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Research Article

ANTIOXIDANT POTENTIAL AND ANTIMICROBIAL SCREENING OF SOME NOVEL IMIDAZOLE DERIVATIVES

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Abstract

Bioactive novel imidazole derivatives have been synthesized under solvent free condition using molecular iodine as the catalyst and they are characterized by NMR spectra, X-ray, mass and CHN analysis. Their antioxidant potential were evaluated using different *in vitro* antioxidant models namely, DPPH (1,1-diphenyl-2-picryl hydroxyl) radical, superoxide anion and hydroxyl radical scavenging activities. The p-tolyl ring at C-3 and p-fluorophenyl at C-2 of the imidazole ring has maximum OH• (fpdmti) when compared with other imidazole derivatives. The low IC_{50} value may be due to the electron donating (+I effect) ability exerted by the methyl substituent and electron withdrawing (-I effect) ability exerted by the fluoro substituent. The 3,5-dimethylphenyl ring at C-2 of the imidazole ring (dmmppi) has maximum DPPH• and superoxide anion radical scavenging activities when compared with other imidazole derivatives and the low IC_{50} value of dmmppi) may be due to the electron donating (+I effect) ability exerted by the two methyl substituent's. Their antibacterial screening against *Staphylococcus aureus*, *Escherichia coli* and *Klbesiella pneumoniae* and antifungal activity against *Aspergillus Niger, Aspergillus flavus* and *Candida-6* were also evaluated.

Keywords: Antioxidant activity; Antimicrobial screening; Free radical scavenging activity; NMR; X-ray.

INTRODUCTION

Organic solvents are high on the list of damaging chemicals, in recent years solid-state organic reactions have caused great interest. Designing of "green" experimental protocol is an enormous challenge to chemists to improve the quality of the environment for present and future generations. Compounds with an imidazole ring system have many pharmacological properties.[1,2] Though there are several methods reported in the literature for the synthesis of imidazoles, they suffer from one or more disadvantages such as harsh reaction conditions, poor yields, prolonged time period, use of hazardous and often expensive acid catalysts.[3,4] Recently, molecular iodine received considerable attention as an inexpensive, nontoxic, readily available catalyst for various organic transformations, affording the corresponding products in excellent yields with high selectivity. Owing to numerous advantages associated with this eco-friendly element, iodine has been explored as a powerful catalyst for various organic transformations.[5] During the course of our studies towards the development of new route to the synthesis of biologically active heterocyclic, we wish to report a simple and an efficient method for the synthesis of substituted imidazoles. Free radicals, the partially reduced metabolites of oxygen and nitrogen, are highly toxic and reactive. Free radicals are linked with the majority of diseases like aging, atherosclerosis, cancer, diabetes, liver cirrhosis, cardiovascular disorders, etc.[6,7] The most common reactive oxygen species are superoxide anion (O_2•-), hydrogen peroxide (H_2O_2), peroxyl radical (ROO•) and highly reactive hydroxyl radical (OH•). The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion (ONOO•). Oxidation process is one of the most important routes for producing free radicals in food, drugs and living systems. Antioxidants are the substances that when present in low concentration significantly delay or reduce the oxidation of the substrate.[8] Antioxidants protect the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body and hindering the process of oxidation. So diseases linked with free radicals can be prevented by antioxidant therapy which gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants particularly of plant origin. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic acid esters have been suspected to cause negative health effects. BHA and BHT are suspected of being responsible for liver toxicity and carcinogenesis.[9-11] Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Traditionally used natural antioxidants from tea, wine, fruits, vegetables, spices, and medicine (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements.[12] Also many other plant species have been investigated in search of novel antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species.[13-16] It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds.[17] Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging,

inhibition of hydrolytic and oxidative enzymes and anti-inflammatory actions.[18-20] The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. Many plant species have been investigated in search for novel antioxidants in the recent time but still the demand to find more novel antioxidant persist. When compared to natural photochemical it is possible to synthesize more potent antioxidants through chemical synthesis. Therefore, the objective of present study was development of new route to the synthesis of heterocyclic with antioxidant potential. These substituted imidazoles were subjected to evaluate *in vitro* antioxidant activity through determining different free radical scavenging assay and their antimicrobial screening were also carried out.

MATERIALS AND METHODS

General procedure for the synthesis of 2-aryl imidazole derivatives

A mixture of aldehyde (1mmol), 1,2-diketone (1mmol), ammonium acetate (2.5 mmol) and iodine (15 mol%) were grained in a mortar at room temperature for appropriate time. The reaction was monitored by TLC and the reaction mixture was treated with aqueous $Na_2S_2O_3$, the formed crude was purified by column chromatography (n-hexane:ethylacetate (9:1) (Scheme.1).[21-23]

4,5-Dimethyl-1-(*p*-tolyl)-2-phenyl-1H-imidazole (dmppi)

Yield: 40 %. mp 125°C, Anal. calcd. for $C_{18}H_8N_2$: C, 82.41; H, 6.92; N, 10.68. Found: C, 82.02; H, 6.69; N, 10.70. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H), 2.31 (s, 3H), 2.02 (s, 3H), 7.02-7.36 (aromatic protons). ^{13}C (100 MHz, $CDCl_3$): δ 9.57, 12.76, 21.19, 125.45, 127.54, 127.63, 127.97, 128.01, 130.11, 130.92, 133.41, 135.34, 138.37, 145.15. MS: m/z 262.00, calcd. 262.35.

2-(4-fluorophenyl)-4,5-dimethyl-1-*p*-tolyl-1H-imidazole (fpdmti)

Yield: 40 %. mp 130°C, Anal. calcd. for $C_{18}H_{17}FN_2$: C, 77.12; H, 6.11; N, 9.99. Found: C, 77.00; H, 5.98; N, 9.03. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01 (s, 3H), 2.29 (s, 3H), 2.43 (s, 3H), 6.87-7.34 (aromatic protons). ^{13}C (100 MHz, $CDCl_3$): δ 9.53, 12.69, 21.18, 114.92, 115.10, 125.44, 127.16, 129.89, 130.20, 133.35, 135.13, 138.57, 144.28, 161.26, 163.23. MS: m/z 280.00, calcd. 280.14.

4,5-dimethyl-1-(3,5-dimethylphenyl)-2-phenyl-1H-imidazole (dmdmppi)

Yield: 40 %. mp 142°C, Anal. calcd. for $C_{19}H_{20}N_2$: C, 82.57; H, 7.29; N, 10.14. Found: C, 82.00; H, 6.89; N, 9.72. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.98 (s, 3H), 2.27 (s, 3H), 2.29 (s, 6H), 6.77-7.36 (aromatic protons). ^{13}C (100 MHz, $CDCl_3$): δ 9.65, 12.77, 21.27, 125.54, 125.59, 127.59, 127.97, 128.03, 130.02, 130.07, 133. 53, 137.88, 139.33, 144.98. MS: m/z 276.00, calcd. 276.16.

In vitro Antibacterial and Antifungal Activity

The *in vitro* activities of bio active imidazole derivatives were tested in Sabourauds dextrose broth (SDB; Hi-media, Mumbai, India) for bacteria by the two fold serial dilution method.[24-26] The compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4-10^5 cfu/ml. The final inoculum size was 10^5 cfu/ml for antibacterial assay and 1.1-1.5 X 10^2 cfu/ml for antifungal assay and testing was performed at pH 7.4 ± 0.2. Exactly 0.2 ml of the solution of each test compound was added to 1.8 ml of seeded broth to form the first dilution. One ml of this was diluted with a further 1 ml of the seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control and likewise solvent controls were also run simultaneously. The tubes were incubated in BOD incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h (for bacteria) and 72-96 h (for fungi) of incubation; ciprofloxacin, Norfloxacin (for antibacterial) and Amphotericin B, Ampicillin (for antifungal) were used as standards.

In vitro Antioxidant Activity

DPPH Radical Scavenging Activity

The stable free radical DPPH method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound. The DPPH radical scavenging activity of imidazole derivatives were determined by the method.[27]

Superoxide Radical Scavenging Activity

Superoxide anion radical scavenging activity of synthetic imidazole derivatives were determined by the method of Nishimiki.[28] The assay was based on the oxidation of Nicotinamide adenine dinucleotide (NADH) by phenazine methosulphate (PMS) to liberate PMS_{red}.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of synthetic imidazole derivatives were determined by the reported method.[29]

Reducing Power Assay

The Fe^{3+} reducing power of imidazole was determined by the method of Oyaizu.[30]

RESULTS AND DISCUSSION

Photophysical Properties of dmppi

Crystalline imidazole derivatives dmppi (1) and fpdpi (2) are monoclinic crystals.[31] dmppi (1) (Figure 1) crystallizes in the space group p_n and cell has dimensions of a = 9.809 Å, b = 7.700 Å, c = 19.907 Å. ORTEP diagram of dmppi (1) shows that the imidazole ring is essentially planar. The imidazole ring makes dihedral angles of 70.91 (19°) and 19.8 (2°) with the *p*-tolyl ring (C11-C16) attached to the nitrogen of the imidazole nucleus and phenyl ring (C_{21}-C_{26}) attached to the carbon of the imidazole nucleus respectively. The dihedral angle between the two phenyl rings is 74.59 (18°). The crystal packing is stabilized by C_{12}-$H_{12}\cdots N_3$ (2-x, 2-y, -z) and C_{16}-$H_{16}\cdots N_3$ (2-x, 1-y, -z) intermolecular hydrogen bonds (Figure 2). This asymmetry of dihedral angles and bond angles reveal the conjugation of the phenyl ring attached to the carbon with the imidazole nucleus. The band appeared at 290.5 nm (λ_{abs}) and 362.0 nm (λ_{em}) is due to the π-π* transition.

Table 1: Selected Bond lengths (Å), Bond angles (°) and torsional angles (°) of dmppi

Bond connectivity	Experimental XRD (Å)	Bond angles	Experimental XRD (°)	Torsional angles	Experimental XRD(°)
N1-C2	1.372 (1.380)	C2-N1-C5	107.2 (107.0)	C5-N1-C2-N3	-0.4 (-0.1)
N1-C5	1.388 (1.407)	C2-N1-C11	128.6 (128.1)	C5-N1-C2-C21	-176.6 (-179.6)
N1-C11	1.443 (1.429)	C5-N1-C11	123.0 (124.5)	C11-N1-C2-N3	-167.5 (-173.4)
N3-C2	1.322 (1.306)	C2-N3-C4	106.4 (107.6)	C11-N1-C2-C21	16.3 (6.2)
N3-C4	1.373 (1.395)	N1-C2-N3	110.4 (110.1)	C2-N1-C5-C4	-0.1 (-0.1)
C2-C21	1.467 (1.474)	N1-C2-C21	126.7 (126.4)	C2-N1-C5-C51	-178.7 (-179.1)
C4-C5	1.359 (1.354)	N3-C2-C21	122.8 (123.4)	C11-N1-C5-C4	167.9 (173.9)
C4-C41	1.498 (1.495)	N3-C4-C5	110.4 (109.4)	C11-N1-C5-C51	-10.7 (-5.5)
C5-C51	1.485 (1.496)	N3-C4-C41	121.3 (120.0)	C2-N1-C11-C12	-119.5 (-111.6)
C11-C12	1.382 (1.384)	C5-C4-C41	128.3 (130.6)	C2-N1-C11-C16	63.6 (68.7)
C11-C16	1.380 (1.385)	N1-C5-C4	105.6 (105.9)	C5-N1-C11-C12	75.3 (75.0)
C12-C13	1.386 (1.387)	N1-C5-C51	123.0 (122.0)	C5-N1-C11-C16	-101.6 (-103.7)
C13-C14	1.387 (1.390)	C4-C5-C51	131.4 (132.1)	C4-N3-C2-N1	0.7 (0.2)
C14-C15	1.385 (1.391)	N1-C11-C12	119.9 (120.1)	C4-N3-C2-C21	177.1 (179.8)
C14-C17	1.511 (1.516)	N1-C11-C16	119.2 (119.9)	C2-N3-C4-C5	-0.8 (-0.3)
C15-C16	1.385 (1.385)	C12-C11-C16	120.8 (120.0)	C2-N3-C4-C41	177.3 (179.4)
C21-C22	1.390 (1.392)	C11-C12-C13	119.2 (119.8)	N1-C2-C21-C22	18.9 (26.3)
C21-C262	1.385 (1.394)	C12-C13-C14	121.2 (120.9)	N1-C2-C21-C26	-164.6 (-156.1)
C22-C23	1.383 (1.387)	C13-C14-C15	118.1 (118.5)	N3-C2-C21-C22	-156.9 (-154.1)
C23-C24	1.380 (1.385)	C13-C14-C17	120.3 (120.9)	N3-C2-C21-C26	19.6 (23.4)
C24-C25	1.372 (1.388)	C15-C14-C17	121.6 (120.6)	N3-C4-C5-N1	0.5 (0.2)
C25-C26	1.373 (1.383)	C14-C15-C16	121.6 (121.0)	N3-C4-C5-C51	179.0 (179.0)
C12-H12	0.930 (1.073)	C11-C16-C15	119.0 (119.8)	C41-C4-C5-N1	-177.3 (-179.4)
C13-H13	0.930 (1.074)	C2-C21-C22	123.8 (123.5)	C41-C4-C5-C51	1.1 (1.3)
C15-H15	0.930 (1.074)	C2-C21-C26	118.1 (117.5)	N1-C11-C12-C13 C16-C11-C12-C13	-176.4 (-179.9)
C16-H16	0.930 (1.073)	C22-C21-C26	118.1 (119.0)	N1-C11-C16-C15	0.4 (0.2)
C17-H17A	0.960 (1.086)	C21-C22-C23	120.7 (120.3)	C12-C11-C16-C15	177.6 (179.8)
C17-H17B	0.960 (1.083)	C22-C23-C24	120.1 (120.4)	C11-C12-C13-C14	0.7 (0.4)
C17-H17C	0.960 (1.084)	C23-C24-C25	119.4 (119.5)	C12-C13-C14-C15	-0.9 (-0.1)
C22-H22	0.930 (1.069)	C24-C25-C26	120.6 (120.3)	C12-C13-C14-C17	0.3 (0.1)
C23-H23	0.930 (1.073)	C21-C26-C25	121.4 (120.5)	C13-C14-C15-C16	179.5 (179.3)
C24-H24	0.930 (1.073)	C11-C12-H12	120.0 (119.7)	C17-C14-C15-C16	0.9 (0.1)
				C14-C15-C16-C11	-178.3 (-179.0)
				C2-C21-C22-C23	-1.4 (-0.4)
					176.8 (178.2)

Table 2: IC50 values (µg/ml) of imidazole derivatives and standard antioxidants by different free radical scavenging methods

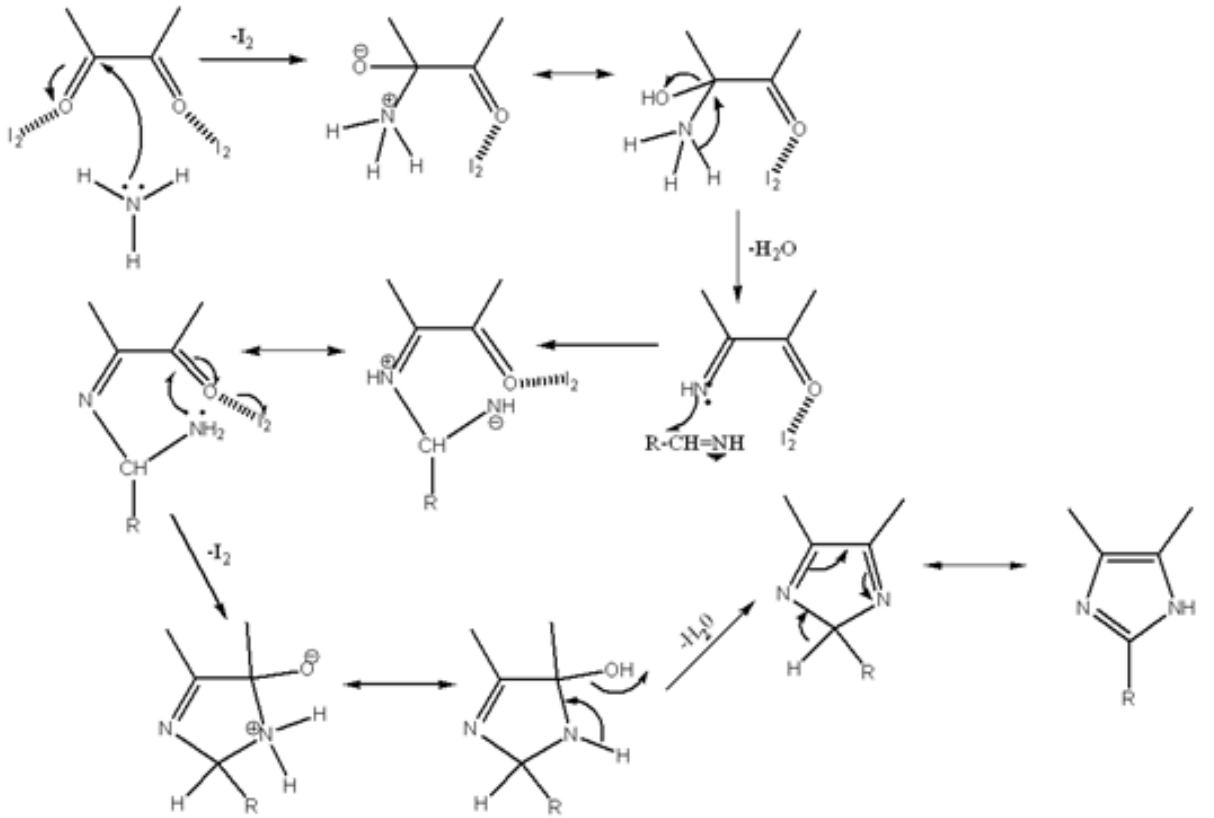
	Hydroxyl radical			
Concentrations (µM)	**Asc**	**dmppi**	**fpdmti**	**dmdmppi**
10	24.26 ± 2.14	19.81 ± 1.56	13.79 ± 3.44	25.22 ± 3.12
20	41.29 ± 3.14	27.02 ± 2.70	28.73 ± 1.99	31.53 ± 1.56
40	48.57 ± 3.7	41.44 ± 4.12	47.12 ± 1.99	48.64 ± 2.70
60	65.32 ± 5	45.04 ± 4.12	55.17 ± 5.97	57.65 ± 5.62
80	70.4 ± 4.9	50.45 ± 4.12	52.87 ± 3.98	64.86 ± 2.70
100	78.46 ± 5.97	54.95 ± 4.12	49.42 ± 1.99	65.76 ± 1.56
IC_{50} value	2.72 ± 0.12	4.73 ± 0.15	4.57 ± 0.16	3.84 ± 0.13
	Superoxide anion			
10	32.56 ± 2.8	33.33 ± 3.29	24.76 ± 3.29	7.61 ± 3.29
20	48.78 ± 3.71	41.90 ± 4.36	38.09 ± 1.64	32.38 ± 3.29
40	55.45 ± 4.22	43.80 ± 4.36	51.42 ± 5.71	39.04 ± 1.64
60	62.11 ± 4.75	49.52 ± 1.64	67.61 ± 3.29	60.95 ± 4.36
80	66.52 ± 2.45	53.33 ± 1.64	65.71 ± 2.85	62.85 ± 4.94
100	73.45 ± 5.56	59.04 ± 1.64	62.85 ± 2.85	65.71 ± 5.71
IC_{50} value	2.80 ± .22	4.26 ± 0.17	3.69 ± 0.15	3.98 ± 0.19
	DPPH radical			
10	26.48 ± 2.08	31.31 ± 1.74	35.35 ± 4.62	16.16 ± 1.74
20	34.47 ± 2.62	38.38 ± 1.74	47.47 ± 1.74	30.30 ± 3.03
30	48.77 ± 3.71	47.47 ± 1.74	50.50 ± 1.74	40.40 ± 1.74
40	66.5 ± 5.06	48.48 ± 3.03	55.55 ± 4.62	50.50 ± 1.74
50	75.39 ± 5.74	46.46 ± 1.74	48.48 ± 3.03	62.62 ± 1.74
IC_{50} value	3.1 ± .24	4.0 ± 0.13	3.68 ± 0.14	3.85 ± 0.13

Table 3: In *vitro* antibacterial activity of imidazole derivatives

Compound	Minimum inhibitory concentration (MIC) in µg/ml		
	S. aureus	***E. Coli***	***pseudomonas***
dmppi	100	50	100
fpdmti	100	50	50
dmdmppi	50	25	25
Ciprofloxalin	50	25	50
Norfloxacin	50	50	50

Table 4: In *vitro* antifungal activity of imidazole derivatives

Compound	Minimum inhibitory concentration (MIC) in µg/ml		
	A. flavus	*A. niger*	*Candida-6*
dmppi	50	25	50
fpdmti	50	25	25
dmdmppi	12.5	12.5	50
Amphotericin-B	25	25	50
Ampicillin	25	50	50



Scheme 1: General schematic representation of synthesis of imidazole derivatives

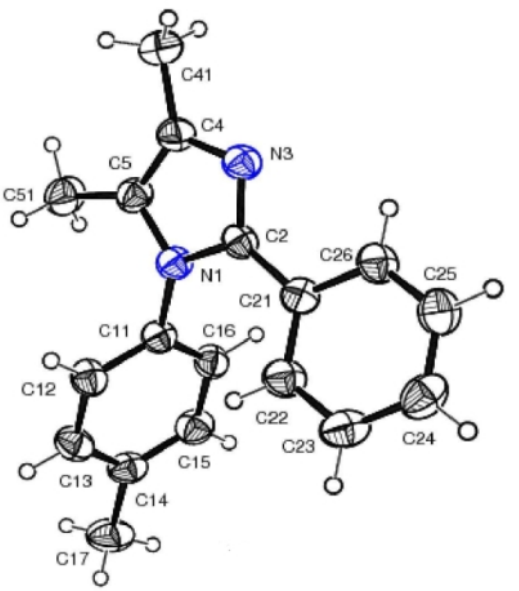


Figure 1: ORTEP diagram for dmppi

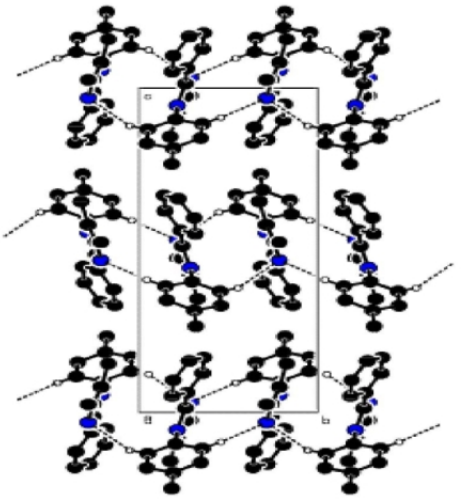


Figure 2: Crystal packing diagram for dmppi

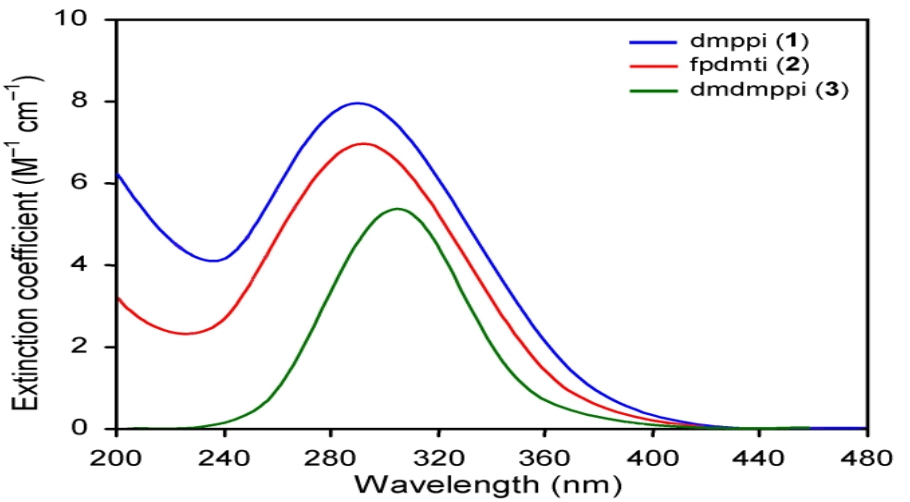


Figure 3: UV-Vis spectrum of imidazole derivatives in dichloromethane for dmppi

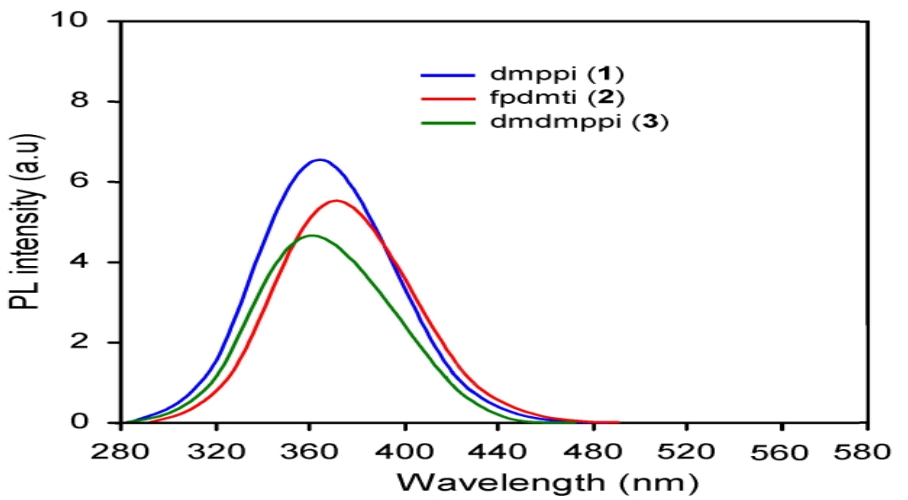


Figure 4: Fluorescence spectrum of imidazole derivatives in dichloromethane

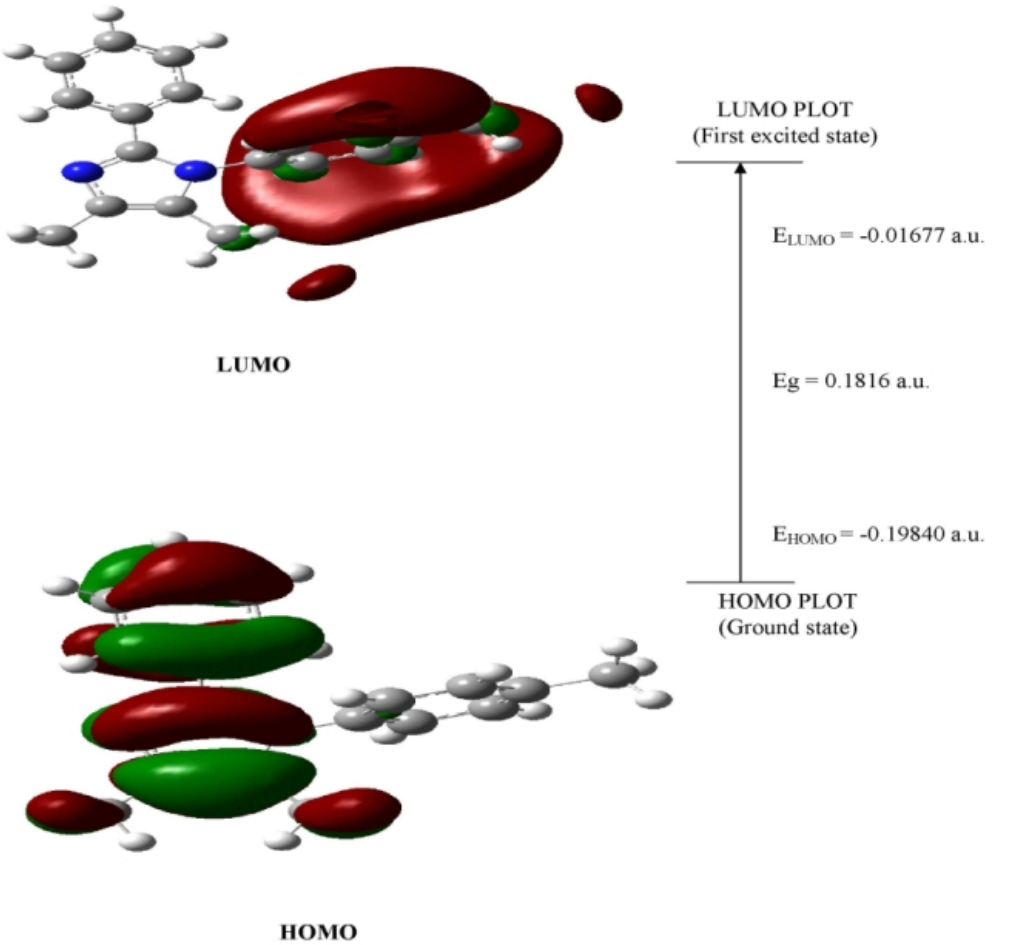


Figure 5: HOMO-LUMO orbital picture of dmppi

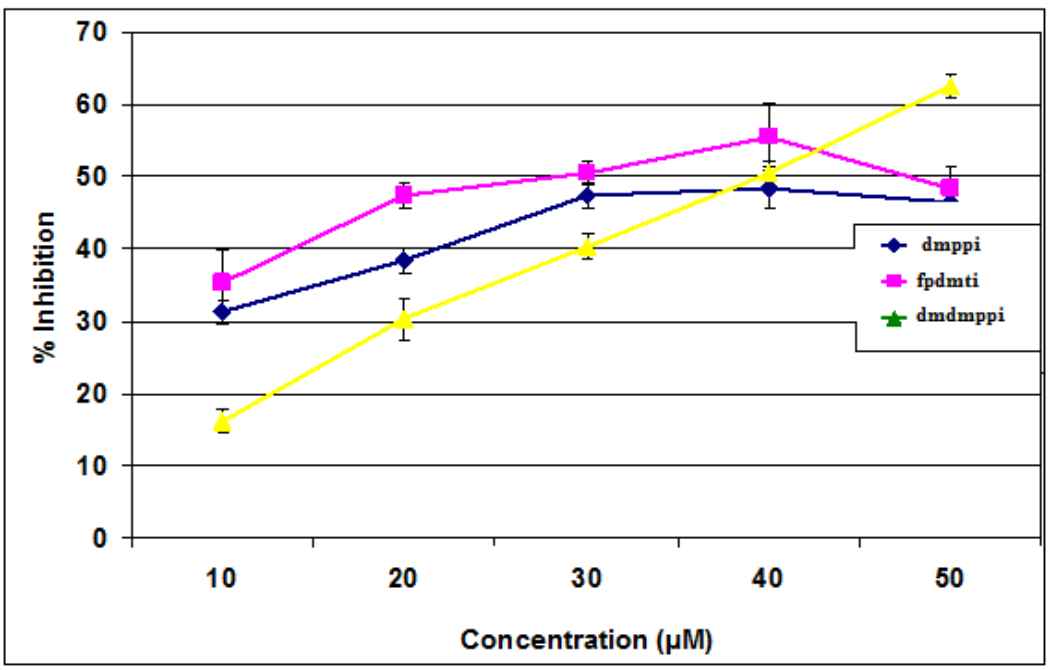


Figure 6: Radical scavenging potential of the imidazole derivatives by DPPH method at different concentrations (µg/mL)

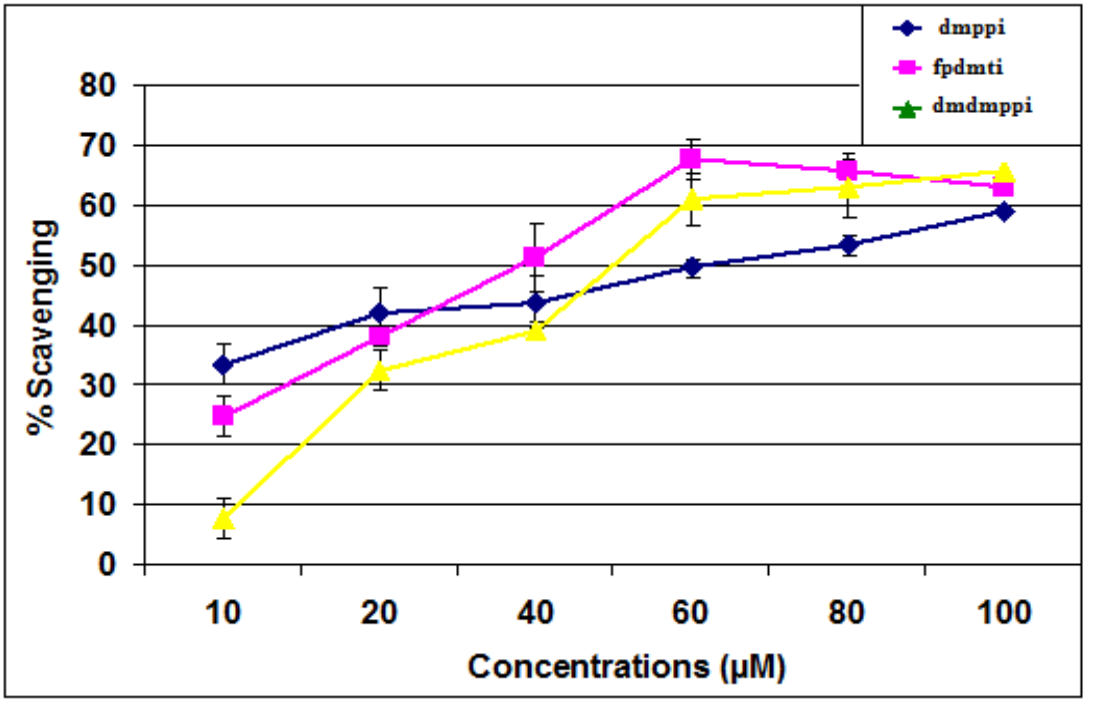


Figure 7: Scavenging potential of the imidazole derivatives at different concentrations (µg/mL) on superoxide radicals generated by the PMS/NADH system.

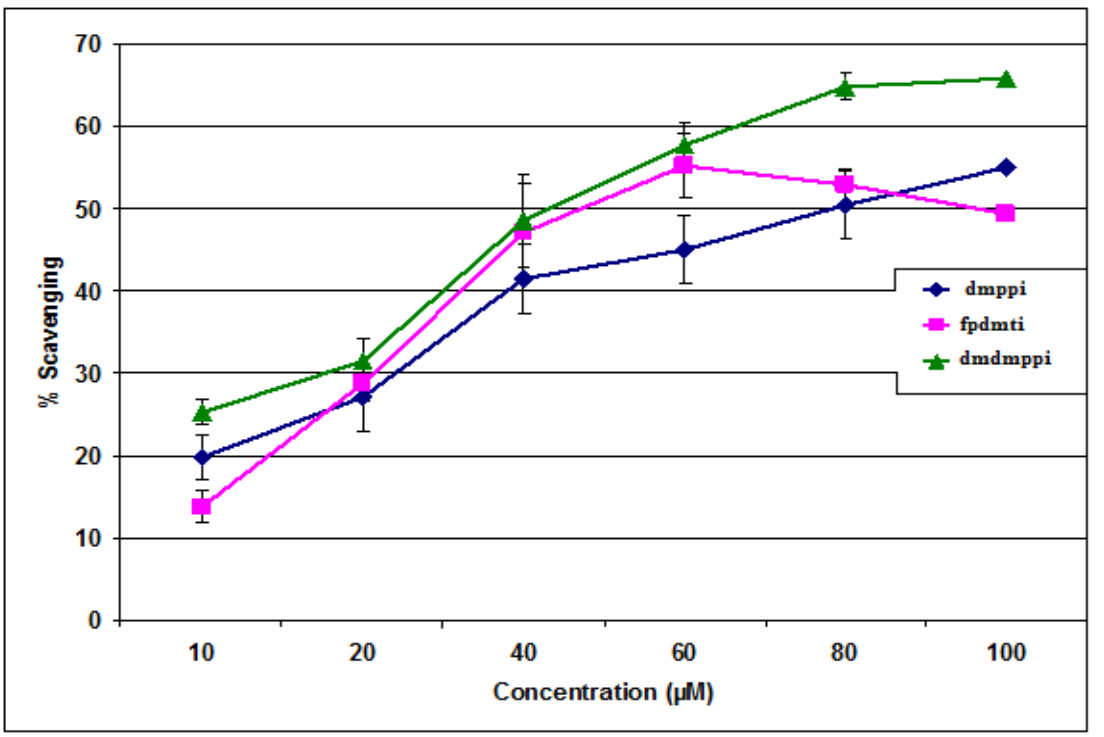


Figure 8: Hydroxy radical scavenging potential of the imidazole derivatives at different concentrations (µg/mL) on deoxyribose degradation method

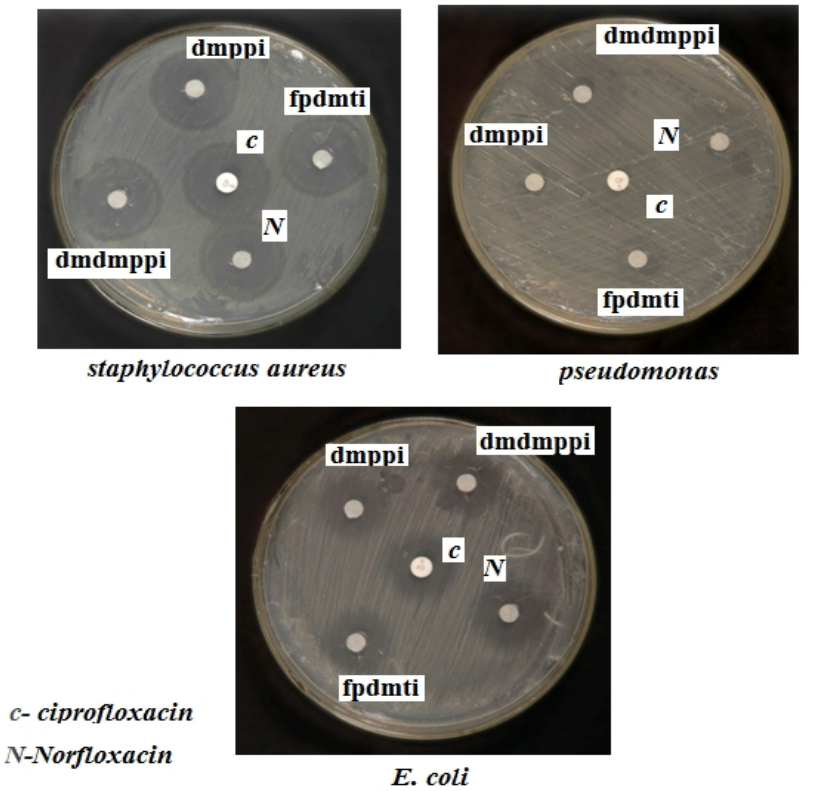


Plate 1: Antibacterial activity of the imidazole derivatives

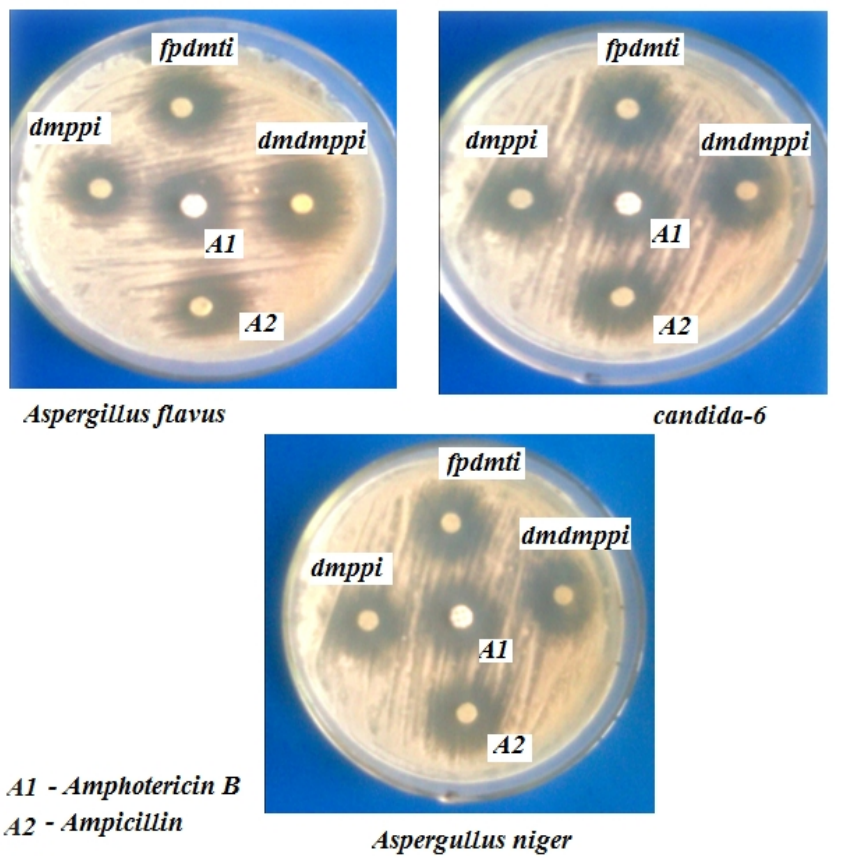


Plate 2: Antifungal activity of the imidazole derivatives

Since in dmppi, n-π* and the π-π* transitions are in close proximity, the low intensity n-π* transition is often completely overlaid by the intensive π-π* transition. The UV-visible (Figure 3) and emission (Figure 4) spectrum of dmppi was also recorded in different solvents such as non-hydroxy solvents and hydroxy solvents and it was observed that the absorption maximum was red shifted in the polar aprotic solvents, may be due to the presence of increased resonance interaction of the π-cloud of the phenyl ring attached to the carbon of the imidazole ring with the lone pair of nitrogen atom (N_3) of the imidazole ring and the blue shift in polar protic solvent is due to hydrogen bonding interactions with the lone pair on nitrogen atom and thus inhibiting from resonance interaction with π-cloud of phenyl ring.[32] The resonance interaction increases if the lone pair and the π-cloud is parallel to each other and in order to understand the coplanarity, optimization of dmppi (1) have been performed by DFT at B3LYP / 6-31G(d,p) using Gaussian-03 (Table 1). From XRD data, the shortening of bond angles C_2-N_1-C_5, C_2-N_3-C_4 and N_1-C_5-C_4 and increase of bond angles C_2-N_1-C_{11}, C_5-N_1-C_{11}, N_1-C_2-C_{21}, N_3-C_2-C_{21}, C_5-C_4-C_{41} and C_2-C_{21}-C_{22} from 120º exactly at the substitution of the imidazole nucleus. All these XRD data are in good agreement with the theoretical values (Table.1). However, from the theoretical values it can be found that most of the optimized bond lengths, bond angles and dihedral angles are slightly higher than that of XRD values. These deviations can be attributed to the fact that the theoretical calculations were aimed at the isolated molecule in the gaseous phase and the XRD results were aimed at the molecule in the solid state. results strongly evidenced the existence of resonance interaction of the π-cloud of the phenyl ring attached to the carbon of the imidazole ring with the lone pair of nitrogen (N_3) of the imidazole ring and also it was found that there is poor coplanarity between the *p*-methylphenyl ring attached to the nitrogen with the imidazole nucleus. The observed fluorescence spectrum is broad and more sensitive to changing the polarity of the solvents. This is in argument with the fact that greater charge transfer takes place from aromatic ring to imidazole nucleus in S_1 state than S_0 state which is evidenced by the decrease in the dihedral angle of N_3-C_2-C_{21}-C_{22} and N_3-C_2-C_{21}-C_{26} from 156.9º to 147.2º and 19.6º to 10.0º, respectively. Moreover, the reduction in the bond distances of C_2-C_{21} from 1.47 Å to 1.36 Å on excitation from S_0 to S_1 state. Since in these imidazole derivatives greater charge transfer takes place from aromatic ring to imidazole nucleus (Figure 5) confirmed by HOMO-LUMO orbital analysis, they are used as fluorophore and are bioactive so that they were tested for antioxidant and antimicrobial activities. There are different models available for evaluation of antioxidant activities. The chemical complexity of compounds could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays for evaluating the antioxidant potential of imidazole derivatives would be more informative and even necessary. In this study, different free radical scavenging activities was measured and all results were compared with standard antioxidant.

In vitro Antioxidant Activity

DPPH radical scavenging activity

Compounds, at various concentrations ranging from 10 to 50 μM, were mixed in 1 mL of freshly prepared 0.5 mM DPPH ethanolic solution and 2 mL of 0.1 M acetate buffer at pH 5.5. The resulting solutions were then incubated at 37°C for 30 minutes and measured at 517 nm in a Shimadzu UV-1601 spectrophotometer. $DPPH^{\bullet}$ scavenging activities of the imidazoles were calculated from the decrease in absorbance from 517 nm in comparison with the negative control (Figure 6). IC_{50} value is the concentration of compound required to inhibit 50 % of $DPPH^{\bullet}$ production.

$$\% \text{ of } DPPH^{\bullet} \text{ scavenging} = [A_0 - A_1 / A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of imidazole derivatives. All the tested compounds showed DPPH Radical quenching activity in a concentration dependent manner, dmdmppi showed maximum activity (IC50 = 16.16 ± 1.74 μg/mL).

Superoxide Anion Scavenging Assay

In the PMS/NADH-NBT system, superoxide anion is generated using a non-enzymatic reaction of phenazine methosulphate in the presence of NADH and molecular oxygen.[33] It is well known that superoxide anions damage bio macromolecules directly or indirectly by forming H_2O_2, $OH^{\cdot}$, peroxylnitrite, or singlet oxygen during pathophysiologic events such as ischemic-reperfusion injury. PMS_{red} (phenazine methosulphate) convert oxidized nitro blue tetrazolium (NBT_{oxi}) to the reduced form (NBT_{red}), which formed a violet coloured complex. The color formation indicates the generation of superoxide anion, which was measured spectrophotometrically at 560 nm. A decrease in the formation of colour after addition of the antioxidant was a measure of its superoxide scavenging activity. The superoxide radical scavenging activities of active imidazoles were evaluated based on their ability to quench the superoxide radical generated from the PMS/NADH reaction. 1 mL of NBT (100 μmol of NBT in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH (468 μmol in 100 mM phosphate buffer, pH 7.4) solution and varying volume of imidazoles (10, 20, 40, 60, 80 and 100 μM) were mixed well. The reaction was started by the addition of 100 μl of PMS (60 μmol/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30 °C for 15 minutes. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the compounds was used as blank. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity (Figure 7). The percentage scavenging was calculated as shown below:

$$\% \text{ of scavenging } [O_2^{\bullet -}] = [A_0 - A_1 / A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of imidazole derivatives. All the tested compounds showed superoxide anion quenching activity in a concentration dependent manner, dmdmppi showed maximum activity (IC50 = 7.61 ± 5.26 μg/mL).

Hydroxyl Radical Scavenging

In this assay, hydroxyl radical was produced by reduction of H_2O_2 by the transition metal (iron) in the presence of ascorbic acid. The generation of $OH^{\bullet}$ is detected by its ability to degrade deoxyribose to form products, which on heating with thiobarbituric acid (TBA) form a pink colour chromogen. Addition of imidazole compound with deoxyribose for $OH^{\bullet}$ and diminishes the colour formation. The incubation mixture in a total volume of 1 mL contained 0.1 mL of buffer, varying volumes of imdazoles (10, 20, 40, 60 80 and 100 μM), 0.2 mL of 500 μM ferric chloride, 0.1 mL of 1 mM ascorbic acid, 0.1 L of 1 M EDTA, 0.1 mL of 10 mM H_2O_2 and 0.2 mL of 2-deoxyribose. The contents were mixed

thoroughly and incubated at room temperature for 60 minutes. Then, 1 mL of 1 % TBA (1 g in 100 mL of 0.05 N NaOH) and 1 mL of 28 % trichloroacetic acid (TCA) were added. All the tubes were kept in a boiling water bath for 30 minutes. The absorbance of the supernatant was observed at 535 nm with reagent blank containing water in place of compounds. Decreased absorbance of the reaction mixture indicates increased hydroxyl radical scavenging activity. The percentage scavenging was calculated as shown below:

$$\% \text{ of scavenging } [OH^{\bullet}] = [A_0 - A_1 / A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of imidazole derivatives.

All the imidazoles suppressed hydroxyl radical mediated deoxyribose degradation in a concentration dependent manner (Figure 8). The hydroxyl radical is a highly potent oxidant that reacts with almost all bio molecules found in living cells when it reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids, lipid hydro peroxides is produced.[34] Lipid hydro peroxide can be decomposed alkoxyl and peroxyl radical and numerous carbonyl products such as malondialdehyde (MDA). The carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases.[35] All the tested compounds showed superoxide anion quenching activity in a concentration dependent manner, fpdmti showed maximum activity (IC50 = 13.79 ± 3.44 µg/mL).

Reducing Power Assay

The imidazole (2.5 mL) at various concentrations was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide $[K_3Fe(CN)_6]$ (1 %, w/v), followed by incubating at 50°C for 20 minutes. The reaction was stopped by adding 2.5 mL of trichloroacetic acid (TCA) solution (10 %) and then centrifuged at 800 × g for 10 minutes. 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1 %, w/v) and the absorbance was measured at 700 nm. Butylated hydroxyl toluene was used as reference standard. Higher absorbance of the reaction mixture indicated greater reducing power. The reducing power assay serves as a significant indicator of potential antioxidant activity. Although, different mechanism was proposed for the antioxidant activity such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.[36] The substituted imidazoles showed concentration-dependant reductive effects. The reducing properties are generally associated with the presence of different reductants.[37] The moderate reducing property was observed for all imidazole derivatives. The antioxidant action of reluctant is based on the breaking of the free radical chain by donating a hydrogen atom. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. The hydroxyl radical scavenging ability of the bioactive imidazole derivatives exhibits inhibition of OH• formation and percentage of inhibition were linearly increased with increasing concentration and the OH• scavenging ability was found to be in the order fpdmti > dmppi > dmmppi. The superoxide anion scavenging ability of imidazole derivatives were also found to be concentration dependent and the percentage of inhibition was linearly increased with increasing concentration and the superoxide anion radical scavenging ability was found to be in the order dmmppi > fpdmti > dmppi. $DPPH^{\bullet}$ with suitable reducing agents and electron becomes paired off and the solution loses colour stoichiometrically with the number of electrons taken up. Such reactivity has been used to test the ability of imidazole derivatives to act as free radical scavengers. The scavenging ability of compounds on $DPPH^{\bullet}$ were linearly increased with increasing concentration and $DPPH^{\bullet}$ scavenging ability was found to be in the order dmmppi > dmppi fpdmti >. The calculated IC_{50} (inhibitory concentration) values of imidazole derivatives are presented in Table 2. From the observed results it was concluded that the imidazole derivatives showed potent scavenging activities and it is evident that p-tolyl ring at C_{-3} and p-fluorophenyl at C_{-2} of the imidazole ring has maximum OH• (fpdmti) when compared with other imidazole derivatives. The low IC_{50} value of fpdmti may be due to the electron donating (+I effect) ability exerted by the methyl substituent and electron withdrawing (-I effect) ability exerted by the fluoro substituent. The 3,5-dimethylphenyl ring at C_{-2} of the imidazole ring (dmmppi) has maximum DPPH• and superoxide anion radical scavenging activities when compared with other imidazole derivatives and the low IC_{50} value of dmmppi) may be due to the electron donating (+I effect) ability exerted by the two methyl substituent's.

ANTIMICROBIAL STUDIES

Antibacterial Activity

All the synthesized imidazole derivatives were tested for their antibacterial activity *in vitro* against *Staphylococcus aureus*, *Escherichia coli* and *Klebesiella pneumoniae*. Ciprofloxacin and Norfloxacin were used as reference drug whose minimum inhibitory concentration (MIC) values were furnished in Table 3 (Plate 1). Imidazole derivatives exerted antibacterial activity *in vitro* at 25-100 µg/ml against all the tested strain. All compounds exerted improved activity against all the tested strains.

Antifungal Activity

The *in vitro* antifungal activity of imidazole derivatives were examined against the fungal strains viz., *Aspergillus niger, Aspergillus flavus* and Candida-6. Amphotericin-B and Ampicillin were used as standard drugs whose minimum inhibitory concentration (MIC) values are furnished in Table 4 (Plate 2). Imidazole derivatives exhibited improved fungal activity at 25-100 µg/ml against all the tested strains.

CONCLUSIONS

A series of substituted imidazoles have been synthesized under solvent free condition in the presence of molecular iodine as the catalyst and characterized by NMR spectra, X-ray, mass and CHN analysis. The novel imidazole derivatives showed free radical scavenging activity when tested in different models. From the observed results it was concluded that the bio active imidazole derivatives showed potent scavenging activities. It is well documented that free radicals are responsible for several diseases. The present result confirms the free radical scavenging activity of the imidazole derivative and it can be used for several diseases. A minute examination of *in vitro* antibacterial and antifungal screening of imidazole derivatives against the tested bacterial and fungal strains provide a better structure activity and thus in future these compounds may be used as templates to generate better drugs to fight against bacterial and fungal infections.

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