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Article

Design, Synthesis, and Biological Evaluation of Benzothiazole Chalcone Conjugates as Antitumor Agents

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ABSTRACT

A series of novel benzothiazole chalcone conjugates (12a-j) have been designed and synthesized by a simple, six-step synthetic procedure. Benzothiazole chalcone conjugates were synthesized by the Claisen-Schmidt condensation of various substituted acetophenones with substituted benzothiazole aldehydes. The structures of final compounds were characterized by infrared, nuclear magnetic resonance, and electrospray ionization mass spectrums. Furthermore, all the benzothiazole chalcone conjugates have been evaluated at the National Cancer Institute USA for their antitumor activity at a single dose (1×10^{-5} M), on a panel of 60 human tumor cell lines. From the screening result, it has been observed that compound **12e** exhibited promising antitumor activity.

Key words: Benzothiazole, Chalcone, Antitumor activity, Anticancer activity.

1. INTRODUCTION

Cancer, the uncontrolled growth of cells, has become one of the major causes of death throughout the world. Every year more than 20% of the population is affected by cancer, and the mortality rate is increasing annually, making it a major area of focus for researchers [1,2]. Benzothiazole, a privileged bicyclic ring system, demonstrated broad spectrum of interesting biological activities such as anti-allergic [3], anti-inflammatory [3,4], and antitumor [5-8] activity. Over the past two decades, various substituted benzothiazole molecules have been extensively studied for their anticancer activity. Among them, compound **1** 2-(4-aminophenyl)-benzothiazole (CJM 126) (Figure 1) has been shown remarkable antitumor activity in *in vitro* assays against MCF-7 and MDA-MB-468 cell lines of breast cancer [9].

Compound **2** 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610) (Figure 1) has been exhibited promising anticancer potential (growth inhibition of 50% [GI_{50}] <0.1 nM) and remarkable *in vitro* antitumor properties particularly in colon, non-small cell lung, and breast cancer cell lines [10]. Mechanistic studies of benzothiazole derivatives reported that benzothiazole derivatives act as tyrosine kinase [11] and topoisomerase inhibitors [12]. Moreover, another class of compounds 1,3-diaryl-2-propen-1-ones known as chalcones. Chalcones are open-chain flavonoids in which two aromatic rings are joined by a three-carbon, α, β-unsaturated carbonyl system. Chalcone derivatives have received a great deal of attention due to their relatively simple structures. Chalcones are known to exhibit a broad range of biological activities such as antioxidant, antibacterial, antifungal, anti-HIV, anti-leishmanial, antimalarial, anti-inflammatory, and anticancer properties [13]. The α, β-unsaturated ketone moiety of chalcones was recognized as a privileged structure [14-18].Chalcones exhibit cytotoxicity by controlling cellular targets such as ornithine decarboxylase [19], NF-κB [20], and proteasome [21] modulating microtubule formation [22]. Chalcone compounds **3** and **4** (Figure 1) showed remarkable anticancer activities by inhibition of tubulin polymerization [22]. Recently, potential hybrid conjugates have been synthesized as novel anticancer agents by the combination of different pharmacophores [23,24]. The remarkable anticancer activity exhibited by these conjugates encouraged us to explore some newer conjugates by linking two pharmacophores such as benzothiazole and chalcone scaffolds to enhance the anticancer activity. In this context, we have designed and synthesized benzothiazole chalcone conjugates (**12a-j**), and these conjugates were evaluated for their anticancer potential by the National Cancer Institute (NCI) USA at a single dose (1×10^{-5} M), on a panel of 60 human tumor cell line. Some of the conjugates such as compounds **12b**, **12d**, **12e,** and **12f,** showed considerable GI_{50} percentage against different human cancer cell lines (Table 1)**.**

2. RESULTS AND DISCUSSION

2.1. Chemistry

Synthetic strategies for the preparation of benzothiazole-chalcone conjugates are depicted in Scheme 1. Initially, esterification of 4-aminobenzoic acid was carried with methanol and catalytic amount of sulfuric acid followed by reaction with potassium thiocyanate in 95% acetic acid and bromine to obtain methyl 4-amino-3-thiocyanobenzoate, which on further treating with sodium sulfite nonahydrate in water to obtain methyl 4-amino-3-mercaptobenzoate. The cyclization of methyl 4-amino-3-mercaptobenzoate was performed by employing various substituted benzoic acids (**8a-e**), and catalytic amount of

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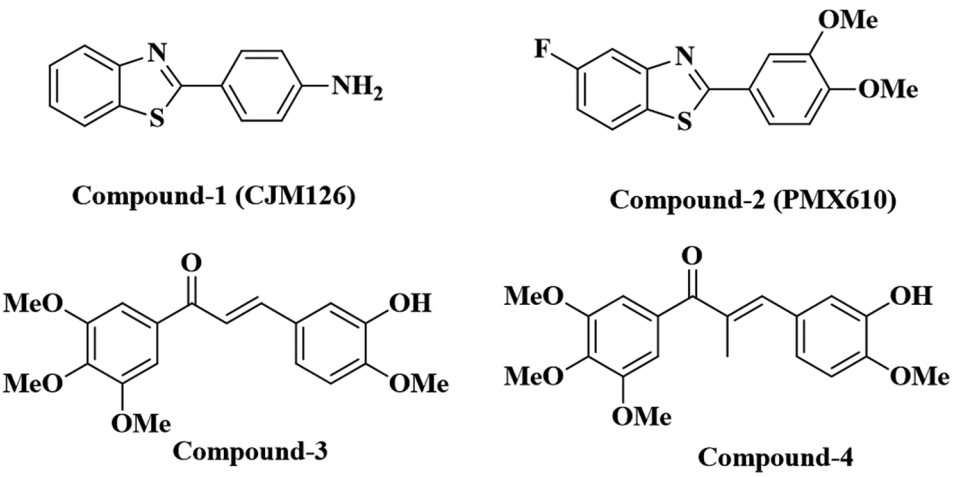
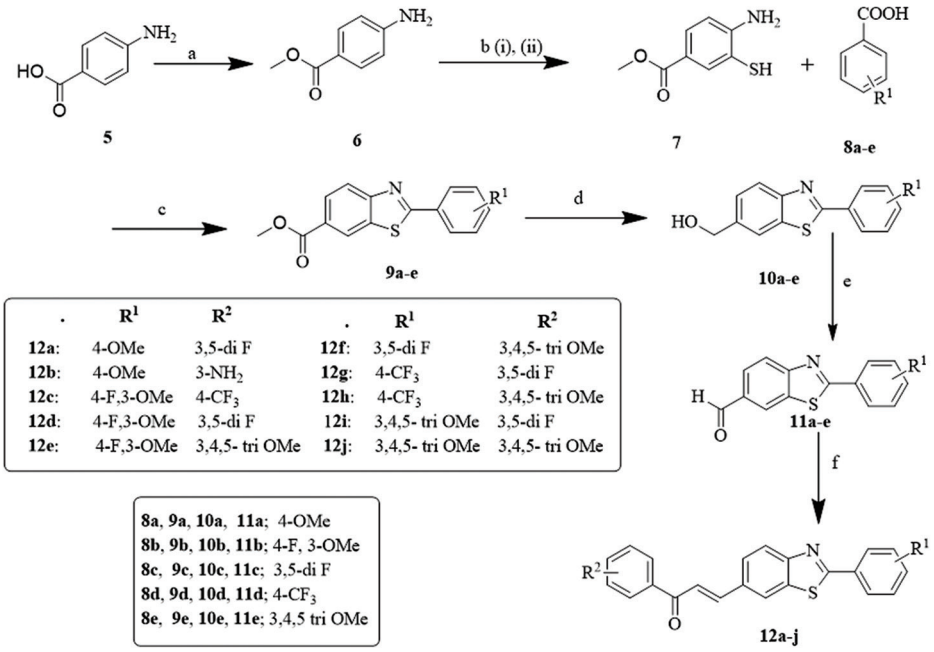


Figure 1: Chemical structures of standard anticancer molecules.



.	R^1	R^2	.	R^1	R^2
12a:	4-OMe	3,5-di F	**12f**:	3,5-di F	3,4,5- tri OMe
12b:	4-OMe	3-NH_2	**12g**:	4-CF_3	3,5-di F
12c:	4-F,3-OMe	4-CF_3	**12h**:	4-CF_3	3,4,5- tri OMe
12d:	4-F,3-OMe	3,5-di F	**12i**:	3,4,5- tri OMe	3,5-di F
12e:	4-F,3-OMe	3,4,5- tri OMe	**12j**:	3,4,5- tri OMe	3,4,5- tri OMe

Scheme 1: Reagents and conditions: (a) H_2SO_4, MeOH, reflux, 4h; (b) (i) potassium thiocyanate, acetic acid, bromine, 0°C, (ii) sodium sulfite nonahydrate, reflux, 2.5 h; (c). (**8a-e**), Zn dust, reflux, 2.5 h; (d) LAH, THF, 0°C-rt, 4 h; (e) DMP, CH_2Cl_2, 0°C–rt, 3 h; (f) NaOH, ethanol, 1–2 h, rt.

zinc dust under reflux conditions afforded the corresponding methyl benzothiazole carboxylate derivatives (**9a-e**). The reduction of esters (**9a-e**) with lithium aluminum hydride (LAH) in dry tetrahydrofuran produced the corresponding alcohols that were taken up for further oxidation by Dess–Martin periodinane in dichloromethane to afford the corresponding benzothiazole carbaldehydes (**11a-e**) in good yields. Finally, the required benzothiazole chalcone conjugates (**12a-j**) were synthesized by employing the Claisen-Schmidt condensation between equimolar mixtures of substituted benzothiazole carbaldehydes (**11a-e**) and various substituted acetophenones, as shown in Scheme 1. The compounds were characterized by means of ^{1}H NMR, ^{13}C NMR, electrospray ionization (ESI) mass, and infrared (IR) spectral data.

2.2. Biology

The benzothiazole chalcone conjugates (**12a-j**) were evaluated for their anticancer activity at a single dose (1×10^{-5} M) by the NCI USA, on a panel of 60 human tumour cell lines derived from nine different cancer types (leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast). The anticancer activity is expressed as a percentage of GI_{50} determined relative to that of untreated control cells. Among them, compound **12b** which contains 4-methoxy substituent on benzothiazole's phenyl ring and 3-amino group on chalcone's phenyl ring showed notable GI of 54.8% and 48.0% against leukemia cell line HL-60 (TB) and melanoma cell line MDA MBA-435, respectively. Compound **12d** which has 3-methoxy and 4-fluro substituents on benzothiazole's phenyl ring and 3,5 di fluoro groups on chalcone's phenyl ring showed considerable GI of 40.8% and 58.4% against leukemia cell lines HL-60 (TB) and MOLT-4, respectively. Compound **12e** which has same substituents on benzothiazole ring similar to compound **12d** and methoxy groups on 3, 4, and 5 position of chalcone ring exhibited promising GI of 49.6%, 79.5%, and 66.0% against leukemia cell lines HL-60 (TB) and SR and breast cancer line MCF7, respectively. Compound **12f** contains fluoro

groups on 3 and 5 position of benzothiazole's phenyl ring and methoxy groups on 3, 4, and 5 position of chalcone ring showed notable GI of 40.8% against HL-60 cell line. Interestingly, the majority of compounds showed considerable GI against leukemia, particularly HL-60 (TB) cancer cell line showed in Table 1.

3. EXPERIMENTAL SECTION

3.1. General

All chemicals and reagents were obtained from local dealers of S.D Fine and Spectrochem Pvt. Ltd., Mumbai, India, and were used without further purification. Reactions were monitored by TLC performed on silica gel coated glass plates containing 60 GF254 with visualization achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H and ^{13}C NMR spectra were recorded on Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (d) are reported in ppm downfield from an internal tetramethylsilane standard. ESI mass spectra were recorded on a Micro mass Quattro LC using ESI+software with a capillary voltage of 3.98 kV and an ESI mode positive ion trap detector.

3.2. Synthesis of methyl 4-aminobenzoate 6

To a solution of 4-aminobenzoic acid **5 (**20 g. 0.13 mol) in methanol, catalytic amount of conc. sulfuric acid (5 mL) was added at 0°C. The reaction mixture was shifted to an oil bath and stirred at 80°C. After 2 h, the reaction mixture was cooled at room temperature and neutralized with a saturated solution of $NaHCO_3$ (150 mL) and extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated to give crude product which was purified by column chromatography to afford pure compound as white solid **6.** mp: 110–112°C; ^{1}H NMR (300 MHz, $CDCl_3$): 3.89 (3H, s, OCH_3), 6.91 (2H, d, *J*=8.7 Hz), 7.78 (2H, d, *J*=8.7 Hz), (ESI, m/z): 152 $[M+H]^+$.

3.3. Synthesis of methyl 4-amino-3-mercaptobenzoate (7)

A solution of potassium thiocyanate (30 g, 0.31 mol) in 95% acetic acid (70 ml) was added to a stirred solution of methyl 4-aminobenzoate 5 (12.4 g, 0.15 mol) in 95% acetic acid (150 ml) at room temperature, the mixture was cooled to below 5°C, and bromine (13.2 g, 0.17 mol) in 95% acetic acid (25 ml) was added with additional stirring. The reaction mixture was poured into water (300 ml), and the resulting precipitate was collected by filtration and washed successively with water (50 ml), aqueous sodium hydroxide, and water this solid was recrystallized from ethanol to give 13.6 g (82%) of methyl 4-amino-3-thiocyanobenzoate as a pale yellow solid.

A mixture of ethyl 4-amino-3-thiocyano benzoate (11.1 g, 0.1 mol) and sodium sulfite nonahydrate (14.5 g, 0.12 mol) in water (30 ml) was refluxed for 1.5 h. After cooling at room temperature, the reaction mixture was filtrated, the filtrate was neutralized with acetic acid, and the resulting precipitate was collected and dissolved with diethyl ether. The solution was washed with water (50 ml) and brine (50 ml), dried over anhydrous sodium sulfate, and concentrated under vacuum to give 4.4 g (44%) of methyl 4-amino-3-Mercaptobenzoate as a yellow amorphous solid. mp: 99–101°C;^{1}H NMR (300 MHz, $CDCl_3$): 2.92 (1H, br s), 3.98 (3H, s, OCH_3), 4.72 (2H, br s), 6.71 (1H, dd, *J*=8.7 Hz and 1.8 Hz), 8.14–7.75 (2H, m); (ESI, m/z): 184 $[M+H]^+$.

3.4. Synthesis of methyl 2-(4-methoxyphenyl)benzo[d]thiazole-6-carboxylate (9a)

A mixture of methyl 4-amino-3-mercaptobenzoate **7 (**2 g, 10 mmol), 4 methoxy benzoic acid **8a** (2.2 g, 15 mmol), and a catalytic amount of zinc dust was refluxed for 2.5 h. After cooling at room temperature, the reaction mixture was poured into ice water (100 ml) and extracted with ethyl acetate. The organic layer was washed with saturated sodium hydrogen carbonate solution (100 ml) and brine (100 ml) and dried over anhydrous sodium sulfate. The solvent was evaporated to give 1.86 g (58%) of methyl 2-(4-methoxyphenyl)benzo[d]thiazole-6-carboxylate **9a** as a pale yellow solid; mp: 231–233°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.98 (d, *J*=8.84 Hz, 2H), 7.12 (d, *J*=8.34 Hz, 1H), 7.92 (d, *J*=9.06, 1H), 8.12 (d, *J*=8.14 Hz, 2H), 8.28 (s, 1H); (ESI, m/z): 300 $[M+H]^+$.

3.5. Synthesis of methyl 2-(4-fluoro-3-methoxyphenyl)benzo[d] thiazole-6-carboxylate (9b)

This compound was prepared according to the method described for compound **9a**, employing methyl 4-amino-3-mercaptobenzoate **7 (**2 g, 10 mmol), and 4 fluro 3 methoxy benzoic acid **8b** (2.55 g, 15 mmol) to obtain 1.92 g (55%) **9b** as a pale yellow solid mp: 204–206°C; ^{1}H NMR (300 MHz, $CDCl_3$) 3.93 (s, 3H), 3.98 (s, 3H), 7.26 (d, *J*=8.14 Hz, 1H), 7.72–7.70 (m, 2H), 7.78 (d, *J*=7.56 Hz, 1H), 7.98 (d, *J*=7.56 Hz, 1H), 8.36 (s, 1H); (ESI, m/z): 318 [M+H].

3.6. Synthesis of methyl 2-(3,5-difluorophenyl)benzo[d]thiazole-6-carboxylate (9c)

This compound was prepared according to the method described for compound **9a**, employing methyl 4-amino-3-mercaptobenzoate **7 (**2 g, 10 mmol), and 3,5 difluoro benzoic acid **8c** (2.37 g, 15 mmol) to obtain 1.96 g (58%) **9c** as a pale yellow solid mp: 243–245°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.98 (s, 3H, OCH_3), 6.92 (s, 1H) 7.61 (s, 2H,), 7.85 (d, *J*=8.36 Hz, 1H), 7.98 (d, *J*=8.34 Hz, 1H), 8.44 (s,1H); (ESI, m/z): 306 $[M+H]^+$.

3.7. Synthesis of methyl 2-(4-(trifluoromethyl) phenyl)benzo[d] thiazole-6-carboxylate (9d):

This compound was prepared according to the method described for compound **9a**, employing methyl 4-amino-3-mercaptobenzoate **7 (**2 g, 10 mmol), and 4 trifluoromethyl benzoic acid **8d** (2.85 g, 15 mmol) to obtain 1.94 g (52%) **9d** as a pale yellow solid. mp: 251–253°C;^{1}H NMR (300 MHz, $CDCl_3$+DMSO) δ 3.96 (s, 3H), 7.34 (d, *J*=8.49 Hz, 2H), 8.04 (d, *J*=8.49 Hz, 1H), 8.17–8.09 (M 3H), 8.58 (s, 1H), (ESI, m/z): 338 $[M+H]^+$.

3.8. Synthesis of methyl 2-(3,4,5-trimethoxy phenyl)benzo[d]thiazole-6-carboxylate (9e)

This compound was prepared according to the method described for compound **9a**, employing methyl 4-amino-3-mercaptobenzoate **7 (**2 g,

Table 1: Growth inhibition activities some of benzothiazole chalcone conjugates.

Compound	Cell line	Growth inhibition %	Cell line	Growth inhibition %	Cell line	Growth inhibition %
12b	HL-60 (TB)	54.8	MDA-MB-435	48.0	-	-
12d	HL-60 (TB)	40.8	MOLT-4	58.4	-	-
12e	HL-60 (TB)	49.6	SR-	79.5	MCF7	66.0
12f	HL-60 (TB)	40.1	-	-	-	-

Growth inhibition at concentration of 1×10^{-5} M), 12b (NSC758372), 12d (NSC758366), 12e (NSC758373), 12f (NSC758355)

10 mmol), and 3,4,5 trimethoxy benzoic acid **8e** (3.18 g, 15 mmol) to obtain 2.14 g (54%) **9e** as a pale yellow solid. mp: 243–245°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.91 (s, 3H), 3.95 (s, 3H), 3.98 (s, 6H), 6.93 (s, 2H), 7.46 (d, *J*=8.17 Hz, 1H), 7.95 (d, *J*=8.17 Hz, 1H), 8.53 (s, 1H); (ESI, m/z): 360 $[M+H]^+$.

3.9. Synthesis of 2-(4-methoxyphenyl) benzo[d] thiazole-6-carbaldehyde (11a)

The compounds (**9a-e** 0.019 mol) taken in dry THF (100 mL) were cooled to −5–0°C in an ice-salt (NaCl) bath. To the reaction mixture LAH (0.076 mol) was added slowly portion wise and continued stirring at room temperature for about 4 h. After completion of the reaction; further, the reaction mixture was cooled to 0°C and the excess of LAH was quenched with Na_2SO_4 paste, filtered on Buchner funnel washed with methanol (2×20 mL). The filtrate was dried over anhydrous Na_2SO_4 concentrated under vacuum. The crude obtained (0.014 mol) was taken in dry CH_2Cl_2 (100 mL). To that DMP (0.021 mol) was added, stirred at room temperature for about 2 h. After the completion of reaction, the reaction mixture was washed with water (2×100 mL) that the organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum. Then, the resulting crude obtained was purified by column chromatography using ethyl acetate/hexane (1:1) to afford products (**11a-e**) with high purity solid: 197–199°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.91 (s,3H), 6.98 (d, *J*=8.30 Hz, 2H), 7.56 (d, *J*=8.30 Hz, 2H), 7.94 (dd, *J*=9.06, 1.15 Hz, 1H), 8.02 (d, *J*=9.06 Hz, 1H), 8.38 (s, 1H), 9.98 (s, 1H); (ESI, m/z): 270 $[M+H]^+$.

3.10. Synthesis of 2-(4-fluoro-3-methoxyphenyl) benzo[d] thiazole-6-carbaldehyde (11b)

This compound was prepared according to the method described for compound **11a**, mp: 190–192°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.96 (s, 3H), 7.24 (d, *J*=8.1 Hz, 1H), 7.78–7.70 (m, 2H), 7.84–7.80 (m, 1H), 8.08 (d, *J*=7.56 Hz, 1H), 8.23 (s, 1H), 9.92. (s, 1H), (ESI, m/z): 288 $[M+H]^+$.

3.11. Synthesis of 2 (3,5difluorophenyl)benzo[d] thiazole-6-carbaldehyde (11c)

This compound was prepared according to the method described for compound **11a**, mp: 186–188°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (s, 1H), 7.52 (s, 2H), 7.68 (d, *J*=7.58 Hz, 1H), 7.82 (d, *J*=7.56 Hz, 1H), 8.30 (s, 1H), 10.14 (s, 1H); (ESI, m/z): 276 $[M+H]^+$.

3.12. Synthesis of 2-(4-(trifluoromethyl) phenyl)benzo [d]thiazole-6-carbaldehyde (11d)

This compound was prepared according to the method described for compound **11a**, mp: 184–182°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, *J*=8.1 Hz, 2H), 7.78–7.67 (m, 3H), 7.86 (d, *J*=7.56 Hz, 1H), 8.33 (s, 1H),10.05 (s, 1H); (ESI, m/z): 308 $[M+H]^+$.

3.13. Synthesis of 2-(3,4,5-trimethoxyphenyl) benzo[d] thiazole-6-carbaldehyde (11e)

This compound was prepared according to the method described for compound **11a**, mp: 187–189°C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO) δ 3.79 (s, 6H), 3.91 (s, 3H), 6.98 (s, 2H), 7.48 (d, *J*=7.42 Hz, 1H),), 7.84 (d, *J*=7.42 Hz, 1H), 8.16 (s, 1H), 10.07 (s, 1H); (ESI, m/z): 330 $[M+H]^+$.

3.14. Synthesis of (E)-1-(3,5-difluorophenyl)-3-(2-(4-methoxyphenyl) benzo[d]thiazol-6-yl)prop-2-en-1-one (12a)

To the stirred solution of 3,5-difluorophenyl)ethanone (156 mg, 1 mmol) and 2-(4-methoxyphenyl)benzo[d]thiazole-6-carbaldehyde (**11a**) (269 mg, 1 mmol) in ethanol (20 ml) 10% aqueous solution of sodium hydroxide was added (5 ml). The reaction mixture was stirred at room temperature 27 ^{0}C for 4 h and the reaction was monitored by TLC using ethyl acetate hexane (5:5) as a solvent system. The solvent was evaporated under vacuum and then the residue was dissolved in ethyl acetate/water. The organic layer was washed with brine and evaporated. This was further purified by column chromatography using ethyl acetate: hexane (5:5) as a solvent system to obtain the pure product as yellow solid (270 mg, 66% yield). mp: 249–251°C; IR (KBr): 3153, 2931, 1681, 1642, 1576, 1474, 1435, 1254, 1179, 1029, 961, 864 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.87 (s, 3H), 7.02 (s, 1H), 7.10 (d, 2H, *J*=8.4 Hz), 7.26 (d, 1H, *J*=8.5) 7.28–7.31 (m, 3H), 7.47 (d, 1H, *J*=8.5) 7.62 (d, 1H, *J*=15.8 Hz), 7.74 (d, 2H, *J*=8.4 Hz), 8.08 (d, 1H, *J*=15.1 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.2, 110.4, 112.6, 115.4, 120.7, 126.2, 128.4, 129.2, 130.2, 134.5, 137.5, 144.6, 146.7, 158.4, 161.8, 178.3; (ESI, m/z): 408 $[M+H]^+$.

3.15. Synthesis of (E)-1-(3-aminophenyl)-3-(2-(4-methoxyphenyl) benzo[d]thiazol-6-yl) prop-2-en-1-one (12b)

This compound was prepared according to the method described for compound **12a**, employing 1-(3-aminophenyl)ethanone (135 mg, 1 mmol) and 2-(4-methoxyphenyl)benzo[d]thiazole-6-carbaldehyde (**11a**) (269 mg, 1 mmol) to obtain the pure product **12b** as a yellow solid (270 mg, 69% yield). mp: 224–226°C; IR (KBr): 3360, 3184, 3094, 2953, 2352, 1671, 1632, 1463, 1420, 1240, 1180, cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.86 (s, 3H), 6.75 (d, 1H, *J*=8.2 Hz), 6.84–6.96 (m, 3H), 7.01 (d, 1H, *J*=8.8 Hz), 7.27 (d, 1H, *J*=16.1 Hz), 7.46–7.54 (m, 2H,) 7.55 (d, 2H, *J*=8.4 Hz), 7.65 (d, 1H, *J*=8.8 Hz), 7.84 (s, 1H), 8.03 (d, 1H, *J*=15.9Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ56.4, 114.7, 116.8, 118.4, 120.7, 123.2, 126.8, 128.7, 130.8, 132.1,136.2, 137.5, 143.2, 145.7, 154.4, 162.2, 176.2; (ESI, m/z): 387 $[M+H]^+$.

3.16. Synthesis of (E)-3-(2-(4-fluoro-3-methoxyphenyl) benzo[d] thiazol-6-yl)-1(4 (trifluoromethyl) phenyl) prop-2-en-1-one (12c)

This compound was prepared according to the method described for compound **12a**, employing 1-(4-(trifluoromethyl)phenyl)ethanone (188 mg, 1 mmol) and 2-(4-fluoro-3-methoxyphenyl) benzo[d] thiazole-6-carbaldehyde (**11b**) (287 mg, 1 mmol) to obtain the pure product **12c** as a yellow solid (290 mg, 63% yield). mp: 242–244°C; (KBr): 3164, 2984, 1691, 1648, 1484, 1274, 1182, 864 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.94(s, 3H), 7.12 (d, 1H, *J*= 8.4 Hz), 7.17 (d, 1H, *J*=7.5 Hz), 7.29–7.16 (m, 2H), 7.54 (d, 1H, *J*=8.2 Hz),7.67 (d, 2H, *J*=8.3 Hz), 7.57 (s, 1H) 7.72 (d, 2H, *J*=9.0, 8.3 Hz), 7.86 (s, 1H,), 8.02 (d, 1H, *J*=15.8 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ55.8, 114.3, 119.5, 120.7, 121.3, 123.1, 124.6, 130.2, 132.4,134.5, 135.8, 137.5, 143.2, 145.7, 156.4, 158.3, 162.2, 176.2; (ESI, m/z): 458 $[M+H]^+$.

3.17. Synthesis of (E)-1-(3,5-difluorophenyl)-3-(2-(4-fluoro-3-methoxyphenyl)benzo[d]thiazol-6-yl)prop-2-en-1-one (12d)

This compound was prepared according to the method described for compound **12a**, employing 1-(3,5-difluorophenyl)ethanone (156 mg, 1 mmol) and 2-(4-fluoro-3-methoxyphenyl) benzo[d] thiazole-6-carbaldehyde (**11b**) (287 mg, 1 mmol) to obtain the pure product **12d** as a yellow solid (290 mg, 68% yield). mp: 238–240°C; (KBr): 3090, 2984, 1684, 1624, 1596, 1465, 1254, 1184, 964, 867 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.96 (s, 3H), 6.91 (s, 1H), 7.18 (d, 1H, *J*=8.3 Hz), 7.24–7.16 (m, 3H), 7.25 (d, 1H, *J*=15.8 Hz), 7.38-7.26 (d, 2H), 7.42 (d, 1H, *J*=8.5 Hz), 7.67 (s, 1H) 8.02 (d, 1H, *J*=15.8 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 56.4, 110.4, 114.8, 120.5, 121.7, 123.8, 124.5, 130.6, 132.7,134.7, 135.6, 137.1, 144.2, 145.3, 156.2, 158.4, 161.4, 177.4; (ESI, m/z): 426 $[M+H]^+$.

3.18. Synthesis of (E)-3-(2-(4-fluoro-3-methoxy phenyl)benzo[d] thiazol-6-yl)-1-(3,4,5-trimethoxy phenyl)prop-2-en-1-one (12e)

This compound was prepared according to the method described for compound **12a**, employing 1-(3,4,5-trimethoxyphenyl)ethanone

(210 mg, 1 mmol) and 2-(4-fluoro-3-methoxyphenyl) benzo[d] thiazole-6-carbaldehyde (**11b**) (287 mg, 1 mmol) to obtain the pure product **12e** as a yellow solid (290 mg, 68% yield). mp: 239–241°C; IR (KBr): 3124, 2968, 1681, 1642, 1576, 1474, 1435, 1254, 1179, 1029, 965 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.89 (s, 3H), 3.94 (s, 3H), 3.98 (s, 6H), 6.95 (s, 2H), 7.18 (d, 1H, *J*=8.2 Hz), 7.28 (s, 1H), 7.32- 7.37 (m, 2H), 7.39 (d, 1H, *J*=15.8 Hz), 7.64 (d, 1H, *J*=8.3 Hz), 7.58 (s, 1H), 8.02 (d, 1H, *J*=15.8 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 56.2, 58.4, 61.3, 102.4, 119.7, 121.6, 122.6, 123.2, 124.6, 130.4, 134.6, 135.4, 137.2, 144.2, 156.2, 161.4, 179.6; (ESI, m/z): 480 $[M+H]^+$.

3.19. Synthesis of (E)-3-(2-(3, 5-difluorophenyl)benzo[d]thiazol-6-yl)-1-(3,4,5-tri methoxyphenyl) prop-2-en-1-one (12f)

This compound was prepared according to the method described for compound **12a**, employing 1-(3,4,5-trimethoxyphenyl)ethanone (210 mg, 1 mmol) and and 2 (3,5difluorophenyl)benzo[d] thiazole-6-carbaldehyde (**11c**) (275 mg, 1 mmol) to obtain the pure product **12f** as a yellow solid (315 mg, 67% yield). mp: 242–244°C; IR (KBr): 3130, 2971, 2352, 1676, 1636, 1576, 1470, 1430, 1252, 1179, 1029, 954, 848 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): d 3.89 (s, 3H), 3.91 (s, 6H), 6.89 (s, 1H), 6.98 (s, 2H), 7.29 (s, 2H), 7.35 (d, 1H, *J*=15.8 Hz), 7.40 (d, 1H, *J*=8.2Hz), 7.54 (d, 1H, *J*=8.2 Hz), 7.83 (s, 1H), 7.96 (d, 1H, *J*=15.8 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 56.4, 60.2, 104.6, 110.6, 118.3, 120.9, 122.3, 123.6, 125.2, 131.4, 133.8, 136.5, 138.6, 142.6, 146.3, 157.9, 161.7, 178.1; (ESI, m/z): 468 $[M+H]^+$.

3.20. Synthesis of (E)-1-(3,5-difluorophenyl)-3-(2-(4-(trifluoromethyl)phenyl)benzo[d]thiazol-6-yl)prop-2-en-1-one (12g)

This compound was prepared according to the method described for compound **12a**, employing 1-(3,5-difluorophenyl)ethanone (156 mg, 1 mmol) and 2-(4-(trifluoromethyl) phenyl)benzo [d]thiazole-6-carbaldehyde (**11d**) (307 mg, 1 mmol) to obtain the pure product **12g** as a yellow solid (310 mg, 69% yield). mp: 238–240°C; IR (KBr): 3141, 2988, 1686, 1647, 1565, 1453, 1254, 1182, 964, 854 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 6.93 (s, 1H), 7.26-7.15 (m, 3H), 7.32 (d, 1H, *J*=7.8 Hz), 7.41 (d, 1H, *J*=15.8 Hz), 7.59 (d, 2H, *J*=8.7, Hz), 7.78 (s, 1H), 7.91 (d, 1H, *J*=15.8 Hz), 7.98 (d, 2H, *J*=8.7 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 110.5, 114.4, 119.4, 120.6, 122.8, 123.4, 124.9, 130.8, 134.2, 136.4, 138.2, 142.3, 146.2,156.2, 157.4, 158.4, 162.6, 176.6; (ESI, m/z): 446 $[M+H]^+$.

3.21. Synthesis of (E)-3-(2-(4-(trifluoromethyl) phenyl)benzo[d] thiazol-6-yl)-1-(3,4,5-tri methoxy phenyl) prop-2-en-1-one (12h)

This compound was prepared according to the method described for compound **12a**, employing 1-(3,4,5-trimethoxyphenyl)ethanone (210 mg, 1mmol) and 2-(4-(trifluoromethyl) phenyl) benzo [d]thiazole-6-carbaldehyde (**11d**) to obtain the pure product **8e** as a yellow solid (324 mg, 64% yield).mp: 235–237°C; IR (KBr): 3124, 2992, 1686, 1638, 1470, 1431, 1182, 860 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): d 3.89 (s, 3H), 3.94 (s, 6H), 6,97 (s, 2H), 7.21 (d, 1H, *J*=8.5 Hz), 7.34 (d, 1H, *J*=15.8 Hz), 7.48 (d, 1H, *J*=8.4 Hz),7.68 (d, 2H, *J*=8.5 Hz) 7.82 (s, 1H), 7.90 (d, 1H, *J*=16.6 Hz), 7.92 (d, 2H, *J*=8.6 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 58.4, 61.4, 106.2, 110.4, 119.4, 121.6, 122.2, 124.8, 131.2, 134.4, 136.3, 138.6,141.3, 145.2, 152.3,158.2, 160.3, 178.4; (ESI, m/z): 500 $[M+H]^+$.

3.22. Synthesis of (E)-1-(3,5-difluorophenyl)-3-(2-(3,4,5-trimethoxyphenyl)benzo[d]thiazol-6-yl)prop-2-en-1-one (12i)

This compound was prepared according to the method described for compound **12a**, employing 1-(3,5-difluorophenyl)ethanone (156 mg, 1 mmol) and 2-(3,4,5-trimethoxyphenyl) benzo[d] thiazole-6-carbaldehyde (**11e**) (329 mg, 1 mmol) to obtain the pure product **12g** as a yellow solid (310 mg, 66% yield). mp: 243–245°C; IR (KBr): 3122, 2989, 1689, 1640, 1570 1472, 1264, 1184, 964, 882 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): d 3.86 (s, 3H), 3.93 (s, 6H), 6.96 (s, 2H), 7.08 (s, 1H), 7.36-7.27(m, 3H), 7.46 (d, 1H, *J*=8.3 Hz), 7.68 (d, 1H, *J*=15.9Hz), 7.84 (s, 1H), 7.92 (d, 1H, *J*=15.8 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.8, 61.8, 103.8, 110.2, 119.4, 120.6, 122.8, 123.4, 124.9, 130.8, 134.2, 136.4, 138.2, 142.3, 146.2, 158.4, 162.6, 176.6; (ESI, m/z): 467 $[M+H]^+$ (ESI, m/z): 468$[M+H]^+$.

3.23. (E)-1-(3,4,5-trimethoxyphenyl)-3-(2-(3,4,5-trimethoxyphenyl) benzo[d]thiazol-6-yl)prop-2-en-1-one (12j)

This compound was prepared according to the method described for compound **12a**, employing 1-(-(4-(trifluoromethyl)phenyl)ethanone (188 mg, 1 mmol) and 2-(3,4,5-trimethoxyphenyl) benzo[d] thiazole-6-carbaldehyde (**11e**) (329 mg, 1mmol) to obtain the pure product **12j** as a yellow solid (340 mg, 65% yield). mp: 240–242°C; IR (KBr): 3140, 2986, 1684, 1640, 1484, 1431, 1233, 1043, 872 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): d 3.91 (s, 9H), 3.93 (s, 6H), 3.94 (s, 3H), 6.95 (s, 2H), 7.02 (s, 2H), 7.22 (d, 1H, *J*=8.2 Hz), 7.38(d, 1H, *J*=8.2 Hz), 7.49 (d, 1H, *J*=17.1 Hz), 7.83 (s, 1H), 8.07 (d, 1H, *J*=15.8 Hz); ^{13}C NMR (75 MHz, DMSO–d_6): δ 56.4, 62.4, 104.2, 109, 118.4, 120.4, 122.6, 124.6, 130.6, 136.2, 138.4,142.3, 146.2, 152.3, 160.3 180.4; (ESI, m/z): 522 $[M+H]^+$.

4. CONCLUSION

We have designed and synthesized some benzothiazole chalcone conjugates that were evaluated for their growth inhibitory potential. Conjugates **12b**, **12d**, **12e,** and **12f** showed significant GI activity against human leukemia cancer cell line (HL-60). Further, conjugate **12e** exhibited promising GI of 66% against breast cancer line MCF7 and inhibition of 79.5% against leukemia cancer line SR. The results suggest that these new benzothiazole chalcone conjugates have considerable potent antitumor potency, and conjugate **12e** is amenable for further structural modifications and development for the antitumor activity of leukemia breast cancer.

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