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Synthesis and Antimicrobial Evaluation of Some Novel Substituted 2-chloroacetanalides

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Abstract: The title compounds were prepared by the reaction of substituted primary amines with chloroacetylchloride in benzene. Structures of all these compounds have been elucidated by their elemental analysis, spectral studies and molecular weights. All the products were assayed for their antimicrobial activities against Staphylococcus aureus and Xanthomonas holcicola bacteria and Aspergillus niger and Fusarium oxysporum fungi and results were compared with that of reference drugs.
Keywords: Substituted chloroacetinilides, antibacterial, antifungal.

1. Introduction

There has been considerable interest in the chemistry of substituted acetanilide derivatives , owing to presence of this moiety in various synthetic pharmaceuticals which display a broad spectrum of biological activities, viz. antimicrobial[1,2], antiviral[3,4], anthelmentic[5], analgesics[6], anti-inflammatory[7], anticytotoxic [8,9] etc. The substituted chloroacetanilides have been explored to a lesser extent, in spite of the presence of different substitutes which provides an additional point of structural diversity in acetanilide a based pharmacophore. The incidence of multi drug resistance against many bacteria and fungi call upon discovery of new drugs effective against pathogenic microbes. This led us to synthesize some novel substituted chloroacetanilides (1) *by* the reaction of substituted primary amines with chloroacetylchloride and evaluate their antimicrobial properties, which have not been reported hitherto (scheme 1).

2. Experimental

2. 1. Materials and Methods

All the substituted primary amines (Aldrich, Germany) and chloroacetylchloride (Spectrochem, India) products and solvents were used in synthetic work and antimicrobial studies as supplied.

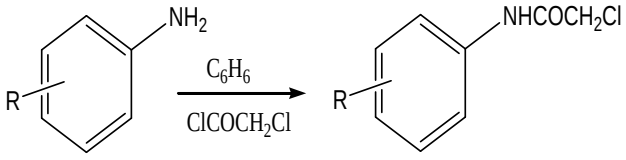
All the melting points determined in open glass capillaries are uncorrected. Purity of synthesized compounds has been checked by thin layer chromatography. Molecular weights were determined by Rast's method using camphor as solvent. Microanalyses for nitrogen, carbon, sulfur and hydrogen elements were done on Vario-el-III Element R. UV spectra of samples were recorded with SP65 UV GALLENK AMP, U.K spectrophotometer in acetone whereas IR spectra were recorded on FTIR-21 spectrophotometer using KBr disc. Brukner Avance spectrometer operating at 400 MHz was used to record ^{1}H NMR and ^{13}C NMR spectra in $CDCl_3$ / DMSO medium using TMS as an internal standard.

2.2. Synthesis of the Compounds

All compounds were synthesized by following the reported procedure [10].

2.2.1.Synthesis of substituted chloroacetanilides (1)

To 0.05 mol of substituted primary amine in benzene, 4.5 ml of chloroacetyl chloride was slowly added dropwise with continuous stirring except for the synthesis of o-iodo-2-chloroacetanilide which was prepared by 0.03 mol of o-iodo-aniline in benzene with 2.5 ml of chloroacetylchloride. Precipitates of substituted chloroacetanilide products obtained during the course of the reaction were filtered under suction and the filtrates were discarded. The products were washed with benzene repeatedly and air dried products were collected (Scheme 1).



1

1a; R= H
1b; R= 3-Br
1c; R= 4-Br
1d; R= 3-Cl
1e; R= 2-I
1f; R= 4-N$(CH_3)_2$

Scheme 1

All compounds **(1)** showed single spot on thin-layers in all the one component developing solvents. Physico-chemical data of newly synthesized compounds are given in Table 1. Theoretically calculated molecular weights are in fair agreement with experimental molecular weight values of the products.

Table 1. Physico-chemical data of synthesized compounds (1a-1f)

Comp	***Mol. Formula***	***Colour***	***M.P. (*** °C ***)***	***Yield %***	***Found% (Calcd)***			***Mol. weight (gm/mol)***		***Error (%)***
					N	***C***	***H***	***Calcd.***	***Found***	
1a	C_8H_8ClNO	Dirty yellow	130	74	8.61 (8.26)	56.20 (56.64)	4.82 (4.72)	168.5	165.42	1.84
1b	$C_8H_7BrClNO$-m	White	201	53	6.06 (5.63)	38.66 (38.63)	3.13 (2.82)	248.5	-	-
1c	$C_8H_7BrClNO$-p	White	176	86	6.19 (5.63)	38.01 (38.63)	3.15 (2.82)	248.5	240.61	3.18
1d	$C_8H_7Cl_2NO$-m	White	212	38	6.85 (6.86)	46.85 (47.06)	3.31 (3.43)	204	-	-
1e	C_8H_7IClNO-o	Light purple	175	34	5.16 (4.74)	32.57 (32.49)	2.78 (2.37)	295.5	294.07	0.49

3. Results And Discussion

3.1 Structural studies

Molecular formulae of the compounds (Table 1) derived from elemental analyses data are supported by their molecular weights.

The structures of the new products are elucidated with the aid of their IR, UV, ^{1}H NMR and ^{13}C NMR spectra. IR spectra of chloroacetanilides (1a-1f) display well defined peaks of their characteristic groups, C=O, C-N, N-H, -CH_2 and H_2C-Cl at 1597-1669 cm^{-1}, 1296-1353 cm^{-1}, 1205-1228 cm^{-1}, 1145-1316 cm^{-1} and 720-758 cm^{-1} respectively. Besides these νC=C, νC-H, νC-Br, νC-Cl, νC-I and νC-N frequencies of aromatic amines present in these compounds are also recorded in their absorption spectra. UV spectra exhibited one doublet or broad band at 210 nm attributed to $\pi \rightarrow \pi^*$ electronic transition and one or two bands in 295-325 nm range of $n \rightarrow \pi^*$ transitions of C=O chromophoric group. ^{1}H and ^{13}C NMR spectra of 1a, 1b and 1f compounds as

typical examples of the series have been examined. Their ^{1}H NMR spectra display four common peaks in δ 7.00-7.35, δ 3.25-3.67, δ 7.51-7.76 and δ 9.95-10.95 corresponding to –NH-, –CH_2-, arom. H and >C-OH (enolic) groups respectively; an additional band of -CH_3 group of –N $(CH_3)_2$ in 1f is observed at δ 4.34. >C-OH (enolic) group peak could be accounted for in terms of tautomerism through their active methylene group involving transfer of one –CH_2 hydrogen to adjacent ketonic group. ^{13}C NMR spectral bands at 164.6-165.5 ppm, 43.5-45.9 ppm, 116.3-138.5 ppm and 164.5-165.5 ppm attributed to >C=O, -CH_2-, arom. C and C-OH (enolic) respectively and an additional signal at 44 ppm in 1f is due to –$N(CH_3)_2$ group support of UV, IR and ^{1}H NMR inferences regarding the formation of chloroacetanilides by interaction of primary amines with chloroacetylchloride.

IR, ^{1}H NMR, ^{13}C NMR and UV spectral bands were assigned by comparing the experimental values with those reported in literature [11, 12].

2-chloroacetanilide 1a: IR (KBr, λ in cm^{-1}): 1597 (C=O), 1317 (C-N), 1528 (N-H), 722 (C-Cl), 1222 (CH_2), 1461, 1602 (arom. C=C), 3147 (C-H arom.); ^{1}H NMR ($CDCl_3$, δ/ppm,): 6.995 (s, 1H, -NH-), 3.25 (s, 2H, CH_2), 7.65 (m, 5H, arom. H), 9.95 (s, 1H, C-OH, enol); ^{13}C NMR ($CDCl_3$, δ/ppm): 164.64 (C=O), 119.86-138.48 (arom. C), 44.53 (CH_2), 164.64 (C-OH, enol); UV (λ in nm): 210 doublet (C=O due to π → π*), 315 (C=O due to n → π*).

m-bromo-2-chloroacetanilide 1b: IR (KBr, λ in cm^{-1}): 1669 (C=O), 1313 (C-N), 1513 (N-H), 728 (C-Cl), 1248 (CH_2), 1595, 1573, 1550, 1487 (arom. C=C), 2923, 3074, 3111 (arom. C-H); 785 (m- substitution), 611(C-Br); UV (λ in nm): 210 doublet (C=O due to π → π*), 315 (C=O due to n → π*).

p-bromo-2-chloroacetanilide 1c: IR (KBr, λ in cm^{-1}): 1668 (C=O), 1330 (C-N), 1505 (N-H), 736 (C-Cl), 1197 (CH_2), 1462, 1486, 1549 (arom. C=C), 2921, 3075, 3111 (C-H arom.); 822 (p-substitution), 615(C-Br); ^{1}H NMR ($CDCl_3$, δ/ppm,): 7.35 (s, 1H, -NH-), 3.40 (s, 2H, CH_2), 7.51 (m, 5H, arom. H), 10.10 (s, 1H, C-OH, enol); ^{13}C NMR ($CDCl_3$, δ/ppm): 164.78 (C=O), 116.34-137.73 (arom. C), 43.45 (CH_2), 164.78 (C-OH, enol); UV (λ in nm): 210 doublet (C=O due to π → π*), 295 (C=O due to n → π*).

m-chloro -2-chloroacetanilide 1d: IR (KBr, λ in cm^{-1}): 1639 (C=O), 1353 (C-N), 1516 (N-H), 720 (C-Cl), 1264 (CH_2), 1478, 1549, 1576 (arom. C=C), 2921, 3059 (arom. C-H); 787 (m-substitution); UV (λ in nm): 210 doublet (C=O due to π → π*), 315 (C=O due to n → π*).

o-iodo-2-chloroacetanilide 1e: IR (KBr, λ in cm^{-1}): 1628 (C=O), 1345 (C-N), 1517 (N-H), 758 (C-Cl), 1234 (CH_2), 1461, 1517, 1577, 1602 (arom. C=C), 2923, 3080 (arom. C-H); 723 (o-substitution), 667 (C-I); UV (λ in nm): 210 doublet (C=O due to π → π*), 325 (C=O due to n → π*).

p-dimethylamino-2-chloroacetanilide 1f: IR (KBr, λ in cm^{-1}): 1617 (C=O), 1296 (C-N), 1514 (N-H), 723 (C-Cl), 1230 (CH_2), 1459, 1528, 1583 (arom. C=C), 2924, 3137 (C-H arom.); 828 (p- substitution); ^{1}H NMR (DMSO, δ/ppm,): 7.25 (s, 1H, -NH-), 3.67 (s, 2H, CH_2), 7.76 (m, 5H, arom. H), 10.95 (s, 1H, C-OH, enol), 4.34 (-CH_3, substituted group); ^{13}C NMR (DMSO, δ/ppm): 165.51 (C=O), 120.76, 121.78 (arom. C), 45.90 (CH_2), 165.51 (C-OH, enol), 43.91 (N-CH_3); UV (λ in nm): 210 doublet (C=O due to π → π*), 315 (C=O due to n → π*).

3.2. Antimicrobial Studies

All the synthesized compounds 1a-1f were tested in vitro against gram positive *Staphylococcus aureus* and gram negative *Xanthomonas holicicola* bacteria and *Aspergillus niger* and *Fusarium oxysporum* fungi using paper disc diffusion method. The zone of inhibition was measured in mm. For comparison chloroamphenicol was used as reference drug against bacteria whereas bavistin was used against fungi. The compounds were tested by using standard solution of 5 mg/ml concentration and taking its 25 µg/disc, 50 µg/disc and 100 µg/disc. The observations show that compounds, 1a-1f, are highly effective against *A. niger* in all three concentrations. But against *F. oxysporum* fungus, except compound 1b which is effective in 10 and 20 µl concentrations, all compounds 1a-1f are inactive. Compounds 1c and 1f which are more inhibitory against *A. niger* in 20 µl concentration than reference drug, could be strongly recommended for their use as drugs.

Against gram positive and gram negative bacteria it is quite evident that except compound 1b which showed moderate activity in all the three concentrations, others are quite inactive. The results of activity are summarized in Table 2.

Table 2. The zone of inhibition in mm of the compound as well as standard drugs tested for antimicrobial activity.

Compounds	*Zone of inhibition (mm)*											
	S. aureus			***X. holicicola***			***A. niger***			***F. oxysporum***		
	5 µl	10 µl	20 µl	5 µl	10 µl	20 µl	5 µl	10 µl	20 µl	5 µl	10 µl	20 µl
1a	-	-	-	-	-	-	17	17	18	-	-	-
1b	-	-	-	10	10	16	14	19	19	-	-	-
1c	-	-	-	-	-	-	18	30	31	-	14	16
1d	-	-	-	-	-	-	-	17	17	-	-	-
1e	-	-	-	-	-	-	-	19	20	-	-	-
1f	-	-	-	-	-	-	-	20	24	-	-	-
Ref.1	11	15	16	18	19	20						
Ref.2							21	22	23	18	18	24

Key: All results are mean of four replications; - = no activity; DMSO did not show any zone of inhibition, Ref.1 = Chloroamphenicol; Ref. 2 = Bavistin

It is interesting to note that almost all substituted chloroacetanilides, irrespective to the nature and position of substituted group in them, showed greater activities than non-substituted product against *A.niger*. In meta-bromo and para-bromo substituted chloroacetanilides all antimicrobial activities in meta ≤ para order clearly reveal adverse effect of steric effect of substituted group in benzene ring on antimicrobial properties irrespective to structural moiety of the compounds. On the basis of structure-activity relationship [13] it has been observed that in para substituted bromo and dimethylamino products of both series antimicrobial, antifungicidal and antibacterial, properties Br > $N(CH_3)_2$, fall in opposite order of their electronegativities.

4. Conclusions

An efficient protocol for substituted primary amine with chloroacetanalides to novel substituted chloroacetanilides reported earlier has been followed owing to its simplicity of the experimental procedure and high yields. The structures of new compounds were confirmed by elemental analysis, UV, IR, ^{1}H NMR and ^{13}C NMR spectra; and spectral results clearly indicate formation of the new products. Molecular weights of the compounds were determined by Rast's method using camphor as solvent where in conformity with the success of the reactions carried out. Antimicrobial assay of the compounds revealed that 1c and 1f exhibiting better fungicidal property against *A. niger* than reference drug, could serve as promising antimicrobial agents for therapeutic use.

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