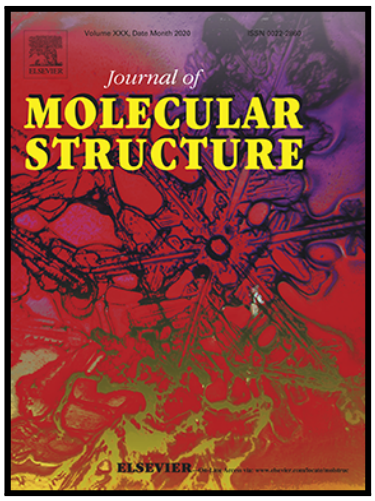


Synthesis of novel cycloheptylbenzothiazole-2-carboxamides and biological evaluation as human estrogen receptor modulators

Purushottam Kapse , Rupesh V. Chikhale ,
Mohammad Rizwan Khan , Saikh M. Wabaidur , Md Ataul Islam

PII: S0022-2860(20)31831-7
DOI: https://doi.org/10.1016/j.molstruc.2020.129516
Reference: MOLSTR 129516

To appear in: *Journal of Molecular Structure*

Received date: 13 June 2020
Revised date: 20 October 2020
Accepted date: 24 October 2020

Please cite this article as: Purushottam Kapse , Rupesh V. Chikhale , Mohammad Rizwan Khan , Saikh M. Wabaidur , Md Ataul Islam , Synthesis of novel cycloheptylbenzothiazole-2-carboxamides and biological evaluation as human estrogen receptor modulators, *Journal of Molecular Structure* (2020), doi: https://doi.org/10.1016/j.molstruc.2020.129516

Highlights

- Novel cycloheptylbenzothiazole-2-carboxamide were designed
- Nine derivatives were successfully synthesised and characterised
- Good inhibitory activity found in TCP1020 bladder cancer cell lines
- Synthesized compounds have good docking score for ligand-protein complex

Synthesis of novel cycloheptylbenzothiazole-2-carboxamides and biological evaluation as human estrogen receptor modulators

Purushottam Kapse[1], Rupesh V. Chikhale[1], Mohammad Rizwan Khan[2], Saikh M. Wabaidur[2], Md Ataul Islam[3*]

[1]*Department of Pharmaceutical Sciences, Rasthrasant Tukadoji Maharaj Nagpur University, Nagpur - 440033, Maharashtra, India*
[2]*Department of Chemistry, P.O. Box 2455, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia*
[3]*Division of Pharmacy and Optometry, University of Manchester, Manchester, M13 9PL, United Kingdom*

[*]To whom correspondence should be addressed: ataul.islam80@gmail.com (MA Islam)

Abstract

Background

Bladder cancer is one of the deadly cancer with 16,390 deaths in 2015-16 alone and 76,960 new cases. The matter of concern is more severe with very limited options of treatment and lack of new drugs, cisplatin and doxorubicin are the only two drugs mostly used in therapy. This situation along with the epidemiological data calls for the development of newer better and safer agents. Herein, we report nine novel benzothiazole derivatives based on structure-based drug discovery and molecular modelling approaches.

Material and methods

Newly designed compounds were synthesized following a four-step reaction and were characterized for structural confirmation. These novel compounds were evaluated on the MTT assay for their in vitro efficiency using the TCP1020 cell lines. These were further analysed for their mechanism of action based on in silico studies.

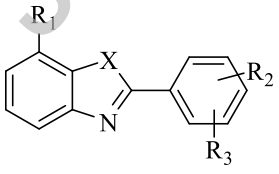
Results

Two compounds of the series exhibit promising results which are in agreement with the in-silico studies. It was found that the methyl group at the seventh position to the nitrogen decreases the electron affinity of the series and is thus responsible for the activity in **4e**. The methyl substituted compound **4e** and fluoro substituted compound **4i** shows the highest activities with an IC_{50} of 10.1 ± 0.2 and 7.8 ± 0.7 respectively.

Keywords: Bladder cancer; estrogen binding receptor; molecular docking; anticancer activity

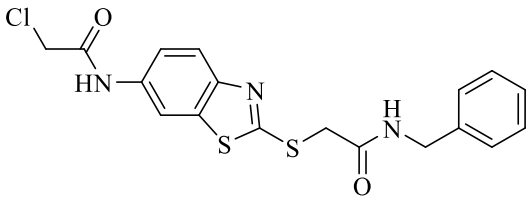
1. Introduction

Urinary bladder cancer (UBC) is one of the deadly heterogeneous diseases and the most common malignant tumor in the urinary tract [1]. In 2015-16 alone, there were 16,390 deaths and 76,960 new cases of UBC [2]. According to the World Health Organization (WHO), the UBC left 199,922 deaths and 549,393 new cases in 2018 globally [3]. In 2018, approximately 81,190 new cases and 17,240 deaths were recorded in the USA only [4]. It is the ninth most widely occurring cancer throughout the world [5]. Moreover, UBC is ranked as the seventh most common cancer among men [5]. In the new cases of UBC, three-quarters are found in men, yet women have higher disease-specific mortality [2]. The hormonal profile differences between men and women are the principal reason behind the disparity in gender incidence and mortality rate [6]. The older women taking estrogen therapy are found to be less vulnerable in comparison to nulliparous women [6]. The causes behind bladder cancer are mostly smoking, chemical contamination of drinking water, arsenic poisoning, and schistosomiasis infection [7]. About 50% of UBC cases are developed due to tobacco smoke and the type of tobacco consumed [5]. The matter of concern is more severe with very limited options of treatment and a lack of new drugs. Currently, few drugs included cisplatin, doxorubicin, budesonide and thiotepa are mostly used in therapy [8–10]. These all are very old drugs, exhibits severe adverse reactions in patients with very little compatibility, and have emerging drug resistance [11–13]. This situation along with the epidemiological data calls for the development of newer better and safer agents. Several attempts were reported for the development of newer agents for the treatment of bladder cancer like the peptides 9-mer bladder cancer-specific peptide (BP), [^{124}I]Y-BP [14] and the 1-(N-methylindolyl)-3-phenylpropenones [15]. Tetracene derivatives have been traditionally used for the treatment of bladder cancer with the doxorubicin as the most important drug of this series [16,17].



I

X = S/O/N; R = H, Alkyl, Aryl

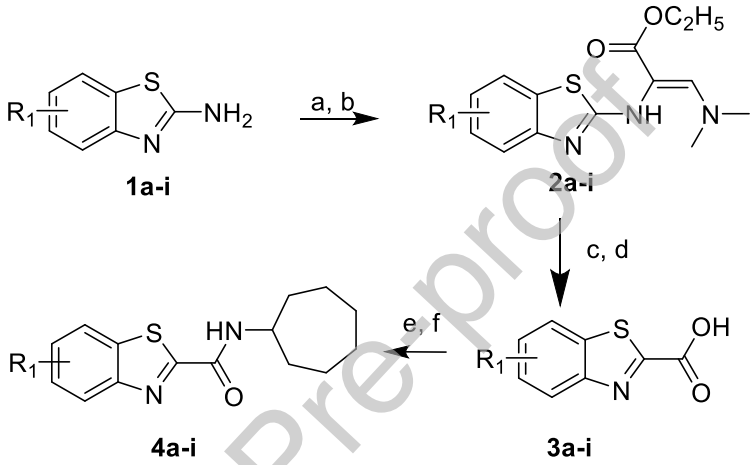


II

SKLB-163

Similarly, the benzazole and benzothiazole derivatives (I-II) are reportedly found to be highly efficacious moiety for the development of newer candidates for the treatment of UBC and other types of cancers [18][19]. Recently the role of estrogen receptor in the progression of

bladder cancer was proved and it was also found that the inhibition of the activity of this receptor or control over its estrogen or progesterone binding region would help in the prevention and cure of the UBC [20,21]. Based on these findings we initiated an attempt to develop lead molecules that would help control the progression and help cure the bladder cancer[22]. Initially, we designed sixteen novel benzothiazolyl derivatives (Scheme I) based on earlier reported benzothiazoles as active moieties against the estrogen binding receptors, then subjected them to molecular docking studies [23]. Based on molecular docking studies nine novel hybrid molecules containing benzothiazolyl and cycloheptyl moiety were synthesized and evaluated for their anticancer study on the TCP1020 cell line [16].



Compound	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**
R_1	4,6-dimethyl	4-CH_3	5-CH_3	6-CH_3	7-CH_3	5,7-dimethyl	6-Br	4,6-Dichloro	4,6-difluro

Reagents and Conditions: a) Ethyl bromoacetate, K_2CO_3, acetone, 60 °C, overnight; (b) DMFDMA, 150 °C, 15 h; (c) $AlCl_3$, CH_2Cl_2, 24 h, rt; (d) LiOH, EtOH, reflux, 3 h; (e) EDC.HCl, HOBt, Et3N, CH_2Cl_2, rt, 12–16 h; (f) $SnCl_2$, MeOH/EtOAc (4:1), H_2.

Scheme I: Synthesis of various substituted *N*-cycloheptylbenzothiazole-2-carboxamides **4a-i**.

2. Experimental and Results

Chemicals were obtained from Sigma Aldrich, USA. Melting points (Mp.) were detected with open capillaries using Thermonik Precision Melting point apparatus and are uncorrected. IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). ^{1}H was obtained using a Bruker Advance-II 400 Spectrometer on 400 MHz using tetramethylsilane (TMS) as an internal standard. All chemical shift values were recorded as δ (ppm), coupling constant value *J* is measured in hertz, the peaks are presented as s (singlet), d (doublet), t (triplet), brs (broad singlet), dd (double doublet), m (multiplet). The purity of

compounds was controlled by thin-layer chromatography (Merck, silica gel, HF254–361, type 60, 0.25 mm, Darmstadt, Germany). Mass spectra (ESI-MS) were recorded at Waters, Q-TOF LC-MS spectrometer (Waters, Micromass LC-MS, USA).

2.1. General procedure for synthesis of ethyl 2-(benzothiazol-2-ylamino)-3-(dimethylamino) acrylate (**2a-i**)

Various substituted 2-aminobenzothiazoles **1a-i** were reacted with ethyl bromoacetate in the presence of potassium carbonate in acetone at 60 ºC overnight. The recovered adduct was obtained as ethyl 2-[(substituted benzothiazolyl)amino]acetate which was reacted in turn with N,N-dimethylformamide dimethyl acetal at 150 ºC for 15 h to yield the ethyl 2-(substituted benzothiazol-2-ylamino)-3-(dimethylamino)acrylates **2a-i**.

2.2. Synthesis of substituted benzothiazole-2-carboxylic acid (**3a-i**)

In the next step, ethyl 2-(substituted benzothiazol-2-ylamino)-3-(dimethylamino) acrylates **2a-i** were treated with Lewis acid ($AlCl_3$) leading to cyclization and yielding cyclized derivative as benzothiazolyl-2-carboxylates, which after basic hydrolysis gave the corresponding acids **3a-i**.

2.3. General procedure for the synthesis of various substituted *N*-cycloheptylbenzothiazole-2-carboxamides **4a-i**.

The benzothiazolyl carboxylic acids **3a-i** were reacted with cycloheptylamine in presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride dissolved in butanol with dichloromethane as a solvent for 12-16 h at room temperature. The adduct obtained was treated with tin chloride under hydrogen leading to final derivatives as substituted *N*-cycloheptylbenzothiazole-2-carboxamides **4a-i**.

2.3.1. Synthesis of N-cycloheptyl-5,7-dimethylbenzo[d]thiazole-2-carboxamide **4a**

Mp: 231-234 ºC; ^{1}H-NMR ($CDCl_3$): δ 1.22-1.62 (m, 12H, cycloheptyl ring), 2.32 (S, 6H, CH_3), 3.58 (m, 1H, methyene), 6.86 (m, 1H, benzothiazole), 7.71 (m, 1H, benzothiazole), 8.1 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 20.1, 21.6, 25.1, 29.0(2), 31.8, 117.70, 118.6, 120.39, 120.72, 125.16, 126.8, 130.88, 133.3(2), 152.49, 167.6.; MS (ESI) m/z: 303.1486; Anal. Calcd for $C_{17}H_{22}N_2OS$: C, 67.51; H, 7.33; N, 9.26; O, 5.29; S, 10.60.

2.3.2. Synthesis of N-cycloheptyl-7-methylbenzo[d]thiazole-2-carboxamide **4b**

Mp: 237-239 ºC; ^{1}H-NMR ($CDCl_3$): δ 1.29-1.63 (m, 12H, cycloheptyl ring), 2.36 (S, 6H, CH_3), 3.58 (m, 1H, methyene), 6.84 (m, 1H, benzothiazole), 7.71 (m, 1H, benzothiazole), 8.1 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 20.4, 21.6, 25.1, 29.1, 31.8, 117.70, 118.6, 120.39, 120.72, 125.16, 126.8, 130.12, 133.3(2), 152.49, 167.7.; MS (ESI) m/z: 288.1296; Anal. Calcd for $C_{16}H_{20}N_2OS$: C, 66.63; H, 6.99; N, 9.71; O, 5.55; S, 11.12

2.3.3. Synthesis of N-cycloheptyl-6-methylbenzo[d]thiazole-2-carboxamide **4c**

Mp: 232-234 ºC; ^{1}H-NMR ($CDCl_3$): δ 1.27-1.67 (m, 12H, cycloheptyl ring), 2.32 (S, 6H, CH_3), 3.57 (m, 1H, methyene), 6.84 (m, 1H, benzothiazole), 7.71 (m, 1H, benzothiazole), 8.1 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 20.4, 21.6, 25.2, 29.0(2), 31.8, 117.10, 118.6, 120.39, 120.72, 125.16, 126.8, 130.81, 133.3(2), 152.49, 167.5.; MS (ESI) m/z: 288.1296; Anal. Calcd for $C_{16}H_{20}N_2OS$: C, 66.63; H, 6.99; N, 9.71; O, 5.55; S, 11.12

2.3.4. Synthesis of N-cycloheptyl-5-methylbenzo[d]thiazole-2-carboxamide **4d**

Mp: 233-235 ºC; ^{1}H-NMR ($CDCl_3$): δ 1.27-1.61 (m, 12H, cycloheptyl ring), 2.33 (S, 6H, CH_3), 3.55 (m, 1H, methyene), 6.86 (m, 1H, benzothiazole), 7.72 (m, 1H, benzothiazole), 8.1 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 20.1, 21.6, 25.1, 29.0, 32.0, 49.8, 117.72, 118.16, 120.39, 120.70, 125.03, 126.8, 130.88, 133.3(2), 152.49, 167.6.; MS (ESI) m/z: 288.1296; Anal. Calcd for $C_{16}H_{20}N_2OS$: C, 66.63; H, 6.99; N, 9.71; O, 5.55; S, 11.12.

2.3.5. Synthesis of N-cycloheptyl-4-methylbenzo[d]thiazole-2-carboxamide **4e**

Mp: 234-236 ºC; ^{1}H-NMR ($CDCl_3$): δ 0.81-0.99 (m, 13H, cycloheptyl ring), 2.50 (S, 3H, CH_3), 7.07-7.23 (m, 1H, benzothiazole), 7.24-7.25 (d, 1H, benzothiazole), 7.28(s, 1H, benzothiazole), 10.78 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.25, 31.34, 31.78, 32.98, 47.04, 114.96, 125.47, 126.89, 128.34, 141.42, 187.73.; MS (ESI) m/z: 288.1330; Anal. Calcd for $C_{16}H_{20}N_2OS$: C, 66.63; H, 6.99; N, 9.71; O, 5.55; S, 11.12

2.3.6. Synthesis of N-cycloheptyl-4,6-dimethylbenzo[d]thiazole-2-carboxamide **4f**

Mp: 244-246 ºC; ^{1}H-NMR ($CDCl_3$): δ 1.22-1.63 (m, 12H, cycloheptyl ring), 2.31 (S, 6H, CH_3), 3.50 (m, 1H, methyene), 6.81 (m, 1H, benzothiazole), 7.71 (m, 1H,

benzothiazole), 8.4 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 19.6, 25.1(2), 29.0(2), 31.8, 33.0, 47.2, 117.70, 118.6, 120.39, 120.72, 125.16, 126.8, 130.88, 133.0, 152.49, 167.6.; MS (ESI) m/z: 302.1455; Anal. Calcd for $C_{17}H_{22}N_2OS$: C, 67.51; H, 7.33; N, 9.26; O, 5.29; S, 10.60

2.3.7. Synthesis of 5-bromo-N-cycloheptylbenzo[d]thiazole-2-carboxamide **4g**

Mp: 212-214 ºC; 1H-NMR (CDCl3): δ 1.27-1.69 (m, 12H, cycloheptyl ring), 3.60 (m, 1H, methyene), 6.80 (m, 2H, benzothiazole), 7.71 (m, 1H, benzothiazole), 8.1 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 19.8, 25.1(2), 29.0, 31.8, 47.2, 117.70, 118.6, 120.39, 122.12, 126.8, 132.11, 150.40, 167.6.; MS (ESI) m/z: 351.0403; Anal. Calcd for $C_{15}H_{17}BrN_2OS$: C, 51.00; H, 4.85; Br, 22.62; N, 7.93; O, 4.53; S, 9.08

2.3.8. Synthesis of 5,7-dichloro-N-cycloheptylbenzo[d]thiazole-2-carboxamide **4h**

Mp: 219-221 ºC; 1H-NMR (CDCl3): δ 1.27-1.63 (m, 12H, cycloheptyl ring), 3.58 (m, 1H, methyene), 6.97 (m, 1H, benzothiazole), 7.71 (m, 1H, benzothiazole), 8.1 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 19.8, 25.1(2), 31.8, 47.2, 117.70, 118.6, 120.39, 122.12, 126.8, 131.09, 151.1, 163.9.; MS (ESI) m/z: 342.0366; Anal. Calcd for $C_{15}H_{16}C_{l2}N_2OS$: C, 52.48; H, 4.70; Cl, 20.66; N, 8.16; O, 4.66; S, 9.34

2.3.9. Synthesis of N-cycloheptyl-5,7-difluorobenzo[d]thiazole-2-carboxamide **4i**

Mp: 119-121 ºC; 1H-NMR (CDCl3): δ 0.72-0.75 (d, 2H, cycloheptyl ring), 0.90 (s, 3H, cycloheptyl ring), 1.04-1.07 (d, 2H, cycloheptyl ring), 1.25-1.28 (d, 2H, cycloheptyl ring), 1.68 (s, 3H, cycloheptyl ring), 2.14 (d, 1H, methyene), 7.43 (s, 1H, benzothiazole), 7.46 (s, 1H, benzothiazole), 11.06 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.25, 31.34, 31.78, 32.98, 47.04, 114.96, 125.47, 126.89, 128.34, 141.42, 187.73.; MS (ESI) m/z: 309.1952; Anal. Calcd for $C_{15}H_{16}F_2N_2OS$: C, 58.05; H, 5.20; F, 12.24; N, 9.03; O, 5.16; S, 10.33

2.4. In vitro screening of compounds **4a-i:**

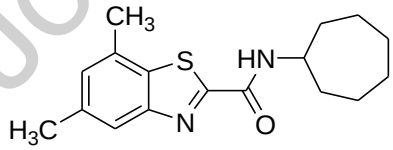
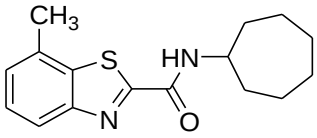
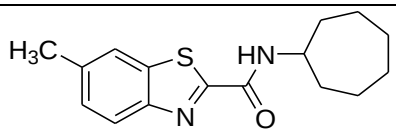
2.4.1. Cell culture

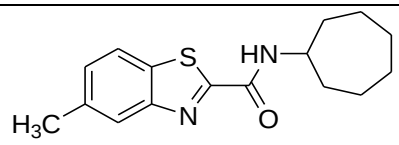
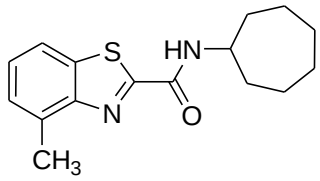
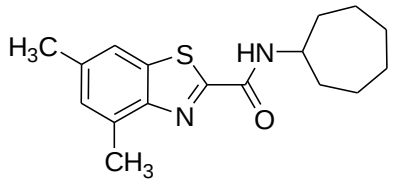
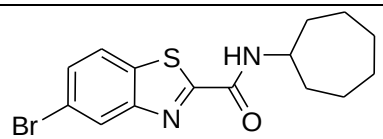
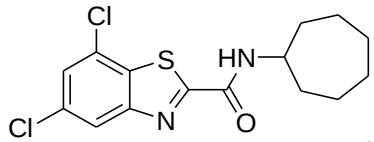
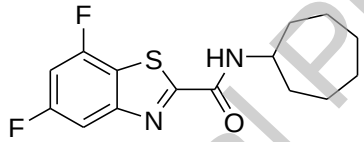
Human bladder cancer cell line TCP1020 was obtained from ATCC, USA. These were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum (FCS) and 2 mM glutamine (Sigma, USA). Cells were maintained at 37 ºC in a 5% CO_2-humidified chamber to ensure freedom from unwanted bacteria.

2.4.2. Cell proliferation assay

Efficacy of the newly synthesized compounds was measured by an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay by a method described earlier.[11] TCP1020 cells were seeded into 96-well plates (3 x103 cells/well) in 100 µl of culture medium. After 24 h, cells were treated with doxorubicin (Sigma-Aldrich, USA) and the cycloheptylbenzothiazole-2-carboxamides at various concentrations. In parallel, a control with DMSO in concentration was employed as a vehicle. The incubation was carried out for 48 h after that 10 µL of an MTT stock solution in PBS at 5 mg/mL was added in each well. These plates were again incubated at 37 ºC for 3 h. These plates were then subjected to centrifugation for 5 min at 1500 rpm. The supernatant medium was discarded and replaced with fresh DMSO to solubilize purple formazan crystals. Following this, the mixture was shaken for 15 min and then the absorbance was measured on an ELISA reader at 570 nm with a reference wavelength of 650 nm. Absorbance observed from control was treated as 100% of cell survival, reading was taken in triplicate for each data point.

Table 1. Antiproliferative profile of the novel *N*-cycloheptylbenzothiazole-2-carboxamides derivatives. The table represents conc. as a result of a 50% loss of cell viability concerning untreated cells (IC_{50}), the results were resolute from dose-response curves. Values are logarithmic from three independent experiments.

Compound no.	**Structure**	**IUPAC name**	**IC_{50} (µM)**	**Dock score (Glide score)**
4a		N-cycloheptyl-5,7-dimethylbenzo[d]thiazole-2-carboxamide	1.77	-5.01
4b		N-cycloheptyl-7-methylbenzo[d]thiazole-2-carboxamide	1.72	-5.13
4c		N-cycloheptyl-6-methylbenzo[d]thiazole-2-carboxamide	0.541	-5.72

4d		N-cycloheptyl-5-methylbenzo[d]thiazole-2-carboxamide	0.65	-4.99
4e		N-cycloheptyl-4-methylbenzo[d]thiazole-2-carboxamide	0.34	-5.78
4f		N-cycloheptyl-4,6-dimethylbenzo[d]thiazole-2-carboxamide	0.53	-5.74
4g		5-bromo-N-cycloheptylbenzo[d]thiazole-2-carboxamide	0.58	-3.24
4h		5,7-dichloro-N-cycloheptylbenzo[d]thiazole-2-carboxamide	0.78	-5.60
4i		N-cycloheptyl-5,7-difluorobenzo[d]thiazole-2-carboxamide	0.27	-6.12
Standard	Doxorubicin	--	0.78	-6.33

2.5. Molecular docking study

The compound library for **4a-i** was prepared for the maximum number of possible conformations using the LigPrep module and docking with estrogen binding receptor (PDB 3ERT) was carried out using Glide docking module of Schrodinger molecular modelling software.[11] F**igure 1** represents the results of molecular docking studies with the best first two molecules from the series for the docking score.

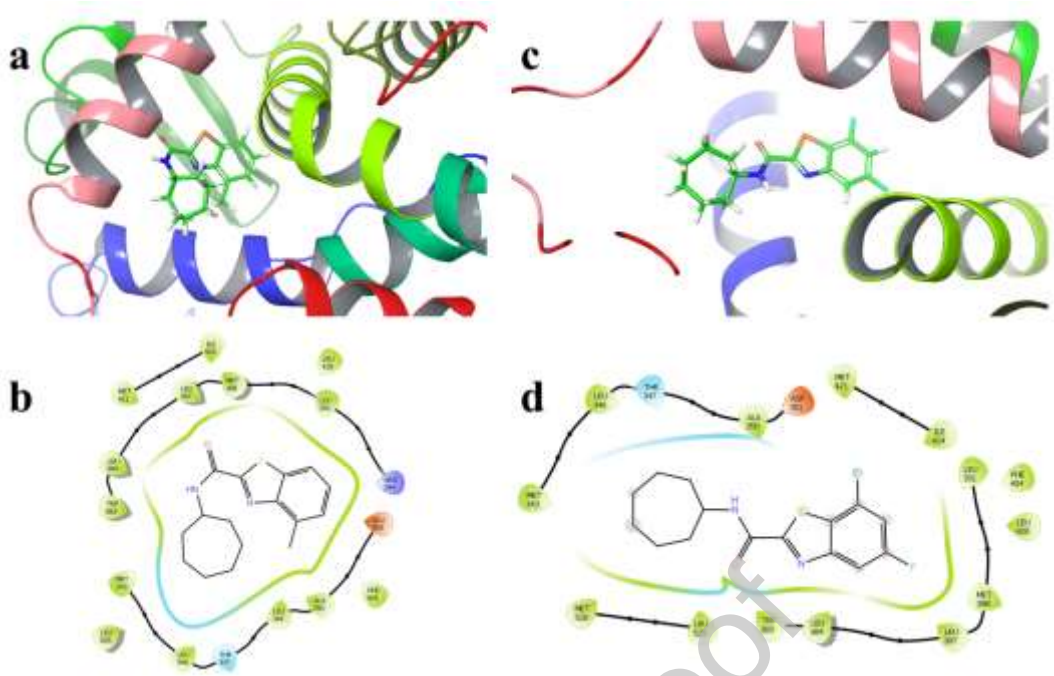


Figure 1. a, b) Molecular docking pose for compound **4i** in 3D and 2D representation showing interaction with the adjacent amino acid residues ASP351 with strong Van der Waals forces. **c**, **d)** Molecular docking pose for compound **4e** in 3D and 2D representation showing interaction with the adjacent amino acid residues ASP351 with strong Van der Waals forces.

3. Discussion

The present investigation addresses the importance and lack of drugs for the treatment of bladder cancer. Herein, we have reported the design and synthesis of novel benzothiazolyl derivatives that show good *in-vitro* activity against the bladder cancer cell lines. Based on the literature, we designed sixteen benzothiazolyl derivatives, these compounds were subjected to molecular docking studies. The molecular docking was carried out on the estrogen binding receptor. To perform the molecular docking studies, we retrieved the crystal structure of the estrogen binding receptor from the protein data bank (PDB 3ERT). It is reported that in most cases of bladder cancer the estrogen binding receptor plays a major role in carcinogenesis, if this receptor is regulated then normal apoptosis will be resumed. The molecular docking experiment was carried out with all the sixteen molecules, but it was found that only nine molecules of the series provided with a significant score, and hence these are only presented in the study (Figure 1, Table 1). Compounds with methyl and halogen-substituted on the benzothiazole nucleus displayed good docking scores in the range of -6.12 to -3.24 with compound **4i** exhibiting the highest score and **4g** with lowest.

Accordingly, nine compounds were synthesized following the appropriate route as mentioned by Bhole et al., in the literature[24]. In the first step, various substituted 2-amino benzothiazolyl **1a-i** derivatives were obtained and further treated with ethyl bromoacetate and DMFDMA to yield the corresponding acrylate **2a-i**. These compounds under the influence of aluminum trichloride and lithium hydroxide yield the substituted benzothiazolyl acids **3a-i**. these were reacted with cycloheptyl amides in the presence of EDC.HCl and further reduced by tin chloride to yield the final derivatives as substituted N-cycloheptylbenzothiazole-2-carboxamides **4a-i**, these compounds were structurally elucidated with help of IR, proton and carbon NMR and mass analysis. The proton NMR exhibited prominent peaks of the cycloheptyl moiety in the upfield region with protons around δ 0.6-1.62 ppm with a multiplet. The methyne proton was observed around δ 2.50 to 3.58 ppm and the amide proton was found around δ 8.0 to 11.0 ppm which is a characteristic feature of these compounds. The compound **4e** and **4i** show typical IR absorption spectra for the aromatic compounds (SI-figure 1 and 4). The Alkane stretch for the C-H bonds was observed between 2990 to 2850 cm^{-1}, aromatic C-H stretch for the compounds was observed between 3110-3000 cm^{-1}, C=C stretch at 1575 cm^{-1}. The amide functional group can be distinctively observed with the absorption of the medium stretch at 3250 cm^{-1} and amide C=O at 1630 cm^{-1}. In the case of compound **4i**, the C-Cl stech was observed at 750 cm^{-1}. The proton NMR of compound **4e** shows the aliphatic cycloheptyl protons between δ 0.5 to 1.15 with a double of doublet at 0.82 ppm, doublet at 0.98 ppm and multiple at 0.87 ppm. The aromatic methyl was observed as singlet at 2.50 ppm and the aromatic protons between 7.07 to 7.28. The amide proton was observed at δ 10.78 as a broad peak. This observation for proton NMR was similar for compounds **4e** and 4**i** with only the difference in the number of protons (SI-figure 2 and 5). The carbon NMR further strengthens the structure elucidation. The 13C-NMR exhibits the carbon atoms in the aliphatic and aromatic regions of the carbon spectra for the molecules **4e** and **4i** (SI-figure 3 and 6). The striking feature of the 13C-NMR was the amide carbon (C=O) at δ 187.73 ppm confirming the formation of the molecules. The ESI-MS spectra for compound 4i shows a molecular ion peak at 309.1952 m/z with a base peak at 178.1761 m/z (SI-figure 7). The IR, NMR and mass analysis confirmed the final derivatives.

These compounds were subjected to the anticancer activity following standard MTT assay protocol using TCP1020 cell line obtained from the ATCC. The compound doxorubicin was used as a standard drug, as this was also used in the molecular docking study. The experimental part is well established and was followed according to the earlier reported

methods. The results were derived after three independent experiments were repeated and the IC_{50} was calculated for each of the molecules including standard drug (Table 1).

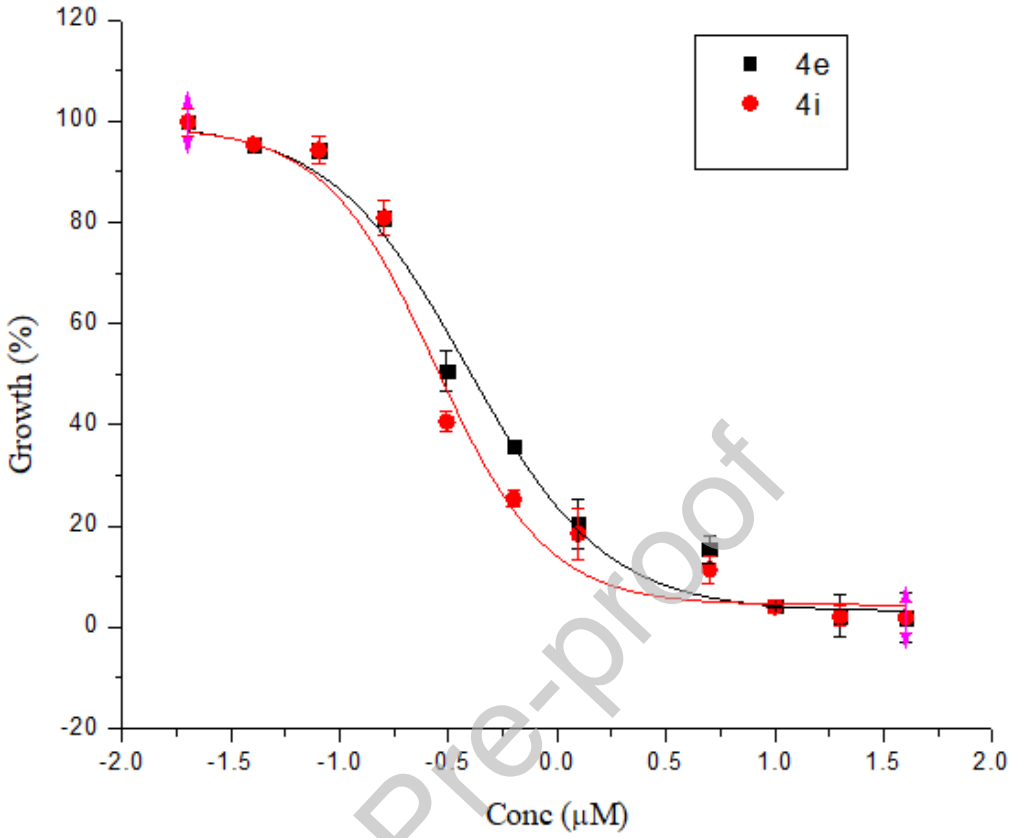


Figure 2. Antiproliferative profile (E=IC_{50}) of the novel *N*-cycloheptylbenzothiazole-2-carboxamides derivatives **4e** (IC_{50} = 0.38491) and **4i** (IC_{50} = 0.27536) calculated by Origin software.

It was found that the standard drug presents IC_{50} of 0.78 uM. In comparison to the experimental compounds, the compound **4i** has the lowest IC_{50} followed by compound **4e** (Table 1). The MTT results were analysed by the Origin software and the logarithmic values were used to calculate the IC_{50} for compounds from the series. The compounds **4e** (IC_{50} = 0.3491) and **4i** (IC_{50} = 0.27536) were the most active compounds from this series (Figure 2). These results are quite significant as these values correspond to our findings from the molecular docking studies. It was found that the compounds with methyl group adjacent to the nitrogen of the benzothiazolyl nucleus have good activity whereas, the molecules with halogens also displayed greater activity, the fluoro substituted molecule displayed the highest activity. It can be derived that the presence of methyl group and halogen on the phenyl ring of the benzothiazolyl moiety could help increase activity.

4. Future Scope

The future scope of this work lies in studying the receptor binding of the hit ligands and druggability analysis by various in vitro and in vivo experiments.

4. Conclusion

The present investigation exhibits the benefits of designing and developing hybrid molecules for their anticancer activity. We have successfully designed, synthesized, and evaluated nine novel derivatives of benzothiazole and cyclic heptylamines. These compounds were found to be highly active on the bladder cancer cell lines, these were found to be comparable to doxorubicin. These compounds can be further improved for their activity and could serve as lead molecules for bladder cancer.

5. Conflict of Interest

None to Declare

Author Contribution

PK and MRK performed the experiment, RVC drafting corrections, SMW and MAI performed data analysis and supervision

Acknowledgments

One of the authors (Mohammad Rizwan Khan) would like to extend his sincere appreciation to the Researchers Supporting Project for funding this work through Research Number (RSP-2019/138), King Saud University, Riyadh, Saudi Arabia

CRediT authorship contribution statement

Purushottam Kapse: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing; **Rupesh V. Chikhale:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing; **Mohammad Rizwan Khan:** Methodology, Writing - original draft, Writing - review & editing; **Saikh M. Wabaidur:** Methodology, Writing - original draft, Writing - review & editing; **Md. Ataul Islam:** Methodology, Writing - original draft, Writing - review & editing

Declaration of competing interest

Authors declare that there is no competing interest.

References

[1] C.Z. Zhu, H.N. Ting, K.H. Ng, T.A. Ong, A review on the accuracy of bladder cancer detection methods, J. Cancer. (2019). https://doi.org/10.7150/jca.28989.

[2] I.A. for R. on C.W.H.O. Iarc., GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012., Globocan. (2012). https://doi.org/10.1002/ijc.27711.

[3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA. Cancer J. Clin. (2019). https://doi.org/10.3322/caac.21551.

[4] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA. Cancer J. Clin. (2018). https://doi.org/10.3322/caac.21442.

[5] M.G.K. Cumberbatch, A.P. Noon, Epidemiology, aetiology and screening of bladder cancer, Transl. Androl. Urol. (2019). https://doi.org/10.21037/tau.2018.09.11.

[6] J. Dobruch, S. Daneshmand, M. Fisch, Y. Lotan, A.P. Noon, M.J. Resnick, S.F. Shariat, A.R. Zlotta, S.A. Boorjian, Gender and Bladder Cancer: A Collaborative Review of Etiology, Biology, and Outcomes, Eur. Urol. (2016). https://doi.org/10.1016/j.eururo.2015.08.037.

[7] S. Janković, V. Radosavljević, Risk factors for bladder cancer, Tumori. (2007). https://doi.org/10.1177/030089160709300102.

[8] J.B. Stone, L.M. DeAngelis, Cancer-treatment-induced neurotoxicity-focus on newer treatments, Nat. Rev. Clin. Oncol. (2016). https://doi.org/10.1038/nrclinonc.2015.152.

[9] H. Ishiguro, T. Kawahara, Y. Zheng, E. Kashiwagi, Y. Li, H. Miyamoto, Differential regulation of bladder cancer growth by various glucocorticoids: Corticosterone and prednisone inhibit cell invasion without promoting cell proliferation or reducing cisplatin cytotoxicity, Cancer Chemother. Pharmacol. (2014). https://doi.org/10.1007/s00280-014-2496-7.

[10] Y.M. Amgaokar, R. V. Chikhale, U.B. Lade, D.M. Biyani, M.J. Umekar, Design, formulation and evaluation of transdermal drug delivery system of budesonide, Dig. J. Nanomater. Biostructures. (2011). https://doi.org/10.4236/pp.2011.23029.

[11] M. Kates, A. Date, T. Yoshida, U. Afzal, P. Kanvinde, T. Babu, N.A. Sopko, H. Matsui, N.M. Hahn, D.J. McConkey, A. Baras, J. Hanes, L. Ensign, T.J. Bivalacqua, Preclinical evaluation of intravesical cisplatin nanoparticles for non–muscle-invasive bladder cancer, Clin. Cancer Res. (2017). https://doi.org/10.1158/1078-0432.CCR-17-1082.

[12] A. Mari, D. D'Andrea, M. Abufaraj, B. Foerster, S. Kimura, S.F. Shariat, Genetic

determinants for chemo- and radiotherapy resistance in bladder cancer, Transl. Androl. Urol. (2017). https://doi.org/10.21037/tau.2017.08.19.

[13] C.J. Cho, C.W. Yang, C.L. Wu, J.Y. Ho, C.P. Yu, S.T. Wu, D.S. Yu, The modulation study of multiple drug resistance in bladder cancer by curcumin and resveratrol, Oncol. Lett. (2019). https://doi.org/10.3892/ol.2019.11023.

[14] Y.S. Ha, H.Y. Lee, G. Il An, J. Kim, W. Kwak, E.J. Lee, S.M. Lee, B.H. Lee, I.S. Kim, T. Belay, W. Lee, B.C. Ahn, J. Lee, J. Yoo, Synthesis and evaluation of a radioiodinated bladder cancer specific peptide, Bioorganic Med. Chem. (2012). https://doi.org/10.1016/j.bmc.2012.05.049.

[15] V. Martel-Frachet, M. Kadri, A. Boumendjel, X. Ronot, Structural requirement of arylindolylpropenones as anti-bladder carcinoma cells agents, Bioorganic Med. Chem. (2011). https://doi.org/10.1016/j.bmc.2011.08.015.

[16] J.F. Liang, V.C. Yang, Synthesis of doxorubicin-peptide conjugate with multidrug resistant tumor cell killing activity, Bioorganic Med. Chem. Lett. (2005). https://doi.org/10.1016/j.bmcl.2005.07.087.

[17] P.J. Burke, T.H. Koch, Design, Synthesis, and Biological Evaluation of Doxorubicin-Formaldehyde Conjugates Targeted to Breast Cancer Cells, J. Med. Chem. (2004). https://doi.org/10.1021/jm030352r.

[18] A. Kamal, M.A.H. Syed, S.M. Mohammed, Therapeutic potential of benzothiazoles: A patent review (2010-2014), Expert Opin. Ther. Pat. (2015). https://doi.org/10.1517/13543776.2014.999764.

[19] R. Chikhale, S. Thorat, R.K. Choudhary, N. Gadewal, P. Khedekar, Design, synthesis and anticancer studies of novel aminobenzazolyl pyrimidines as tyrosine kinase inhibitors, Bioorg. Chem. (2018). https://doi.org/10.1016/j.bioorg.2018.01.008.

[20] E.A. Vladusic, A.E. Hornby, F.K. Guerra-Vladusic, J. Lakins, R. Lupu, Expression and regulation of estrogen receptor ß in human breast tumors and cell lines, Oncol. Rep. (2000). https://doi.org/10.3892/or.7.1.157.

[21] R. Mashhadi, G. Pourmand, F. Kosari, A. Mehrsai, S. Salem, M.R. Pourmand, S. Alatab, M. Khonsari, F. Heydari, L. Beladi, F. Alizadeh, Role of steroid hormone receptors in formation and progression of bladder carcinoma: A case-control study, Urol. J. (2014). https://doi.org/10.22037/uj.v11i06.2617.

[22] P.R. Murumkar, R. V. Chikhale, Vicinal Diaryl Triazoles and Tetrazoles, in: Vicin. Diaryl Substituted Heterocycles, 2018. https://doi.org/10.1016/b978-0-08-102237-5.00006-7.

[23] R. Chikhale, S. Thorat, A. Pant, A. Jadhav, K.C. Thatipamula, R. Bansode, G. Bhargavi, N. Karodia, M.V. Rajasekharan, A. Paradkar, P. Khedekar, Design, synthesis and pharmacological evaluation of pyrimidobenzothiazole-3-carboxylate derivatives as selective L-type calcium channel blockers, Bioorganic Med. Chem. 23 (2015). https://doi.org/10.1016/j.bmc.2015.09.009.

[24] R.P. Bhole, Y.B. Zambare, C.G. Bonde, Synthesis and evaluation of novel n-cycloheptyl-substituted -2,3- dihydro-1,3- benzothiazole-2-carboxamide targeting the estrogen binding receptor, Jordan J. Pharm. Sci. (2019).