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A Case Study :

Synthesis, spectral characterization and antimicrobial evaluation of some heterocyclic hydroxamic acid compounds

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ABSTRACT

Synthesis of N-benzyl thiophene hydroxamic acid, p-chloro benzyl thiophene hydroxamic acid and p-methyl benzyl thiophene hydroxamic acid, have been carried out by coupling of thiophene -2 carbonyl chloride with benzyl hydroxyl amine, p-chloro benzyl hydroxylamine and p- methyl benzyl hydroxylamine which have been synthesized by hydrolysis of nitrone in acidic medium(con. HCl) by steam distillation which in turn have been synthesized by oxidation of di benzyl hydroxylamine, di chloro benzyl hydroxyl amine, and di methyl benzyl hydroxylamine in presence of mercuric oxide(yellow),diderivative have been obtained from reaction of hydroxylamine hydrochloride and benzyl chloride, p-chloro benzyl chloride, p- methyl benzyl chloride in ethereal medium, while N- methyl thiophene hydroxamic acid was synthesized from N- methyl hydroxyl amine obtained from reduction of nitromethane with Zn dust. Structural assignments were done on the basis of elemental analysis, IR and ^{1}H NMR.All the synthesized compounds were screened for their antimicrobial activity by paper-disc agar-plate method.

KEY WORDS : Hydroxamic acid, Anti microbial activity, Paper-disc agar-plate method

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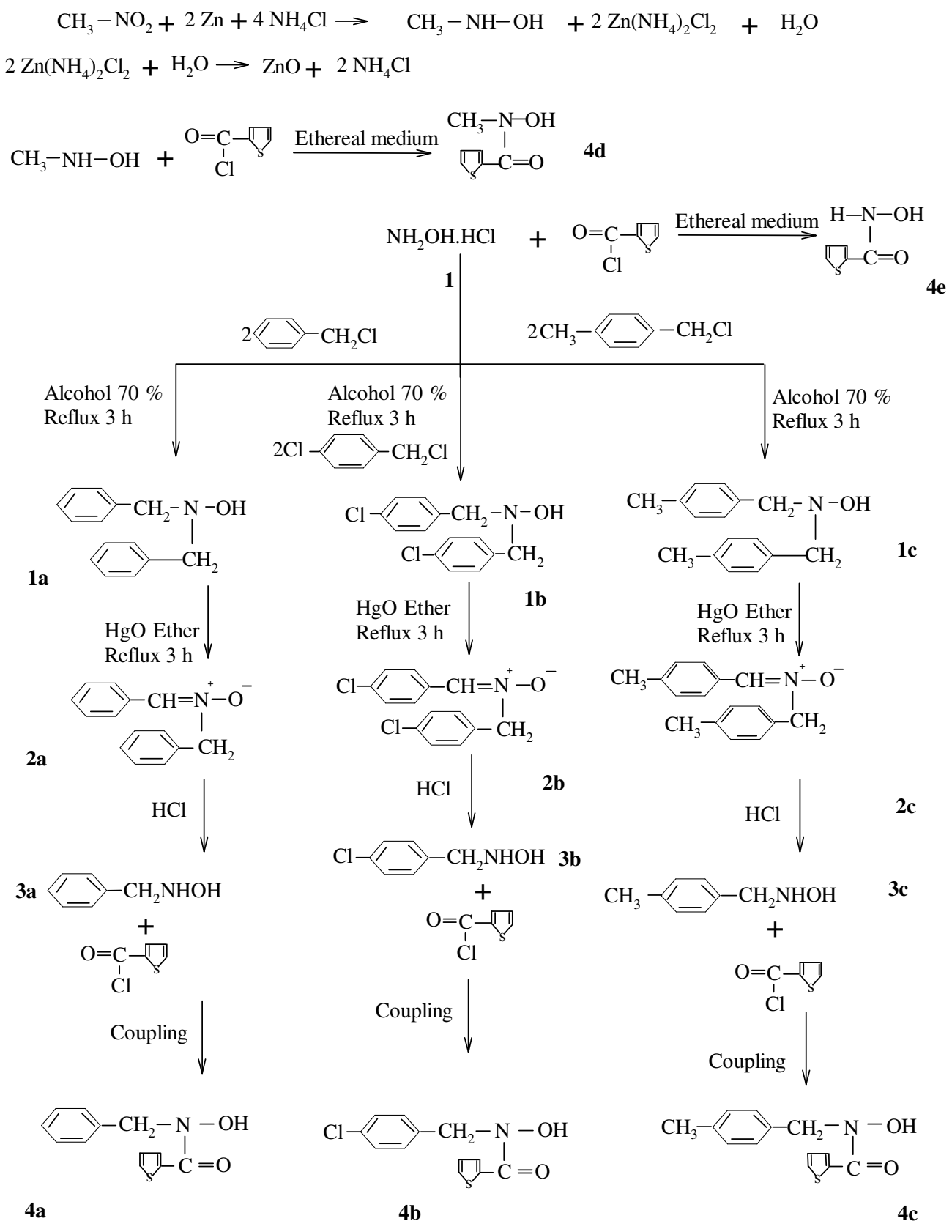
Hydroxamic acids refers to a class of chemical organic compounds having general formula RC (=O) NR'OH, Instead of Sophisticated chemistry of hydroxamic acids they shows a wide range of applications in various fields of analytical, pharmaceutical, biological, medical molecular modeling[1] , docking[2] , technical and nuclear chemistry.

Hydroxamic acids possess antibacterial and antifungal activities including anticancer [3], antimalarial [4], hypotensive [5] anti neoplastic [6], antihistamine [7], antipsychotic [8] ,anti-inflammatory[9] and antitumor properties[10], inhibitors of various enzymes such as peroxidases[11], ureases [12], matrix metalloproteases [13], hydrolases [14] cycloxygenases [15], lipoxygenases [16], and peptide deformilases [17] this make hydroxamic acid drug design. Number of synthetic hydroxamic acids has been reported to be active as soil enhancers [18] herbicides [19] pesticides [20] plant growth promoters [21] in agriculture. A docking protocol using gold software was developed to predict the binding disposition of HDAC inhibitors.

The enormous potentialities of these compounds led to the exploration of some new derivatives of thiophene hydroxamic acid *viz*, 2-thiohene hydroxamic acid, N-benzyl thiophene hydroxamic acid, N-methyl thiophene hydroxamic acid, p-chloro benzyl thiophene hydroxamic acid, p-methyl benzyl thiophene hydroxamic acid synthesized by reaction of thiophene 2- carbonyl chloride with hydroxylamine hydrochloride, N- benzyl hydroxyl amine, methyl hydroxylamine, p-chloro benzyl hydroxylamine, p-methyl benzyl hydroxylamine. All the compounds were characterized by elemental analysis, and spectroscopic studies *viz*, IR, ^{1}H NMR.

The synthesis of thiophene hydroxamic acid was performed following the main steps shown in reaction scheme 1, the required compound 4a has been prepared in four steps reaction of hydroxylamine hydrochloride with Na_2CO_3, and 50 ml benzyl chloride in 70 per cent alcoholic medium and refluxed for 3h diderivative was precipitated it was crystallized with benzene and petroleum ether. Dibenzyl hydroxylamine 1a was oxidized with yellow HgO in ethereal medium refluxed for 3h at 30^{0}C to obtain nitrone 2a, extracted with acetone. Further 1b was hydrolyzed in acidic medium conc. HCl was used to obtain N-Benzyl hydroxylamine 3a, aldehyde removed by steam distillation neutralized with cold Na_2CO_3 solution 3a was further coupled with thiophene-2-carbonyl chloride to


CH3 – NO2 + 2 Zn + 4 NH4Cl → CH3 – NH– OH + 2 Zn(NH4)2Cl2 + H2O
2 Zn(NH4)2Cl2 + H2O → ZnO + 2 NH4Cl
CH3–NH–OH + O=C–Cl Ethereal medium CH3–N–OH C=O 4d
NH2OH.HCl + O=C–Cl Ethereal medium H–N–OH C=O 4e
1
2 CH2Cl
2CH3 – CH2Cl
2Cl – CH2Cl
Alcohol 70 % Reflux 3 h
Alcohol 70 % Reflux 3 h
Alcohol 70 % Reflux 3 h
CH2– N –OH CH2 1a
Cl–CH2–N–OH Cl–CH2 1b
CH3–CH2– N –OH CH3–CH2 1c
HgO Ether Reflux 3 h
HgO Ether Reflux 3 h
HgO Ether Reflux 3 h
CH=N+–O– CH2 2a
Cl–CH=N+–O– Cl–CH2 2b
CH3–CH=N+–O– CH3–CH2 2c
HCl
HCl
HCl
3a CH2NHOH
Cl–CH2NHOH 3b
CH3 –CH2NHOH 3c
O=C–Cl
Coupling
Coupling
Coupling
CH2– N –OH C=O 4a
Cl–CH2– N –OH C=O 4b
CH3–CH2– N –OH C=O 4c

obtain required compound N- benzyl thiophene hydroxamic acid 4a in ethereal medium at temperature below 0^0C. Compound 4b and 4c were also obtained in the same manner, corresponding hydroxylamines were prepared as above steps. Compound 4d was obtained by reduction of nitromethane with Zn dust, obtained hydroxylamine was coupled with thiophene-2-carbonyl chloride in ethereal medium to obtain 4d.

In the IR spectra of compound 4a,4d and 4e showed N-H stretching vibration in the region 3254-3092 cm^{-1} where as OH stretching vibration were examined between 3103-3027 cm^{-1} in all the compounds, the absorption bands due to O-H stretching when free appears around 3600 cm^{-1}, but due to hydrogen bonding wavelength shifts towards lower frequencies [22] and the C=O group appears as strong band in the region 1740-1613 cm^{-1} due to intermolecular bonding these bands shifts towards lower frequencies, bands between 1528-1547cm^{-1} and 1238-1492 cm^{-1} were assigned to C=C stretching (aromatic) and C-N stretching vibrations, respectively and appearance of bonds in the region 1000-1042 cm^{-1} were due to N-O stretching, C-S-C linkage in all thiophene compounds had strong bands in the region 811-853 cm^{-1}.MTHA showed peculiar behavior for C=O peak is shifted into two closely placed peaks, this splitting is explained due to Fermi resonance [23]. The band for C-Cl group 4b was observed at 780 cm^{-1}. In the ^{1}HNMR spectra of compounds signal for OH appeared in the region 11.3-8.3 d the - NH proton signal in the 4e appeared at 2.57 δ Where as CH_3-N signal in 4d at 3.45 δ-CH_2 protons in 4a, 4b and 4c showed a singlet in the region 4.87-4.9 δ. A peak due to – CH_3 proton in 4c showed the singlet at 2.31 δ were as thiophene ring proton and aromatic proton appeared in the region 5.0-7.9 δ, thus giving the conformation for the compound containing alcohol group, aromatic, thiophene as well as CH_2, CH_3 proton.

Antimicrobial activity:

All the synthesized compounds were screened for their antibacterial as well as antifungal activities at 1000µg/ml concentration in agar media , the antimicrobial activities was assayed by using paper-disc agar-plate method [24] by measuring the zone of inhibition in (mm) compounds were screened against bacterial stains such Lactobacillus (gram-positive),Rhizobium, Escherichia Coli and Pseudomonas (gram-negative) and fungi Aspergillus niger, A.sp.(un),Fusarium Oxysporium, standard drugs Amoxycillin 30 mcg/disc and Griseofulvin were used for comparison purpose the results are given in Table 2.

Experimental section:

Melting points were determined on electro thermal apparatus using open capillaries and are uncorrected.IR spectra were recorded on Shimadzu IR-8400S FTIR using KBr pellets ^{1}H NMR spectra were recorded on a Bruker Avance II (400 MHz) spectrometer (SAIF, PU Chandigarh) in CDCl3 and DMSO using TMS as internal standard, chemical shift expressed in δ.

Table 1 : Physical and analytical characterization data of compounds 4a-4e

Compounds	Name	Mol. Formula	Mol. Wt.	m.p.°C	Yield %
4a	N-BTHA	$C_{12}H_{11}O_2NS$	233.28	59	61
4b	N.p-Cl-BTHA	$C_{12}H_{10}ClO_2NS$	267.73	108	54
4c	N.p-CH_3-BTHA	$C_{13}H_{13}O_2NS$	247.31	124	58
4d	MTHA	$C_6H_7O_2NS$	157.19	135	66
4e	THA	$C_5H_5O_2NS$	143.16	115	68

Table 2 : Antibacterial and antifungal screening results of compounds 4a-e and (Activity index)*

Compounds	Zone of inhibition (mm) 1000 g/ml after 72 hr.						
	Antibacterial activity				Antifungal activity		
	Gram positive	Gram negative			A.niger	Fusarium oxysporium	A.Sp. (un)
	Lactobacillus	Rhizobium	E.Coli	Pseudomonas			
4a	12(0.48)	16(0.64)	15(0.62)	08(0.32)	19.5(0.50)	14(0.39)	08(0.26)
4b	18(0.72)	23(0.92)	22(0.88)	17(0.68)	24(0.62)	20(0.55)	09(0.29)
4c	17(0.68)	21(0.84)	20(0.80)	19(0.76)	22(0.56)	19(0.53)	-
4d	08(0.32)	13(0.52)	19(0.76)	14(0.56)	20.2(0.51)	18(0.5)	12(0.33)
4e	11(0.44)	07(0.28)	14(0.56)	16(0.64)	21(0.53)	22(0.61)	-
Griseofulvin	-	-	-	-	39	36	31
Amoxycillin	25	27	28	26	-	-	-

*Activity index = (Inhibition area of sample/Inhibition area of standard)

Synthesis of N- Benzyl thiophene hydroxamic acid (N-BTHA) 4a:

A mixture of hydroxylamine hydrochloride 1 (0.2mole), Na_2CO_3 (0.6mole) and 50 ml benzyl chloride in 200 ml 70 per cent alcohol was refluxed for 3 hr. decant mother liquor on cooling dibenzyl hydroxylamine 1a white crystals appears wash with petroleum ether, (0.159mole) diderivative was oxidized to nitrone 2a with HgO (0.341mole) in ethereal medium refluxed for 3 hr. at 30 ^{0}C decant nitrone obtained recrystallized with acetone further (8.5 g) nitrone added to 17 ml con. HCl reflux for 40 min. steam distillate till benzaldehyde completely removed cool and neutralize with cold saturated Na_2CO_3, keep for 1 hr. benzyl hydroxylamine 3a precipitates out couple (0.1mole) of it with (0.1) mole of (thiophene-2 carbonyl chloride (T.C.C) and (0.1mole) of Na_2CO_3 in ethereal medium 50 ml at below 0^{0}C compound 4a precipitates out dried and purified by recystallization with hot benzene and petroleum ether.IR(KBr) : 3209 (-NH str), 3033(-OH), 1630 (-C=O), 1528 (-C=C), 1300 (-C-N), 1000 (N-O), 848 cm $^{-1}$ (C-S-C); ^{1}HNMR ($CDCl_3$): δ 9.5 (N-OH), 6.9-7.6(Ar-H and Thiophene Ring), 4.9 (Ar.-CH_2).

Synthesis of p-Cl benzyl thiophene hydroxamic acid (p Cl BTHA) 4b:

Compound1 (0.2mole), Na_2CO_3 (0.6mole), and 50 ml p- Cl benzyl chloride were refluxed for 3 hr. in alcoholic medium (200 ml 70 %) on cooling di chloro benzyl hydroxylamine 1b crystals are obtained, washed with P.E .Oxidation of 1b (0.159mole) with HgO (0.341mole) yields nitrone 2b in ethereal medium, in (8.5g) 2b add 17 ml con. HCl and reflux for 40-50 min. steam distillate it now solution is heated to less than half and neutralized with cold and saturated Na_2CO_3, keep for 1 hr. p- chloro benzyl hydroxylamine 3b precipitates out filter it and (0.1mole) of 3b is coupled immediately with (0.1mole) of thiophene -2 carbonyl chloride in separating funnel prepared in 50 ml ether on evaporating ether compound 4b comes out and is isolated using hot ethyl acetate and petroleum ether. IR(KBr): 3188(-NH str), 2987 (-OH), 1622 (-C=O), 1520 (-C=C), 1308 (-C-N), 1010 (N-O), 779 (C-Cl) 845 cm $^{-1}$ (C-S-C); ^{1}HNMR ($CDCl_3$): δ 9.5 (N-OH), 6.9-7.6 (Ar-H and Thiophene Ring), 4.9 (Ar.-CH_2).

Synthesis of p-CH_3 benzyl thiophene hydroxamic acid (p- CH_3 BTHA) 4c:

Compound 1 (0.2mole), p- methyl benzyl chloride 50 ml and Na_2CO_3 (0.6mole) were refluxed for 3 hr. in alcohol 200ml 70 per cent decant the liquor and di methyl benzyl hydroxylamine 1c obtained now 1c (0.159mole) was oxidized with HgO (0.341mole) refluxing for 3 hr. yields nitrone 2c in ethereal medium, now in (8.5g) 2c add 17 ml con. HCl and reflux for 40-50 min. steam distillate it now solution is heated to less than half and neutralized with cold and saturated Na_2CO_3, keeping for 1hr. yields p - methyl benzyl hydroxylamine 3c filter it and now couple (01mole) of 3c along with (0.1mole) Na_2CO_3 to (0.1mole) thiophene-2 carbonyl chloride in separating funnel in 50ml ether as evaporation occurs compounds 4c precipitates out and is isolated using hot benzene and petroleum ether. IR(KBR): 3428(-NH str), 3103(-OH), 1740 (-C=O), 1547 (-C=C), 1238 (-C-N), 1042 (N-O), 811 cm $^{-1}$ (C-S-C); ^{1}HNMR (DMSO): ä 8.3 (N-OH), 7.06-7.97 (Ar-H &Thiophene Ring), 4.87 (Ar.-CH_2), 2.31(-CH_3).

Synthesis of N- CH_3 thiophene hydroxamic acid (N-MTHA) 4d:

(0.23mole) of Zn dust was mechanically added to stirred mixture of nitromethane (0.09mole),ammonium chloride(0.063mole) and 800 ml distilled water decant the solution and add NaCl to it N-methyl hydroxylamine was precipitated wash it and (0.1mole) of it along with (0.1mole) Na_2CO_3 is coupled with(0.1mole) thiophene-2 carbonyl chloride on evaporating ether N- CH_3 thiophene hydroxamic acid precipitates out extracted with ethyl acetate and petroleum ether. IR(KBr): 3093(-NH str), 3029(-OH), 1613 (-C=O), 1538 (-C=C), 1421 (-C-N), 1033 (N-O), 853 cm $^{-1}$ (C-S-C); ^{1}HNMR ($CDCl_3$): ä 8.2 (N-OH), 5.0-7.75 (thiophene Ring), 3.45(CH_3 -N).

Synthesis of 2-thiophene hydroxamic acid (2-THA) 4e:

Mixture of hydroxylamine hydrochloride(0.1mole) and Na_2CO_3(0.1mole) in 50 ml ether is coupled with thiophene 2 carbonyl chloride (0.1mole) in ethereal medium contained in separating funnel now evaporate ether 2-thiophene hydroxamic acid 4e precipitates out wash with water and recrystallized with ethyl acetate and petroleum ether. . IR(KBr): 33254(-NH str), 3027(-OH), 1613 (-C=O), 1542(-C=C), 1492 (-C-N), 1003 (N-O), 843 cm $^{-1}$ (C-S-C); ^{1}HNMR ($CDCl_3$): ä 11.3 (N-OH), 7.01-7.67 (thiophene Ring), 2.57(-N-H).

Conclusion:

The structures of synthesized compounds were assigned on the basis of spectral data like IR and ^{1}HNMR; all the newly synthesized compounds were in full agreement with the proposed structures. From the results of antibacterial activities it was seen that compound 4b

and 4c were found to be active while 4a, 4d and 4e were moderately active. Compounds 4b, 4c and 4e were more active for antifungal activities.

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Abbreviation:

THA–Thiophene hydroxamic acid, MTHA–Methyl thiophene hydroxamic acid, N-BTHA–N-benzyl thiophene hydroxamic acid, p-Cl–BTHAp-chloro benzyl thiophene hydroxamic acid, p-CH_3BTHA–p- methyl thiophene hydroxamic acid, HDAC–Histone deacetylase, FTIR–Fourier transform infra red, NMR–Nuclear magnetic resonance, DMSO–Dimethyl sulphoxide, TMS–Tetra methyl silane, SAIF,PU–Sophisticated analytical instrument facility, Punjab university.

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