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SYNTHESIS OF SOME NOVEL 2-SUBSTITUTED NITROIMIDAZOLE AS POTENT ANTI-ANAEROBIC AGENT

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ABSTRACT

The nitroheterocyclic compounds have proved to be very effective antimicrobial agents and very valuable member of limited armamentarium of antiprotozoal agents. A number of 5-nitoimidazole with substitution at 2-position with various substitutent are found to modify the antibacterial spectrum. Some 2-substituted nitroimidazole analogues were designed, synthesized and characterized. The synthesized compounds were tested for their antibacterial sensitivity against Gram-positive anaerobe (*C. sporogenus*) and Gram-negative anaerobe (*B.fragilis*) using macro broth dilution method. All the synthesized compounds exhibit good activity at 10 mcg/ml against Gram-negative anaerobe compared to standard control tinidazole and metronidazole, while remain inactive against Gram-positive anaerobe.

Keywords: nitroimidazole, reduction, acid chloride, macro broth dilution.

INTRODUCTION

The nitroheterocyclic compounds have proved to be very effective antimicrobial agents and very valuable member of limited armamentarium of antiprotozoal agents. The mechanism of action of nitroheterocyclic agents depend upon the reduction of the nitro group to toxic radicals and binding to the protein and DNA. The two families of nitroheterocyclic, the nitroimidazole and nitrofurans are very different in their reduction characteristics.

Drug resistance to the nitroheterocyclic drugs and cross resistance leads to the development of new derivatives. A number of 5-nitoimidazole with substitution at 2-position with various substitutes like beta lactam ring, dioxane, 2-hexahyropyrimidine, 1-formyl and 2-formyl ring, pyridinium, imidolium, tetrahyropyridine derivatives and nitroimidazole with a trisubstituted ethylenic double bond are reported recently. Two of

beta lactam substituted imidazole compounds exhibited 100 and 50-fold increase in activity than metronidazole against *Trichomonas* and *Entamoeba histolytica* while one of the dioxane derivative was more effective than metronidazole against *Entamoeba histolytica* and *Trichomonas vaginalis* [1]. The formyl derivative exhibited an antiparasitic spectrum with significant activity against *Entamoeba histolytica* and *Trichomonas vaginalis* [2] . Such studies indicate the thrust for the future development of new nitroimidazole drugs. In the present study the 5- nitroimidazoles like tinidazole and dimetridazole molecule is thought to be modified at 2- position.

MATERIALS AND METHODS

Materials

Metronidazole, tinidazole, triethylamine, toluene, acetonitrile, methanol and Acid chlorides of LR grade are used after purification. All the melting points were determined in open capillaries and are uncorrected. Thin layer chromatography was performed on microscopic slides (2x7.5cm) coated with silica gel G and spots were visualized by normal TLC and exposure to iodine vapor. IR spectra were recorded in KBr on SHIMADZU Fourier Transform Infrared 8400S spectrophotometer. Mass spectra were recorded on Micromass Q-T , TOF MS $ES^{+}4.73e^{3}$. Nuclear Magnetic Resonance spectra (^{1}H NMR) were recorded in DMSO-d_6 on BRUKER AVANCE II at 400 MHz and the chemical shift are given in parts per million, downfield from Tetramethyl silane (TMS) was used as internal standard. Gram-positive anaerobe (*C. Sporogenus*) and Gram-negative anaerobe (*B.fragilis*) were cultured at laboratory scale.

Methods

General method for Synthesis of 2-(1-(substituted)-5-nitro-1H-imidazol-2-yl)-1-(substituted) ethanone [3]

To a mixture of 4.94 g (0.020 mole) of 1-(2-(ethylsulfonyl)ethyl)-2-methyl-5-nitro-1H-imidazole , 17.5 ml, of toluene, and 8.09g (0.080mole) of triethylamine was added with occasional cooling (0.55 mole) of acid chloride. The mixture was stirred for 22-24 hours, diluted with 10 ml of ether and chilled. The mixture was filtered and the solid washed with three 10 ml portions ether and four 10 ml portion water. The whole crude product is taken in to 10 ml of water, 15 ml of ethanol and 10 ml of concentrated hydrochloric acid and refluxed for 2-4 hours. The solution was chilled and poured on to

the ice. The solid was filtered and washed with water to give 2-(1-(2-(ethylsulfonyl) ethyl)-5-nitro-1H-imidazol-2-yl)-1- phenylethanone. Recrystallization from ethanol gave yellow crystals.

Anti-anaerobic Screening

The synthesized compounds were tested for their antibacterial sensitivity against Gram-positive anaerobe (*C. sporogenus*) and Gram-negative anaerobe (*B.fragilis*) using macro broth dilution method. A volume of inoculums equal to the amount of broth containing the drug is added, and tests are incubated at 37^{o}C in Gas Pak jars for approximately 48 hours. All inoculated broth containing no antimicrobial agent is included as a growth control for each strain tested and a tube of uninoculated broth is also included with each day's tests. The turbidity is measured after 2 days. Metronidazole and Tinidazole were used as standard control .

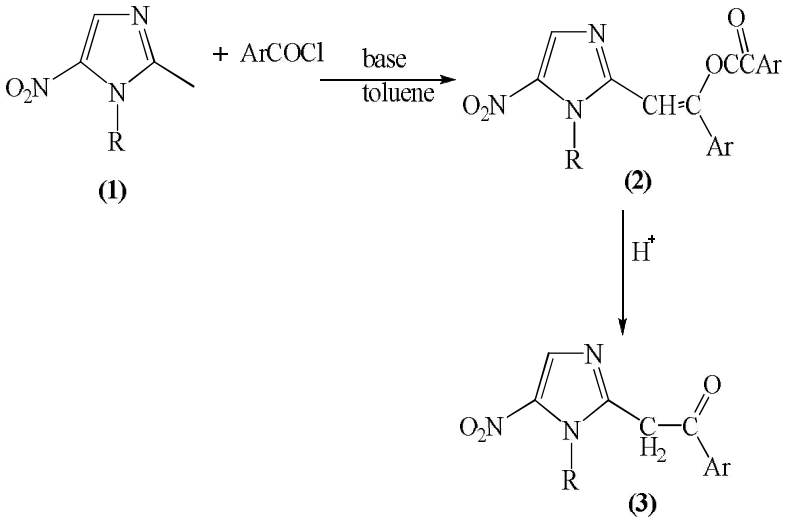
Procedure:

Serial two-fold dilutions of the antimicrobial agent are prepared in Brucella broth containing fildes enrichment (5%) or hemin (5µg/ml) and vitamin K_1 (0.1 µg/ml). Three or four colonies or a 3mm loopful of the strain to be tested are picked from an overnight culture on a blood agar plate and inoculated in to a tube of 5 to 6 ml supplemented thioglycollate medium without indicator (BBL 135 C). This medium is enriched with hemin (5µg/ml) and vitamin K_1 (0.1 µg/ml) prior to sterilization plus $NaHCO_3$ (1mg/ml) added just prior to use. This medium will henceforth be referred to as THCK. After a 4 to 9 h incubation at 37^{o}C, the culture is diluted in Brucella broth (supplemented as above) to the turbidity of the 0.5 McFarland standard (10^8CFU/ml), and is then further diluted 1:200. With slower growing strains, colonies are picked from a 2 to 3-days-old blood agar plate culture, inoculated into THCK and incubated overnight prior to dilution for the test. Tests on control strains of known susceptibility should also be included. The minimum inhibitory concentration (MIC) is read as the lowest concentration of drug showing no visible growth. Bactericidal endpoints are determined by streaking 0.1 ml of material from each tube to a blood agar plate. Plates are incubated anaerobically for 48 hours and the minimum bactericidal concentration (MBC) is read as the lowest concentration of drug resulting in fewer than 25 colonies (99.9% killing rate) [4].

RESULTS AND DISCUSSION

Chemistry

2-(1-(substituted)-5-nitro-1H-imidazol-2-yl)-1-(substituted) ethanone **(3)** was synthesized by activation of 2-position methyl group of **(1)** by base (triethylamine) and subsequent reaction with acid chlorides afforded **(2)** as intermediate which upon acidic hydrolysis yields the desired product [5]

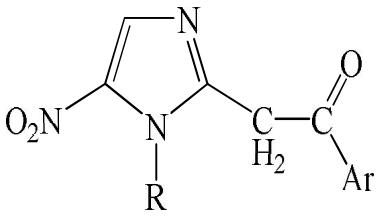


Scheme I : 2-(1-(substituted)-5-nitro-1H-imidazol-2-yl)-1-(substituted) ethanone

All the compounds were yellow to pale yellow in color and are soluble in dichloromethane, chloroform and partially soluble in methanol and ethanol. All the compounds were recrystallized from ethanol. The C-H streching (3096-2800 cm^{-1}), >C=O (1720-1680cm^{-1}) , C=N (1665 cm^{-1}), NO_2 (1570-1540 and 1366-1358cm^{-1}), is observed in IR spectra. The ^1H NMR (DMSO-d_6) spectra of 2-(1-(substituted)-5-nitro-1H-imidazol-2-yl)-1-(substituted) ethanone, displays the triplet of methyl group (-$SO_2CH_2\underline{CH_3}$) resonates at δ 1.28-1.40 ppm, quartet of methylene group (-$SO_2\underline{CH_2}CH_3$) resonates at δ 3.15 ppm, triplet for(- $CH_2\underline{CH_2}SO_2CH_2CH_3$) resonates at δ 3.66-3.89 ppm, singlet for (-N=C-$\underline{CH_3}$) resonates at δ 4.26-4.30, triplet for (imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$) resonates at δ 4.96 ppm, all the aromatic proton resonates between δ 6.54-7.94 , the singlet for (1H nitroimidazole ring) observed between δ 7.87-8.1ppm. The

molecular ions, M+1, M+2 were observed. The fragmentation routes primarily involved losses of NO (M-30), NO_2 (M-46) and HNO_2 (M-47) from the molecular ion, which are characteristic of compounds.

TABLE 1: PHYSICAL CHARACTERISTICS OF 2-(1-(SUBSTITUTED)-5-NITRO-1H-IMIDAZOL-2-YL)-1-(SUBSTITUTED) ETHANONE



(3)

Code	R	Ar	M.P. (^{0}C)	*Rf***	Yield (%)*	Molecular formula	Mol. Wt.
3-a	$-CH_2CH_2SO_2C_2H_5$	phenyl	146-148 (146-148)[3]	0.67	80	$C_{15}H_{17}N_3O_5S$	351.38
3-b	$-CH_2CH_2SO_2C_2H_5$	*p-cl* phenyl	152-154	0.69	72	$C_{15}H_{16}ClN_3O_5S$	385.82
3-c	$-CH_2CH_2SO_2C_2H_5$	*m-cl* phenyl	156-158	0.62	65	$C_{15}H_{16}ClN_3O_5S$	385.82
3-d	$-CH_2CH_2SO_2C_2H_5$	*o- cl* phenyl	180-182	0.60	45	$C_{15}H_{16}ClN_3O_5S$	385.82
3-e	$-CH_2CH_2SO_2C_2H_5$	furoyl	165-166 (165-168)[3]	0.56	75	$C_{13}H_{15}N_3O_6S$	341.34
3-f	$-CH_3$	furoyl	130-132	0.50	78	$C_{10}H_9N_3O_4$	235.20

*Solvent of recrystallization; methanol – chloroform

** *Rf,* Toluene: acetonitrile; 4:1

TABLE 2: SPECTRAL DATA OF 2-(1-(SUBSTITUTED)-5-NITRO-1H-IMIDAZOL-2-YL)-1-(SUBSTITUTED) ETHANONE

Code	Nomenclature	IR (Cm^{-1})	^{1}HNMR (DMSO-d6) (ppm)	MASS (m/z)
3-a	*2-(1-(2-(ethylsulfonyl) ethyl)-5-nitro-1H-imidazol-2-yl)-1-phenylethanone*	2977-2800 (CH_2), 1720 (C=O), 1541,1345 (NO_2)	δ 1.28(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.89 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 4H, Ar-H), δ 7.81 (s,1H, nitroimidazole ring)	352 (M+1)
3-b	*1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1H-imidazol-2-yl)ethanone*	2977-2850 (CH_2), 1700(C=O),1565, 1365 (NO_2)	δ1.28(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.20 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.72 (t, 2H,-$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.30 (s, 3H, N=C-$\underline{CH_3}$), δ 4.90 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 5H, Ar-H), δ 7.87 (s,1H, nitroimidazole ring)	387 (M+2)
3-c	*1-(3-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1H-imidazol-2-yl)ethanone*	2977-2850 (CH_2), 1700(C=O),1565, 1365 (NO_2)	δ1.30(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.18 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.28 (s, 3H, N=C-$\underline{CH_3}$), δ 4.90 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.50-7.86 (m, 4H, Ar-H), δ 7.87 singlet for (1H, imidazole ring).	387 (M+2)
3-d	*1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1H-imidazol-2-yl)ethanone*	2977-2850 (CH_2), 1700(C=O),1565, 1365 (NO_2)	δ1.38(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.18 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.70(t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.30 (s, 3H, N=C-$\underline{CH_3}$), δ 4.92(t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.44-7.88 (m, 5H, Ar-H), δ 7.87 (s,1H, nitroimidazole ring)	387 (M+2)
3-e	*2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1H-imidazol-2-yl)-1-(furan-2-yl)ethanone*	2977-2850 (CH_2), 1690(C=O),1550, 1360 (NO_2)	δ 1.40 (t,3H,-$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.754 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 8.1 (s,1H, nitroimidazole ring)	341.34 (M)
3-f	*1-(furan-2-yl)-2-(1-methyl-5-nitro-1H-imidazol-2-yl)ethanone*	2977-2850 (CH_2), 1700(C=O),1555, 1362 (NO_2)	δ 4.30 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (s, 3H, imidazole-$\underline{CH_3}$), δ 6.54 (d, 1H, 3-furoyl), δ 6.754 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.98 (s,1H, nitroimidazole ring)	235.20 (M)

Microbiological Screening

TABLE 3: INHIBITION CUT OFF VALUE AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE ANAEROBE

Sr. No.	Compound Code	Organism tested (Sensitivity)	
		Clostridium sporogenus (Gram positive anaerobe) Inhibition cut off value	*Bacteriodes fragililis* (Gram negative anaerobe) Inhibition cut off value
1	**3-a**	>20 µg/ml	10 µg/ml
2	**3-b**	>20 µg/ml	10 µg/ml
3	**3-c**	>20 µg/ml	10 µg/ml
4	**3-d**	>20 µg/ml	10 µg/ml
5	**3-e**	>20 µg/ml	10 µg/ml
6	**3-f**	>20 µg/ml	10 µg/ml
7	**Metronidazole (Control)**	20 µg/ml	10 µg/ml
8	**Tinidazole (Control)**	10 µg/ml	10 µg/ml

All the compounds shows good activity at 10 µg /ml against Gram-negative anaerobe similar to tinidazole and metronidazole, while remain inactive against Gram-positive anaerobe.

CONCLUSION

The modification of 5-nitroimidazoles at 2-position, leads to selective activity against the Gram-negative anaerobic bacteria.

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