IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2021 Vol.:22, Issue:4

Chemical Features Responsible for The Antioxidant Property in 7-Azaindoles and Related Compounds

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Ekta Singh[1], Gurubasavaraja Swamy Purawarga Matada[1]*

[1]Integrated Drug Discovery Centre, Department of Pharmaceutical Chemistry, Acharya & BM Reddy College of Pharmacy, Bengaluru 560107, Karnataka, India

Submitted: 20 October 2021
Accepted: 25 October 2021
Published: 30 November 2021

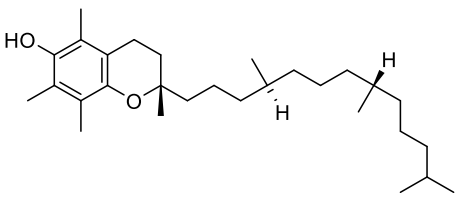
Keywords: Azaindoles, Antioxidants, Oxidative stress, Phenolic hydroxyl group, Conjugated double bond

ABSTRACT

Background: The pro-oxidant molecules are reactive towards the cell organelles due to instability in their electronic composition. Thus, pro-oxidants interact with and cause damages to cell organelles. This damage can be prevented by antioxidant molecules as they neutralize the pro-oxidants and render them harmless. The ability of molecules to act as an antioxidant is exhibited by its chemical feature. Alpha tocopherol and its analogs are well established antioxidants. The antioxidants can be designed based on the structure of alpha tocopherol. Some research articles have compared the antioxidant properties of alpha tocopherol and small molecules with different heterocylic rings. In continuation to the development of small antioxidant molecules azaindole analogs have also been compared with alpha tocopherol. Azaindoles are a novel class of compounds that can be developed as antioxidant molecules based on their similarity with heterocyclic rings present in reported antioxidants. In the current research we have studied some azaindole related compounds and their antioxidant properties. **Objective**: The objective of this study is to study some 7-azaindole related compounds as antioxidants. Materials and Methods: In this research work, small substituted azaindoles and related structures have been studied for their antioxidant properties by DPPH assay method. **Results**: The structure with Ketone and double bond functional group show antioxidant property in DPPHA assay. The presence of chlorine on aromatic ring increases the antioxidant property of azaindoles. **Conclusion**: Azaindole scaffold can be used to design and develop novel antioxidant molecules.

INTRODUCTION

Antioxidants are the molecules that can quench the electronic imbalance of the pro-oxidants and render them nonreactive [1]. Antioxidants have been classified based on their mechanism of action[2], chemical features [3], and sources [4]. These may neutralize pro-oxidants by hydrogen atom transfer, single electron transfer, sequential transfer of electrons, or chelation. The mechanism of antioxidants is dependent on their chemical features [5-7]. Chemically antioxidants are classified into phenols, naphthols, hydroquinone, aromatic amines, aminophenols, quinines, nitrones, iminoquinones, nitroxyl radicals, metals, sulfides, phosphide, thiophosphate, diamines, hydroxy acids, hydroperoxides. Biochemically these work by different mechanisms inside a cell[8]. Phenols[9], naphthols, hydroquinones, aromatic amines, and aminophenols have a proton that can be dissociated in a cellular setup. These antioxidants donate a proton and the liberated proton breaks the reactions initiated by pro-oxidants. Such hydrogen donor antioxidants can be studied for their hydrogen bond dissociation energy. This relates to their efficacy as antioxidants. Quinines[10], nitrones, and iminoquinones donate electrons to free radicals and stop the free radical chain reactions induced by pro-oxidants. Antioxidants containing sulfide[11], phosphide, and thiophosphate neutralize hydrogen peroxide. They accept a proton from hydrogen peroxide and lead to the formation of a water molecule. Diamines, hydroxyl acids[12], and dihydroxy compounds show antioxidant properties by chelating the pro-oxidants. Carotenoids[13] and conjugated olefins neutralize reactive oxygen species (free radicals such as singlet oxygen ($O^{\bullet}$), superoxide radicals ($O_2^{\bullet}$), peroxyl ($ROO^{\bullet}$) and hydroxyl ($^{\bullet}OH$)by delocalizing the electron across the double bond[14]. Alpha-tocopherol, an established antioxidant was compared with the heterocyclic ring analogs[15]. Figure no.1 shows the structure of alpha tocopherol.



(*S*)-2,5,7,8-tetramethyl-2-((4*S*,8*S*)-4,8,12-trimethyltridecyl)chroman-6-ol

Figure no. 1 Alpha tocopherol

The natural antioxidant alpha-tocopherol acts by inhibiting free radical chain reaction in lipid peroxidation. It transfers a proton to the free radicals and thereby stops the chain reaction of

lipid peroxyl radical formation. Lipid peroxyl free radicals and lipid radicals are the major contributors to cellular damage. The structural composition is depicted in Figure 2.

General components of most of the phenolic antioxidant molecules

Figure no. 2 Structural features of phenolic antioxidants

The hydrogen from the phenolic hydroxyl group is the key atom in the neutralizing pro-oxidants. Different phenolic hydroxyl group/s containing compounds such as alpha-tocopherol, **4**, **5**, **6**, and **7** inhibit chain propagation. The structures are exhibited in as shown in Figure no. 3. The ability of the phenolic group to transfer hydrogen can be considered as the efficiency of antioxidants in inhibiting chain propagation of free radicals in the biological system. This can be expressed in terms of H bond dissociation enthalpy. The antioxidants with lower O-H BDE (Bond Dissociation Energy) are generally more effective than the compounds with higher BDE. The effect of substitutes in the proximity of the phenolic OH group affects BDE. For example, electron-donating groups lower BDE as evident in structure **4** of Figure no. 3. Simultaneously these electron-rich species decrease the ionization potential of phenols and improve their stability in air. Also, the results indicate that when electron density on the ring increases it increases the O-H bond dissociation enthalpy.

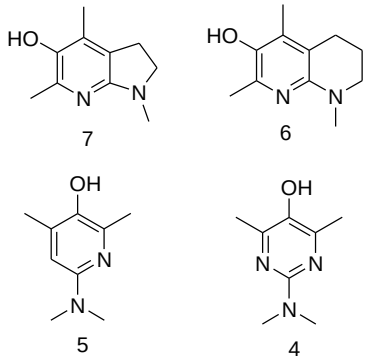


Figure no. 3 Different scaffolds studied for antioxidant effect[15]

Another study reported that the presence of pyridine ring imparts basicity to the structures[16]. The basicity of the pyridinols of Figure no.4 was correlated to their antioxidant activities.

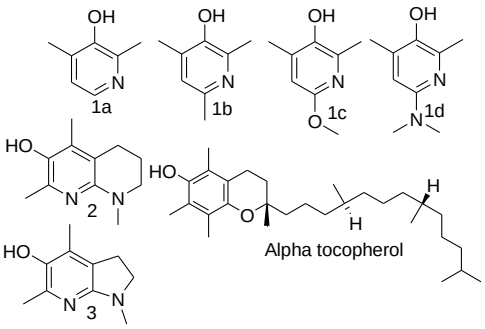


Figure no. 4 Compounds studied for their ability to inhibit autoxidation of styrene[16]

The introduction of nitrogen in the structure has been compared with ability of Pyridinols to release phenolic hydrogen. The introduction of Nitrogen increases the electron density of the rings and affects the basicity of the pyridinols. If the basicity of the compound increases, its BDE decreases. The dissociation constants of **1a**, **1b**,**1c**,**1d**, **2**, and **3** indicate that there is an increase in basicity from one ring system to a fused ring system. This increases the antioxidant activities of mono pyridinols and fused pyridinols which can b observed as an increase in Kinh activity on methyl styrene autoxidations. But the compounds **2** and **3** have decreased aerobic stability due to increased electron density on the ring. This leads to decomposition even on slight heating. Despite having less stability their Kinh was better than compound **1a**[16].

The antioxidant studies on azaindoles have been reported in a very few research works despite having a potent scaffold[15,16]. The antioxidant activity of azaindole derivatives shown in Figure no. 5 was reported by 2,2-diphenyl-1-picryl hydrazine (DPPH) assay. The oxidant scavenging potency of the azaindole derivatives followed the order **9h**>**9g**>**9c**. Compounds with the electron-withdrawing group on the benzene ring show more activity than the compounds without it. Also, carbamates derivatives are better antioxidants than sulfonamides[17].

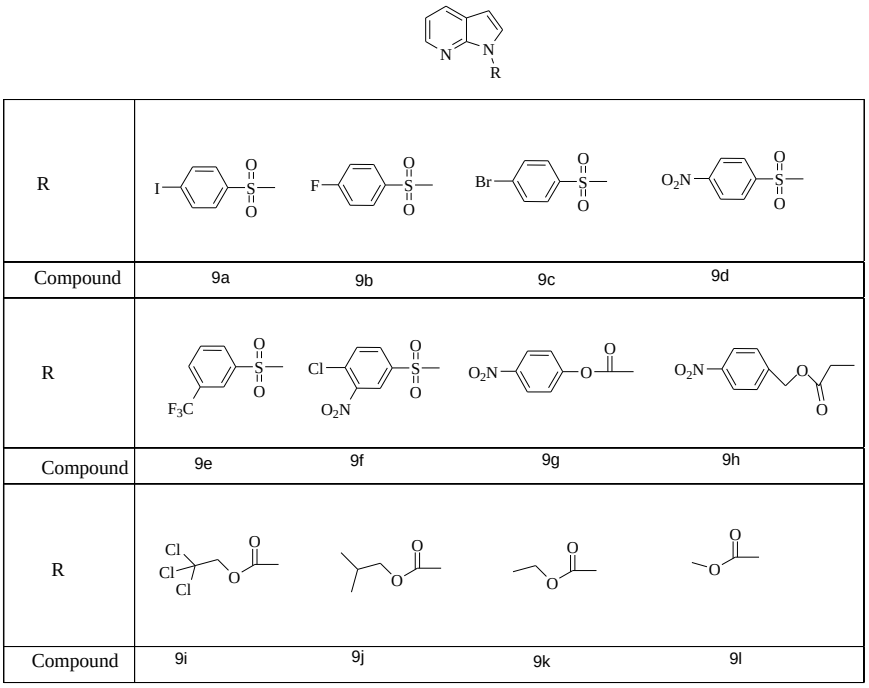


Figure no. 5 Azaindole derivatives as antioxidants[17]

Antioxidant properties of 7-Azaindoles in oxidative stress model has been reported using SH-SY5Y neuroblastoma cells[18]. These cells were subjected to oxidative stress by administration of a stressor cocktail formed by rotenone plus oligomycin A (R/O). The antioxidant property of azaindoles was studied as the protection from oxidative stress. Figure no. 6 shows the molecules studied.

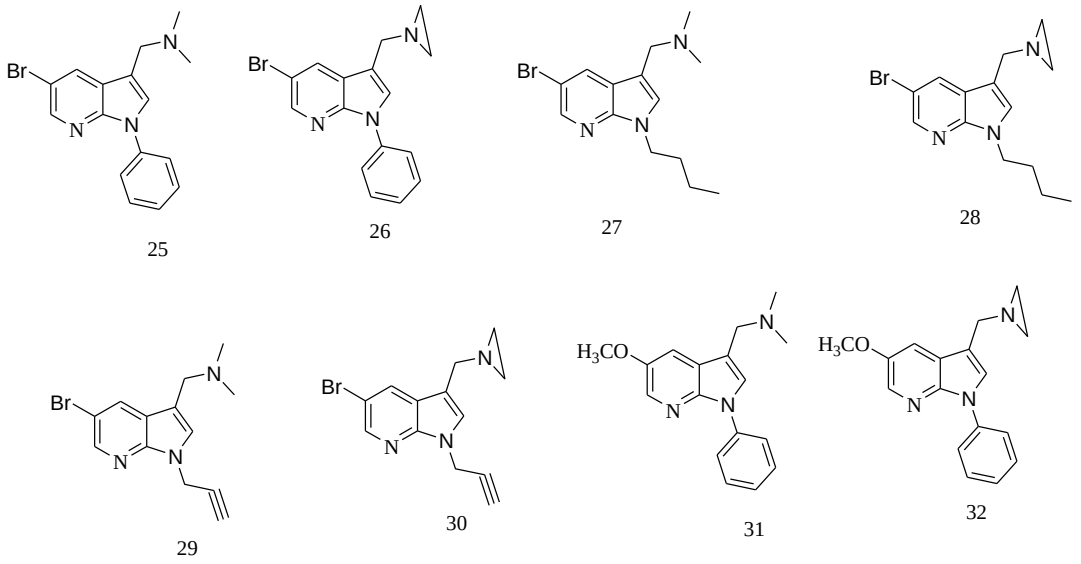


Figure no. 6 Structures studied for an antioxidant property[18]

Protection of SH-SY5Y neuroblastoma cells from oxidative stress gives a comparative antioxidant effect of these molecules. When compared to the standard drug melatonin, compounds **28** and **32** showed an improved protective effect. These structures have an N-methylene aziridine group in common apart from the azaindole scaffold. The presence of bromine and methoxy groups both are electronegative and capable of electron-withdrawing effect and donating lone pair to the resonance stabilization. Methoxy group with 1 position phenyl substitution makes the compound **32** a better antioxidant than compound **28** and melatonin.

The antioxidant properties of N-substituted derivatives of octahydro 6-azaindole has been reported in an *in-vitro* study[19]. Figure no. 7 shows tha compounds under study.

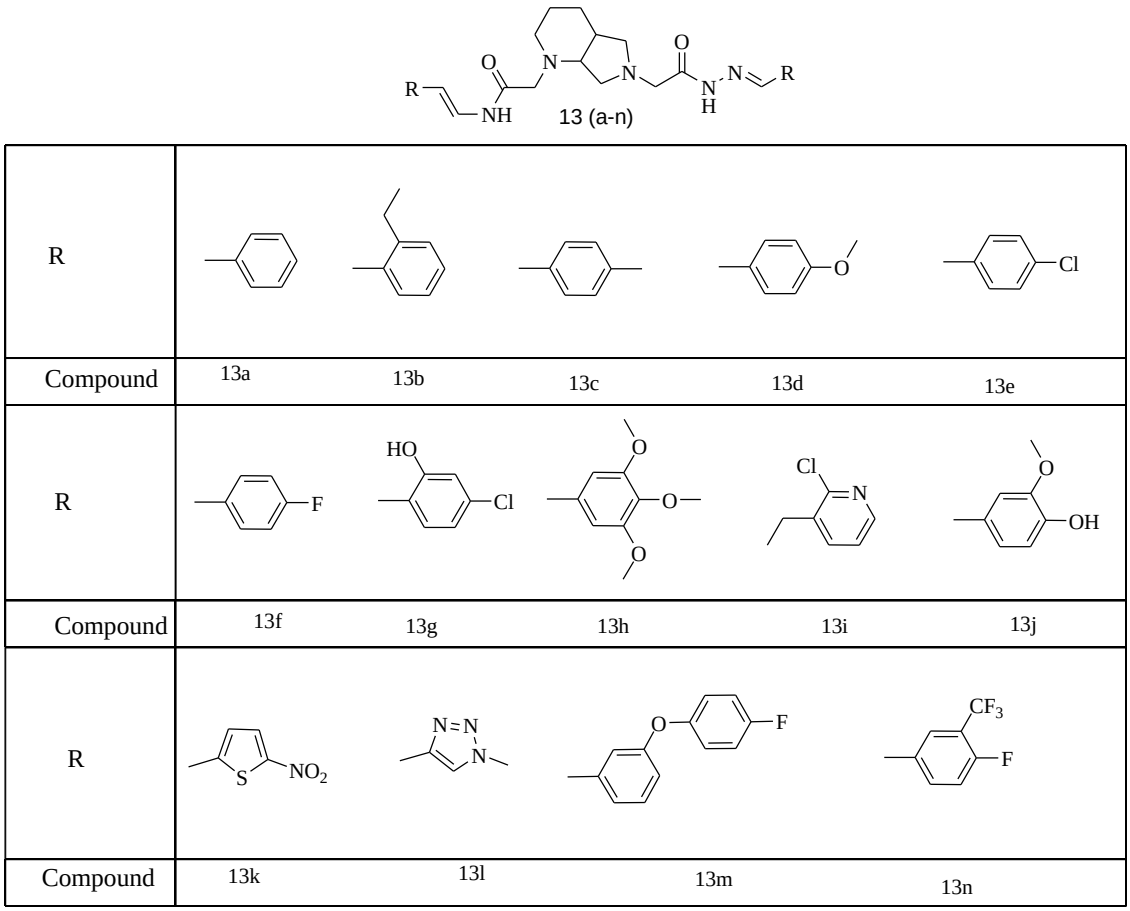


Figure no. 7 Compounds studied by Sekhar *et al* [19]

As reported the compound **13j** shows comparable antioxidant properties and it can be related to the presence of the hydroxyl group. The phenolic hydroxyl group has been reported to play a role in antioxidant activity by dissociating hydrogen. But, if the hydroxyl group is present

along with a halogen the antioxidant activity diminishes. This can be seen in **13g**. Though it has a hydroxyl group its antioxidant property is low.

The above mentioned studies provide a strong reason for exploring antioxidant properties of Azaindole related compounds. Hence, Azaindole and its analogs which have not been reported in the above mentioned studies have been analyzed for their antioxidant property in this article.

MATERIALS AND METHODS

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

DPPH assay was used to determine the free radical scavenging capacity of samples. DPPH solution (0.06% w/v) was prepared in 95% methanol with 24 hours incubation. The stock solution of samples (100mg/ml) was prepared using 95% methanol and water.100ul of freshly prepared DPPH solution and 2.5ul and 5 of the samples were pipetted out into separate test tubes. The test tubes were incubated for 30 minutes at room temperature, in the dark. The color change was measured by reading the absorbance 517nm. The experiment was performed in duplicates. The reference standard used was ascorbic acid and DPPH without any extract, was used as the control[20].Table no.1 enlists the compounds analyzed for antioxidant property as compared to ascorbic acid.

Table no. 1 Details of compounds for which DPPH Assay was performed

S.N	Code	Compound	Mol Wt
1.	a	7-Azaindole	118.17
2.	b	5-Bromo-7-azaindole	197.03
3.	c	7-Azaindole-3-carboxaldehyde	146.05
4.	d	Imidazole-2-carboxaldehyde	96.09
5.	e	5-Bromor-7-azaindole-N-sufanilamide	352.21
6.	f	3-(3,4-dimethoxyphenyl)-1-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)prop-2-en-1-one	308.33
7.	g	3-Acetyl-7-azaindole	160.17
8.	h	3-(1*H*-indol-3-yl)-1-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)prop-2-en-1-one	287.11
9.	i	3-(2-chlorophenyl)-1-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)prop-2-en-1-one	282.72

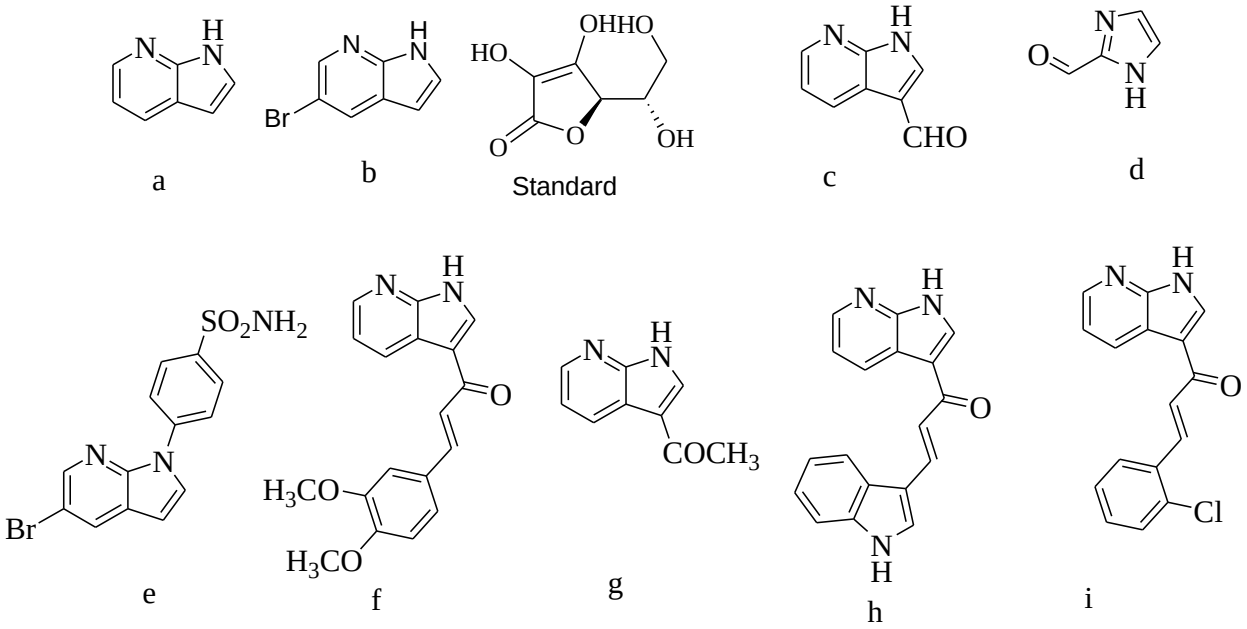


Figure no. 8 Structures of compounds for which DPPH assay was performed

RESULTS

The results of DPPH assay of 7-Azaindole and its analogs are shown in Table no. 2. Imidazole-2-carboxaldehyde was included in the library of compounds to find out whether one ring or fused ring system is better for the activity. 7-Azaindole showed a better antioxidant effect in aqueous extract than methanolic extract. Compounds with various substituents at different positions were taken for this study so that the effect of substituents can be compared.

Table no. 2 DPPH assay of compounds a-i

	Methanolic Extract				Aqueous Extract			
	Sample (250ng)		Sample (500ng)		Sample (250ng)		Sample (500ng)	
Sample	Absorbance (Avg)	% inhibition	Absorbance (Avg)	% inhibition	Absorbance (Avg)	% inhibition	Absorbance (Avg)	% inhibition
a	1.1545	42.275	1.23	38.5	0.954	52.3	0.9575	52.125
b	1.1195	44.025	0.7335	63.325	1.133	43.35	1.181	40.95
c	0.734	63.3	1.1495	42.525	1.1875	40.625	1.2435	37.825
d	1.0785	46.075	1.2355	38.225	1.3395	33.025	1.264	36.8
e	0.8565	57.175	0.9345	53.275	1.5145	24.275	1.287	35.65
f	1.129	43.55	1.3865	30.675	1.1105	44.475	0.949	52.55
g	1.1725	41.375	1.298	35.1	0.87	56.5	0.4865	75.675
h	1.3185	34.075	1.555	22.25	1.362	31.9	1.525	23.75
i	1.3945	30.275	0.7755	61.225	1.4345	28.275	0.837	58.15

DISCUSSION

Compounds **b, c, e** and showed an antioxidant effect in methanolic extract. The antioxidant effect for **c** and **e** decreased on increasing the concentration. Compounds **a, g** and **i** showed antioxidant activity in aqueous extract. The antioxidant effect of **g** and **i** increased upon increasing the concentrations. On comparing the results of aqueous and methanolic extract compounds **i** showed antioxidant activity in both solutions. This can be attributed to the ketone functional group and the double bond. These two functional groups are there in the compound as well but the presence of chlorine in the compound makes it a better antioxidant than **h**. So, the presence of an electron-withdrawing group plays an important role in the antioxidant activity.

The ability to show antioxidant properties depends on the chemical features of the compounds. Pyridinols show antioxidant activity but are less effective as an antioxidant than hydroxyl azaindoles. The fused pyridinol scaffold was compared with the heterocyclic ring of alpha-tocopherol. This information along with the effect of substituents on the antioxidant property of azaindole can be used for designing novel antioxidants. Further studies on 7-Azaindole without phenolic hydroxyl group reveal that though antioxidant property becomes

less but still can be retained by a combination of functional groups other than hydroxyl group. Derivatives of reduced 6-Azaindole show antioxidant properties due to the functional groups present. Here, the structure with hydroxyl group shows good antioxidant activity but the effect of halogen contributes adversely.

CONCLUSION

From this study, we conclude that the compound **i** shows antioxidant property in DPPH assay. The substituents on the 7-Azaindole can be modified to achieve antioxidant activity. The structures with a ketone functional group adjacent to a double bond show the antioxidant property. This may be due to tautomeric forms which cause stabilization of resonance structures. This research work encourages the scientists to endeavor in the area of developing antioxidants with different chemical features on 7-Azaindoles. Hence, this information can be used as the foundation for further ligand-based designing of novel 7-Azaindole antioxidants.

REFERENCES

1. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by an antioxidants-an overview on their chemistry and influences on health status. Eur. J. Med. Chem. 2020;112891. https://doi.org/10.1016/j.ejmech.2020.112891
2. Fischer N, Seo EJ, Efferth T. Prevention from radiation damage by natural products. Phytomedicine. 2018;47:192-200. https://doi.org/10.1016/j.phymed.2017.11.005
3. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assay. J. Agric. Food Chem. 2005;53:1841-56. https://doi.org/10.1021/jf030723c
4. Pisoschi AM, Pop A, Cimpeanu C, Predoi G. Antioxidant Capacity Determination in Plants and Plant-Derived Products: A Review. Oxid. Med. Cell Longev. 2016;2016:9130976. https://doi.org/10.1155/2016/9130976
5. Galano A, Mazzone G, Alvarez-Diduk R, Marino T, Alvarez-Idaboy JR, Russo N. Food Antioxidants: Chemical Insights at the Molecular Level. Annu Rev Food Sci Technol. 2016;7:335-52. https://doi.org/10.1146/annurev-food-041715-033206 .
6. Ivanova A, Gerasimova E, Gazizullina E. Study of Antioxidant Properties of Agents from the Perspective of Their Action Mechanisms. Molecules. 2020;25(18):4251. https://doi.org/10.3390/molecules25184251
7. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assay. J. Agri. Food Chem. 2005;53:1841-56. https://doi.org/10.1021/jf030723c
8. Gulcin İ. Antioxidants and antioxidant methods: an updated overview. Arch Toxicol. 2020;94(3):651-715. doi: 10.1007/s00204-020-02689-3. https://doi.org/10.1007/s00204-020-02689-3
9. Santos-Sánchez NF, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. Antioxidant compounds and their antioxidant mechanism. London, UK: IntechOpen; 2019. https://www.intechopen.com/chapters/66259
10. Michalík M, Poliak P, Lukeš V, Klein E. From phenols to quinones: Thermodynamics of radical scavenging activity of para-substituted phenols. Phytochemistry. 2019;166:112077. https://doi.org/10.1016/j.phytochem.2019.112077
11. Chu JW, Trout BL. On the mechanisms of oxidation of organic sulfides by H2O2 in aqueous solutions. J. Am. Chem. Soc. 2004;126(3):900-8. https://doi.org/10.1021/ja036762m

12. Chen J, Yang J, Ma L, Li J, Shahzad N, Kim CK. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. Sci. Rep. 2020;10(1):1-9. https://doi.org/10.1038/s41598-020-59451-z
13. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 2014;6(2):466-88. https://doi.org/10.3390/nu6020466
14. Flora SJ. Structural, chemical, and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxid. Med. Cell. Longev. 2009;2(4):191-206. https://doi.org/10.4161/oxim.2.4.9112
15. Wijtmans M, Pratt DA, Valgimigli L, DiLabio GA, Pedulli GF, Porter NA. 6-Amino-3-Pyridinols: Towards Diffusion-Controlled Chain-Breaking Antioxidants. Angew. Chem. Int. Ed. 2003;42(36):4370-3. https://doi.org/10.1002/anie.200351881
16. Wijtmans M, Pratt DA, Brinkhorst J, Serwa R, Valgimigli L, Pedulli GF, Porter NA. Synthesis and reactivity of some 6-substituted-2, 4-dimethyl-3-pyridinols, a novel class of chain-breaking antioxidants. J. Org. Chem. 2004;69(26):9215-23. https://doi.org/10.1021/jo048842u
17. Sudhamani H, Basha ST, Reddy SM, Sreedhar B, Adam S, Raju CN. Synthesis and evaluation of biological activities of new sulfonamide and carbamate derivatives of 1H-pyrrolo [2, 3-b] pyridine (7-azaindole). Res. Chem. Intermed. 2016;42(10):7471-86. https://link.springer.com/article/10.1007/s11164-016-2547-2
18. Lajarín-Cuesta R, Arribas RL, Nanclares C, García-Frutos EM, Gandía L, de los Ríos C. Design and synthesis of multipotent 3-aminomethylindoles and 7-azaindoles with enhanced protein phosphatase 2A-activating profile and neuroprotection. Eur. J. Med. Chem. 2018;157:294-309. https://doi.org/10.1016/j.ejmech.2018.07.030
19. Sekar S, Naik N, Mahalingappa SA, Peethamber SK. Synthesis and pharmacological evaluation of certain Schiff bases of octahydro-1H-pyrrolo [3, 4-b] pyridine derivatives. Int. J. Pharm. Pharm. Sci. 2015;7:3-10. https://innovareacademics.in/journals/index.php/ijpps/article/view/7472
20. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci. Technol. 2011;48(4):412-22. https://doi.org/10.1007/s13197-011-0251-1

Ekta Singh
Integrated Drug Discovery Centre
Department of Pharmaceutical Chemistry
Acharya & BM Reddy College of Pharmacy
Bengaluru 560 107, Karnataka, India

Author Name– Corresponding Author
Gurubasavaraja Swamy Purawarga Matada (Corresponding author)
Integrated Drug Discovery Centre
Department of Pharmaceutical Chemistry
Acharya & BM Reddy College of Pharmacy
Bengaluru 560 107, Karnataka, India