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Novel Schiff Bases of 4-Hydroxy 6-Carboxhydrazino Benzofuran Analogs: Synthesis and Pharmacological Study

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Abstract: The present investigation was aimed to study the effect of bioisosteric replacement of sulfur with oxygen with regard to antibacterial activity. Hence some new bioisosteric analogues of benzothiophen derivatives namely schiff bases of 4-hydroxy 6-carboxhydrazino benzofuran for antibacterial activity were carried out. The title compounds were characterized on the basis of spectroscopic techniques viz. IR, ^{1}H-NMR, Mass spectral studies and evaluated for their qualitative and quantitative antibacterial activity by agar cup plate method and micro titration method, respectively. From the biological activity it was possible to observe that some of the substituents such as chloro, dimethylamino, hydroxy and methyl on the phenyl ring of the benzofuran analogs influenced the biological activity.

Key words: 4-Hydroxy 6-carboxhydrazino benzofuran, schiff bases, antibacterial activity, minimum inhibitory concentration

INTRODUCTION

The benzofuran derivatives have been reported for their various biological activities such as insecticidal, fungicidal, antioxidant (Zhang *et al*., 2003), antimicrobial (Sangapure and Basawaraj, 2004) properties and also as A β-aggregate-specific imaging agents for Alzeimer's disease (Masahiro *et al*., 2002). Since schiff bases are reported to possess antibacterial activity (Pawar *et al*., 1999) and based on our ongoing research activities on development of efficient protocols for the preparation of substituted benzothiophenes and other bioactive heterocycles (Gopal Krishna Rao *et al*., 2005; Venugopala *et al*., 2004), we herewith describe a simple, novel and high yielding method for the synthesis of the title compounds containing divalent oxygen atom as bioisostere as in the case of divalent sulphur atom in schiff bases of 4-hydroxy 6-carboxhydrazino benzothiophene analogs (Venugopala *et al*., 2007) by microwave method in comparison with conventional method and screened them for their *in vitro* qualitative and quantitative antibacterial activity by agar cup plate method and micro titration method, respectively.

MATERIALS AND METHODS

Melting points of all the compounds synthesized in laboratory were determined in open capillary tubes and were found uncorrected. IR spectra were recorded on Fourier Transform IR spectrophotometer (Shimadzu 8700) using KBr (ν_{max} in cm^{-1}) disc method at Al-Ameen College of Pharmacy, Bangalore. ^{1}H-NMR spectra were recorded in $CDCl_3$-d and DMSO-d_6 on AMX-400 liquid state NMR spectrometer using TMS as an internal reference standard, mass spectra were recorded on JEOL JMS DX303 Mass spectrometer with Electron Impact Ionization (EII) at 70 ev and

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Elemental analysis was performed on Thermo Finnigan FLASH EA 1112 CHNS analyzer at Indian Institute of Science, Bangalore. The purity of the test compounds was determined by thin layer chromatography using Merck silica gel 60 F_{254} coated aluminium plates of several solvent systems of different polarity. All the chemicals used were of AR grade and were procured from Sigma-Aldrich. Minimum inhibitory concentration of the title compounds was determined using micro-titration method with Luria broth medium as medium for MIC determination at GKVK, University of Agricultural Science, Bangalore.

Synthesis of 3-methoxycarbonyl-*cis*-4-(2-furyl)but-3-enoic acid (1a) and α, β-difurfurylidenesuccinic acid (1b)

These compounds were prepared as per the procedure described by Abdel-Wahhab and El-Assal (1968). Potassium metal (4.29 g, 0.11 mol) in small pieces was dissolved in anhydrous *tert.* butanol (80 mL) with intermittent warming to give potassium *tert.* butoxide. To this a mixture of 2-furfuraldehyde (9.6 g, 0.1 mol), dimethyl succinate (17.5 g, 0.12 mol) and 10 mL of *tert.* butanol were added gradually over 1 h with stirring at 5-10°C. The reaction mixture was stirred at room temperature for 4 h. Then it was acidified with 4 N HCl (congo red), 50-70 mL of distilled water was added and *tert.* butanol was distilled under reduced pressure. The flask was cooled to room temperature and reddish oil separated, which was extracted with ether. The acidic portion was extracted into sodium bicarbonate solution from ether phase. The sodium bicarbonate layer was separated and acidified cautiously with 4 N HCl. The precipitated orange red heavy oil was taken into ether (while extracting, a yellow fine compound α, β- difurfurylidenesuccinic acid (1b) was precipitated. The solution was filtered to separate the compound). The ether layer was separated and dried over anhydrous sodium sulfate. The ether was removed by distillation, the residue (1a and 1b) was digested with boiling benzene to separate a further amount of the benzene insoluble α, β- difurfurylidenesuccinic acid (Scheme 1) (1b). The insoluble product was treated with dilute hydrochloric acid for 30 min and then filtered off. On crystallization from acetone-benzene, it gave α, β- difurfurylidenesuccinic acid (1b) as yellow rhombic crystals to give 2.1 g (7.66%), mp. 232°C (Lit 231-232°C). $C_{14}H_{10}O_6$; Elemental analysis found (expected) in %: C 61.36 (61.32), H 3.72 (3.68).

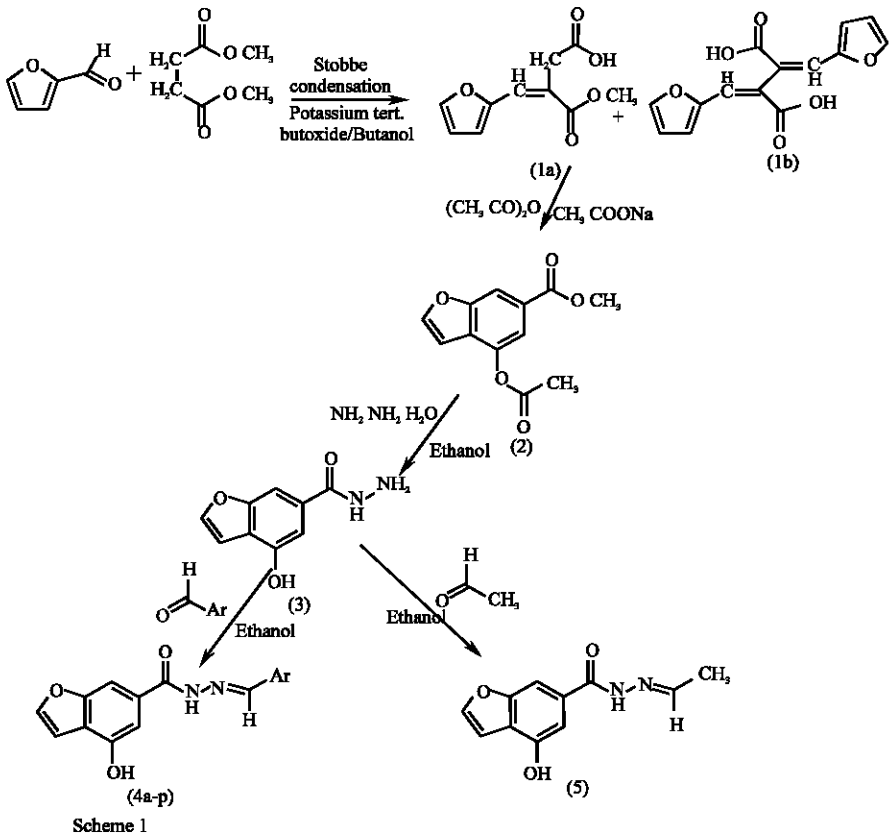
IR (KBr, ν in cm^{-1}): 3056 (Ar-H), 1674 (C = O) and 1607 (Ar-C = C).

Evaporation of the benzene mother liquor left dark brown viscous oil which was repeatedly extracted with boiling light petroleum (bp 60-80). Concentration and cooling (ice box) gave the half-ester 3-methoxycarbonyl-*cis*-4-(2-furyl) but-3-enoic acid (1a) as yellow rhombic crystals. Yield 17.01 g (81%) mp. 102°C (Lit 103-104°C).

IR (KBr, ν in cm^{-1}): 3142 (Ar-H), 1687 (C = O) and 1583 (Ar-C = C). $C_{10}H_{10}O_5$; Elemental analysis found (expected) in %: C 57.32 (57.14), H 4.85 (4.80).

Synthesis of methyl 4-acetoxybenzofuran-6-carboxylate (2)

This compound was prepared as per the procedure described by Abdel-Wahhab and El-Assal (1968). The acid ester 3-methoxycarbonyl-*cis*-4-(2-furyl)but-3-enoic acid 1a, 21 g (0.1 mol) was added to a mixture of sodium acetate (8.2 g) and acetic anhydride (65 mL) and left over night at room temperature with stirring using a magnetic stirrer. The temperature was then gradually raised to 70-80°C over a period of 2 h and maintained for another 4 h with stirring. Then it was allowed to cool to room temperature and the reaction mixture was poured into warm water. The neutral portion was extracted into ether layer (200 mL) and washed with cold sodium bicarbonate solution (3×50 mL). Ether layer was dried over anhydrous sodium sulphate and distilled off. The semisolid product was recrystallized from light petroleum ether (bp. 60-80°C) to give methyl 4-acetoxybenzofuran-6-carboxylate as yellow crystals with yield of 9.36 g (40%) mp. 64°C. (Lit 64-65°C) from methanol. $C_{12}H_{10}O_5$; Elemental analysis found (expected) in %: C 61.50 (61.54), H 4.28 (4.30).



Scheme 1

IR (KBr, ν in cm^{-1}): 3119 (Ar-H), 1713 (-$OCOCH_3$), 1590(-$COOCH_3$) and 1525 (Ar-C = C).

^{1}H-NMR (400MHz, $CDCl_3$): δ 3.68 (s, 3H, $OCOCH_3$), 3.71 (s, 3H, -$COOCH_3$), 7.13 (s, 1H, Ar-H), 7.33 (d, 1H, Ar-H), 7.49 (d, 1H, Ar-H), 7.96 (s, 1H, Ar-H). MS: m/z 235(M^{+1}), 203, 193, 188, 168, 160, 149 and 121.

Synthesis of 4-hydroxy benzofuran-6-carboxhydrazide (3)

Methyl 4-acetoxybenzofuran-6-carboxylate 23.4 g (0.1 mol) and hydrazine hydrate 8 mL were placed in a round bottomed flask fitted with a reflux condenser and the mixture was heated gently under reflux for 10 min sufficient quantity of absolute alcohol was added through the condenser to get a clear solution (about 8 mL). This was refluxed for 2.0 h and ethanol was distilled off and residue cooled. The crystals of acid hydrazide were filtered and recrystallized from ethanol. The product was isolated as white fluffy mass with yield of 18.01 g (93.84%) and the same reaction when carried out by microwave method yield observed was 18.95 g (98.71%), mp 211°C.

IR (KBr, ν in cm^{-1}): 3341 (-OH), 3341 and 3281 (-NH NH_2), 1686 (C = O), 1598 (Ar C = C).

^{1}H-NMR (400MHz, DMSO-d_6): δ 5.89 (s, 2H, -NH_2), 6.52 (s, 1H, -NH), 7.21 (s, 1H, Ar-H), 7.36 (d, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 10.32 (s, 1H, phenolic OH), MS: m/z 193(M^{+1}), 185, 171, 164, 156, 147, 128, 111 and 107.

Synthesis of schiff bases of 4-hydroxy benzofuran-6-carboxhydrazide (4a-p) and (5)

4-Hydroxy benzofuran-6-carboxhydrazide 3 (0.01 mol) was refluxed with substituted aromatic aldehydes (a-p) (0.011 mol) and acetaldehyde (0.011 mol) in ethanol at different time intervals to get

schiff bases 4a-p and 5, respectively. Both conventional and microwave methods were employed to synthesize title compounds which were recrystalized using alcohol as recrystalizing solvent and the results are tabulated in Table 2. In case of microwave method different solvents were used to enhance the products yield and the same is represented in Table 1.

Table 1: Intermediate (3) was made to react with substituted araldehyde in different solvents and yields are found as follows

Solvent	Yield (%)
PEG-400	77.53
PEG-200	80.12
Ethylene glycol	80.71
Diethylene glycol	72.84
Isopropyl alcohol	73.59
Dimethyl sulfoxide	67.28
No solvent	35.35
Absolute ethanol	96.00

Present method reaction condition: p-methoxybenzaldehyde (10 mmol), intermediate 3 (10 mmol) and absolute ethanol (60 mL) were irradiated to microwaves for 60 sec to afford product 4b

Table 2: Reaction parameters of schiff bases of 4-hydroxy 6-carboxhydrazino benzofurans (4a-p) and (5)

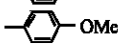
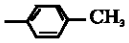
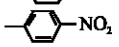
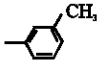
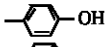
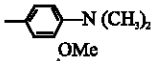
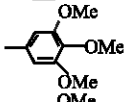
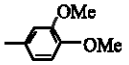
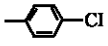
		Reaction time		Yield (%)		
Comp.	Ar	Conventional(h)	M. W (sec)	Conventional	M. W (sec)	M.P (°C)
1a	-	3.0	--	81.00	--	102
1b	-	3.0	--	07.66	--	232
2	-	6.0	--	40.00	--	64
3	-	2.0	135	93.84	98.71	211
4a		2.0	80	61.14	77.96	130-132
4b		2.0	60	80.51	96.00	158-160
4c		2.0	70	68.57	95.02	204-206
4d		2.0	100	56.12	86.25	180
4e		2.0	85	67.65	95.03	176-178
4f		2.0	115	74.59	91.45	208-210
4g		2.0	130	68.57	79.02	214-216
4h		2.0	140	52.89	71.83	220-222
4i		2.0	112	76.80	90.09	198-200
4j		2.0	135	69.41	91.56	236
4k		2.0	110	68.10	92.75	186
4l		2.0	90	77.27	95.10	240-242
4m		2.0	95	54.48	95.48	182
4n		2.0	130	77.64	90.35	176
4o		2.0	125	72.00	85.71	158
4p		2.0	98	81.56	93.39	171
5	--	2.0	170	49.99	68.77	164

Table 3: Qualitative antibacterial activity of schiff bases of 4-hydroxy 6-carboxhydrazino benzofuran analogs (4a-p) and 5

Comp.	Control	*B. s*	*E. c*	*S. a*	*K. p*
4a	-	++	++	+++	+++
4b	-	+	++	+	+
4c	-	++	+++	++	+++
4d	-	++	+	++	+++
4e	-	+	++	+	++
4f	-	++	+++	++	++
4g	-	+	+	+	+
4h	-	+	+	+	+
4i	-	+	+	+++	+
4j	-	+	++	++	+
4k	-	+	+	+	+
4l	-	++	++	+++	+
4m	-	++	++	+	++
4n	-	+	+	+	+
4o	-	+	+	+	+
4p	-	+++	+++	++	+++
5	-	+	++	+	+
Standard					
Streptomycin	-	+++++	+++++	+++++	+++++
Ampicillin	-	+++++	+++++	+++++	+++++

+: Less than 12 mm; ++: 12-15 mm (least active); +++: 15-21 mm (moderately active); ++++: 21-27 mm (highly active); +++++ : >27 mm (standards) and -: Control *B. s: Bacillus subtilis*; *E. c*: *Escherichia coli*, *S. a: Staphylococcus aureous* ; *K. p: Klebshella pneumonia*

Spectral Data

4-Hydroxy benzofuran-6-[N (2'-methylbenzaldimino)] carboxamide (4 g)

IR (KBr, ν in cm^{-1}): 3404(-OH), 3221 (-NH), 1682 (C = O), 1627 (ArC = C).

^{1}H-NMR (400 MHZ, DMSO-d_6): δ2.37 (s, 3H, Ar-CH_3), 5.61 (s, 1H, NH), 6.38 (s, 1H, CH), 7.14-8.30 (m, 8H, Ar-H), 11.49 (s, 1H, Phenolic OH).

MS: m/z 295(M^{+1}), 227, 213, 135, 118 and 107.

Antibacterial Activity

The antibacterial activity (Parmar *et al*., 1992) of the test samples (4a-p) and (5) were determined by agar cup plate method using four organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* and two standard drugs Ampicillin and Streptomycin. This method was based on diffusion of antibacterial component from reservoir bore to the surrounding inoculated nutrient agar medium so that the growth of microorganisms was inhibited as circular zone around the bore. The concentration of test compounds was 100 µg, 100 μL^{-1} and was prepared in Dimethyl Sulfoxide (DMSO). The test samples and standard drugs were placed in a bore made in petridishes which contains different organisms and were incubated at 37°C for 24 h. The zone of inhibitions around the bore was measured after 24 h. The antibacterial activity was classified as standards (>27 mm), highly active (21-27 mm), moderately active (15-21 mm), least active (12-15 mm) and less than 12 mm was taken as inactive. All the samples were tested in triplicate. The antibacterial activity data are recorded in Table 3.

Determination of Minimum Inhibitory Concentration (MIC)

The determination of minimum inhibitory concentration (Elisabeth Lowdin *et al*., 1993; Kotretsou *et al*., 1995) was done with four isolates of *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* which were inoculated into Luria broth medium which contains 1% tryptone, 0.5% yeast extract and 0.5% sodium chloride. The pH of the medium was adjusted to 7.2 with sterile phosphate buffered saline and incubated at 37°C for 24 h. The optical

Table 4: Minimum inhibitory concentration of schiff bases of 4-hydroxy 6-carboxhydrazino benzofuran analogs

	Minimum Inhibitory Concentration (μg/50 μL)			
Compound	*B. s*	*E. c*	*S. a*	*K. p*
4a	521	518	416	428
4c	498	461	531	424
4f	506	432	527	540
4p	408	453	524	430
Streptomycin	196	170	194	164
Ampicillin	164	194	168	200

density of the bacteria from mid log phase of growth was measured at 540 ηm and diluted in fresh medium so as to get an optical density of 0.004 (corresponding to 5×10^5 colony forming units/mL). To each well of the ELISA plate (Corning, USA), 200 μL of diluted bacterial suspension was added. Graded concentrations (0.2-500 μg/50 μL) of the synthesized promising compounds and two standard antibiotics (Streptomycin and Ampicillin) in dimethyl sulfoxide were added and incubated at 37°C for 24 h. At the end of incubation the effect of the drugs on the growth of organisms were monitored by measuring the optical density at 540 ηm using ELISA reader (Multiscan MS, Labsystems, Helsinki, Finland). The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. Determination of minimum inhibitory concentration was performed in triplicate and the results are presented in Table 4.

RESULTS AND DISCUSSION

The parent compound, 4-hydroxy benzofuran-6-carboxhydrazide was prepared by treating methyl-4-acetoxybenzofuran-6-carboxylate and hydrazine hydrate in alcohol medium for 2 h. The former was prepared by cyclization of 3-methoxycarbonyl-*cis*-4-(2-furyl) but-3-enoic acid in presence of sodium acetate and acetic anhydride at room temperature and processed to obtain 4-hydroxy benzofuran-6-carboxhydrazide. The intermediate 3-methoxycarbonyl-*cis*-4-(2-furyl) but-3-enoic acid was prepared by Stobbe condensation method using 2-furfuraldehyde and dimethyl succinate in presence of potassium *tert.* butoxide as catalyst in *tert.* butanol. The parent compound was treated with various aromatic aldehydes to obtain schiff bases of substituted benzofuran (4a-p and 5).

The purified compounds were characterized by IR, ^{1}H-NMR, Mass spectral studies and elemental analysis. The spectral evidences in compound 4 g confirms the presence of –OH, -NH-, –CO- and fused benzene ring, (IR at 3404, 3221, 1682 and 1627, respectively) similarly ^{1}H-NMR multiplet in the range of 7.14-8.30 ppm of 8H also confirms the presence of aromatic rings. The synthetic scheme of schiff bases of 4-hydroxy 6- carboxhydrazino benzofuran are mentioned in Scheme 1 and the effect of solvent, reaction parameters, qualitative and quantitative antibacterial activity are showed in Table 1-4, respectively. Out of several solvents tried for microwave irradiated synthesis of schiff bases of 4-hydroxy benzofuran-6-carboxhydrazide though ethylene glycol and PEG 200 showed satisfactory yield, maximum yield was observed with absolute ethanol.

Some of the schiff bases of 4-hydroxy benzofuran-6-carboxhydrazides (4a-p) and (5) with functional groups such as *meta* chloro, *para* dimethylamino, *ortho* hydroxy, *para* methyl and *para* nitro have exhibited moderate to high degree of antibacterial activity when compared to standard drugs, whereas other compounds showed least antibacterial activity.

However, amongst all the derivatives, analog having chlorine atom at *para* position of the phenyl ring exhibited significant minimum inhibitory concentration compared to standard drugs.

Interestingly benzofuran analogues with chloro, hydroxyl, methyl and nitro substitution exhibited moderate activity compared to their bioisosteric benzothiophen analogues.

CONCLUSIONS

Some novel schiff bases of 4-hydroxy 6-carboxhydrazino benzofuran derivative were synthesized and compounds (4a-p) and (5) were tested for their qualitative antibacterial activity using agar cup plate method against two G+ve pathogenic organisms *Bacillus subtilis* and *Staphylococcus aureus* and two G-ve pathogenic organisms *Escherichia coli* and *Klebsiella pneumoniae* using two standard antibiotics Ampicillin and Streptomycin. Some of the test compounds exhibited significant antibacterial activity when compared to standards (Table-3). The promising test samples were also subjected for the determination of minimum inhibitory concentration (MIC) using same strains of organisms and same standard drugs. Some of the compounds such as 4-hydroxy benzofuran-6-[N(2'-chlorobenzaldimino)] carboxamide, 4-hydroxy benzofuran-6-[N (4'-methylbenzaldimino)] carboxamide, 4-hydroxy benzofuran-6-[N (2'-hydroxybenzaldimino)] carboxamide and 4-hydroxy benzofuran-6-[N (4'-chlorobenzaldimino)] carboxamide have shown good minimum inhibitory concentration when compared to standard Ampicillin and Streptomycin. In contrast to earlier study aper it is evident that schiff bases of 4-hydroxy 6-carboxhydrazino benzothiophene analogs were better antibacterial agents than schiff bases of 4-hydroxy 6-carboxhydrazino benzofuran analogs.

Replacement of sulphur atom by divalent oxygen in benzothiophen led to the development of schiff bases of 4-hydroxy benzofuran-6-carboxhydrazide. Schiff bases of benzofuran analogs reported revealed different spectrum of antibacterial activity. However, from the above observation it was possible to conclude that the schiff bases of 4-hydroxy benzothiophene-6-carboxhydrazide seem to possess better antibacterial activity than their benzofuran counterparts.

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