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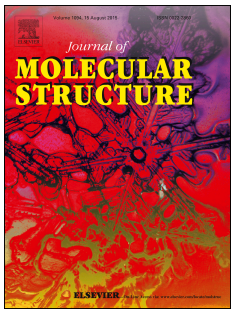

Synthesis, anticancer activity and docking studies of *N*-phenyl-2-(2-((4-phenyl piperazin-1-yl) methyl)-1*H*-benzo [*d*] imidazol-1-yl) acetamides

Lingaiah Boddu, Ashok Kumar Pagudala, Durgaiah Gandamalla, Saikrishna Balabadra, Vijjulatha Manga, Yellu Narsimha Reddy, N.J.P. Subhashini

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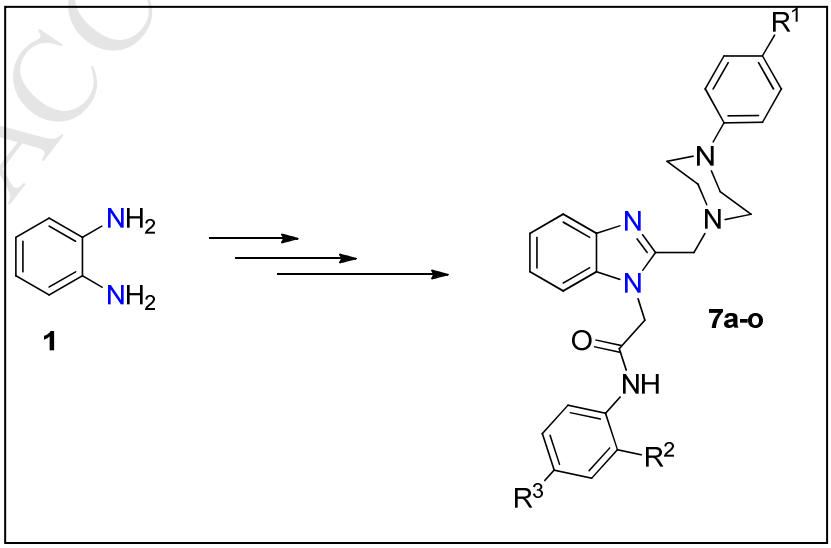
Abstract

A series of novel N-phenyl-2-(2-((4-phenyl piperazin-1-yl) methyl)-1*H*-benzo [*d*] imidazol-1-yl) acetamides (**7a-o**) have been synthesized in multiple steps with suitable reaction procedures and well characterized by various analytical techniques. All the synthesized compounds were evaluated for their in vitro anticancer activity against three human cancer cell lines includes human cervical carcinoma (HeLa), human breast carcinoma (MCF-7) and human embryonic kidney (HEK 293) cell lines at various concentrations. The results were shown in terms of percentage cell viability reduction and IC_{50}values were compared against standard anti cancer drug doxorubicin. Among all the synthesized compounds, compound **7k** has shown highest activity against HeLa and MCF-7. The compounds 7**b**, 7**l**, 7**m**, 7**n** and 7**o** also showed significant activity over HeLa and MCF-7. Furthermore, the structure and anticancer activity relationship was supported by molecular docking study of the active compounds against quinone reductase-2 (PDB ID 4ZVM) protein.



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Abstract

A series of novel N-phenyl-2-(2-((4-phenyl piperazin-1-yl) methyl)-1*H*-benzo [*d*] imidazol-1-yl) acetamides (**7a-o**) have been synthesized in multiple steps with suitable reaction procedures and well characterized by various analytical techniques. All the synthesized compounds were evaluated for their in vitro anticancer activity against three human cancer cell lines includes human cervical carcinoma (HeLa), human breast carcinoma (MCF-7) and human embryonic kidney (HEK 293) cell lines at various concentrations. The results were shown in terms of percentage cell viability reduction and IC_{50} values were compared against standard anti cancer drug doxorubicin. Among all the synthesized compounds, compound **7k** has shown highest activity against HeLa and MCF-7. The compounds **7b**, **7l**, **7m**, **7n** and **7o** also showed significant activity over HeLa and MCF-7. Furthermore, the structure and anticancer activity relationship was supported by molecular docking study of the active compounds against quinone reductase-2 (PDB ID 4ZVM) protein.

Key words: *o*-phenylene diamine, phenyl piperazines, acid-amide coupling, anticancer activity, docking.

Introduction

Cancer is a generic term used for the collection of related diseases involving uncontrolled cell growth beyond their limit, invade and spread to distinct parts of the body. There by healthy cells are destroyed and displaced by cancer cell tissues and can have severe health consequences and leading to cause death. In order to reduce the unhealthy cell growth, so many chemical agents available for various cancer types includes lung, prostate, colorectal, stomach, liver, breast and uterine cervix cancer common for human. It is one of

the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases in 2012 and the number expected raise by 2030[1]. Globally, one in six deaths due to cancer and it was responsible for 8.8 million deaths in 2015. Cancer causing infections hepatitis and human papilloma virus (HPV) are responsible up to 25% in low and middle income countries[2]. The most important risk factor for cancer is tobacco products[3] and other risk factors also include overweight or obesity, physical inactivity and consuming unhealthy diet. The primary goal is cure cancer or considerably prolongs life. In order to cure the cancer, diagnosis and identification of the stage of cancer is crucial. At initial stage, it can be cured and final stage it may not be cured but prolongs life using certain drugs. Even though many chemo therapeutic agents are exists, they fail to stop the proliferation resistance developed in them by the existing drugs. So, some other new chemo therapeutic agents are required to prevent the cell growth or destroy the cancer cell.

Benzimidazole structure is a privileged structure of heterocyclic compounds and it is essential moiety in medicinal chemistry, which shows broad spectrum of biological activity including antimicrobial[4], antiprotozoal[5], antihypertensive[6], antiulcer[7], anti diabetics[8] and anticancer[9-12]. Most of anti hypertension and antiulcer drugs were incorporated with benzimidazole moiety as a substructure and marketed under various trade names, viz. nocodazole (anticancer), candesartan, telmisartan (antihypertension), omeprazole, pantoprazole, lansoprazole, dexlansoprazole and rabeprazole (antiulcer).

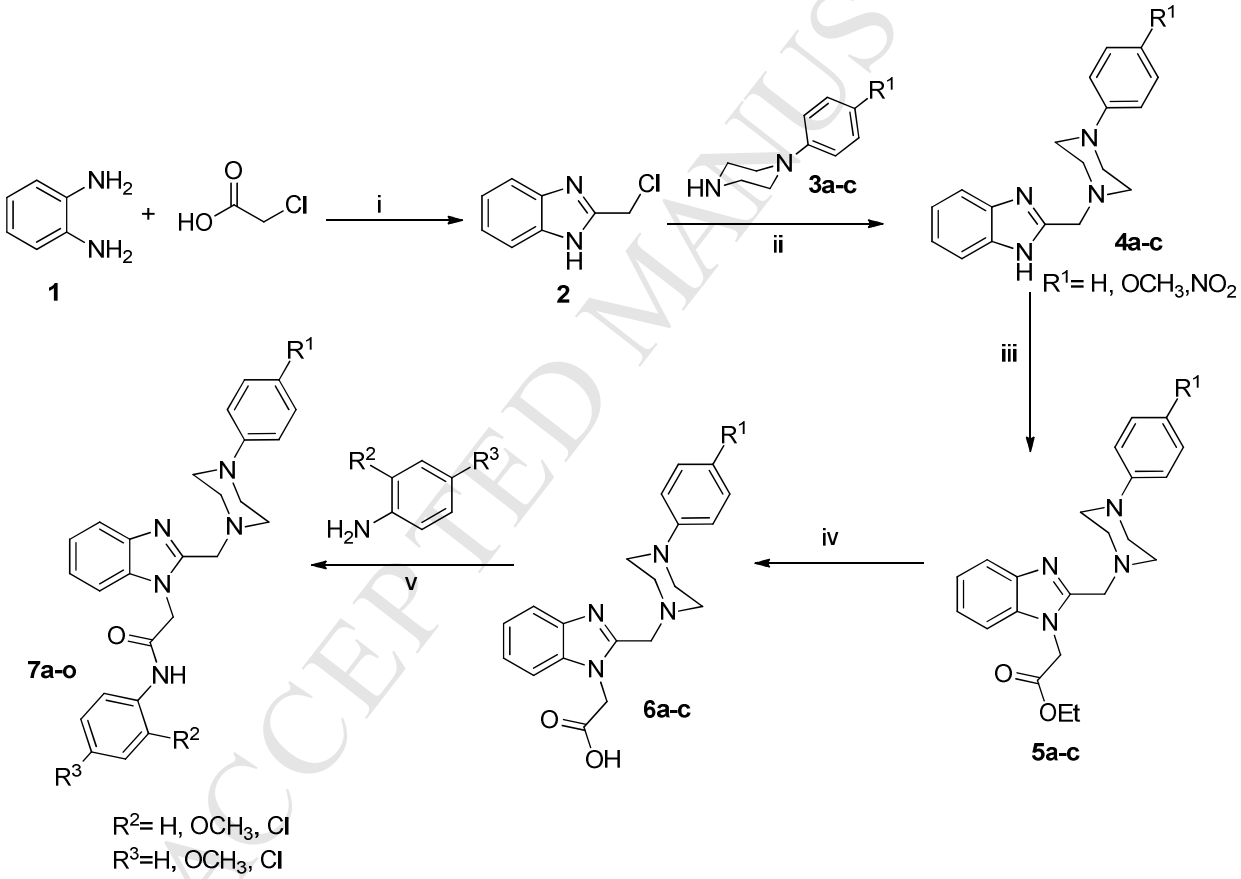
In present work, we describe the synthesis of *N*-Phenyl-2-(2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazol-1-yl) acetamides derivatives (**7a-o**) with widely utilized procedures, but benzimidazole framework was appeared in most of the drugs for numerous diseases. So, we planned to synthesize new molecules containing additional rings or side chain with functional group on benzimidazole moiety. In addition to this, all these compounds were evaluated for their in vitro anticancer activity against 3 cell lines, viz. human cervical carcinoma (HeLa), human breast carcinoma (MCF-7) and human embryonic kidney (HEK 293) cell lines was performed at various concentrations. The results of cell viability in terms of IC_{50} values were compared with the standard anticancer drug doxorubicin. Furthermore, all these compounds were explored for molecular docking studies.

Results and discussion

Chemistry

The synthetic route for the desired N-phenyl-2-(2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo [*d*] imidazol-1-yl) acetamides (**7a-o**) were summarised in scheme 1.

Benzimidazole core was constructed by commercially available *o*- phenylene diamine and chloro acetic acid in presence of 4N HCl at harsh dehydrating conditions and it forms 2-(chloromethyl)-1*H*-benzo[*d*]imidazole (**2**). N- alkylation of phenyl pipirazine (**3**) with 2-(chloromethyl)-1*H*-benzo[*d*]imidazole produced 2-((4-phenylpiperazin-1-yl)methyl)-1*H*-benzo[*d*] imidazole (**4**) in presence of sodium hydride, which on further alkylation with bromo ethyl acetate produce (2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazol-1-yl) acetate derivatives (**5a-c**), upon hydrolysis produce 2-(2-((4-phenylpiperazin-1-yl)methyl)-1*H*-benzo[*d*]imidazol-1-yl)acetic acid (**6a-c**) in presence of $LiOH.H_2O$. The titled amide compounds (**7a-o**) obtained with 2-(2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazol-1-yl) acetic acid (**6**) and corresponding amines in presence of EDC.HCl in good yields.



Scheme 1: Conditions. (i) 4N HCl, Reflux, 3-4h. (ii) Sodium hydride, 0ºC to r.t., DMF, 2h. (iii) Sodium hydride, 0ºC to r.t., Bromo ethyl acetate, 2h. (iv) $LiOH.H_2O$, THF: H_2O (4:1), r.t., 2h. (v) EDC.HCl, HoBt, TEA, r.t., 5-6h.

All the synthesized compounds were confirmed by analytical and spectroscopic data. The compound **7b** was confirmed by representative peaks in ^{1}H NMR, δ 3.44 due to methoxy protons and ^{13}C NMR, δ 164.7 due to amide carbonyl, which is not present in acid

compound (^{13}C NMR of acid, δ 170.4), and it was further confirmed by IR spectrum, which shows that, peaks at 3306 cm^{-1}(amide I) and 1667 cm^{-1}(amide II) were not coincidence with acid peaks at 3388 cm^{-1}(acid OH) and 1647 cm^{-1}(acid carbonyl). Finally it also confirmed by mass spectrum of the compound have molecular mass 455 (m/z. 456)

Anticancer activity

Synthesized novel N-Phenyl-2-(2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazol-1-yl) acetamide derivatives (**7a-o**) were evaluated for their in vitro anticancer activity against a panel of human cancer cell lines namely HeLa, MCF-7 and HEK 293 by MTT colorimetric assay as per ATCC protocol and the results were performed in Table 1 as percentage of inhibition and in terms of IC_{50} values and also presented in graphical form (Fig.1). Cell viability of all the three human cell lines was also presented separately as shown in fig.2, 3 and 4. Fig.2 demonstrates cell viability on HeLa cell lines; Fig.3 reveals the cell viability on MCF 7 and Fig.4 showed the cell viability on HEK 293 cells at various concentrations. The whole thing was presented in Fig.1. From the results, the amide compound **7k** shows highest activity against HeLa and MCF-7 among all the tested compounds. By close examination of data reveals that **7b**, **7l**, **7m**, **7n** and **7o** shown superior activity and the rest of the compounds shown moderate activity against HeLa and MCF-7. Few products shown medium activity and remaining products shows less activity towards HEK 293. So, it is indicating that these products were selectively works towards particular type of cell lines only. By analysing the results, compounds embedded nitro (NO_2) substitution shown highest activity, where as other substitutions containing compoundss shown moderate activity. Either of the groups (OCH_3, Cl) slightly reduces the activity of the unsubstituted amide compound **(7k)** compare with amides **7l**, **7m**, **7n** and **7o**.

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Table 1: Anticancer activity of synthesized compounds (**7a-o**) with cell viability (in terms of IC_{50} values)

S.No	Compound	IC_{50} Values (µM)*		
		HeLa[A]	MCF 7[B]	HEK 293[C]
1	**7a**	57.57 ± 2.04	65.48 ±3.20	57.34 ± 3.19
2	**7b**	**40.43** ± 2.39	**43.36** ±3.49	80.82 ± 3.30
3	**7c**	68.03 ± 3.20	64.88 ± 3.26	61.0 ± 3.28
4	**7d**	59.65 ± 3.19	63.97 ± 3.60	73.34 ± 3.42
5	**7e**	55.92 ± 3.50	58.86 ± 3.62	72.63 ± 3.15
6	**7f**	59.97 ± 3.73	56.82 ± 3.92	62.47 ± 3.05
7	**7g**	64.03 ± 3.28	68.38 ± 3.83	71.74± 3.29
8	**7h**	64.27 ± 3.10	56.77 ± 3.20	93.14 ± 2.70
9	**7i**	87.11 ± 3.27	95.04 ± 4.28	84.77 ± 2.14
10	**7j**	54.80 ± 3.20	71.02 ± 3.60	70.14 ± 2.59
11	**7k**	**14.05** ± 3.40	**17.64** ± 3.29	90.89 ± 3.82
12	**7l**	**26.40** ± 3.21	**22.86** ± 3.25	**46.38** ± 3.71
13	**7m**	**22.39** ± 2.37	**25.02** ± 2.14	64.0 ± 3.20
14	**7n**	**25.35** ± 2.05	**24.71** ± 2.30	**52.51** ± 3.60
15	**7o**	**28.95** ± 2.40	**26.59** ± 3.19	**50.78** ± 3.91
	Doxorubicin	3.56 ± 3.17	4.94 ± 3.28	65.61 ± 2.30

* Values are expresses as mean±SD (n=4).

1. HeLa: Human cervical cancer cells
2. MCF-7: Human Breast cancer cells
3. HEK 293: Human embryonic kidney cells

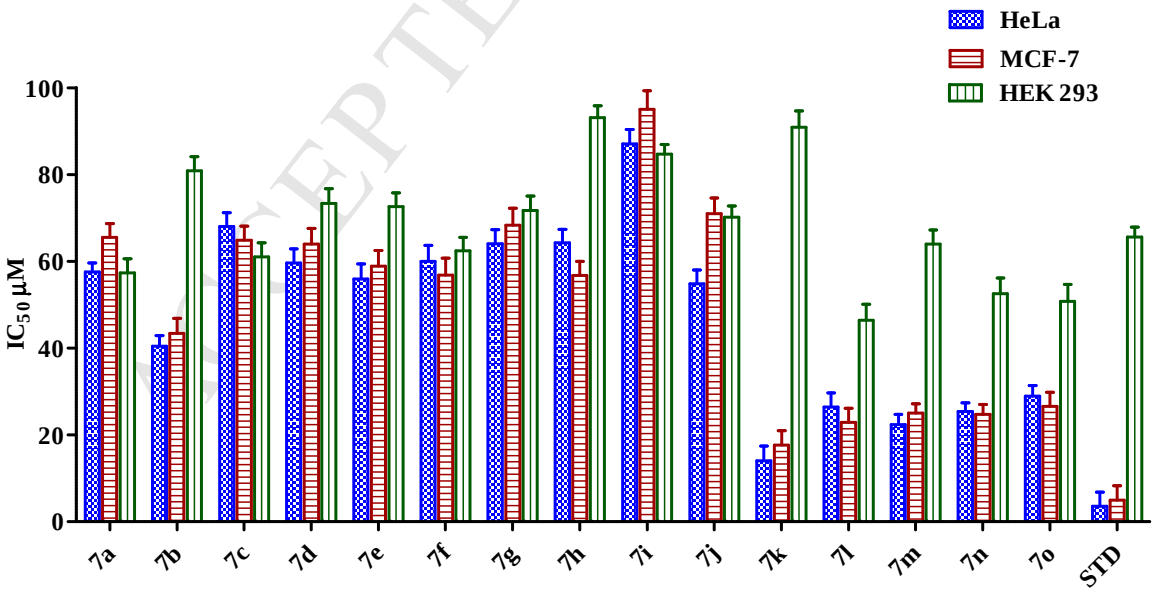

Fig. 1: Percentage Cell viability reduction, IC_{50} Values (µM) of compounds (**7a-o**) on three human cell lines

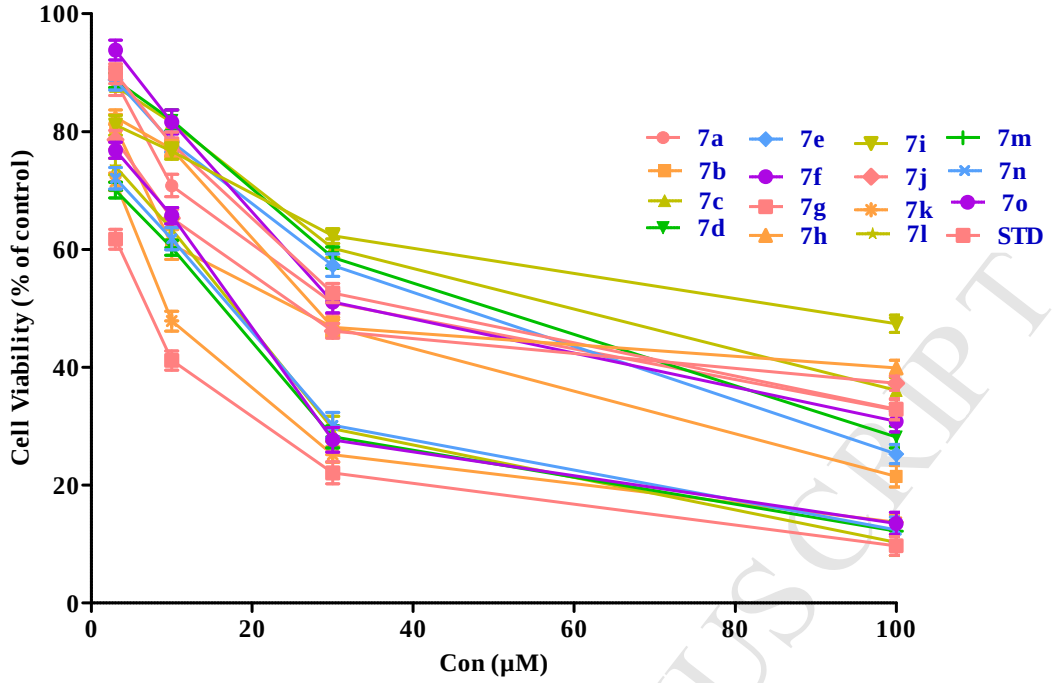

Fig. 2: Anticancer activity of compounds (**7a-o**) measured by MTT assay on Human Cervical Cancer (HeLa) with concentrations (1 - 100 µM) for 48 h. Data as mean ± standard deviation (S.D.) n = 4. Stastical analysis was performed using one-way ANOVA followed by Bonferroni post-hoc test. *$p<0.05$, $^{\$}p<0.01$, $^{\#}p<0.001$versus Standard; $^{a}p<0.001$versus Control.

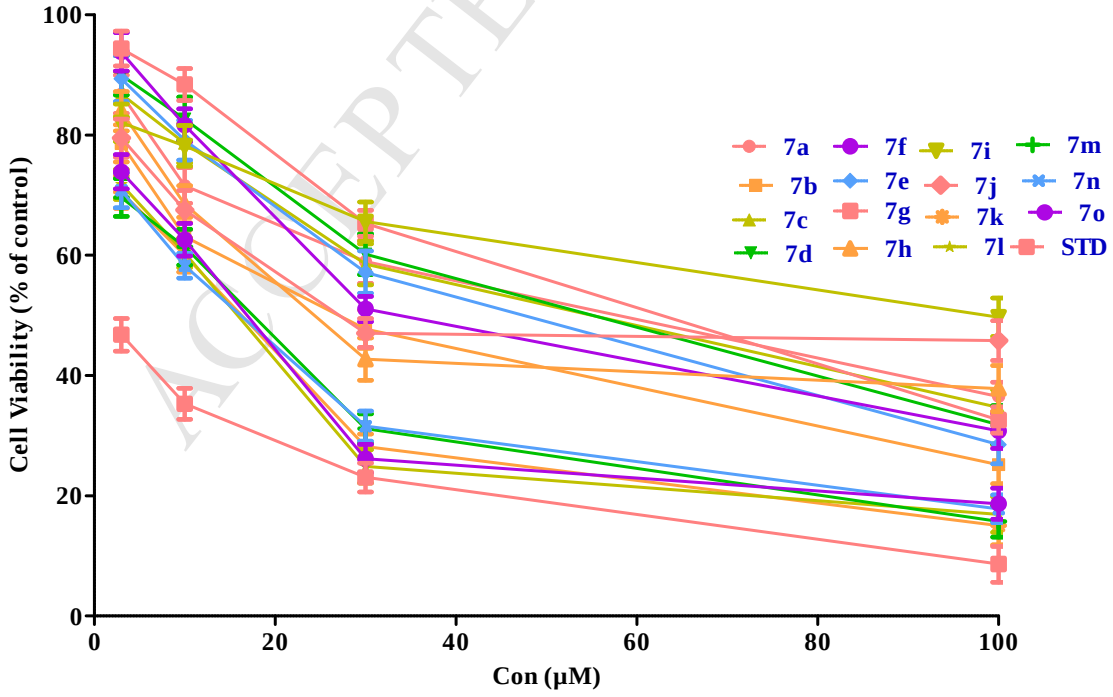

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Fig. 3: Anticancer activity of compounds (**7a-o**) measured by MTT assay on Human breast cancer cells with concentrations (1 - 100 µM) for 48 h. Data as mean ± standard deviation (S.D.) n = 4. Stastical analysis was performed using one-way ANOVA followed by Bonferroni post-hoc test. *p<0.05, $p <0.01, #p<0.001versus Standard; ap<0.001 versus Control

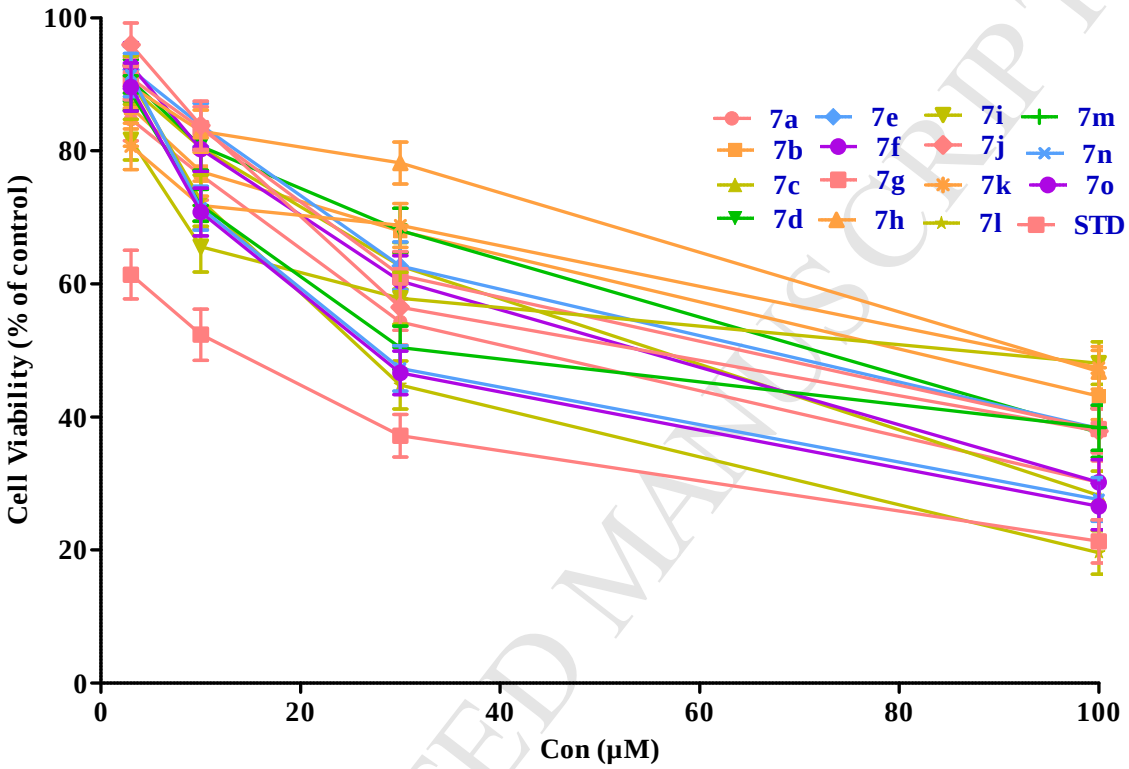

Fig. 4: Anticancer activity of compounds (**7a-o**) measured by MTT assay on Human kidney cells (HEK 293) with concentrations (1 - 100 µM) for 48 h. Data as mean ± standard deviation (S.D.) n = 4. Stastical analysis was performed using one-way ANOVA followed by Bonferroni post-hoc test. *p<0.05, $p <0.01, #p<0.001versus Standard; ap<0.001 versus Control.

Docking Studies

GLIDE 5.6[13] was used for molecular docking studies. Protein quinone reductase-2 (PDB ID 4ZVM) [14-15] was prepared using protein preparation wizard in Maestro 9.0 applying default parameters; a grid was generated around the active site by selecting the co-crystallized ligand. Receptor van der Waals scaling for non polar atoms was kept at 0.9. Molecules were built using Maestro build panel and prepared by LigPrep 2.0 application. Low energy conformation of the ligands were selected and docked into the grid generated for the protein using an extra precision (XP) docking mode. Dock pose of each ligand was

analyzed for interactions with the receptor. From the docking studies, it was observed that the standard Doxorubicin showed hydrogen bond interactions with Leu 103, Gly 149, Gly 150, Tyr 155 and Glu 193 and one hydrophobic/π-π stacking interaction with Tyr 104 residues. The best active compounds **7k** showed hydrogen bond interactions with Leu 103 (1.635 Å bond length), Gly 149 (2.364 Å) and Asn 161(1.92 Å) and **7m** showed hydrogen bond interactions with Leu 103 (1.732 Å), Gly 149 (2.121 Å), Tyr 155 (2.461 Å) and Asn 161 (2.429 Å) residues. Figure A, B, C represents the docked poses with ligand interaction diagrams of **7k**, **7m** and Doxorubicin molecules in the target 4ZVM active site. The dock scores, Emodels and Glide energies were given in Table 2.

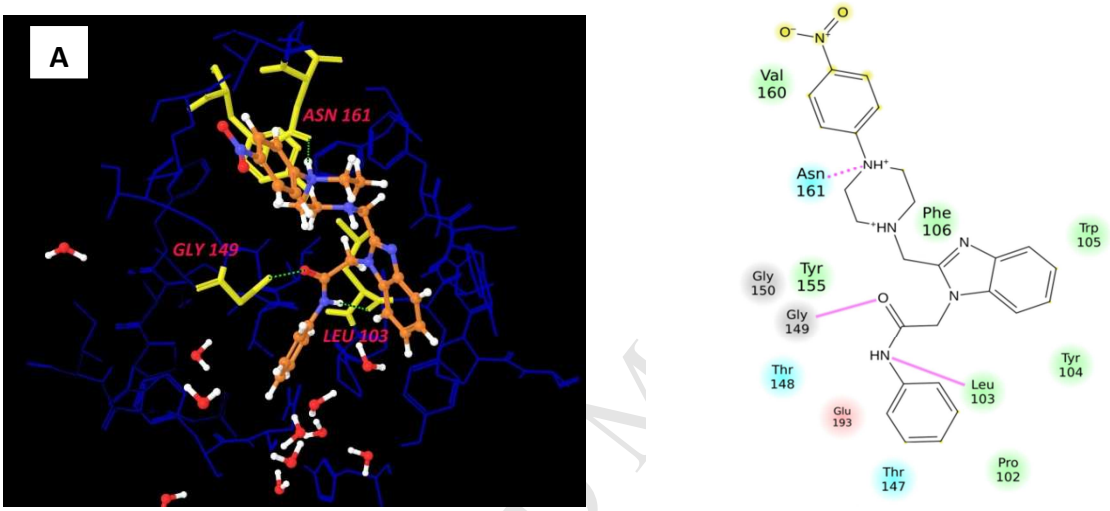

Fig. A: Ligand interaction diagram of **7k** with active site of PDB ID 4ZVM (Protein Quinone reductase-2)

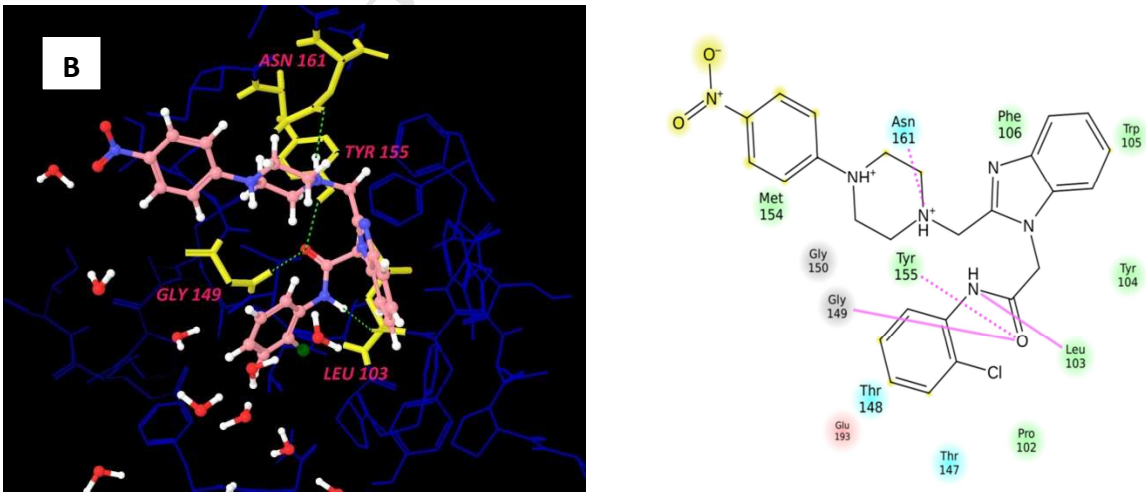

Fig. B: Ligand interaction diagram of **7m** with active site of PDB ID 4ZVM (Protein Quinone reductase-2)

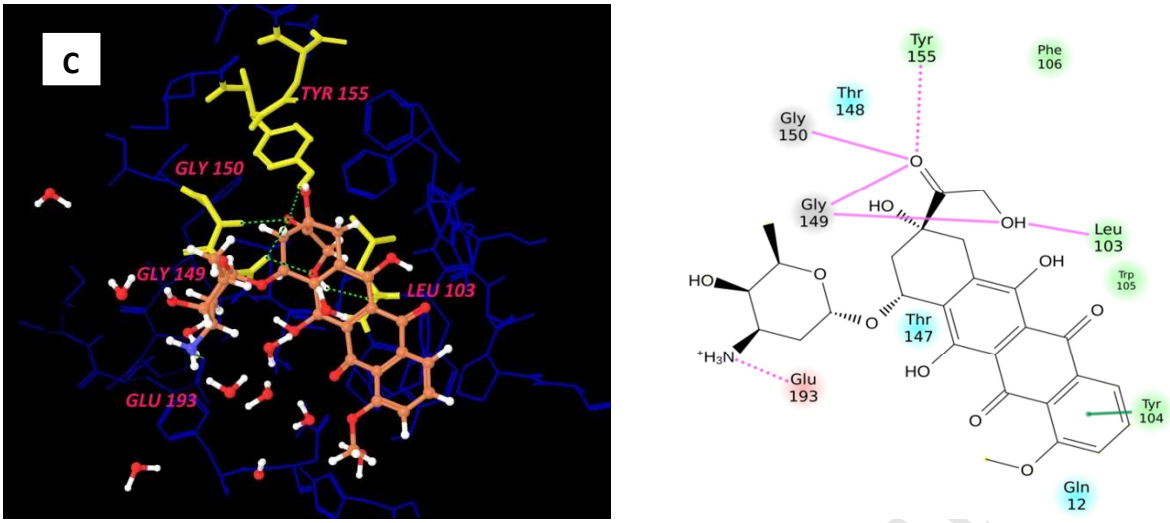

Fig. C: Ligand interaction diagram of Doxorubicin with active site of PDB ID 4ZVM (Protein Quinone reductase-2)

From the docking results we have observed that the most potent compounds **7k** and **7m** showed common hydrogen bond interactions (Leu 103, Gly 149, Tyr 155) as compared to Doxorubicin which is in agreement with experimental anticancer activities. The other synthesized compounds also showed good and comparable binding interactions with target protein.

Table 2. Glide scores, Glide energies and Glide emodels of synthesized compounds

Compound	GScore (XP)	glide energy	glide emodel
7a	-3.67	-36.1	-46.78
7b	-3.71	-37.45	-50.1
7c	-3.67	-33.44	-48.8
7d	-3.82	-37.49	-53.45
7e	-1.92	-37.45	-49.02
7f	-3.6	-33.06	-48.36
7g	-3.76	-35.33	-49.97
7h	-3.53	-36.05	-47.6
7i	-4.15	-33.11	-44.15
7j	-3.98	-34.12	-40.08
7k	**-4.08**	**-40.68**	**-52.68**
7l	-3.82	-38.6	-53
7m	**-4.31**	**-41.41**	**-57.28**
7n	-3.62	-35.56	-40.99
7o	-3.53	-38.62	-50.82
Doxorubicin	**-7.94**	**-45.28**	**-64.1**

ADME Calculations

ADME (Drug like) properties of all synthesized ligands were checked using QikProp4.0. ADME explains about Absorption, Distribution, Metabolism and Excretion of drug candidates. These are considered as drug like properties need to fulfil by the drug molecule to pass through phase-I clinical trials. QikProp helps in analyzing pharmacokinetics and pharmacodynamics of the ligand by accessing drug like properties. It predicts both physically significant descriptors and pharmaceutically relevant properties. It also evaluates an acceptability of the analogues based on the Lipinski's rule of 5 (number of violations of Lipinski's rule of five) that is essential for rational drug design. Poor absorption or permeation are more likely when a ligand molecule violates Lipinski's rule of five i.e., more than 5 hydrogen bond donors, the molecular weight over 750, the log P over 5 and the sum of N's and O's over 10. The calculated values for the synthesized molecules ranged within the acceptable limits, indicating that the compounds may be considered for further development (Table 3).

Table 3. ADME properties of synthesized compounds

Compound	M.Wt	QPlogPo/w[a]	QPlogS[b]	QPPCaco[c]	QPlogBB[d]	QPPMDCK[e]	%Human Oral Absorption[f]
7a	425.5	4.93	-5.78	900.5	0.15	488.7	100
7b	455.6	4.83	-5.43	816.7	0.06	439.7	100
7c	460	5.13	-5.78	917.6	0.27	814.1	100
7d	455.6	4.95	-5.83	899.4	0.08	488.1	100
7e	460	5.33	-6.41	829.4	0.28	1104	100
7f	455.6	5.01	-5.98	900.4	0.08	488.6	96.179
7g	485.6	4.95	-5.69	828.2	-0.01	446.4	100
7h	490	5.17	-6	866.5	0.18	783.1	96.859
7i	485.6	5.02	-6.01	878.4	0	475.7	96.085
7j	490	5.53	-6.62	1189	0.39	1629	100
7k	470.5	3.83	-4.36	187.8	-0.55	89.78	90.052
7l	500.6	4.15	-5.61	99.15	-1.09	45.01	74.007
7m	505	4.41	-5.9	109.9	-0.86	82.16	76.365
7n	500.6	4.29	-6.11	107.3	-1.13	49.04	75.443
7o	505	4.73	-6.54	142.5	-0.73	164.5	80.24

[a] Predicted octanol/water partition coefficient log P (Acceptable range⁻2.0 to 6.5).

[b] Predicted aqueous solubility's in mol/L (Acceptable range⁻6.5 to 0.5).

[c] Predicted BBB permeability (Acceptable range⁻3 to 1.2).

[d] Predicted Caco cell permeability in nm/s (Acceptable range: < 25 is poor and >500 is great).

[e] Predicted apparent MDCK cell permeability in nm/s (Acceptable range in nm/s (Acceptable range: <25 is poor and>500 is great).

[f] Percentage of human oral absorption (Acceptable range: <25 is poor and >80% is high).

Experimental

All the reagents and solvents purchased from commercially available sources. Analytical TLC was performed on Merck Silica Gel GF254 plates and visualization by I_2 vapours / UV light. Melting points were determined in open capillary tubes on SISCO electrical melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer spectrophotometer using potassium bromide optics. ^{1}H and ^{13}C NMR spectra recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C) in $CDCl_3$/DMSO using TMS as internal standard. ESI Mass Spectra recorded on Quatro LC Micromass (Waters Manchester, UK) mass Spectrometer.

The *o*- phenylene diamine (**1,** 18.5 mmol, 2g) reacts with chloroacetic acid (27.75 mmol, 2.6g) in presence of 4N HCl at reflux condition for 3-4 h yields 2-(chloromethyl)-1*H*-benzo[*d*]imidazole (**2,** 9.03 mmol, 1.5g), which is subsequently treated with substituted phenyl piperazine (**3a**, 9.03 mmol, 1.46g) in presence of sodium hydride gives sustituted 2-((4-phenylpiperazin-1-yl)methyl)-1*H*-benzo[d]imidazole (**4a).** Further, Substituted 2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazole (**4a,** 6.84 mmol, 2g) treated with bromo ethylacetate (8.21 mmol, 1.36g) in presence of sodium hydride to obtain corresponding (2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazol-1-yl) acetate derivatives (**5a**). Then, hydrolysis of (2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazol-1-yl) acetate derivative (**5a,** 3.96 mmol,1.56g) with LiOH.H_2O (5.95 mmol, 244mg) in THF: H_2O (4:1) produces substituted 2-(2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazol-1-yl) acetic acid (**6a**). The amide coupling reactions of substituted 2-(2-((4-phenylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazol-1-yl) acetic acid (**6a,** 0.571 mmol, 200mg) with o- anisidine (**7a,** 0.571 mmol, 69 mg) in presence of EDC.HCl and HoBt in TEA, DMF gives the titled compounds in good yields (73-86%).

Anticancer activity

MTT assay Method

In vitro anti cancer activity of the compounds was tested using MTT colorimetric assay as per ATCC protocol. Cell lines used for testing in-vitro cytotoxicity are HeLa, MCF-7 and HEK 293 being received with job number 1769 from NCCS, Pune. Cell lines were maintained at 37 ^{0}C in a humidified 5% CO_2 incubator (Thermo scientific) using suitable

media prescribed in NCCS Protocol. Decontaminated flasks were incubated for subculture. Cells were passed by 14 numbers, After getting 70% confluence, from culture flasks take 100µL cell suspension and make a cell count using haemocytometer and found 8,000 – 10,000 per well in a 96-well plate. The cell suspension was mixed thoroughly by pipetting several times to get a uniform single cell suspension. Different dilutions of solutions 3, 10, 30, 100 µg/ mL were made in media DMEM, 0.2% DMSO and ultrapure water H_20. 100µl of cell suspension was transferred aseptically to each well of a 96 well plate and to it 100µl of nanoparticle solution (in quadruplicate) in media was added. The plate was then incubated at 37^0C for 72 h in CO_2 incubator. After 48 h of incubation, 20µl of MTT was added to each well aseptically. The plate was again incubated for 2 h. 80µl of lyses buffer was added to each well the plate was wrapped in aluminium foil to prevent the oxidation of the dye and the plate was placed on a shaker for 30 minutes. The absorbance was recorded on the ELISA reader (Biotech EL x 800) at 570 nm wavelength. We have calculated the % inhibition by following formula % inhibition = Control ODs – Test ODs ÷ Control ODs × 100 and finally IC_{50} values to asses anticancer activity. Doxorubicin was used as the standard drug in the assay.

Principle

The cytotoxicity assay was performed using MTT reagent. In this colorimetric assay MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) a water soluble yellow coloured tetrazolium salt, was converted to an insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase present within the mitochondria. The formazan product being impermeable to the cell membranes it accumulates in healthy cells. Since reduction of MTT could only occur in metabolically active cells, the intensity of purple colour was used as a measure of the viability of the cells. The intensity was measured by spectrophotometer after dissolving formazan crystal in DMSO.

Conclusion

A series of novel N-phenyl-2-(2-((4-phenyl piperazin-1-yl) methyl)-1*H*-benzo [*d*] imidazol-1-yl) acetamides (**7a-o**) were synthesized and well characterized by using various spectral techniques. All the synthesized compounds were examined for their anticancer activity against three human cell lines, viz. Hela, MCF-7 and HEK-293 at various concentrations and the results were performed by means cell viability with reference to standard anticancer drug doxorubicin. Among all the compounds, compound **7k** showed highest activity against HeLa and MCF-7. The compounds **7b**, **7l**, **7m**, **7n** and **7o** also showed good activity over HeLa and MCF-7. Based on the anticancer activity, docking and ADME

studies, the synthesized molecules can be considered as promising lead molecules for the development of new anticancer agents.

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ACCEPTED MANUSCRIPT

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Date: 14.04.2018

To

The Editor,

Journal of Molecular Structure

Dear Sir/Madam,

Kindly find enclosed our revised manuscript entitled "Synthesis, anticancer activity and docking studies of *N*-phenyl-2-(2-((4-phenyl piperazin-1-yl) methyl)-1*H*-benzo [*d*] imidazol-1-yl) acetamides" for publication in your esteemed journal, the "Journal of Molecular Structure". This manuscript has not been published elsewhere or submitted to another journal and all authors are familiar with and agree with the contents of the manuscript.

Highlights of the manuscript are

- A series of novel N-phenyl-2-(2-((4-phenyl piperazin-1-yl) methyl)-1*H*-benzo [*d*] imidazol-1-yl) acetamides were synthesize in conventional methods.
- The newly synthesized compounds were screened for anticancer activity against 3 cell lines. Viz, HeLa, MCF-7 and HEK 293. Compound **7k** had shown excellent activity against HeLa and MCF-7 and compounds 7**b**, 7**l**, 7**m**, 7**n** and 7**o** also showed significant activity over HeLa and MCF-7.
- *In-silico* molecular docking studies were performed using Schrodinger software. **(**quinone reductase-2 (PDB ID 4ZVM) protein.

Thank you.

With best reguards

Yours Sincerely,

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