

Synthesis and antioxidant activity of some novel Fluorobenzothiazolopyrazoline

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ABSTRACT

A series of 7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-substituted phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine (5a-f) have been synthesized by treating 4-fluoro-3-chloro aniline with KSCN in presence of bromine in glacial acetic acid and ammonia to get 7-chloro-6-fluorobenzo[d]thiazol-2-amine (1), which was treated with hydrazine hydrate in HCl in presence of ethylene glycol to get 7-chloro-6-fluoro-2-hydrazinylbenzo[d]thiazole (2). Alternatively compound 1 was treated with acetic anhydride to get N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)acetamide (3) which were used to synthesized a series of chalcones via Claisen-Schmidt condensation by using six different substituted aromatic aldehydes to get N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-substituted phenyl acrylamide (4a-f). Finally the above chalcones were refluxed with compound 2 to get the novel targeted compound (5a-f). All the compounds were characterized by melting point, TLC and their chemical structures were proved by means of IR, ¹H NMR, mass spectroscopy, and elemental analysis data. All the final compounds and chalcones have been evaluated in vitro for their antioxidant activity by ferric ion reduction and DPPH methods. The final compounds have shown significant antioxidant activity compared to chalcones.

Key Words: Benzothiazole, Pyrazoline, Antioxidant activity, Chalcones.

INTRODUCTION

The study of benzothiazole derivatives is of considerable current interest as a result of their important biological and biophysical properties such as antitumor [1], antimicrobial [2], antifungal agents [3] as well as imaging agents for β -amyloid [4]. Several substituted benzothiazoles [5-8] have been identified as potent anthelmintic drugs. Aminobenzothiazoles have manifested a large scale of biological activities such as antiparkinsonian, dopamine antagonist [9,10], antibacterial [11] and antihistaminic agents [12].

The pyrazoline derivatives constitute an interesting class of organic compounds, which are associated with diverse chemical and pharmacological properties [13-14].

Fluorine has been incorporated into the molecule, being the second smallest substituent next to hydrogen, closely mimics hydrogen in enzyme receptor interactions. The substitutions of hydrogen by fluorine increases lipid solubility which in turn increases the transport and absorption of drug *in vivo*, and a strong electron withdrawing inductive effect of fluorine can significantly influence reactivity and stability of functional groups and the reactivity of neighbouring reaction centres [15].

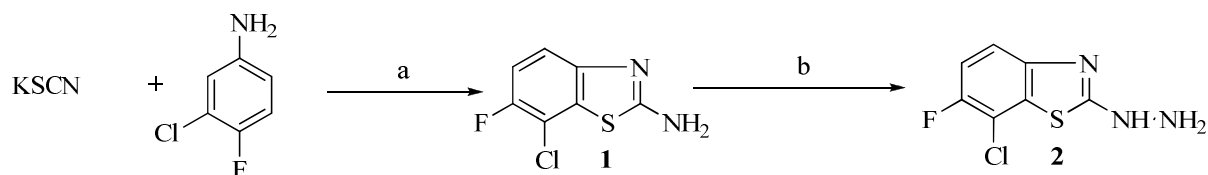
Antioxidants are any substance that when present in low concentration compared to those of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate [16]. They may be synthetic or natural origins, which protect the biomembrane against reactive oxygen species (ROS), mediated tissue damage [17]. Antioxidants are of great importance because they provide electrons that neutralize free radicals, i.e. molecules with unpaired electrons which have capability to cause degenerative and life threatening diseases.

The role of free radicals and reactive oxygen species (ROS) in the pathogenesis of human diseases such as cancer, aging, inflammatory response syndrome, respiratory diseases, liver diseases, and atherosclerosis, has been widely recognized [18]. Electron acceptors, such as molecular oxygen, readily reacts with free radicals to become free radicals themselves, also referred as ROS, with chemical species such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl free radicals ($\cdot OH$), which are known to induce damage to biomembranes, proteins and DNA [19]. Prominent manifestation of free radical activity in biological systems is lipid peroxidation and it is involved in the development of different diseases. The primary targets of free radical attack on lipids are polyunsaturated fatty acids (PUFA). Lipid peroxidation usually proceeds as a chain reaction: alkyl radicals are formed during the initiation step by the attack of lipids by free radicals or other reactive species, followed in the propagation phase by the formation of alkylperoxyl ($ROO\cdot$) and alkoxy ($RO\cdot$) radicals, and terminating with the formation of lipid hydroperoxides ($ROOH$) [20]. However, these products readily decompose to other relatively more stable substances such as aldehydes (malondialdehyde, hydroxynonenal, dienals, etc.) or isoprostanes, which have been used to assess lipid peroxidation *in vivo*.

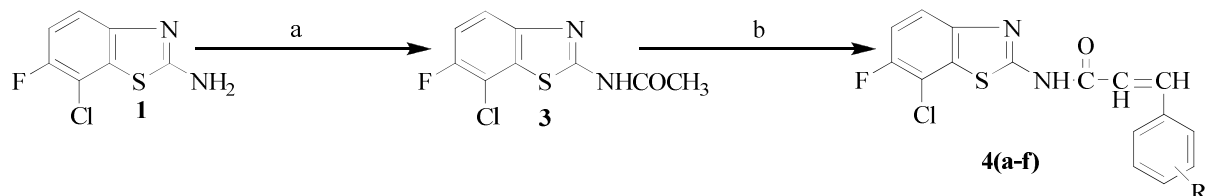
Drawing on structural knowledge obtained from our past work we herein synthesized six chalcones (**4a-f**) and six fluorobenzothiazolopyrazolines (**5a-f**) for their antioxidant activity.

MATERIALS AND METHODS

The melting points were determined with an electrothermal melting point apparatus and are uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8400 Shimadzu and the frequencies were expressed in cm^{-1} . 1H NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard; the chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hertz (Hz). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and br s (broad singlet). Mass spectra were recorded on ESI-MS, Thermo, Finnigan LCQ deca xp max. Elemental analyses were performed on Perkin-Elmer 2400 CHN elemental analyser. Analyses indicated by the symbols of the elements of functions were within $\pm 0.4\%$ of the theoretical values. The purity of the compounds was checked on Merck precoated silica gel 60 F-254. Column chromatography was performed using P.D. fine chem. silica gel (100-200 mesh). Yields were not optimized. All the solvents and reagents were used without further purification.

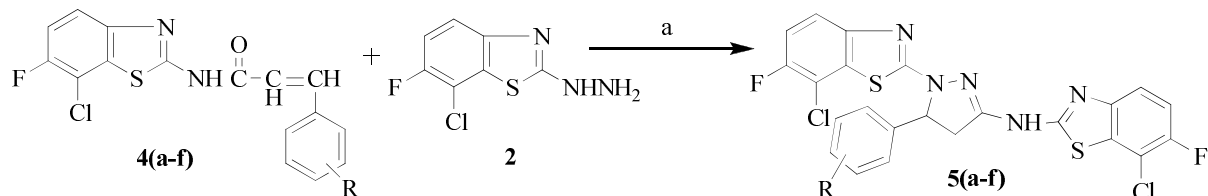


Scheme 1. Reagents and conditions: (a) Br_2 , CH_3COOH , 5°C , NH_3 ; (b) $\text{NH}_2.\text{NH}_2.\text{H}_2\text{O}/\text{HCl}$, Ethylene glycol, reflux 2h.



$R = 4a = 4\text{-N}(\text{CH}_3)_2$, $4b = 4\text{-OH}$, $4c = 4\text{-OH}$, 3-OCH_3 , $4d = 4\text{-OCH}_3$, $4e = 2\text{-OH}$, $4f = \text{H}$.

Scheme 2. Reagents and conditions: (a) $(\text{CH}_3\text{CO})_2\text{O}$, reflux 1h; (b), Substituted aromatic aldehydes, 10% NaOH , stir rt. 12h;



$R = 5a = 4\text{-N}(\text{CH}_3)_2$, $5b = 4\text{-OH}$, $5c = 4\text{-OH}$, 3-OCH_3 , $5d = 4\text{-OCH}_3$, $5e = 2\text{-OH}$, $5f = \text{H}$.

Scheme 3. Reagents and conditions: (a) Ethanol, 3,4-drops glacial acetic acid, reflux 8h.

Synthesis of 7-chloro-6-fluorobenzo[d]thiazol-2-amine (1)

To a stirred mixture of glacial acetic acid (40 ml) precooled at 5°C were added 40 g (0.4123 mol) potassium thiocyanate and 7.25 g (0.0498 mol) of 4-fluoro-3-chloro aniline. To that 6 ml of bromine in 24 ml of glacial acetic acid was added slowly at such a rate that the temperature should not rise beyond 5°C , for a period of 2 h. Stirring was continued for an additional 2 h at the same temperature and at room temperature for 10 h. It was allowed to stand overnight, add 30 ml of water and heated at 85°C , filtered hot. The filtrate was cooled and neutralized with ammonia solution to pH 6. A light yellow precipitate was collected, washed with water and recrystallized by toluene.

Yield 76%; slight yellowish crystalline; mp $180\text{--}182^\circ\text{C}$; IR (ν_{max} , cm^{-1} , KBr): 3480 (N-H), 1199 (C-F), 681 (C-Cl); ^1H NMR (400MHz, DMSO-d_6), δ (ppm): 4.21 (s, 2H, NH_2), 7.62–7.60 (d, 1H, $J = 7.22$, ArH), 7.70–7.68 (d, 1H, ArH, $J = 8.59$ Hz); ESI-MS, m/z : 201.98 $[\text{M}]^+$, 203.86 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_7\text{H}_4\text{ClFN}_2\text{S}$: C, 41.49; H, 1.99; N, 13.82. Found: C, 41.51; H, 2.03; N, 13.81.

Synthesis of 7-chloro-6-fluoro-2-hydrazinylbenzo[d]thiazole (2)

10 ml of conc. HCl was added drop-wise with stirring to 10 ml (0.3 mol) hydrazine hydrate at $5\text{--}10^\circ\text{C}$ followed by ethylene glycol 40 ml. To the above solution 2.025 g (0.01 mol) of compound (1) in portion was added and the resulting mixture was refluxed for 2 h. On cooling solid separated out, was filtered and washed with water, dried and recrystallized by ethanol.

Yield 66%, mp 217-219 °C, light brown needle shaped crystals; IR ($\nu_{\max}$, cm^{-1} , KBr): 3380 (N-H), 1200 (C-F), 683 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 5.09 (s, 2H, NH_2), 7.42-7.41 (d, 1H, ArH, $J = 6.64$ Hz), 7.81-7.79 (d, 1H, ArH, $J = 9.24$ Hz), 9.20 (s, 1H, NH); ESI-MS, m/z : 217.02 $[\text{M}]^+$, 217.05 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_7\text{H}_5\text{ClFN}_3\text{S}$: C, 38.63; H, 2.32; N, 19.31. Found: C, 38.61; H, 2.34, N, 19.31.

Synthesis of N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)acetamide (3)

A mixture of compound (1) 2.025 g (0.01 mol) and 10 ml of acetic anhydride was refluxed for 1 h. Then the reaction mixture was cooled, the separated solid was heated with water, filter and washed with water. The product was then recrystallized by ethanol.

Yield 98%, mp 232-233 °C, whitish needle shaped crystal; IR ($\nu_{\max}$, cm^{-1} , KBr): 3318 (N-H), 1681 (C=O), 1189 (C-F), 652 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 2.34 (s, 3H, CH_3), 7.26-7.24 (d, 1H, ArH, $J = 7.57$ Hz), 7.75-7.73 (d, 1H, ArH, $J = 7.60$ Hz), 9.05 (s, 1H, NH); ESI-MS, m/z : 244.03 $[\text{M}]^+$, 246.02 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_9\text{H}_6\text{ClFN}_2\text{OS}$: C, 44.18; H, 2.47; N, 11.45. Found: C, 44.17; H, 2.49; N, 11.44

Synthesis of N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-substituted phenyl acrylamide (4a-f).

A mixture of compound (3) 2.445 g (0.01 mol) in ethanol (25 ml) and 8 ml of 10% NaOH was stirred in room temperature. To this mixture equimolar quantity of substituted aromatic aldehyde (0.01 mol) was added in small portions. Stirring was continued for overnight in room temperature. The excess of solvent was distilled off, cool, filter and residue was thoroughly washed with cold water. The product was then recrystallized by acetone-water mixture.

N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-dimethylamino)phenyl)acrylamide (4a).

Yield 89%; mp 140-142 °C; yellowish needle shaped crystal; IR ($\nu_{\max}$, cm^{-1} , KBr): 3306 (N-H), 1664 (C=O), 1170 (C-F), 667 (C-Cl); ^1H NMR (200MHz, CDCl_3), δ (ppm): 3.09 (s, 6H, CH_3), 4.58-4.35 (dd, 2H, CH), 6.73-6.68 (d, 2H, ArH, $J = 8.88$ Hz), 7.39-7.35 (d, 1H, ArH, $J = 8.35$ Hz), 7.56-7.54 (d, 1H, ArH, $J = 7.50$ Hz), 7.76-7.72 (d, 2H, ArH, $J = 8.88$ Hz), 9.74 (s, 1H, NH); ESI-MS, m/z : 375.09 $[\text{M}]^+$, 377.09 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_{18}\text{H}_{15}\text{ClFN}_3\text{OS}$: C, 57.52; H, 4.02; N, 11.18. Found: C, 57.53; H, 4.03; N, 11.17

N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-hydroxyphenyl) acrylamide (4b).

Yield 83%; mp 227-229 °C; buff coloured powder; IR ($\nu_{\max}$, cm^{-1} , KBr): 3480 (N-H), 3152 (O-H), 1647 (C=O), 1189 (C-F), 664 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 4.15 (s, 1H, OH), 4.54-5.52 (d, 1H, CH, $J = 7.32$ Hz), 4.68-4.66 (d, 1H, CH, $J = 7.30$ Hz), 7.19-6.92 (d, 2H, ArH, $J = 8.71$ Hz), 7.49-7.25 (d, 2H, ArH, $J = 8.78$ Hz), 7.56-7.52 (d, 1H, ArH, $J = 8.50$ Hz), 7.78-7.76 (d, 2H, ArH, $J = 8.82$ Hz), 9.65 (s, 1H, NH); ESI-MS, m/z : 348.07 $[\text{M}]^+$, 350.05 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_{16}\text{H}_{10}\text{ClFN}_2\text{O}_2\text{S}$: C, 55.10; H, 2.89; N, 8.03. Found: C, 55.12; H, 2.90; N, 8.02.

N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-hydroxy-3-methoxyphenyl)acryl-amide (4c).

Yield 81%; mp 185-187 °C; white powder; IR ($\nu_{\max}$, cm^{-1} , KBr): 3485 (N-H), 3095 (OH), 1685 (C=O), 1250 & 1050 (C-O), 1200 (C-F), 685 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 3.02 (s, 3H, OCH_3), 4.22 (s, 1H, OH), 5.03-4.90 (d, 1H, $J = 7.44$ Hz, CH), 5.16 (d, 1H, CH, $J = 7.40$ Hz), 7.76-7.73 (m, 3H, ArH), 7.96-7.94 (d, 2H, ArH, $J = 8.26$ Hz), 9.85 (s, 1H, NH); ESI-MS, m/z : 378.06 $[\text{M}]^+$, 380.07 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_{17}\text{H}_{12}\text{ClFN}_2\text{O}_3\text{S}$: C, 53.90; H, 3.19; N, 7.40. Found: C, 53.92; H, 3.21; N, 7.39.

N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-methoxyphenyl)acrylamide (4d).

Yield 87%; mp 175-178 °C; orange powder; IR ($\nu_{\max}$, cm^{-1} , KBr): 3485 (N-H), 1680 (C=O), 1270 (C-F), 675 (C-Cl), 1260 & 1050 (C-O); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 3.20 (s, 3H, OCH₃), 4.62-4.58 (d, 1H, J = 7.93 Hz, CH), 5.28-5.26 (d, 1H, CH, J = 7.49 Hz), 7.78-7.52 (m, 4H, ArH), 7.82-7.80 (d, 2H, ArH, J = 7.08 Hz), 9.87 (s, 1H, NH); ESI-MS, m/z : 362.08 $[\text{M}]^+$, 364.09 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_{17}\text{H}_{12}\text{ClFN}_2\text{O}_2\text{S}$: C, 56.28; H, 3.33; N, 7.72. Found: C, 56.29; H, 3.35; N, 7.71.

N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(2-hydroxyphenyl) acrylamide (4e).

Yield 56%; mp 178-181 °C; ash coloured powder; IR ($\nu_{\max}$, cm^{-1} , KBr): 3487 (N-H), 3095 (O-H), 1695 (C=O), 1210 (C-F), 680 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 9.25 (s, 1H, NH), 7.86-7.84 (d, 2H, ArH, J = 7.42 Hz), 7.46-7.42 (m, 4H, ArH), 4.77- 4.73 (d, 1H, CH, J = 7.85 Hz), 5.72 (d, 1H, CH), 4.09 (s, 1H, OH); ESI-MS, m/z : 348.09 $[\text{M}]^+$, 350.11 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_{16}\text{H}_{10}\text{ClFN}_2\text{O}_2\text{S}$: C, 55.10; H, 2.89; N, 8.03. Found: C, 55.11; H, 2.91; N, 8.02.

N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-phenylacrylamide (4f).

Yield 58%; mp 212-214 °C; orange crystalline solid; IR ($\nu_{\max}$, cm^{-1} , KBr): 3489 (N-H), 1705 (C=O), 1203 (C-F), 676 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 4.40-4.36 (d, 1H, CH, J = 7.31 Hz), 4.49-4.43 (d, 1H, CH, J = 7.29 Hz), 7.56-7.55 (m, 5H, ArH), 7.78-7.77 (d, 2H, ArH, J = 8.22 Hz), 9.64 (s, 1H, NH); ESI-MS, m/z : 332.08 $[\text{M}]^+$, 334.07 $[\text{M}+2]^+$; Anal. calcd. for $\text{C}_{16}\text{H}_{10}\text{ClFN}_2\text{OS}$: C, 57.75; H, 3.03; N, 8.42. Found: C, 57.77; H, 3.06; N, 8.41.

Synthesis of 7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-substituted phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine (5a-f)

A mixture of compound (4a-f) (0.002 mol) and 7-chloro-6-fluoro-2-hydrazinylbenzo[d] thiazole (2) (0.002 mol) in ethanol (25 ml) containing 3-4 drops of glacial acetic acid was heated under reflux for 8 h. After completion, the reaction mixture was concentrated under reduced pressure. On cooling, solid separated out, was collected and recrystallized by DMF-water mixture.

7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-(4-(dimethylamino)phenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine (5a).

Yield 82%; mp 240-242 °C; buff coloured powder; IR ($\nu_{\max}$, cm^{-1} , KBr): 3440 (N-H), 1100 (C-F), 640 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 2.05 (s, 6H, CH₃), 3.27 [1H, dd, J = 5.8, 18.20 Hz, H-4 trans (pyrazoline)], 4.04 [1H, dd, J = 12.10, 18.20 Hz, H-4 cis (pyrazoline)], 5.06 (s, 1H, NH), 5.22 [1H, dd, J = 5.80, 12.00 Hz, H-5 (pyrazoline)], 7.23-7.19 (m, 4H, ArH), 7.68- 7.64 (m, 4H, ArH); ESI-MS, m/z : 574.06 $[\text{M}]^+$, 576.05 $[\text{M}+2]^+$, 578.05 $[\text{M}+4]^+$; Anal. calcd. for $\text{C}_{25}\text{H}_{18}\text{Cl}_2\text{F}_2\text{N}_6\text{S}_2$: C, 52.18; H, 3.15; N, 14.60. Found: C, 52.23; H, 3.21; N, 14.52.

4-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(7-chloro-6-fluorobenzo[d]thiazol-2-ylamino)-4,5-dihydro-1H-pyrazol-5-yl)phenol (5b).

Yield 78%; mp 170-171 °C; yellowish crystal; IR ($\nu_{\max}$, cm^{-1} , KBr): 3482 (N-H), 3120 (O-H), 1160 (C-F), 638 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 3.31 [1H, dd, J = 5.7, 18.4 Hz, H-4 trans (pyrazoline)], 4.04 [1H, dd, J = 12.3, 18.5 Hz, H-4 cis (pyrazoline)], 4.49 (bs, 1H, OH), 5.12 (s, 1H, NH), 5.25 [1H, dd, J = 5.83, 12.07 Hz, H-5 (pyrazoline)], 7.43-7.40 (m, 4H, ArH), 7.72- 7.69 (m, 4H, ArH); ESI-MS, m/z : 547.01 $[\text{M}]^+$, 549 $[\text{M}+2]^+$, 551 $[\text{M}+4]^+$; Anal. calcd. for $\text{C}_{23}\text{H}_{13}\text{Cl}_2\text{F}_2\text{N}_5\text{OS}_2$: C, 50.37; H, 2.39; N, 12.77. Found: C, 50.37; H, 2.39; N, 12.77.

4-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(7-chloro-6-fluorobenzo[d]thiazol-2-ylamino)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol (5c).

Yield 72%; mp 185-186 °C; buff coloured powder; IR ($\nu_{\max}$, cm^{-1} , KBr): 3490 (N-H), 3037 (O-H), 1251 & 1050 (C-O), 1178 (C-F), 660 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 3.27 [1H, dd, $J = 5.8, 18.2$ Hz, H-4 trans (pyrazoline)], 3.63 (s, 3H, OCH_3), 4.03 [1H, dd, $J = 12.30, 18.50$ Hz, H-4 cis (pyrazoline)], 4.51(bs, 1H OH), 5.03 (s, 1H, NH), 5.23 [1H, dd, $J = 5.9, 12.04$ Hz, H-5 (pyrazoline)], 7.58-7.55 (m, 3H, ArH), 7.77- 7.73 (m, 4H, ArH); ESI-MS, m/z : 577.02 $[\text{M}]^+$, 579.05 $[\text{M}+2]^+$, 581.03 $[\text{M}+4]^+$; Anal. calcd. for $\text{C}_{24}\text{H}_{15}\text{Cl}_2\text{F}_2\text{N}_5\text{O}_2\text{S}_2$: C, 49.83; H, 2.61; N, 12.11. Found: C, 49.92; H, 2.69; N, 12.16.

7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine (5d).

Yield 80%; mp 180-181 °C; light yellowish crystal IR ($\nu_{\max}$, cm^{-1} , KBr): 3489 (N-H), 1252 & 1055 (C-O), 1178 (C-F), 643 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 3.29 [1H, dd, $J = 5.80, 18.2$ Hz, H-4 trans (pyrazoline)], 3.64 (s, 3H, OCH_3), 4.07 [1H, dd, $J = 11.99, 18.0$ Hz, H-4 cis (pyrazoline)], 5.04 (s, 1H, NH), 5.23 [1H, dd, $J = 5.7, 11.97$ Hz, H-5 (pyrazoline)], 7.48-7.45 (m, 4H, ArH), 7.73- 7.69 (m, 4H, ArH); ESI-MS, m/z : 561.04 $[\text{M}]^+$, 563.06 $[\text{M}+2]^+$, 565.01 $[\text{M}+4]^+$; Anal. calcd. for $\text{C}_{24}\text{H}_{15}\text{Cl}_2\text{F}_2\text{N}_5\text{OS}_2$: C, 51.25; H, 2.69; N, 12.45. Found: C, 51.31; H, 2.76; N, 12.41.

2-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(7-chloro-6-fluorobenzo[d]thiazol-2-ylamino)-4,5-dihydro-1H-pyrazol-5-yl)phenol (5e).

Yield 62%; mp 175-177 °C; white crystal; IR ($\nu_{\max}$, cm^{-1} , KBr): 3475 (N-H), 3119 (O-H), 1176 (C-F), 641 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 3.32 [1H, dd, $J = 5.86, 18.19$ Hz, H-4 trans (pyrazoline)], 4.12 [1H, dd, $J = 12.03, 18.06$ Hz, H-4 cis (pyrazoline)], 4.41 (bs, 1H OH), 5.06 (s, 1H, NH), 5.25 [1H, dd, $J = 5.70, 12.02$ Hz, H-5 (pyrazoline)], 7.38-7.35 (m, 4H, ArH), 7.84-7.80 (m, 4H, ArH); ESI-MS, m/z : 547 $[\text{M}]^+$, 549.02 $[\text{M}+2]^+$, 551 $[\text{M}+4]^+$; Anal. calcd. for $\text{C}_{23}\text{H}_{13}\text{Cl}_2\text{F}_2\text{N}_5\text{OS}_2$: C, 50.37; H, 2.39; N, 12.77. Found: C, 50.49; H, 2.45; N, 12.81.

7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine (5f).

Yield 69%; mp 190-192 °C; gray colour powder; IR ($\nu_{\max}$, cm^{-1} , KBr): 3465 (N-H), 1188 (C-F), 633 (C-Cl); ^1H NMR (400MHz, DMSO- d_6), δ (ppm): 3.27 [1H, dd, $J=5.8, 18.2$ Hz, H-4 trans (pyrazoline)], 4.07 [1H, dd, $J = 11.99, 18.0$ Hz, H-4 cis (pyrazoline)], 4.98 (s, 1H, NH), 5.23 [1H, dd, $J = 5.70, 11.97$ Hz, H-5 (pyrazoline)], 7.26-7.23 (m, 5H, ArH), 7.73-7.68 (m, 4H, ArH); ESI-MS, m/z : 531 $[\text{M}]^+$, 533.01 $[\text{M}+2]^+$, 535 $[\text{M}+4]^+$; Anal. calcd. for $\text{C}_{23}\text{H}_{13}\text{Cl}_2\text{F}_2\text{N}_5\text{S}_2$: C, 51.89; H, 2.46; N, 13.15. Found: C, 51.96; H, 2.52; N, 13.11.

Antioxidant activity [21,22]

DPPH method: 5 to 50 μl (5 to 50 μg) of ascorbic acid and synthesized compounds were taken in different test tubes. Then the volume was adjusted to 1000 μl with methanol. To this 4 ml of methanolic solution of DPPH was added, shaken well and the mixture was allowed to stand at room temperature for 20 minutes. The control was prepared as above without compound. The readings were taken for blank (methanol), control and sample at 517 nm.

Anti-oxidant activity by ferric ion reduction method was calculated by the following formula:

$$\% \text{ Anti radical activity} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \times 100$$

Ferric ion reduction method: The reaction mixture containing 1, 10-o-phenanthroline (0.5 mL), ferric chloride 1mL (0.02 mM) and test compound (solution of different concentration of synthesised compounds and ascorbic acid as standard drug) in a final volume of 5 mL with methanol was incubated for 15-20 min at ambient temperature and absorbance was measured at 510 nm. In another set, sodium dithionite (0.3 mM) was added instead of the compounds and absorbance was taken as equivalent to 100% reduction of all the ferric ions present.

Anti-oxidant activity by ferric ion reduction method can be calculated by the following formula:

$$\% \text{ Activity} = [A_t / A_s] \times 100$$

Where, A_s = absorbance by standard drug solution at 510 nm.

A_t = absorbance by the sample solution at 510 nm.

Table: 1 Antioxidant activities (IC_{50} mM) of synthesised compounds by DPPH and Ferric ion reduction method

Compound	IC_{50} (mM)	
	DPPH	Ferric ion reduction
4a	0.263	25.276
4b	0.187	13.618
4c	0.193	14.519
4d	0.205	22.739
4e	0.141	15.697
4f	0.182	22.988
5a	0.031	4.869
5b	0.018	2.689
5c	0.015	2.333
5d	0.010	1.866
5e	0.032	4.608
5f	0.054	5.963
Ascorbic acid	0.001	0.087

Absorbance by Sodium dithionite (300 μ g/ml) at 510 nm. = 0.571 (Standard, 100%) for ferric ion reduction.

Note: IC_{50} values were not detected at the highest concentration. It was determined by extrapolating the graph.

RESULTS AND DISCUSSION

The preparation of final compounds **5a-f** was accomplished by synthetic sequence illustrated in **Scheme 1, 2** and **3**. The compound **1** was synthesized from 4-fluoro-3-chloro aniline and potassium thiocyanate with bromine in acetic acid. The presence of two aromatic doublet at δ 7.6 to 7.7 ppm and a primary amine peak at δ 4.21 ppm in NMR spectra indicates the formation of basic ring. This was treated with hydrazine hydrate and conc. HCl in the presence of ethylene glycol yielded compound **2**. A singlet peak at δ 9.2 ppm for a secondary amine indicates the formation of hydrazino derivative. Alternatively the compound **1** was treated with acetic anhydride to get compound **3**. The presence of methyl proton at δ 2.34 ppm accounting 3 proton and sharp peak appeared in IR at ν 1681 cm^{-1} confirmed the formation of compound **3**. This was treated with different aromatic aldehydes in basic medium as in Claisen-Schmidt condensation yielded **4a-f** (chalcones). The presence of aromatic peak in the aromatic region, vinylic peak at δ 4.5 to 5.5 ppm and absence of CH_3 peak in between δ 2 to 3 ppm showed the formation of chalcones. The synthesis of target compounds **5a-f**, were carried out by refluxing **4a-f** with 7-chloro-6-fluoro-2-hydrazinylbenzo[d]thiazole (**2**) in alcohol. Presence of singlet in between δ 3 to 5.5 ppm for pyrazoline hydrogen showed the formation of target compounds and all the

compounds structure were further confirmed by mass spectra and there estimated CHN elemental analysis.

The free radical scavenging potentials of the synthesised compounds by DPPH method were tested against a methanolic solution of α, α diphenyl- β -picryl hydrazyl (DPPH) [22]. Antioxidants react with DPPH and convert it to α, α diphenyl- β -picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant activity. The change in the absorbance produced at 517 nm has been used as a measure of antioxidant activity [21]. Whereas in case of ferric ion reduction method, Fe^{2+} reacts rapidly with 1, 10-o-phenanthroline and forms red coloured complex which is exceptionally stable. This complex has strong absorption in the visible spectrum at a wavelength of 510 nm. The synthesised compounds react with Fe^{3+} to reduce and convert it to Fe^{2+} . The degree of coloration indicates the reduction potential of the compounds. The changes in the absorbance produced at 510 nm have been used as a measure of ferric ions reducing potency. The reaction is measured taking sodium dithionite instead of compounds and considered as equivalent to 100% reduction of all the ferric ions present and ascorbic acid was used as a standard drug.

In both the methods compound **5a-f** has shown better Antioxidant activity compared to compound **4a-f**, probably the presence of pyrazoline ring along with two benzothiazole nucleus enhanced the activity. Substitution with electron donating properties at 4th position in the phenyl ring (**5b**, **5c** and **5d**) enhanced the activity.

CONCLUSION

Compound N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-substituted phenyl acrylamide (Chalcones) (**4a-f**) and 7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-substituted phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine (**5a-f**) have been synthesized with good yield. Both series of compounds (**4a-f** & **5a-f**) were tested for antioxidant activity by DPPH and ferric ion reduction methods. Compounds **5a-f** has shown better antioxidant activity than **4a-f** in both the methods.

Acknowledgement

Authors are thankful to Director and Principal, Nargund College of Pharmacy, Bangalore, for providing the necessary facilities for carrying out the project work and also to Indian Institute of Science, Bangalore, for providing spectral data.

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