Med Chem Res (2012) 21:1850–1860
DOI 10.1007/s00044-011-9702-5

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antioxidant potential and antimicrobial screening of some novel imidazole derivatives: greenway efficient one pot synthesis

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Received: 3 February 2011 / Accepted: 9 June 2011 / Published online: 25 June 2011

Abstract A series of substituted imidazoles have been synthesized under solvent-free condition by grinding 1,2-diketone, aromatic aldehyde, and ammonium acetate in the presence of molecular iodine as the catalyst. The short reaction time and easy workup make this protocol practically and economically attractive and 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-picrylhydrazyl) radical, superoxide anion, and hydroxyl radical scavenging activities. 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. Among all, dimethoxyphenyl substituent at N3 of the imidazole derivatives exhibited the highest hydroxy and superoxide anion radical scavenging activities, whereas dimethoxyphenyl substituent at N3 and fluorophenyl at C2 of the imidazole derivatives exhibited the highest DPPH radical scavenging activity.

Keywords Antioxidant activity · Antimicrobial screening · Free radical scavenging activity · NMR · X-ray

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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 (Lambardino and Wiseman, 1974; Maier *et al.*, 1989). Though there are several methods reported (Lantos *et al.*, 1993; Zhang *et al.*, 1996) 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. 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 (Jianwei *et al.*, 2004). During the course of our studies toward the development of new route to the synthesis of biologically active heterocycles, 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. (Gutteridgde 1995; Aruoma 1998). 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

peroxy nitrite 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 (Halliwell, 2000). 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 toward finding naturally occurring antioxidants particularly of plant origin. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy 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 (Branen, 1975; Wichi, 1986; Grice, 1988). 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 as nutritional supplements (Schuler, 1990). Also many other plant species have been investigated in search of novel antioxidants (Mantle *et al.*, 2000; Koleva *et al.*, 2002; (Oke and Hamburger, 2002; Parejo *et al.*, 2003), but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). 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 (Frautchy *et al.*, 2001; Wang *et al.*, 2006; Clavin *et al.*, 2007). 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 persists. When compared with natural phytochemicals, it is possible to synthesize more potent antioxidants through chemical synthesis. Therefore, the objective of present study was a development of new route to the synthesis of heterocycles 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.

Experimental

Materials and methods

General procedure for the synthesis of 2-aryl imidazole derivatives

A mixture of aldehyde (1 mmol), 1,2-diketone (1 mmol), ammonium acetate (2.5 mmol), and iodine (15 mol%) were grined in a mortar at room temperature for 2 h. The completion of 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 (Jayabharathi *et al.*, 2009, 2010a, b; Saravanan *et al.*, 2010) (*n*-hexane:ethyl acetate (9:1) (Scheme 1).

4,5-Dimethyl-1-(3′,5′-dimethoxyphenyl)-2-(p-fluorophenyl)-1H-imidazole (dmdmpfpi)

Yield: 60%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H), 1.99 (s, 3H), 3.85 (s, 3H), 6.85–7.35 (aromatic protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.50, 12.69, 55.48, 114.71, 114.93, 115.14, 128.90, 129.80, 130.42, 133.21, 144.36, 159.45. Anal. calcd. for $C_{18}H_{17}N_2OF$: C, 72.95; H, 5.78; N, 9.45. Found: C, 72.24; H, 5.36; N, 8.98. MS: m/z 296.5, calcd. 296.35.

4,5-Dimethyl-1-(4′-methoxyphenyl)-2-phenyl-1H-imidazole (dmmppi)

Yield: 60%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.29 (s, 3H), 2.02 (s, 3H), 3.85 (s, 3H), 6.91–7.10 (aromatic protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.39, 12.59, 55.32, 114.50, 125.51, 127.41, 127.87, 128.78, 130.48, 130.74, 133.12, 145.08, 159.23. Anal. calcd. for $C_{18}H_{18}N_2O$: C, 77.67; H, 6.52; N, 10.06. Found: C, 77.14; H, 6.32; N, 9.87. MS: m/z 278.2, calcd. 278.36.

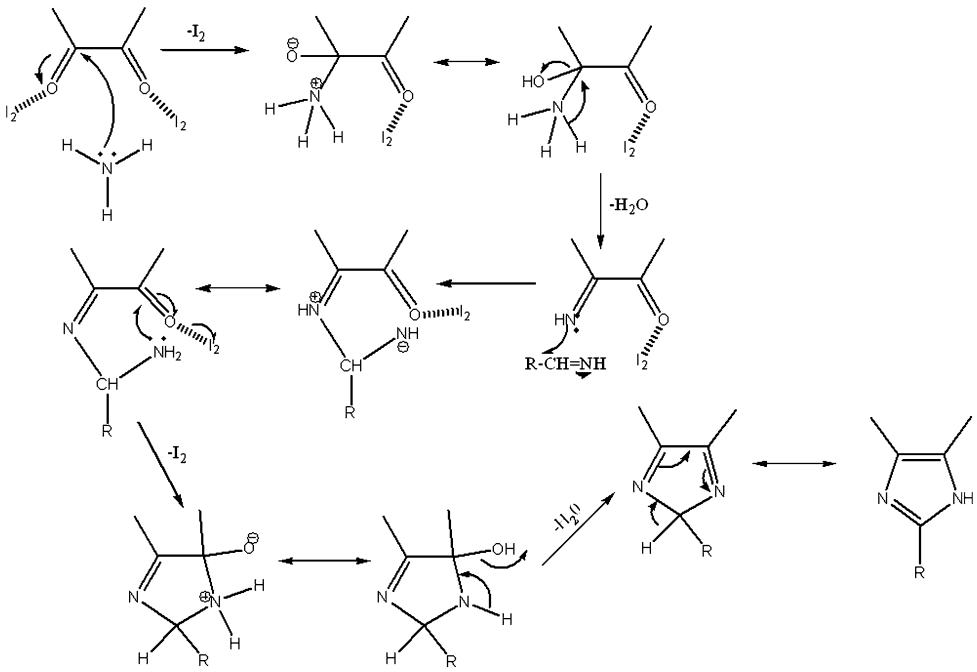
4,5-Dimethyl-1-(3′,5′-dimethoxyphenyl)-2-(p-fluorophenyl)-1H-imidazole (dmdmpfpi)

Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H), 2.04 (s, 3H), 3.74 (s, 6H), 6.30–7.40 (aromatic protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.40, 12.58, 55.44, 100.32, 106.25, 114.85, 115.06, 125.23, 127.00, 129.55, 133.38, 139.27, 143.98, 161.18. Anal. calcd. for $C_{19}H_{19}N_2O_2F$: C, 69.92; H, 5.87; N, 8.58. Found: C, 69.12; H, 5.37; N, 8.24. MS: m/z 326.3, calcd. 326.37.

4,5-Dimethyl-1-(3′,5′-dimethoxyphenyl)-2-phenyl-1H-imidazole (dmdmppi)

Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.29 (s, 3H), 2.05 (s, 3H), 3.73 (s, 6H), 6.32–7.43 (aromatic protons).

Scheme 1 General schematic representation of synthesis of imidazole derivatives



^{13}C NMR (100 MHz, $CDCl_3$): δ 9.36, 12.58, 55.32, 100.21, 106.20, 125.13, 127.45, 127.65, 127.83, 130.68, 133.34, 139.38, 144.79, 161.01. Anal. calcd. for $C_{19}H_{20}N_2O_2$: C, 73.93; H, 6.54; N, 9.08. Found: C, 73.23; H, 6.18; N, 8.79. MS: m/z 308.3, calcd. 308.38.

In Vitro Antibacterial and Antifungal Activity

The in vitro activities of imidazole derivatives were tested in Sabourauds dextrose broth (Jayabharathi *et al.*, 2007, 2008, 2010a, b) (SDB; Hi-media, Mumbai) for bacteria by the two fold serial dilution method. 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 $\times$ 10^2 cfu/ml for antifungal assay, and testing was performed at pH 7.4 $\pm$ 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. 1 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 similarly solvent controls were also run simultaneously. The tubes were incubated in BOD incubators at 37 $\pm$ 1 °C for bacteria and 28 $\pm$ 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* and *Amphotericin B* 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 of Baricevic *et al.*, 2001.

Superoxide radical scavenging activity Superoxide anion radical scavenging activity of synthetic imidazole derivatives were determined by the method of Nishimiki (Nishimiki *et al.*, 1972). 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 (Elizabeth and Rao, 1990).

Reducing power assay The Fe^{3+} reducing power of imidazole was determined by the method of Oyaizu (1986).

Results and discussion

Photophysical properties of dmdmpfpi

Crystal growth of dmdmpfpi was carried out by Slow Evaporation Solution Growth Technique (SEST) and the crystalline imidazole derivative dmdmpfpi was a monoclinic crystal (Fig. 1) crystallizes in the space group p_{21}/c, and cell has dimensions of $a = 8.5132$ (1) Å, $b = 9.5128$ (2) Å, $c = 19.2610$ (3) Å, $\beta = 96.798$ (2). ORTEP diagram (Gayathri *et al.*, 2010) of dmdmpfpi shows that the imidazole ring makes dihedral angles of 76.46 (7) and 40.68 (7)° with the methoxyphenyl and fluorophenyl rings, respectively. The dihedral angle between the two benzene rings is 71.25 (6)°. The band appeared at 292.8 nm (λ_{abs}) and 364.4 nm (λ_{em}) is due to the π–π^* transition. Since in dmdmpfpi, 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 spectrum (Fig. 2) of dmdmpfpi was 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 (N3) of the imidazole ring (Ren *et al.*, 2003) 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. The resonance interaction increases if the lone pair and the π-cloud are parallel to each other and to understand the coplanarity, optimization of dmdmpfpi DFT at B3LYP/6-31G(d,p) have been performed using Gaussian-03 (Table 1). The observed dihedral angle from both XRD [N3–C2–C21–C22(-137.6°); N3–C2–C21–C26 (40.2) and C5–N1–C11–C12 (84.2°); C5–N1–C11–C16 (−94.7°)] and DFT [N3–C2–C21–C22 (−139.1°); N3–C2–C21–C26 (39.0) and C5–N1–C11–C12 (71.3°); C5–N1–C11–C16 (−84.1°)] 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 (N3) of the imidazole ring and also it was found that there is poor coplanarity between the *p*-methoxyphenyl ring attached to the nitrogen with the imidazole nucleus (Fig. 3). The observed fluorescence spectrum (Fig. 4) 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 N3–C2–C21–C22 and N3–C2–C21–C26 from 139.1° to 127.2° and 38.9° to 10.0°, respectively. Moreover, the reduction in the bond distances of C2–C21 from 1.47 to 1.36 Å on excitation from S_0 to S_1 state (Table 1). Since these imidazole derivatives are used as fluorophore, 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 were measured and all the 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 min 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 (Fig. 5). IC_{50} value is the concentration of the compound required to inhibit 50% of $DPPH^{\bullet}$ production.

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

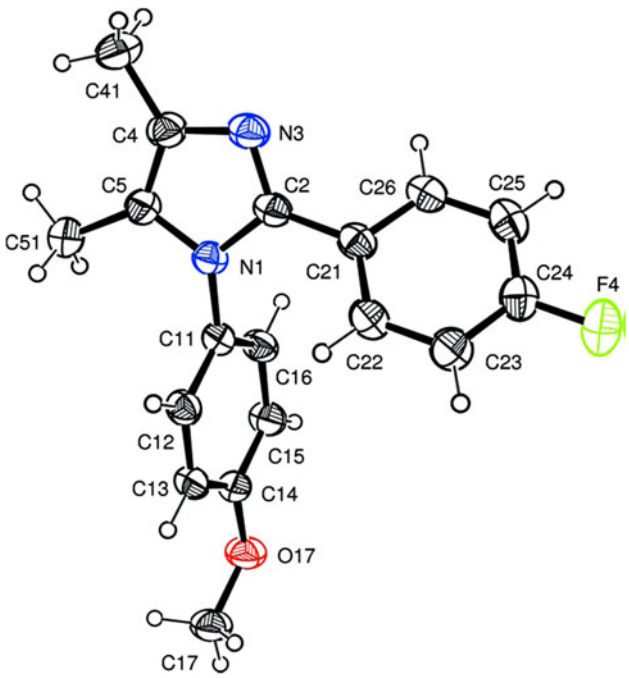


Fig. 1 ORTEP diagram for dmmpfpi

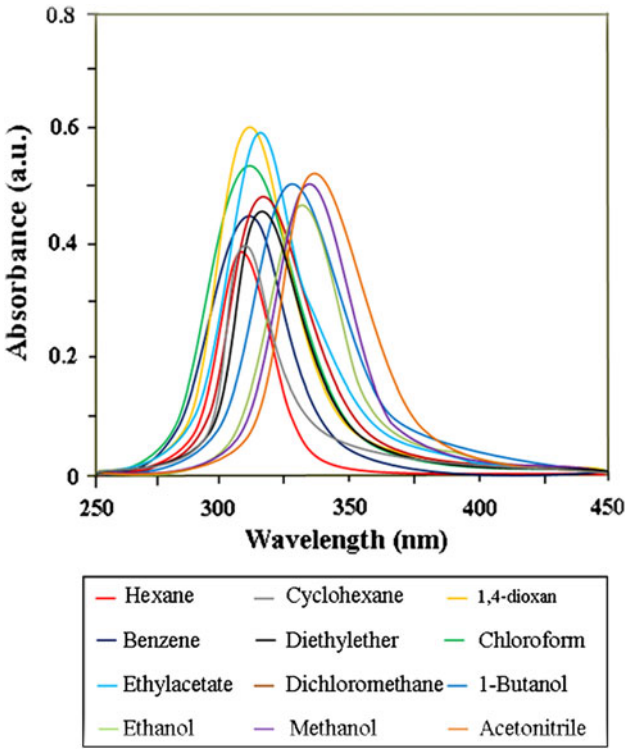


Fig. 2 UV–Vis Spectrum of dmdmppi in various solvents

Table 1 Calculated parameters of dmdmpfpi in the ground and excited states

Characteristics	dmdmpfpi
S_0 state	
E (k cal/mole)	0.00
μg (D)	4.17
Excited state DFT	
E (k cal/mol)	1.00
μl (D)	10.02
Dihedral angle (°)	
Ground state	
(N3–C2–C21–C22)	139.1
(N3–C2–C21–C26)	38.9
Excited state	
(N3–C2–C21–C22)	-127.2
(N3-C2-C21–C26)	10.0
Bond length (Å)	
C2–C21 (Ground state)	1.47
C2–C21 (Excited state)	1.36

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, dmmpfpi showed maximum activity (IC50 = 11.11 ± 1.74 μg/mL L).

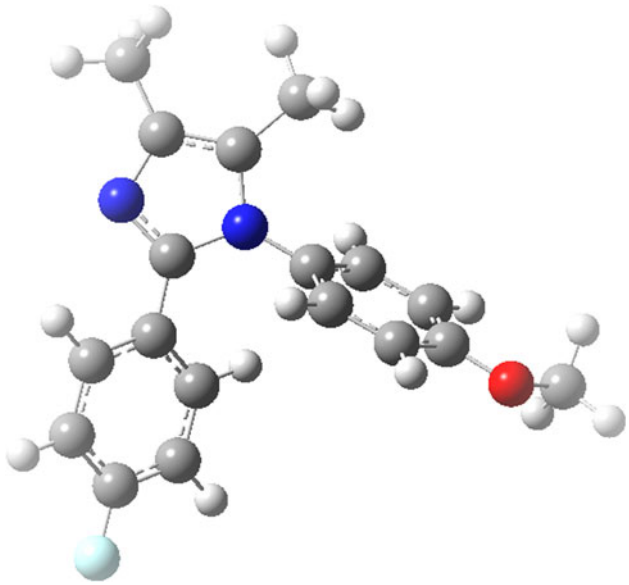


Fig. 3 Energy minimized modeling of dmmpfpi

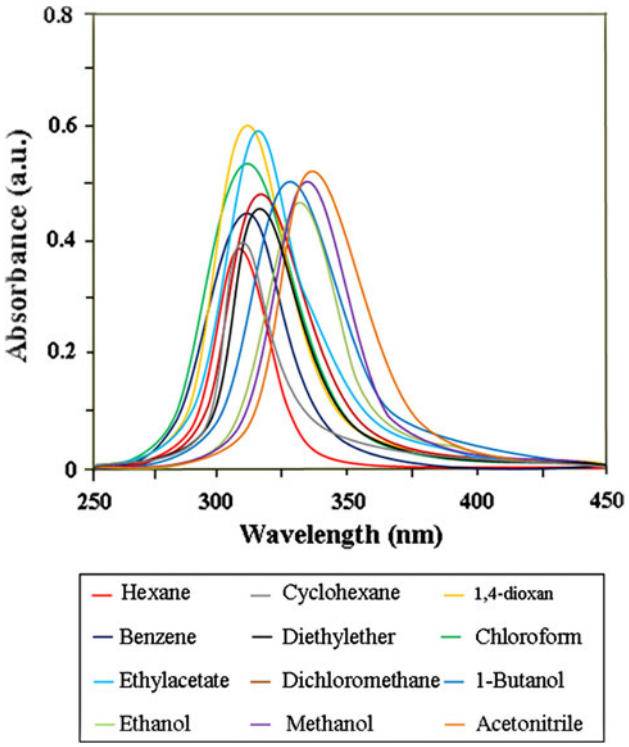


Fig. 4 Fluorescence spectrum of dmdmppi in various solvents

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 (Robak and Gryglewski, 1998). It is well known that superoxide anions damage biomacromolecules directly or indirectly by forming H_2O_2, OH•, peroxylnitrite, or

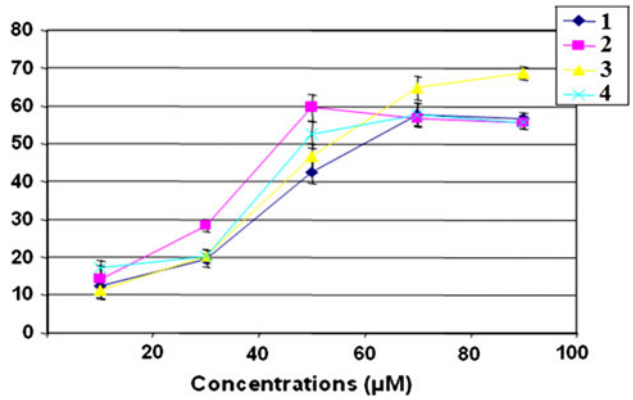


Fig. 5 Radical scavenging potential of the imidazole derivatives by DPPH method at different concentrations (μg/mL)

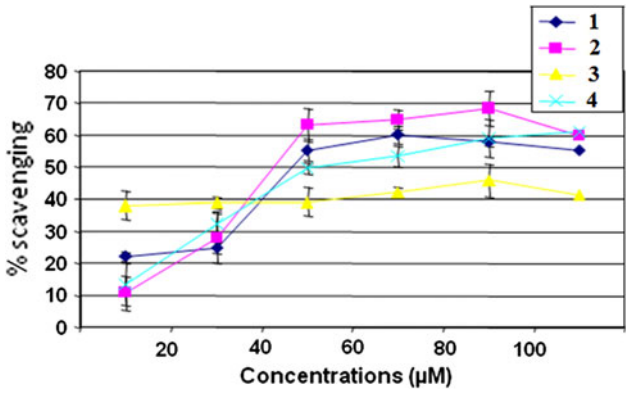


Fig. 6 Scavenging potential of the imidazole derivatives at different concentrations (μg/mL) on superoxide radicals generated by the PMS/NADH system

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-colored complex. The color formation indicates the generation of superoxide anion, which was measured spectrophotometrically at 560 nm. A decrease in the formation of color after the 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 min. 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 (Fig. 6). The percentage scavenging was calculated as shown below:

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

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, dmmppi showed maximum activity (IC50 = 10.52 ± 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-colored chromogen. The addition of imidazole compound with deoxyribose for $OH^{\bullet}$ diminishes the color 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 min. 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 min. 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 (Fig. 7). 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 hydroperoxides is produced (Valentao *et al.*, 2002). Lipid hydroperoxide can be decomposed alkoxyl and peroxyl radicals and numerous carbonyl products such as malondialdehyde (MDA). The carbonyl products are responsible for DNA damage, generation of cancer and aging-related diseases (Okhawa *et al.*, 1979). All the tested compounds showed

superoxide anion quenching activity in a concentration dependent manner, dmdmppi showed maximum activity (IC50 = 34.48 ± 3.44 μg/mL L).

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 [K3Fe(CN)6] (1%, w/v), followed by incubating at 50 °C for 20 min. The reaction was stopped by adding 2.5 mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 800×*g* for 10 min. 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

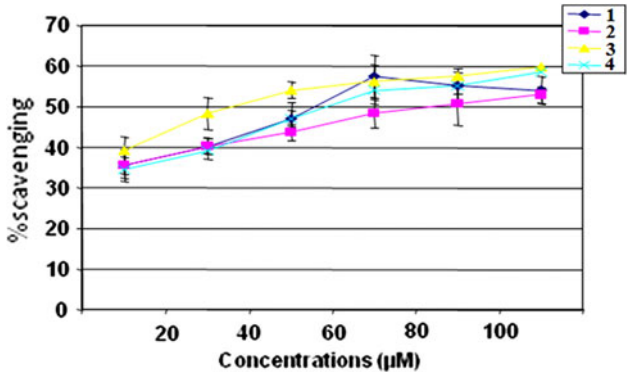


Fig. 7 Hydroxy radical scavenging potential of the imidazole derivatives at different concentrations (μg/mL) on deoxyribose degradation method

Table 3 In *vitro* antibacterial activity of imidazole derivatives

Compound	Minimum inhibitory concentration (MIC) in μg/mL		
	S. aureus	*E. Coli*	*K. pneumoniae*
dmdmpfpi	50	50	100
dmmppi	50	25	100
dmmpfpi	50	25	50
dmdmppi	25	50	100
Ciprofloxacin	25	50	100

Table 2 IC_{50} values (μg/ml) of imidazole derivatives (**1**–**4**) and standard antioxidants by different free radical scavenging methods

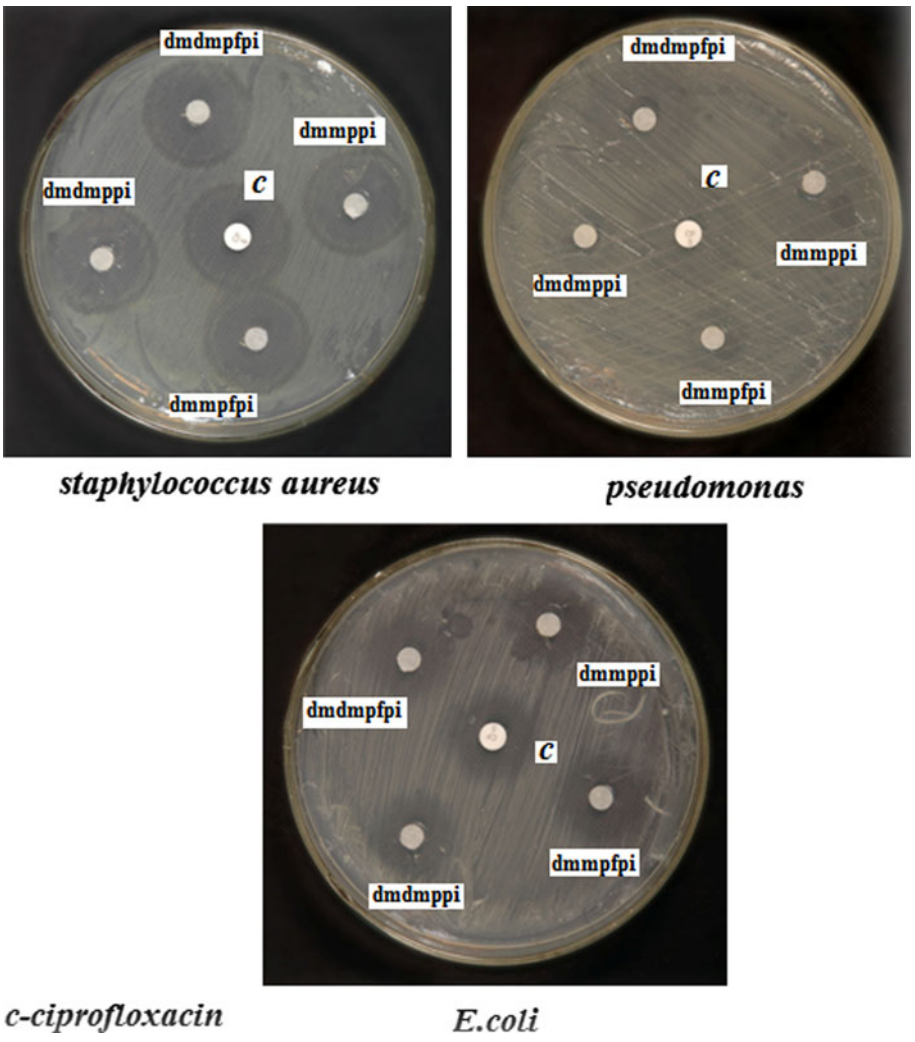
Concentrations (μm)	Ascorbic acid	dmdmpfpi	dmmppi	dmmpfpi	dmdmppi
Hydroxyl radical method					
10	24.26 ± 2.14	35.63 ± 1.99	35.63 ± 3.98	39.08 ± 1.99	34.48 ± 3.44
20	41.29 ± 3.14	40.22 ± 1.99	40.22 ± 1.99	48.27 ± 1.99	39.08 ± 1.99
40	48.57 ± 3.7	47.12 ± 1.99	43.67 ± 1.99	43.67 ± 1.99	47.12 ± 1.99
60	65.32 ± 5	57.47 ± 5.26	48.27 ± 3.44	48.27 ± 3.44	54.02 ± 3.98
80	70.4 ± 4.9	55.17 ± 3.44	50.57 ± 5.26	50.57 ± 5.26	55.17 ± 3.44
100	78.46 ± 5.97	54.02 ± 1.99	52.87 ± 1.99	52.87 ± 1.99	58.62 ± 3.44
IC_{50} value	2.72 ± 0.12	4.18 ± 0.16	4.50 ± 0.15	3.84 ± 0.0.11	4.14 ± 0.14
Superoxide anion method					
10	32.56 ± 2.8	21.90 ± 1.64	10.52 ± 5.26	38.09 ± 4.36	13.33 ± 6.59
20	48.78 ± 3.71	24.76 ± 1.64	28.07 ± 8.03	39.04 ± 1.64	32.38 ± 3.29
40	55.45 ± 4.22	55.23 ± 3.29	63.15 ± 5.26	39.04 ± 4.36	49.52 ± 1.64
60	62.11 ± 4.75	60.0 ± 2.85	64.91 ± 3.03	41.90 ± 1.64	53.33 ± 3.29
80	66.52 ± 2.45	58.09 ± 1.64	68.42 ± 5.26	45.71 ± 4.94	59.04 ± 5.94
100	73.45 ± 5.56	55.23 ± 4.36	59.64 ± 3.03	40.95 ± 1.64	60.95 ± 1.64
IC_{50} value	2.80 ± .22	4.16 ± 0.14	3.74 ± 0.12	5.20 ± .18	4.16 ± 0.16
DPPH radical method					
10	26.48 ± 2.08	12.12 ± 3.03	14.14 ± 3.49	11.11 ± 1.74	17.17 ± 1.74
20	34.47 ± 2.62	19.19 ± 1.74	28.28 ± 1.74	20.20 ± 1.74	20.20 ± 1.74
30	48.77 ± 3.71	42.42 ± 3.03	59.59 ± 3.49	46.46 ± 3.49	52.52 ± 3.49
40	66.5 ± 5.06	57.57 ± 3.03	56.56 ± 1.74	64.64 ± 1.74	57.57 ± 3.03
50	75.39 ± 5.74	56.56 ± 1.74	55.55 ± 1.74	68.68 ± 1.74	55.55 ± 1.74
IC_{50} value	3.1 ± .24	3.98 ± 0.18	3.64 ± 0.12	3.46 ± 0.16	3.80 ± 0.12

as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Gordon 1990). The substituted imidazoles showed concentration-dependant reductive effects. The reducing properties are generally associated with the presence of different reductants (Duh 1998). 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 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 dmdmppi > dmmppi > dmdmpfpi > dmmpfpi. The superoxide anion scavenging ability of imidazole derivatives were 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 dmdmppi > dmmppi > dmdmpfpi > dmmpfpi. $DPPH^{\bullet}$ with suitable reducing agents and electron becomes paired off and the solution loses color stoichiometrically with the number of electrons taken up. Such reactivity has been employed 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 dmmpfpi > dmdmpfpi > dmmppi > dmdmppi. 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 dimethoxyphenyl at C3 of the imidazole ring (dmdmppi) has maximum $OH^{\bullet}$ and superoxide

Plate 1 Antibacterial activity of the imidazole derivatives



anion radical scavenging activities when compared with other imidazole derivatives. The low IC_{50} value of dmdmppi may be due to the electron donating (+I effect) ability exerted by the two methoxy substituents. The *p*-fluoro phenyl group at C2 of the imidazole ring (dmmpfpi) has maximum $DPPH^{\bullet}$ radical scavenging activity when compared with other imidazole derivatives and the low IC_{50} value of dmmpfpi may be due to the electron withdrawing (−I effect) ability exerted by the fluoro substituent.

Table 4 In vitro antifungal activity of imidazole derivatives

Compound	Minimum inhibitory concentration (MIC) in µg/mL		
	Candida-6	*A. niger*	*A. flavus*
dmdmpfpi	50	50	50
dmmppi	25	100	25
dmmpfpi	25	25	25
dmdmppi	25	25	25
Amphotericin-B	12.5	50	25

Antimicrobial studies

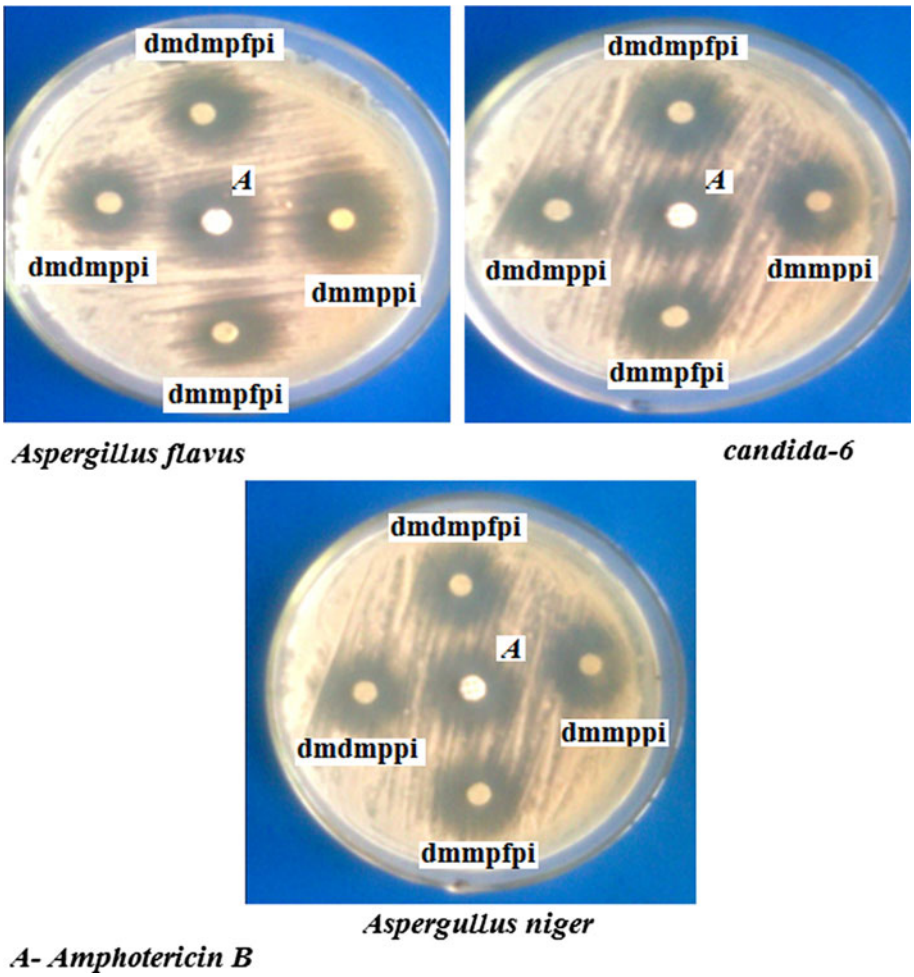
Antibacterial activity

All the synthesized imidazole derivatives were tested for their antibacterial activity in vitro against *Staphylococcus aureus*, *Escherichia coli* and *Klebesiella pneumoniae*. *Ciprofloxacin* was used as reference drug the minimum inhibitory concentration (MIC) values of which 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 was used as a standard drug the minimum inhibitory concentration (MIC) values of which are furnished in Table 4 (Plate 2). Imidazole derivatives exhibited improved fungal activity at 25–100 µg/ml against all the tested strains.

Plate 2 Antifungal activity of the imidazole derivatives



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. Among all, dimethoxyphenyl substituent at N3 of the imidazole derivatives exhibited the highest hydroxy and superoxide anion radical scavenging activities whereas dimethoxyphenyl substituent at N3 and fluorophenyl at C2 of the imidazole derivatives exhibited the highest DPPH radical scavenging activity. It is well documented that free radicals are responsible for several diseases. The present result confirms the free radical scavenging activity of the imidazole derivatives 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.

Acknowledgments One of the authors, Dr. J. Jayabharathi, Associate Professor in Chemistry, Annamalai University is thankful to the Department of Science and Technology (No. SR/S1/IC-07/2007), the University Grants Commission (F. No. 36-21/2008 (SR)) for providing fund to this research study, and Prof. C.H. Cheng, Chairman, the National Science Council, the National Tsing Hua University for her post-doctoral research study.

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