Synthesis and Evaluation of Novel Benzothiazole Derivatives against Human Cervical Cancer Cell Lines

SUVARNA KINI*, S. P. SWAIN AND A. M. GANDHI
Dept. of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, MAHE, Manipal-576 104, India.

The 2-arylsubstituted benzothiazole derivatives were synthesized by refluxing o-aminothiophenol with substituted benzoic acids in the presence of polyphosphoric acid at 220°. 2-Mercaptothiazole was used along with thionyl chloride to get the carbothioates. The physical and spectral data such as mp, R_f, IR, NMR was obtained for the synthesized compounds and the structures were confirmed. The screening for antitumour activity was done as per the National Cancer Institute drug screening strategy 3 compounds were found to be significantly cytotoxic as compared to [2-(3-bromo-4-aminophenyl) benzothiazole] against the human cervical cancer cell lines.

The substituted benzothiazole derivatives have antitumor[1], vasodilator[2], antitubercular[3], antifungal[4], CNS[5] activities. The 2-(4-aminophenyl) benzothiazoles are a novel class of potent and selective antitumor agents and display a characteristic profile of cytotoxicity response across the cell lines; sensitive cell lines show GI_{50} values $<10^{-8}$ M and insensitive cell lines $>10^{-4}$ M[6]. Since they display potent and selective antitumor activity against interalia breast, ovarian, colon and renal cell lines, we thought it worthwhile to check for their antitumor activity against human cervical cancer cell lines. A compound NCB [2-(3-bromo-4-aminophenyl) benzothiazole showed substantial *in-vitro* activity against breast cancer cell lines MCF-7 (ER^+)[6] NCB, 2-arylsubstituted benzothiazoles and their carbothioates with various substituents on the phenyl ring were therefore synthesized. Comparison of antitumor activities of the synthesized compounds against human cervical cancer cell lines was performed by *in vitro* cytotoxicity study on Ehrlich Ascitic Carcinoma (Tryphan blue exclusion assay) and MTT cell proliferation assay according to the protocol of ATCC (American Type Cell Culture collection) using human cervical cancer cell lines (SiHa). *In vivo* cytotoxicity study on Ehrlich Ascitic Carcinoma induced Swiss albino mice was also performed.

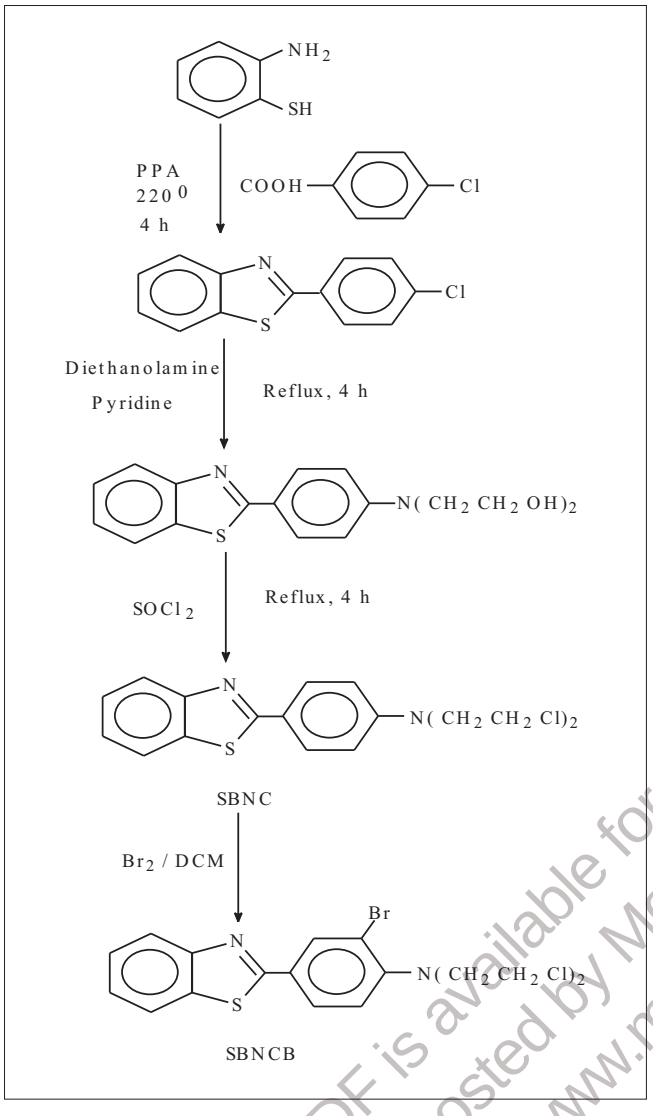
MATERIALS AND METHODS

Melting points were determined on a Toshniwal Scientific melting point apparatus and are uncorrected; UV spectra were recorded on UV-1601 PC, UV/Vis spectrophotometer (Shimadzu). IR spectra were recorded in KBr disc on a FTIR 8300, KBr Press (Shimadzu) spectrometer at MCOPS, Manipal. ^{1}H NMR spectra (DMSO-d_6) were obtained from IISc Bangalore. The physical data of the derivatives are listed in Table 1. The scheme of synthesis is given in Schemes 1, 2 and 3.

2-(2-chloro-4-fluorophenyl)-benzothiazole (SBCF):
Equimolar quantities of o-aminothiophenol (0.04 mol) and 2-chloro-4-fluorobenzoic acid were added to 15 g of polyphosphoric acid and refluxed for 4 h at 220°. The reaction mixture was cooled and poured in ice cold 10% sodium carbonate solution. The precipitate was filtered and recrystallized from methanol. IR (KBr) cm^{-1}: 3050.0 (ArH), 1593.1 (C=C), 1311.5 (C-N), 1691.5 (C=N), 1490.90 (C-C), 1047.3 (C-F), 719.4 (C-Cl), ^{1}H NMR (DMSO-d_6): δ ppm 7.18- 8.26 (m, 7H, ArH).

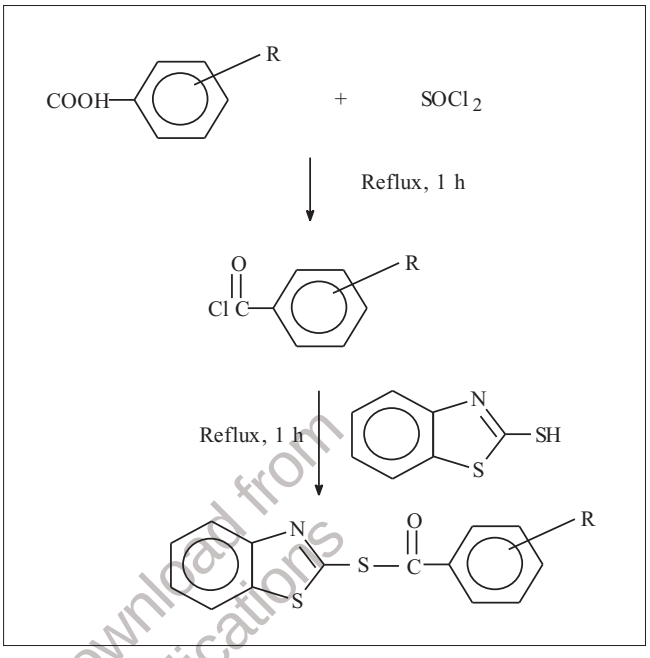
2-[4-(N,N-dichloroethylamino) phenyl] benzothiazole (SBNC):
Equimolar quantities of o-aminothiophenol (0.04 mol) and 4-chlorobenzoic acid were added to 15 g of polyphosphoric acid and refluxed for 4 h at 220°. The reaction mixture was cooled and poured in ice cold 10% sodium carbonate solution. The precipitate was filtered and recrystallized from methanol to get 2-(4'-chlorophenyl)-benzothiazole. 0.01 mol of 2-(4'-chlorophenyl)-benzothiazole and 0.01 mol of

***For correspondence**
E-mail: suvarna.gk@manipal.edu



Scheme 1: Synthesis of novel benzothiazole derivatives

diethanolamine were dissolved in 25 ml of pyridine and refluxed for 4 h, cooled and poured in cold water. The mixture was filtered after 1 h and the precipitate recrystallized from methanol to get N-[4-(1,3-benzothiazol-2-yl)phenyl]-N,N-bis-(2-hydroxyethyl)amine. 0.01 mol of N-[4-(1,3-benzothiazol-2-yl)phenyl]-N,N-bis-(2-hydroxyethyl)amine was refluxed with 0.03 mol of thionyl chloride for 4 h. The excess of thionyl chloride was removed by distilling with benzene. After



Scheme 2: Synthesis of novel benzothiazole derivatives

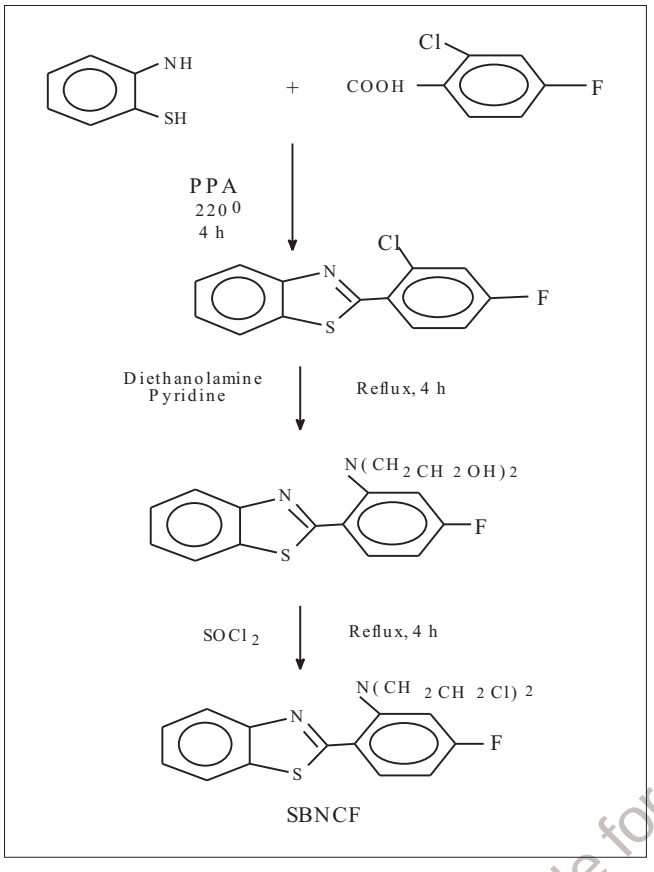
distillation, the residue was collected, washed with cold water and recrystallized from ethanol. IR (KBr) cm^{-1}: 3053.1 (ArH), 1544.9 (C=C), 1313.4 (C-N), 1649.0 (C=N), 1433.0 (C-C), 2854.5 (C-H), 2920.0 (C-Cl). ^1H NMR (DMSO-d_6): δ ppm 7.20- 8.23 (m,8H, ArH), 2.5-3.5 [m, 8H, 2 (CH_2 CH_2)]

2-[3-bromo-4-(N,N-dichloroethylamino) phenyl] benzothiazole (SBNCB):

Equimolar quantity of 2-[4-(N,N-dichloroethylamino) phenyl] benzothiazole (0.04 mol) was dissolved in 50 ml of dichloromethane and bromine in 10 ml of dichloromethane was added to it. The reaction mixture was vigorously stirred for 2 min at -5°. Then 400 ml of cold water was added to it and stirred for 1 h the organic layer was shaken with sodium thiosulfate solution, washed several times with water and evaporated to get the crude product which was recrystallized from ethanol. IR (KBr) cm^{-1}: 3057.1 (ArH), 1560.3 (C=C), 1311.5 (C-N), 1652.9 (C=N), 1425.3 (C-C), 2856.4 (C-H), 719.4 (C-Cl), 530.4 (C-Br). ^1H NMR (DMSO-d_6): δ ppm 7.15-8.27 (m,7H, ArH), 2.5-3.8 [m, 8H, 2 (CH_2 CH_2)].

TABLE 1: PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS

Compound name	Yield (%)	M.P. (°)	R_f	λ_{max}	Molecular formula
SBCF	74	105	0.9	295	$C_{13}H_7NSCl_2F$
SBNCF	82	110	0.88	295	$C_{17}H_{16}N_2SCl_2F$
SBNC	63	111	0.91	341	$C_{17}H_{16}N_2SCl_2$
SBNCB	72	114	0.89	304	$C_{17}H_{16}N_2SCl_2Br$
SBSNO	76	180	0.62	257	$C_{14}H_8N_2S_2O_3$
SBSNH	42	140	0.5	306	$C_{14}H_{10}N_2S_2O$



Scheme 3: Synthesis of novel benzothiazole derivatives

2-[2-(N,N-dichloroethylamino)-4-fluorophenyl)-benzothiazole (SBNCF):

Equimolar quantities of o-aminothiophenol (0.04 mol) and 2-amino-4-fluorobenzoic acid were added to 15 g of polyphosphoric acid and refluxed for 4 h at 220°. The reaction mixture was cooled and poured in ice cold 10% sodium carbonate solution. The precipitate was filtered and recrystallized from methanol to get 2-(2'-amino-4'-fluorophenyl)-benzothiazole. 2-(2'-Amino-4'-fluorophenyl)-benzothiazole (0.01 mol) and 0.01 mol of diethanolamine were dissolved in 25 ml of pyridine and refluxed for 4 h, cooled and poured in cold water. The mixture was filtered after 1 h and the precipitate recrystallized from methanol to get 2-[2-(N,N-dihydroxyethylamino)-4-fluorophenyl)-benzothiazole. 2-[2-(N,N-Dihydroxyethylamino)-4-fluorophenyl)-benzothiazole (0.01 mol) was refluxed with 0.03 mol of thionyl chloride for 4 h. The excess of thionyl chloride was removed by distilling with benzene. After distillation, the residue was collected, washed with cold water and recrystallized from ethanol. IR (KBr) cm^{-1}: 3058.9 (ArH), 1595.0 (C=C), 1311.5 (C-N), 1695.3 (C=N), 1425.3 (C-C), 2922.0, 2822.5 (C-H), 1047.3 cm^{-1} (C-F). ^{1}H NMR (DMSO-d_6): δ ppm 7.13- 8.22 (m,7H, ArH), 2.5-3.9 [m, 8H, 2 (CH_2 CH_2)].

1,3-benzothiazol-2-yl-4-nitrobenzene carbothiaote (SBSNO):

A quantity equivalent to 0.01 mol of 4-nitrobenzoic acid and 0.04 mol of thionyl chloride were magnetically stirred and refluxed at 70^0 for 1 h. The excess of thionyl chloride was removed from the reaction mixture by distilling with benzene to get the acid chloride. The acid chloride (0.01 mol) and 0.01 mol of 2-mercaptobenzothiazole were dissolved in 25 ml pyridine and heated on water-bath for 15 min. The reaction mixture was cooled and poured in ice-cold water to get the precipitate that was later recrystallized from methanol. IR (KBr) cm^{-1}: 3050.0 (ArH), 1602.7 (C=C), 1348.1 (C-N), 1425.3 (C-C), 1693.4 (C=O), 1320.0, 1531.4 (N-O). ^{1}H NMR (DMSO-d_6): δ ppm 7.13-8.28 (m, 8H, ArH).

1,3-benzothiazol-2-yl-4-aminobenzene carbothiaote (SBSNH):

A quantity equivalent to 0.01 mol of 4-aminobenzoic acid and 0.04 mol of thionyl chloride were magnetically stirred and refluxed at 70° for 1 h. The excess of thionyl chloride was removed from the reaction mixture by distilling with benzene to get the acid chloride. 0.01 mol of the acid chloride and 0.01 mol of 2-mercaptobenzothiazole were dissolved in 25 ml pyridine and heated on water-bath for 15 min. The reaction mixture was cooled and poured in ice-cold water to get the precipitate that was later recrystallized from methanol. IR (KBr) cm^{-1}: 3024.2 (ArH), 1596.9 (C=C), 1336.6 (C-N), 1444.6 (C-C), 1703.0 (C=O), 3159.2, 3234.4 cm^{-1} (N-H). ^{1}H NMR (DMSO-d_6): δ ppm 7.14-8.22 (m, 8H, ArH).

***In vitro* cytotoxicity of compounds on EAC (Ehrlich Ascitic Carcinoma):**

All the synthesized compounds were screened for antitumor activity[7] by tryphan blue exclusion method. The compounds were dissolved in DMSO to obtain the concentration of 1000 µg/ml. An aliquot (500 µl) of the EAC cell suspension in phosphate buffer saline (1×10^{-6}cells per ml) was taken and 50 µl of the solution of the compounds was added to it. It was incubated at 37° for 4 h in 5% CO_2 atmosphere. Then 25 µl of tryphan blue solution was added to it. The dead (blue coloured) and live (no colour) cells were counted in haemocytometer. The results are expressed as % cell death (Table 2).

***In vitro* cytotoxicity studies on human cervical cell lines:**

In vitro cytotoxicity studies were carried out on human cervical cell lines (SiHa). Cell suspension (100 µl$\times10^{-6}$ cells per ml) was transferred to each well of a 96 well flat

TABLE 2: *IN VITRO* CYTOTOXICITY STUDIES ON EAC

Compound	% Cell death
SBCF	88
SBNCF	81
SBNC	91
SBNCB	92
SBSNO	44
SBSNH	90

bottom micro plate. It was incubated at 37° for 24 h in 5% CO_2. Then 10 µl of concentration of drug at different concentrations were added and incubated for 44 h. MTT solution (20 µl) was added to each well and incubated for 4 h. Purple color was developed after incubation. Isopropyl alcohol: HCl (4 N) (1:100) mixture (100 µl) was added to each well. The absorbances were noted at 570 nm and at 630 nm and compared with control. The results are expressed as % cell death at different conc. viz. 1000, 500, 250, 100 µg/ml (Table 3)

Acute toxicity studies:

The compounds were weighed and formulated into a suspension using 2% acacia. The solvent used was double distilled water. The required dose of the drugs was given daily for 9 d intraperitonially. The LD_{50} for all the compounds was found out.

***In vivo* studies on EAC induced Swiss Albino mice:**

The ascitic fluid from ascitic tumor bearing mice (donor) was injected intraperitonially to obtain ascitic tumor in the Swiss albino mice. The drug administration was started 24 h of the tumor inoculation. The drug was administered daily for 9 d and the mice were weighed on every day. The tumor response was assessed on the basis of mean survival time (MST) and % increase in life span (%ILS). %ILS={MST(treated)–MST(control)}×100/MST(control). As per the literature survey if the %ILS is more than 125% then the drug is considered to be an effective antitumor agent. For statistical analysis one way ANOVA with post hoc Scheffe's test was applied to all the parameters. (Table 4)

TABLE 3: *IN VITRO* CYTOTOXICITY STUDIES ON HUMAN CERVICAL CANCER CELL LINES

Compound	% Cell death at different conc.			
	1000 µg/ml	500 µg/ml	250 µg/ml	100 µg/ml
NCB	10	0	0	0
SBNCB	38	18	0	0
SBCF	18	2	0	0
SBSNH	25	5	0	0
SBNCF	11	0	0	0
Control	0	0	0	0

% cervical cell death produced by the benzothiazole derivatives at different concentrations. Zero indicates that no cells have died at that concentration

TABLE 4: RESPONSE OF EHRLICH ASCITIC CARCINOMA IN MICE TO BENZOTHIAZOLE DERIVATIVES

Compound	MST (Days) Mean± S.E.M	% ILS
Control	18.83 ± 0.30	100
Cisplatin	37.33 ± 0.33[a]	198.24
SBCF	25.00 ± 0.36[a]	132.76
SBNCB	32.16 ± 0.30[a]	170.79
SBSNH	29.33 ± 0.49[a]	155.76

One way ANOVA by using SPSS 9.0 computer package. [a] - values in mean±SEM, $p< 0.05$

RESULTS AND DISCUSSION

Screening for antitumor activity included measurement of % cell death of EAC by tryphan blue exclusion assay. The compounds SBCF, SBNCB, SBSNH were found to be significantly cytotoxic compared to the other derivatives. So, these were selected for *in vivo* screening of antitumor activity using Swiss mice. Cisplatin was the standard drug for comparison and it was found that SBNCB, SBCF, SBSNH had significantly increased the %ILS i.e., ILS>125%. The difference in the mean between cisplatin, SBCF, SBNCB, SBSNH groups and control groups was found to be significant ($p> 0.05$). At 1000 µg/ml, only SBNCB showed good activity on cell growth inhibition of cervical cancer cell lines. SBSNH and SBCF showed moderate cytotoxicity whereas others showed poor activity. At 500 µg/ml, the activity of all the compounds was reduced and at 250 µg/ml, no drug showed cytotoxicity.

The cytotoxicities of the compounds were compared with [2-(3-bromo-4-aminophenyl) benzothiazole] (NCB) due to similarity in structure and which is a potent antitumor drug against mammary cancer cell lines according to literature survey[1]. SBNCB, SBSNH, SBCF showed higher cytotoxicity than NCB.

The cytotoxicity of the compound containing -$N(CH_2CH_2Cl)_2$ at para position is more than the compounds having -NH_2 at para position on the phenyl ring attached to the benzothiazole. Replacing chlorine in SBCF by -$N(CH_2CH_2Cl)_2$ reduced the activity in *in vitro* assay on EAC cells to one half the original activity. Compounds with -NH_2 at meta position on the phenyl ring decrease the cytotoxicity. Compounds with more than one halogen exhibited better cytotoxicity. Meta substituted derivatives were less cytotoxic. Nitro-substituted derivatives were the least cytotoxic of the compounds synthesized.

ACKNOWLEDGEMENTS

We acknowledge the Animal Ethical Committee for giving permission to carry out the antitumor activity on animals. We are grateful to Dr. Satyamurthy, Head of Biotechnology Dept., Science Centre, Manipal for helping us with the anticancer activity against the Human cervical cancer cell lines. We also acknowledge IISc, Bangalore for providing spectral details in time.

REFERENCES

1. Dong- Fang S., Bradshaw, T.D., Wrigley, S., McCall, C.J., Lelieveld, P., Fitchner, I. and Stevens, M.F.G., **J. Med. Chem.**, 1996, 39, 3375.
2. Kochichiro, Y., Katsumi, G., Kazuya, Y., Tominori, M. and Goro, T., **J. Med. Chem.**, 1990, 33, 2192.
3. Bhusari, K.P., Khadekar, P.B., Umathe, S.N., Bahekar, R.H. and Rao, A.R., **Indian. J. Heterocycl. Chem.**, 2000, 9, 213.
4. Klein, L.L., Yeuns, C.M., Weissing, D.E., Lartey, P.A., Tonaka, S.K., Plattner, J.J. and Mulford, D.J., **J. Med. Chem.,** 1994, 37, 572.
5. Verma, R.S. and Singh, A.P., **Indian. J. Chem.**, 1998, 27B, 438.
6. Mei-Sze C., Dong- Fang S., Wrigley, S., Bradshaw, T.D., and Hutchinson, I., Shaw, P.N., Barrett, D.A., Stanley, L.A. and Stevens, M.F.G., **J. Med. Chem.,** 1999, 42, 381.
7. Goldin, A., Schepartz, S.A., Venditti, J.M. and Devita, V.T., In; Methods in Cancer Research, Vol.16, Academic Press, 1979, 165.

Accepted 21 January 2007
Revised 19 July 2006
Received 2 September 2005
Indian J. Pharm. Sci., 2007, 69 (1): 46-50