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Biologically Active Azoles: Synthesis, Characterization and Antimicrobial Activity of Some 1-substituted imidazoles

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Abstract

A few 1-substituted imidazoles 1(a-d) and 2(a-d) have been synthesized and characterized on the basis of elemental analysis, FT-IR, ^{1}H-NMR, DART-MS spectral data. The synthesized compounds were screened for their in vitro growth inhibiting activity against different strains of bacteria and fungi viz., Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Aspergillus niger and Penicillium chrysogenum (10 and 50µg/ml) and the results were compared with the standard antibiotic Ciprofloxacin and ketoconazole (10µg/ml) using agar diffusion technique.

Key words: 1-substituted imidazoles, Phenacyl bromides, antimicrobial evaluation, agar well diffusion method.

INTRODUCTION

Fungal infections (mycoses), though not as frequent as bacterial or viral infections, have nonetheless been increasing in incidence in the human population over the last 15 years. A number of fungal infections can be difficult to treat even when the offending organism is identified and appropriate therapy is applied. On the other hand, like bacteria, fungi have unique characteristics, distinct from their mammalian hosts, allowing for selective targeting of therapeutic drugs. Fungi are, however, much more complex organisms in comparison to bacteria, are in fact eukaryotic and often grow fairly slowly. Consequently, only a few drugs are aimed at interfering with cell division and have limited use. Most antifungal drugs are targeted to the cell membrane.

Imidazoles are a versatile lead molecule for designing potential bioactive agents. The 1-substituted imidazole derivatives have been found to exhibit diverse biological activities such as antiprotozoal, mutagenic properties, anticancer, antiviral, enzyme inhibition, H_2-antagonism, α-adrenergic agonism and β-blocking, anticonvulsant, broad spectrum antibacterial and antifungal activities [1-11]. It is well known that imidazoles are effective on antimicrobial studies especially in the case of azole antifungals viz., imidazole antifungals: miconazole, ketoconazole, econazole, bifonazole, butoconazole, fenticonazole, isoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole all were used for the treatment of candidiasis (yeast infection or thrush), athlete's foot, ringworm and jock itch like various worm infections both internal and external in decades.

Keeping this context in mind an attempt has been made to synthesis and ***in vitro*** **antimicrobial activity** of some novel 1-substituted 2-methyl imidazole (1a-1d) and 1-substituted imidazole (2a-2d) derivatives. This possibly led to the development of compounds with probable antimicrobial activity especially in antifungal study to overcome the strains those are resistant with earlier imidazole derivatives by developing structural modifications.

MATERIALS AND METHODS

Experimental

Melting points were determined in open glass capillaries using an Electro thermal IA 9000 SERIES digital melting point apparatus (Electrothermal, UK) and are uncorrected. IR spectra (KBr wafers) were taken on Shimadzu FT-IR Spectrophotometer, Model No.8400S (Japan). ^{1}H NMR spectra were recorded on Bruker 300 MHz NMR spectrometer (Switzerland) using CDCl3 as solvent. Mass spectra were documented on Joel SX-102(EI/CI/DART/MS) (USA). Microanalysis was done on a Vario EL III Elementor C, H, N analyzer (Germany). Iodine vapour was used as the developing agent and the solvent system used was benzene: ethanol (8:2). Imidazole, 2-methyl imidazole and para substituted phenacyl bromides were procured from sigma-Aldrich, USA. All other chemicals used in the present studies were either of A.R or G.R quality. Anhydrous sodium sulphate was used as the drying agent. The purity of all compounds was established by a single spot on the TLC plates (Merck, Germany). Para substituted phenacyl bromides (Aldrich), dimethyl formamide (Sigma), ciprofloxacin; ketoconazole (pure drug) was used. All the prototypes were dissolved in dimethyl sulfoxide for making the concentration of 10µg/ml and 50µg/ml.

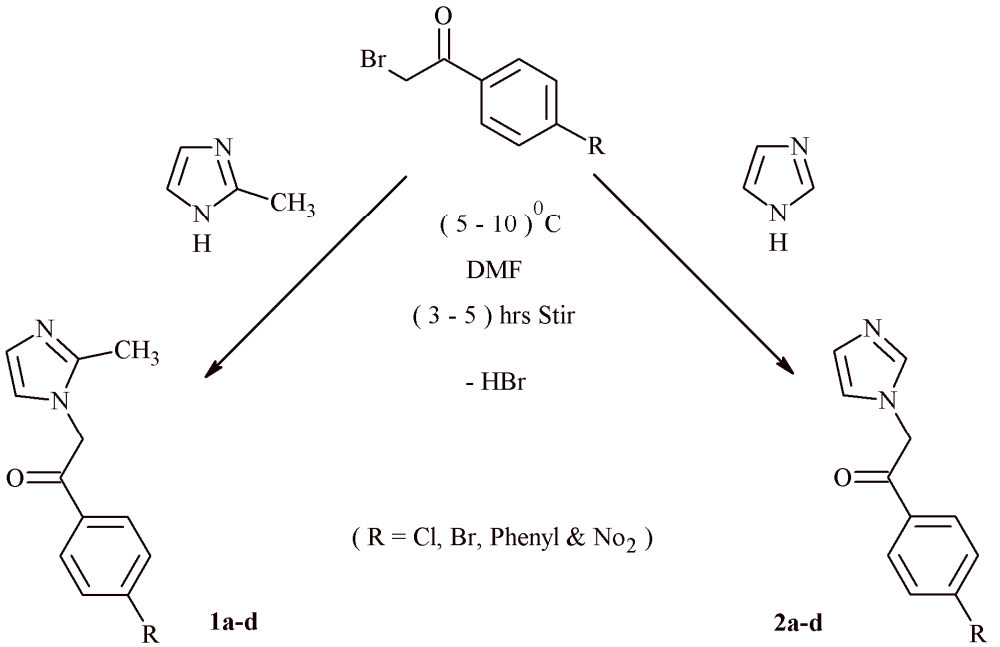
Chemical Synthesis

Synthesisof 1-(4-substitutedphenyl)-2-(2-methyl-1*H*-imidazol-1-yl) ethanone (1a-d)

In the present scheme [12], 1-phenacyl 2-Methyl Imidazole derivatives 1a-d (Scheme:1) have been synthesized by treating 2.46 g (0.03mol) of 2-methyl imidazole and 0.46g(0.002 mol) of appropriate para substituted phenacyl bromides (chloro, bromo, phenyl and nitro) in presence of dry DMF (dimethylformamide) with the cold stirring (5-10°C) for about (3-6) hrs. Next, the mixture was poured into cold water (20 ml) and stirred for further one hour. The precipitate was collected and then treated with benzene and filtered. The benzene soluble portion on removal of solvent yielded a solid mass, which was recrystallised from benzene-ethanol. The purity of all compounds was established by single spot on the TLC plates as described above.

Synthesis of 1-(4-substituted phenyl)-2-(1*H*-imidazol-1-yl) ethanone (2a-d)

1-phenacyl Imidazole derivatives 2a-d (Scheme:1) have been synthesized by treating 2.04 g (0.03mol) of imidazole and 0.46g (0.002 mol) of appropriate para substituted phenacyl bromides (chloro, bromo, phenyl and nitro) in presence of dry DMF (dimethylformamide) with the cold stirring (5-10°C) for about 3-6 hrs [12]. Next, the mixture was poured into cold water (20ml) and stirred for further one hour. The precipitate was collected and then treated with benzene and filtered. The benzene soluble portion on removal of solvent yielded a solid mass, which was recrystallised from benzene-ethanol. The purity of all compounds was established by a single spot on the TLC plates.



Scheme 1: Represents the synthesis of 1-substituted imidazoles.

Table 1. Physico-chemical data of synthesised compounds

Compd	R	MF	Mr	Y (%)	MP (ºC)	Elemental analysis Calcd. / found (%)		
						C	H	N
1a	Cl	$C_{12}H_{11}ClN_2O$	234.68	55	149-152	61.41/ 61.38	4.72/4.66	11.94/11.86
1b	Br	$C_{12}H_{11}BrN_2O$	279.13	41	186-188	51.63/51.49	3.97/3.87	10.04/09.94
1c	C_6H_5	$C_{18}H_{16}N_2O$	276.33	58	182-185	78.24/78.13	5.84/5.76	10.14/10.11
1d	NO_2	$C_{12}H_{11}N_3O_3$	245.23	64	194-196	58.77/58.59	4.52/4.42	17.13/17.09
2a	Cl	$C_{11}H_9ClN_2O$	220.65	52	132-135	61.41/61.39	4.72/4.67	11.94/11.83
2b	Br	$C_{11}H_9BrN_2O$	265.10	59	142-144	49.84/49.79	3.42/3.35	10.57/10.43
2c	C_6H_5	$C_{17}H_{14}N_2O$	262.30	66	164-166	77.84/ 77.76	5.38/5.25	10.68/10.56
2d	NO_2	$C_{11}H_9N_3O_3$	231.20	62	152-154	57.14/57.11	3.92/3.79	18.17//18.03

MF: Molecular formula, Mr: Molecular weight, Y: Yield, MP: Melting point

Table 2. Spectral data of synthesised compounds

Compd	IR (ν, cm^{-1})	^1H NMR (300 MHz, δ, ppm) (DMSO-d_6)	DART-MS (m/z)
1a	2964, 1288, 1496, 1691, 827, 669.	2.3 (s, 3H, - CH_3), 5.27 (s, 2H, -CH_2), 7-7.4 (m, 4H, Ar-H), 7.5 (d , 1H, Het-H) 7.9 (d, 1H, Het-H)	235 $(M+H)^+$
1b	2960, 226, 1436, 1691, 823, 563.	2.3 (s, 3H, -CH_3), 5.26 (s, 2H, CH_2), 7-7.6 (m, 4H, Ar-H), 7.7 (d, 1H, Het-H) 7.8 (d, 1H, Het-H)	279 $(M+ H)^+$
1c	2960, 1224, 1693, 1494, 846, 767.	2.44 (s, 3H, - CH_3), 5.348 (s, 2H, -CH_2), 6.8-7.2 (m, 9H, Ar-H), 8.1 (d, 1H, Het-H), 8.3 (d, 1H, Het-H)	277 (M+ H, bp)$^+$
1d	3088, 1217, 1435, 1691, 825, 1517.	2.34 (s, 3H, - CH_3), 5.34 (s, 2H, CH_2), 7.2-7.5 (m, 4H, Ar-H), 7.6 (d,1H, Het-H), 7.7(d, 1H, Het-H)	246 (M+ H, bp)$^+$
2a	3018, 1493, 1680, 842, 684.	5.37 (s, 2H, -CH_2), 6.9-7.4 (m, 4H, Ar-H), 7.5 (d, 1H, Het-H), 7.9 (1H, d, Het-H)	221 $(M+ H)^+$
2b	2999, 1458, 1595, 831, 578.	5.364 (s, 2H, -CH_2), 6.9-7.4 (m, 4H, Ar-H), 7.7 (d, 1H, Het-H), 7.8 (d, 1H, Het-H)	265 $(M+ H)^+$
2c	3159, 1514, 1593, 815, 748.	5.43 (s, 2H, -CH_2), 7.3-7.5 (m, 9H, Ar-H), 7.6 (d, 1H, Het-H), 7.7 (d, 1H, Het-H), 8.0 (s, 1H, Het-H)	263 $(M+ H)^+$
2d	3020, 1464, 1678, 828, 1522.	5.66 (2H, s, -CH_2), 7.3-7.5 (m, 4H, Ar-H), 7.6 (d, 1H, Het-H), 8.2 (d, 1H, Het-H), 8.3 (s, 1H, Het-H)	232 $(M+ H)^+$

Screening for Antimicrobial activity

The antimicrobial activity of all the newly synthesized compounds 1(a-d) and 2(a-d) was determined by well plate or agar diffusion method [13]. The medium used were double strength nutrient broth (Hi-Media) for antibacterial activity and double strength malt yeast extract (Hi-Media) for antifungal activity. The *in vitro* antimicrobial activity was carried out against 24h old cultures of bacterial and 72h old cultures of fungal strain. The different strains of bacteria and fungi viz., *Staphylococcus aureus* (NCIM 2122), *Bacillus subtilis* (NCIM 2193), *Streptococcus faecalis* (NCIM 5024) (Gram positive bacteria's), *Escherichia coli* (NCIM 2809), *Pseudomonas aeruginosa* (NCIM 2036), *Salmonella typhi* (NCIM 2501) (Gram negative bacteria's), *Candida albicans* (NCIM 3471), *Aspergillus niger* (NCIM 1056) and *Penicillium chrysogenum* (NCIM 723) (fungal strains) were used. Pure cultures of the test microorganisms were procured from Institute of Microbial Technology, Chandigarh and National Chemical Laboratory, Pune. The compounds were tested at the concentrations of 10 and 50 µg/ml and solutions were prepared by dissolving in dimethyl sulfoxide (DMSO). The petridishes used for antibacterial screening were incubated at 37±1ºC for 24h, while those used for antifungal activity were incubated at 28ºC for 48-72h. The results were compared to Ciprofloxacin (10µg/ml) and ketoconazole (10µg/ml) for antibacterial and antifungal activity respectively by measuring zone of inhibition in mm. The antibacterial and antifungal screening results were presented in Table 3 and Table 4.

Table: 3 Evaluation of *In Vitro* antibacterial activity of 1-Substituted Imidazoles

Compounds	Zone of inhibition (mm)											
	Gram positive						Gram negative					
	Staphylococcus Aureus		*Bacillus Subtilis*		*Streptococcus Faecalis*		*Escherichia Coli*		*Salmonella Typhi*		*Pseudomonas Aeruginosa*	
	10[a]	50[a]	10[a]	50[a]	10[a]	50[a]	10[a]	50[a]	10[a]	50[a]	10[a]	50[a]
1a	-	-	-	-	-	-	-	-	-	-	-	-
1b	-	06	-	06	-	06	07	07	-	-	-	06
1c	06	06	-	07	06	07	-	05	-	06	05	07
1d	-	05	-	-	-	05	-	07	-	-	-	05
2a	-	-	-	-	-	-	-	-	-	-	-	-
2b	05	06	05	05	-	-	05	06	05	07	-	-
2c	09	08	-	07	05	07	-	08	-	07	05	07
2d	07	06	-	05	-	04	-	05	-	-	-	04
Ciprofloxacin[b]	31		32		39		32		34		38	

[a] µg/ml, [b] Ciprofloxacin (10µg/ml) was used as positive reference standard antibiotic.

Table 4. Evaluation of *in vitro* antifungal activity of 1-Substituted Imidazoles

Compounds	Zone of inhibition (mm)					
	Candida albicans		*Aspergillus niger*		*Penicillium chrysogenum*	
	10[a]	50[a]	10[a]	50[a]	10[a]	50[a]
1a	10	24	07	11	14	21
1b	15	27	09	14	17	26
1c	16	25	11	15	18	27
1d	19	23	8	13	15	22
2a	17	23	9	13	19	24
2b	21	28	11	15	21	26
2c	22	27	10	11	20	27
2d	20	25	09	18	19	23
Ketoconazole[b]	24		17		21	

[a] µg/ml, [b] Ketoconazole (10µg/ml) was used as positive reference standard antibiotic.

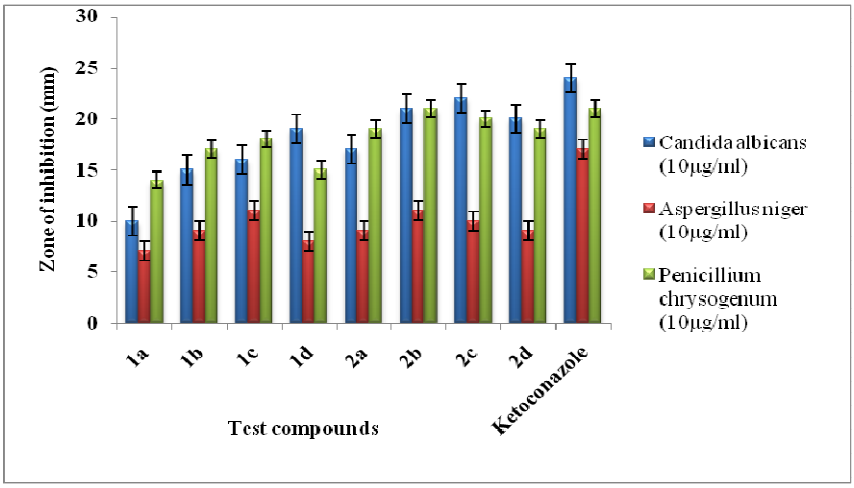


Fig.1: Effect of prototypes (10µg/ml) and Ketoconazole (10µg/ml) on *in Vitro* antifungal activity.

RESULTS AND DISCUSSION

The results of antimicrobial activity indicated that all the compounds 1(a-d) and 2(a-d) were found to be very less or no activity against all bacterial strains used in this *in vitro* bioassay at the concentration range of 10-50 µg/ml whereas in case of anti fungal activity all the compounds exhibit an excellent activity at 50µg/ml. The test compounds 1b, 1c, and 2b, 2c and 2d exhibited highest activity against *Candida albicans* and *Penicillium chrysogenum* but moderate activity against *Aspergillus niger* at 10µg/ml concentration. In fact, these compounds exhibited comparatively equipotent activity at 50µg/ml concentration with that of standard

ketoconazole (10µg/ml) which has an imidazole moiety (Fig.1). Rather the test compounds 1a [1-(p-chlorophenacyl imidazole)] and 2a [1-(p-chlorophenacyl)-2-methyl imidazole] showed comparatively lesser activity than other compounds.

It was noticed that 2-methyl derivatives (1a-1d) were found to possess comparatively less activity than imidazole derivatives (2a-2d) due to the presence of methyl moiety in its second position. Introduction of p-bromophenacyl, p-phenylphenacyl and p-nitrophenacyl at first position of the imidazole nucleus has significantly improved potency. Moreover from a perusal of the results, it was evident that the compounds substituted with p-bromophenacyl, p-phenylphenacyl and p-nitrophenacyl at first position of the imidazole nucleus showed higher activity against all the tested fungi strains *Candida albicans* , *Aspergillus niger* and *Penicillium chrysogenum*.

CONCLUSION

In the present attempt, all the newly synthesized 1-substituted imidazoles 1(a-d) and 2(a-d) are related to azoles. Therefore the probable mechanism of antifungal activity is to inhibit the fungal cytochrome P-450-3-A dependent enzyme 14-alpha demethylase, thereby interrupting the synthesis of ergosterol. Inhibition of this critical enzyme in the ergosterol synthesis pathway leads to the depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth. However, further revision is necessary to ascertain the molecular mechanism of antifungal activity of test compounds.

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