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Synthesis of novel 7-fluoro-3-substituted-1,2,4-triazolo[3,4-b]benzothiazoles (FTBs) as potent antifungal agents: molecular docking and in silico evaluation

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Abstract A novel series of fluorinated 1,2,4-triazolo[3,4-b]benzothiazoles was synthesized by fusion of proven antifungal lead 1,2,4-triazole with flourinated benzothiazole nucleus exploiting lead hybridization strategy. Their in vitro antifungal assay against various phytopathogenic fungi revealed that a 2- or 3-chlorinated aryl group at the 3-position of the fused system yielded outstanding and remarkable results of fungitoxicity. Compounds **3b** and **3c** were found to inflict the best fungitoxicity against most of the test fungi (EC_{50} value as low as 0.24 mmoles/L) with results better than or comparable to standards. In silico molecular docking and Lipinskii indices were in agreement with the observed trend of antifungal activity. Moreover, the toxicity analysis showed that the compounds belong to class III of toxicity which is the same as that of the recommended standards used.

Keywords 7-Fluoro-3-substituted-1,2,4-triazolo[3,4-b]benzothiazoles · Toxtree analysis · Molecular docking · Lipinski filtration · Antifungal evaluation

Introduction

Lead hybridization-based synthesis [1] of combinational heterocycles is a matter of immense interest and an important source for the discovery of new bioactive molecules which are likely to have diverse and augmented biological activities. The novel molecules thus formed inflict multisite modes of action, lower doses of usage, effects of the same molecules at different stages of growth, high bioavailability and low or zero resistance.

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Triazole and their analogues, considered as bioisosteres of imadazole [2], belongs to the most rapidly expanding group as fungicides [3–8]. They have well-known inhibitiory effects on the cytochrome P450-dependent lanosterol 14α-demethylase (CYP51) which affects the sterol biosynthesis of fungal cell membranes [8]. They have additional advantages of low toxicity [9], high bioavailability [10] and a broad spectrum of activity [11] against several fungi. So far, more than 20 triazole fungicides have been commercialized in the agricultural field. On the other hand, 1,3-benzothiazoles have already been extensively reported for their antifungal [12, 13], antibacterial [14], antiparasitic [15], antimicrobial [16], anticancer [17], antitubercular [18], analgesic [19], antitumor [20], antihelmintic [21], anti-inflammatory [22], and anti-oxidant [23] activities, and are also part of commercially available fungicides [24], herbicides [25], plant desiccants and defoliant [26] compounds. The combination of 1,2,4-triazole and benzothiazol have shown synergestic effects in combination with each other [1]. So, there is overwhelming attention on their mutual derivatization with each other for synergistic and augmented fungi-toxicological profiles of the molecules.

Most of the organic molecules have failed to reach commercialization due to low tissue uptake and, thus, low bioavalability [27–29]. The presence of fluorine in the molecules is known to induce improved pharmokinetic profiles of the bioactive molecule,s improving lipophilicity and suppressing the metabolic detoxification by increasing the in vivo lifetime of the molecules and, thus, increasing the tissue bioavailability. So, the share of commercial fluorinated compounds is on the increase nowadays.

The vitality of pharmokinetic profiles and biofunctionality of 1,2,4-triazoles and 1,3-benzothiazoles along with the importance of the introduction of fluorine in biologically active compounds, impelled us to take up the synthesis of series of novel 7-fluoro-3-substituted-1,2,4-triazolo[3,4-b]benzothiazoles (FTBs) to check the effect of fusion of these leads on the antifungal potential of the compounds. Due to the development of resistance to 1,2,4-triazole fungicides (reduced risk fungicides), various phytopathogenic fungi have become the optimal candidates for the potential evaluation of 1,2,4-triazolo analogues as resistance measures. In continuity with our work, with remarkable results of the combination of 1,2,4-triazole and 1,3-benzothiazole with spacers between them [1], this paper reports the synthesis of novel 7-fluoro-3-substituted-1,2,4-triazolo[3,4-b]benzothiazoles having these moieties in the form of fused heterocycles and their antifungal evaluation against various phytopathogenic fungi. In silico Toxtree analysis, docking studies in the active site of lanosterol 14α-demethylase [30] and Lipinski characters [31] have been further performed to rationalize the trends of the observed bioactivity.

Experimental

General information

All chemicals were of analytical grade and used directly. Melting points were determined in scientific melting point apparatus and were uncorrected. The purity of the compounds were checked by TLC using Merck silica gel 60 F254 and visualized

by exposure to iodine vapors or UV light. IR spectra were recorded on a Perkin Elmer RX1 FTIR spectrophotometer, using potassium bromide pellets, and the frequencies are expressed in cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance II 400 NMR spectrometer, using tetramethylsilane as the internal reference, with $CDCl_3$ and DMSO-d_6 as solvents. The chemical shifts are reported in parts per million (d ppm). Elemental analyses were performed on a Perkin Elmer 2400 Series II CHNS/O elemental analyzer. The mass spectra were recorded on Perkin Elmer Clarus 500 Mass Spectrometer. All spectral data were consistent with the proposed structure.

*General procedure for the synthesis of 6-flourbenzothiazol-2-amines (**1**)*

4-fluoroaniline (0.1 mol) and potassium thiocyanate (0.4 mol) were dissolved in glacial acetic acid (40 ml) and cooled. Bromine (0.04 mol) mixed with glacial acetic acid (24 ml) was added from a dropping funnel at such a rate that temperature did not rise above 5–6 °C. After all the bromine had been added, the solution was stirred with a glass rod for an additional 2 h at low temperature. The mixture was allowed to stand overnight, during which period orange–yellow precipitates settled at the bottom. Water was added and the slurry formed was heated at 85 °C on a steam bath for 20 min and filtered hot. The filtrate was cooled and neutralized with ammonia solution to pH 6. Off-white precipitates obtained were collected, washed with water and recrystallized from benzene to get pure crystals. IR (KBr) ν_{max}: 3452 (N–H str), 3080 and 813 (C–H aromatic str), 3010 (=C–H str), 1680 (C=C str), 1350 (C=N str). The ^{1}HNMR (DMSO-d_6) spectrum of this product showed signals: δ 6.5–7.4 (3H, m, Ar C–H) and at 5.4 (2H, br, NH_2) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 108.0, 113.9, 117.8, 131.6, 148.8, 158.5 and 166.3 ppm.

*Synthesis of 6-flourohydrazinylbenzothiazol (**2**)*

Concentrated hydrochloric acid (0.067 mol) was added dropwise with stirring to hydrazine hydrate (0.12 mol) at 5–6 °C followed by ethylene glycol (30 mL); thereafter, 6-fluorobenzo[d]thiazol-2-amine (**1**) (20 mmol) was added in portions and the resultant mixture was refluxed for 2–3 h and cooled at room temperature. The reaction progress was monitored by TLC using toluene:ethylacetate (75:25) as mobile phase. The reaction mixture was filtered and the resulting precipitates were washed with distilled water. The resulting crude was recrystallized from ethanol. IR (KBr) ν_{max}: 3010 (=C–H str), 1645 (C=C str), 1398 (C=N str). The ^{1}HNMR (DMSO-d_6) spectrum of this product showed signals: δ 7.2–7.4 (3H, m, Ar C–H), at 4.0 (1H, br, NH) and 4.75 (2H, br, NH_2) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 106.1, 112.4, 115.5, 129.7, 145.5, 157.5, 171.2 ppm.

*General procedure for the synthesis of 7-flouro-3-phenyl-1,2,4-triazolo[3,4-b]benzothiazoles (**3a–3t**)*

The mixture of 6-fluoro-2-hydrazinylbenzo[d]thiazole (**2**) (0.01 mol) and benzaldehyde/substituted benzaldehyde (0.01 mol) was refluxed in ethanol (15 ml) at

70–80 °C for 3 h. The separated product obtained was filtered off, washed with distilled water and recrystallized from methanol to give the corresponding hydrazone. The product obtained was further dissolved in acetic acid (20 ml) at room temperature followed by the addition of sodium acetate (0.5 g). Bromine (2 mmol) in acetic acid (10 ml) was added dropwise to the refluxing reaction mixture. After 1 h, the mixture was poured onto crushed ice (100 g). The precipitate obtained was filtered off and crystallized from ethanol-dimethylformamide (1:1) to give crystals of (**3a**–**3t**).

7-flouro-3-phenyl-1,2,4-triazolo[3,4-b]benzothiazole (**3a**) IR (KBr) ν_{max}: 1600 (>C=N str), 1522 (C=C str), 1245 (C–F str) and 852 (C–S str) cm^{-1}. The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.0–7.6 (3H, m, fused benzothiazole ring) and 7.7–8.0 (5H, m, phenyl ring) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 106.1, 112.4, 115.5, 129.7, 145.5, 157.5, 171.2 ppm.

3-(2′Chlorophenyl)-7-flouro-1,2,4-triazolo[3,4-b]benzothiazole (**3b**) IR (KBr) ν_{max}: 2853 (C–H str), 1610 (>C=N str), 1531(C=C str), 1356 (C–F str), 825 (C–Cl str) and 714 (C–S str) cm^{-1}. The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.9–8.3 (3H, m, fused benzothiazole ring) and 7.3–7.5 (4H, m, phenyl ring) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 107.8, 114.4, 123.4, 127.5, 128.0, 129.4, 130.4, 131.7, 132.3, 136.7, 138.1, 151.2, 158.6, 159.9.

3-(3′-Chlorophenyl)-7-flouro-1,2,4-triazolo[3,4-b]benzothiazole (**3c**) IR (KBr) ν_{max}: 2853 (C–H str), 1623 (>C=N str), 1531 (C=C str), 1398 (C–F str), 755 (C–Cl str) and 601 (C–S str) cm^{-1}. The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.2–8.1 (3H, m, fused benzo ring) and 7.4–8.1 (4H, m, chlorophenyl ring) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 106.4, 113.4, 124.3, 127.5, 128.0, 129.4, 130.4, 131.5, 133.3, 137.7, 139.1, 153.2, 159.3, 161.2.

3-(4′-Chlorophenyl)-7-flouro-1,2,4-triazolo[3,4-b]benzothiazole (**3d**) IR (KBr) ν_{max}: 2956 (C–H str), 1595 (>C=N str), 1563 (C=C str), 1265 (C–F str), 985 (C–Cl str) and 814 (C–S str) cm^{-1}. The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.2–7.5 (3H, m, fused benzo ring) and 7.9–8.4 (4H, m, chlorophenyl ring) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 105.2, 116.3, 125.1, 127.8, 129.5, 129.9, 130.8, 132.6, 134.1, 136.3, 140.1, 151.3, 156.6, 157.3.

7-Flouro-3-(4′-flourophenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3e**) IR (KBr) ν_{max}: 2893 (C–H str), 1600 (>C=N str), 1516 (C=C str), 1322 (C–F str) and 714 (C–S str) cm^{-1}. The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.6–7.9 (3H, m, fused benzo ring) and 7.1–7.4 (4H, m, fluorophenyl ring) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 108.4, 113.2, 116.7, 116.6, 123.3, 128.7, 128.9, 130.3, 132.2, 136.5, 150.4, 157.9, 162.3, 163.5.

7-Flouro-3-(2′-hydroxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3f**) IR (KBr) ν_{max}: 3301 (O–H str), 2913 (C–H str), 1576 (>C=N str), 1501 (C=C str), 1321 (C–F str), 1176 (>C–OH str) and 714 (C–S str) cm^{-1}. The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.6 (3H, m, fused benzothiazole ring), 8.8 (1H, br, phenolic proton) and between 7.0 and 7.2 (4H, m, substituted phenyl

ring) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 108.8, 113.4, 117.9, 118.9, 121.3, 123.6, 130.9, 132.3, 133.4, 136.5, 151.3, 154.3, 158.8, 161.2.

7-Flouro-3-(3′-hydroxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3g**) IR (KBr) ν_{max}: 3313 (O–H str), 2799 (C–H str), 1583 (>C=N str), 1512 (C=C str), 1287 (C–F str), 1216 (>C–OH str) and 797 (C–S str) cm^{-1}. The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.5–7.6 (3H, m, fused benzothiazole ring), 7.2–7.4 (4H, m, substituted pheyl group) and 9.30 (1H, br, phenolic proton) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 107.5, 112.5, 116.4, 118.5, 120.4, 122.4, 131.9, 132.4, 134.5, 138.9, 152.4, 155.4, 158.6, 164.6.

7-Flouro-3-(4′-hydroxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3h**) IR (KBr) ν_{max}: 3312 (O–H str), 2879 (C–H str), 1644 (>C=N str), 1531 (C=C str), 1343 (C–F str), 1299 (>C–OH str) and 723 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of the product showed signals: δ 7.4–7.7 (3H, m, fused benzothiazole ring), 7.0–7.2 (4H, m, substituted phenyl ring) and 9.33 (1H, br, phenolic proton) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 107.2, 111.9, 115.4, 117.4, 121.4, 122.4, 131.9, 132.4, 134.5, 140.1, 153.3, 154.4, 158.6, 166.9.

7-Flouro-3-(4′-methoxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3i**) IR (KBr) ν_{max}: 2875 (C–H str), 1676 (O–C str), 1564 (>C=N str), 1502 (C=C str), 1376 (C–F str), 1096 (C–O–C str) and 713 (C–S str) cm^{-1}. The ^{1}HNMR ($CDCl_3$) spectrum of the product showed signals: δ 7.2–7.6 (3H, m, fused benzothiazole ring), 7.9–8.1 (4H, m, substituted phenyl ring) and 3.13 (3H, s, –OCH_3) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 57.6, 108.8, 113.4, 114.6, 115.5, 123.7, 126.0, 130.1, 130.1, 132.9, 137.9, 150.3, 159.8, 161.7, 163.4.

7-Flouro-3-(2′-nitrophenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3j**) IR (KBr) ν_{max}: 2875 (C–H str), 1564 (>C=N str), 1528 (C=C str), 1501 (NO_2 str), 1386 (C–F str) and 845 (C–S str) cm^{-1}.The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.0–7.2 (3H, m, Ar C–H) and 7.9–8.4 (4H, m, nitrophenyl group) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 105.7, 112.4, 113.4, 118.0, 124.5, 126.7, 131.1, 131.1, 135.3, 139.5, 154.3, 159.8, 160.1, 161.4.

7-Flouro-3-(3′-nitrophenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3k**) IR (KBr) ν_{max}: 2793 (C–H str), 1614 (>C=N str), 1578 (C=C str), 1494 (NO_2 str), 1345 (C–F str) and 898 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.3–7.5 (3H, m, Ar C–H) and 7.7–8.3 (4H, m, nitrophenyl group) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 109.2, 111.1, 114.3, 116.3, 123.0, 126.7, 133.4, 133.4, 137.1, 142.5, 154.3, 159.8, 164.2, 169.4.

7-Flouro-3-(4′-nitrophenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3l**) IR (KBr) ν_{max}: 2793 (C–H str), 1614 (>C=N str), 1588 (C=C str), 1523 (NO_2 str), 1335 (C–F str) and 816 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.7–8.0 (3H, m, fused benzothiazole ring) and 8.1–8.3 (4H, m, nitrophenyl ring) ppm. The peaks in its ^{13}CNMR (DMSO-d_6) δ: 102.3, 117.1, 119.3, 121.3, 125.0, 129.7, 135.4, 135.4, 139.1, 149.5, 158.3, 162.8, 168.2, 169.4.

7-Flouro-3-(3′,4′-dimethoxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3m**) IR (KBr) ν_{max}: 2832 (C–H str), 1614 (>C=N str), 1594 (C=C str), 1376 (C–F str), 1105 (C–O–C str) and 915 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.0–7.2 (3H, m, benzothiazole ring), 6.9–8.3 (3H, m, phenyl group) and δ 3.3 (6H, s, OCH_3 groups) ppm. The peaks in its $^{13}CNMR$ (DMSO-d_6) δ: 59.4, 59.4, 105.7, 112.4, 113.4, 118.0, 124.5, 126.7, 131.1, 131.1, 135.3, 139.5, 154.3, 159.8, 160.1, 161.4.

7-Flouro-3-(4′-hyroxy-3′-methoxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3n**) IR (KBr) ν_{max}: 3306 (O–H str), 2912 (C–H str), 1667 (>C=N str), 1611 (C=C str), 1322 (C–F str), 1087 (C–O–C str) and 878 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.7–8.0 (3H, m, benzothiazole), 6.9–7.1 (3H, m, phenyl group), 3.8 (3H, s, CH_3 group) and 9.83 (1H, br, phenolic proton) ppm. The peaks in its $^{13}CNMR$ (DMSO-d_6) δ: 57.9, 108.3, 113.3, 119.6, 123.4, 125.9, 135.3, 135.3, 139.7, 142.2, 148.8, 151.6, 156.8, 160.0, 163.4.

7-Flouro-3-(4′-ethoxy-3′-methoxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3o**) IR (KBr) ν_{max}: 3243 (O–H str), 2832 (C–H str), 1614 (>C=N str), 1599 (C=C str), 1324 (C–F str), 1176 (>C–OH str), 996 (C–O–C str) and 754 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.6–8.0 (3H, m, benzothiazole ring), 6.9–7.2 (3H, m, substituted phenyl group), 4.0 (2H, m, CH_2), 1.32 (3H, m, CH_3) and 9.83 (1H, br, OH group) ppm. The peaks in its $^{13}CNMR$ (DMSO-d_6) δ: 17.8, 67.9, 107.2, 119.7, 121.6, 124.4, 129.6, 138.5, 138.5, 140.1, 144.6, 149.2, 153.6, 157.0, 161.4, 164.0.

3-(4′-dimetylaminophenyl)-7-Flouro-1,2,4-triazolo[3,4-b]benzothiazole (**3p**) IR (KBr) ν_{max}: 2874 (C–H str), 1601 (>C=N str), 1531(C=C str), 1354 (C–F str) and 899 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.3–7.5 (3H, m, benzothiazole ring), 6.7–7.0 (4H, m, substituted phenyl group) and 3.1 (6H, m, CH_3) ppm. The peaks in its $^{13}CNMR$ (DMSO-d_6) δ: 43.7, 43.7, 105.7, 112.4, 112.4, 115.9, 123.5, 125.7, 128.9, 128.9, 132.9, 138.7, 150.7, 156.3, 159.8, 161.7.

7-Flouro-3-(3′,4′,5′-trimethoxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3q**) IR (KBr) ν_{max}: 2753 (C–H str), 1645 (>C=N str), 1603 (C=C str), 1354 (C–F str), 1123 (C–O–C str) and 769 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.0–7.2 (3H, m, fused benzo ring), 3.0–3.3 (9H, s, CH_3) and 6.9 (2H, m, substituted phenyl group) ppm. The peaks in its $^{13}CNMR$ (DMSO-d_6) δ: 56.7, 58.1, 62.4, 108.1, 113.4, 113.4, 119.4, 121.5, 123.7, 128.4, 128.4, 132.9, 138.7, 152.3, 157.7, 160.1, 164.5.

7-Flouro-3-(4′hydroxy-3′,5′-dimethoxyphenyl)-1,2,4-triazolo[3,4-b]benzothiazole (**3r**) IR (KBr) ν_{max}: 3218 (O–H str), 2873 (C–H str), 1598 (>C=N str), 1531 (C=C str), 1395 (C–F str), 1054 (C–O–C str) and 715 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.8–8.1 (3H, m, fused benzo ring), 6.7 (2H, s, substituted phenyl ring), 8.7 (1H, br, OH) and 3.8 (6H, s, CH_3) ppm. The peaks in its $^{13}CNMR$ (DMSO-d_6) δ: 56.7, 56.7, 103.8, 117.5, 119.5, 120.4, 124.4, 126.7, 129.2, 129.2, 135.3, 138.7, 151.3, 159.7, 165.1, 169.5.

7-Flouro-3-furanyl-1,2,4-triazolo[3,4-b]benzothiazole (**3s**) IR (KBr) ν_{max}: 2897 (C–H str), 1612 (>C=N str), 1601 (C=C str), 1376 (C–F str) and 829 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.2–7.5 (3H, m, fused benzo ring) and 7.7–8.3 (3H, s, furan ring) ppm. The peaks in its $^{13}CNMR$ (DMSO-d_6) δ: 106.7, 109.3, 112.2, 114.8, 123.9, 134.6, 138.9, 145.4, 149.8, 154.4, 159.9, 163.0.

7-Flouro-3-styrenyl-1,2,4-triazolo[3,4-b]benzothiazole (**3t**) IR (KBr) ν_{max}: 2793 (C–H str), 1602 (>C=N str),1589 (C=C str), 1357 (C–F str) and 763 (C–S str) cm^{-1}. The ^{1}H NMR ($CDCl_3$) spectrum of product showed signals: δ 7.8–8.0 (3H, m, benzothiazole ring), 7.3–7.6 (5H, s, phenyl ring) and 6.5 (2H, s, CH) ppm. The peaks in its $^{13}CNMR$ (DMSO-d_6) δ: 108.9, 114.5, 123.4, 127.9, 128.9, 128.9, 130.1, 130.1, 133.4, 134.6, 137.9, 138.7, 142.2, 148.3, 158.8, 162.3.

In silico analysis

Toxtree analysis

Different derivatives were screened for different physico-chemical properties by using different software. Toxtree v.2.6.6 is an open-source software application that places chemicals into categories and predicts various kinds of toxic effects by applying various decision-tree approaches. Toxtree was developed by IdeaConsult (Sofia, Bulgaria) under the terms of an ECB contract. The software is made freely available by ECB as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity. The new module with the revised list of SAS also includes quantitative structure–activity relationship (QSAR) models that enable the toxicity evaluations for a number of chemical classes to be fine-tuned.

In order to find out the toxic hazards of all the synthesized compounds, two-dimensional models of the compounds were first converted into its simplified molecular-input line-entry system (SMILES format). Then, simply putting the SMILES code into the chemical identifier row available in the Toxtree software, we can easily obtain the toxic characters.

Docking procedure

The compounds were built using the builder tool kit of the software package ArgusLab 4.0.1 and the geometry was optimized using the semi-empirical quantum mechanical method, PM_3. Due to the non-availability of fungal lanosterol demethylase, the crystal coordinates of human lanosterol 14α-demethylase (PDB ID 2 VKU) were downloaded from the protein data bank (www.rcsb.org) and the active site of the enzyme was located, carrying a 4,4′-dihydroxybenzophenone (DHBP) as a ligand in the active site. The molecule to be docked in the active site of the protein was pasted in the work space carrying the structure of the enzyme. The docking programme implements an efficient grid-based docking algorithm which approximates an exhaustive search within the free volume of the binding site cavity.

The conformational space was explored by the geometry optimization of the flexible ligand (rings were treated as rigid) in combination with the incremental construction of the ligand torsions. Thus, docking occurs between the flexible ligand parts of the compound and enzyme. The final positions of the compounds were ranked by lowest interaction energy values (docking score in Kcal/mol). H-bond interactions between the compound and enzyme were explored.

Lipinski filtration

Fungitoxicity profiles were further rationalized for their potential bioactivity as antifungals or pro-antifungals by theoretically calculating the Lipinski parameters of all the synthesized molecules using Molinspiration which is a web-based software. It is already referred to as the Pfizer's rule of five or simply the rule of five (RO5). This rule was formulated by Christopher A. Lipinski in 1997. According to Lipinski's rule of five, molecules should have log *P* values of ≤ 5 in order to be readily bioavailable. Lipinski's rule says that the molecular weight should be below 500, the number of hydrogen bond acceptors should not be more than 5 and also the number of hydrogen bond donors should not be more than 5. These numbers are the upper limits for the biomolecules to be able to penetrate through biomembranes. In order to calculate the molecular properties, first we have either to draw the molecule online (www.molinspiration.com) or simply to input the SMILES code, then all the Lipinski characters along with the bioactivity and 3D structure of the molecule are generated.

Antifungal assays

The in vitro antifungal activity of all the title compounds were evaluated against five phytopathogenic fungi viz. *Puccinia triticina, Ustilago tritici, Erysiphe graminis*, *Puccinia striiformis* and *Alternaria alternata* which are often encountered, and compared with the standard fungicides Tilt (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole) and Vitavax (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide). The isolates of phytopathogenic fungi were provided by the Plant Pathology Department of the Punjab Agricultural University and the standards Tilt and Vitavax, serving as the positive control, were obtained from their respective manufacturers.

Five micromoles per litre of each of the test compounds and standards used were made by dissolving the appropriate amount of the compound in 1.0 ml of dimethyl sulfoxide (DMSO) and making the final volume 10 ml. These were capped and refrigerated to be used as stock solutions. Serial dilution of the stock solutions up to 0.1 mmoles per litre was done as and when required.

The in vitro effective concentrations of the compounds were determined by the spore germination inhibition technique [35]. The EC_{50} refers to the concentration which induces a response halfway. For assays, 0.02 ml of the title compounds to be tested and 0.02 ml of spore suspension were seeded in the cavity of a cavity slide and incubated at 15 ± 2 °C. Growth was determined at 24 h for all the

phytopathogenic fungi. The results of assays in terms of EC_{50} are summarized in Table 2. The data points are the means of triplicates.

Results and discussion

Synthetic chemistry

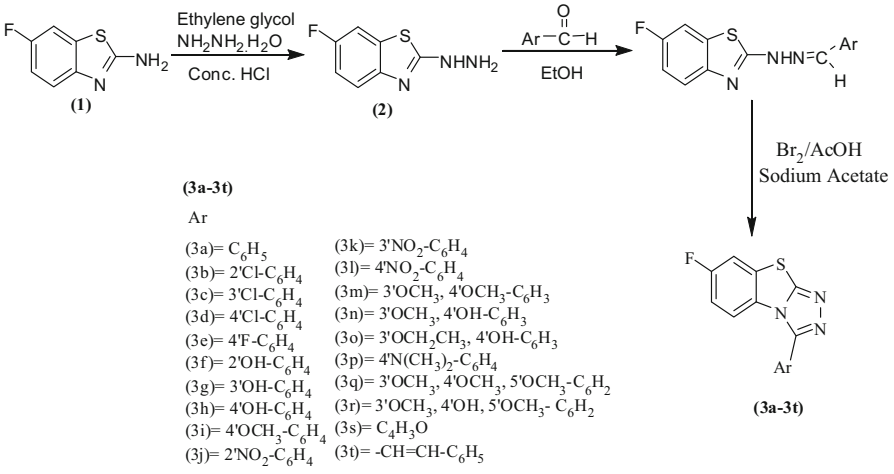
Scheme 1 shows the schematic representation of synthetic route for the preparation of the target compounds. Compound **1** was synthesized by the reaction of aldehydes with 4-flouroaniline followed by bromocyclization.

The reaction of compound **1** with hydrazine hydrate in ethylene glycol yielded 6-fluoro-2-hydrazinylbenzo[d]thiazole **2**. Further, 7-fluoro-3-substituted-1,2,4-triazolo[3,4-b]benzothiazoles **3a–3t** were prepared by refluxing the mixture of compound **2** and a suitable aromatic aldehyde in ethanol. The substituted hydrazones obtained were further bromocyclised to give corresponding FTBs, **3a–3t**. The structures of the compounds were confirmed by IR, ^{1}H NMR and ^{13}C NMR and elemental analysis (Table 1) which were correlated with the proposed structures, confirming the formation of the compounds.

In silico analysis

Toxtree analysis

Estimation of toxic hazards by Cramer rules was carried out using Toxtree v.2.6.6 software [32], which showed that the compounds belong to the class III level of toxicity, which was same as that of the toxicity level standard fungicides used.



Scheme 1 General synthesis of 7-flouro-3-phenyl-1,2,4-triazolo[3,4-b]benzothiazoles

Table 1 Physical characteristics and elemental analysis of all the synthesized compounds

Compds	Yield (%)	M.P (°C)	Mol. formula	Elemental analysis (calculated %/found %)		
				C	H	N
1	89	195	$C_7H_5FN_2S$	(48.33/49.99)	(2.12/3.00)	(15.67/16.66)
2	84	223	$C_7H_6FN_3S$	(44.79/45.89)	(2.98/3.30)	(21.97/22.94)
3a	75	235	$C_{14}H_8FN_3S$	(61.77/62.44)	(2.12/2.99)	(14.87/15.60)
3b	63	239	$C_{14}H_7ClFN_3S$	(54.57/55.36)	(1.78/2.32)	(13.21/13.83)
3c	61	252	$C_{14}H_7ClFN_3S$	(54.36/55.34)	(2.99/2.32)	(13.23/13.83)
3d	66	247	$C_{14}H_7ClFN_3S$	(54.43/55.34)	(2.23/2.32)	(13.00/13.83)
3e	54	267	$C_{14}H_7F_2N_3S$	(57.32/58.34)	(2.39/2.86)	(14.42/14.83)
3f	62	234	$C_{14}H_8FN_3OS$	(58.24/58.94)	(2.12/2.83)	(13.97/14.73)
3g	67	247	$C_{14}H_8FN_3OS$	(57.44/58.94)	(2.09/2.83)	(13.74/14.73)
3h	63	243	$C_{14}H_8FN_3OS$	(57.94/58.94)	(2.42/2.83)	(13.87/14.73)
3i	69	268	$C_{15}H_{10}FN_3OS$	(59.77/60.19)	(3.22/3.37)	(13.87/14.04)
3j	54	285	$C_{14}H_7FN_4O_2S$	(52.76/53.50)	(2.74/2.24)	(17.67/17.83)
3k	51	292	$C_{14}H_7FN_4O_2S$	(52.34/53.50)	(2.22/2.24)	(16.99/17.83)
3l	54	287	$C_{14}H_7FN_4O_2S$	(52.16/53.50)	(2.44/2.24)	(17.23/17.83)
3m	64	247	$C_{16}H_{12}FN_3O_2S$	(57.33/58.35)	(3.24/3.67)	(12.33/12.76)
3n	63	241	$C_{15}H_{10}FN_3O_2S$	(57.22/57.54)	(2.98/3.20)	(12.89/13.33)
3o	64	246	$C_{16}H_{12}FN_3O_2S$	(57.39/58.35)	(3.22/3.67)	(12.34/12.76)
3p	51	249	$C_{16}H_{13}FN_4S$	(60.33/61.52)	(3.87/3.19)	(17.22/17.94)
3q	53	257	$C_{17}H_{14}FN_3O_3S$	(56.12/56.82)	(3.22/3.93)	(11.22/11.69)
3r	62	252	$C_{16}H_{12}FN_3O_3S$	(55.22/55.65)	(3.21/3.50)	(11.8/12.17)
3s	52	265	$C_{12}H_6FN_3OS$	(55.21/55.59)	(2.12/2.33)	(16.27/16.21)
3t	49	261	$C_{16}H_{10}FN_3S$	(64.23/65.02)	(3.12/3.41)	(14.19/14.23)

Docking studies

Molecular docking scores of all the synthesized compounds were performed in the active site of the enzyme lanosterol 14α-demethylase (PDB ID 2VKU), using ArgusLab 4.0.1. All the compounds get concealed in the interior of the enzyme and none of these compounds exhibited H-bond interactions with active sites of lanosterol 14α-demethylase because of no labile hydrogens (–NH, –OH or HF) present in the molecule, but they still have high favorable free energies of binding because of the close strong interactions with amino acid residues, viz. glycine, alanine, phenylalanine, cystein and threonine (Fig. 1). Compounds **3b**, **3c, 3g** and **3t** had the most favourable free energies of binding, viz. −11.12, −11.76, −10.99 and −10.74 kcal/mol, respectively. Significant differences in their respective docking scores were observed which was probably due to hydrophobic and other dipole–dipole interactions [33, 34] of the test compound with amino acid residues in the active site. Docking scores of most of the synthesized compounds were better than the compounds having these two leads separated by spacers [1], and their bioactive assay, therefore, followed the trend.

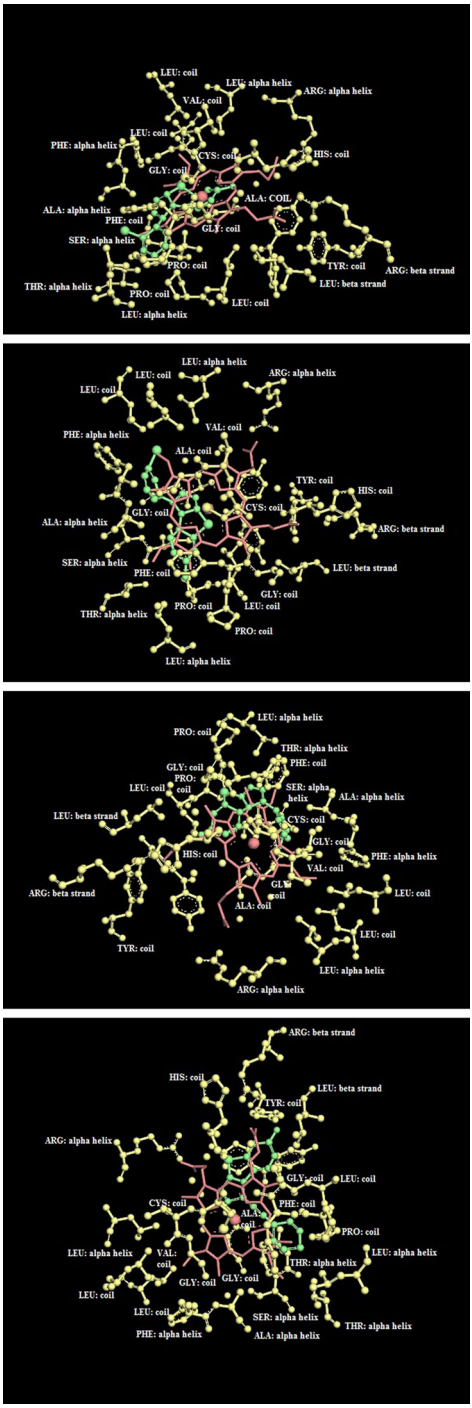


Fig. 1 Docking poses of the compounds **3b**, **3c**, **3g** and **3t** (shown in *green*), respectively, in the active site (shown in *red*) of cytochrome P450 lanosterol 14α-demethylase

Lipinski filtration

Lipinski parameters of the synthesized compounds are presented in Table 2. All the compounds had molecular weights less than 360. Compounds **3q** and **3r** had H-bond acceptors more than 5 but all of them had the number of hydrogen bond donors (i.e. the sum of OH and NH groups) less than 5. The log p value was unfavorable (more than 5) in the case of compounds **3j**, **3k**, **3l** and **3t**. Combining all the factors, compounds **3j, 3k, 3l**, **3q**, **3r** and **3t** were expected to be non-relevant for bioactivity. But for real results, all the compounds were evaluated for fungitoxicity.

Antifungal assay

Fungicidal evaluation of the series of title compounds was performed against various phytopathogenic fungi by a spore germination inhibition technique representing their results in terms of EC_{50} values. The five fungi used in the

Table 2 The docking scores and Lipinski characters

Compds	Final docked energy (kcal/mol)	Log p	Molecular weight	*n* ON	*n* OHNH
1	−8.62	2.59	168	2	1
2	−9.19	2.10	183	3	2
3a	−10.46	4.37	269	3	0
3b	−11.12	4.93	303	3	0
3c	−11.76	4.93	303	3	0
3d	−10.73	4.93	303	3	0
3e	−10.15	4.53	287	3	0
3f	−9.51	3.99	285	4	1
3g	−10.99	3.99	285	4	1
3h	−9.97	3.99	285	4	1
3i	−9.76	4.29	295	4	0
3j	−9.54	5.16	314	5	0
3k	−9.43	5.16	314	5	0
3l	−9.68	5.16	314	5	0
3m	−9.59	4.12	329	5	0
3n	−9.02	3.86	315	5	1
3o	−9.99	4.20	329	5	1
3p	−8.95	4.66	312	4	0
3q	−8.14	4.00	359	6	0
3r	−8.67	3.73	345	6	1
3s	−8.45	2.96	259	4	0
3t	−10.74	5.16	295	3	0

Log p logarithm of the octanol–water partition coefficient; *n ON* number of hydrogen bond acceptors (sum of O and N atoms); *n OHNH* number of hydrogen bond donors (sum of OH and NH groups)

fungicidal bioassay were *Puccinia triticina, Ustilago tritici, Erysiphe graminis*, *Puccinia striiformis* and *Alternaria alternata*. Commercial agricultural fungicide Vitavax (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide) against *Ustilago tritici* and Tilt (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole) against the rest of the fungi were used as standards. For the sake of comparison of fungitoxicity of the molecules with standard fungicides, the results were appropriately expressed in terms of mmoles/L.

Investigations of antifungal screening listed in Table 3 clearly revealed that most of the synthesized fluorinated heterocylics showed significant inhibition of germination against all the test fungi. This series were found to be most active against *A. alternata*. Ten out of 20 compounds had antifungal potential better than the standard compound Tilt (ED_{50} 0.69 mmoles/L) with compound **3c** the best with ED_{50} 0.39 m moles/L which was much more favorable than the standard. Against *E.*

Table 3 Antifungal potential of the synthesized compounds against various phytopathogenic fungi in terms of EC_{50} (mmoles/L)

Compounds	*P. triticina*	*U. tritici*	*E. graminis*	*P. striiformis*	*A. alternata*
EC_{50} (m moles/L)					
1	4.64	2.08	4.37	3.86	2.85
2	2.19	0.55	0.59	3.01	1.21
3a	0.92	0.65	0.92	1.67	0.46
3b	0.49	0.33	0.27	0.47	0.54
3c	0.47	0.60	0.24	0.36	0.39
3d	0.82	0.79	0.37	1.81	1.32
3e	1.74	0.31	1.74	2.26	1.21
3f	1.22	0.25	0.84	1.57	0.86
3g	0.46	0.81	0.27	0.87	0.82
3h	1.75	0.87	0.70	1.92	0.70
3i	1.69	0.33	0.84	1.11	0.42
3j	1.59	1.47	0.40	0.66	0.41
3k	1.53	1.27	0.59	1.12	0.54
3l	2.31	0.76	0.89	2.26	0.61
3m	1.31	0.30	0.75	1.20	0.45
3n	0.79	0.63	1.12	1.09	0.66
3o	1.51	0.60	0.40	1.36	0.75
3p	1.60	0.80	1.60	1.12	0.80
3q	1.32	0.69	1.81	1.25	0.52
3r	2.11	0.72	1.76	1.30	1.43
3s	1.93	1.54	0.96	1.73	1.92
3t	0.83	0.73	0.41	0.64	0.62
Tilt[a]	0.65	–	0.40	0.50	0.69
Vitavax[b]	–	0.31	–	–	–

[a] Standard fungicide against *A.alternata, E. graminis, P. triticina and P.striiformis*

[b] Standard fungicide against *U. tritici*

graminis, compounds **3b**, **3c** and **3d** have antifungal potential with EC_{50} values of 0.27, 0.24 and 0.27 mmoles/L, respectively, which were again lower than the standard fungicide Tilt (EC_{50} = 0.40 mmoles/L). Similar trends were repeated against *P. triticina*, where compounds **3b**, **3c** and **3g** showing promising results (EC_{50} values 0.49, 0.47 and 0.46 mmoles/L, respectively) better than the standard (Tilt EC_{50} values 0.60 mmoles/L). Against *P. striiformis*, compounds **3b** and **3c** showed EC_{50} values of 0.47 and 0.36 m moles/L, lower than the Tilt (EC_{50} values 0.50 m moles/L). In the case of *U.tritici*, compounds **3e** and **3f** were the most effective compounds with EC_{50} values of 0.31 and 0.25, respectively, which were comparable to the standard fungicide Vitavax (EC_{50} values 0.31 mmoles/L).

All the 7-fluoro-3-substituted-1,2,4-triazolo[3,4-b]benzothiazoles exhibited antifungal activity very much better than the precursors **1** and **2**. The presence of aryl groups at the 3-position was important for favorable fungitoxicity as the compounds **3a** has lower fungitoxicity than the rest of the series. Compounds **3b**, **3c** and **3d** exhibited appreciable fungitoxicity followed by compounds **3f** and **3t**, which evidently endorsed that the presence of chloro, flouro and vinyl phenyl groups at the 3-position was important for appreciable results of antifungal potential. Compounds **3b** and **3c** were the best against all the fungi with significant inhibition of germination in comparison to the standards, indicating the importance of the chloro group in the phenyl ring at position 3. These results also corresponded well to the best docking scores in these molecules. All the compounds mentioned also performed best in silico docking scores. Their other drug-like indices along with log *p* values were in the approved range of the Lipinskii rule. The results of antifungal potential of the fused 1,2,4-triazole with benzothiazole were better than the non-fused analogues separated by spacers [1], endorsing the ring fusion of the leads than their presence in the same molecule separated by the spacers.

Conclusion

A novel series of 7-fluoro-3-substituted-1,2,4-triazolo[3,4-b]benzothiazoles synthesized on the basis of the lead hybridization strategy gave synergistic effects of heterocyclic fusion on their antifungal potential against various test fungi. Their EC_{50} values in some cases were lower than the standard fungicides used. Favorable in silico and in vitro antifungal evaluation studies provides an impetus to the further exploration of compounds **3b**, **3c**, **3d**, **3g**, and **3f** as potential leads for in vivo trials, in place of existing 1,2,4-triazoles, as reduced risk fungicides. Further designing, synthesis and structural optimization are in progress.

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