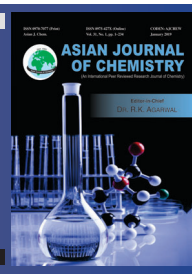
Asian Journal of Chemistry; Vol. 31, No. 11 (2019), 2448-2452

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.22107



Synthesis, Molecular Docking and Radical Scavenging Activity of 1,2,4,5-Tetrasubstituted Imidazole Derivatives

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Received: 30 March 2019; *Accepted*: 3 May 2019; *Published online*: 28 September 2019; AJC-19567

A series of 1,2,4,5-tetrasubstituted imidazoles (**2a-g**) were synthesized using 1,2-diketone, 1-naphthaldehyde, substituted aromatic amine and ammonium acetate in the presence of ceric ammonium nitrate as a catalyst. The synthesized compounds were characterized by FT-IR, ^{1}H NMR, Mass spectra and explored for their antioxidant activity by DPPH free radical scavenging assay method. Among the synthesized compounds **2a**, **2e** and **2f** exhibit good antioxidant activities. Molecular docking study was also been performed to know the possible interactions between the synthesized compound and antioxidant receptor 3MNG.

Keywords: 1,2,4,5-Tetrasubstituted imidazoles, Ceric ammonium nitrate, Antioxidant activity, Molecular docking.

INTRODUCTION

Imidazole and its analogues are important class of compounds and being the constituent of many naturally occurring substances and drugs. Compound containing imidazole moieties have attracted many medicinal chemists due to their wide range of biological applications. Literature review revealed that many new imidazole containing drugs have been synthesized and explored their biological activity. The series of 1,2,4,5-tetrasubstituted imidazoles acts as an anti-bacterial agent [1], antifungal agents [2], antioxidant agents [3], anti-inflammatory agents [4], analgesic agents [5], antiviral agents [6], anti-tuberculosis agents [7], anticonvulsant agents [8], anticancer agents [9], antiurease [10] and also ATP-mimetic inhibitors of P38 MAP Kinase [11].

The development of new antioxidant agents has been very important because free radicals produced by various endogeneous process controls crucial cellular metabolism and mediate many critical biochemical reactions and induce physiological effects. The antioxidants play an important role in the treatment of many chronic and degenerative diseases such as brain dysfunction, immune system decline, atherosclerosis and cancer. Many methods have been reported for the synthesis of 1,2,4,5-tetrasubstituted imidazole derivatives which includes conventional methods and solvent free microwave assisted synthesis. In recent years, extensive work has been carried out on the development of methods for the efficient synthesis imidazole derivatives. Particularly, four component one-pot synthesis involving aldehyde, 1,2-diketone, primary amines and ammonium acetate in acetic acid and in the presence of variety of catalysts such as $FeCl_3/I_2$ [12], nanocrystalline $MgAl_2O_4$ [13], $InCl_3{\cdot}3H_2O$ [14], Ph_3P/CCl_4 [15], *p*-toluene sulfonic acid [16], nano-$TiCl_4{\cdot}SiO_2$ [17], ZnO nanorods [18], Co_3O_4 nanoparticles [19], SBA-Pr-SO_3H [20], silica supported $SbCl_3$ [21], sodium benzenesulfonate [22], ceric ammonium nitrate [23], Fe^{3+}-K10 [24], sodium dihydrogen phosphate [25], L-proline [26], erbium triflate [27] and molecular iodine under solvent free condition [28].

Considering the bioprofile and synthetic methods, a series of 1,2,4,5-tetrasubstituted imidazoles were synthesized and screened for their antioxidant activity by DPPH free radical scavenging assay method. A molecular docking study was also

carried out to understand the interaction sites of receptor with the synthesized molecules.

EXPERIMENTAL

All reagents used in the synthesis were purchased from Sigma-Aldrich, USA. Solvents were purchased from S.D. Fine Chemicals, India and distilled once before usage. The progress of the reaction and purity of the samples were checked by TLC using mobile phase petroleum ether and ethyl acetate (4:1). For TLC stationary phase, silica gel coated aluminum sheets (silica gel 60 F_{254}) procured from Merck, India were employed. Yields were recorded after isolation of the products. IR spectra of the products were obtained on a JASCO FTIR4100 spectrophotometer using KBr pellet method. ^{1}H NMR spectra were recorded using JEOL 500 MHz instrument by using TMS as internal standard and chemical shift values are expressed in δ ppm. Shimadzu LC-2010EV with ESI probe was used for recording mass of the sample. Melting points of the synthesized compounds were determined by open capillary method by using Mvtec melting point apparatus and are uncorrected.

Synthesis of 1,2,4,5-Tetrasubstituted imidazole derivatives (2a-g): A mixture of 1-naphthaldehyde (10 mmol), 1,2-diketone (10 mmol), ammonium acetate (50 mmol), aromatic amine (10 mmol) and ceric ammonium nitrate (10 mol %) as catalyst were taken in a round bottom flask. Methanol (20 mL) was added as a solvent and the reaction mixture was refluxed for 10-12 h. The progress of the reaction was monitored by TLC using petroleum ether and ethyl acetate (4:1) as eluent. After completion of the reaction, the mixture was poured into ice-cold water and the solid obtained was filtered and dried (**Scheme-I**). The crude solid product was then subjected to purification by column chromatography.

1-(4-Methylphenyl)-2-(naphthalen-1-yl)-4,5-diphenyl-1*H*-imidazole (2a): Off white solid; Yield: 76.18 %; m.f.: $C_{32}H_{24}N_2$; m.p.:162-163 ºC; IR (KBr, λ_{max}, cm^{-1}): 3051.80 (Ar-H), 2998.77 (C-CH_3), 1598.70 (C=N), 1580.38 (C=C),1336.43 (C-N); ^{1}H NMR (500 MHz,$CDCl_3$) δ ppm: 2.14 (s, 3H, -CH_3), 6.76 (d, J_1 = 8.3 Hz, 2H, ArH), 6.81 (d, J_1 = 8.3 Hz, 2H, ArH), 7.20 (m, 3H, ArH), 7.25 (t, J_1 = 7.2 Hz, 6H, ArH), 7.31-7.80 (m, 7H, ArH), 8.20 (q, J_1 = 4.4 Hz, 1H, ArH); MS (ESI) *m/z*: 437.21 (M^+), calcd: 436.19.

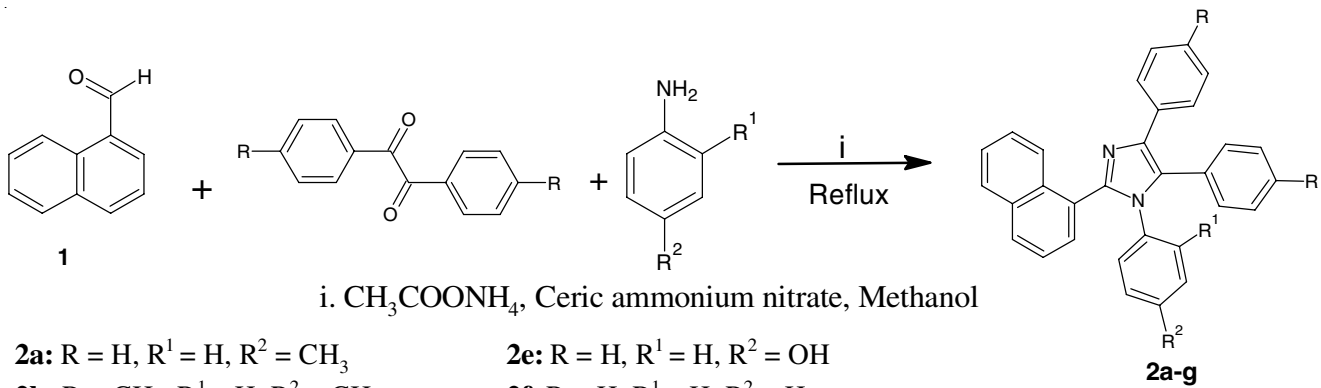
1-(4-Methylphenyl)-4,5-*bis*(4-methylphenyl)-2-(naphthalen-1-yl)-1*H*-imidazole (2b): Pale brown solid; Yield: 89.40 %; m.f.: $C_{34}H_{28}N_2$; m.p.: 216-218 ºC; IR (KBr, λ_{max}, cm^{-1}): 3044.09 (Ar-H), 2918.73 (C-CH_3), 1607.38 (C=N), 1585.20 (C=C), 1335.46 (C-N); ^{1}H NMR (500 MHz,$CDCl_3$) δ ppm: 2.16 (s, 3H, -CH_3), 2.320 (S, 3H, -CH_3), 2.328 (s, 3H, -CH_3), 6.75 (d, J_1 = 8.3 Hz, 2H, ArH), 6.82 (d, J_1 = 7.6 Hz, 2H, ArH), 7.05-7.46 (m, 10H, ArH), 7.54 (d, J_1 = 8.3 Hz, 2H, ArH), 7.81 (m, 2H, ArH), 8.20 (q, J_1 = 3.2 Hz, 1H, ArH); MS (ESI) *m/z*: 465.01 (M^+), calcd: 464.22.

1-(4-Bromophenyl)-2-(naphthalen-1-yl)-4,5-diphenyl-1*H*-imidazole (2c): Brown solid; Yield: 83.24 %; m.f.: $C_{31}H_{21}N_2Br$; m.p.: 155-157 ºC; IR (KBr, λ_{max}, cm^{-1}): 3057.58 (Ar-H),1598.70 (C=N),1556.27 (C=C), 1363.36 (C-N), 776.20 (C-Br); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 6.72-7.64 (m, 21H, ArH); MS (ESI) *m/z*: 501.13 (M^+), calcd: 500.08

1-(2-Chlorophenyl)-4,5-*bis*(4-methylphenyl)-2-(naphthalen-1-yl)-1*H*-imidazole (2d): Off white solid; Yield: 68.28 %; m.f.: $C_{33}H_{25}N_2Cl$; m.p.: 227-228 ºC; IR (KBr, λ_{max}, cm^{-1}): 3047.94 (Ar-H), 1613.16 (C=N), 1583.27 (C=C), 1336.43 (C-N), 670.14 (C-Cl); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 2.32 (s, 3H, -CH_3), 2.34 (s, 3H, -CH_3), 6.73 (td, J_1 = 5.9 Hz, J_2 = 3.4 Hz, 2H, ArH), 7.0-7.54 (m, 12H, ArH), 7.5 (d, J_1 = 8.3 Hz, 2H, ArH), 7.83 (td, J_1 = 5.3 Hz, J_2 = 3.4 Hz, 2H, ArH), 8.14 (t, J_1 = 4.8 Hz, 1H, ArH); MS (ESI) *m/z*: 485.21 (M^+), calcd:484.17.

1-(4-Hydroxyphenyl)-2-(naphthalen-1-yl)-4,5-diphenyl-1*H*-imidazole (2e): Brown solid; Yield: 80.22 %; m.f.: $C_{31}H_{22}N_2O$; m.p.: 305-308 ºC; IR (KBr, λ_{max}, cm^{-1}): 3061.15 (Ar-H), 1612.86 (C=N),1596.27 (C=C),1336.18 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 1.70 (s,1H, OH), 6.32 (t, J_1 = 10.0 Hz, 2H, ArH), 6.6 (d, J_1 = 9.0 Hz, 2H, ArH), 7.1-7.5 (m, 14H, ArH), 7.6 (d, J_1 = 7.6 Hz, 2H, ArH), 8.07 (d, J_1 = 8.3 Hz, 1H, ArH); MS (ESI) *m/z*: 439.20 (M^+), calcd:438.17.

2-(Naphthalen-1-yl)-1,4,5-triphenyl-1*H*-imidazole (2f): Off white solid; Yield: 75.81 %; m.f.: $C_{31}H_{22}N_2$; m.p.: 168-169 ºC; IR (KBr, λ_{max}, cm^{-1}): 3055.66 (Ar-H), 1621.86 (C=N), 1596.77 (C=C), 1332.57 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 6.88-7.20 (m, 8H, ArH), 7.24 (t, J_1 = 4.5 Hz, 2H, ArH), 7.27-7.47 (m, 7H, ArH), 7.6 (d, J_1 = 6.9 Hz, 2H, ArH), 7.79 (m, 2H, ArH), 8.20 (t, J_1 = 4.8 Hz, 1H, ArH); MS (ESI) *m/z*: 423.16 (M^+), calcd:422.18.



i. CH_3COONH_4, Ceric ammonium nitrate, Methanol

2a: R = H, R^1 = H, R^2 = CH_3
2b: R = CH_3, R^1 = H, R^2 = CH_3
2c: R = H, R^1 = H, R^2 = Br
2d: R = CH_3, R^1 = Cl, R^2 = H
2e: R = H, R^1 = H, R^2 = OH
2f: R = H, R^1 = H, R^2 = H
2g: R = CH_3, R^1 = H, R^2 = Br

Scheme-I: General synthesis of 1,2,4,5-tetrasubstituted imidazole analogues

1-(4-Bromophenyl)-4,5-*bis*(4-methylphenyl)-2-(naphthalen-1-yl)-1*H*-imidazole (2g): Pale brown; Yield: 79.10 %; m.f.: $C_{33}H_{25}N_2Br$; m.p: 225-228 ºC; IR (KBr, λ_{max}, cm^{-1}): 3048.91 (Ar-H), 2943.80 (C-CH_3), 1613.16 (C=N), 1588.09 (C=C), 1335.46 (C-N), 773.31 (C-Br); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 2.30 (s, 3H, -CH_3), 2.32 (s, 3H, -CH_3), 6.72-7.64 (m, 16H, ArH) 7.75 (m, 2H, ArH, 8.18 (q, 1H, ArH); MS (ESI) *m/z*: 529.18 (M^+), calcd:528.11.

Molecular docking study: *in silico* Molecular docking study was performed by previously reported methods and using receptor PDB ID: 3MNG, which obtained from RCSB Protein Data Bank [29]. The receptor structure was prepared prior to use in docking study using protein preparation module of HEX modeling package 8.0. During the protein preparation, all hetero and water molecules were removed from the crystal structure except water molecules within 5 Å from the ligand. All the molecules docked at the binding sites of the receptor structures. The 3D structure of each ligand with the receptor and binding interactions were visualized to optimization quality by discovery studio 3.2. The *in silico* molecular docking scores give useful information concerning the capability of the newly synthesized compounds to bind the active sites of the receptor. Thus the obtained docking score values guided us for performing antioxidant activity by DPPH assay method.

Free radical scavenging activity by DPPH assay method: Free radical scavenging activities of 1,2,4,5-tetrasubstituted imidazoles (**2a-g**) were determined as per the reported method [30]. Compounds have been dissolved first in DMSO and diluted with methanol to get the concentrations 50, 100, 150, 200, 500 and1000 µg/mL. Methanolic solution (0.1mM, 5 mL) of 1,1-diphenyl-2-picryl hydrazyl was added to the above stock solutions and shaken vigorously. The solutions were allowed to stand at room temperature for 20 min. The absorbance of the samples was measured at 517 nm using butylated hydroxy anisole (BHA) as a reference standard. Free radical scavenging activities were calculated using the following formula:

$$\text{Free radical scavenging (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Sample OD}} \times 100$$

RESULTS AND DISCUSSION

The synthesis of 1,2,4,5-tetrasubstituted imidazole derivatives (**2a-g**) involves four components one pot reaction of 1-naphthaldehyde, aromatic amine, benzil analogue and ammonium acetate in presence of ceric ammonium nitrate as a catalyst. Methanol was found to be good solvent for the synthesis and product yield in the range 68.28 to 89.40 % has been achieved after the isolation. The structures of the synthesized compounds were established on the basis of FT-IR, ^{1}H NMR and mass spectral data. All the title compounds showed a characteristic absorption bands in the range between 1613-1595 cm^{-1} for C=N, 1337-1332 cm^{-1} for C-N and 1598-1583 cm^{-1} for C=C stretching frequencies of imidazole ring. ^{1}H NMR spectrum supported the structures of 1,2,4,5-tetrasubstituted imidazole derivatives and exhibits a multiplet between 6.32-8.20 ppm for aromatic protons. Further, the structures were confirmed from the mass analysis in which the calculated mass values were well correlated with the observed data.

The molecular docking of the synthesized compounds (**2a-g**) was done with antioxidant receptor (PDB ID: 3MNG). The binding energies of the compounds **2a-g** with the receptors were calculated using computational docking studies with HEX 8.0 engine. These docking energy values and possible interactions are listed in Table-1. The antioxidant docking results showed that there are possible good interactions between **2a**, **2e** and **2f**. The compound **2e** showed a stronger interaction with 3MNG

TABLE-1
BINDING INTERACTION OF LIGANDS OF 3MNG WITH COMPOUNDS **2a-g**

Compounds	Docking score (Kcal/mol)	Ligand interaction			
		Hydrogen bond	π-Lonepair	π-Alkyl	Alkyl-alkyl
2a	-291.56	LEU 97, CYS 72, PHE 128, GLY38, MET130, ALA, 71, HE37, GY38	PHE 128	VAL 35, ARG 127, VAL39, LEU 73, CYS 72	VAL 35, LEU 36, MET 130
2b	-289.42	SER 74, LEU 97, ARG95, VAL70, MET 130, LEU36, VAL 71, ARG 95, CYS72	LEU 73, PHE 37	CYS72, PHE 37, ALA 71	ARG 95, LEU 36, VAL94, LEU73, LEU125, PHE 55, VAL 94, CYS 72
2c	-277.43	LEU 127, LEU 110, ARG 95, VAL 35, PHE55, VAL 70, VAL 94	CYS72, PHE37, GLY38	PHE 37, PHE128, SER 129	LEU 36, LEU110, ALA71, CYS72, VAL70
2d	-280.48	PHE 15, ALA 98, VAL 39, LEU73, CYS72, ARG 95, SER 74, LEU 97, GLY38	CYS72, PHE 37, PHE 55, ASP99, SER 74, MET 130	VAL 35, LEU 36, GLY 38 PHE 37, CYS 72, SER74	ASN 76, LYS 126, LEU110, ASN 141, SER 129
2e	-298.42	ALA 71, VAL 35, CYS 72, ARG 95, LEU 97, VAL 70, VAL 35	SER 129, LEU 36, PHE 37, LEU 125, LEU 110	SER 74, PHE 128, SER 129, LEU 36	ALA 98, PHE 37, ALA 71, CYS 72, ARG 95, SER 74
2f	-292.09	ILE 9, VAL 14, PHE 15, LEU 25, PHE 29, VAL 35, SER 74, LEU 97	LEU 36, PHE 37, GLY 38, VAL 39	CYS 47, HIS 51, PHE 55, ALA 71, CYS 72, LEU 73	LEU 125, VAL131, ARG127, PHE 128, SER 129, MET 130
2g	-288.48	ALA71, CYS 72, LEU 73, SER 74, VAL 75, TRP 84, LEU 96, LEU 97	MET30, SER 129, GLY 38, LEU 25	PHE 37, SER 129, LEU 36, ALA 71	VAL 94, SER 129, LEU 36
Standard (BHT)[a]	-183.02	–	–	–	–

receptor with lowest binding energy −298.42 kcal mol^{-1}. The compounds **2a**, **2e** and **2f** are hydrogen bonded to LEU97, CYS72, PHE128, GLY38, MET130, ALA71 and GLY 38 as shown in Fig. 1.

The antioxidant activity of the title compounds was studied by using DPPH scavenging assay method. The results of the activity of compounds **2a-g** are presented in Table-2. The antioxidant activity has been compared with reference standard BHA, compounds **2a**, **2e** and **2f** exhibit good antioxidant activity and moderate activity with respect to others.

TABLE-2
PERCENTAGE OF FREE RADICAL SCAVENGIG ACTIVITIES OF COMPOUNDS **2a-g**

Compd.	Concentration (µg/mL)					
	50	100	150	200	500	1000
2a	27.41	32.26	33.5	35.64	33.99	35.31
2b	19.34	20.25	21.15	22.72	25.84	29.38
2c	14.24	18.6	17.28	17.45	17.53	19.42
2d	24.03	20.33	20.82	21.15	21.48	20.66
2e	29.55	34.16	37.94	41.32	50.95	69.96
2f	28.56	33.58	33	33.09	34.65	36.5
2g	27.73	31.11	32.26	31.11	31.77	32, 51
BHA	27.98	42.8	60.08	95.47	100	100

Conclusion

In this study, the synthesis of 1,2,4,5-tetrasubstituted imidazoles from one-pot synthesis using 1-naphthaldehyde, aromatic

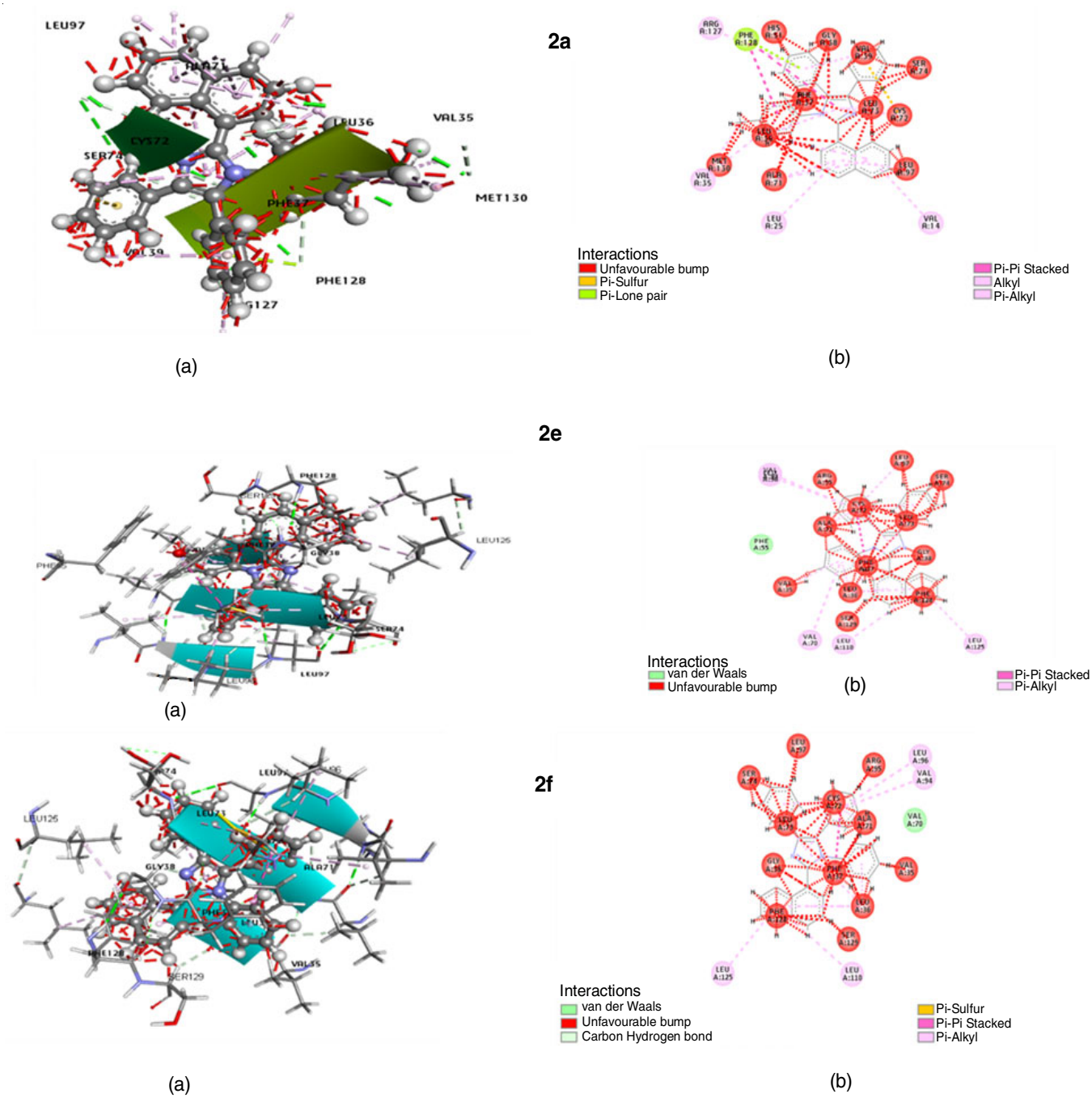


Fig. 1. (a) Three dimension and (b) Two dimension binding interaction of the compounds **2a**, **2e**, **2f** with ligands of receptor 3MNG

primary amine, 1,2-diketones in the presence of ceric ammonium nitrate as a catalyst in methanol solvent is reported. All the synthesized imidazoles are characterized by ^{1}H NMR, FTIR and mass spectra. Antioxidant activities are performed by DPPH free radical scavenging assay method and title compounds **2a**, **2e** and **2f** exhibits good and others with moderate antioxidant activity when compared with reference standard BHA.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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