Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (3): 248-255, 2007
ISSN 1816-496X

Synthesis and Pharmacological Study of Some Novel Schiff Bases of 4-Hydroxy 6-Carboxhydrazino Benzothiophene Analogs

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Abstract: A versatile method for the synthesis of novel schiff bases of 4-hydroxy 6-carboxhydrazino benzothiophene derivative was described. The title compounds were characterized on the basis of spectroscopic techniques 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 on the phenyl ring of the benzothiophene analogs influenced the biological activity.

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

INTRODUCTION

Novel benzothiophene analogs are a privileged structures present in many biologically active compounds. Benzothiophene derivatives constitute an important class of compounds, some analogs are synthesized and evaluated for NSAIDs activity (Mahesh *et al*., 2004) and many possess diverse biological activity like pesticidal activity, antimicrobial, analgesic, antiexudative, anti-inflammatory, diuretic and enzyme inhibition (Grazia *et al*., 2002). In addition, schiff bases are also known for their antibacterial activity (Pawar *et al*., 1999). In the present investigation, the strategy adapted was keeping in view the diverse therapeutic activities of benzothiophene derivatives and as a part of our ongoing development of efficient protocols for the preparation of bioactive heterocycles (Rao *et al*., 2005; Venugopala *et al*., 2004) and study of polymorphism in heterocycles (Munshi *et al*., 2004). Herewith, we describe a simple, novel, high yielding synthesis of the title compounds by microwave method in comparison with conventional method and screened them for their *in vitro* antibacterial activity by qualitative and quantitative antibacterial activity by agar cup plate method and micro titration method, respectively.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and were found uncorrected. Infra Red spectra were recorded on Fourier Transform IR spectrophotometer (Shimadzu 8700) using KBr (ν_{max} in cm^{-1}) disc method. ^{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 70ev and

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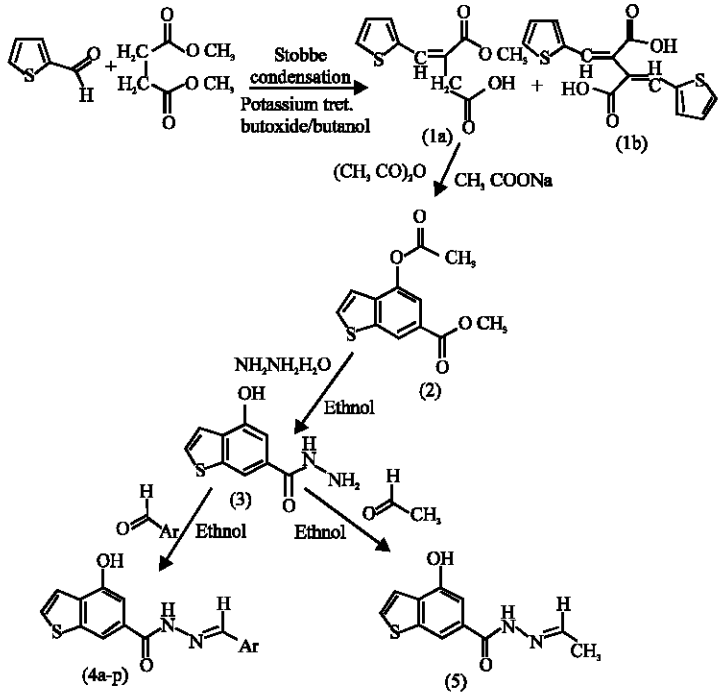
Elemental analysis was performed on Thermo Finnigan FLASH EA 1112 CHNS analyzer at Indian Institute of Science, Bangalore. The purity of the test compounds was determined by thin layer chromatography using Merck silica gel 60 F_{254} coated aluminium plates using several solvent systems of different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

Synthesis of Trans-3-Methoxycarbonyl-4-(2-Thienyl) But-3-Enoic Acid (1a) and α, β-Dithenylidinesuccinic Acid (1b)

These compounds were prepared as per the procedure described by Abdel-Wahhab and El-Rayyes (1971). 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 thiophene-2-carbaldehyde (11.2 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-dithenylidenesuccinic 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 b) was



Scheme 1

digested with boiling benzene to separate a further amount of the benzene insoluble α, β-dithenylidene succinic acid (1b). The insoluble product was treated with dilute hydrochloric acid for 30 min and then filtered off. On crystallization from 70% acetic acid, it gave α, β-dithenylidene succinic acid (1b) as yellow lustrous crystals, further the compound α, β-dithenylidene succinic acid (1b) was recrystallized with methanol to give 1.7 g (5.5%), mp. 254-256 (Lit 255-256°C). $C_{14}H_{10}O_4S_2$; Elemental analysis found (expected) in %: C 54.82 (54.89), H 3.32 (3.29), S 20.89 (20.93).

IR (KBr, ν in cm^{-1}): 3107 (Ar-H), 1664 (C = O) and 1591 (Ar-C = C)

Evaporation of the benzene mother liquor left dark brown viscous oil which was repeatedly extracted with boiling light petroleum (b.p 60-80). Concentration and cooling (ice box) gave the half-ester *trans*-3-methoxycarbonyl-4-(2-thienyl) but-3-enoic acid (1a) as yellow rhombic crystals. Yield 18.99 (84.07%) mp. 116-118°C (Lit 117-118°C)

IR (KBr, ν in cm^{-1}): 3115 (Ar-H), 1710 (C = O) and 1622 (Ar-C = C)

Synthesis of Methyl 4-Acetoxybenzothiophen-6-Carboxylate (2)

This compound was prepared as per the procedure described by Abdel-Wahhab and El-Rayyes (1971). The acid ester *trans*-3-methoxycarbonyl-4-(2-thienyl) but-3-enoic acid 1a, 22.6 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 (3x50 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-acetoxybenzothiophen-6-carboxylate as yellow needles with yield of 23.2 g (92.80%) mp. 84-85°C. (Lit 84-85°C)

IR (KBr, ν in cm^{-1}): 3115 (Ar-H), 1710 (-$OCOCH_3$), 1620(-$COOCH_3$) and 1560 (Ar-C = C)

^{1}H-NMR (400MHz, $CDCl_3$): δ 3.72 (s, 3H, $OCOCH_3$), 3.81 (s, 3H,-$COOCH_3$), 7.13 (s, 1H, Ar-H), 7.32 (d, 1H, Ar-H), 7.52 (d, 1H, Ar-H), 7.97 (s, 1H, Ar-H).
MS: m/z 250(M^+), 231, 207, 185, 156 and 107.

Synthesis of 4-Hydroxy Benzothiophen-6-Carboxhydrazide (3)

Methyl 4-acetoxybenzothiophen-6-carboxylate 25.0 g (0.1 mol) and Hydrazine hydrate 8 mL ware 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 2.5 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 18.3 g (87.98%) and the same reaction when carried out by microwave method yield observed was 19.4 g (93.26%), mp. 234°C.

IR (KBr, ν in cm^{-1}): 3300 (-OH), 3300 and 3211 (-NH NH_2), 1708 (C = O), 1545 (Ar C = C).

^{1}H-NMR (400MHz, DMSO-d_6): δ 6.12 (s, 2H,-NH_2), 6.59 (s, 1H,-NH), 7.20 (s, 1H, Ar-H), 7.37 (d, 1H, Ar-H), 7.63 (d, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 10.42 (s, 1H, phenolic OH),
MS: m/z 209(M+1), 185, 151, 135, 123 and 110.

Synthesis of Schiff Bases of 4-Hydroxy Benzothiophen-6-Carboxhydrazide (4a-p) and (5)

4-Hydroxy benzothiophen-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 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

Solvent	Yield (%)
PEG-400	83.64
PEG-200	81.00
Ethylene glycol	82.57
Diethylene glycol	76.44
Isopropyl alcohol	77.30
Dimethyl sulfoxide	70.08
No solvent	40.81
Absolute ethanol	94.69

Present method reaction condition: *o*-chlorobenzaldehyde (10 m mol), intermediate 3 (10 m mol) and absolute ethanol (60 mL) were irradiated to microwaves for 96 sec to afford product 4a

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

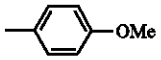
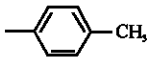
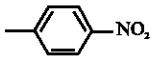
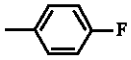
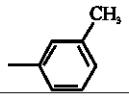
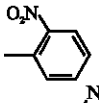
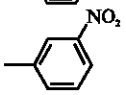
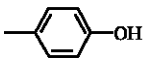
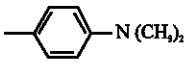
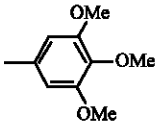
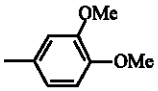
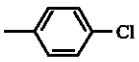
		Reaction time		Yield (%)		
Comp.	Ar	Conven.	MW (sec)	Convent.	MW (sec)	MP (°C)
1a	-	3.0 h	--	84.07	--	116-118
1b	-	3.0 h	--	5.50	--	254-256
2	-	6.0 h	--	92.80	--	84-85
3	-	2.5 h	100	87.98	93.26	233
4a		2.0 h	96	75.75	94.69	150-151
4b		2.0 h	100	80.52	95.85	182
4c		2.0 h	145	72.50	95.06	217
4d		1.3 h	115	73.31	91.64	208-210
4e		2.0 h	120	71.71	95.61	180
4f		2.0 h	130	64.25	84.33	220-222
4g		2.0 h	120	74.59	94.75	218
4h		2.0 h	140	68.54	84.67	188

Table 2: Continued

Comp.	Ar	Reaction time		Yield (%)		MP (°C)
		Conven.	MW (sec)	Convent.	MW (sec)	
4i		2.0 h	115	62.31	84.31	194-195
4j		2.0 h	135	60.48	89.80	194-196
4k		2.0 h	120	64.10	88.14	208
4l		2.0 h	95	70.05	88.49	256-258
4m		2.0 h	100	68.00	92.61	190-192
4n		2.0 h	105	77.24	94.80	189
4o		2.0 h	150	67.56	89.10	172-174
4p		2.3 h	90	88.02	95.50	164
5	--	1.3 h	160	58.21	71.30	168

SPECTRAL DATA

4-Hydroxy Benzothiophene-6-[N (3'-Methylbenzaldimino)] Carboxamide (4h)

IR (KBr, ν in cm^{-1}): 3431(-OH), 3203 (-NH), 1676 (C = O), 1608 (ArC = C)

^{1}H-NMR (400 MHZ, DMSO-d_6): δ 2.34 (s, 3H,-CH_3), 5.80 (s, 1H, NH), 6.97 (s, 1H, CH), 7.07-8.27 (m, 8H, Ar-H), 11.35 (s, 1H, Phenolic OH).
MS: m/z 310(M^+), 295, 268, 201, 177 and 161.

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 *B. subtilis*, *S. aureus*, *E. coli* and *K. 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 petri dishes 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

Table 3: Qualitative antibacterial activity of schiff bases of 4-hydroxy 6-carboxhydrazino benzothiophene 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 (standard) -: Control; *B.s*: *Bacillus subtilis*; *E.c*: *Escherichia coli*; *S.a*: *Staphylococcus aureous*; *K.p*: *Klebshella pneumonia*

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

	Minimum Inhibitory Concentration (μg/150 μL)			
Compound	*B.s*	*E.c*	*S.a*	*K.p*
4c	408	358	332	430
4d	418	432	358	342
4e	424	308	518	488
4p	356	388	446	330
Streptomycin	196	170	194	164
Ampicillin	164	194	168	200

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 (Lowdin *et al*., 1993; Kotretsou *et al*., 1995) was done with four isolates of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *K. 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 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^{-1}). 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 shown in Table 4.

RESULTS AND DISCUSSION

The parent compound 4-hydroxy benzothiophen-6-carboxhydrazide was prepared by treating methyl-4-acetoxybenzothiophene-6-carboxylate and hydrazine hydrate in alcohol medium for 2 h. The former was prepared by cyclization of *trans-3*-methoxy carbonyl-4-(2-thienyl)but-3-enoic acid in presence of sodium acetate and acetic anhydride at room temperature and processed to obtain 4-hydroxy-benzothiophene-6-carboxhydrazide. The intermediate *trans-3*-methoxy carbonyl-4-(2-thienyl)but-3-enoic acid was prepared by Stobbe condensation method using thiophene 2-carbaldehyde 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 benzothiophene. The purified compounds were characterized by IR, ^{1}H-NMR, Mass spectral studies and elemental analysis. The spectral evidences in compound 4h confirms the presence of-OH,-NH-,-CO-and fused benzene ring, (IR at 3431, 3202, 1676 and 1608, respectively) similarly ^{1}H-NMR multiplet in the range of 7.07-8.27 ppm of 8H also confirms the presence of aromatic rings. The synthetic scheme of schiff bases of 4-hydroxy 6-carboxhydrazino benzothiophene are mentioned in scheme-1 and the effect of solvent, reaction parameters, qualitative and quantitative antibacterial activity are shown in Table 1-4, respectively. Out of several solvents tried for microwave irradiated synthesis of schiff bases of 4-hydroxy benzothiophen-6-carboxhydrazide though PEG 400 and ethylene glycol showed satisfactory yield, maximum yield was observed with absolute ethanol.

CONCLUSIONS

Some novel schiff bases of 4-hydroxy 6-carboxhydrazino benzothiophene 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*, *S. 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 determination of Minimum Inhibitory Concentration (MIC) using same strains of organisms and same standard drugs. Some of the compounds such as 4-hydroxy benzothiophene-6-[N (4'-methylbenzaldimino)] carboxamide, 4-hydroxy benzothiophene-6-[N (4'-nitrobenzaldimino)] carboxamide, 4-hydroxy benzothiophene-6-[N (4'-fluorobenzaldimino)] carboxamide and 4-hydroxy benzothiophene-6-[N (4'-chlorobenzaldimino)] carboxamide have shown good minimum inhibitory concentration when compared to standard Ampicillin and Streptomycin.

ACKNOWLEDGMENTS

The authors are thankful to Prof. B.G. Shivananda, Principal, Al-Ameen College of Pharmacy, Bangalore for facilities and Dr. Chandrashekaran, Department of Organic Chemistry, Indian Institute of Science, Bangalore for ^{1}H-NMR, Mass spectra and Elemental analysis.

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