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Synthesis and characterization of new thiourea and urea derivatives of 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole: *In vitro* Antimicrobial and Antioxidant activity

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Abstract. A new class of antimicrobial and antioxidant agents, based on thiourea and urea derivatives of 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole were synthesized by adopting a simple and efficient method. Synthesized compounds were characterized by spectral data of IR, ^{1}H, ^{13}C NMR and mass spectra. The compounds were evaluated for their efficacy as antimicrobial and antioxidant agents. A few compounds showed good antibacterial, antifungal and antioxidant activity when compared to standard drugs and thus represent a new class of promising lead compounds.

Keywords. Isothiocyanates; Isocyanates; 6-Fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole; Antimicrobial and Antioxidant activity.

1. Introduction

The treatment for microbial infectious diseases still remains an important and challenging problem for researchers worldwide. Despite the development of several new antimicrobial agents, their clinical value is limited to treat an increasing array of life threatening systemic infections because of their relatively high risk of toxicity, developing resistance to existing drugs by altering their gene sequence, pharmacokinetic differences and/or insufficiencies in their antimicrobial activity.[1] Hence, the scientific communities are highlighting the need for the search and discovery of new and more effective antimicrobial agents. Nowadays the aim of the researchers is to develop new motifs which are structurally modified from the basic structure and that can effectively inhibit the growth of microorganisms. Therefore, the basic pharmacophoric unit structure is altered by linking various functional groups like amides, imides and large alkyl groups which will bring different mode of action that could be beneficial for the treatment of microbial pathogens.

Isoxazole is one of the basic structural scaffolds in many pharmacologically active drugs such as Zonisamide, (**1**) Ibotenic acid, (**2**) Valdecoxib (**3**) and Paliperidone (**4**) (figure 1). Hence, it became a valuable scaffold in medicinal chemistry as well as a useful synthon in natural products synthesis.[2] Among the various biologically active isoxazole derivatives, substituted 1,2-benzisoxazoles occupy an extremely important role in the pharmaceutical and medicinal fields.[3,4] 1,2-Benzisoxazoles substituted derivatives were used as anticonvulsant,[5,6] anti-psychotic,[7] anti-cancer,[8,9] antimicrobial,[10] anti-thrombotic,[11] uricosuric,[12] anti-inflammatory,[13] tuberculostatic,[14] sedative,[15] analgesic[16] and neuroleptic agents.[17] In addition, *N*-benzylpiperidine-benzisoxazole derivatives are selective inhibitors of the enzyme acetyl cholinesterase (AChE), used for the treatment of Alzheimer's disease.[18] Particularly, 3-(4-piperidyl)-6-fluoro-1,2-benzisoxazole is an important intermediate for the synthesis of paliperidone which is the primary active metabolite of the older antipsychotic risperidone.[19]

The recent literature is enriched with compounds containing benzoisoxazole, amide bond and fluorine substituent which can alter the chemical properties and biological activity of drugs. The fluorine substitution can also have a profound effect on drug disposition, in terms of distribution, drug clearance route(s) and extent of drug metabolism.[20] For example, 3-(4-piperidyl)-6-fluoro-1,2-benzisoxazole was conjugated to various amino acids to form amide linkage which exhibited enhanced antimicrobial activity.[21] Benaka Prasad and his coworkers reported the synthesis

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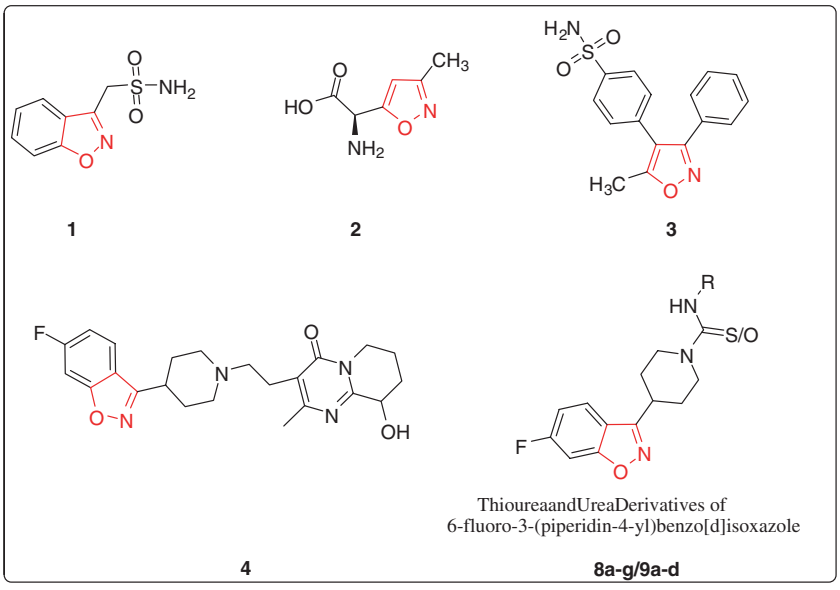


Figure 1. Biologically active drugs containing isoxazole moiety.

of a novel series of 3-(4-piperidyl)-6-fluoro-1,2-benzisoxazole amide derivatives as potent anti-proliferative agents in cancer cell lines.[22]

Bearing these results in mind, we have designed and synthesized the urea and thiourea derivatives of 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole. Their antimicrobial and antioxidant activities were also evaluated.

2. Experimental

2.1 *Materials and methods*

All the required chemicals were purchased from Sigma Aldrich and the solvents from Merck and were used without further purification. The completion and purity of the reactions were monitored by TLC, performed on silica gel aluminum 60 F-254 thin layer plates procured from Merck, and visualization on TLC was achieved by UV light and iodine indicator. Melting points of the compounds were determined on Guna digital melting point apparatus using open capillary tubes and are uncorrected. Infrared spectra were recorded on FT-IR Bruker ALPHA Interferometer and wave numbers are given in cm^{-1}. NMR spectra were recorded on a Bruker instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$. TMS was used as an internal standard. Assignments of the signals are based on the chemical shifts and intensity patterns. Chemical shift (δ) and coupling constant (J) were expressed in ppm and Hertz respectively. The following abbreviations were used to indicate the peak multiplicity: s-singlet, d-doublet, t-triplet, m-multiplet. LC mass spectra were recorded on Agilent LCMS- model 2010A Shimadzu instrument in positive mode.

2.2 *General procedure for the synthesis of thiourea and urea derivatives of (**8a-g/9a-d**)*

The thiourea and urea derivatives of 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole (**8a-g/9a-d**) were synthesized using 6-fluoro-3-(piperidin-4-yl)benzo[*d*]isoxazole hydrochloride as starting material. The starting material (255 mg, 1 mmol) was dissolved in dry THF (15 mL) in a 50 mL round bottomed flask, to this solution triethylamine (0.14 mL, 1 mmol) and pyridine (2 mL) was added and then stirred the reaction mixture for 3 h at refluxing temperature. The reaction mixture was filtered through Buchner funnel to remove $Et_3N.HCl$. The filtrate containing (6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole) (**5**) was used for the reaction with various isothiocyanates and isocyanates.

The filtrate 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole (1 mmol) (**5**) was allowed to cool to 10°C and 4-nitrophenyl isothiocyanate (180 mg, 1 mmol)(**6a**) was added, stirred for 3 h at 40°C. The progress of the reaction was monitored by TLC (n-hexane: ethyl acetate 3:1). After completion of reaction, the reaction mixture was diluted with ethyl acetate and washed twice with water. The organic layer was dried over anhydrous sodium sulphate(Na_2SO_4) and concentrated under reduced pressure. The crude product was subjected to silica gel column chromatography

using increasing amounts of ethyl acetate in hexane (EtOAc/n-hexane) as eluent to obtain the final product (**8a**). Same procedure was adopted for the synthesis of remaining compounds (**8b-g**) and urea derivatives (**9a-d**).

2.3 *Spectral data*

*4-(6-Fluorobenzo[**d**]isoxazol-3-yl)-N-(4-nitrophenyl) piperidine-1-carbothioamide (**8a**):* Yield: 82%, Yellow solid, M.p. 180–183°C. IR(υ_{max}, cm^{-1}):3339 (NH), 1490 (NO_2), 1296 (C=S); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 7.98–7.81(2H, m, Ar-H), 7.62-7.53(1H, m, Ar-H), 7.24–7.15(2H, m, Ar-H), 6.87–6.76(2H, m, Ar-H), 6.42(1H, s, NH), 4.71–4.68(1H, m, $\underline{CH}(CH_2)_2$), 3.45–3.25(4H, m, $(\underline{CH_2})_2N$), 2.27–2.12(4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm):δ 183.1(C_{11}), 165.4(C_7), 164.8(d, J = 140.2, C_5), 145.7(C_1), 140.9(C_{16}), 135.6(C_{13}), 129.8($C_{14,18}$), 126.3($C_{15,17}$), 123.1(C_3), 117.2(C_2), 113.3(C_4), 97.6(C_6), 44.5($C_{10,10'}$), 33.6($C_{9,9'}$), 29.3(C_8); LCMS calculated for $C_{19}H_{17}FN_4O_3S$: 400.43 Found 401.2 $[M+H]^+$.

*4-(6-Fluorobenzo[**d**]isoxazol-3-yl)-N-phenylpiperidine-1-carbothioamide (8b):* Yield: 75%, Brown solid, M.p. 163–166°C. IR($\upsilon_{max}cm^{-1}$):3224 (NH),1307 (C=S); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 7.82–7.75(2H, m, Ar-H), 7.71–7.68(1H, m, Ar-H), 7.32–7.29(2H, m, Ar-H), 7.01–6.85(3H, m, Ar-H), 6.38(1H, s, NH), 4.72–4.59(1H, m, $\underline{CH}(CH_2)_2$), 3.75–3.51(4H, m, $(\underline{CH_2})_2N$), 2.22–2.04(4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR(100 MHz, $CDCl_3$, ppm):δ 185.0(C_{11}), 165.2(C_7), 164.3(d, J = 146.0, C_5), 146.0(C_1), 135.9(C_{13}), 129.8($C_{15,17}$), 128.5(C_{16}), 125.8($C_{14,18}$), 122.4(C_3), 117.5(C_2), 112.4(C_4), 97.8(C_6), 47.2($C_{10,10'}$), 33.9($C_{9,9'}$), 29.1(C_8); LCMS calculated for $C_{19}H_{18}FN_3OS$: 355.43 Found 356.1$[M+H]^+$.

*4-(6-Fluorobenzo[**d**]isoxazol-3-yl)-N-(4-fluorophenyl) piperidine-1-carbothioamide (8c):* Yield: 80%,Brown solid, M.p. 197–200°C. IR($\upsilon_{max}cm^{-1}$):3227 (NH),1322 (C=S); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 7.60–7.55(1H, m, Ar-H), 7.21–7.18(2H, m, Ar-H), 7.02–6.97(4H, m, Ar-H), 6.38(1H, s, NH), 4.61–4.51(1H, m, $\underline{CH}(CH_2)_2$), 3.39–3.05(4H, m, $(\underline{CH_2})_2N$), 2.13–1.92 (4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm):δ 183.4(C_{11}), 165.1(C_7), 164.0(d, J = 147.6, C_5), 161.5(d, J = 165.5,C_{16}), 145.5(C_1), 136.0(C_{13}), 134.2($C_{14,18}$), 122.1(C_3), 117.0(C_2), 115.8($C_{15,17}$), 112.9(C_4), 97.7(C_6), 48.9($C_{10,10'}$), 33.6($C_{9,9'}$), 29.7(C_8); LCMS calculated for $C_{19}H_{17}F_2N_3OS$: 373.42 Found 374.2$[M+H]^+$.

*N-(3-Bromophenyl)-4-(6-fluorobenzo[**d**]isoxazol-3-yl) piperidine-1-carbothioamide (8d):* Yield: 74%, White solid, M.p. 208–211°C. IR(υ_{max} cm^{-1}):3273 (NH),1315 (C=S);^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 7.57–7.42 (1H, m, Ar-H), 7.21–7.03(2H, m, Ar-H), 6.92–6.74 (4H, m, Ar-H), 6.54 (1H, s, NH), 4.49–4.35(1H, m, $\underline{CH}(CH_2)_2$), 3.62–3.49(4H, m, $(\underline{CH_2})_2N$), 2.25–2.14(4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 184.6(C_{11}), 165.3(C_7), 164.6(d, J = 146.2, C_5), 145.8(C_1), 135.5(C_{13}), 130.3(C_{17}), 127.8(C_{16}), 126.2 (C_{18}), 125.5(C_{14}), 124.8(C_{15}), 122.7(C_3), 118.0(C_2), 112.8(C_4), 97.2(C_6), 48.3($C_{10,10'}$), 34.2($C_{9,9'}$), 29.7(C_8); LCMS calculated for $C_{19}H_{17}BrFN_3OS$: 434.33 Found 434.9$[M+H]^+$.

*N-(3,4-Dichlorophenyl)-4-(6-fluorobenzo[**d**]isoxazol-3-yl)piperidine-1-carbothioamide (8e):* Yield: 76%, White solid, M.p. 219–222°C. IR(υ_{max} cm^{-1}):3278 (NH),1310 (C=S);^{1}H-NMR(400 MHz, $CDCl_3$,ppm):δ 7.66–7.62(1H, m, Ar-H), 7.41–7.38(1H, m, Ar-H), 7.32–7.26(2H, m, Ar-H), 7.09–7.06(2H, m, Ar-H), 6.76(1H, s, NH), 4.66–4.56(1H, m, $\underline{CH}(CH_2)_2$), 3.49–3.35(4H, m, $(\underline{CH_2})_2N$), 2.21–2.11(4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm):δ 184.9(C_{11}), 165.8(C_7), 164.0(d, J = 144.0, C_5), 146.2(C_1), 136.2 (C_{13}), 135.9(C_{14}), 132.1(C_{15}), 129.5(C_{16}), 128.7(C_{18}), 123.2(C_3), 121.5(C_{17}), 117.1(C_2), 111.5(C_4), 97.0(C_6), 48.0($C_{10,10'}$), 33.5($C_{9,9'}$), 29.3(C_8); LCMS calculated for $C_{19}H_{16}Cl_2FN_3OS$: 424.32 Found 424.9$[M+H]^+$.

*N-(3-Chlorophenyl)-4-(6-fluorobenzo[**d**]isoxazol-3-yl) piperidine-1-carbothioamide (8f):* Yield: 75%, White solid, M.p. 194-197°C. IR($\upsilon_{max}cm^{-1}$):3285 (NH), 1332 (C=S); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 7.51–7.25 (2H, m, Ar-H), 7.13–7.09(1H, m, Ar-H), 6.85–6.67(4H, m, Ar-H), 6.47(1H, s, NH), 4.54–4.31(1H, m, $\underline{CH}(CH_2)_2$), 3.71–3.54(4H, m, $(\underline{CH_2})_2N$), 2.27–2.16(4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm):δ 183.7(C_{11}), 165.2(C_7), 163.9(d, J = 140.6(C_5), 145.9 (C_1), 135.7(C_{13}), 134.2(C_{15}), 132.9(C_{17}), 129.1(C_{16}), 127.2(C_{14}), 124.0(C_{18}), 122.8(C_3), 117.5(C_2), 111.7(C_4), 97.4(C_6), 47.9($C_{10,10'}$), 34.1($C_{9,9'}$), 29.6(C_8); LCMS calculated for $C_{19}H_{17}ClFN_3OS$: 389.87 Found 390.8 $[M+H]^+$.

*4-(6-Fluorobenzo[**d**]isoxazol-3-yl)-N-(3-(trifluoromethyl)phenyl)piperidine-1-carbothioamide (8g):* Yield: 75%, Brown solid, M.p. 196–199°C. IR($\upsilon_{max}cm^{-1}$): 3233 (NH), 1319 (C=S),1113 (CF_3); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 7.49–7.21(2H, m, Ar-H),

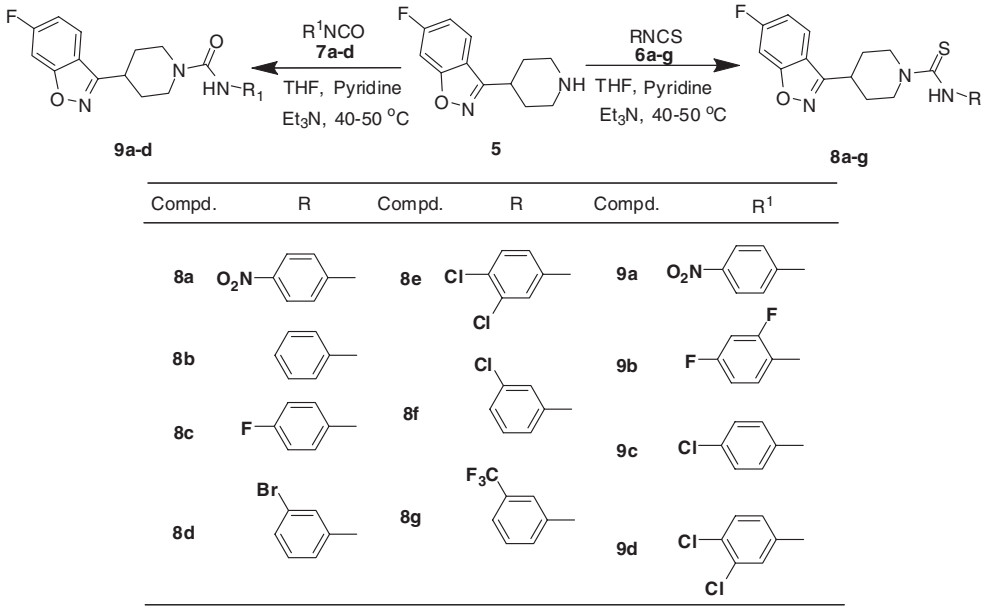
7.15–6.94(2H, m, Ar-H), 6.89–6.57(3H, m, Ar-H), 6.48(1H, s, NH), 4.68–4.59(1H, m, $\underline{CH}(CH_2)_2$), 3.58–3.43(4H, m, $(\underline{CH_2})_2N$), 2.28–2.12(4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 183.5(C_{11}), 165.9(C_7), 164.2(d, J = 144.9, C_5), 145.5(C_1), 135.3(C_{13}), 132.8 (C_{15}), 131.2(C_{14}), 129.2(C_{18}), 129.0(C_{17}), 123.8(C_{19}), 122.5(C_3), 120.3(C_{16}), 117.2(Cl_2), 111.5(C_4), 97.8(C_6), 47.1($C_{10,10'}$), 33.2($C_{9,9'}$), 29.4(C_8); LCMS calculated for $C_{20}H_{17}F_4N_3OS$: 423.43 Found 424.4$[M+H]^+$.

*4-(6-Fluorobenzo[**d**]isoxazol-3-yl)-N-(4-nitrophenyl) piperidine-1-carboxamide (9a):* Yield: 80%, Yellow solid, M.p. 198–201°C. IR(υ_{max}cm^{-1}): 3344 (NH), 1587 (C=O), 1487 (NO_2); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 8.19–7.89(4H, m, Ar-H), 7.49–7.21(2H, m, Ar-H), 6.84(1H, m, Ar-H), 6.54(1H, s, NH), 4.64–4.53(1H, m, $\underline{CH}(CH_2)_2$), 3.68–3.51(4H, m, $(\underline{CH_2})_2N$), 2.37–2.15(4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$,ppm): δ 166.7(C_7), 164.8(d, J = 152.4, C_5), 154.1(C_{11}), 146.8(C_1), 142.5(C_{16}), 137.9(C_{13}), 125.9 ($C_{15,17}$), 122.8(C_3), 120.2($C_{14,18}$), 117.8(C_2), 111.2(C_4), 97.2(C_6), 44.8($C_{10,10'}$), 33.4($C_{9,9'}$), 29.1(C_8); LCMS calculated for $C_{19}H_{17}FN_4O_4$: 384.36 Found 385.4 $[M+H]^+$.

*N-(2,4-Difluorophenyl)-4-(6-fluorobenzo[**d**]isoxazol-3-yl)piperidine-1-carboxamide :* Yield: 79%, White solid, M.p. 213–216°C. IR(υ_{max} cm^{-1}): 3270 (NH), 1598 (C=O); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm): δ 7.81–7.54 (2H, m, Ar-H), 7.24–7.19(1H, m, Ar-H), 6.91–6.64 (3H, m, Ar-H), 6.50(1H, s, NH), 4.71–4.58(1H, m, $\underline{CH}$ $(CH_2)_2$), 3.69–3.52(4H, m, $CH(\underline{CH_2})_2$), 2.26–2.02(4H, m, $(\underline{CH_2})_2N$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 166.1(C_7), 164.9(C_{14}), 164.3(d, J = 146.0, C_5), 161.8 (C_{16}),154.12(C_{11}), 146.5(C_1), 125.6(C_{18}), 122.5(C_3), 117.1(C_2), 114.5(C_{13}), 113.2(C_{17}), 111.8(C_4), 105.2 (C_{15}), 97.1(C_6), 44.5($C_{10,10'}$), 33.9($C_{9,9'}$), 29.8(C_8); LCMS calculated for $C_{19}H_{16}F_3N_3O_2$: 375.34 Found 378.2$[M+H]^+$.

*N-(4-Chlorophenyl)-4-(6-fluorobenzo[**d**]isoxazol-3-yl) piperidine-1-carboxamide (9c):* Yield: 77%, White solid, M.p. 204–207°C. IR(υ_{max} cm^{-1}):3286 (NH), 1625 (C=O); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 7.79–7.48 (4H, m, Ar-H), 7.37–7.24(1H, m, Ar-H), 7.19–6.95 (2H, m, Ar-H), 6.46(1H, s, NH), 4.69-4.54(1H, m, $\underline{CH}$ $(CH_2)_2$), 3.75–3.68(4H, m, $(\underline{CH_2})_2N$), 2.19–1.98 (4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm):δ 166.5(C_7), 164.7(d, J = 152.5, C_5),154.6(C_{11}), 147.2 (C_1), 138.0(C_{13}), 134.2(C_{16}), 129.5($C_{15,17}$), 122.2(C_3), 120.7($C_{14,18}$),117.5(C_2), 111.5(C_4), 97.8(C_6), 44.5 ($C_{10,10'}$), 33.9($C_{9,9'}$), 29.8(C_8); LCMS calculated for $C_{19}H_{17}ClFN_3O_2$: 373.81 Found 374.6$[M+H]^+$.

*N-(3,4-Dichlorophenyl)-4-(6-fluorobenzo[**d**]isoxazol-3-yl)piperidine-1-carboxamide (9d):* Yield: 78%, White solid, M.p. 189–193°C. IR(υ_{max} cm^{-1}): 3288 (NH), 632



Scheme 1. Synthesis of bioactive thiourea and urea derivatives of 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole.

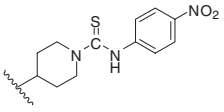
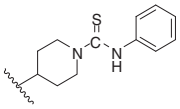
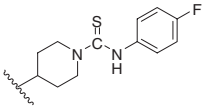
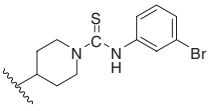
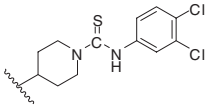
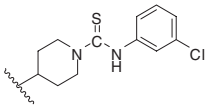
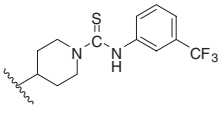
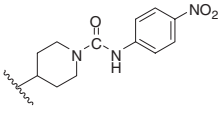
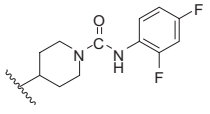
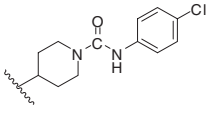
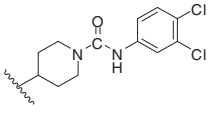
(C=O); ^{1}H-NMR(400 MHz, $CDCl_3$, ppm):δ 7.59–7.54 (2H, m, Ar-H), 7.32–7.21(1H, m, Ar-H), 7.19–7.13 (2H, m, Ar-H), 7.04–7.02(1H, m, Ar-H), 6.50(1H, s, NH), 4.11–4.04(1H, m, $\underline{CH}(CH_2)_2$), 3.31–3.10(4H, m, $(\underline{CH_2})_2N$), 2.14–1.97(4H, m, $CH(\underline{CH_2})_2$); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 166.3(C_7), 164.2(d, J = 145.0, C_5), 154.2(C_{11}), 147.9(C_1), 138.4(C_{13}), 131.7 (C_{14}), 130.3(C_{15}), 128.8(C_{16}), 126.4(C_{18}), 122.1(C_3), 121.5(C_{17}), 119.1(C_2), 112.8(C_4), 97.8(C_6), 44.2 ($C_{10,10'}$), 33.9($C_{9,9'}$), 29.9(C_8); LCMS calculated for $C_{19}H_{16}Cl_2FN_3O_2$: 408.25 Found 409.0$[M+H]^+$.

3. Results and Discussion

3.1 *Chemistry*

6-Fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole was treated with various biopotentisothiocyanates(**6**) and isocyanates(**7**) in the presence of a base Et_3N in THF-Pyridine as solvent mixture at 40–50°C to obtain the final products (**8a-g** and **9a-d**). This method has the advantages of easier work-up, mild reaction conditions and high yields, 74 to 82% (scheme 1).

Table 1. Physical data of the title compounds (**8a-g/9ad**).

Compd.	Structure	Time (h)	Yield (%)	Melting points (°C)
8a		4	82	180–183
8b		2.5	75	163–166
8c		3	80	197–200
8d		3.5	74	208–211
8e		4	76	219–222
8f		3.5	75	194-197
8g		3	75	196-199
9a		3.5	80	198-201
9b		4	79	213-216
9c		2.5	77	204-207
9d		3	78	189-193

In the IR spectra of compounds **8a-g** and **9a-d**, the following bands were detected: (i) NH vibrations at 3344–3224 cm^{-1}; (ii) for urea (C=O) at 1632–1587 $cm^{-1}$and for thiourea derivatives (C=S) at 1332–1296 cm^{-1}. In the 400 MHz ^{1}H-NMR spectra of derivatives (in $CDCl_3$), the following signals were detected. A peak at δ 8.19–6.57 for aromatic protons, the amino group (NH) resonated as a broad singlet at δ 6.76–6.38 and multiplet peaks for $\underline{C}H(CH_2)_2$, $(\underline{C}H_2)_2N$) and $CH(\underline{C}H_2)_2$) appeared at 4.72–4.04, 3.75–3.05 and 2.37–1.90 ppm respectively. ^{13}C-NMR (100 MHz, $CDCl_3$, ppm), the following signals were detected. A peak at δ 185.0–183.4 and 154.6–154.1 ppm correspond to C=S of thiourea and C=O of urea respectively. Signals in the range of δ 164.9–163.9 ppm for C-F (C_5) as a doublet, signal at 138.0–135.3 ppm is due to (C_{14}) and aromatic carbon signals appeared in the range of 166.0–97.1 ppm. Besides NMR studies, mass spectra displayed the exact molecular ion peaks in positive mode. The yields and physical data of the final compounds are given in table 1.

3.2 *Biological assay*

3.2a *Antibacterial activity*: *In vitro* antibacterial activity was evaluated against human pathogens of both gram positive *Bacillus subtilis* and *Staphylococcus aureus* and gram negative namely *Escherichia coli* and *Pseudomonas aeruginosa* by Disc diffusion method.[23,24] Tetracycline was used as a standard drug. The results showed that the 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole thiourea/urea derivatives **8b, 8g** and **9c** showed significant inhibition against Gram positive bacteria. The significant inhibition against Gram negative bacteria was shown by the compounds **8g** and **9c** which might be due to the presence of electron withdrawing groups like trifluoromethyl and chloro groups present in the benzene ring. The high inhibition may be due to the presence of trifluoromethyl group, the most lipophilic group, which can exert effect compared to a phenyl ring (table S1, in Supplementary Information). The title compounds showed their potential to serve as a good platform for further investigation in order to discover new derivatives having an improved overall biological profile with a special emphasis on resistant bacterial strains.

3.2b *Antifungal activity*: Antifungal activity of the title compounds was evaluated by Disc diffusion method[25] and the results are shown in table S2. *Candida albicans* and *Candida nonalbicans* were included in the assay as these are wide spread fungi. Ampotericin-B, a well known compound possessing strong antifungal activity has been included as a standard in this assay. Almost all the title compounds exhibited moderate level of activity. However, compounds **8e>9c=8b** exhibited good activity against *Candida albicans*. Compounds **8e>9a>9b** exhibited good activity against *Candida non albicans*. The results of antifungal activity illustrate that the presence of electron withdrawing (groups chloro and nitro) on the phenyl ring exhibited good activity. Antifungal activity observed against *Candida non albicans* species is encouraging in comparison with *Candida albicans*.

3.2c *Antioxidant activity*: In H_2O_2 method,[26,27] all the newly synthesized thiourea/urea derivatives of

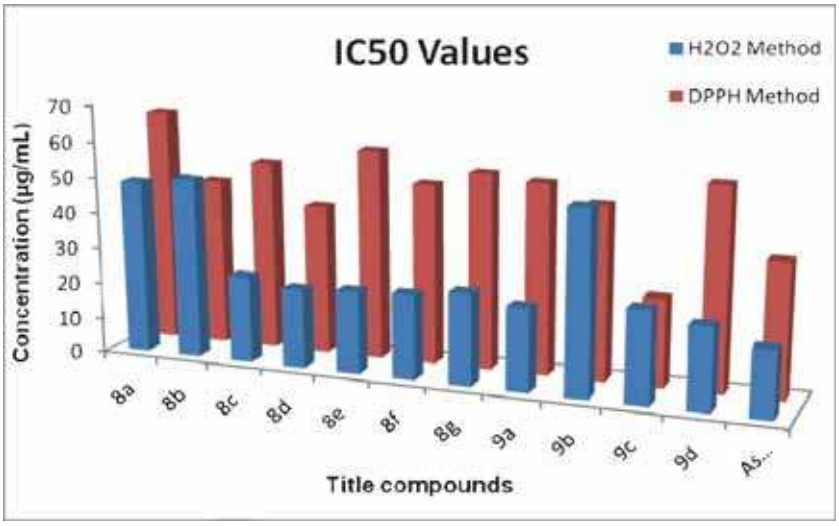


Figure 2. Half-maximal inhibitory concentration of the title compounds by H_2O_2 and DPPH methods.

6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole were tested at different concentrations (25, 50, 75, 100 μg/mL) and showed potential to moderate activity. Among the compounds **8c**, **8e** and **8g** proved to exhibit potent antioxidant activity, the reason might be the presence of electron withdrawing groups (fluoro, chloro and trifluoromethyl groups) on phenyl ring of thiourea derivatives as shown in table S3. In DPPH method,[28,29] the compounds **8c, 8g** and **9c** exhibited high activity due to the presence of electron withdrawing groups (table S4). In H_2O_2 method, compounds **8d** and **8e** showed the lowest IC_{50} values as shown in figure 2. In DPPH method, compound **9c** having the least IC_{50} value of 24.64 μ g/mL and it was followed by **8d** and **8b** in the activity test. In both the methods, IC_{50} values were found to vary from 22.14 to 65.25 μ g/mL showing a wide range of variations in the reactivity of the samples. The wide variations in free radical scavenging activities may be due to the presence of various substituents on the phenyl ring.

4. Conclusions

In summary, the objective of the present study was to synthesize and investigate the antimicrobial and antioxidant activities of the novel series of 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole thiourea/urea derivatives by adopting a simple and versatile synthetic methodology. From the bio-assay results, it was established that both thiourea and urea derivatives of compound **5** showed noticeable *in vitro* antimicrobial and antioxidant activities. The most striking feature of the study is that compounds **8b** and **9c** exhibited excellent antimicrobial activity and **8c, 8g** and **9c** exhibited good antioxidant activity. Most of the synthesized compounds showed potent activity with low IC_{50} values, **9c** having the least IC_{50} value. Thus, this novel class of thiourea and urea derivatives of 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole core represents the worthy hit compounds, which can be a basis for further investigations and development of novel potent antimicrobial agents.

Supplementary Information

Experimental details and tables of results of antibacterial, antifungal and antioxidant (DPPH and H_2O_2 methods) activities, numbering of the title compound and scanned spectra of ^1H and ^{13}C of **8c** and **9d** compounds are given in the Supplementary Information which is available at www.ias.ac.in/chemsci.

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