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Biologically active hydroxymoyl chlorides as antifungal agents†

Tabasum Ismail[a], Syed Shafi[a], Parvinder Pal Singh[a], Naveed Ahmed Qazi[a], Sanghapal D Sawant[a], Intzar Ali[b], Inshad Ali Khan[b], H M S Kumar*[a], Ghulam Nabi Qazi[d] & M Sarwar Alam[c]

[a]Departments of Synthetic Chemistry & [b]Biotechnology,
[c]Department of Chemistry, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi, 110 062

[d]Indian Institute of Integrative Medicine (formerly RRL, Jammu), Canal Road, Jammu Tawi 180 001, India

E-mail: hmskumar@yahoo.com

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Several oximes and oxime ethers have been developed as antimicrobial agents. A series of chlorooximes (hydroximoyl chlorides) have been synthesized and tested for antifungal activity under *in-vitro* conditions against *Candida albicans, Candida parapsilosis, Candida glabrata, Candida krusei, Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger.* The derived antifungal activity has been compared with the corresponding oximes. The results show that most of the chlorooximes exhibit potent antifungal activity with *anti*-isomers showing better activity. It is observed that most of the chlorooximes show interesting antifungal activity (MICs $\leq$ 32 μg/mL) compared to oximes. Compound **3q** (2,3-dimethoxy phenyl hydroxymoyl chloride) is the most active compound. This compound is active against all the *Candida* species (MIC 0.5 μg/mL) as well as filamentous fungi with MIC range of 2-4 μg/mL. This series of compounds are fungicidal in nature as evident from the MFC results.

Keywords: Hydroxymoyl chloride (chlorooxime), oximes, hydroxylamine hydrochloride, *N*-chlorosuccinimide, antifungal activity

It is well known that fungi cause many diseases of plants, animals, and humans and often acquire drug resistance during treatment. Since fungal infections are caused by eukaryotic organisms, for this reason they generally present more difficult therapeutic problems than do bacterial infections. Fungal infections have emerged as a major cause of morbidity and often of mortality in immunocompromised and debilitated patients over the past two decades[1,2]. Many of the currently available drugs are toxic, produce recurrence because they are fungi static and not fungicidal or lead to the development of resistance due in part to the prolonged periods of administration of the available antifungal drugs. The usage of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of present treatments of bacterial and fungal infections and drug side-effects[3-6]. Although the use of a new generation of triazoles, the available polyenes in lipid formulations, the use of echinocandins or the combination therapy have been introduced as alternatives in the last ten years, fungal infections remain difficult to eradicate[7]. There is, therefore, a clear need for the discovery of new chemical entities with antifungal properties, which could lead to the development of new drugs for the management of fungal infections. Oximes and their derivatives have attracted considerable attention since the past few decades due to their chemotherapeutic value. Many oximes are found to be anti-hyperglycemic[8], anti-neoplastic[9], anti-inflammatory[10], anti-leishmanial[11], and VEGFR-2 kinase inhibitors[12]. Oximes also possess transcriptional activity[13]. Besides this, several oximes and oxime-ethers have been developed as anti-microbial agents[14]. The current study attempted to assess particularly the antifungal effects of various chlorooximes on different strains of fungi such as *Candida albicans*, *C. parapsilosis, C. glabrata, C. krusei, Aspergillus fumigatus, A. flavus* and *A. niger* to explore their therapeutic potential. Chemically, chlorooximes are important intermediates for the synthesis of nitrile oxides which in turn are used in a number of chemical reactions such as dipolar cyloaddition reactions and lead to the synthesis of a variety of heterocycles like isoxazoles, isoxazolines, *etc*. Even though oximes were used as

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pharmacophoric groups for the generation of highly effective anti-microbials, no efforts has been hitherto made to explore the biological potential of their halogenated analogs, *i.e*., chorooximes. In the present paper, is reported the synthesis of a focused library of chlorooximes to evaluate their antifungal activity including minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) against standardized ATCC isolates along with an analysis of the structure-activity relationship (SAR) in comparison to the corresponding parent oximes.

Results and Discussion

Chemistry

Herein is reported a library of oximes and their corresponding chlorooximes acting as antifungal agents. The oximes and chlorooximes were synthesized as per the literature procedure (**Table I**) (ref. 15). To the neutralized solution of hydroxylamine hydrochloride, aldehyde **1** was added and the reaction mixture was stirred for 1 hr at RT. Excess of water was added to the reaction-mixture and organic compound was extracted with ethylacetate (2×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford pure oxime (*syn* and *anti*) in 99% yield.

Oximes **2**, when treated with *N*-chlorosuccinimide in DMF led to the synthesis of corresponding chlorooximes **3** in good yields (>85%). All these synthesized compounds (**Table I**) were screened for anti-fungal activity against seven fungal strains (*Candida albicans*, *Candida parapsilosis, Candida glabrata, Candida krusei, Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger*) using microdilution technique.

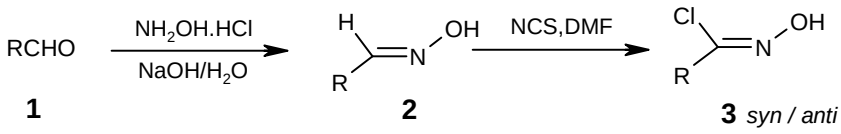
Antifungal activity and structure activity relationship studies

The MIC and MFC of the oximes and chlorooximes is described in **Table II**. Among the main observations, it can be stated that most of the chlorooximes showed interesting antifungal activity (MICs $\leq$ 32 μg/mL) whereas oximes showed no such attractive MICs. Among these derivatives, compounds **3a, 3b, 3i, 3m, 3q, 3r, 3s, 3t** and **3v** showed significant activity against *C. albicans, C. parapsilosis, C. glabrata, C. krusei, A. fumigatus, A. flavus* and *A. niger* with MICs in single digits. Compound **3q** was the most active compound. This compound was very potent against all the *Candida* species (MIC 0.5 μg/mL). It was also active against filamentous fungi with MIC range of 2-4 μg/mL. This series of compounds were fungicidal in nature as evident from the MFC results.

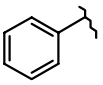
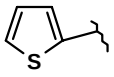
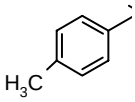
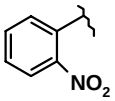
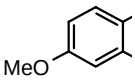
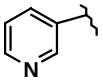
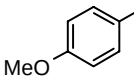
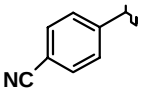
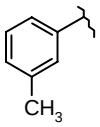
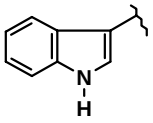
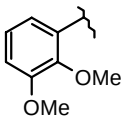
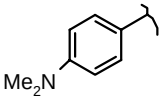
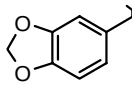
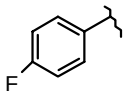
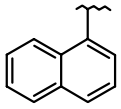
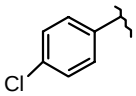
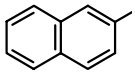
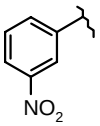
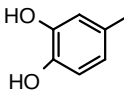
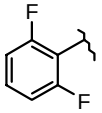
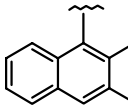
SAR studies on these compounds revealed that oximes are less potent than chlorooximes. Among chlorooximes, compound **3q** (2,3-dimethoxy phenyl hydroximoyl chloride) exhibited highest activity (MIC 0.5 μg/mL and MFC 0.5 μg/mL), showed to be the most potent fungicides amongst all the substrates studied and was active against all the strains of *Candida* and *Aspergillus* species. Even though a variety of chlorooximes derived from phenyl, substituted phenyl and heteroaromatic oximes exhibited potent antifungal activity, both electron donating and electron withdrawing groups on aromatic nucleus have shown no appreciable effect on the MIC and MFC values. Similarly, bulky aromatic rings like napthyl and anthracyl oximes did not have profound effect on the antifungal activity. Chlorooximes derived from aliphatic oximes have shown lower activity in comparison with their aromatic counterparts. Since these compounds exist in two isomeric forms *i.e*, *syn*- and *anti*-isomers, the need to examine the antifungal activity of each isomeric form was felt. Thus, compound **3q** being the most potent antifungal derivative, was subjected to column chromatography (silica gel 230-400 mesh as stationary phase, hexane/ethylacetate as mobile phase) and both geometrical isomers were isolated in pure form. Both the isomers were identified on the basis of their coupling constant values, *syn*-isomer having coupling constant values of 8.01 Hz and 8.06 Hz whereas *anti*-isomer having coupling constant values of 8.93 Hz and 8.94 Hz. The distinction between *syn* and *anti* could also be made on the basis of polarity (*syn* being more polar than *anti*) and their respective melting point differences (*syn*-isomer having melting point of 108.8°C and *anti*-isomer having melting point of 92°C). Each isomer thus isolated was screened for anti-fungal activity against the above mentioned seven strains (**Table III**). It was found that *anti*-isomer was more potent than *syn*-isomer.

It is now well established that the zinc and calcium dependent family of proteins called the MMPs (matrix metalloproteinases) which are secreted by fungus such as *Candida albicans*, hydrolyses the collagen proteins on skin and consequently causes fungal infections under physiological conditions. These are selectively regulated by endogenous inhibitors.

Table I — Synthesis of various oximes and their corresponding chlorooximes



1 **2** **3** *syn / anti*

Compd	m.p (°C)	R	Compd	m.p (°C)	R
2a	130		**2l**	liquid	
3a	50		**3l**	50	
2b	133		**2m**	74-75	
3b	102		**3m**	71.5-72.5	
2c	100-02		**2n**	107-08	
3c	95-98		**3n**	153-54	
2d	146-48		**2o**	89-91	
3d	142-45		**3o**	89	
2e	127-28.5		**2p**	63	
3e	151-52		**3p**	49	
2f	197-200		**2q**	96.5	
3f	180		**3q**	111.8-13	
2g	144		**2r**	111-12	
3g	semisolid		**3r**	126-27	
2h	85-87		**2s**	98	
3h	72-73		**3s**	97	
2i	96-97		**2t**	147-4	
3i	76-77		**3t**	semisolid	
2j	119-20		**2u**	157	
3j	99-100		**3u**	semisolid	
2k	114.5-15		**2v**	146.5	
3k	104-06		**3v**	114-15	

a) Products characterised by IR, ^{1}H NMR and mass spectral analysis

Table II — The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of oximes, chlorooximes and the control drug. MIC and MFC are expressed in μg/mL

Entry	Test organisms													
	Yeast								Filamentous Fungi					
	C. albicans		*C.parapsilosis*		*C. glabrata*		*C. krusei*		*A. fumigatus*		*A. flavus*		*A. niger*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Ampho-tericin-B	0.5	0.5	0.5	1.0	0.5	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5	1.0
2a	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3a	4.0	4.0	2.0	4.0	2.0	4.0	2.0	2.0	8.0	8.0	4.0	4.0	4.0	4.0
2b	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3b	8.0	8.0	4.0	8.0	4.0	8.0	4.0	4.0	32	64	32	32	16	16
2c	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3c	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2d	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3d	64	>64	64	>64	64	64	64	64	>64	>64	>64	>64	64	>64
2e	16	32	16	16	16	16	8.0	16	>64	>64	>64	>64	64	>64
3e	32	64	32	32	32	32	32	32	>64	>64	>64	>64	>64	>64
2f	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3f	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2g	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3g	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2h	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3h	32	32	16	32	16	32	16	16	64	64	64	64	32	32
2i	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3i	8.0	8.0	8.0	8.0	8.0	8.0	4.0	4.0	16	16	8.0	16	16	16
2j	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3j	64	>64	64	>64	64	>64	64	64	64	>64	64	>64	64	>64
2k	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3k	32	32	16	32	16	16	16	16	32	32	32	32	16	32
2l	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3l	16	32	16	16	16	16	8.0	16	32	32	16	16	16	16
2m	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3m	8.0	8.0	4.0	8.0	4.0	4.0	2.0	2.0	16	16	16	16	8.0	8.0
2n	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3n	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2o	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3o	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2p	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3p	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2q	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3q	1.0	1.0	1.0	1.0	1.0	1.0	0.5	0.5	4.0	4.0	2.0	2.0	2.0	2.0
2r	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3r	16	16	8.0	8.0	8.0	8.0	4.0	4.0	32	32	16	32	16	16
2s	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3s	8.0	8.0	8.0	8.0	8.0	8.0	4.0	4.0	16	16	16	16	16	16
2t	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	64	>64

— Contd

Table II — The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of oximes, chlorooximes and the control drug. MIC and MFC are expressed in μg/mL — *Contd*

Entry	Test organisms													
	Yeast								Filamentous Fungi					
	C. albicans		*C.parapsilosis*		*C. glabrata*		*C. krusei*		*A. fumigatus*		*A. flavus*		*A. niger*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Ampho-tericin-B	0.5	0.5	0.5	1.0	0.5	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5	1.0
2t	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	64	>64
3t	4.0	8.0	4.0	4.0	4.0	4.0	2.0	2.0	16	16	8.0	16	8.0	16
2u	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3u	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2v	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3v	4.0	8.0	4.0	4.0	4.0	4.0	2.0	2.0	16	16	16	16	8.0	8.0

Table III — The comparative MIC and MFC values for each isomer of compound **3q**. MIC and MFC are expressed in μg/mL.

Entry	Test organisms													
	Yeast								Filamentous Fungi					
	C. albicans		*C. parapsilosis*		*C. glabrata*		*C. krusei*		*A. fumigatus*		*A. flavus*		*A. niger*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
3q *syn*	4.0	4.0	4.0	4.0	4.0	4.0	2.0	2.0	8.0	16	8.0	8.0	8.0	8.0
3q *anti*	1.0	1.0	1.0	1.0	1.0	1.0	0.5	1.0	4.0	8.0	4.0	8.0	4.0	8.0

Imbalances between the active enzymes and their natural inhibitors lead to the fungal disease. The potential for using specific enzyme inhibitors to redress this balance has led to intensive research focused on the design, synthesis[16], and molecular deciphering of low molecular mass inhibitors of this family of proteins. Moreover, certain derivatives, such as oximes and hydrazides, also possess selective chelating or binding properties with the zinc active-site of MMPs. Such small molecule MMP inhibitors act either as competitive substrates or distort the geometry of one of zinc centers in MMPs by binding with such zinc cations in the form of a five or six-member ring with one or two double bonds, respectively, in a bidentate structure form. After distorting the geometry of such zinc cations, these MMP inhibitors appear to move away from this "deactivated" active-site and go to the next active-site to deactivate it[17]. Thus, a similar mechanism of antifungal action is envisaged by chloroximes and oximes as they are strong ligands for zinc binding. A detailed mechanistic evaluation is currently in progress to investigate the actual role of this novel class of anti-fungal compounds.

Materials and Methods

Antifungal activity of all compounds was performed using microdilution method (NCCLS M27 A, NCCLS M38 P) against four yeast strains (*Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258) and three filamentous fungi (*Aspergillus fumigatus* LSI-II, *Aspergillus niger* ATCC 16404, *Aspergillus flavus* MTCC 2799). The ATCC cultures used for this study were purchased from American Type Culture Collection, Manassas, VA 20108 USA. RPMI supplemented with 0.165 M MOPS was used as test media. The MIC (Minimum Inhibitory Concentration) was determined by serial 2-fold dilution of the test compound in the above-mentioned media in 100 μL volume in a 96 well U bottom microtitre plate. Yeast inoculums were prepared by growing isolates on Sabouraud Dextrose Agar plates overnight at 37°C. The isolated colonies were picked up and suspensions

were prepared in sterile normal saline with 0.05% (vol/vol) Tween 80 (NST). The density of these suspensions was adjusted to 1 McFarland (1-5 × 10^6 CFU/mL), further diluted to 1:50 in NST and 1:20 in RPMI 1640 media with 0.165 M MOPS to get 2 times the final inoculum (1-5 × 10^3 CFU/mL). For filamentous fungi, the inoculums were prepared from the spores of the cultures, which were sporulated on Potato Dextrose Agar (PDA) after an incubation of 7 days at 28ºC. The density of the spore suspension was adjusted to an optical density of 0.09 to 0.11. These suspensions were diluted 1:50 in RPMI 1640 media with 0.165 M MOPS to get the final inoculum (0.4 × 10^4 to 5 × 10^4 CFU/mL). 100 µL of this 2 × inoculum of yeast and fungi was added to each well of the microtitre plate. The plates were incubated at 37°C for 48 hr. The plates were read visually and the minimum concentration of the compound showing no turbidity was recorded as MIC. The MFC was determined by spotting 10 µL volume on Sabouraud Dextrose Agar plate from the wells showing no visible growth. The plates were incubated at 37ºC for 48 hr (ref. 18,19). Minimum concentration of compound showing absence of growth was recorded as MFC. Amphotericin-B was taken as a standard.

Experimental Section

All chemicals (reagent grade) used were commercially available. Melting points were measured on a Buchi D-545 melting point apparatus and are uncorrected. IR spectra were recorded on a Bruker Vector 22 instrument using KBr pellets and in chloroform. ^{1}H NMR were recorded on a Bruker DPX 200 instrument in $CDCl_3$ using TMS as internal standard for protons. ^{1}H NMR chemical shifts and coupling constants *J* are given in ppm and Hz respectively. Mass spectra were recorded on EIMS (Shimadzu) instrument. Mass-spectrometric (MS) data is reported in *m/z*. Elemental analysis was carried out using Elemental Vario EL III elemental analyser. Elemental analysis data is reported in % standard. Silica gel 230-400 mesh was supplied by Loba Chemie. Homogeneity of the compounds was checked by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV 254-366 nm.

Typical procedure for the synthesis of 2,3-dimethoxybenzaldoxime, 2q: In a typical procedure hydroxylamine hydrochloride (0.50 g, 7.22 mmole) was dissolved in water and neutralized with NaOH. To the neutralized solution of hydroxylamine hydrochloride, 2,3-dimethoxybenzaldehyde (1.00 g, 6.02 mmole) was added and the reaction mixture was stirred for 1 hr at RT. Excess of water was added to the reaction mixture and the organic compound was extracted with ethylacetate (2 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford pure oxime (*syn* and *anti*) in 99% yield.

Typical procedure for the synthesis of 2,3-dimethoxy phenyl hydroxymoyl chloride, 3q: 2,3-Dimethoxybenzaldoxime (1.00 g, 5.52 mmole) was dissolved in DMF (20 mL). *N*-chlorosuccinimide (0.95 g, 7.18 mmole) was added to the above solution and the reaction-mixture was stirred for 8-10 hr. Excess of water was added and extracted with diethylether (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford pure chlorooxime (*syn* and *anti*). The chlorooxime so formed was subjected to column chromatography (silica gel 230-400 mesh as stationary phase, hexane/ethylacetate as mobile phase) and both geometrical isomers were isolated in pure form.

Phenyl hydroxymoyl chloride, 3a: (white solid); m.p. 50°C; IR (KBr): 3210.53, 3061.35, 2927.53, 1706.75, 1654.93, 1493.41, 1446.39, 1387.57, 1236.53, 1181.87, 1101.72, 935.50, 763.39 and 691.90 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.42 (3H, m, Ar-H), 7.85 (2H, m, Ar-H); ESI-MS: *m/z* 155.58 (M^+). Anal. Calcd. for C_7H_6ClNO: C, 54.04; H, 3.89; N, 9.00. Found: C, 53.98; H, 4.00; N, 9.11%.

Thiophene-2-hydroxymoyl chloride, 3b: (brownish solid); m.p. 102°C; IR (KBr): 3321.07, 3106.69, 2923.94, 1649.09, 1597.62, 1422.54, 1237.42, 993.23, 877.32, 857.10, 836.72, 800.16 and 710.33 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.13 (1H, s, N-OH), 6.88 (2H, m, Ar-H), 7.05 (1H, m, Ar-H); ESI-MS: *m/z* 184.61 (M+Na). Anal. Calcd. for C_5H_4ClNOS: C, 37.16; H, 2.49; N, 8.67. Found: C, 36.97; H, 2.44; N, 8.99%.

4-Chloro phenyl hydroxymoyl chloride, 3i: (white solid); m.p. 76-77°C**;** IR (KBr): 3292.05, 2924.02, 2852.54, 1650.53, 1595.24, 1488.68, 1401.63, 1245.38, 1093.31, 1014.93, 936.59, 828.95 and 665.98 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.70 (1H, s, N-OH), 7.38 (2H, d, *J* = 8.74 Hz, Ar-H), 7.80 (2H, d, *J* = 8.77 Hz, Ar-H); ESI-MS: *m/z* 191.03 (M+1). Anal. Calcd. for $C_7H_5Cl_2NO$: C, 44.24; H, 2.65; N, 7.37. Found: C, 44.39; H, 2.85; N, 7.12%

4-Methyl phenyl hydroxymoyl chloride, 3m: (yellow solid); m.p. 71.5-72.5°C; IR (KBr): 3384.87, 2924.57, 2360.95, 2341.82, 1655.30, 1558.76, 1387.98, 1097.87, 1019.00, 896.45, 832.93 and 663.91 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.24 (1H, s, N-OH), 2.40 (3H, s, 4-CH_3), 7.24 (2H, d, *J* = 8.67 Hz, Ar-H), 7.42 (2H, d, *J* = 8.68 Hz, Ar-H); ESI-MS: *m/z* 169.62 (M^+). Anal. Calcd. for C_8H_8ClNO: C, 56.65; H, 4.75; N, 8.26. Found: C, 56.53; H, 4.99; N, 8.32%.

2,3-Dimethoxy phenyl hydroxymoyl chloride, 3q: (white solid); m.p. 111.8-13°C**;** IR (KBr): 3252.47, 3020.13, 2975.25, 19998.04, 1578.64, 1477.46, 1424.85, 1320.11, 1221.47, 1173.56, 1004.04, 984.11, 972.00, 768.58 and 738.88 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.86 (3H,s, -OCH_3), 3.88 (3H, s, -OCH_3), 6.93 (2H, d, *J* = 8.06 Hz, Ar-H), 7.35 (1H, d, *J* = 7.78, Ar-H); ESI-MS: *m/z* 215.65 (M^+). Anal. Calcd. for $C_9H_{10}ClNO_3$: C, 50.13; H, 4.67; N, 6.50. Found: C, 50.25; H, 4.12; N, 6.66%.

2,3-Dimethoxy phenyl hydroxymoyl chloride, 3q (*syn*): (white solid); m.p.108.8°C; IR (KBr): 3405.15, 3081.34, 2923.19, 2358.08, 2309.30, 1573.95, 1471.44, 1431.18, 1419.14, 1333.27, 1297.75, 1265.69, 1055.33, 1018.42, 1005.68, 931.46, 858.41, 786.97 and 669.36 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.93 (3H, s, -OCH_3), 4.02 (3H, s, -OCH_3), 6.7 (2H, d, *J* = 8.06 Hz, Ar-H), 7.1(1H, d, *J* = 8.01 Hz, Ar-H); ESI-MS: *m/z* 215.65 (M^+). Anal. Calcd. for $C_9H_{10}ClNO_3$: C, 50.13; H, 4.67; N, 6.50. Found: C, 50.00; H, 4.78; N, 6.39%.

2,3-Dimethoxy phenyl hydroxymoyl chloride, 3q (*anti*): (white solid); m.p 90.2°C; IR (KBr): 3355.48, 3081.94, 2924.55, 2359.81, 2310.31, 1574.78, 1477.03, 1420.21, 1339.35, 1274.44, 1232.36, 1095.31, 1045.41, 1006.95, 901.59, 836.46, 808.68, 675.46 and 617.49 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.87 (3H, s, -OCH_3), 3.98 (3H, s, -OCH_3), 6.93 (2H, d, *J* = 8.93 Hz, Ar-H), 7.12 (1H, d, *J* = 8.94 Hz, Ar-H); ESI-MS: *m/z* 216.65 (M+1). Anal. Calcd. for $C_9H_{10}ClNO_3$: C, 50.13; H, 4.67; N, 6.50. Found: C, 50.28; H, 4.97; N, 6.44%.

2-Naphthyl hyroxymoyl chloride, 3t: gummy liquid; IR ($CHCl_3$): 3238.21, 3058.80, 2925.55, 1701.82, 1630.42, 1602.23, 1503.74, 1404.30, 1271.58, 1185.20, 1125.23, 1095.29, 861.78, 820.19, 750.37, 657.34, 616.48, 581.31 and 474.61 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.92 (1H, s, N-OH), 7.54 (2H, m, Ar-H), 7.80-7.95 (3H, m, Ar-H), 8.00 (1H, d, *J* = 8.69 Hz, Ar-H), 8.34 (1H, s, Ar-H); ESI-MS: *m/z* 229 (M+Na). Anal. Calcd. for $C_{11}H_8ClNO$: C,64.25; H, 3.92; N, 6.81. Found: C, 64.18; H, 3.99; N, 6.65%.

9-Anthracyl hydroxymoyl chloride, 3v: (yellow solid); m.p. 114-15°C; IR (KBr): 3374.73, 3054.03, 2924.61, 2286.76, 1672.39, 1623.60, 1442.59, 1418.93, 1376.02, 1293.92, 1266.64, 1248.18, 1016.85, 955.19, 892.23, 842.21, 779.28, 733.50, 611.38, 592.52 and 539.22 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.51-7.70 (4H, m, Ar-H), 8.04 (2H, d, *J* = 8.36 Hz, Ar-H), 8.27 (2H, d, *J* = 8.71 Hz, Ar-H), 8.54 (1H, s, Ar-H); ESI-MS: *m/z* 278 (M+Na). Anal. Calcd. for $C_{15}H_{10}ClNO$: C, 70.46; H, 3.94; N, 5.48. Found: C, 70.54; H, 3.88; N, 5.55%.

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