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Synthesis and Anticancer Evaluation of Novel Benzothiazole Derivatives

V. SEKAR*, P. PERUMAL, S. GANDHIMATHI, S. JAYASEELAN and V. RAJESH
Department of Pharmaceutical Chemistry, J.K.K. Nataraja College of Pharmacy, Komarapalayam-638 183, India
E-mail: vsekar74@gmail.com

In present study novel series of benzothiazole derivatives were synthesized and their anticarcinogenic efficacy was analyzed using cancer cell lines (human small cell lung carcinoma, mouse melanoma cell line and human larynx epithelial carcinoma). The compounds were synthesized by simple and facile procedures and characterized by IR, ^{1}H NMR and mass spectra study. The synthesized compounds were screened for anticarcinogenic activity using *in vitro* assays. The compounds were found to exhibit moderate anticarcinogenic activities in all cell lines. Among the synthesized six compounds, **T2** showed significant activity in all cell lines.

Key Words: Benzothiazole, Anticancer, MTT assay.

INTRODUCTION

In view of the fact, that a large number of derivatives of benzoxazoles, benzothiazoles and benzimidazoles have been found to exhibit a wide variety of pharmacological activities. From these derivatives the anticancer agents discovered in recent years, the identification of various 2-(4-aminophenyl) benzothiazoles as potent and selectively antitumor drugs against breast, ovarian, colon and renal cell lines has stimulated remarkable interest[1-10]. Moreover, there is experimental evidence suggesting a novel mechanism of action for these benzothiazole compounds and our interest in the benzothiazole nucleus, it was considered worthwhile to synthesize compounds bearing benzothiazole nucleus and their anticancer activities were screened.

EXPERIMENTAL

The melting range of the synthesized compounds was performed by LAB India visual melting point apparatus and is uncorrected. The IR spectra of the compounds were recorded on Perkin-Elmer FT-IR spectrometer with potassium bromide pellets. Mass spectra were recorded on Shimadzu GCMS QP 5000. The ^{1}H NMR and spectra of the synthesized compounds were recorded on a Bruker 300 NMR spectrometer in deuterated methanol.

General procedure for preparation of compounds (T1-T6)

Step I: A mixture of different 4-substituted aniline (0.01 mol) and potassium thiocyanate (0.01 mol) in glacial acetic acid (20 mL) was cooled and stirred. To this solution bromine (0.01 mol) was added drop wise at 10 ºC. Stirring was continued for an additional 3 h and the separated hydrobromide salt was filtered, washed with acetic acid and dried. It was dissolved in hot water and neutralized with aqueous ammonia solution (25 %), filtered, washed with water and dried, recrystallized with benzene to obtain 6- substituted-1,3-benzothiazol-2-amine[11].

Step II: To the solution of sodium cyanate (0.5 g) in minimum quantity of water, glacial acetic acid (5 mL) was added. This solution was heated with respective 6-substituted-1,3-benzothiazol-2-amine (**I**) (1.7 g, 0.01 mol) in alcohol, until the contents of the mixture became turbid and volume remained half of the original volume. The contents were added to ice cold water. The solid (**II**) obtained was filtered off and dried[12].

Step III: To a solution of compound **II** (0.01 mol) in ethanol/glacial acetic acid (25 mL) was added hydrazine hydrate (99 %, 0.01 mol). The reaction mixture was heated at reflux for 6 h and the solvent was removed under reduced pressure. The separated solid was recrystallized from ethanol (**III**)[13].

Step IV: To a solution containing 95 % ethanol and 0.05 mol potassium hydroxide (dissolved in 10 mL water) was added 0.05 mol compound **III** and 0.8 mL of carbon disulfide. The mixture was refluxed for 5-6 h, contents were concentrated and the residue obtained was dissolved in water and poured into ice cold water-acetic acid (1:1). The solidified product thus obtained was filtered, dried and recrystallized from ethanol (**IV**)[14].

Step V: Equimolar quantities of compound **IV** and thiosemicarbazide in glacial acetic acid (40 mL) was refluxed for 4-6 h. After cooling, the contents were poured into crushed ice. The resulting solid was washed with distilled water, filtered, dried in vacuum and recrystallized from warm ethanol (**V**)[13].

Compound T1 {1-(3-(6-chlorobenzo[d]thiazol-2-ylamino)-5-thioxo-1*H*-1,2,4-triazol-4(5*H*)-yl) thiourea}: m.p. 188-190 ºC; IR (KBr, ν_{max}, cm^{-1}): 3436 (Ar-CH), 2854 (C-N), 1050 (C-N-C), 997 (N-C=S), 684 (C-Cl); EI-MS m/z: 357.82; ^{1}H NMR (MeOD) (δ ppm): 9.1 (1H, s, Hetero - NH), 7.4 - 7.9 (3H, m, Ar-CH), 5.2 (1H, s, C-NH), 5.0 (1H, s, N-NH), 4.6 (2H, s, $-NH_2$).

Compound T2 {1-(3-(6-fluorobenzo[d]thiazol-2-ylamino)-5-thioxo-1*H*-1,2,4-triazol-4(5*H*)-yl) thiourea}: m.p. 148-150 ºC; IR (KBr, ν_{max}, cm^{-1}): 3412 (Ar-CH), 2921 (C-N), 1090 (C-N-C), 802 (N-C=S); EI-MS m/z: 341.37; ^{1}H NMR (MeOD) (δ ppm): 8.5 (1H, s, Hetero -NH), 7.0-7.5 (3H, m, Ar-CH), 5.2 (1H, s, C-NH), 5.0 (1H, s, N-NH), 4.6 (2H, s, $-NH_2$).

Compound T3 {1-(3-(6-bromobenzo[d]thiazol-2-ylamino)-5-thioxo-1*H*-1,2,4-triazol-4(5*H*)-yl) thiourea}: m.p. 100-102 ºC; IR (KBr, ν_{max}, cm^{-1}): 3415 (Ar-CH), 2854 (C-N), 1068 (C-N-C), 860 (N-C=S), 548 (C-Br); EI-MS m/z: 402.02; ^{1}H NMR (MeOD) (δ ppm): 9.1 (1H, s, Hetero -NH), 7.5-7.8 (3H, m, Ar-CH), 5.1 (1H, s, C-NH), 4.9 (1H, s, N-NH), 4.7 (2H, s, $-NH_2$).

Compound T4 {1-(3-(6-nitrobenzo[d]thiazol-2-ylamino)-5-thioxo-1*H*-1,2,4-triazol-4(5*H*)-yl) thiourea}: m.p. 178-180 ºC; IR (KBr, ν_{max}, cm^{-1}): 3427 (Ar-CH), 2854 (C-N), 1090 (C-N-C), 789 (N-C=S), 1452 (C-NO_2); EI-MS m/z: 368.58; ^{1}H NMR (MeOD) (δ ppm): 8.7 (1H, s, Hetero -NH), 7.0-7.2 (3H, m, Ar-CH), 5.0 (1H, s, C-NH), 4.8 (1H, s, N-NH), 4.6 (2H, s, -NH_2).

Compound T5 {1-(3-(6-methoxybenzo[d]thiazol-2-ylamino)-5-thioxo-1*H*-1,2,4-triazol-4(5*H*)-yl) thiourea}: m.p. 136-138 ºC; IR (KBr, ν_{max}, cm^{-1}): 3435 (Ar-CH), 2852 (C-N), 1254 (C-N-C), 728 (N-C=S); EI-MS m/z: 353.35; ^{1}H NMR (MeOD) (δ ppm): 9.4 (1H, s, Hetero -NH), 6.7 - 7.5 (3H, m, Ar-CH), 5.1 (1H, s, C-NH), 4.9 (1H, s, N-NH), 4.8 (2H, s, -NH_2), 3.3 (3H, s, -OCH_3).

Compound T6 {1-(3-(4,6-dinitrobenzo[d]thiazol-2-ylamino)-5-thioxo-1*H*-1,2,4-triazol-4(5*H*)-yl) thiourea}: m.p. 142-144 ºC; IR (KBr, ν_{max}, cm^{-1}): 3450 (Ar-CH), 2923 (C-N), 1126 (C-N-C), 837 (N-C=S), 1496 (C-NO_2); EI-MS m/z: 413.58; ^{1}H NMR (MeOD) (δ ppm): 9.0 (1H, s, Hetero -NH), 7.0 - 8.2 (2H, m, Ar-CH), 5.2 (1H, s, C-NH), 4.8 (1H, s, N-NH), 4.6 (2H, s, -NH_2).

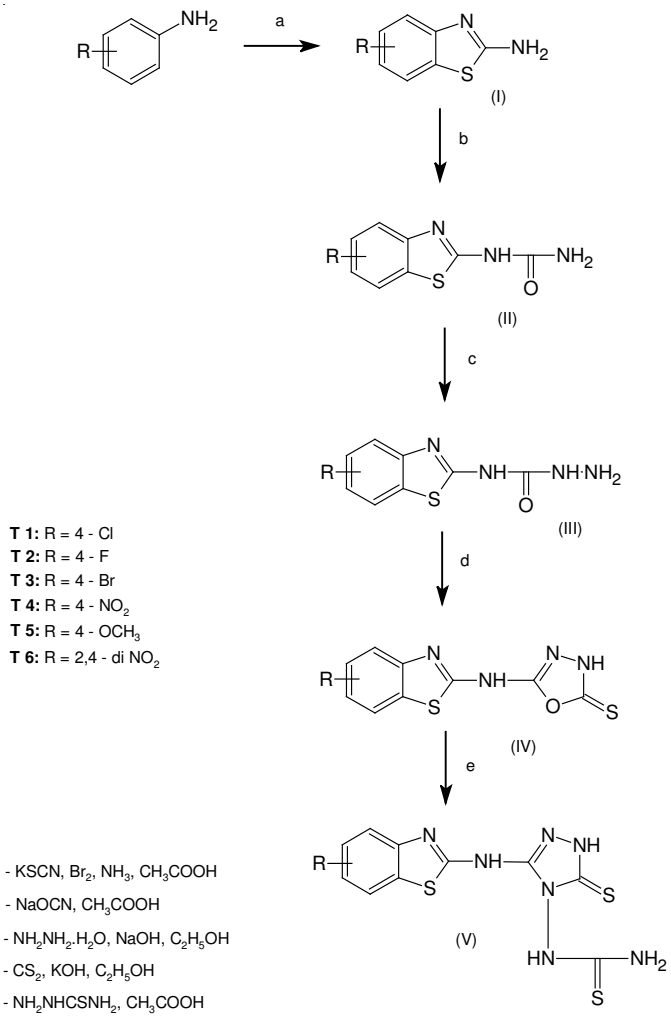
Biological assay: Biological evaluation of the compounds was performed to test a potent anticancer activity. In this study, the influence of a different concentration of each compound on proliferation of cancer and normal cell lines was examined. 0.1 mL of the cell suspension (containing 5×10^6 cells/100 µL) and 0.1 mL of the test solution (6.25-100 µg in 1 % DMSO such that the final concentration of DMSO in media is less than 1 %) were added to the 96 well plates and kept in 5 % CO_2 incubator at 37 ºC for 72 h. Blank contains only cell suspension and control wells contain 1 % DMSO and cell suspension.

After 72 h, 20 µL of MTT was added and kept in carbon dioxide incubator for 2 h followed by propanonl 100 µL. The plate was covered with aluminum foil to protect it from light. Then the 96 well plates are kept in rotary shaker for 10-20 min.

After 10-20 min, the 96 well plates were processed on ELISA reader for absorption at 562 nm. Each result is a mean value from three separate experiments, performed in triplicate. The readings were averaged and viability of the test samples was compared with DMSO control.

RESULTS AND DISCUSSION

Scheme-I summarizes the general synthetic approach that we employed for the synthesis of novel benzothiazole derivatives (**T1-T6**). All the compounds (**T1-T6**) were initially evaluated in MTT assay using three cancer cell lines consisting of A-549 (human small cell long carcinoma), $B_{16}F_{10}$ (mouse melanoma cell line), Hep-2 (human larynx epithelial carcinoma) and their antcancer activity data (GI_{50}) are provided in Table-1. Among these compounds, the highest activity was observed with compound **T2**, but moderate activity was observed with compound **T5**.



Scheme-I: Synthesis of novel benzothiazole derivatives

TABLE-1
GI_{50} OF THE SYNTHESIZED COMPOUNDS

Sample	A-549			$B_{16}F_{10}$			Hep-2			Mean GI_{50}
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	
T1	22.8	49.5	>100.0	15.5	46.6	91.5	22.1	48.8	79.2	52.33
T2	25.2	55.5	91.6	22.5	47.6	98.0	32.6	71.0	>100.0	46.67
T3	24.8	59.2	96.9	26.9	51.4	99.2	32.1	76.9	>100.0	59.16
T4	22.5	63.5	>100.0	41.6	61.9	>100	26.8	61.7	>100.0	49.00
T5	18.3	29.5	68.0	18.4	43.8	81.0	29.7	63.7	>100.0	47.00
T6	25.2	58.5	98.7	27.7	48.0	97.7	31.3	75.5	>100.0	57.33

The final results are in GI_{50} (growth inhibition 50), TGI (total growth inhibition), LC_{50} (lethal concentration 50). Concentration used (μm-micro molar) 100, 10, 1, 0.1 and 0.01 μm.

Biological activity: All the synthesized compounds (**T1-T6**) were screened for their anticancer activity against the cell lines (human small cell lung carcinoma, mouse melanoma cell line, human larynx epithelial carcinoma). The GI_{50} of the synthesized compounds was determined using MTT assay method. DMSO was used as solvent and blank. It was observed that compound **T2** was more effective against all the cell lines. The best mean GI_{50} values were achieved with compound **T2** and **T5** with slight difference among them. Other examined compounds exhibited a moderate inhibitory effect, depending on type of the cells. All the compounds (**T1-T6**) were found to exhibit mild to moderate anticancer activities in all cell lines and the results were summarized in Table-1.

Conclusion

The anticancer screening of synthesized compounds (**T1-T6**) were evaluated against cancer cell lines and the synthesized compounds were found to exhibit mild to moderate anticancer activities in all cell lines.

In conclusion, **T2 > T5 > T4 > T1 > T6 > T3**.

Fluoro, $-OCH_3$ pharmacophore containing compounds shows significant lead optimization. So further beneficial pharmacophore modifications in the design of benzothiazole as promising anti cancer agent. Generally the anticancer activity of the synthesized compounds may be due to the presence of versatile pharmacophores like fluoro, $-OCH_3$.

All the synthesized compounds containing $-NH_2$ as part of the structure, due to high hydrogen bonding and delocalization of π electrons may be the reason for promising activity.

Sulfur containing compounds have promising in biopotency, especially thio (C=S) group as part of structure. So, this also may chance for increasing the lipophilicity of the synthesized compounds, automatically potency also.

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REFERENCES

1. E. Kashiyama, I. Hutchinson, M.S. Chua, S.F. Stinson, L.R. Phillips, G. Kaur, E.A. Sausville, T.D. Bradshaw, A.D. Westwell and M.F.G. Stevens, *J. Med. Chem.*, **42**, 4172 (1999).
2. D.F. Shi, T.D. Bradshaw, S. Wrigley, J. Carol, P.L. Mccall and F.G. Stevens, *J. Med. Chem.*, **39**, 3375 (1996).
3. I. Caleta, M. Grdisa, D. Mrvos-Sermek, M. Cetina, V. Tralic-Kulenovic, K. Pavelic and G. Karminski-Zamola, *IL Farmaco*, **59**, 297 (2004).
4. I. Hutchinson, M.S. Chua, H.L. Browne, V. Trapani, T.D. Bradshaw, A.D Westwell and M.F.G. Stevens, *J. Med. Chem.*, **44**, 1446 (2001).
5. J. Das, J. Lin, R.V. Moquin, Z. Shen, S.H. Spergel, J. Wityak, A.M. Doweyko, H.F. DeFex, Q. Fang, S. Pang, S. Pitt, D.R. Shen, G.L. Schieven, J.C. Barrish, *Bioorg. Med. Chem. Lett.*, **13**, 2145 (2003).

6. I. Hutchinson, T.D. Bradshaw, C.S. Matthews, F.G. Malcolm Stevens and A.D. Westwell, *Bioorg. Med. Chem. Lett*., **13**, 471 (2003).
7. M.S. Chua, D.F. Shi, S. Wrigley, T.D. Bradshaw, I. Hutchinson, P.N. Shaw, D.A. Barrett, L.A. Stanley and M.F.G. Stevens, *J. Med. Chem*., **42**, 381 (1999).
8. P. Sharma, A. Mandloi and S. Pritmani, *Indian J. Chem.*, **38B**, 1289 (1999).
9. T.D. Bradshaw and A.D. Westwell, *Curr. Med. Chem*., **11**, 1009 (2004).
10. T.D. Bradshaw, S. Wrigley, D.F. Shi, R.J. Schultz, K.D. Paul and M.F.G. Stevens, *Br. J. Cancer*, **77**, 745 (1998).
11. A. Rana, N. Sidduiqi, S.A. Khan and A.M. Bhat, *Eur. J. Med. Chem*., **43**, 1114 (2008).
12. A. Rana, N. Sidduiqi, S.A. Khan and A.M. Bhat, *Bioorg. Med. Chem. Lett*., **17**, 4178 (2007).
13. P. Pannerselvam, R.V.P. Chandran and S.K. Sridhar, *Indian J. Pha. Sci*., **65**, 268 (2003).
14. R.S. Varma, V. Bajpai and A. Kapil, *Indian J. Heterocycl. Chem*., **10**, 17 (2000).

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