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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, cytotoxic evaluation, and in silico studies of substituted *N*-alkylbromo-benzothiazoles

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Abstract In efforts to develop a new class of anticancer agents with improved efficacy and selective action, a series of *N*-alkylbromo-benzothiazoles were synthesized and evaluated for in vitro cytotoxic activity against various human cancer cell lines such as lung (A-549), prostate (PC-3), leukemia (THP-1), and colon (Caco-2). They were found to be highly active against prostate (PC-3) and leukemia (THP-1) cancer cells, moderately active against colon (Caco-2) cancer cells and less active against lung (A-549) cancer cells. Of the 12 compounds, two (**11d**, **11j**) exhibit IC_{50} values of ≤ 1 μM against leukemia (THP-1) cancer cell lines. Compound **11l** showed significant cytotoxic activity against the PC-3 ($IC_{50} = 0.6$ μM), THP-1 ($IC_{50} = 3$ μM) and Caco-2 cell lines ($IC_{50} = 9.9$ μM), respectively. Docking study of the synthesized ligand was done on epidermal growth factor receptor using ArgusLab flexible docking, to determine their observed activity. Further QSAR investigations with stepwise multiple linear regression analysis were applied to find correlation between various physicochemical parameters and anticancer activity. The QSAR results showed that anticancer activity could be modeled with descriptors. The predictive ability of models was cross-validated by observation of the low residual activity values and adjusted coefficient of variation (r^2_{adj}) obtained by leave-one-out technique.

Keywords 2-Aminobenzothiazole · Dibromoalkane · *N*-Alkylation · Cytotoxic activity · Sulforhodamine assay

Introduction

Cancer is a renegade system of growth that originates within a patient's biosystem, which are of different types but all share one hallmark characteristic, unchecked growth that progresses toward limitless expansion (www.cancer.gov., 2012). Currently, one in four deaths in the United States is due to cancer (Siegel *et al.*, 2011). Despite the treatment efficacy, cytotoxicity of anticancer drugs to the normal cells is the major problem in cancer therapy that engenders the risk of inducing secondary malignancy (Saeed *et al.*, 2010). To improve this, in the recent years, there has been a concerned research for the discovery and development of novel selective anticancer agents that impede the progression of malignant tumors or prevent their recurrence.

A number of nitrogen and sulfur-containing heterocyclic compounds such as indole (Singh *et al.*, 2009), isatin (Solomon *et al.*, 2009), benzothiazole, oxadiazole (Zhang *et al.*, 2011), etc., show anticancer activities. Among them, benzothiazole moiety shows interesting anticancer activity by inhibiting tyrosine kinase (Bhuva and Kini, 2010), aurora kinase (Tasler *et al.*, 2009), topoisomerase II (Pinar *et al.*, 2004), and ubiquitin proteasome system (Guedat and Colland, 2007). In addition, benzothiazole derivatives also exhibit a variety of biological functions such as anticonvulsant (Siddiqui *et al.*, 2007), antitubercular (Palmer *et al.*,

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1971), antimalarial (Burger and Sawhney, 1968), antidiabetic (Moreno-Diaz *et al.*, 2008), and antimicrobial activities (Bondock *et al.*, 2010). *N*-Alkylated indoles (Liu *et al.*, 2003; Bacher *et al.*, 2001) and isatins (Singh *et al.*, 2011) are also well known to exhibit anticancer activity.

Some of the benzothiazole derivatives as anticancer agents (Fig. 1) include 2-acetyl-3-(6-methoxy-benzothiazol)-2-yl-amino-acrylonitrile (AMBAN, compound **1**) induced apoptosis in HL60 and U937 cells through mitochondrial/caspase 3-dependent pathway (Repicky *et al.*, 2009). Research on benzothiazole moiety has been further extended through introduction of amide (compound **2**) (Song *et al.*, 2008), urea substituents (compound **3**) (Song *et al.*, 2008), isopropylamidino (compound **4**) (Caleta *et al.*, 2009), and imidazolinyl groups (compound **5**) (Caleta *et al.*, 2009) which increased the antiproliferative effect on the wide range of tumor cell lines. Benzothiazole linked with pyrrolobenzodiazepine conjugates (compound **6**) reported to have significant cytotoxicity due to promising DNA-binding ability and apoptosis caused by G0/G1 phase arrest at sub-micromolar concentrations (Kamal *et al.*, 2010).

In silico studies play an important role in the drug design and discovery, as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule in a non-covalent fashion. Probable mode of action could be analyzed by prediction of correct binding geometry of each ligand at the active site, which reveals the docking score with surrounding amino acids. The docking score of a protein/ligand complex is estimated in the term of binding free energy using ArgusLab (ArgusLab 4.0.1, 2011). It consists of a user interface that displays the graphical structure of the molecules and runs quantum mechanics calculation using Argus Computing Server.

Taking cognizance and our interest toward the synthesis of novel heterocyclic compounds with diverse medicinal potential, we report herein the synthesis and cytotoxic evaluation of novel *N*-alkylbromo-benzothiazoles against four different human cancer cell lines namely lung (A-549), prostate (PC-3), leukemia (THP-1), and colon (Caco-2). Then several attempts have been made in generating the predictive QSAR models on different cancer cell lines using various types of descriptors. The alkyl chain length and substitution at 4, 5, and 6 positions have been varied on benzothiazole moiety to ascertain the quantitative structure–activity relationship of the resulting scaffolds.

Results and discussion

Chemistry

The compounds **11a–11l** described in this study were prepared as shown in Scheme 1. The substituted 2-aminobenzothiazoles (compounds **10a–10l**) were synthesized by treating substituted aniline with ammonium thiocyanate and bromine in chloroform (Saeed *et al.*, 2010). This reaction involves the nucleophilic attack at the electrophilic carbon of the thiocyanate ion by the amine. The arylthiourea synthesized, was then cyclized in the presence of bromine and chloroform to form 2-aminobenzothiazole (compounds **10a–10l**). Finally, synthesis of *N*-alkylbromo-benzothiazole derivatives (compounds **11a–11l**) was achieved by *N*-alkylation of substituted 2-aminobenzothiazole (compound **10**) with appropriate dibromoalkane in the presence of potassium carbonate in DMF. The completion of reaction was monitored

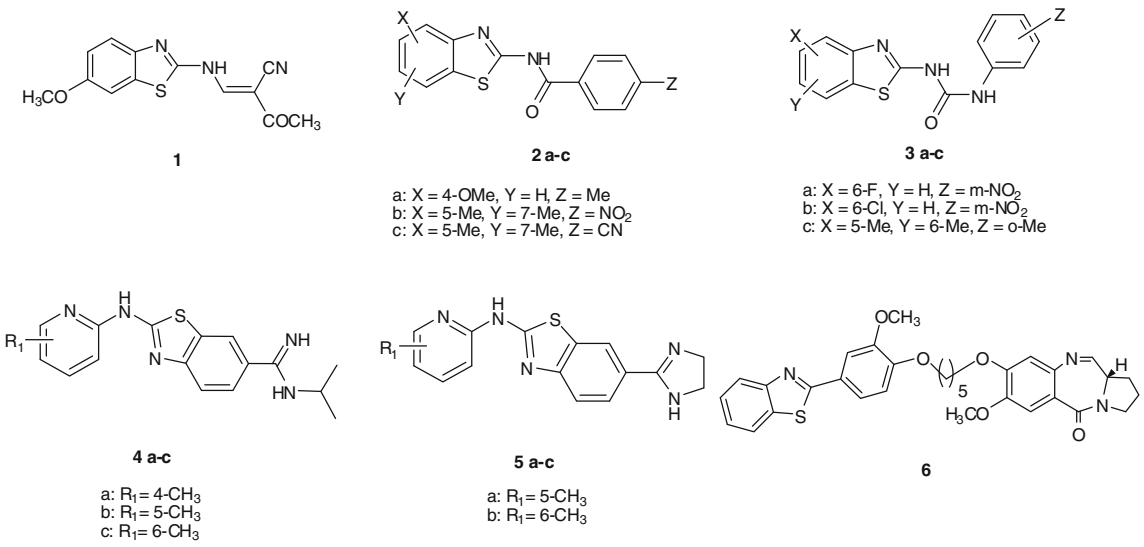


Fig. 1 Some benzothiazole analogs as anticancer agents

by thin layer chromatography. All the compounds were separated and purified by column chromatography.

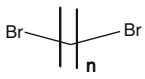
All the structures of novel *N*-alkylbromo-benzothiazoles were characterized by rigorous spectroscopic analysis i.e., ^{1}H & ^{13}C NMR, IR, and Mass. The IR (KBr) spectra of compound **11a** compound has strong N–H absorptions at about 3465.88 cm^{-1}, and displayed absorptions at about 1585.38 cm^{-1} and 1230.50 cm^{-1}which indicates the formation of imine bond (C=NH) and CH_2Br, respectively. In ^{1}H-NMR-spectra, two triplets were observed confirmed by their coupling constants. A triplet at δ 4.49 ($J = 7.5$ Hz) is observed for CH_2Br and at δ 4.66 ($J = 7.5$ Hz) for CH_2–NH. Peak of NH protons are extremely variable and weak, therefore, confirmed by exchange of NH proton with D_2O. Reaction time and yield (%) of various compounds are summarized in Table 1.

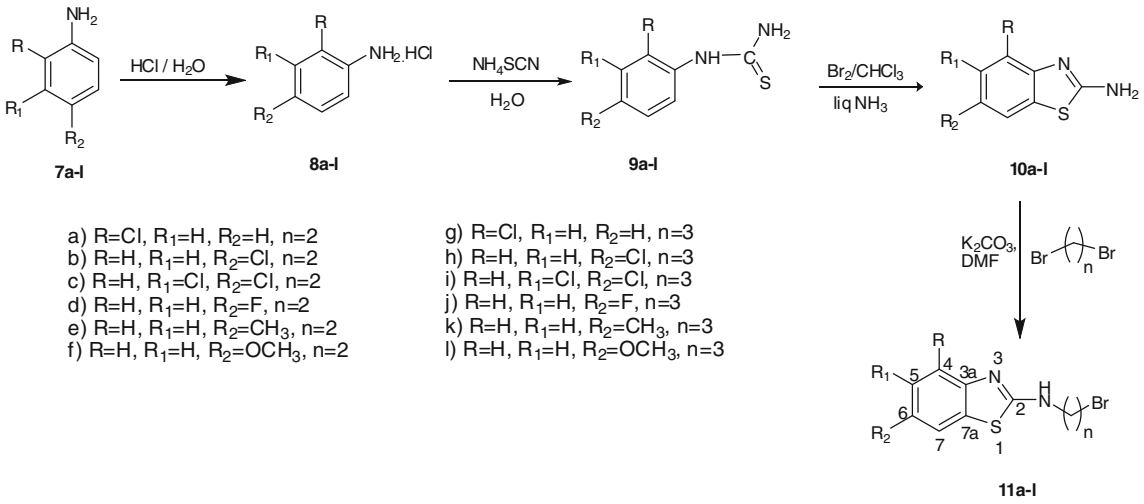
In vitro cytotoxicity

In vitro cytotoxicity of all the synthesized compounds (**11a**–**11l**) was assessed by Sulforhodamine-B (SRB) assay against various human cancer cell lines. The SRB assay was performed to assess growth inhibition. This is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye. Initially cancer cells were treated with each compound for 48 h, followed by measuring cell growth rates by SRB-based spectrophotometry as described (Skehan *et al.*, 1990). Percentage growth inhibition of all the synthesized compounds were evaluated at 10, 50, and 100 µM concentration against lung (A-549), prostate (PC-3), leukemia (THP-1), and colon (Caco 2) cancer cell lines using paclitaxel, mitomycin-C, and 5-fluorouracil as positive controls. The concentration dependent cytotoxicity of these compounds against human cancer cell lines are shown in Fig. 2 and the final results expressed in terms of IC_{50} (µM, concentration that required to inhibit cancer cell proliferation by 50 % after exposure of cells to test compounds) values as shown in Table 2.

Present study reveals that among the human cancer cell lines tested, prostate (PC-3) and leukemia (THP-1) cancer cell lines are more sensitive to all the tested compounds than colon (Caco-2) and lung (A-549) cancer cell lines. As shown in Table 2, most of the tested compounds exhibit significant cytotoxic activities against all the tested cell lines. Compound **11a** bearing chlorine group at 4th position of benzothiazole ring was active only against colon (Caco-2) cancer cell line with IC_{50} value 26.9 µM. However, by increasing chain length as in compound **11g** having the same substitution, shows significant cytotoxic activity against prostate

Table 1 Reaction time and yield (%) of various products (compounds **11a**–**11l**) are:

S. no.	Compounds	Substituted benzothiazole				Reaction time (h)	Yield (%) of various products
		R	R_1	R_2	$n = 2, 3$		
1	**11a**	Cl	H	H	2	18	68
2	**11b**	H	H	Cl	2	18	70
3	**11c**	H	Cl	Cl	2	24	72
4	**11d**	H	H	F	2	18	67
5	**11e**	H	H	CH_3	2	40	66
6	**11f**	H	H	OCH_3	2	34	61
7	**11g**	Cl	H	H	3	18	70
8	**11h**	H	H	Cl	3	18	65
9	**11i**	H	Cl	Cl	3	24	74
10	**11j**	H	H	F	3	18	65
11	**11k**	H	H	CH_3	3	40	71
12	**11l**	H	H	OCH_3	3	34	65



Scheme 1 Synthesis of substituted *N*-alkylbromo-benzothiazoles

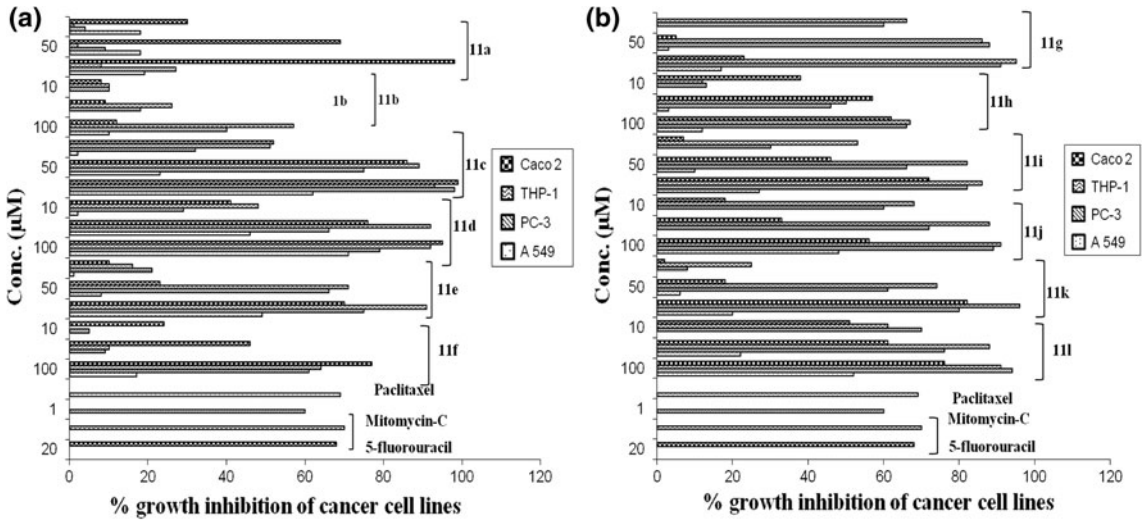


Fig. 2 Cytotoxicity of **a** *N*-ethylbromo-benzothiazoles (compounds **11a–11f**); **b** *N*-propylbromo-benzothiazoles (compounds **11g–11l**)

Table 2 IC_{50} determination of cytotoxicity of compounds (**11a–11l**) against human cancer cell lines

Compounds	IC_{50} value (μM)			
	Lung (A549)	Prostate (PC-3)	Leukemia (THP-1)	Colon (Caco-2)
11a	>100	110	110	26.9
11b	>100	99	97.8	>100
11c	85.5	24	8.46	8.4
11d	67.6	9.9	1	16.8
11e	>100	40.9	39.9	67.7
11f	>100	100	110	47.9
11g	>100	3.1	1.72	>100
11h	>100	62.8	60	33
11i	>100	28	7	63
11j	>100	2.5	0.9	93.7
11k	>100	53.3	30.3	81.6
11l	89.9	0.6	3	9.9
Paclitaxel	2.7	NT	NT	NT
5-Fluorouracil	NT	NT	NT	21
Mitomycin-C	0.4	1.5	1.5	NT

NT Not tested

(PC-3) and leukemia (THP-1) cancer cell line (IC_{50} = 3.1 and 1.72 μM, respectively); whereas it was found to be not active against colon cancer cell line. Dichloro substituted compound **11c** displayed IC_{50} = 8.4 μM against colon (Caco-2) cancer cell line, which is comparable to 5-Fluorouracil (IC_{50} = 21 μM). Compound **11c** and **11i** also possess moderate activity against leukemia (THP-1) cancer cell line with IC_{50} = 8.46 and 7 μM, respectively. Furthermore, the activity of compound **11d** and **11j** against THP-1 cell line (IC_{50} = 1 and 0.9 μM) were higher than that of Mitomycin-C (IC_{50} = 1.5 μM). Compound **11j** also exhibit significant cytotoxic activity against prostate (PC-3) cancer cell line with IC_{50} = 2.5 μM. The difference in cytotoxic activity of compounds **11g** and **11h** against prostate (PC-3) cancer cell line (IC_{50} = 3.1 and 62.8 μM, respectively) and leukemia (THP-1) cancer cell line (IC_{50} = 1.72 and 60 μM, respectively) is attributed to the change in position of chlorine group at 4th and 6th position of benzothiazole derivatives. When comparing compounds **11k** and **11l** (6-methyl vs 6-methoxy), **11l** bearing methoxy group showed good cytotoxic activity against all the tested cell lines with IC_{50} value of 0.6, 3, and 9.9 μM on prostate (PC-3), leukemia (THP-1), and colon (Caco-2) cancer cell lines, respectively. Most interestingly their activity against colon (Caco-2) cell line was higher than 5-fluorouracil. Present study showed that on elongation of the alkyl chain, cytotoxic activity of the compound increases. However, the differences in the IC_{50} values may be attributable to such factors as the nature of the *N*-alkylbromides and hydrophilic or hydrophobic substitution on 4, 5, or 6th position of benzothiazole ring.

Although in most of the cases, the exact mechanism of cytotoxic activity is not known for benzothiazole derivatives; however, the varied modes of action of benzothiazole derivatives have been reported. Previous studies have shown that the purposed mechanism of cytotoxicity of benzothiazoles is mediated via activation of arylhydrocarbon receptor (AhR) signaling pathway by translocation from the cytosol to the nucleus. Therein the induced cytochrome P450 CYP1A1 enzyme activity leads to the generation of a reactive electrophilic species that selectively generates DNA adducts, cause DNA damage, ultimately resulting in cell death by activation of apoptic machinery (Loaiza-Perez *et al.*, 2002; Trapani *et al.*, 2003;

Leong *et al.*, 2004). Docking studies confirmed that benzothiazole containing hydroxamic acid act as inhibitior of histone deacetylases (HDAC) that could be a prominent action mechanism for the cytotoxicity (Oanh *et al.*, 2011). Various benzothiazole derivatives were proposed as inhibitors of fatty acid amide hydrolase (FAAH) (Wang *et al.*, 2009), Raf kinase (Raf-1) (Song *et al.*, 2008), and B-cell lymphoma protein BCL-2 (Zheng *et al.*, 2007).

Docking study

Tyrosine kinases of the epidermal growth factor receptor (EGFR) family are involved in cancer proliferation suggest that an inhibitor which blocks the tyrosine kinase activity of the entire EGFR family, could play significant role in cancer (Mendelsohn and Baselga, 2000).

So EGFR was selected as a biological target for carrying out the docking study of our synthesized compounds to know probable mode of action. The crystal structure of EGFR kinase domain in complex with an irreversible inhibitor (PDB ID: 2J5F) was obtained from the protein data bank. The protein structure with co-crystal was given in the Fig. 3a.

The co-crystallized ligand lying within the receptor was isolated. The receptor was optimized and saved as pdb file, which was used for docking simulation. Structures of ligands were sketched by means of Chem-Draw Ultra 8.0 (http://www.cambridgesoft.com, 2012) and were exported to Weblab Pro (http://weblab-viewer-pro.fyxm.net/, 2012). Three-dimensional structures of all molecules were generated. The 3-D structures were then energetically minimized up to the rms gradient of 0.01 using Universal Force Field (UFF). Binding site was determined with the help of Ligplot (Fig. 3e). The parameter fixed for docking simulation were 50 population size, 1000 maximum generation, 0.8 crossover rate, 0.2 mutation rate, 5 elitism, 0.0015936 kcal/mol convergence, 0.06 local search rate, 0.00015936 kcal/mol local search convergence, 20 local search max steps, 0.4 grid resolution, and 15 × 15 × 15 angstroms binding site box size. The docking simulation was done using ArgusDock including flexible docking, lamarckian genetic algorithm and AScore scoring function. Docking was carried out to determine the probable mode of action of the synthesized ligands.

Docking score clears the importance of electron-releasing methoxy group and carbon linkers (Table S1, supplementary). The proposed interaction modes of the ligand with the 2J5F binding site were determined as the highest scored conformation among the thousand conformations. Hypothetical binding motif of all ligands in the enzyme pocket of 2J5F was given in the Fig. 3b. Significant hydrogen bond interaction of methoxy group of most active compound was observed in the binding pocket (Fig. 3c). Comparison of most active and least active compound shows the importance of hydrogen bond interactions with Cys797 amino acid present on binding site contributing the inhibitory activity toward the target (Fig. 3d).

Quantitative structure activity relationship

QSAR analysis applies statistical methods to describe the relationship between chemical structure and biological activities of a series of *N*-alkylbromo-benzothiazoles quantitatively. In the present study, we report the synthesis and QSAR studies of substituted benzothiazoles. The groups of calculated thermodynamic descriptors included bend energy, heat of formation, torsion energy, boiling point, melting point, Gibbs free energy, Henry's Law constant, ideal gas thermal capacity, exact mass, and molecular weight. Steric descriptors derived were steric energy, connolly accessible area, connolly molecular area, connolly solvent excluded volume, molar refractivity, and ovality apart from this partition coefficient calculated as Log *P*. Electronic descriptors included kinetic energy, potential energy, and total energy were calculated. Molecular topology descriptors included balaban index, cluster count, molecular topological index, num rotatable bonds, polar surface area, radius, shape attribute, sum of degrees, sum of valence degrees, topological diameter, total connectivity, total valence connectivity, and wiener index.

It is important to note that all these models were developed using the entire set ($n = 12$), since no outliers were identified. The quality of the models is indicated by the following parameters: r—correlation coefficient; F—Fisher's statistics; and s—standard error of estimation, r^2_{adj}—adjusted coefficient of variation r^2 obtained by 'leave-one-out' (LOO) method. Stepwise multiple linear regression analysis method was used to perform QSAR analysis employing Minitab software (Darlington, 1990). The best model was selected on the basis of various statistical parameters such as correlation coefficient (r), standard error of estimation (SEE) and sequential Fischer test (F).

The main objective of this work is to generate quantitative models using different physicochemical descriptors which can be useful in identifying potent anticancer agents. The reference drugs were not included in model generation as they belong to different structural series. The co-crystal natural substrate was taken out of the active site. The data presented in Table S2 demonstrates the feeble correlation of molecular descriptors of different substituted benzothiazoles with their anticancer activity. It will prompt us to develop QSAR model using consensus method by statistical approach.

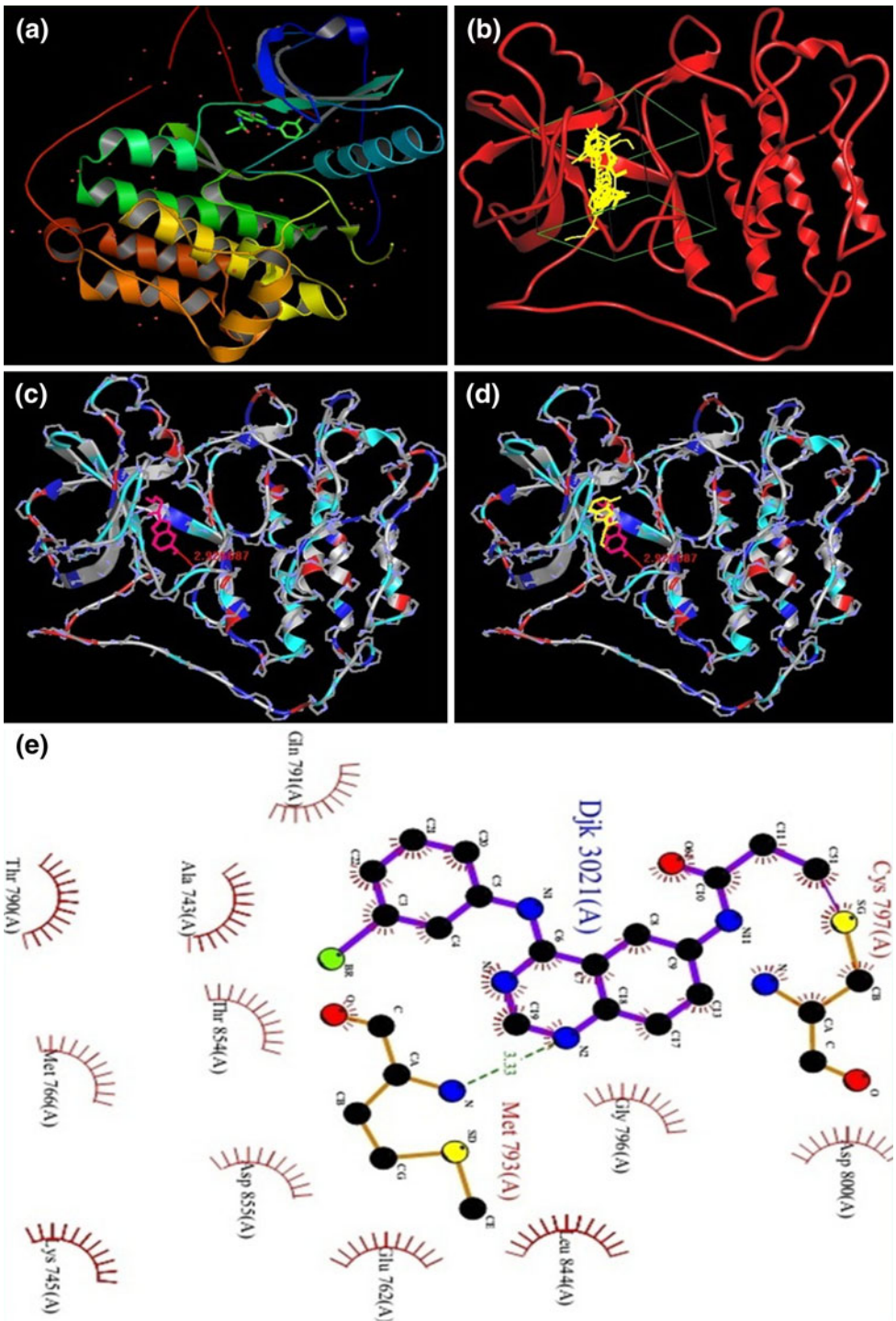


Fig. 3 **a** The 3-D structure of Tyrosine kinase in the ribbon form with co-crystal (PDB ID: 2J5F); **b** hypothetical binding motif of all ligands in the enzyme pocket of 2J5F; **c** binding orientation of compound **11l** (most active) showing hydrogen bond interaction with Cys797 at distance of 2.9 Å; **d** comparison of most active (**11l**, *pink color*) and least active (**11a**, *yellow color*) compound in the enzyme pocket of 2J5F; **e** *ligplot* of 2J5F showing the active amino acid present at the binding site (Color figure online)

$$
\begin{aligned}
p\mathrm{IC}_{50} = {} & (3.542 - (0.3166 \times \text{Torsion energy}) \\
& - (1.296 \times \text{Topological diameter}) \\
& + (0.2111 \times \text{Sum of valence degrees}) \\
& + (0.8112 \times \text{Num rotatable bonds}) \\
& + (0.0076 \times \text{Ideal gas thermal capacity})) \quad \text{(M1)}
\end{aligned}
$$

$$
s = 0.0425, \quad r^2 = 99.87, \quad r^2_{\text{adj}} = 99.72,
$$
$$
\text{PRESS} = 0.062391, r^2_{\text{pred}} = 99.13
$$

The consensus of six types of descriptors torsion energy, topological diameter, sum of valence degrees, radius, num rotatable bonds, and ideal gas thermal capacity was found to be very significant in generating the predictive equation.

As shown in the Model 1 (M1), the inhibitory activity is proportional to the sum of valence degrees, it means higher the sum of valence degrees more the inhibitory activity as in the case of ligand number **11a**, **11b**, **11c**, and **11d**. Torsion energy has not favorable contribution toward the binding affinity i.e., high torsion energy leads to repress the activity. Topological diameter has an unfavorable contribution as evidenced by negative regression coefficient. Num rotatable bonds have a favorable contribution toward the binding affinity as in the case of ligand number **11i**, **11j**, and **11k**.

The above model explains 99.72 % of variance (adjusted coefficient of variation-r^2_{adj} and 99.87 % of r^2 which indicates the robustness of fit (Fig. 4a) and the predicted potential was further validated by predicted r^2 (r^2_{pred}) (Table S3).

$$\begin{aligned} p\mathrm{IC}_{50} = {} & (1.819 - (0.179 \times \text{Torsion energy}) \\ & + (0.236 \times \text{Sum of valence degrees}) \\ & - (1.68 \times \text{Topological diameter}) \\ & + (1.86 \times \text{Radius}) + (1.27 \times \text{Num rotatable bonds}) \\ & + (0.007 \times \text{Ideal gas thermal capacity}) \\ & - (0.00121 \times \text{Molecular topological index})) \end{aligned} \quad \text{(M2)}$$

$$s = 0.205, \quad r^2 = 97.18, \quad r^2_{adj} = 92.25, \quad \mathrm{PRESS} = 0.823715, \quad r^2_{pred} = 86.19$$

As shown in the Model 2 (M2), torsion energy and topological diameter has adverse contribution toward binding affinity. Sum of valence degree has a positive contribution, can be understood as in the case of ligand number **11j**, **11k**, and **11l**. Num rotatable bond and radius has played a positive role toward binding affinity as evidenced by positive correlation. Molecular topological index has decreased the activity as in the case of ligand number **11f** and **11g**. The low residual activity values observed (Table S4), justify the selection of the linear regression model expressed by Eq. M2. Further the plot of linear regression predicted values against the observed values also favors the model expressed by Fig. 4b.

The r^2, r^2_{adj} and r^2_{pred} was found to be 97.18, 92.25, and 86.19 respectively, shows the reliability of above explained model.

QSAR models could not be developed for A549 cancer cell line, while for the Caco-2 cancer cell line there is low quality of predictive ability. In this case, the influences of the synthesized compounds are quite different, making the unsatisfactory QSAR models.

The statistical quantities of different models are given in Table-S5, explain the model quality, internal and external validation parameters.

On comparing the compounds **11b** (R_2 = Cl), **11d** (R_2 = F), **11h** (R_2 = Cl), and **11j** (R_2 = F), QSAR models suggest that cytotoxic activity increases in the order of **11b** < **11d** and **11h** < **11j**, which is the same order as the substituent's electron withdrawing ability as shown in the Fig. 5. However, two chlorine atoms substituted compound **11c** and **11i** exhibited increased activity in PC-3, THP-1, and Caco-2 cancer cell lines than compounds **11b** and **11h**. Among the electron-releasing group-substituted compounds such as **11k** with a methyl group at the 6-position did not show good cytotoxic activity; however, compound **11l** having a stronger electron-releasing methoxy group showed highest activity. Whereas, when compounds **11l** and **11j** (6-methoxy vs 6-fluorine) were compared, **11l** displayed higher cytotoxic activity against prostate and colon cancer cell lines and **11j** displayed highest cytotoxic activity against leukemia cancer cell line. These results suggest that compounds bearing stronger electron-releasing (methoxy) or withdrawing (fluorine) substituents show improved cytotoxic activity against their particular cancer cell lines. Furthermore, the cytotoxic activity increases by increasing carbon linkers between nitrogen and bromo group as evidenced by compounds **11g**, **11h**, **11i**, **11j**, **11k**, and **11l** which showed maximum growth inhibition in case of prostate (PC-3) and leukemia (THP-1) cancer cell lines while the compounds **11a**, **11b**, **11c**, **11d**, **11e**, and **11f** presented moderate growth inhibition.

Validation methods

QSAR modeling is done to develop model, which should be strong enough to be capable for reliable predictions of biological activities of new ligands. Robustness of QSAR

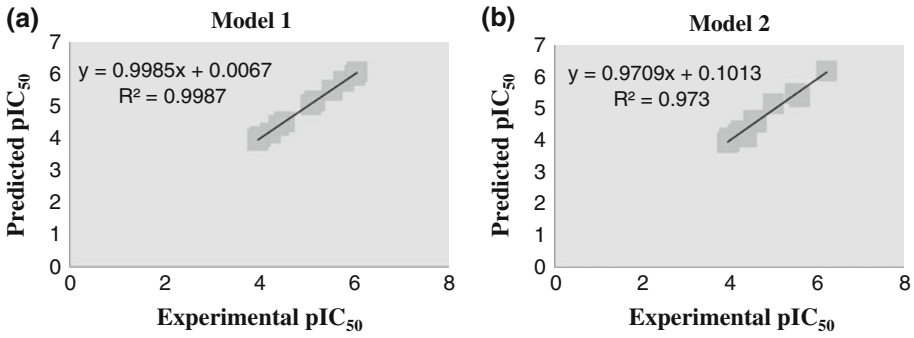


Fig. 4 Correlation between experimental and predicted activity using **a** model 1 and **b** model 2

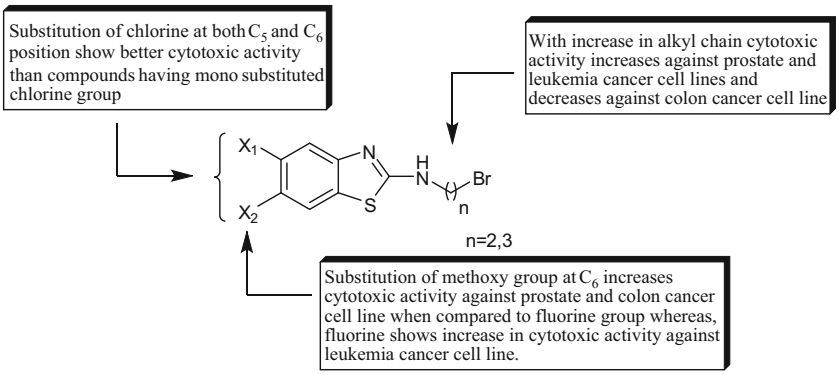


Fig. 5 Structure activity relationship of *N*-alkylbromo-benzothiazoles (**11a–11l**)

models is generally verified by means of different types of validation criteria such as (i) internal validation or cross-validation, (ii) validation by dividing the dataset into training and test compounds, (iii) data randomization or Y-scrambling, and (iv) true external validation by application of model on new external data (Roy, 2007). Prediction error sum of squares (PRESS) is a standard index to measure the accuracy of a modeling method based on the cross-validation technique (Roy, 2007; Roy and Roy, 2008)

Conclusion

In present report, we describe synthesis and in vitro cytotoxic evaluation of a series of *N*-alkyl-bromo benzothiazoles. Some of these derivatives exhibit potent cytotoxic activity. The benchmark active compound was **11l** (3-bromo-propyl-6-methoxy-benzothiazol-2-yl-amine) shows significant cytotoxic activity on PC-3, THP-1, and Caco-2 cancer cell lines confirmed by their IC_{50} values as 0.6, 3, and 9.9 μM, respectively. Other compounds exhibited IC_{50} value of ≤ 1 μM were **11d** and **11j** against leukemia (THP-1) cancer cell line. These molecules shall serve as useful "lead" for further anticancer drug development.

On the comparison of docking score of synthesized ligand with inhibitory activity, it may be concluded that ligand may be acted by inhibiting tyrosine kinase of EGFR family to have anticancer activity.

Further the analysis of multiple stepwise linear regression model give an insight into characteristic feature and the result suggests that the inhibitory potency of benzothiazole analogs is increased by augmenting the carbon chain length. The models were validated using Leave-one-out procedure. The QSAR methodology can greatly help this effort being firmly founded on physicochemical and statistical basis. Therefore, developed QSAR models could be used to predict the range of activities for new anticancer agents and play a significant role to facilitate the process of design of new potent anticancer agents.

Experimental

General

Melting points (mp) were taken in open capillaries on the Veego VMP-PM melting point apparatus. The ^{1}H and ^{13}C NMR-spectra were recorded on a JEOL-JNM—300-spectrometer at 300 and 75 MHz respectively, in $CDCl_3$ as solvent. Chemical shifts were expressed as δ values relative to TMS as internal standard. IR spectra were recorded on Shimadzu 8400 S FT-IR spectrophotometer with KBr pellets. Mass spectra were reported on Shimadzu GCMS-QP-2000A mass spectrograph. The progress of the reaction was monitored on silica-gel plates using hexane: ethyl acetate as solvent. Iodine was used as a developing agent. Chromatographic purification was carried out using silica gel (60–120 mesh).

Chemistry

*General procedure for the synthesis of N-γ-bromo-alkyl-(benzothiazol-2-yl)-amines (compound **11a–11l**)*

All the synthesized compounds were prepared according to the following two-step procedures:

Step 01: The intermediates **10a–10l** was prepared according to the previous reported procedure with certain modifications (Saeed *et al.*, 2010). Equimolar quantities of substituted aniline (25 ml) and concentrated hydrochloric acid (25 ml) were warmed for 30 min. A saturated solution of ammonium thiocyanate in water (30 g in 60 ml) was added slowly in above solution, and then the mixture was boiled until the solution got turbid. The turbid solution was poured in cold water. The resulting precipitate was filtered and re-crystallized from aqueous ethanol to get pure phenylthiourea. The solution of substituted phenylthiourea (26 mmol) in chloroform (50 ml) was brominated using bromine solution in chloroform (5 %) till the orange-yellow color appeared. After completion of total addition of

bromine stirring was continued for a period of 4 h. The slurry was kept overnight in freezer. The precipitate obtained was filtered and washed with sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The precipitate, as hydrobromide, was dissolved in rectified spirit (150 ml) and basified with ammonia solution. The precipitate was filtered, washed with water, dried and re-crystallized using rectified spirit to afford 2-aminobenzothiazole (compound **10a**–**10l**).

Step 02: To a solution of 2-aminobenzothiazoles (compound **10a**–**10l**) in DMF, K_2CO_3 (1.6 equiv.) was added slowly and the mixture was stirred vigorously for 15 min at room temperature. To the resulting solution, terminally substituted dibromoalkane (1 equiv.) in 2 ml of DMF was added drop wise. The reaction mixture was heated at 60 °C, and the progress of the reaction was monitored by TLC. The reaction mixture was quenched by addition of water and extracted with ethyl acetate. The organic layer was washed with brine two times and dried over anhydrous Na_2SO_4. After filtration, organic layer is concentrated under reduced pressure. Then, the crude product was purified by column chromatography using ethyl acetate/hexane (20:80) to afford the desired product compound **11**.

Characterization data for all synthesized compounds

*(2-Bromo-ethyl)-(4-chloro-benzothiazol-2-yl)-amine (compound **11a**)*

Yield: 68 %, mp: 149–153 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.79 (d, 1H, J = 4.7 Hz, ArH), 7.66 (dd, 1H, J = 8.4 Hz, 4.7 Hz, ArH), 7.42 (d, 1H, J = 8.2 Hz, ArH), 4.66 (t, 2H, J = 7.5 Hz, CH_2–NH), 4.49 (t, 2H, J = 7.5 Hz, CH_2Br). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.91 (C_2), 147.48 (C_{3a}), 129.49 (C_4), 126.54 (C_6), 124.56 (C_5), 122.44 (C_{7a}), 119.84 (C_7), 44.97 (CH_2–NH), 35.67 (CH_2Br). IR (KBr, cm^{-1}) υ: 3465.84, 1332.72 (C–NH), 1517.87, 1585.38 (C=N), 1230.50 (CH_2Br), 1033.77 cm^{-1} (Aryl-Cl). MS (*m*/*z*): 290.8 (M^+). Anal. Calcd for $C_9H_8BrClN_2S$: C, 37.07; H, 2.77; N, 9.61. Found: C, 37.11; H, 2.74; N, 9.64.

*(2-Bromo-ethyl)-(6-chloro-benzothiazol-2-yl)-amine (compound **11b**)*

Yield: 70 %, mp: 154–158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.66 (s, 1H, ArH), 7.38 (d, 1H, J = 2.4 Hz, ArH), 7.35 (d, 1H, J = 2.3 Hz, ArH), 4.65 (t, 2H, J = 8.2 Hz, CH_2–NH), 4.40 (t, 2H, J = 8.4 Hz, CH_2Br). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.28 (C_2), 138.56 (C_{3a}), 122.98 (C_4), 128.27 (C_6), 125.21 (C_5), 123.45 (C_{7a}), 121.76 (C_7), 49.28 (CH_2–NH), 32.26 (CH_2Br). IR (KBr, cm^{-1}) υ: 3396.41, 1332.72 (C–NH), 1590.19 (C=N), 1230.50 (CH_2–Br), 1101.28 cm^{-1} (Aryl-Cl). MS (*m*/*z*): 292 (M^+). Anal. Calcd for $C_9H_8BrClN_2S$: C, 37.07; H, 2.77; N, 9.61. Found: C, 37.09; H, 2.73; N, 9.57.

*(2-Bromo-ethyl)-(5,6-dichloro-benzothiazol-2-yl)-amine (compound **11c**)*

Yield: 72 %, mp: 162–166 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.48–7.21 (m, 2H, ArH), 4.29 (t, 2H, J = 7.4 Hz, CH_2–NH), 3.52 (t, 2H, J = 7.5 Hz, CH_2Br). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.21 (C_2), 142.16 (C_{3a}), 131.02 (C_6), 126.65 (C_5), 123.23 (C_7), 121.46 (C_4), 106.95 (C_{7a}), 48.75 (CH_2–NH), 29.80 (CH_2Br). IR (KBr, cm^{-1}) υ: 3343, 1321.44 (C–NH), 1641.31 (C=N), 1248.01 (CH_2Br), 1113.03 cm^{-1} (Aryl-Cl). MS (*m*/*z*): 292 (M^+). Anal. Calcd for $C_9H_7BrCl_2N_2S$: C, 33.15; H, 2.16; N, 8.59. Found: C, 33.12; H, 2.19; N, 8.63.

*(2-Bromo-ethyl)-(6-fluoro-benzothiazol-2-yl)-amine (compound **11d**)*

Yield: 67 %, mp: 142–145 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.31–7.06 (m, 3H, ArH), 4.78 (t, 2H, J = 6.8 Hz, CH_2–NH), 4.53 (t, 2H, J = 6.9 Hz, CH_2Br). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.91 (C_2), 155.92 (C_6), 141.56 (C_{3a}), 115.76 (C_{7a}), 112.62 (C_4), 109.93 (C_5), 100.01 (C_7), 57.21 (CH_2–NH), 35.02 (CH_2Br). IR (KBr, cm^{-1}) υ: 3360.11, 1312.46 (C–NH), 1625.88 (C=N), 1230.50 (Aryl-F), 1211.22 cm^{-1} (CH_2Br). MS (*m*/*z*): 276 (M^+). Anal. Calcd for $C_9H_8BrFN_2S$: C, 39.29; H, 2.93; N, 10.18. Found: C, 39.32; H, 2.95; N, 10.15.

*(2-Bromo-ethyl)-(6-methyl-benzothiazol-2-yl)-amine (compound **11e**)*

Yield: 66 %, mp: 132–135 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.63 (d, 1H, J = 3.6 Hz, ArH), 7.57 (s, 1H, ArH), 7.51 (d, 1H, J = 3.4 Hz, ArH), 4.65 (t, 2H, J = 6.9 Hz, CH_2–NH), 4.44 (t, 2H, J = 6.8 Hz, CH_2Br), 2.46 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.72 (C_2), 138.13 (C_{3a}), 133.85 (C_6), 127.56 (C_5), 121.13 (C_7), 120.88 (C_{7a}), 120.40 (C_4), 49.94 (CH_2–NH), 35.37 (CH_2Br), 21.13 (CH_3). IR (KBr, cm^{-1}) υ: 3310.05, 1323.81 (C–NH), 2923.45 (sp^3-C), 1618.17 (C=N), 1235.11 cm^{-1} (CH_2Br). MS (*m*/*z*): 270 (M^+). Anal. Calcd for $C_{10}H_{11}BrN_2S$: C, 44.29; H, 4.09; N, 10.33. Found: C, 44.32; H, 4.11; N, 10.35.

*(2-Bromo-ethyl)-(6-methoxy-benzothiazol-2-yl)-amine (compound **11f**)*

Yield: 60 %, mp: 158–160 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.89–7.62 (m, 3H, ArH), 4.65 (t, 2H, J = 7.8 Hz, CH_2–NH), 4.34 (t, 2H, J = 7.8 Hz, CH_2Br),

3.74 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.32 (C_2), 154.27 (C_6), 143.04 (C_{3a}), 121.88 (C_{7a}), 120.31 (C_4), 111.17 (C_5), 96.21 (C_7), 56.95 (O–CH_3), 56.63 (CH_2–NH), 38.69 (CH_2Br). IR (KBr, cm^{-1}) υ: 3431.13, 1328.86 (C–NH), 2937.38 (sp^3-C), 1598.88 (C=N), 1267.14 cm^{-1} (CH_2Br). MS (*m/z*): 286.9 (M^+). Anal. Calcd for $C_{10}H_{11}BrN_2OS$: C, 41.82; H, 3.86; N, 9.75. Found: C, 41.86; H, 3.80; N, 9.81.

*(3-Bromo-propyl)-(4-chloro-benzothiazol-2-yl)-amine (compound **11g**)*

Yield: 70 %. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.61 (d, 1H, $J = 4.5$ Hz, ArH), 7.50 (dd, 1H, $J = 7.8$ Hz, 4.8 Hz, ArH), 7.41 (d, 1H, $J = 7.6$ Hz, ArH), 4.45 (t, 2H, $J = 5.4$ Hz, CH_2–NH), 4.26 (t, 2H, $J = 6.0$ Hz, CH_2Br), 2.35–2.28 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.24 (C_2), 142.58 (C_{3a}), 125.56 (C_4), 125.11 (C_6), 122.24 (C_5), 120.63 (C_{7a}), 119.24 (C_7), 49.93 (CH_2–NH), 37.24 (CH_2), 29.98 (CH_2Br). IR (KBr, cm^{-1}) υ: 3350.08, 1282.14 (C–NH), 1607.67 (C=N), 1242.43 (CH_2Br), 1042.28 cm^{-1} (Aryl-Cl). MS (*m/z*): 305 (M^+). Anal. Calcd for $C_{10}H_{10}BrClN_2S$: C, 39.30; H, 3.30; N, 9.17. Found: C, 39.34; H, 3.27; N, 9.15.

*(3-Bromo-propyl)-(6-chloro-benzothiazol-2-yl)-amine (compound **11h**)*

Yield: 65 %. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.25 (s, 1H, ArH), 7.22 (d, 1H, $J = 2.3$ Hz, ArH), 7.20 (d, 1H, $J = 2.4$ Hz, ArH), 4.48 (t, 2H, $J = 5.4$ Hz, CH_2–NH), 4.25 (t, 2H, $J = 6.0$ Hz, CH_2Br), 2.33–2.24 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.51 (C_2), 144.24 (C_{3a}), 131.42 (C_6), 123.45 (C_5), 123.13 (C_{7a}), 122.91 (C_4), 122.05 (C_7), 49.10 (CH_2–NH), 35.24 (CH_2), 29.26 (CH_2Br). IR (KBr, cm^{-1}) υ: 3361, 1300.26 (C–NH, 1627.81 (C=N), 1220.18 (CH_2Br), 1120.06 cm^{-1} (Aryl-Cl). MS (*m/z*): 306 (M^+). Anal. Calcd for $C_{10}H_{10}BrClN_2S$: C, 39.30; H, 3.30; N, 9.17. Found: C, 39.33; H, 3.32; N, 9.20.

*(3-Bromo-propyl)-(5,6-dichloro-benzothiazol-2-yl)-amine (compound **11i**)*

Yield: 74 %. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.51 (s, 1H, ArH), 7.35 (s, 1H, ArH), 4.16 (t, 2H, $J = 6.3$ Hz, CH_2–NH), 4.40 (t, 2H, $J = 7.5$ Hz, CH_2Br), 2.26–2.18 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 156.58 (C_2), 141.28 (C_{3a}), 129.90 (C_6), 127.73 (C_5), 121.98 (C_7), 121.52 (C_4), 106.20 (C_{7a}), 46.26 (CH_2–NH), 35.23 (CH_2), 29.75 (CH_2Br). IR (KBr, cm^{-1}) υ: 3348.19, 1319.22 (C–NH), 1618.17 (C=N), 1213.14 (CH_2Br), 1100.28 cm^{-1} (Aryl-Cl). MS (*m/z*): 341 (M^+). Anal. Calcd for $C_{10}H_9BrCl_2N_2S$: C, 35.32; H, 2.67; N, 8.24. Found: C, 35.29; H, 2.64; N, 8.20.

*(3-Bromo-propyl)-(6-fluoro-benzothiazol-2-yl)-amine (compound **11j**)*

Yield: 65 %. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.46–7.09 (m, 3H, ArH), 4.46 (t, 2H, $J = 6.1$ Hz, CH_2–NH), 4.26 (t, 2H, $J = 6.3$ Hz, CH_2Br), 2.38–2.29 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.82 (C_2), 156.03 (C_6), 139.71 (C_{3a}), 120.45 (C_{7a}), 117.42 (C_4), 112.85 (C_5), 107.26 (C_7), 45.87 (CH_2–NH), 34.24 (CH_2), 30.90 (CH_2Br). IR (KBr, cm^{-1}) υ: 330.42, 1294.91 (C–NH), 1598.95 (C=N), 1273.61 (CH_2Br), 1145.23 cm^{-1} (Aryl-F). MS (*m/z*): 288 (M^+). Anal. Calcd for $C_{10}H_{10}BrFN_2S$: C, 41.54; H, 3.49; N, 9.69. Found: C, 41.57; H, 3.43; N, 9.72.

*(3-Bromo-propyl)-(6-methyl-benzothiazol-2-yl)-amine (compound **11k**)*

Yield: 71 %. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.37–7.07 (m, 3H, ArH), 3.72 (t, 2H, $J = 6.7$ Hz, CH_2–NH), 3.47 (t, 2H, $J = 6.7$ Hz, CH_2Br), 2.92–2.89 (m, 2H, CH_2), 2.39 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.21 (C_2), 142.46 (C_{3a}), 135.49 (C_6), 122.47 (C_5), 121.67 (C_7), 121.22 (C_{7a}), 120.98 (C_4), 44.94 (CH_2–NH), 29.93 (CH_2), 21.47 (CH_2Br), 20.76 (CH_3). IR (KBr, cm^{-1}) υ: 3362.45, 1341.12 (C–NH), 2800.08 (sp^3–C), 1605.28 (C=N), 1219.32 cm^{-1} (CH_2Br). MS (*m/z*): 286 (M^+). Anal. Calcd for $C_{11}H_{13}BrN_2S$: C, 46.32; H, 4.59; N, 9.82. Found: C, 46.28; H, 4.54; N, 9.93.

*(3-Bromo-propyl)-(6-methoxy-benzothiazol-2-yl)-amine (compound **11l**)*

Yield: 65 %. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.69–6.98 (m, 3H, ArH), 3.59 (t, 2H, $J = 7.0$ Hz, CH_2–NH), 3.33 (t, 2H, $J = 7.2$ Hz, CH_2Br), 2.70–2.66 (m, 2H, CH_2), 2.35 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.93 (C_2), 149.93 (C_6), 132.21 (C_{3a}), 120.64 (C_{7a}), 120.49 (C_4), 108.24 (C_5), 96.53 (C_7), 56.80 (O–CH_3), 46.18 (CH_2–NH), 34.73 (CH_2), 29.63 (CH_2Br). IR (KBr, cm^{-1}) υ: 3421.48, 1379.01 (C–NH), 2848.67 (sp^3–C), 1618.17 (C=N), 1267.14 (CH_2Br), 1215.07 (C–O). MS (*m/z*): 300.8 (M^+). Anal. Calcd for $C_{11}H_{13}BrN_2OS$: C, 43.86; H, 4.35; N, 9.30. Found: C, 43.82; H, 4.32; N, 9.34.

Biological assay

For evaluating cytotoxicity, the compounds were dissolved in DMSO and stock solution of 2×10^4 μM was prepared. Stock solutions were further diluted with complete growth medium supplemented with 50 μg/ml gentamicin to obtain test concentrations of 10, 50, and 100 μM. 5-fluorouracil and paclitaxel were dissolved in DMSO and stock solution of 2×10^3 μM was prepared. Mitomycin-C was dissolved

in double-distilled water and stock solution of 2×10^3 μM was prepared. Stock solutions were further diluted with complete growth medium supplemented with 50 μg/ml gentamicin to obtain desired concentrations. All the cells were maintained in RPMI-1640 medium, supplemented with fetal bovine serum (10 %), 100 U/ml penicillin and 100 μg/ml streptomycin (complete medium). The cells were seeded into 96 well cell culture plates (1×10^4 cells/100 μl/well) and incubated in CO_2 incubator (37 °C, 5 % CO_2, 95 % relative humidity) for 24 h. After 24 h, compounds **11a–11l** and positive controls (100 μl/well) were added in quadruplets, and the plates were further incubated in CO_2 incubator for 48 h. Suitable controls were also included in each experiment. After 48 h chilled trichloroacetic acid (50 % w/v, 50 μl) was laid gently on top of the medium in all the wells. The plates were incubated at 4 °C for 1 h to fix the cells. All the contents of the wells were gently pipette out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium, low molecular weight metabolites, and serum proteins, etc. The plates were air-dried. Sulforhodamine-B (0.4 % SRB in 1 % acetic acid, 100 μl/well) was added to each well of the 96 well plates for 30 min. Excess of the dye was washed off using 1 % acetic acid, and the plates were air-dried. Tris buffer (10 mM, pH 10.5, 100 μl/well) was added to each well and plates were shaken on a mechanical stirrer for 10 min and O. D. was recorded on ELISA reader at 540 nm. Viability of cells was evaluated by trypan blue exclusion method immediately before setting up the experiment for cytotoxicity determination. Cells with >98 % viability were used in the assay.

Binding energy calculation using ArgusLab

It is the software used for calculating the binding energies of the ligands with receptors i.e., proteins. Dock score is obtained in the form of binding energy (kcal/mol). Docking is done in two steps: Optimization of geometry and then docking with Argusdock. After adding hydrogen atoms, ligand molecules were minimized using the Universal Force Field (UFF) implemented in Arguslab. For docking tests, ArgusDock was evaluated using Ascore scoring function with grid resolution 0.4 Å. Furthermore, size of the binding site bounding box was determined automatically using Arguslab (22.351 × 15.765 × 13.957 Å). Docked poses with energy were displayed in the molecule tree view window.

Minitab software

The prediction models were developed using the stepwise multiple linear regression method (MINITAB software) based on forward selection and backward elimination techniques for inclusion and rejection of descriptors. The selection of the significant descriptors for developing the model was done using "stepping criteria" (F) with $F = 4$ for inclusion and $F = 3.9$ for exclusion (Darlington, 1990; Mitra *et.al.*, 2009).

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