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A study on synthesis, antioxidant, anticancer activities and docking study of novel benzimidazoloyl thiazole derivatives

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2-(Substituted-amino)-5-(1-methylbenzimidazol-2-oyl)thiazoles have been synthesized by the reaction of 2-(2-bromoacetyl)-1-methylbenzimidazole and 1-(substituted-amino)-3-(N,N-dimethylimidoyl)thioureas in the presence of triethyl amine. The structure of the newly synthesized benzmidazoloyl thiazole analogues have been confirmed by spectroscopic techniques. The *in vitro* antioxidant activity has been studied using DPPH assay and anticancer activity has been studied using MTT assay against human colon adenocarcinoma cells. *In silico* studies have been performed to predict the binding modes of the compounds with the cyclin-dependent kinase protein 5FGK. It is evident from the current results that all the synthesized compounds exhibit remarkable antioxidant and anticancer potential.

Keywords: Antioxidant, DPPH, Docking, Adenocarcinoma, MTT assay

The nitrogen heterocycles of benzimidazole which are electron rich, posses broad spectrum of pharmacological activities[1]. The discovery and routine administration of drugs revolutionized the therapeutic paradigm. Unfortunately, the emergence of drug resistance threatens the therapeutic accomplishment, jeoparadizing the successful outcomes of critically ill patients. Moreover, many of today's diseases are caused due to oxidative stress that results from an imbalance between the formation and neutralization of prooxidants. This process produces free radicals which interact with the molecules within our cells resulting in damage to nearby cells.

The action of antioxidants prevents this oxidative damage. Several synthetic antioxidants such as thiols, xanthine oxidase inhibitors, iron ion chelators and probucol may be of limited relevance as antioxidants for human use and their antioxidant ability is not widespread. Hence, there is a need for development of alternative synthetic antioxidants.

Benzimidazole has drawn considerable interest as an important scaffold in drug discovery[2]. The minimal toxicity effect of benzimidazole has made it an excellent heterocyclic scaffold in anti-cancer drug development[3]. Many benzimidazolyl compounds were found to be associated with a wide range of biological activities, such as antimicrobial[3], anticancer[4], anti-ulcer[5], anti-HIV [6], anti-convulsant[7], anti-inflammatory[8], anthelmintic[9] and antiprotozoal[10] activities. Linking Benzimidazole scaffold toother hetrocyclic moieties produced improved pharmacological compounds[11]. These recent observations prompted us to synthesize 2-substituted benzmidazolyl thiazolederivativesinorder to investigate their anti-oxidant and anti-canceractivities.

Experimental Section

All the reagents and solvents used were purchased from Sigma-Aldrich Co. andwere used without further purification. Melting points were determined using Digital Program Rate melting point apparatus which were uncorrected. ^{1}H NMR spectra were recorded using Bruker Avance III, 400MHz NMR spectrometer, mass spectra on Waters UPLC - TQD mass spectrometer (ESI – MS) and IR spectra were recorded using KBr pellets in a Nicolet 400D Fourier Transform IR spectrometer (Central Drug Research Institute, Lucknow, India). The progress of the reactions were checked by thin layer chromatography (TLC) on pre-coated silica gel plates (Merck Co.) and visualized under UV light (254/366 nm).

The novel benzimidazolyl thiazole derivatives were synthesized according to Scheme 1.

Synthesis of 3,5-dimethyl-1-thiocarboxamidopyrazole, 1

Thiosemicarbazide (0.01 mol) was dissolved in hot water (35 mL). The solution was filtered to remove insoluble residues. To this hot solution, acetyl acetone (0.01 mol) was added with stirring. Concentrated hydrochloric acid (0.06 mL) was added to the mixture and stirred continuously for 2 hours at RT. The white crystalline solid obtained was filtered, washed and dried to afford **1**.

Synthesis of substituted-thioureas, 2a-d

An equimolar mixture of 3,5-dimethyl-1-thiocarboxamidopyrazole (**1**) (0.01mol) and different primary amines (0.01mol) was stirred well and kept at RT for 15 hours. The excess amine was removed under reduced pressure. The crude material (0.75g, 90%) was then purified by column chromatography (silica gel 60 – 150 mesh) using chloroform as the eluent to obtain pure colourless crystals of thioureas.

Synthesis of 1-(substituted-amino)-3-(N,N-dimethy limidoyl)thioureas, 3a-d

An equimolar mixture of N,N-dimethylformamide dimethylacetal (0.01mol) and thiourea (**2a-d**) (0.01mol) was taken in a stoppered flask and heated at 90-95°C for 2 hours. On evaporation under reduced pressure at 50-60°C, a white crystalline solid was collected by filtration and the dry residue was recrystallised using ethanol.

Synthesis of 2-(2-bromoacetyl)-1-methylbenzimida zole, 4

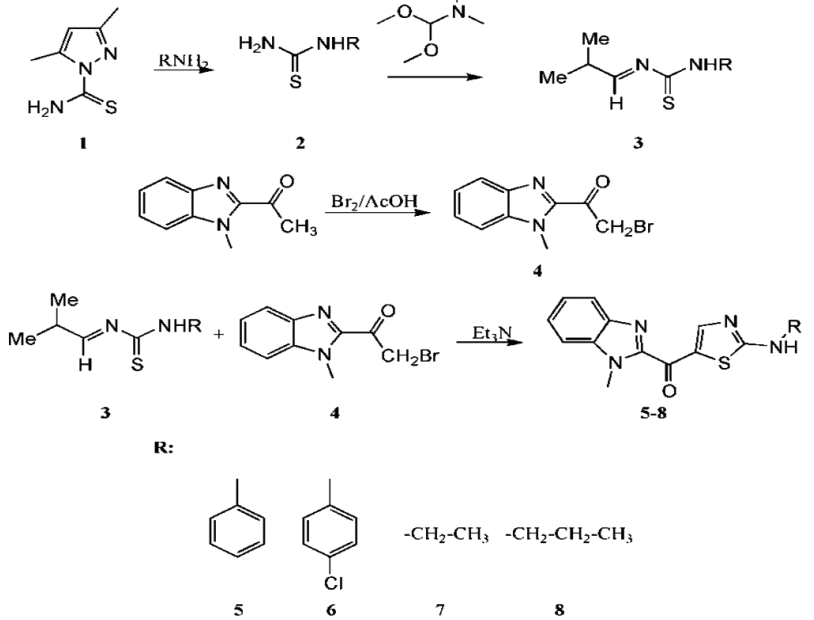
A solution of bromine (0.01mol) in acetic acid (20 mL) was added to 2-acetyl-1-methylbenzimidazole (0.01mol) with continuous stirring. The reaction mixture was stirred for two hours at 80°C. The solution is cooled and filtered. The insolubles were washed successively with acetic acid and diethyl ether. It is then suspended in water and neutralized with sodium bicarbonate solution to obtain **4**.

Synthesis of 2-alkyl/arylamino-5-(1-methylbenzimidazol-2-oyl)thiazoles, 5-8

An equimolar mixture of 1-substituted-3-(N,N-dimethylimidoyl)thiourea (**3a-d**) (0.01mol), 2-(2-bromoacetyl)-1-methylbenzimidazole (**4**) (0.01mol) in DMF (2 mL) and triethylamine (0.01mol) was heated in a water bath for 10minutes at 80-85°C. Then the reaction mixture was poured into ice-cold water. The orange yellow precipitate was filtered and recrystallised from ethanol. The purity of the compound was checked on TLC.

2-Phenylamino-5-(1-methylbenzimidazol-2-oyl) thiazole, 5

Yield 61.5%. m.p.223°C. IR (KBr): 1106 (C-S), 1686 (C=O), 1565 (C=C), 3451 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 3.55(s, 3H, N-CH_3), 6.93-7.38(m, 5H, Ar-H),



Scheme 1 — Synthesis of compounds **5-8**

7.48(s, 1H, thiazolyl), 7.53-8.44(m, 4H, Ar-H), 10.35(s, 1H, NH); LC-MS (ESI): *m/z* 335[M^+]. Anal. Calcd for $C_{18}H_{14}N_4OS$: C, 64.68; H, 4.53; N, 16.71. Found: C, 64.65; H, 4.22; N, 16.75%.

2-(4-Chlorophenylamino)-5-(1-methylbenzimidazol-2-oyl)thiazole, 6

Yield 59.9%. m.p.187°C. IR (KBr): 1106 (C-S), 773 (C-Cl), 1674 (C=O), 1608 (C=C), 3442 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 3.85 (s, 3H, N-CH_3), 7.51 (s, 1H, H-4 of thiazolyl), 7.10-7.47 (m, 5H, ArH), 7.78-8.01 (m, 4H, ArH), 10.4 (s, 1H, NH); LC-MS (ESI): *m/z* 369[M^+]. Anal. Calcd for $C_{18}H_{13}ClN_4OS$: C, 58.87; H, 3.29; N, 15.44. Found: C, 58.61; H, 3.55; N, 15.19%.

2-Ethylamino-5-(1-methylbenzimidazol-2-oyl)thiazole, 7

Yield 60.7%. m.p.204°C. IR (KBr): 1127 (C-S), 1697 (C=O), 1513 (C=C), 3406 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 0.96 (t, 7.4Hz, 3H, CH_3), 3.53 (q,7.4Hz, 2H, CH_2), 3.64 (s, 3H, N-CH_3), 4.39 (s, 1H, NH), 7.52 (s, 1H, H-4 of thiazolyl), 7.55-8.11 (m, 4H, ArH); LC-MS (ESI): *m/z* 287[M^+]. Anal. Calcd for $C_{14}H_{14}N_4OS$: C, 58.81; H, 5.09; N, 19.14. Found: C, 58.72; H, 4.93; N, 19.5%.

2-Propylamino-5-(1-methylbenzimidazol-2-oyl)thiazole, 8

Yield 70.3%. m.p.187°C. IR (KBr): 1104 (C-S), 1653 (C=O), 1574(C=C), 3406 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 1.04(t, 7.3Hz, 3H, CH_3), 1.76(m, 2H, CH_2), 3.21(t, 7.2Hz, 2H, CH_2), 3.63(s, 3H, N-CH_3), 4.53(s, 1H, NH), 7.48(s, 1H, H-4 of thiazolyl), 7.49-8.10(m, 4H, ArH); LC-MS (ESI): *m/z* 301[M^+]. Anal. Calcd for $C_{15}H_{16}N_4OS$: C, 59.67; H, 5.16; N, 18.82. Found: C, 59.98; H, 5.37; N, 18.65%.

1-(Substituted-amino)-3-(N,N-dimethylimidoyl)thiourea, 3

1-(Substituted-amino)-3-(N,N-dimethylimidoyl) thiourea **3** was synthesized by the reaction of alkyl/arylthiourea **2** with 3,5-dimethyl-1-thiocarboxamid opyrazole **1**. Reaction of thiourea **3** with 2-(2-bromoacetyl)-1-methylbenzimidazole **4** forms the 2-(substituted-amino)-5-(1-methylbenzimidazol-2-oyl)thiazoles **5-8**. The structure of the target compounds **5-8** was in accordance with spectral and elemental analysis data.

Antioxidant activity of 2-substitutedamino-5-(1-methylbenzimidazol-2-oyl)thiazoles, 5-8

DPPH assay was conducted for the evaluation of antioxidant activity of the newly synthesized benzimidazoloyl thiazoles against the stable free radical a,a-diphenyl-β-picrylhydrazyl (DPPH)[11,12]. A solution of DPPH was prepared at the concentration of 10^{-5}M in methanol. Various concentrations (50 μM, 100 μM, 250 μM, 500 μM and 750 μM) of methanolic solutions of benzmidazoloyl thiazole derivatives **5-8** (1 mL) were added to 2 mL of DPPH solution, mixed thoroughly and left to stand in darkness for 30 min. The absorbance of the test samples was read at 517 nm against methanol as the blank. The percentage reduction of the DPPH radical concentration with different concentrations of the test compounds was calculated using the equation[26,29].

$$\%\ Inhibition = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance}\ 100\% \quad (1)$$

and was compared with standard butylated hydroxyanisole BHA. The % inhibition was plotted against the concentration of the tested samples. The DPPH free radical scavenging activity was expressed as IC_{50} values, obtained from the scavenging curves.

***In vitro* anticancer activity of 2-substitutedamino-5-(1-methylbenzimidazol-2-oyl)thiazoles, 5-8**

Cell culture conditions

HT-29 (Human colon adenocarcinoma cell line) cells were cultured in 25 cm^2 tissue culture, maintained in Dulbecos modified Eagles medium (DMEM) supplemented with 10% v/v fetal bovine serum (FBS), L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100 U/mL), Amphoteracin B (2.5 μg/mL) and Streptomycin (100 μg/mL). The culture was kept at 37°C in a humidified 5% CO_2 incubator. The tripsinized two days old confluent monolayer of cells was suspended in 10% growth medium for seeding[19].

Cytotoxicity assay

The anticancer effect was measured using MTT method. A suspension of 5×10^4 cells/well was seeded in a 96-well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After incubation, the cells were treated with different concentrations (100 μg, 50 μg, 25 μg, 12.5 μg, 6.25 μg in 100 μL of 5% DMEM) of the synthesized benzimidazoloyl thiazole derivatives **5-8** and incubated for 72 h. A solution of 15 mg MTT in 3 mL PBS was prepared and added to each well, then incubated at 37°C in a humidified 5% CO_2 incubator for 4 h to evaluate cell survival. The medium in each well was then replaced

with 100 µL of DMSO, pipetted up and down to solubilise the formazan crystals. The absorbance of the wells was measured with a microplate reader at a wavelength of 570 nm. The % viability was measured using DMSO as control.

$$\% \, survival = \frac{A_{well} - A_{culture\ medium}}{A_{culture\ medium}} \times 100 \qquad (2)$$

The anticancer activity was recorded as concentration causing 50% growth inhibition (IC_{50}) of HT-29 cells[23,25].

Results and Discussion

Antioxidant activity

The synthesized thiazoles **5-8** were tested for *in vitro* antioxidant activity by subjecting to DPPH free radical scavenging assay. The percentage inhibition of the compounds was measured at various concentrations and IC_{50} values determined (Fig. 1). All the benzimidazoloyl thiazole derivatives (**5-8**) have shown moderate to potent antioxidant activity compared to the standard BHA[26,29].

The IC_{50} values were 330 µM, 51 µM, 195 µM, 292 µM and 624 µM for the synthesized benzimidazoloyl thiazoles **5**, **6**, **7**, **8** and the standard butylated hydroxyanisole (BHA) respectively. Compound 2-(4-chlorophenylamino)-5-(1-methyl-benzimidazol-2-oyl)thiazole **6** showed potent inhibitory activity when compared to the standard BHA (IC_{50} = 51 µM). 2-Phenylamino-5-(1-methylbenzi-midazol-2-oyl)thiazole **5** is the least active (IC_{50}=330µM).

In vitro cellular cytotoxicity and cell imaging

The compounds were evaluated for their anticancer potential against Human colon adenocarcinoma cell lines (HT-29) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. Different concentrations of benzimidazoloyl thiazole derivatives **5-8** were treated with HT-29 cells (Fig. 2) and the IC_{50} is found from the dose response cure. The IC_{50} of the compounds **5-8** was 35, 25, 30 and 63 µg/mL respectively in HT-29 cell.

Fig. 3 shows the percentage cytotoxicity of the newly synthesized benzimidazoloyl thiazole derivatives on HT-29 cells. All the tested compounds exhibit significant inhibitory activity. Maximum activity is exhibited by 2-(4-chlorophynylamino)-5-(1-methylbenzimidazol-2-oyl)thiazole **6** whose proliferative activity is shown in Fig. 2.

Molecular Docking Studies

In order to rationalize the biological data and to study the possible interactions of the synthesized compounds with the target, docking studies were performed on each compound using Dockthor portal which predicts the best ligand poses and binding free energy[12,14].

Kinase inhibitors are efficient candidates in cancer therapy, targeting specifically mutations leading to

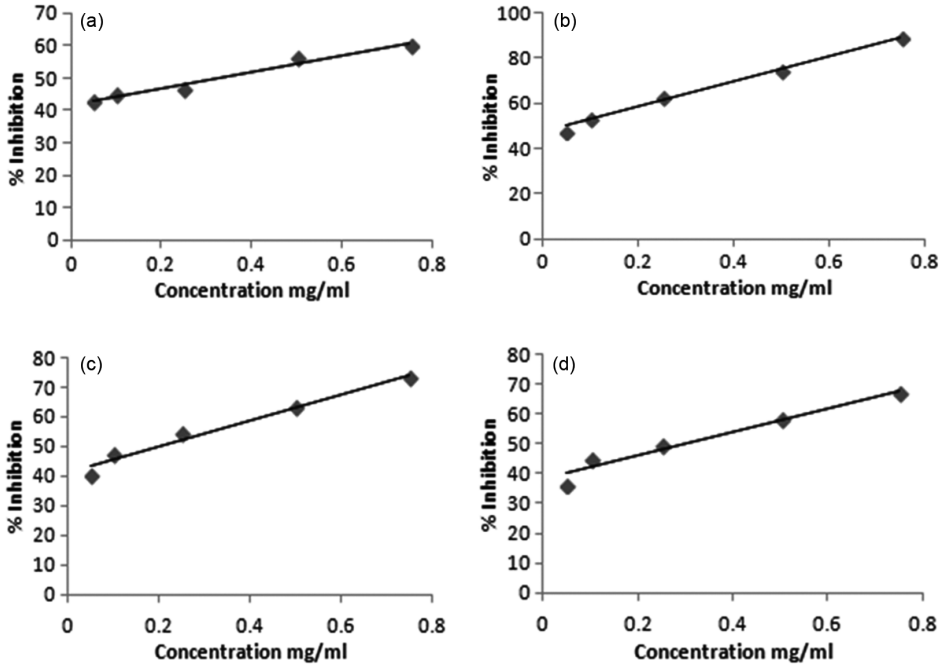


Fig. 1 — (a) DPPH free radical scavenging activity of compound **5**; (b) DPPH free radical scavenging activity of compound **6**; (c) DPPH free radical scavenging activity of compound **7**; (d) DPPH free radical scavenging activity of compound **8**.

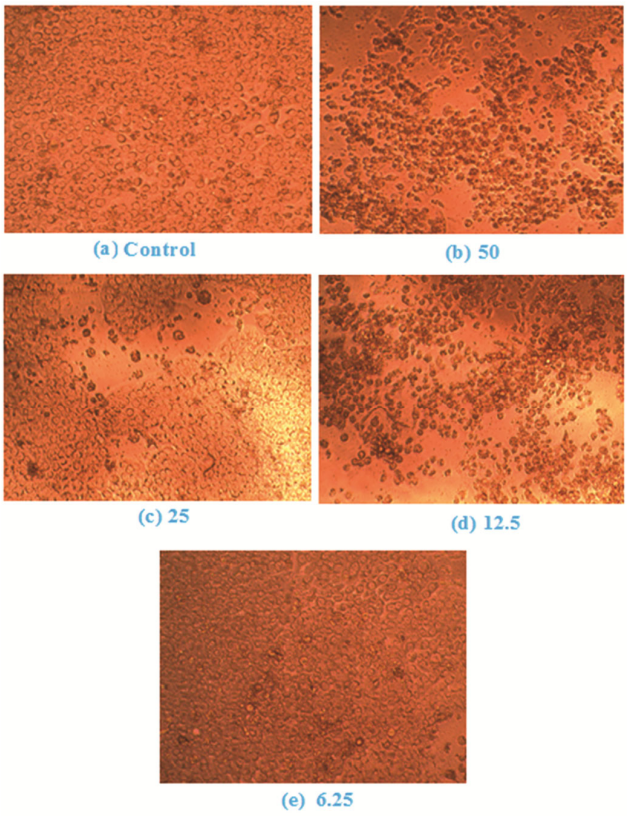


Fig. 2 — Effect of compound **6** on viability of HT – 29 cancer cells (a) Control; (b) 50 µg/mL; (c) 25 µg/mL; (d) 12.5 µg/mL; (e) 6.25 µg/mL

tumorigenesis[15]. Hence we have selected the cyclin-dependent kinase CDK-8 (PDB ID: 5FGK) as the target for carrying out docking.

The crystal structure of the selected kinase domain was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/home.home.do). PdbThorBox module of Dockthor was used to prepare the crude protein for docking by adding the lost hydrogen atoms, changing the amino acid protonation states and completing the missed side chains. The optimized target protein was then used for docking analysis. The 3D structures of the ligands **5-8** were generated using ACD/ChemSketch 2016. 1.1 and minimization of energy done using MMFF94 force field. The synthesized compounds were docked at the active site of the receptor (Fig. 4). The result of the

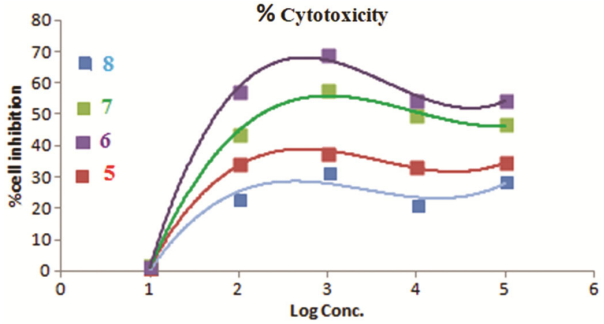


Fig. 3 — Effect of compounds **5-8** on HT-29 cells metabolic activity as estimated by MTT assay

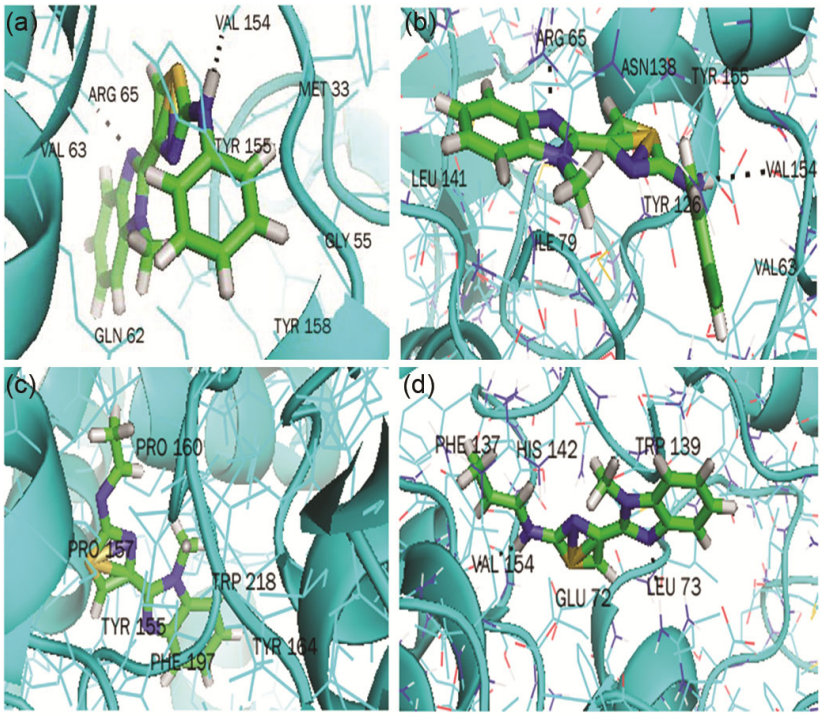


Fig. 4 — (a) Docking pose of compound **5** with 5FGK; (b) Docking pose of compound **6** with 5FGK; (c) Docking pose of compound **7** with 5FGK; (d) Docking pose of compound **8** with 5FGK

Table 1 — Molecular docking evaluations of compounds 5-8

Compd	Dock score	Hydrogen bonded residues	Vdw energy°[a] (Kcal/mol	Total energy (Kcl/mol)
5	– 7.668	VAL 154, ARG 165	–23.894	–30.783
6	–7.95	VAL 154, ARG 65	–26.646	–24.821
7	–7.894	–	–24.446	–24.821
8	–7.25	VAL 154, LEU 73	–20.335	–26.461

[a] van Der Waals energy

study was reported as CDK-8 (5FGK) binding free energy (Table 1). The higher negative values correspond to higher spontaneity of bindings[16,19].

From the docking results, the compound **6** with the *p*-chlorophenyl substituent possesses the highest negative binding energy score (–7.95kcal/mol). The lowest energy docking pose analysis of the compound **6** showed an overall binding energy of –35.11 kcal/mol, wherein the electrostatic interaction (–6.889 kcal/mol) was found to be less than the van Der Waals interaction (–23.894 kcal/mol). This reveals that the compound **6** forms the drug-receptor complex of highest stability and forms strong hydrogen bonds with ARG 65 and VAL 154 residues of the enzyme. All the other compounds also exhibit promising CDK-8 inhibitory activity with docking scores very close to compound **6**.

The biological activity has close relationship with electronic property of the compounds. The *p*-chloro and phenyl substituted compounds exhibited higher activity (**5** and **6**) than the electron donating ethyl and propyl substituents. This confirms that suitable functional groups are necessary in drug design to exhibit better biological activities[41].

Conclusion

The synthesis, antioxidant activity and anticancer activity of novel benzimidazoloyl thiazole analogs have been discussed. The compounds exhibited moderate to potent DPPH free radical scavenging activity. It is evident that the compound 2-(4-chlorophenylamino)-5-(1-methylbenzimidazol-2-oyl)thiazole **6** with the lowest IC_{50} posseses the highest antioxidant activity as compared to the standard BHA. From the MTT assay, it is clear that the compounds exhibited excellent anticancer activity against Human colon adenocarcinoma cells and ranking as 6>7>**5**>8. Further, the molecular docking of the synthesized thiazoles revealed their high affinity towards the kinase enzyme active site. This study reveals that the novel benzimidazoloyl thiazole compounds synthesized might have wide application as anti-oxidant and anticancer drugs and can be considered after confirmation by *in vivo* experiments.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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