SYNTHESIS, CHARACTERISATION AND BIOCIDAL STUDIES OF SOME HYDROXAMIC ACIDS

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ABSTRACT: The synthesis and characterization of a series of hydroxamic acids derived from benzoylation or acylation of substituted aryl hydroxylamine or hydroxylamine hydrochloride are reported. Elemental analysis, ^{1}H NMR, ^{13}C NMR, and IR spectral data of the compounds are discussed. All the compounds have been tested in vitro against a number of microorganisms in order to assess their antimicrobial properties.
Key words: Hydroxamic acid, Benzoylation, Acylation, Biocidal Studies.

INTRODUCTION

The chemistry of hydroxamic acids and polyhydroxamic acids has received considerable attention in view of their pharmacological, toxicological and pathological properties, promising biological implications as well as their role as iron chelators and microbial siderophores[1-13]. Antibacterial, antifungal, antitumor and anti-inflammatory activities of hydroxamic acids are connected with their ability to inhibit various enzymes, viz., matrix metalloproteinases[14, 15], urease[16, 17] or ribonucleotid reductase[18]. The mode of action of these structures at the molecular level is not well understood, but it has been associated to the electrophilic character of the hydroxamic moiety. The electrophilicity allows the molecule to react with nucleophilic centers present in enzymes involved in fundamental processes[19-21]. The nucleophile-electrophile interactions should be mainly determined by the electrophilic character of the nitrogen atom of the hydroxamic group which is a function of the leaving capability of the substituents at the nitrogen atom[22] and electronic effects of the substituent in the aromatic ring[23, 24]. This hypothesis is sustained mainly from the products obtained when these molecules react with model nucleophiles structurally similar to those present in biomolecules[25].
In view of the above applications, the present work relates to the synthesis and characterisation of hydroxamic acids such as N-phenylbenzohydroxamic acids (PBHA), N-*p*-tolylbenzohydroxamic acid (PTBHA), N-*o*-tolylbenzohydroxamic acid (OTBHA), N-*m*-chlorophenylbenzohydroxamic acid (MCBHA), N-*p*-carboxyphenylbenzohydroxamic acid (PCBHA), acetohydroxamic acid (AHA), benzohydroxamic acid (BHA) and salicylhydroxamic acid (SHA) and reports the results of the undertaken antimicrobial evaluation.

Experimental

Reagents and chemicals

All chemicals used in the present investigation were of analytical grade and SHA was purchased from Sigma-Aldrich. All the solvents were dried and then distilled out. Doubly distilled water was used to prepare the required solutions.

Physical Measurements

Elemental analyses (C, H, N) of hydroxamic acids were performed at Central Drug Research Institute, Lucknow. The melting points were determined in open capillary tubes using Prefit model. The molecular weight was determined by cryoscopic method using glacial acetic acid as solvent. The spectral studies of hydroxamic acids were carried out for its characterization using FTIR spectrophotometer (model 8400S, Schimadzu) and 300.4 MHz FT NMR spectrometer (model Jeol AL 300). The infrared spectra were recorded in KBr wafer phase and ^{1}HNMR were recorded in $CDCl_3$ using TMS as an internal standard.

Synthesis

A procedure similar to that described by Priyadarshini and tandon[26] was used. The details of the synthesis are described below which involves two stages:

Preparation of substituted N-phenyl hydroxylamine

Ammonium chloride (0.23 mol), substituted nitrobenzene (0.2 mol) and water (500 ml) were taken in a one litre beaker fitted with a thermometer and a mechanical stirrer. The mixture was stirred vigorously. Zinc dust (0.51 mol) was added in small amounts. The reaction being exothermic, the addition of zinc dust was so adjusted that the temperature did not exceed 55–60 ◦C. The stirring was further continued for about 20 min for complete reduction, till the temperature began to fall. The reaction mixture was filtered under suction and the filtrate was saturated with common salt in a conical flask. It was cooled in an ice bath for about 45 min to ensure maximum crystallization of substituted N-phenyl hydroxylamine.

Preparation of substituted hydroxamic acid

0.1 mol of the prepared hydroxylamine in 25 ml of benzene, distilled water and sodium bicarbonate 0.02 mol were taken in a 250 ml Erlenmayer's flask. Benzoyl chloride, 0.25 mol, was gradually added to the solution, with constant shaking, till effervescence ceased. The water layer was kept alkaline to litmus by the gradual addition of sodium bicarbonate. Addition of benzoyl chloride changed the colour of the reaction mixture from yellow to pink near completion of the reaction. Approximately 90 minutes are required for completion of the reaction. Both mono- and di-substituted derivatives are formed. The solution was filtered, and the solid was washed with water. The residue was purified using aqueous ammonia to remove di-substituted derivatives. The filtered ammonical solution, which was generally yellow or green, was added dropwise to slight excess of dilute sulphuric acid containing some crushed ice to yield the hydroxamic acid which was filtered, washed with water, and dried. The product was crystallized from 60:40 (v/v) ethyl alcohol-water mixture. For BHA direct benzoylation of hydroxylamine hydrochloride and for AHA the acylation of hydroxylamine hydrochloride was carried out. The physical and analytical data obtained for these compounds are shown in table 1.

Table 1: Physical and Analytical data of substituted hydroxamic acid

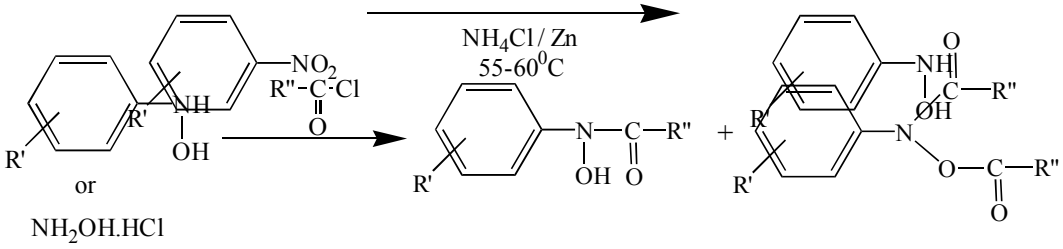
S. N.	R′	R"	Molecular formula	Molecular weight		Yield	m.p.	Elemental analysis						R_f
				Found	Calcd.	%	°C	% Found			%Calculated			
								C	H	N	C	H	N	
1.	C_6H_5	C_6H_5	$C_{13}H_{11}NO_2$	226	213	60	116	73.96	4.78	7.06	73.24	5.16	6.57	0.466
2.	C_6H_5 (*p*-CH_3)	C_6H_5	$C_{14}H_{13}NO_2$	241	227	65	102	74.53	6.32	5.65	74.0	5.73	6.17	0.493
3.	C_6H_5 (*o*-CH_3)	C_6H_5	$C_{14}H_{13}NO_2$	244	227	55	104	73.45	5.21	6.58	74.0	5.73	6.17	0.533
4	C_6H_5 (*m*-Cl)	C_6H_5	$C_{13}H_{10}ClNO_2$	263	247.5	57	106	63.96	3.62	6.32	63.03	4.04	5.66	0.506
5.	C_6H_5 (*p*-COOH)	C_6H_5	$C_{14}H_{11}NO_4$	269	257	44	230	70.55	4.02	4.84	65.37	4.28	5.45	0.374
6.	H	CH_3	$C_2H_5NO_2$	97	75.00	62	82	32.68	5.47	19.36	32.0	6.67	18.67	0.465
7.	H	C_6H_5	$C_7H_7NO_2$	152	137.14	57	123	60.48	4.51	11.35	61.27	5.10	10.21	0.533

Antimicrobial activity

The *in vitro* biological screening effect of the compounds were tested against the bacteria *Enterobacter cloacae, Escherichia coli*, *Pseudomonas aeuruginosa*, *Proteus vulgaris, Staphylococcus aureus* (coagulase positive and coagulase negative) and the fungi *Candida albicans* by disc diffusion method[27, 28]. The microorganism were cultured in nutrient agar medium in Petri plates and used as inoculums for the study. Measured quantities of the test compounds were dissolved in DMF to get final concentrations of 250 ppm and soaked in filter paper discs of 5mm diameter. These discs were placed on the previously seeded plates and incubated at 35±0.5^{0}C. The diameter (millimetre) of inhibitory zone around each disc was measured after 24 hours. Filter paper disc treated with DMF served as control and Amoxyclav (30 mcg/disc for *Staphylococcus aureus*), Ceftazidime (30 mcg/disc for *Pseudomonas aeuruginosa*), Ciprofloxacin (5 mcg/disc for *Proteus vulgaris*, *Enterobacter cloacae*, *Escherichia coli*) and ketoconozole (100 units/disc for fungi) used as reference drugs separately.

RESULTS AND DISCUSSION

The air- and light-stable compounds were synthesized by the reactions of benzoyl chloride with the appropriate hydroxylamine as shown by the scheme below.



Where, R´ = H, (*p*-CH_3), (*o*-CH_3), (*m*-Cl), (*p*-COOH), (*p*-OH);
R" = C_6H_5, CH_3, C_6H_5 (*o*-OH)

Infrared spectra

The most characteristic bands associated with the hydroxamic acid functional group are due to the O–H and C=O stretching vibrations and these can be assigned rather unambiguously. Hydroxamic acids are characterized in the solid state by the bands between 3200-3150 cm^{-1} (O–H)[29], a band near 1640 cm^{-1} (C=O)[30], a band near 1599 cm^{-1} (C–N–C), a variable intensity band at 1440–1360 cm^{-1} (C–N), and a strong band[29] near 900 cm^{-1} (N–O).

Assignment of the bands is given in table 2, in which the most intense bands are analyzed. There is large conjugation in the molecule under study, some deviations have been observed from the expected values. Hydroxamic acids are involved in strong hydrogen bonding which causes a large shift (of the order of 500 cm^{-1}) in the absorption band to lower frequencies, and may be ascribed to resonance stabilization. A consequence of this resonance stabilization should be to increase the contribution of the single bond form, thereby causing a fall in the frequency of the C=O stretching vibration.

Nuclear Magnetic Resonance Spectra

The ^{1}H NMR spectra of hydroxamic acids under investigation show the characteristic singlet of the proton of the hydroxyl group attached to the nitrogen atom in the region 10.5-11.5 ppm. The shifting of the resonance signal of hydroxyl proton to lower field supports intermolecular hydrogen bonding. The proton of –COOH in PCBHA is off scale which gives singlet between 10-11 ppm. A broad signal in the region 10.4-11.4 ppm which evidently belonged to the NH and OH protons of the hydroxylamine unit[31]. This suggests that OH and NH groups can undergo rapid proton exchange with each other.

Table 2: Infrared spectral data of substituted hydroxamic acid

S.N.	R´	R"	ν_{O-H} (cm^{-1})	$\nu_{C=O}$ (cm^{-1})	ν_{N-O} (cm^{-1})	ν_{N-C} (cm^{-1})	$\delta_{C=C}$ (cm^{-1})	
1.	C_6H_5	C_6H_5	3110 (br)	1630	920	1400	765	690
2.	C_6H_5 (*p*-CH_3)	C_6H_5	3100 (br)	1640	920	1400	830	_
3.	C_6H_5 (*o*-CH_3)	C_6H_5	3100 (br)	1640	920	1390	770	_
4.	C_6H_5 (*m*-Cl)	C_6H_5	3250 (s)	1620	940	1450	780	720
5.	C_6H_5 (*p*-COOH)	C_6H_5	3300-2750 (br)	1690	940	1420	840	_
6.	H	CH_3	3400-2750 (br)	1660	990	1450	_	_
7.	H	C_6H_5	3180 (br)	1650	900	1450	795	680

The ^{13}C-NMR spectra exhibit absorption signal due to carbonyl, C=O carbon nearby 165 ppm. The chemical shifts of aromatic carbon appear in the region 138-121 ppm. Beside these signals, a singlet nearby 21 ppm and a singlet at 179 ppm appeared which correspond to the carbon atom of alkyl group and carboxy group respectively. Above NMR spectral data are summarized in table 3 and 4.

Antimicrobial activity

The all synthesized hydroxamic acids have been tested for the *in vitro* growth inhibitory activity against the bacteria *Enterobacter cloacae, Escherichia coli*, *Pseudomonas aeuruginosa*, *Proteus vulgaris, Staphylococcus aureus* (coagulase positive and coagulase negative) and the fungi *Candida albicans* by using the disc-diffusion method. From the results (Table 5), it is concluded that out of six bacterial cultures tested, acetohydroxamic acid (AHA) and benzohydroxamic (BHA) acid exhibited wide spectrum of activity, as they were highly active against all cultures and the next one was salicylhydroxamic acid (SHA), as it was also active against all cultures. All of the other tested compounds exhibit moderate antimicrobial activity against all species of bacteria used in this study.

Table 3: ^{1}H NMR spectral data of substituted hydroxamic acid

S.N.	R´	R"	δppm	Hydrogen	Multiplicity	Assignment
1.	C_6H_5	C_6H_5	10.73 7.44-7.19	1 10	Singlet Multiplet	O-H proton Aromatic protons
2.	C_6H_5 (*p*-CH_3)	C_6H_5	10.67 7.43-7.02 2.32	1 9 3	Singlet Multiplet Singlet	O-H proton Aromatic protons CH_3 protons
3.	C_6H_5 (*o*-CH_3)	C_6H_5	10.32 7.55-7.13 2.38	1 9 3	Singlet Multiplet Singlet	O-H proton Aromatic protons CH_3 protons
4.	C_6H_5 (*m*-Cl)	C_6H_5	10.89 7.45-7.01	1 9	Singlet Multiplet	O-H proton Aromatic protons
5.	C_6H_5 (*p*-COOH)	C_6H_5	11.23 10.73 8.09-7.42	1 1 9	Singlet Singlet Multiplet	O-H proton Carboxy proton Aromatic protons
6.	H	CH_3	10.42 (br) 2.58	1 3	Singlet Singlet	O-H & N-H proton Methyl proton
7.	H	C_6H_5	11.19 (br) 7.90-7.26	1 5	Singlet Multiplet	O-H & N-H proton Aromatic protons

Among all the N-aryl benzohydroxamic acids no significant activity has been recorded against *Pseudomonas aeuruginosa* whereas, in case of *Escherichia coli* and *Proteus vulgaris* only OTBHA and in case of *Enterobacter cloacae* only PBHA has exhibited activity. PBHA, PTBHA and OTBHA were effective against both strains of *Staphylococcus aureus*. PCBHA and MCBHA showed no antimicrobial activity on the most of the Gram-positive and Gram-negative bacteria whereas, in the case of *Staphylococcus aureus* (coagulase negative), they were effective moderately.

The antifungal activity of the compounds was studied on *Candida albicans*. The results of fungicidal screening (Table 5) show that among all the tested hydroxamic acids, BHA and AHA have shown good activity while other compounds except PCBHA and SHA were effective moderately. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or the difference in ribosomes of microbial cells[32].

Table 4: ^{13}C NMR spectral data of substituted hydroxamic acid

S.N.	R´	R"	^{13}C NMR data
1.	C_6H_5	C_6H_5	δ 165.26 (C-7), δ 139.42 (C-1), δ 132.05 (C-8), δ 131.03 (C-2, C-6), δ 129.10 (C-9, C-13), δ 128.87 (C-3, C-5), δ 128.19 (C-10, C-12), δ 125.98 (C-4, C-11)
2.	C_6H_5 (*p*-CH_3)	C_6H_5	δ 165.20 (C-7), δ 138.48 (C-1), δ 136.92 (C-8), δ 132.15 (C-2, C-6), δ 130.89 (C-9, C-13), δ 129.75 (C-3, C-5), δ 128.89 (C-10, C-12), δ 128.13 (C-4), δ 126.15 (C-11), δ 21.14 (C-14)
3.	C_6H_5 (*o*-CH_3)	C_6H_5	δ 165.18 (C-7), δ 138.01 (C-1), δ 134.69 (C-8), δ 131.33 (C-2, C-6), δ 130.01 (C-13), δ 128.15 (C-3, C-5), δ 128.54 (C-12), δ 127.89 (C-10), δ 127.57 (C-4, C-11), δ 126.87 (C-9), δ 21.10 (C-14)
4.	C_6H_5 (*m*-Cl)	C_6H_5	δ 166.01 (C-7), δ 140.84 (C-10), δ 139.05 (C-8), δ 134.62 (C-1), δ 131.34 (C-9), δ 129.81 (C-11), δ 128.76 (C-12), δ 128.36 (C-6, C-2), δ 127.86 (C-13), δ 125.26 (C-5, C-3), δ 123.43 (C-4)
5.	C_6H_5 (*p*-COOH)	C_6H_5	δ 179.14 (C-14), δ 167.18 (C-7), δ 145.60 (C-11), δ 131.89 (C-10, C-12), δ 130.48 (C-8), δ 129.81 (C-9, C-13), δ 128.53 (C-1), δ 127.67 (C-2, C-6, C-3, C-5), δ 120.05 (C-4)
6.	H	CH_3	δ 166.03 (C-1), δ 17.85 (C-2)
7.	H	C_6H_5	δ 165.37 (C-1), δ 132.11 (C-2), δ 131.25 (C-3, C-7), δ 128.57 (C-4, C-6), δ 127.71 (C-5)

Table 5: Antimicrobial screening data of the hydroxamic acids

S. No.	Compounds	Diameter of inhibition zone (mm)						
		E. coli	*P. aeuruginosa*	*S. aureus*	*S. aureus*	*E. cloacae*	*C. albicans*	*P. Vulgaris*
					- ve		+ ve	
1.	PBHA	_	_	8	9	_	8	10
2.	PTBHA	_	_	8	10	_	_	9
3.	OTBHA	9	_	15	15	11	_	9
4.	MCBHA	_	_	10	_	_	_	8
5.	PCBHA	_	_	10	_	_	_	_
6.	BHA	15	11	12	19	15	17	24
7.	AHA	17	25	18	10	19	24	19
8.	SHA	13	13	15	10	13	14	_

Conclusions

Seven hydroxamic acids have been prepared and characterized on the basis of analytical and spectral data. Furthermore, all the compounds were found to be potential bioactive material against the pathogenic microorganism.

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REFERENCES

1. H.R. Bravo and W. Lazo (1996). Journal of Agricultural and Food Chemistry: Vol. 44(6) 1569-71.
2. H.R. Bravo and W. Lazo (1993). Phytochemistry: Vol. 33(3) 569-71.
3. E.M.F. Muri, M.J. Nieto, R.D. Sindelar and J.S. Williamson (2002). Current Medicinal Chemistry: Vol. 9(17) 1631-53.
4. W.Y. Goa, H. Mitsuya, J.S. Driscoll and D.G. Johns (1995). Biochemical Pharmacology: Vol. 50(2) 274-76.
5. T.J. Gora and T. Robak (1995). Acta haematologica Polonica: Vol. 26(1) 39-45.
6. L.H. Coutinho, M.L. Brereton, A.M. Santos, W.D. Ryder, J. Chang, C.J. Harrison, J.A. Yin, T.M. Dekter and N.G. Testa (1996). British journal of haematology: Vol. 93(4) 869-77.
7. S. Nand, W. Stock, J. Godwin and S.G. Fisher (1996). American Journal of Hematology: Vol. 52(1) 42-46.
8. P. Bruce and B.J. Kennedy (1970). Proceedings of the American Association for Cancer Research: 11, 63.
9. E.C. Moore (1969). Cancer Research: Vol. 29(2) 291-95.
10. H.L. Elford, G.L. Wampler and B.V. Riet (1971). Cancer Research: Vol. 39, 844.
11. H.K. Kehl (1982). Chemistry and Biology of Hydroxamic Aids, Karger: Basel.
12. R.J. Bergeron (1984). Chem. Rev: Vol. **84,** 587-602.
13. S. Hanessian and S. Johnstone (1999). Journal of Organic Chemistry: Vol. **64,** 5896-03.
14. M. Whittaker, C.D. Floyd, P. Brown and A.J.H. Gearing (1999). Chemical Reviews (Washington, D. C.): Vol. 99(9) 2735-76.
15. T. Supuran and A. Scozzafava (2002). In *Recent Potential Targets for Drug Development*, Taylor & Francis, London, 35-61.
16. S. Odake, T. Morikava, M. Tsuchiya, L. Imamura and K. Kobashi (1994). Biological & Pharmaceutical Bulletin: Vol. 17(10) 1329-32.
17. M.A. Pearson, L.O. Michel, R.P. Hausinger and P.A. Karplus (1997). Biochemistry: Vol. 36 (26) 8164-72.
18. P. Nandy, E.J. Lien and V.I. Avramis (1999). Anticancer Research: Vol. 19 (3A) 1625-33.
19. C.B. Queirolo, C.S. Andreo, H.M. Niemeyer and L.J. Corcuera (1983). Plant Physiology: 27, 2455.
20. C.B. Queirolo, C.S. Andreo, H.M. Niemeyer and L.J. Corcuera (1981). Plant Physiology: Vol. 68(4) 941-43.
21. F.J. Perez and H.M. Niemeyer (1989). Phytochemistry: Vol. 28(7), 1831-34.
22. Y. Hashimoto, T. Ishizaki and K. Shudo (1991). Tetrahedron: Vol. 47(10-11) 1837-60.
23. H.R. Bravo and W. Lazo (1993). Phytochemistry: Vol. 33(3) 569-71.
24. L.B. Weiss and H.R. Bravo (1994). Heterocycles: Vol. 38(1) 9-16.
25. F.J. Perez and H.M. Niemeyer (1985). Phytochemistry: Vol. 24(12) 2963-66.
26. Priyadarshini and S.G. Tandon (1967). Journal of Chemical and Engineering Data: Vol. 12, 143.
27. L.L. Fish and A.L.Crumbliss (1985). Inorganic Chemistry: Vol. 24, 2198.
28. H.A. Tang, L.F. Wang and R.D. Yang (2003). Transition Metal Chemistry: Vol. 28(4) 395-98.
29. D. Hadzi and D. Prevorsek (1957). Spectrochimica Acta: Vol. 10, 38-51.
30. W. Kliegel, U. Schumacher, M. Tajerbashi, S.J. Rettig and J. Trotter (1991). Canadian Journal of Chemistry: Vol. **69**, 545-49.
31. V.N. Yarovenko, O.V. Lysenko and M.M. Krayushkin (1993). Chemistry of heterocyclic compounds: Vol. **29**(4) 452-54.
32. Z.H.A. El-Wahab, M.M. Mashaly, A.A. Salman, B.A. El-Shetary and A.A. Faheim (2004). Spectrochimica Acta Part A: vol. 60 (12) 2861-73.