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Synthesis, molecular docking, molinspiration and anti-oxidant studies of novel N-ethylbenzimidazolylisoxazole derivatives

J Harsha & T F Abbs Fen Reji*

Department of Chemistry and Research Centre, Nesamony Memorial Christian College, Marthandam 629 165, Tamil Nadu, India

(Affiliated to Manonmaniam Sundaranar University, Tirunelveli 627 012, Tamil Nadu, India)

E-mail: abbsfen@gmail.com

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A pandemic of acute respiratory disease known as "coronavirus disease 2019" (COVID-19) has been caused by coronavirus 2 (SARS-CoV-2), a highly transmissible and pathogenic coronavirus that first appeared in late 2019. This disease poses a hazard to public health and safety. A series of novel benzimidazolylisoxazoles have been synthesized from 1,2-diaminobenzene and lactic acid. It is expected that they will exhibit a wide range of biological activities. Synthesised compounds have been characterised by FT-IR, ^{1}H NMR and mass spectroscopy. Docking studies have also been carried out by using PyRx and visualized by PyMoL software. Molecular properties and bioactivity of the compounds have been predicted using molinspiration online software. Anti-oxidant activities of the synthesised compounds have been determined using DPPH scavenging assay.

Keywords: SARS-CoV-2, Docking, Molinspiration, Anti-oxidant, DPPH

SARS-CoV-2 is a strain of coronavirus that causes COVID-19 (coronavirus disease 2019), the respiratory illness responsible for the COVID-19 pandemic. SARS-CoV-2 was transmitted easily human to human *via* respiratory droplets from coughs and sneezes within a range of about 1.8 metres (6 ft). During human-to-human transmission, between 200 to 800 infectious SARS-CoV-2 viruses are thought to initiate a new infection. The aim of this research work is to investigate the interaction between novel benzimidazolylisoxazoles with SARS-CoV-2 through molecular docking approach.

Benzimidazole is the most prevalent and significant heterocyclic compound in the field of medicinal chemistry. They play a crucial role in numerous pharmaceutical formulations that are present in a variety of drugs, the majority of vitamins and other natural items[1]. One of the major property of benzimidazole moiety was to be acts as antioxidant. The chain reaction caused by free radicals can be stopped by antioxidants. Therefore, researchers in the field of drug design and discovery must devote significant attention to the creation of efficient antioxidant agents[2]. Benzimidazole derivatives also have numerous biological activities such as antifungal[3], antimicrobial[4], antiulcer[5], anti-inflammatory[6], antitubercular[7], antiparasitic, antitumor, antihypertensive, antidiabetic[8], antibacterial[9] and also antiviral properties against picornavirus, poliovirus, enterovirus and 2-substituted benzimidazole have activity against Tobacco mosaic virus[9].

Isoxazoles are a class of heterocyclic compounds that contain a five-membered ring consisting of three carbon atoms, one oxygen atom, and one nitrogen atom. They belong to the broader category of azoles, which also includes compounds like imidazoles, pyrazoles, and oxazoles. Isoxazoles can be found naturally in various sources, including plants, fungi and marine organisms, and they also serve as important building blocks in the synthesis of pharmaceuticals and agrochemicals. Like benzimidazole is oxazoles also exhibits various biological activities such as antimicrobial[10], anti-inflammatory[11], antioxidant, anticancer, antitubercular[12], antiviral, antidepressant, anticonvulsant[13]. A compound with increased biological activity was created when two biodynamic heterocyclic systems were connected. Due to their improved biological activity, the chemistry of these connected biheterocycles has been a fascinating area of study in medicinal chemistry[14].

Experimental Section

All the reagents used were purchased from E.Merck and was purified for further use. Proton NMR analysis

were done in Bruker advance III, 400MHz NMR Spectrometer and the chemical shift values were given in δ (ppm). Mass spectral studies are also done.IR spectra were recorded using FT-IR spectrometer. Anti-oxidant studies of the prepared compounds are done by using DPPH scavenging assay.

General procedure

A mixture of corresponding chalcone (0.03mol) and hydroxylamine hydrochloride (0.02mol) was taken. Then sodium acetate in ethanol(10mL) was added and refluxed for about 6 hours. After the reaction was completed the reaction mixture was poured into ice-cold water (50mL) with continuous stirring. The precipitate obtained was collected, dried and recrystallised using ethanol.

Molecular docking studies

Docking studies of the compounds were done using AutoDock Vina in PyRx virtual screening tool. Suitable protein was downloaded from Protein Data Bank (PDB). PDB id of the protein is 7EN8. All the water molecules and the ligands were removed from the preotein and the optimized protein structure was saved as PDB file. The 2D structures of the compounds were converted to 3D using ACD\ChemSketch 1.1. From the molecular docking studies docking score and the interaction between the protein and the compound was obtained.

Anti-oxidant activity

The newly synthesised benzimidazoloylisoxazoles were tested for their anti-oxidant activity against the stable free radical (DPPH) using the DPPH assay. At a concentration of 10^{-5}M, DPPH was produced as a solution in ethanol. To 2.8 mL of DPPH solution, various concentrations (0.1, 0.25, 0.5, 0.75, 1 mM) of ethanolic solutions (0.5mL) of benzmidazoloylisoxazole derivatives were added, properly mixed, and allowed to stand in the dark for about 30 minutes. At 517 nm, the absorbance of the test samples was measured using ethanol as the reference.

$$\% \text{ inhibition} = \frac{\text{Control absorbance-Sample absorbance}}{\text{Control absorbance}} \times 100$$

Using the equation, it was possible to determine how much the concentration of DPPH radicals decreased at various test substance concentrations and was compared with standard butylated hydroxyanisole BHA. The percentage inhibition was plotted *versus* the sample concentration under test. The DPPH free radical scavenging activity (IC_{50}) was derived from the scavenging curves.

Results and Discussion

Spectral data

3-(1-Ethylbenzimidazol-2-yl)-5-phenylisoxazole, 2a: IR (KBr): 840 (N-O), 1278 (C-N), 1453 (C=C), 1687 (C=N Str), 3057 cm^{-1} (CH aromatic); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 3H, CH_3 of ethyl), 3.35 (s, 2H, CH_2 of ethyl), 4.05 (s, 1H, CH), 7.-7.40 (m, 5H, ArH), 7.50-7.78 (m, 4H, ArH); ESI-MS: *m/z* 290 [M+]. Anal. Calcd for $C_{18}H_{15}N_3O$: C, 74.72; H, 5.23; N, 14.52; O, 5.53. Found: C, 73.92; H, 5.08; N, 14.19; O, 5.05%.

3-(1-Ethylbenzimidazol-2-yl)-5-(4-chlorophenyl) isoxazole, 2b: IR (KBr): 959 (N-O), 1356 (C-N), 1480 (C=C), 1780 (C=N Str), 670 (C-Cl), 3055 cm^{-1} (CH aromatic); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 3H, CH_3 of ethyl), 3.34 (s, 2H, CH_2 of ethyl), 4.05 (s, 1H, CH), 7.20-7.40 (m, 4H, ArH), 7.50-7.72 (m, 4H, ArH); ESI-MS: *m/z* 324 [M+]. Anal. Calcd for $C_{18}H_{14}ClN_3O$: C, 66.77; H, 4.36; Cl, 10.95; N, 12.98; O, 4.94. Found: C, 66.89; H, 4.08;Cl, 10.76; N, 12.19; O, 4.65%.

3-(1-Ethylbenzimidazol-2-yl)-5-(4-methylphenyl)isoxazole, 2c: IR (KBr): 860 (N-O), 1310 (C-N), 1453 (C=C), 1687 (C=N Str), 3055 cm^{-1} (CH aromatic); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 3H, CH_3 of ethyl), 2.28 (s, 3H, CH_3), 3.33 (s, 2H, CH_2 of ethyl), 4.31 (s, 1H, CH), 7.02-7.22 (m, 4H, ArH), 7.36-7.67 (m, 4H, ArH); ESI-MS: *m/z* 304 [M+]. Anal. Calcd for $C_{19}H_{17}N_3O$: C, 75.23; H, 5.65; N, 13.85; O, 5.27. Found: C, 74.98; H, 5.09; N, 13.05; O, 5.63%.

3-(1-Ethylbenzimidazol-2-yl)-5-(4-methoxyphenyl)isoxazole, 2d: IR (KBr): 840 (N-O), 1280 (C-N), 1470 (C=C), 1700 (C=N Str), 3057 cm^{-1} (CH aromatic); ^{1}H NMR (DMSO-d_6): δ 1.26 (s, 3H, CH_3 of ethyl), 3.48 (s, 2H, CH_2 of ethyl), 3.73 (s, 3H, OCH_3), 4.98 (s, 1H, CH), 6.80 (s, 2H, ArH), 7.39 (s, 2H. ArH), 7.42-7.70 (m, 4H, ArH); ESI-MS: *m/z* 320 [M+]. Anal. Calcd for $C_{19}H_{17}N_3O_2$: C, 71.46; H, 5.37; N, 13.16; O, 10.02. Found: C, 71.86; H, 4.98; N, 13.55; O, 10.43%.

Molecular docking studies

Molecular docking studies for the synthesised compounds were carried out using AutoDock Vina in

PyRx to explore the binding affinity and interaction between the ligand and the active site of the target and Pymol software is used for visualization. SARS-CoV-2 protein (PDB code: 7EN8) is docked with the compounds. Based on the docking scores obtained, we are able to conclude that the compounds are more active against 7EN8. Higher negative values equate to more spontaneous bonding. Compound 2a (–7.9kcal/mol) has a greater anti-protein activity when compared to the other scores. This demonstrates that compound 2a interacts with the enzyme's THR 21 and THR 26 residues to produce the drug-receptor complex with the highest degree of stability. The docking scores of all the other compounds are extremely similar to compound 2a, and they all demonstrate promising SARS-CoV-2 inhibitory action. The docking scores and H-bonding between the compounds and protein are shown in Table 1. The interaction between the protein and the compound is shown in Fig. 1.

Table 1 — Docking scores of 3-(1-ethylbenzimidazol-2-yl)-5-(aryl)isoxazoles

Compd	Docking Score (Kcal/mol)	Residue involved in Hydrogen bonding
2a	–7.9	THR21, THR26
2b	–7.7	THR21, THR26, GLN69
2c	–7.8	THR21, THR26, GLN69
2d	–7.7	THR21, THR26, GLN69

Anti-oxidant activity of the synthesized compounds 2a-d

The *in vitro* anti-oxidant activity of the synthesised isoxazoles 2a-2d was evaluated using the DPPH free radical scavenging assay (Fig. 2). The % inhibition of the substances were calculated for various concentration and IC_{50} value is measured. In comparison to standard BHA all of the benzimidazoloylisoxazole derivatives (2a-2d) demonstrated moderate to powerful antioxidant activity. The IC50 values for the synthesised

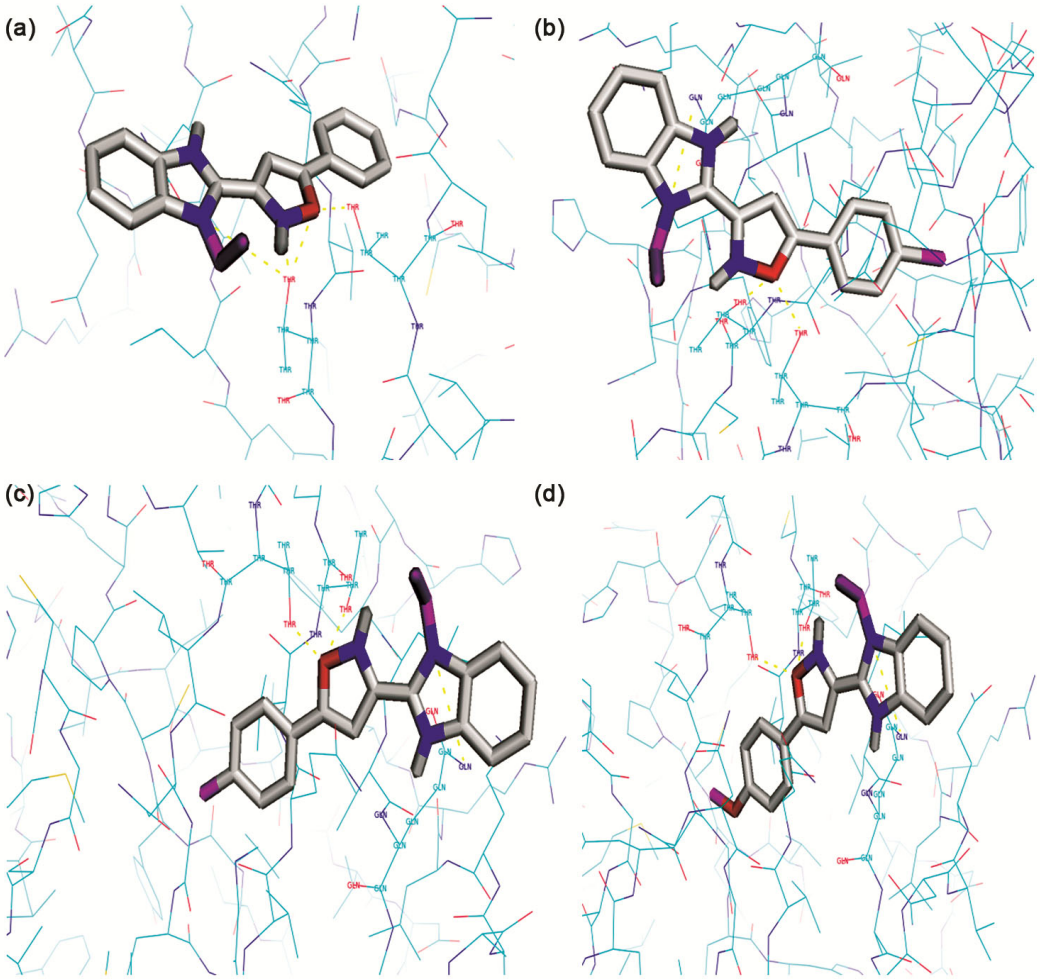

Fig. 1 — Docking images of 3-(1-ethylbenzimidazol-2-yl)-5-(aryl)isoxazoles

benzimidazoloylisoxazoles 2a, 2b, 2c, 2d and the reference butylated hydroxyanisole (BHA) were 297μM, 135μM, 351μM, 190μM, and 624 □M, respectively. When compared to the reference BHA, compound 3-(1-ethylbenzimidazol-2-yl)-5-(4-chlorophenyl)isoxazole (2b) exhibited strong inhibitory action (IC_{50} = 135 M). The least active compound is 3-(1-ethylbenzimidazol-2-yl)-5-(4-methylphenyl)isoxazole (2c) (IC_{50}=351) (Table 2).

Table 2 — Anti-oxidant activity of 3-(1-ethylbenzimidazol-2-yl)-5-(aryl)isoxazoles

Compd	IC_{50} Value (μM)
2a	297
2b	135
2c	351
2d	190

Molinspiration analysis

Molinspiration is a free online software used for the calculation of important molecular properties such as logP, polar surface area, number of hydrogen bond donors and acceptors and others, as well as prediction of bioactivity score for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors). The datas are given in Table 3 and Table 4.

Table 3 — Molinspiration molecular descriptors prediction

Compd	miLogP	TPSA	nAtoms	MW	nON	nOHNH	n violations	nrotb	volume
2a	4.27	43.86	22	289.34	4	0	0	3	262.84
2b	4.95	43.86	23	323.78	4	0	0	3	276.37
2c	4.72	43.86	23	303.37	4	0	0	3	279.40
2d	4.33	53.09	24	319.36	5	0	0	4	288.38

Table 4 — Molinspiration bioactivity scores prediction

Compd	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
2a	0.03	0.18	0.19	–0.12	–0.30	0.09
2b	0.04	0.16	0.17	–0.12	–0.31	0.05
2c	–0.00	0.09	0.14	–0.13	–0.32	0.02
2d	–0.00	0.08	0.15	–0.11	–0.29	0.03

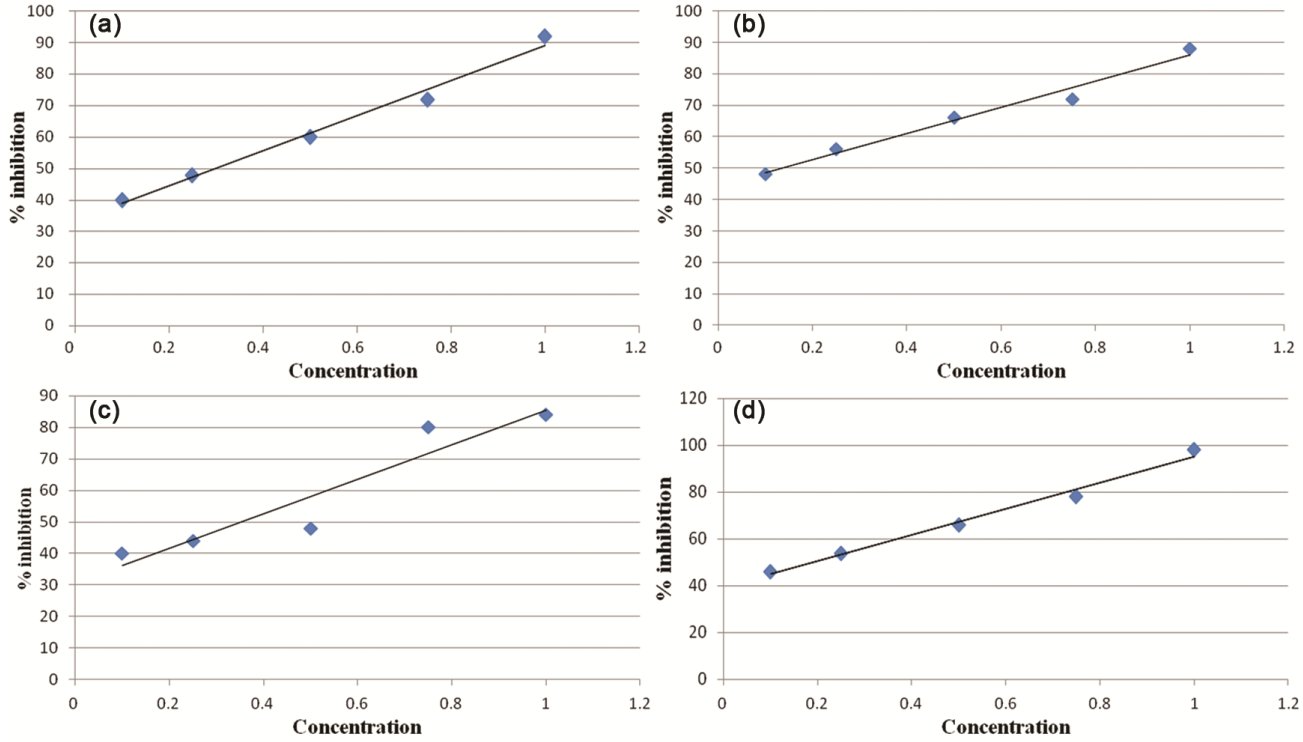

Fig. 2 — DPPH free radical scavenging activity of compounds **2a-d**

Conclusion

Novel benzimidazoloylisoxazole analogues have been explored in relation to their production, antioxidant activity and docking studies. The compounds displayed moderate to very good DPPH free radical scavenging activity. The compound 3-(1-ethylbenzimidazol-2-yl)-5-(4-chlorophenyl)isoxazole (2b) with the lowest IC_{50} value explore the very good antioxidant activity when compared to the standard BHA. To learn more about the interaction between the ligand and the protein, docking studies were also carried out. We may conclude that the compounds have more activity against SARS-CoV-2 from the docking score. Compared with all the compounds 2a has better activity against SARS-CoV-2. Molinspiration analysis also reveals the compounds molecular properties and bioactivity. According to this study, newly synthesised benzimidazoloyl isoxazoles may find widespread use as anti-oxidant and potent pharmacophore against SARS-CoV-2. From molinspiration analysis we know about the molecular properties and bioactivity of the synthesized compounds.

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