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Synthesis of some 2-methyl-5-nitroimidazole derivatives as potential antimicrobial agents

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ABSTRACT

The nitroimidazole class of compounds has yielded several antimicrobial agents that are currently in use. 5-nitroimidazoles are widely used in the treatment of diseases caused by protozoa and anaerobic bacteria. The simplest structural requirement for biological activity is the 1-alkyl-5-nitro-imidazole unit as exemplified by analogs of metronidazole such as tinidazole, ronidazole, secnidazole, ipronidazole. It has been known that the isopropyl alcoholic functional group in the secnidazole molecule is suitable for various reactions. Hence it is substituted with different groups by replacing the hydroxyl group with several groups and other effective secnidazole derivatives were obtained. Using these features, ester derivatives of 1-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol were synthesized and their antibacterial and antifungal activities were assayed.

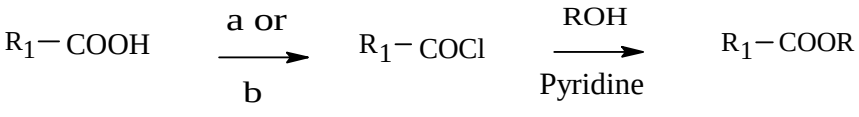
Key words: secnidazole, nitroimidazole, antibacterial and antifungal activity, synthesis.

INTRODUCTION

Metronidazole and related N-1 substituted like 5-nitroimidazoles ornidazole, secnidazole and tinidazole are widely used in the treatment of diseases caused by protozoa and anaerobic bacteria[1-3]. For biological activity of nitroimidazoles, the nitro group in the 5-position of imidazole ring is essential and so it has to be sterically unhampered. So the steric protection of

NO_2 group by substituents in positions 1 and 2 appears to be necessary. This configuration not only influence the metabolism but also physicochemical properties of such compounds [4]. Thus the regions of 5-nitroimidazole structure involving C_2, C_5, and N_1 positions are important in interaction with receptors in anaerobic organisms. It was shown that a lipophilic NO_2 group may be important for tissue penetration. Thus the simplest structural requirement for biological activity is the 1-alkyl-5-nitro-imidazole unit as exemplified by analogs of metronidazole such as tinidazole, ronidazole, secnidazole, ipronidazole [4] . It has been speculated that a reactive intermediate formed in the microbial reduction of the 5-nitro group of nitroimidazoles covalently binds to the DNA of the microorganism, triggering the lethal effect [5]. The isopropyl alcoholic functional group in the secnidazole molecule is suitable for various reactions. Hence it is substituted with different groups by replacing the hydroxyl group with several groups and other effective secnidazole derivatives were obtained. Using these features, ester derivatives of 1-(2-methyl-5-nitro-1*H*-imidazol-1-yl) propan-2-ol were synthesized and their antibacterial and antifungal activities were assayed.

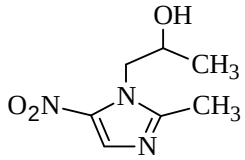
Scheme-I Alcoholysis of acyl halides

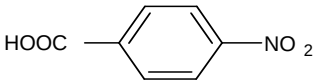
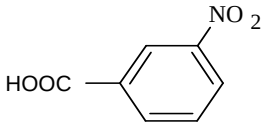
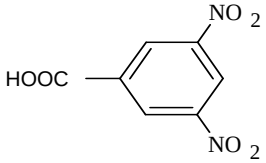


a = $SOCl_2$

b = PCl_5

R =



Compound	R_1
A	$C_{17}H_{35}$
B	$C_{15}H_{31}$
C	$C_{13}H_{27}$
D	
E	
F	

EXPERIMENTAL SECTION

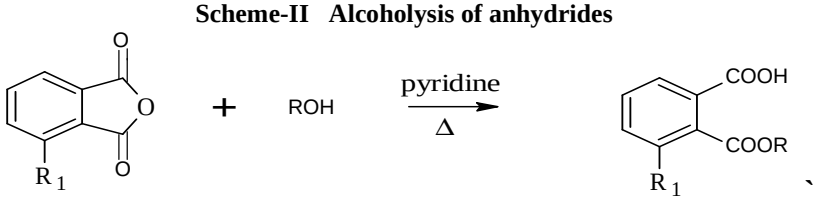
Drug secnidazole was obtained as gift sample from Cipla Laboratories, Mumbai. Following chemicals were used for the study: Thionyl chloride, pyridine, benzene sulphonyl chloride, p-toluene sulphonyl chloride and methanol (Rankem Laboratories, Mumbai), p-nitrobenzoic acid, 3,5-dinitrobenzoic acid, m-nitrobenzoic acid, pthalic acid, 3-nitropthalic acid, stearic acid, palmitic acid, myristic acid (S.D.Fine-Chem Ltd, Mumbai).

Synthesis of esters

The general schemes for synthesis of esters involves reactions between acid chlorides or anhydrides with alcohol in presence of suitable catalyst.

As per scheme-I acid chlorides are prepared by reacting acids with thionyl chloride or phosphorous pentachloride. These are further reacted with mixture of alcohol in presence of pyridine, to obtain esters[6-11] .

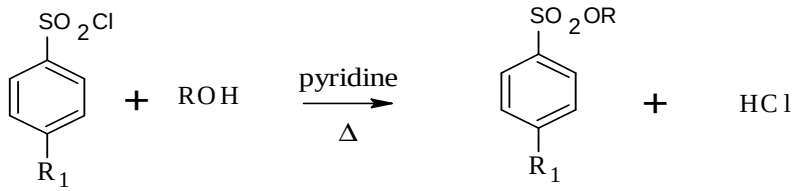
Scheme-II Alcoholysis of anhydrides



Compound	R_1
G	H
H	NO_2

As per scheme-II anhydrides are reacted with alcohol in presence of pyridine, reaction mixture is heated till completion of reaction, to obtain the ester[12-15] .

Scheme-III Alcoholysis of sulphonyl chlorides



Compound	R_1
I	H
J	CH_3

As per scheme-III alcohol and aromatic substrates like p-toluene sulphonyl chloride, benzene sulphonyl chloride are refluxed in presence of pyridine to obtain ester[16] .
Using these general methods esters of secnidazole were synthesized.

Method for antimicrobial activity

The synthesized derivatives were analyzed for antimicrobial activity by agar cup plate method. The organisms used for the antimicrobial study were gram positive organism *Staphylococcus aureus*, *Bacillus subtilis*. The gram negative organisms used for the study were *E. coli*, *Pseudomonas aeruginosa*. The fungi used were *Aspergillus niger* and *Candida albicans*. The concentration of synthesized compounds were 100 μg/ml. The standard drugs used for the study were Streptomycin and Griseofulvin for antibacterial and antifungal activity respectively. Control test with solvents were performed for every assay but showed no inhibition of the microbial growth. The results are shown in Table 3.

All the synthesized compounds were purified by recrystallisation with suitable solvents. The purity of all the synthesized compounds were checked by their melting point and thin layer chromatographic pattern. The melting points were determined in open capillaries by using Expo-HiTech melting point apparatus. The Rf values were found out by thin layer chromatography using precoated silica gel G plates as stationary phase and a suitable mobile phase. Detection was done by exposure to UV radiation and iodine vapours. The structures of synthesized compounds were characterized by their infrared spectra using Jasco FTIR-4100 and Jasco FTIR-5300. Mull method and KBr disc method was used to record IR spectra. The structures of synthesized compounds were characterized and confirmed by recording their NMR spectra using VNMRS-300 spectrometer, 300MHz. Deuteriated chloroform carbon tetrachloride and dimethyl sulphoxide were the solvents used for recording NMR spectra. Tetramethyl silane was used as the reference standard. The structures of some synthesized compounds were also confirmed by Mass spectrum analysis. Table 1 and 2

Synthesis of 1-methyl-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl octadecanoate (Compound A)

Mixture of Stearic acid (1g, 3.52 mmoles) and thionyl chloride (0.5ml, 6.87 mmoles) was refluxed at 65^{o}C for one hour by placing calcium chloride guard tube on reflux condenser to absorb the hydrogen chloride gas formed during reaction. Stearoyl chloride thus formed was highly fuming and immediately reacted with mixture of secnidazole (1g, 5.4 mmoles) and pyridine (0.5ml, 6.20mmoles). The reaction beaker was intermediately kept on ice bath to cool the beaker heated by reaction between stearoyl chloride, secnidazole and pyridine. The solid separated after stirring for 5-10 minutes was cooled and recrystallised using hot methanol.

Synthesis of 1-methyl-2-(2-methyl-5-nitro-1H imidazol-1-yl) ethyl hexadecanoate (Compound B) and Synthesis of 1-methyl-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethyl tetradecanoate (Compound C) were carried out in similar manner.

Synthesis of 1-methyl-2-(2-methyl-5-nitro-1*H* imidazol-1-yl) ethyl 4-nitrobenzoate (Compound D)

Mixture of p-Nitrobenzoic acid (1.0g, 6mmoles) and phosphorous pentachloride (1.3g, 6mmoles) was refluxed at 70^{o}C for one hour by placing calcium chloride guard tube on reflux condenser to absorb the hydrogen chloride gas formed during reaction. A pale yellow homogenous liquid of p-Nitrobenzoyl chloride which solidifies and separates as fine yellow needles melting at 71-73^{o}C was immediately reacted with mixture of secnidazole (1g, 5.4 mmoles) and pyridine (0.8ml, 10mmoles). The reaction beaker was intermediately kept on ice bath to cool the beaker heated by reaction between p-Nitrobenzoyl chloride, secnidazole and pyridine. After completion of reaction 10-15ml of 2% sodium hydrogen carbonate solution was added to the reaction mixture and solid thus separated was washed with water, filtered and dried. Recrystallised using hot methanol giving yellow colored crystals.

Synthesis of 1-methyl-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethyl 3-nitrobenzoate (Compound E) and Synthesis of 1-(2-methyl-5-nitro-1*H*-imidazol-1-yl)propan-2-yl 3,5-dinitrobenzoate (Compound F) were carried out in similar manner.

Synthesis of 2-{[1-methyl-2-(2-methyl-5-nitro-1*H*-imidazol-1yl)ethoxy]carbonyl}benzoic acid (Compound G)

Mixture of Secnidazole (1.0g, 5.4mmoles) and pyridine (1.0ml, 12.4mmoles) were placed in porcelain dish and stirred on water bath to dissolve secnidazole. To it phthalic anhydride (1.0g,

6.75mmoles) was added slowly with constant stirring and heating on water bath maintained at 100°C. When phthalic anhydride dissolves completely, it was cooled and to it 5-10ml hot water was added , stirred and allowed to cool. Crude product was washed with water, dried and recrystallised from hot water.

Table 1: Physical data of synthesized compounds

Compd	MF	% Yield	M P °C	MW	R_f value	Solubility	Elemental Analysis				
							%C	%H	%N	%O	%S
A	$C_{25}H_{45}N_3O_4$	78.19	46-47	451.643	0.51*	a,b,c,d,e	66.48	10.02	9.30	14.15	-
B	$C_{23}H_{41}N_3O_4$	84.21	45-47	423.589	0.56*	a,b,c[1],d,e	65.21	9.76	9.90	15.10	-
C	$C_{21}H_{37}N_3O_4$	75.24	42-44	395.538	0.53*	a,b,c[1],d,e	63.77	9.43	10.60	16.15	-
D	$C_{14}H_{14}N_4O_6$	91.67	161-163	334.285	0.46*	a,b,c,d,e	50.28	4.22	16.75	28.72	-
E	$C_{14}H_{14}N_4O_6$	68.76	126-128	334.285	0.44*	a,b,c,d,e	50.30	4.22	16.76	28.70	-
F	$C_{14}H_{13}N_5O_8$	78.81	152-154	379.283	0.47*	a,b,c,d,e	44.32	3.45	18.45	33.74	-
G	$C_{15}H_{15}N_3O_6$	83.88	197-199	333.296	0.69**	d,e	54.05	4.53	12.60	28.80	-
H	$C_{15}H_{14}N_4O_8$	85.27	233-235	378.294	0.52**	d,e	47.62	3.72	14.80	33.84	-
I	$C_{13}H_{15}N_3O_5S$	75.80	131-133	325.340	0.41*	a,b,c,d,e	47.97	4.65	12.92	24.59	9.85
J	$C_{14}H_{17}N_3O_5S$	81.96	133-136	339.367	0.42*	a,b,c,d,e	49.55	5.05	12.38	23.57	9.45

** Toluene: Glacial acetic acid (8:2), * * Chloroform: Hexane: Ethanol (8:1.5:0.5), a – Methanol, b- Ethanol, c- Chloroform, d- DMSO, e- DMF, 1- Slightly soluble*

Table 2 : Infrared /H^1 NMR/ MS data of synthesized compounds

Compd	IR wavenumbers cm^{-1}	NMR protons (δ ppm)	m/z
A	2916, 2851 (C-H), 1736 (C=O), 1541 (NO_2), 1471 (CH_3), 1267 (C-N), 1170, 1194 (C- O)	2.52 (s, 3H, CH_3), 5.2-5.3 (m, 1H, CH), 4.62 (d, 2H, CH_2), 1.3 (d, 3H, CH_3), 7.95 (s, 1H, Ar-CH), 1.2-1.4 (m, 35H, $C_{17}H_{35}$)	
B	2916, 2851 (C-H), 1736 (C=O), 1541 (NO_2), 1471 (CH_3), 1267(C-N), 1194 (C-O)	1.28 (d, 3H, CH_3), 2.46 (s, 3H, CH_3), 4.53 (d, 2H, CH_2), 5.15-5.18 (m, H, CH), 7.85 (s, 1H, Ar-CH), 1.18-1.29 (m,31H,$C_{15}H_{31}$)	-
C	2918, 2851 (C-H), 1739 (C=O), 1541 (NO_2), 1471 (CH_3), 1267 (C-N), 1107, 1194 (C-O)	1.01-1.12 (m, 23H, $C_{13}H_{23}$), 2.18-2.21 (t, 2H, CH_2), 1.41 (d, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.37 (d, 2H, CH_2), 5.0 (m, 1H, CH), 7.88 (s,1H, Ar-H)	-
D	3072 (Ar C-H), 1718 (C=O), 1601(C=C), 1523 (NO_2), 1461 (CH_3), 1274 (C-N), 1103, 1191 (C-O)	2.48 (s, 3H, CH_3), 1.58 (d, 3H, CH) 4.65 (d, 2H, CH_2), 5.3-5.6 (m, 1H, CH) 7.9-8.3 (m, 5H, Ar C-H)	334.9 (M+1) 208,149.9
E	3087 (Ar C-H), 1718 (C=O), 1614 (C=C), 1524 (NO_2), 1461 (CH_3), 1277 (C-N), 1073, 1186 (C-O),	1.56 (d, 3H, CH_3), 2.5 (s, 3H, CH_3) 4.7 (d, 2H, CH_2), 5.58-6.2 (m, H, CH) 7.6-8.8 (m, 5H, Ar C-H).	334 (M+1) 208, 150
F	3099 (Ar C-H) ,1731 (C=O), 1544 (NO_2) 1282 (C-N) 1077, 1194 cm^{-1} (C-O)	1.45 (d, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.70 (d, 2H, CH_2), 5.42-5.5 (m, H, CH), 8.0-8.78 (m,4H, Ar-H).	379 (M +1) 253, 168
G	3425 (OH), 2923 (C-H), 1730 (C=O), 1542 (NO_2), 1465 (CH_3), 1268 (C-N),1076, 1195 (C-O)	2.30 (s, 3H, CH_3), 1.40 (d, 3H, CH_3) 4.62 (d, 2H, CH_2), 5.3-5.5 (m, 1H, CH) 7.2-8.1 (m, 5H, Ar C-H), 13.3 (s, 1H, COOH)	333.9 (M+1) 207,149
H	3448 (O-H), 2923 (C-H), 1730 (C=O), 1524 (NO_2), 1465 (CH_3), 1268 (C-N), 1075,1191 (C-O)	1.45 (d, 3H, CH_3), 2.50 (s, 3H, CH_3) 4.7 (d, 2H, CH_2), 5.4-5.6 (m, 1H, CH),8.0 – 8.78 (m,4H,Ar C-H), 13.28 (s,1H, COOH)	379 (M +1) 252, 194, 168.

I	3096 (ArC-H), 2962, 2923 (C-H) 1525 (NO_2), 1461(CH_3), 1363 (S=O_2, Asym), 1263 (C-N), 1190 (S=O_2 Sym),	1.52 (d, 3H, CH_3), 2.48 (s, 3H, CH_3) 4.5 (d, 2H, CH_2), 4.8-4.9 (m, 1H, CH) 7.4-7.65 (m, 5H, Ar- H)	-
J	3078 (ArC-H), 2955, 2918 (C-H), 1527 (NO_2), 1462 (CH_3), 1376, (S=O_2 Asym), 1263 (C-N),1175 (S=O_2 Sym)	1.52 (d, 3H, CH_3), 2.42 (s, 3H, CH_3) 4.45 (d, 2H, CH_2), 4.76-4.88 (m, 1H, CH) 7.18-7.60 (m, 5H, Ar-CH)	339.9 (M+1) 168

Table 3 : antibacterial and antifungal activity of synthesized compounds

Compound	Zone of inhibition in mm at 100µg/ml					
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
A	23	23	21	22	20	21
B	23	20	23	19	21	19
C	18	17	19	18	12	14
D	22	20	23	20	20	19
E	23	22	23	21	14	15
F	24	20	22	22	13	15
G	14	16	17	18	15	17
H	16	15	13	15	14	18
I	24	22	20	21	21	20
J	23	24	21	22	20	21
Secnidazole	22	21	21	22	18	19
Ampicillin	23	23	22	21	-	-
Griseofulvin	-	-	-	-	23	23

** Including diameter of the well 12 mm*

Synthesis of 2-{[1-methyl-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethoxy] carbonyl}-3-nitrobenzoic acid (Compound H)

First 3-nitrophthalic anhydride was synthesized by refluxing a mixture of 3-nirtophthalic acid (2.5 g 11.8 mmoles) and acetic anhydride (2.48 g, 24.3 mmoles) for one hour at 80˚C. The hot mixture was poured in porcelain dish, allowed to cool and crystallize. The crystals of 3-nitrophthalic anhydride were grinded in mortar with 15 ml of sodium dried ether. The product was filtered, once more washed with 15 ml of ether and dried and used for synthesis of ester in manner similar to compound G.

Synthesis of 1-methyl-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethyl benzenesulfonate (Compound I)

Mixture of Secnidazole (1 g, 5.4 mmoles), pyridine (1.0ml, 12.4 mmoles) and benzenesulfonyl chloride (2 gm, 11.3 mmoles) was refluxed at 60˚C for one hour. The reaction mixture was poured into 25 ml cold 2% sodium bicarbonate solution and stirred vigorously until the oil solidifies. The crude product was recrystallized from methanol.

Synthesis of l-(2-methyl-5-nitro-1H imidazol-1-yl) ethyl-4-methylbenzenesulfonate (Compound J) was in similar manner.

RESULTS AND DISCUSSION

Ester derivatives of 1-(2-methyl-5-nitro-1*H*-imidazol-1-yl) propan-2-ol were synthesized and the structures of the compounds were established by means of IR, ^{1}H-NMR, MS and elemental analysis. All the compounds were evaluated for antibacterial and antifungal activity by cup-plate method. Compounds A, B, D, E, F, I and J have shown significant antibacterial activity. Remaining compounds have also shown moderate or weak antibacterial activity. Compounds A,

B, D, I and J have shown significant antifungal activity. Remaining compounds have also shown moderate or weak antifungal activity. The synthesized compounds are novel molecules and can prove as potent antimicrobial agents in future.

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