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IMINE OXIMES : SYNTHESIS, CHARACTERIZATION, THERMAL AND BIOLOGICAL STUDIES

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ABSTRACT

The imines with amino substitutent groups were synthesized. It was shown that derivatives of NH_2 / NH containing compounds at the carbonyl group of 3-hydroxyimino-5-methyl-2-hexanone are formed as a result of these reactions. The major distinctive physicochemical characteristics and thermal stabilities of the imino oximes were determined. All compounds were preliminary scanned against various strains of microbes to study their biological activity.

Keywords: Imines, 3-Hydroxyimino-5-methyl-2-hexanone, Physicochemical characteristics, Thermal stabilities.

INTRODUCTION

The interest in the study of imine oxime derivatives possessing potential donor sites has been intensively increasing owing to their coordinating capability[1], their pharmacological activity[2] and their uses in analytical chemistry as metal extracting agent[3]. A number of imino oximes, for example imipenem[4], panipenem[5] and meropenem[6] are currently in clinical use due to their broad antibacterial spectra and potent bactericidal effects. Oximes are extensively used as preferred derivatives for purification and characterization of carbonyl compounds and they play an important role as protecting[7] and selectively α-activating groups in synthetic organic chemistry[8]. Furthermore, their synthesis from non-carbonyl compounds provides a valid alternative pathway to carbonyl compounds[9]. It is also been reported that the oxime derivatives with amides, amines, semicarbazones, thiosemicarbazones finds wide applications in pharmaceutical industries[2, 10-13].

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During the past decade, extensive synthetic efforts have been made to confer anti-microbial activity of oximes and compounds containing NH_2 group[14,15]. Thus, we postulated that a combination of these two factors in a single side chain might lead to agents with a broader spectrum of microbial activity. The carbonyl group in the isonitroso oximes is an interesting linker in the construction of oximino systems[16]. We conceived that introduction of an oxime and NH_2 containing moiety into the aliphatic side chain was responsible for the improvements in biological activity, because the compounds having oxime moiety has shown to enhance drug activity in general. It was therefore; worthwhile to synthesise some derivatives of oxime 3-hydroxyimino-5-methyl-2-hexanone (HIMH) with ethylene diamine (in 1 : 1 and 1 : 2 proportions), salicylamide, anthranilamide, semicarbazide and thiosemi-carbazide with a view to see their combined activity and thermal stability. The names of the products obtained are bis[3-hydroxyimino-5-methyl-N-methyl]-2-imine (**1**), 3-hydroxyimino -5-methyl-2-hexanone-ethyleneamine-2-imine (**2**), 3-hydroxyimino-5-methyl-2-hexanone semicarbazone (**3**) and 3-hydroxyimino-5-methyl-2-hexanone thiosemicarbazone (**4**), 3-hydroxyimino-5-methyl-2-hexanone-N-salicoyl-2-imine (**5**) and 3-hydroxyimino-5-methyl-2-hexanone-N-anthraniloyl-2-imine (**6**).

EXPERIMENTAL

The reactions were carried out with analytical grade chemicals. The 3-hydroxyimino-5-methyl-2-hexanone (HIMH, oxime) was prepared by following the procedure reported in the literature[17]. The C. P. grade chemicals, whenever used were purified by standard methods. The organic solvents were redistilled before use. FTIR, spectra were recorded on Spectrum One (Perkin-Elmer) in KBr pellets, ^1H NMR spectra were recorded on BRUKER 300 AVANCE^{II} in $CDCl_3$ and DMSO. Thin layer chromatographic (TLC) analyses were performed to establish the purity of the compounds on the plates coated with silica gel G (Merck). TG-DTA were recorded on Pyris Dimond TG/DTA Thermogravimetric / Differential Thermal Analyzer

General method of synthesis of compounds

The compounds were prepared by treating stoichiometric quantities of the appropriate ethanolic solution of ethylene diamine / salicylamide / anthranilamide / semicarbazide and / thiosemicarbazide with an ethanolic solution of HIMH and 5-6 drops of conc. HCl. For the reaction of semicarbazide, 1-2 g of sodium acetate was added instead of Conc. HCl. The resulting mixture was refluxed for 3-4 hours in water bath. Hot reaction mixture was poured into crushed ice. The crystals obtained were separated by filtration and recrystalized from redistilled ethyl alcohol.

Compound characterization

Compound 1: Yield, 86%; Light yellow amorphous solid, mp 175ºC, IR ν_{NOH} = 3292 cm^{-1}, $\nu_{C=N}$ = 1467 cm^{-1}, ν_{N-O} = 1365 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.744-0.766 (d-2 x CH_3), 2.319 (s-CH_3), 1.820-1.933 (m-CH), 2.441-2.466 (d-CH_2), 11.293 (s-NOH), 3.402 (-CH_2-CH_2-); Anal. Calc. for $C_{16}H_{30}N_4O_2$: C, 58.731; H, 9.677; N, 21.344; O, 10.248, Found C, 58.557; H, 9.471; N, 21.085; O, 10.887. Molecular weight Calcd. 310; Found 310.

Compound 2: Yield, 88%; Light brown amorphous solid, mp 158ºC, IR ν_{NOH} = 3285 cm^{-1}, $\nu_{C=N}$ = 1431 cm^{-1}, ν_{N-O} = 1293 cm^{-1}, ν_{-NH_2} = 3213 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.898-0.908 (d-2 x CH_3), 2.259 (s-CH_3), 1.872-1.988 (m-CH), 2.593-2.608 (d-CH_2), 10.909 (s-NOH), 3.852 (-CH_2-CH_2-), 1.699 (d-NH_2); Anal. Calc. for $C_9H_{19}N_3O$: C, 58.38; H, 10.27; N, 22.70; O, 8.65 Found C, 58.47; H, 10.84; N, 22.13; O, 8.56. Molecular weight Calcd. 185; Found 185.

Compound 3: Yield, 96%; Light brown crystalline solid, mp 186ºC, IR ν_{NOH} = 3477.79 cm^{-1}, $\nu_{C=N}$ = 1434 cm^{-1}, ν_{N-O} = 965.92 cm^{-1}, $\nu_{C=O}$ = 1671.01, ν_{-NH_2} = 3203 cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 0.915-0.938 (d-2 x CH_3), 1.997-2.088 (m-CH), 2.020 (s-CH_3), 2.574-2.599 (d-CH_2), 1.659 (s-NH_2), 7.265(s-NH), 7.912 (s-NOH). Anal. Calc. for $C_8H_{16}N_4O_2$: C, 48.0; H, 8.0; N, 28.0; O, 16 Found C, 47.38; H, 8.17; N, 28.41; O, 16.04. Molecular weight Calcd. 200; Found 200.

Compound 4: Yield, 89%; White amorphous solid, mp 218ºC, IR ν_{NOH} = 3402.2 cm^{-1}, $\nu_{C=N}$ = 1585 cm^{-1}, ν_{N-O} = 954.7cm^{-1}, ν_{-NH_2} = 3207, $\nu_{C=S}$ = 1072, ^{1}H NMR ($CDCl_3$) δ 0.907-0.930 (d-2 x CH_3), 2.009-2.084 (m-CH), 2.106 (s-CH_3), 2.573-2.597 (d-CH_2), 1.762 (s-NH_2), 8.135 (s-NOH). Anal. Calc. for $C_8H_{16}N_4OS$: C, 44.44; H, 7.41; N, 25.93; O, 7.41; S, 14.81; Found C, 45.02; H, 7.35; N, 25.02; O, 7.92; S, 14.69. Molecular weight Calcd. 216; Found 216.

Compound 5: Yield, 92%; Light pink crystalline solid, mp 205ºC, IR ν_{NOH} = 3401.91 cm^{-1}, $\nu_{C=N}$ = 1590 cm^{-1}, $\nu_{C=O}$ = 1673.75, ν_{N-O} = 908.89 cm^{-1}, Ar-OH = 3193.95 cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 0.100 (d-2xCH_3), 0.878-0.974 (m-CH), 1.253 (s-CH_3), 2.046 (d-CH_2), 6.444 (Ar-OH), 6.825-6.958 (Benzene), 12.459(NOH). Anal. Calc. for $C_{14}H_{18}N_2O_3$: C, 64.12; H, 6.87; N, 10.69; O, 18.32; Found C, 64.37; H, 6.91; N, 10.85; O, 17.87. Molecular weight Calcd, 262; Found 262.

Compound 6: Yield, 93%; Pinkish brown crystalline solid, mp 187ºC, IR ν_{NOH} = 3473.37 cm^{-1}, $\nu_{C=N}$ = 1419.95 cm^{-1}, $\nu_{C=O}$ = 1672.19, ν_{N-O} = 916.52 cm^{-1}, Ar-NH_2 = 3373 cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 0.072 (d-2 x CH_3), 0.835-0.920 (m-CH), 1.251 (s-CH_3), 2.212 (d-CH_2), 6.678 (d-NH_2), 7.259-7.345 (Benzene), 7.918 (s-OH); Anal. Calc. for $C_{14}H_{19}N_3O_2$: C, 64.37; H, 7.28; N, 16.09; O, 12.26; Found C, 63.84; H, 7.53; N, 16.12; O, 12.51. Molecular weight Calcd. 261; Found 261.

RESULTS AND DISCUSSION

The ^{1}H NMR resonances and IR data given in the experimental section and the elemental analysis results confirm the identity of the compounds. All bands are assigned by comparing the spectrum with the standard values reported in the literature[18]. The physical characteristics of compounds have been studied and discussed. All the compounds synthesized here are solid substances, stable at room temperature and not affected by atmospheric conditions over at least in two weeks. On the other hand, as these compounds contain oximino functions in their molecules, they seem to be suitable candidates for further chemical modifications and may be pharmacologically active and useful as ligands in coordination chemistry.

Table 1: Thermal analysis data

Compd.	TG Temp. range (ºC)	DTA peak temp. (ºC)	Experimental loss (%)	Calculated loss (%)	Moiety lost
1	40-1000	175	84.624	84.52	$C_{16}H_{30}N_4O_2$
2	30-1000	158	84.458	84.41	$C_9H_{19}N_3O$
3	30-1000	186	92.458	92.50	$C_8H_{16}N_4O_2$
4	30-1000	220	53.522	53.70	$C_8H_{16}N_4OS$
5	30-1000	204	89.056	88.55	$C_{14}H_{18}N_2O_3$
6	40-1000	188	83.931	83.52	$C_{14}H_{19}N_3O_2$

For the biological evaluation of the compounds prepared, some representative examples were screened against nine different microorganisms (six bacteria and three fungi) using serial tube dilution method. The solvent used was DMF, and the sample concentrations were 200, 100, 50, 25 and 12.5 ppm. The test results obtained are listed in Table 2.

Antibacterial and antifungal data for tetracycline and amphotericine are also included in this Table for the purpose of comparison. The data show that these compounds generally exhibited moderate toxicity in the selected concentrations towards many of the biological strains tested.

From the physicochemical investigations, the chemical structures of compounds have been proposed as shown in the reaction scheme. All the compounds revealed good / moderate activities against several microorganisms (Table 2).

Table 2: Results of antimicrobial activities in ppm

Compd.	Antibacterial activity					Antifungal activity			
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**
1	25	50	50	50	50	25	25	25	50
2	50	50	100	25	50	50	100	100	100
3	25	25	25	12.5	50	12.5	25	50	25
4	50	12.5	25	50	12.5	25	25	50	25
5	25	100	25	50	100	100	50	100	100
6	25	200	50	25	50	25	100	50	100
Standard	25	12.5	12.5	25	12.5	25	12.5	25	12.5

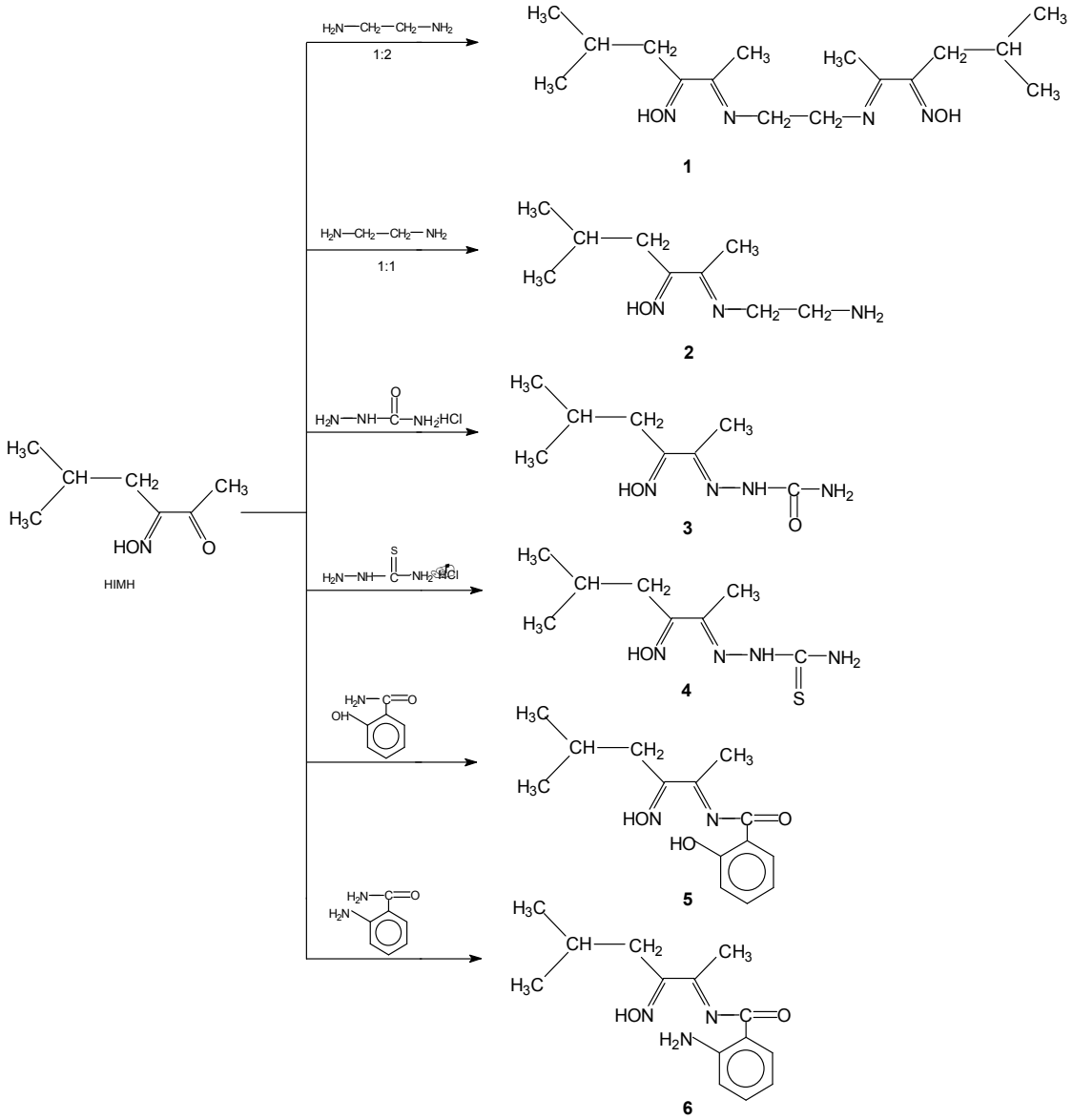
a. *C. diphtheriae*, **b.** *E. coli*, **c.** *S. dysenteriae*, **d.** *V. cholerae*, **e.** *S. typhi*; **f.** *S. aureus*; **g.** *C. albicans*; **h.** *A. niger*; **i.** *S. cerevisiae*, **Standard**: Tetracycline for anti bacterial activities and Amphotericine for anti fungal activities.

Thermal study

This study demonstrated that the compounds are crystalline solids; however, all decompose exothermically at about their mp. Polycrystalline mixtures or undesirable solvates were not detected.

The thermogravimetric analysis of compound synthesized was carried out between 28°C and 1000°C at a heating rate of 10°C per minute in the nitrogen atmosphere. All the compounds investigated show similar behavior in their TG and differential thermal analysis (DTA) studies. The thermogram and the differential thermogram show that the major weight

loss occurs in single stage between 30°C and 1000°C. This weight loss corresponds to the loss of complete molecule. The single stage decomposition of the compounds was also confirmed and compared with the endothermic peak in differential thermograms.



Scheme

Antimicrobial screening

The compounds synthesized in the present investigation have been subjected to various biological screening program based on their structural features so as to ascertain their suitability as potential chemotherapeutic agents. The various screening program conducted have been in the *in vitro* study by the serial tube dilution technique[19].

Serial tube rilution method

This method was used to study the biological activity of the compounds against some of the pathogenic fungi and bacteria. Microbial inoculum was prepared inoculating the selected strains into sterilized Sabouraud's broth to which 0.1 mg/mL of streptomycin was added to prevent bacterial contamination. After sporulation, the spores were harvested in the same media by gentle stirring using a magnetic stirrer and the spore suspension was poured into another sterile flask. To a 5 mL of Sabouraud's broth contained in a 15 mL Corning test-tube, 0.1 mL of the test solution of the compound in DMF was added. It was autoclaved at 15 lb pressure for 15 minutes. The tubes were then cooled and were inoculated with 0.1 mL spore suspension. The tubes were then kept on a rotary shaker and incubated at room temperature. The growth was measured after 24 h in case of antibacterial activity and 48 h for antifungal activity. The percentage growth of the fungi was calculated after determining the optical density (OD) of the solution on a spectrophotometer at 530 nm with inoculated Sabouraud's broth as blank. The growth of the microbes in the tube, which contained none of the antifungal / antibacterial agent, was assumed as 100%. The results were compared against those of the control tetracycline for anti bacterial activities and amphotericine for antifungal activities, which were screened simultaneously.

Biological activities

The biological activity data of compounds and the standards are shown in Table 2. It has been observed that the compounds used for current investigation show moderate antibacterial and antifungal activity at 12.5, 25, 50, 100 and 200 ppm. The study shows that the synthesized derivatives of imine oxime HIMH show moderate activity against all the organisms under study. The compound **3** shows good activity against all selected microbes. A bacteriostatic effect has been observed in a number of cases, which show that the compounds inhibit protein synthesis and act by binding to the ribosome[20]. The binding, however, is not tight, and when the concentration of the compound is lowered, the compound becomes free from the ribosome and growth is resumed. The more bulky group reduces considerably the polarity of the compounds[21]. This is due mainly to the partial sharing of its positive charge with the donor group and possible p-electron delocalization

over the whole compounds through *pp–pp* or *dp–dp* interactions of the orbitals of the compounds, which in turn increases the hydrophobic character of the compounds and thus, enables its permeation through the cell membrane of microorganisms. Compared to tetracycline, the present compounds are much less active against the representative strains of microorganisms. The antifungal activity of the compounds against *Candida albicans* and *Aspergillus niger* was studied by the tube dilution method[22]. The results have been expressed as percentage inhibition. The data show that the antifungal activity of compounds is significantly enhanced on addition of more bulky group. All the compounds show moderate antifungal activity against both fungi. Generally, the compounds show higher activity against *Aspergillus niger* than against *Candida albicans*[23]. Compared to standard antifungal compound amphotericin, the present compounds are much less active.

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