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Docking, Synthesis and Evaluation of Antioxidant Activity of 2,4,5-Triaryl Imidazole

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Abstract

A series of nitrogen-containing heterocyclic compounds such as substituted 2,4,5-triaryl imidazole were synthesized by benzyl, ammonium acetate and aromatic/heteroaromatic aldehyde, evaluated for their antioxidant activity by DPPH method. Among the screened compounds, electron rich imidazole exhibited significant antioxidant activities.

Keywords: Auto dock; Synthesis; Antioxidant activity; DPPH; 2,4,5-triaryl substituted imidazole

Introduction

Nitrogen containing heterocyclic compounds especially imidazole and imidazolium compounds are indispensable structural subunit in many polycyclic natural products [1,2] and various medicinal leads [3]. The imidazole ring is present in the nucleotides adenine and guanine (Figure 1) in DNA and also in biotin (also known as Co-enzyme R), a member of the B group of vitamins. The imidazole nucleus is found in a number of important natural products such as histidine, and the purines, while 5,6-dimethyI-l-(α-D-ribofuranosyl) benzimidazole is an integral part of the structure of vitamin B12. It is also found as an entity in natural compounds, such as theophylline (Figure 1) [4] which is a stimulant found in tea and coffee. Differently substituted imidazole moieties are known to show antiedema, anti-inflammatory [5,6], antibacterial [7], antifungal [8], antihelimnitic [9,10], analgesic [11], antiviral [12,13], antitubercular [14], antihistamine, anticancer [15], activities and COX-2/LOX inhibitor.

In the view of the facts mentioned above, free radical scavenged antioxidant activity of substituted imidazole is considered relevant. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources such the regular metabolism or external sources [16,17]. The action of free radicals is counteracted by free radicals endogenous or exogenous or synthetic route. Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl, and nitric oxide radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases. Antioxidants act as a major defense against radical mediated toxicity by trapping the free radicals.

Free radical scavenging is one of the best known mechanisms and offer rapid techniques for screening the radical scavenging activity (RSA) of specific compounds. Antioxidant activity is governed by the following method such as DPPH, ORAC, ABTS, DMPD, FRAP, TRAP, TBA, superoxide radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, xanthine oxidase, cytochrome C, reducing power method, etc. The DPPH method is very common and proved as the best [18]. It is revealed from the literature that a very little attention has been given to the antioxidant activity of hetero-aromatic imidazole compound. In view of this observation synthesis and evaluation of antioxidant activity of variously substituted imidazoles are considered relevant. The free radicals and reactive oxygen species cause an phenomena called oxidative stress and that plays a decisive role in the development of various diseases, chronicle and degenerative cancer [19], atherosclerosis [20], arthritis, viral infection stroke, myocardial infarction, pulmonary condition, inflammatory bowel disease, neurogenerative disease [21] and others may be produced by reactive oxygen species, for example, hydrogen peroxide scavenging (H_2O_2); hypochlorous acid scavenging (HOCl); hydroxyl radical scavenging (HO radical); peroxyl radical scavenging (ROO radical).

Results and Discussion

Chemistry

Substituted 2,4,5-triaryl imidazoles are synthesized by three-component reaction between benzil, ammonium acetate and aromatic/heteroaromatic aldehyde at 110°C without a catalyst, in solvent free condition. The reaction is completed within 5 minutes having excellent yield. The simple work-up procedure, mild reaction conditions and good yields make this methodology ecofriendly (Scheme 1).

Docking studies of antioxidant activity

Prior to the simulations, all bound ligands, cofactors, and water molecules were removed from the proteins. The macromolecule was checked for polar hydrogen, and torsion bonds of the inhibitors were selected and defined. Gasteiger charges were computed, and the Auto Dock atom types were defined using Auto Dock version 4.2, the graphical user interface of Auto Dock supplied by MGL Tools [22]. The Lamarckian genetic algorithm (LGA), which is considered one of the best docking methods available in Auto Dock, was employed [23,24] This algorithm yields superior docking performance compared to simulated annealing or the simple genetic algorithm and the other search algorithms available in Auto Dock version 4.0. Then, the three-dimensional grid boxes were created by the Auto Grid algorithm to evaluate the binding energies on the macromolecule coordinates. The grid maps representing the intact ligand in the actual docking target site were calculated with Auto Grid (part of the Auto Dock package).

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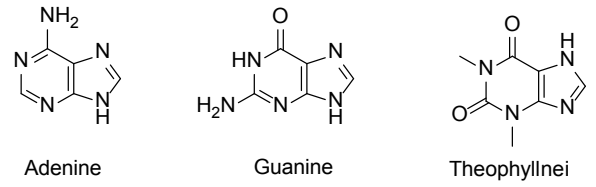
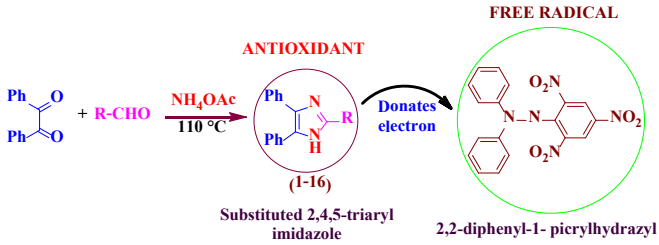


Figure 1: Adenine, Guanine and Theophylline.



Scheme 1: Synthesis of 2,4,5- trisubstituted imidazoles and their mechanism for antioxidant activity.

Eventually, cubic grids encompassed the binding site where the intact ligand was embedded.

Finally, Auto Dock was used to calculate the binding free energy of a given inhibitor in the macromolecular structure. Finally, Auto Dock was used to calculate the binding free energy of a given inhibitor conformation in the macromolecular structure. It evaluates how small molecule (substrate, inhibitor, drug or drug candidate) and the target macromolecule (receptor, enzyme or nucleic acid) fit together. This can be useful for developing better drug candidates and also for understanding the nature of the binding. Therefore molecular docking studies were carried out in order to explain *in silico* antioxidant studies, a specific protein tyrosine kinase (2HCK) [25] and peroxiredoxin 5 (1HD2) [26,27] were identified as the target for antioxidant compounds. Their PDB file was obtained from the protein data bank and used after removal of all bound water, ligands and cofactors. To investigate the ability for anti-oxidant agent, the molecular docking was first conducted with α-Tocopherol used as reference ligand. The molecular docking studies have been carried out to evaluate the binding affinity of substituted 2,4,5-triaryl imidazole (1-16) with these enzymes. The important binding interactions of the actively docked conformations of ligand with the target proteins are identified one by one all amino acids within 5 Å of the active site of the target protein. The binding interactions of all compounds have shown strong hydrogen bonding and hydrophobic interactions with the target protein. Table 1 shows the docking scores of α-Tocopherol and 1-16 compounds within the active sites of above protein. From the structure-activity relationship (SAR) perspective, straight comparison of inhibitory potency and selectivity index profiles of all compound 1-16, revealed that compound 1, 3, 6, 10 and 16 shown better binding affinity with (2HCK) protein while 1, 2, 3, 4, 10 and 16 shown better binding affinity with (1HD2) protein in comparison to α-Tocopherol (Figure 2).

Antioxidant activity by DPPH method

Antioxidant behaviour of these imidazole derivatives (1-16) is measured *in vitro* by the inhibition of generated stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the end point that is used for the determination. The DPPH solution was prepared by dissolving accurately weighed 22 mg of DPPH in 100 ml of ethanol. From this stock solution, 18 ml was diluted to 100 ml with ethanol to obtain 100 µM DPPH solutions. The sample solution was prepared by accurately weighed 2.1 mg of each of the compounds and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 2.1 mg/ml concentration and the standard solution of was prepared by accurately weighed 10.5 mg of α-Tocopherol and dissolved in 1 ml of freshly distilled DMSO to get 10.5 mg/ml concentration. A solution of test compound in ethanol (500 µl) was added to the ethanolic solution of DPPH radical. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the corresponding blank solution. The final concentration of the samples and standard α-Tocopherol solutions used is 100 µg/ml. The percentage scavenging DPPH radical inhibitions were calculated by using the following formula:

$$\text{DPPH radical scavenging activity } (\%) = [(Abs_{control} - Abs_{sample})/(Abs_{control})] \times 100$$

Where, $Abs_{control}$ was the absorbance of DPPH radical and ethanol, Abs_{sample} was the absorbance of DPPH radical and sample/standard. The scavenging activity was expressed in terms of IC50, the concentration of the samples required to give a 50% reduction in the intensity of the signal of the DPPH radical. The results were done at least in triplicate.

The structure of DPPH and its reduction by an antioxidant are shown in Scheme 2. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The molar absorptivity of the DPPH radical at 517 nm decreases when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

The free radical scavenging activity of the synthesized substituted 2,4,5-triaryl imidazole derivatives arise either from phenolic hydroxyl groups or from the imine unit of the imidazole moiety. A reactive free radical can undergo electron transfer or abstract H atom from

PDB ID	2HCK		1HD2	
Sample	Binding energy Kcal/mol	Estimated Inhibition Constant, (Ki) in µM	Binding energy Kcal/mol	Estimated Inhibition Constant, (Ki) in µM
1	-7.13	5.98	-6.05	36.45
2	-6.54	16.10	-6.13	32.15
3	-7.20	5.28	-6.00	39.73
4	-6.68	12.77	-6.21	27.98
5	-6.37	31.58	-5.49	94.09
6	-7.12	6.04	-5.60	78.77
7	-5.72	64.49	-5.54	86.72
8	-6.96	7.95	-5.70	66.77
9	-6.68	12.69	-5.65	71.94
10	-7.99	1.39	-6.74	11.53
11	-6.88	9.09	-5.77	59.03
12	-6.74	11.47	-5.92	45.77
13	-6.80	10.43	-5.77	58.58
14	-6.22	27.74	-5.45	101.28
15	-6.85	9.51	-5.40	110.96
16	-7.07	6.59	-5.95	43.57
α-Tocopherol	-5.60	78.46	-3.91	1.35 mM

Table 1: Molecular docking of new hexahydroindazole derivatives of curcumin with glucosamine-6-phosphate synthase.

either of these two sites. Recently there are some reports published in the literature supporting the two possible sites of attack viz. NH or OH by the free radicals. Our aim is to increase activity, stability and possibly to get better insight into structure-activity relationships. The antioxidant experiments testify that these compounds exhibit very good antioxidant activity in comparison to standard α-Tocopherol. The plot of % DPPH scavenging capacity against a range of concentrations for each antioxidant gave the IC50 values. IC50 values of the synthesized molecules were tested for the DPPH radical scavenging capacities and were recorded in Table 2. As it is clear from the Table 2 and Figure 3, the average scavenging effect decreased in the order 10>16>15>4>1>8>9>3>12. However the samples 5, 14 and 13 did not show radical scavenging capacities effectively as shown by the standard α-Tocopherol. Sample 10 and 16 exhibited highest scavenging capacity with IC50 value 465.5 g/ml concentration. The reason for higher radical scavenging capacity of the compound can be explained by the mechanism of radical scavenging by imidazoles. It has been reported that the presence of indole, pyrazole group adjacent to imidazole ring that can stabilize an unpaired electron in general boost up the antioxidant capacity of the molecule. But, the compounds 15, 4 and 1 have shown moderate antioxidant activity. Whereas, compounds 5, 14 and 13 do not show any significant activities.

Conclusion

From the results of *in vitro* antioxidant activity, it is concluded that these molecules can be designed as potential drugs with a slight modification in the structure of the molecules. The DPPH radical scavenging activity was undertaken to evaluate the effect of substituent on the antioxidant activities of the all synthesized compounds and shows promising activity. Among all synthesized compounds, 10 and 16 exhibited good radical scavenging activities compared to α-Tocopherol, which are also, supported by docking studies with tyrosine kinase (2HCK) and peroxiredoxin 5 (1HD2) proteins. The reason for higher antioxidant activity of compound 10 and 16 are due to presence of indole, pyrazole group adjacent to imidazole ring that can stabilize an unpaired electron in general boost up the antioxidant capacity of the molecule. There for, these molecules could be developed for antioxidant agent.

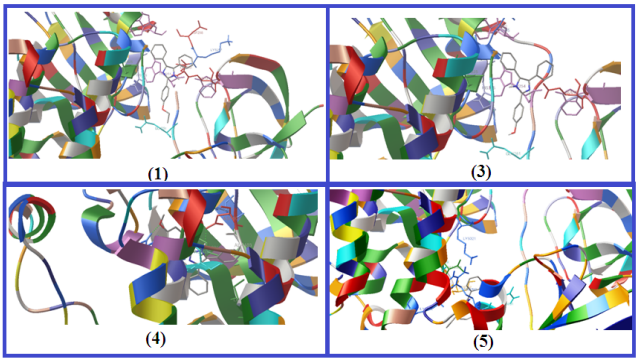
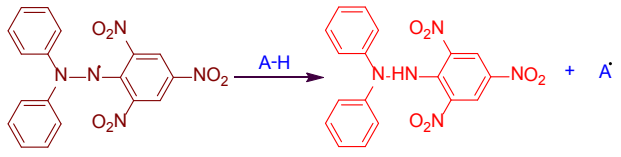
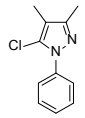


Figure 2: Best affinity mode of docked compounds (1, 3, 4 and 5) with 2HCK.



Scheme 2: Mechanism of DPPH free radical with anti-oxidant compound.

	R	0 mins	30 mins	1 hr	2 hr	Average IC_{50}
Sample 1	4-OC_2H_5-C_6H_4	1.68	2.98	4.58	6.88	4.03
Sample 2	2-OCH_3-C_6H_4	11.70	5.88	5.07	4.80	6.86
Sample 3	4-OH-C_6H_4	4.90	4.34	4.57	4.02	4.46
Sample 4	4-OCH_3-C_6H_4	2.20	4.34	4.11	4.76	3.85
Sample 5	2,6-(Cl)-C_6H_3	21.71	8.78	5.13	3.86	9.87
Sample 6	2-OH-C_6H_4	5.27	5.77	5.54	6.34	5.73
Sample 7	3-OC_2H_5-4-OH-C_6H_3	2.98	5.32	5.87	6.06	5.06
Sample 8	3-OH-C_6H_4	1.43	3.24	5.85	5.66	4.05
Sample 9	3-OC_2H_5-C_6H_4	3.04	4.18	4.77	4.25	4.06
Sample 10		2.29	2.58	3.32	3.96	3.04
Sample 11	4-OCH_2Ph-C_6H_4	9.03	7.71	5.37	3.82	6.48
Sample 12	C_6H_5	4.53	5.54	4.56	4.13	4.69
Sample 13	4-Cl-C_6H_4	9.11	6.71	6.65	5.92	7.10
Sample 14	3,4,5-$(OCH_3)_3$-C_6H_2	5.38	9.51	7.61	7.38	7.47
Sample 15	3,5-$[(CH_3)_3]_2$-4-OH-C_6H_2	3.31	3.69	3.86	4.02	3.72
Sample 16		3.14	3.42	3.33	3.21	3.28
α-Tocopherol		3.99	3.82	3.03	3.19	3.51

[a]IC_{50}=the concentration (IM) exhibiting 50% DPPH radical scavenging activity.

Table 2: IC_{50}[a] of different 2,4,5-triaryl substituted imidazoles at different concentration.

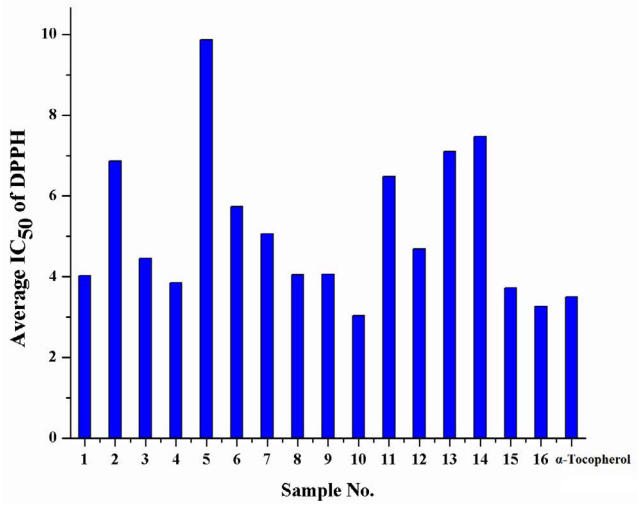


Figure 3: Effects of α-Tocopherol and the synthetic compounds 1-16 against DPPH radical.

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References

1. Williams DE, Davies J, Patrick BO, Bottriell H, Tarling T, et al. (2008) Cladoniamides A-G, tryptophan-derived alkaloids produced in culture by Streptomyces uncialis. Org Lett 10: 3501-3504.
2. Raheem IT, Thiara PS, Peterson EA, Jacobsen EN (2007) Enantioselective Pictet-Spengler-type cyclizations of hydroxylactams: H-bond donor catalysis by anion binding. J Am Chem Soc 129: 13404-13405.
3. Le Quement ST, Nielsen TE, Meldal M (2007) Scaffold diversity through intramolecular cascade reactions of solid-supported cyclic N-acyliminium intermediates. J Comb Chem 9: 1060-1072.
4. Sedgwick NV, Miller IT, Springall HD (1996) Clarendon Press, Oxford University, Oxford, England.
5. Breslin HJ, Cai C, Miskowski TA, Coutinho SV, Zhang SP, et al. (2006) Identification of potent phenyl imidazoles as opioid receptor agonists. Bioorg Med Chem Lett 16: 2505-2508.
6. Yadav MR, Puntambekar DS, Sarathy KP, Vengurlekar S, Giridhar R (2005) Quantitative structure activity relationships studies of diarylimidazoles as selective COX-2 inhibitors. Ind J Chem 45B: 475-482.
7. Matysiak J, Niewiadomy A, Krajewska-Kułak E, Macik-Niewiadomy G (2003) Synthesis of some 1-(2,4-dihydroxythiobenzoyl)imidazoles, -imidazolines and -tetrazoles and their potent activity against Candida species. Farmaco 58: 455-461.
8. Dahiya R (2008) Synthesis, Characterization and Antimicrobial Studies on Some Newer Imidazole Analogs. Sci Pharm 76: 217-239.
9. Kapoor VK, Dubey S, Mahindroo N (2000) Ind J Chem. 39B, 27.
10. Takakura Y, Kitajima M, Matsumoto S, Hashida M, Sezaki H (1987) Polymeric Materials Encyclopedia. Int J Pharm 37: 135.
11. Khabnadideh S, Rezaei Z, Khalafi-Nezhad A, Bahrinajafi R, Mohamadi R, et al. (2003) Synthesis of N-alkylated derivatives of imidazole as antibacterial agents. Bioorg Med Chem Lett 13: 2863-2865.
12. Van Tyle JH (1984) Ketoconazole. Mechanism of action, spectrum of activity, pharmacokinetics, drug interactions, adverse reactions and therapeutic use. Pharmacotherapy 4: 343-373.
13. Cheng J, Xie J, Luo X (2005) Synthesis and antiviral activity against Coxsackie virus B3 of some novel benzimidazole derivatives. Bioorg Med Chem Lett 15: 267-269.
14. Narayanan S, Vangapandu S, Jain R (2001) Regiospecific synthesis of 2,3-disubstituted-L-histidines and histamines. Bioorg Med Chem Lett 11: 1133-1136.
15. Nair SC, Panikkar B, Akamanchi KG, Panikkar KR (1991) Inhibitory effects of Ixora javanica extract on skin chemical carcinogenesis in mice and its antitumour activity. Cancer Lett 60: 253-258.
16. Navidpour L, Shadnia H, Shafaroodi H, Amini M, Dehpour AR, et al. (2007) Design, synthesis, and biological evaluation of substituted 2-alkylthio-1,5-diarylimidazoles as selective COX-2 inhibitors. Bioorg Med Chem 15: 1976-1982.
17. Suthakaran R, Kavimani S, Venkapayya P, Suganthi K (2008) synthesis and antimicrobial activity of 3 -(2-(4z) - 4 - substituted benzylidene-4,5-dihydro-5-oxo-2-phenyl imidazol-1-yl)ethyl)-6,8- un /dibromo subtituted-2-substituted quinazoline-(3h)-one. Int J Pharmco Bio Sci 1: 22-29.
18. Hossain SU, Bhattacharya S (2007) Synthesis of O-prenylated and O-geranylated derivatives of 5-benzylidene2,4-thiazolidinediones and evaluation of their free radical scavenging activity as well as effect on some phase II antioxidant/detoxifying enzymes. Bioorg Med Chem Lett 17: 1149-1154.
19. Davies KJ (1995) Oxidative stress: the paradox of aerobic life. Biochem Soc Symp 61: 1-31.
20. Ames BN (1983) Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. Science 221: 1256-1264.
21. Berliner JA, Heinecke JW (1996) The role of oxidized lipoproteins in atherogenesis. Free Radic Biol Med 20: 707-727.
22. Sanner MF (1999) Python: a programming language for software integration and development. J Mol Graph Model 17: 57-61.
23. Huey R, Morris GM, Olson AJ, Goodsell DS (2007) A semiempirical free energy force field with charge-based desolvation. J Comput Chem 28: 1145-1152.
24. Morris GM, Goodsell DS, Hallidayetal RSJ (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Comput Chem 19: 1639-1662.
25. Khalid A, Kalsoom S, Riaz N (2013) IOSR Journal of Pharmacy and Biological Sciences 5: 75.
26. Declercq JP, Christine E, Clippe AA, Stricht DV, Bernard A, et al. (2001) J Mol Biol 311: 751.
27. Soares Netto LE, de Oliveira MA, Monteiro GD, Demasi AP, Rosa Cussiol JR, et al. (2007) Comparative Biochemistry and Physiology. Part C. pp: 146-180.

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