schiff's base derivative resistant bacteria which have necessitated the search for newer drugs with efficacy against such resistant strains[2]. In this regard, as health care providers and responsible citizens we have to acknowledge the issue of increasing resistance and to develop the strategies to combat this challenge for the management and treatment of the infectious diseases. Hence continued efforts are being taken to get potent, non-toxic, broad spectrum antibacterial agents.Formazans are one of such important chemical compounds widely studied by Von Pechmann and Runge and Friese [3]. In recent years these have been eliciting interest in the chemistry and industrial technology. They are widely applied in different branches of biological science viz, medicine, pharmacology, immunology, botany, biochemistry and histochemistry etc., The substituted formazans have shown various biological activities such as antimicrobial[4-7], anti-inflammatory[8], antiviral[9-11], anticonvulsant[12], anticancer and anti HIV[13] etc., Encouraged by different findings of these reports and to cope up with current requirements of developing newer safer and broad spectrum antibacterial agents, the present work was undertaken in continuation of our earlier report onSchiff's bases of sulphonamides which were prepared by thecondensation of 4-amino benzene sulfonamide with different aromatic aldehydes in the presence of glacial acetic acid in ethanol[14]. In the present paper, Schiff's bases of sulfonamideswere converted into their respective formazans and their antimicrobial activity was studied by using agar-diffusion method against pathogenic strains of bacteria and fungi[15].

*Address for correspondence: chaluvarajukc@gmail.com

Materials and Methods

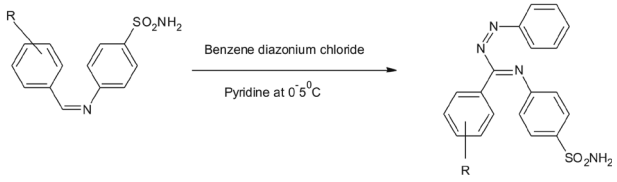
Melting points of synthesized compounds were determined by open capillary and are uncorrected. Purity of compounds was checked using pre-coated TLC plates (MERCK, 60F) using chloroform: methanol (8:2) solvent system. The developed chromatographic plates were visualized under UV at 254nm[16].IR spectra of compounds were recorded using KBr on Shimadzu FTIR model 8400 spectrophotometer[17]. The record of^{1}H NMR spectra in DMSO was obtained using BRUKER FT-NMR instrument using TMS as an internal standard[18].

General procedure for the synthesis of substituted 4-aminosulfinyl phenyl-N'(phenylimino) benzamidines (2a to 2i)

Concentrated HCl (3ml) was added to aniline (0.1mol) dissolved in glacial acetic acid (5ml) at 0-5^{0}C. A solution of sodium nitrite (0.1mol, 1g in 5ml of water) was then added drop-wise to it. The diazonium salt solution thus prepared was added into a solution of compounds1a to 1f (0.1 mol)

in ethanol drop-wise with constant stirring in pyridine (25ml) below 0^0C. The reaction mixtures were kept at room temperature for 2-3 days and then poured into ice cold water (250ml). The resulting solids were washed with water and recrystallized from ethanol.

Scheme



1a to1i 2a to 2i

Compound	R
2a	Hydrogen
2b	2-Chloro
2c	2-Hydroxy
2d	4-dimethylamino
2e	4-methoxy
2f	3, 4, 5-trimethoxy
2g	4-chloro
2h	3-hydroxy
2i	3-nitro

Where,

Compound 2a: N(4-aminosulfanyl) Phenyl-N' (phenylimino) benzamidine % yield: 90; molecular formula: $C_{19}H_{16}N_4SO_2$; molecular wt.: 364; m.p: 95-96^{0}C;R_f:0.71 ;IR (KBr cm-1): 3296(NH_2), 2995(-CH aromatic), 1616(CN), 1575(N=N), 1151(-SO sulfonamide);^{1}HNMR (DMSO δ ppm): 7.9-6.8 (m, 14H, ArH), 2.1(s, 2H, SO_2NH_2).

Compound 2b: 2-chloro- N(4-aminosulfanyl)Phenyl-N' (phenylimino) benzamidine % yield: 70; molecular formula: $C_{19}H_{15}N_4SO_2Cl$; molecular wt.: 398; m.p: 92-94^{0}C; R_f :0.62;IR (KBr cm-1): 3180(NH_2), 2914(-CH aromatic), 1710(CN), 1583(N=N), 1242(-SO sulfonamide), 752(C-Cl) ;^{1}HNMR (DMSO δ ppm):7.7-7.1 (m, 13H, ArH), 2.0(s, 2H, SO_2NH_2).

Compound 2c: 2 hydroxy- N(4-aminosulfanyl)Phenyl-N' (phenylimino) benzamidine % yield: 80; molecular formula: $C_{19}H_{16}N_4SO_3$; molecular wt.: 380; m.p: 80-84^{0}C; R_f :0.6 ;IR (KBr cm-1): 3024(NH_2), 2914(-CH aromatic), 1674(CN), 1539(N=N), 1273(-SO sulfonamide), 3271(Ar-OH);^{1}HNMR (DMSO δ ppm): 7.8-6.7 (m, 13H, ArH),3.87 (s, 1H, ArOH), 2.15(s, 2H, SO_2NH_2).

Compound 2d: 4-dimethyl amino- N(4-aminosulfanyl) Phenyl-N' (phenylimino) benzamidine % yield: 72; molecular formula: $C_{21}H_{21}N_5SO_2$; molecular wt.:407; m.p: 90-92^{0}C; R_f:0.59;IR (KBr cm-1): 3271(NH_2), 3024(-CH aromatic), 1674(CN), 1539(N=N), 1273(-SO sulfonamide), 1356(N-CH_3 bending);^{1}HNMR (DMSO δ ppm): 8.4-6.9 (m, 13H, ArH),3.36 {s, 6H, $N(CH_3)_2$}, 2.09(s, 2H, SO_2NH_2).

Compound 2e: 4-methoxy-N(4-aminosulfanyl)Phenyl-N' (phenylimino) benzamidine % yield: 60; molecular formula: $C_{20}H_{18}N_4SO_3$; molecular wt.: 394; m.p:86^{0}C; R_f :0.82;IR (KBr cm-1): 3271(NH_2), 2914(-CH aromatic), 1674(CN), 1539(N=N), 1273(-SO sulphonamide);^{1}HNMR (DMSO δ ppm): 7.9-6.8 (m, 13H, ArH),3.83 (s, 3H, OCH_3), 2.15 (s, 2H, SO_2NH_2).

Compound 2f: 3,4,5-trimethoxy-N (4-aminosulfanyl Phenyl-N' (phenylimino) benzamidine % yield: 74; molecular formula: $C_{22}H_{22}N_4SO_5$; molecular wt.: 454; m.p: 87-88^{0}C; R_f :0.68 ;IR (KBr cm-1): 3484(NH_2), 3249(-CH aromatic), 1618(CN), 1570 (N=N), 1159 (-SO sulfonamide);^{1}HNMR (DMSO δ ppm): 7.7-6.5 (m, 11H, ArH),3.7 (s, 9H, OCH_3), 2.0 (s, 2H, SO_2NH_2).

Compound 2g: 4-chloro-N(4-aminosulfanyl)Phenyl-N' (phenylimino) benzamidine % yield: 65; molecular formula: $C_{19}H_{15}N_4SO_2Cl$; molecular wt.: 398; m.p: 90^{0}C; R_f :0.76; IR (KBr cm-1): 3300(NH_2), 3086(-CH aromatic), 1620(CN), 1577 (N=N), 1153 (-SO sulfonamide);^{1}HNMR (DMSO δ ppm): 7.7-7.1 (m, 13H, ArH), 2.0(s, 2H, SO_2NH_2).

Compound 2h: 3-hydroxy-N(4-aminosulfanyl)Phenyl-N' (phenylimino) benzamidine % yield: 68; molecular formula: $C_{19}H_{16}N_4SO_3$; molecular wt.: 380; m.p: 92-94^{0}C;R_f :0.65 IR (KBr cm^{-1}): 3332(NH_2), 3055(-CH aromatic), 1618(CN), 1581 (N=N), 1153 (-SO sulfonamide).^{1}HNMR (DMSO δ ppm): 7.8-6.7 (m, 13H, ArH),3.87 (s, 1H, ArOH), 2.15(s, 2H, SO_2NH_2).

Compound 2i: 3-nitro-N(4-aminosulfanyl)Phenyl-N' (phenylimino) benzamidine % yield: 60; molecular formula: $C_{19}H_{15}N_5SO_4$; molecular wt.: 409; m.p: 96-98^{0}C;R_f :0.63;IR (KBr cm^{-1}): 3349(NH_2), 3248(-CH aromatic), 1618(CN), 1570 (N=N), 1159 (-SO sulfonamide), 1570($ArNO_2$); ^{1}HNMR (DMSO δ ppm): 7.9-6.8 (m, 13H, ArH), 2.1(s, 2H, SO_2NH_2).

Antimicrobial Activity

All synthesized compounds were purified, characterized and screened for their possible antibacterial and antifungal activity by cup-plate method. They were tested against four species of bacteria namely *B.subtilis, S.aureus, E.coli, S.typhi* and two species of fungi- *A.niger, C.albicans* against the standard drugs ciprofloxacin and ketoconazole respectively. The diameter of zone of inhibition was measured in mm from one end to another end of inhibition zone in triplicate and the average value was recorded **(Table-1).**

Table-1
Antimicrobial activity of the synthesized compound 2a to 2i

Compound	Zone of inhibition in mm					
	B.subtilis	***S.aureus***	***E.coli***	***S.typhi***	***A.niger***	***C.albicans***
2a	13	15	13	11	14	15
2b	14	18	14	10	16	13
2c	12	17	17	12	17	15
2d	15	12	15	13	14	18
2e	17	18	14	14	17	14
2f	22	22	20	19	16	22
2g	23	20	19	15	21	15
2h	20	21	20	16	13	16
2i	24	24	22	20	21	23
Ciprofloxacin	26	25	26	27	-	-
Ketoconazole	-	-	-	-	27	24

Results and Discussions

Substituted (4-aminosulfonyl) phenyl-n' (phenylimino) benzamidine 2a to 2i were synthesized by treating Schiff's bases benzylidine-4-sulfonamide benzenamines 1a to 1i with benzene diazonium chloride in pyridine at 0-5^{0}C. IR spectrum of the compound displayed absorption at wave number 1151-1273 cm^{-1} indicated the presence of sulphonamide group, absorption at wave number 740 cm^{-1} and 1570 cm^{-1} in compounds 2b, 2h and 2i indicated the presence of C-Cl and NO_2 respectively.^{1}H NMR spectrum of compounds 2a to 2i exhibitedmultiplet at δ 7.9-6.5 ppm indicated the presence of aromatic hydrogens and a singlet at δ 2.1-2.0 ppm represents hydrogens in sulphonamide groups. The signal at δ 3.8 and 3.7 ppm corresponds to methoxy groups in compound 2c and 2f respectively. All these facts collectively suggested that these assigned structures were in good agreement with the proposed one. Results of antibacterial and antifungal activities are summarised in table-1. These results revealed that compounds show considerable and varied activity because of variation in the substitution. The compound 2i substituted with nitro group showed higher activity than compounds with other substitutions. It can be concluded that the activity of compound depends on the electron withdrawing nature of the substituted group on the basis of SAR studies and therefore the sequence of antimicrobial activity is in the following order among reported compounds i.e. Nitro> chloro> hydroxyl> methoxy>dimethyl> methyl> hydrogen substitutions on the phenyl ring.

Conclusion

Objectives of the proposed study was achieved by synthesizing nine (4-aminosulfonyl) phenyl-n' (phenylimino) benzamidine 2a to 2i with different substituent groups. The structures of these synthesized compounds were confirmed by using physical and spectral data and were screened for their possible antimicrobial activity. Data of the antimicrobial activity revealed that the compound **2i** displayed good activity among the compound tested whencompared with standard drugs used. In general all compounds 2a to 2i showed better antimicrobial activity in comparison with compounds 1a to 1i reported in our previous study.

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