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Research Article

Synthesis, characterization and biological evaluation of some novel 9-benzooxazol-4-yl-3,4-diphenyl-1-oxa-6-thia-2,4,9-triaza-spiro [4.4]non-2-en-8-ones

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Abstract

A series of various novel 4-benzooxazol-4-yl-9-phenyl-1,6-dithia-4,9-diaza-spiro[4.4]nonane-3,8-diones (5a-e) and 9-benzooxazol-4-yl-3,4-diphenyl-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-ones (6a-e) were synthesized in good yields by using 4-nitro-1*H*-benzooxazole (1) as starting material and 4-amino-1*H*-benzooxazole (2), *N*-benzooxazol-4-yl-2-chloro-acetamide (3) and 3-benzooxazol-4-yl-2-phenylimino-thiazolidin-4-ones (4a-e) as intermediates. After structural confirmation, the title compounds were screened for their antimicrobial and anti-inflammatory activity.

Keywords: Benzooxazole, Thiatriazaspirononones, antimicrobial and anti-inflammatory activity

1. Introduction

A literature search revealed that, thiazolidinone derivatives may exhibit antibacterial,[1] antituberculosis,[2] antiviral[3] and anticancer[4] properties. In addition, some thiazolidinones were recently reported as novel inhibitors of mycobacterial rhamnose synthetic enzymes.[5] Small ring heterocycles containing nitrogen, sulfur and oxygen have been under investigation for a long time because of their important medicinal and biological properties. Among these types of heterocyclic molecules, spirothiazolidin based compounds have been shown to have various important biological activities such as antimicrobial,[6] antileukemic,[7] anti-helminthic, anticonvulsant,[8] antibacterial,[9] antifungal,[10] anti-tubercular,[11] anticancer[12] activity. Recent observations suggest that substituted benzoxazoles possess potential activity with lower toxicities in the chemotherapeutic approach in man.[13] Careful literature survey revealed that targets containing benzoxazole moiety have remarkable biological activities like antibacterial,[14] antihistaminic,[15] antiparasitics,[16] antiviral[17] and antifungal activity[18].

2. Materials and methods

All reagents and solvents were used as purchased without further purification. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60–120 mesh. IR spectra were obtained on a Perkin-Elmer BX serried FTIR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a varian 300 MHz spectrometer for ^{1}H NMR and 100 MHz spectrometer ^{13}C NMR. The chemical shifts were reported as ppm down field using TMS as an internal standard. Mass spectra were recorded on a VG-Micromass 7070H spectrometer operating at 70 eV.

2.1 General procedure for the preparation of 4-amino-1*H*-benzooxazole (2)

A solution of 4-nitro-1*H*-benzooxazole **(1)** (0.01 mol) in ethanol (20 mL) containing $SnCl_2.2H_2O$ (10 mmol) was refluxed on steam-bath for 45 min. A usual work-up, followed by crystallisation of the product from dichloro methane furnished pure 4-amino-1*H*-benzooxazole **(2)**.

2.2 General procedure for the preparation of *N*-benzooxazol-4-yl-2-chloro-acetamide (3)

4-Amino-1*H*-benzooxazole **(2)** (0.01 mol) and chloro acetylchloride (0.01 mol) were dissolved in tri ethyl amine (3 mL) and stirred at room temperature for 3 h (10 mL). After completion of the reaction (monitored by TLC), the solvent was evaporated *in vacuo* and the residue on purification by column chromatography using hexane–EtOAc (9:1) to give *N*-benzooxazol-4-yl-2-chloro-acetamide **(3)**.

2.3 General procedure for the preparation of 3-benzooxazol-4-yl-2-phenylimino-thiazolidin-4-ones (4a-e)

N-Benzooxazol-4-yl-2-chloro-acetamide **(3)** (0.01 mol) was treated with phenyl isothiocyanate (0.01 mol) at room temperature in the presence of K_2CO_3 (0.5 g) in acetonitrile (15 mL). The reaction mixture was refluxed while stirring for 8-9 h. After completion of the reaction (monitored by TLC), followed by conventional work up, the product was purified by recrystallization from methanol afforded 3-benzooxazol-4-yl-2-phenylimino-thiazolidin-4-ones **(4a-e)** as product.

2.4 General procedure for the preparation of 4-benzooxazol-4-yl-9-phenyl-1,6-dithia-4,9-diaza-spiro[4.4]nonane-3,8-diones (5a-e)

An equimolar mixture of 3-benzooxazol-4-yl-2-phenylimino-thiazolidin-4-ones **(4a-e)** (0.01 mol) and mercapto acetic acid was refluxed in dioxane for 8-9 h. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool and was poured over crushed ice. The organic layer was extracted with ethyl acetate (20 mL), washed with 10% sodium bicarbonate solution (1 X 20 mL) and dried over anhydrous sodium sulphate. The solvent was removed under vacuum and the residue was recrystallized from methanol to give 4-benzooxazol-4-yl-9-phenyl-1,6-dithia-4,9-diaza-spiro[4.4]nonane-3,8-diones **(5a-e)**.

2.5 General procedure for the preparation of 9-benzooxazol-4-yl-3,4-diphenyl-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-ones (6a-e)

A solution of 4-benzooxazol-4-yl-9-phenyl-1,6-dithia-4,9-diaza-spiro[4.4]nonane-3,8-diones **(5a-e)** in dry chloroform (15 mL) cooled in ice-salt bath, benzhydroxamoyl chloride (0.01 mol) in chloroform (10 mL) was added to this reaction mixture at $0C^0$ during 15 mins with constant stirring and the reaction continued for another 4-5 h at $0C^0$. After completion of the reaction (monitored by TLC), followed by removal of Et_3N, HCl, the solvent was distilled off under reduced pressure and the resulting crude product was recrystallized from benzene to offered 9-benzooxazol-4-yl-3,4-diphenyl-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-ones **(6a-e)** in pure form.

2.6. Physical and spectral data

2.6.1 4-Amino-1*H*-benzoxazole (2)

Gray solid; yield 70%; mp 111-113 °C; IR (KBr) 3112 (N-H), 3024 (C-H, Ar), 1580 (C=C, Ar), 1428 (C=N), 1128 (C-O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.76-7.34 (m, 3H, Ar-H), 5.84 (s, 1H, CH), 3.87 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.3, 150.7, 139.7, 136.8, 126.7, 123.4, 117.4; MS: *m/z* 134 (M^+); Anal. Calcd. for $C_7H_6N_2O_3$: C-51.23, H-2.46, N-17.07, O-29.25. Found: C-50.12, H-2.35, N-16.45, O-28.42.

2.6.2 *N*-Benzooxazol-4-yl-2-chloro-acetamide (3)

Yellow solid; yield 74%; mp 156-158 °C; IR (KBr) 3320 (N-H), 3030 (C-H, Ar), 1680 (C=O), 1568 (C=C, Ar), 1451 (C=N), 1142 (C-O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.72-7.31 (m, 3H, Ar-H), 7.60 (s, 1H, NH), 5.74 (s, 1H, CH), 4.20 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.1, 153.1, 149.7, 132.8, 130.3, 124.8, 117.6, 106.3, 47.3; MS: 210 *m/z* (M^+); Anal. Calcd. for $C_9H_7ClN_2O_2$: C-51.32, H-3.35, Cl-16.83, N-13.30, O-15.19. Found: C-50.02, H-3.12, Cl-15.81, N-12.10, O-14.28.

2.6.3 3-Benzooxazol-4-yl-2-phenylimino-thiazolidin-4-one (4a)

Pale yellow solid; yield 72%; mp 125-127 °C; IR (KBr) 3012 (C-H, Ar), 2985 (C-H,CH_2), 1679 (C=O), 1584 (C=C, Ar), 1454 (C=N), 1142 (C-O), 684 (C-S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.78-7.39 (m, 3H, Ar-H), 7.65-7.21 (m, 5H, Ar-H), 5.81 (s, 1H, CH), 4.17 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.8, 162.0, 151.7, 148.5, 144.6, 133.8, 131.7, 128.3 (2), 126.2, 125.3, 123.2 (2), 119.1, 109.4, 35.6; MS: 309 *m/z* (M^+);

Anal. Calcd. for $C_{16}H_{11}N_3O_2S$: C-62.12, H-3.58, N-13.58, O-10.34, S-10.37. Found: C-61.23, H-3.24, N-12.10, O-9.58, S-9.68.

2.6.4 3-Benzooxazol-4-yl-2-(4-methoxy-phenylimino)-thiazolidin-4-one (4b)

White solid; yield 70%; mp 136-138 °C; IR (KBr) 3032 (C-H, Ar), 2982 (C-H,CH_2), 1675 (C=O), 1570 (C=C, Ar), 1456 (C=N), 1140 (C-O), 686 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.69-7.34 (m, 3H, Ar-H), 7.65 (d, 2H, *J* = 7.4 Hz, Ar-H), 7.54 (d, 2H, J = 7.4 Hz, Ar-H), 5.71 (s, 1H, CH), 4.11 (s, 2H, CH_2), 3.70 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 163.7, 158.3, 153.2, 150.4, 146.3, 137.1, 134.5, 128.2, 124.3 (2), 121.0, 117.3 (2), 111.2, 58.3, 36.3; MS: 339 *m/z* (M^+); Anal. Calcd. for $C_{17}H_{13}N_3O_3S$: C-60.17, H-3.86, N-12.38, O-14.14, S-9.45. Found: C-59.65, H-3.21, N-11.14, O-13.62, S-8.45.

2.6.5 3-Benzooxazol-4-yl-2-(4-chloro-phenylimino)-thiazolidin-4-one (4c)

Brown solid; yield 76%; mp 141-143 °C; IR (KBr) 3045 (C-H, Ar), 2978 (C-H,CH_2), 1673 (C=O), 1584 (C=C, Ar), 1452 (C=N), 1142 (C-O), 680 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.75-7.41 (m, 3H, Ar-H), 7.62 (d, 2H, J = 7.2 Hz, Ar-H), 5.69 (s, 1H, CH), 4.01 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 164.1, 155.4, 151.2, 147.8, 136.5, 134.2, 132.8, 130.2 (2), 128.5, 121.7 (2), 117.4, 113.4, 37.6; MS: 343 *m/z* (M^+); Anal. Calcd. for $C_{16}H_{10}ClN_3O_2S$: C-55.90, H-2.95, Cl-10.31, N-12.22, O-9.31, S-9.33. Found: C-54.12, H-2.32, Cl-9.68, N-11.85, O-8.75, S-8.45.

2.6.6 3-Benzooxazol-4-yl-2-(4-bromo-phenylimino)-thiazolidin-4-one (4d)

Gaeenish yellow solid; yield 71%; mp 150-152 °C; IR (KBr) 3030 (C-H, Ar), 2981 (C-H,CH_2), 1675 (C=O), 1570 (C=C, Ar), 1464 (C=N), 1154 (C-O), 692 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70-7.38 (m, 3H, Ar-H), 7.65 (d, 2H, J = 7.0 Hz, Ar-H), 7.58 (d, 2H, J = 7.0 Hz, Ar-H), 5.74 (s, 1H, CH), 4.22 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 160.2, 154.1, 146.3, 143.4, 137.1, 136.1, 133.5 (2), 125.2, 122.1 (2), 121.0, 120.4, 107.1, 32.7; MS: 388 *m/z* (M^+); Anal. Calcd. for $C_{16}H_{10}BrN_3O_2S$: C-49.50, H-2.60, Br-20.58, N-10.82, O-8.24, S-8.26. Found: C-48.12, H-2.21, Br-19.68, N-9.84, O-7.84, S-7.95.

2.6.7 3-Benzooxazol-4-yl-2-(4-nitro-phenylimino)-thiazolidin-4-one (4e)

Yellow solid; yield 73%; mp 136-138 °C; IR (KBr) 3031 (C-H, Ar), 2982 (C-H,CH_2), 1676 (C=O), 1584 (C=C, Ar), 1435 (C=N), 1141 (C-O), 681 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76-7.48 (m, 3H, Ar-H), 7.52 (d, 2H, J = 7.4 Hz, Ar-H), 7.05 (d, 2H, J = 7.4 Hz, Ar-H), 5.69 (s, 1H, CH), 4.05 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 160.2, 153.4, 147.3, 146.7, 145.2, 137.4, 133.2, 128.3, 126.3 (2), 125.4 (2), 121.0, 112.4, 38.6; MS: 354 *m/z* (M^+); Anal. Calcd. for $C_{16}H_{10}N_4O_4S$: C-54.23, H-2.84, N-15.81, O-18.06, S-9.05. Found: C-53.46, H-2.36, N-14.61, O-17.48, S-8.49.

2.6.8 4-Benzooxazol-4-yl-9-phenyl-1,6-dithia-4,9-diaza-spiro[4.4]nonane-3,8-dione (5a)

Pale yellow solid; yield 70%; mp 122-124 °C; IR (KBr) 3028 (C-H, Ar), 2974 (C-H,CH_2), 1684 (C=O), 1568 (C=C, Ar), 1470 (C=N), 1162 (C-O), 684 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.89-7.45 (m, 5H Ar-H), 7.74-7.52 (m, 3H, Ar-H), 5.51 (s, 1H, CH), 4.41 (s, 2H), 4.19 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.3, 168.4, 152.6, 149.7, 142.7, 134.1, 132.7, 128.7 (2), 126.7, 124.3, 122.4 (2), 118.4, 104.8, 77.6, 34.5, 32.7; MS: 383 *m/z* (M^+); Anal. Calcd. for $C_{18}H_{13}N_3O_3S_2$: C-56.38, H-3.42, N-10.96, O-12.52, S-16.72. Found: C-55.23, H-3.14, N-9.87, O-11.85, S-15.74.

2.6.9 4-Benzooxazol-4-yl-9-(4-methoxy-phenyl-1,6-dithia-4,9-diaza-spiro[4.4]nonane-3,8-dione (5b)

Brown solid; yield 73%; mp 140-142 °C; IR (KBr) 3036 (C-H, Ar), 2985 (C-H,CH_2), 1668 (C=O), 1584 (C=C, Ar), 1474 (C=N), 1165 (C-O), 682 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65-7.48 (m, 3H, Ar-H), 7.50 (d, 2H, J = 7.1 Hz, Ar-H), 7.14 (d, 2H, J = 7.1 Hz, Ar-H), 5.49 (s, 1H, CH), 4.47 (s, 2H CH_2), 4.26 (s, 2H, CH_2), 3.70 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 71.3, 167.5, 157.3, 154.6, 151.7, 144.6, 136.7, 134.7, 127.9, 123.6 (2), 120.1, 114.3 (2), 106.7, 79.3, 55.3, 35.6, 33.7; MS: 413 *m/z* (M^+); Anal. Calcd. for $C_{19}H_{15}N_3O_4S$: C-55.19, H-3.66, N-10.61, O-15.48, S-15.51. Found: C-54.12, H-3.21, N-9.87, O-14.23, S-14.71.

2.6.10 4-Benzooxazol-4-yl-9-(4-chloro-phenyl-1,6-dithia-4,9-di-aza-spiro[4.4]nonane-3,8-dione (5c)

White solid; yield 75%; mp 155-157 °C; IR (KBr) 3026 (C-H, Ar), 2975 (C-H,CH_2), 1682 (C=O), 1586 (C=C, Ar), 1479 (C=N), 1168 (C-O), 682 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70-7.42 (m, 3H, Ar-H), 7.59 (d, 2H, J = 7.2 Hz, Ar-H), 7.14 (d, 2H, J = 7.2 Hz, Ar-H), 5.54 (s, 1H, CH), 4.32 (s, 2H, CH_2), 4.04 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3, 167.4, 150.2, 147.8, 141.4, 133.2, 130.7, 128.3 (2), 126.3, 124.9,

121.8 (2), 117.4, 103.2, 75.7, 33.6, 31.7; MS: 417 *m/z* (M^+); Anal. Calcd. for $C_{18}H_{12}ClN_3O_3S_2$: C-51.73, H-2.89, Cl-8.48, N-10.06, O-11.49, S-15.35. Found: C-50.21, H-2.35, Cl-7.89, N-9.47, O-10.74, S-14.69.

2.6.11 4-Benzooxazol-4-yl-9-(4-bromo-phenyl-1,6-dithia-4,9-di-aza-spiro[4.4]nonane-3,8-dione (5d)

Gray solid; yield 77%; mp 162-164 °C; IR (KBr) 3036 (C-H, Ar), 2975 (C-H,CH_2), 1668 (C=O), 1582 (C=C, Ar), 1474 (C=N), 1168 (C-O), 684 (C-S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.67-7.42 (m, 3H, Ar-H), 7.58 (d, 2H, J = 7.4 Hz, Ar-H), 7.12 (d, 2H, J = 7.4 Hz, Ar-H), 5.54 (s, 1H, CH), 4.47 (s, 2H, CH_2), 4.28 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.4, 169.4, 151.7, 147.8, 141.4, 133.6, 131.4, 129.6 (2), 125.8, 123.4 (2), 119.7, 117.4, 105.6, 78.4, 35.3, 33.2; MS: 460 *m/z* (M^+); Anal. Calcd. for $C_{18}H_{12}BrN_3O_3S_2$: C-46.76 H-2.62, Br-17.28, N-9.09, O-10.38, S-13.87. Found: C-45.36 H-2.24, Br-16.85, N-8.74, O-9.86, S-12.23.

2.6.12 4-Benzooxazol-4-yl-9-(4-nitro-phenyl-1,6-dithia-4,9-di-aza-spiro[4.4]nonane-3,8-dione (5e)

Yellow solid; yield 72%; mp 128-130 °C; IR (KBr) 3028 (C-H, Ar), 2979 (C-H,CH_2), 1682 (C=O), 1568 (C=C, Ar), 1469 (C=N), 1165 (C-O), 678 (C-S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.69-7.58 (m, 3H, Ar-H), 7.48 (d, 2H, J = 7.6 Hz, Ar-H), 7.21 (d, 2H, J = 7.6 Hz, Ar-H), 5.47 (s, 1H, CH), 4.51 (s, 2H, CH_2), 4.32 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 167.4, 153.6, 150.4, 146.3, 144.1, 136.2, 134.7, 125.6, 123.0 (2), 121.3 (2), 116.5, 103.2, 75.7, 34.7, 31.7; MS: 428 *m/z* (M^+); Anal. Calcd. for $C_{18}H_{12}N_4O_5S_2$: C-50.46, H-2.82, N-13.08, O-18.67, S-14.97. Found: C-49.68, H-2.25, N-12.74, O-17.65, S-13.94.

2,6.13 9-Benzooxazol-4-yl-3,4-diphenyl-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-one (6a)

Yellow solid; yield 70%; mp 120-122 °C; IR (KBr) 3045 (C-H, Ar), 1674 (C=O), 1576 (C=C, Ar), 1484 (C=N), 1158 (C-O), 669 (C-S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.89-7.32 (m, 13H Ar-H), 5.45 (s, 1H, CH), 4.36 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 165.2, 153.2, 151.4, 142.5, 134.6, 133.6, 131.0, 130.2, 129.6 (2), 128.6 (2), 126.3 (2), 125.6, 118.4, 116.3, 114.5 (2), 106.7, 103.5, 28.4; MS: 428 *m/z* (M^+); Anal. Calcd. for $C_{23}H_{16}N_4O_3S$: C-64.47, H-3.76, N-13.08, O-11.20, S-7.48. Found: C-62.36, H-3.12, N-12.56, O-10.58, S-6.87.

2.6.14 9-Benzooxazol-4-yl-3-(4-chloro-phenyl)-4-phenyl-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-one (6b)

Pale yellow solid; yield 73%; mp 135-137 °C; IR (KBr) 3035 (C-H, Ar), 1685 (C=O), 1574 (C=C, Ar), 1462 (C=N), 1160 (C-O), 684 (C-S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.63-7.32 (m, 8H, Ar-H), 7.36 (d, 2H, J = 7.0 Hz, Ar-H), 7.24 (d, 2H, J = 7.0 Hz, Ar-H), 5.36 (s, 1H, CH), 4.45 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.1, 164.3, 154.3, 152.8, 141.0, 136.2, 135.8, 132.8, 131.5 (2), 130.5, 127.4 (2), 125.6 (2), 124.1, 120.4, 118.5, 116.4 (2), 108.7, 105.2, 29.7; MS: 476 *m/z* (M^+); Anal. Calcd. for $C_{23}H_{15}ClN_4O_3S$: C-60.44, H-3.59, Cl-7.43, N-11.75, O-10.06, S-6.72. Found: C-59.12, H-3.02, Cl-6.98, N-10.84, O-9.95, S-5.97.

2.6.15 9-Benzooxazol-4-yl-4-(4-methoxy-phenyl)-3-phenyl-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-one (6c)

White solid; yield 75%; mp 144-146 °C; IR (KBr) 3038 (C-H, Ar), 2965 (C-H,CH_3), 1688 (C=O), 1570 (C=C, Ar), 1475 (C=N), 1163 (C-O), 682 (C-S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.69-7.28 (m, 8H, Ar-H), 7.42 (d, 2H, J = 7.2 Hz, Ar-H), 7.18 (d, 2H, J = 7.2 Hz, Ar-H), 5.52 (s, 1H, CH), 4.51 (s, 2H, CH_2), 3.60 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 165.2, 153.2, 151.4, 148.6, 142.5, 134.6, 133.6, 131.0, 129.3 (2), 128.2, 126.3 (2), 125.6, 118.4, 116.3 (2), 114.5 (2), 106.7, 103.5, 58.3, 28.4; MS: 458 *m/z* (M^+); Anal. Calcd. for $C_{24}H_{18}N_4O_4S$: C-62.87, H-3.96, N-12.22, O-13.96, S-6.99. Found: C-60.84, H-3.41, N-11.65, O-12.84, S-6.12.

2.6. 16 9-Benzooxazol -4-yl-4- (4-chloro-phenyl) -3- (4-methoxy-phenyl) -1-oxa-6-thia- 2,4,9- triaza- spiro [4.4] non-2-en-8-one (6d)

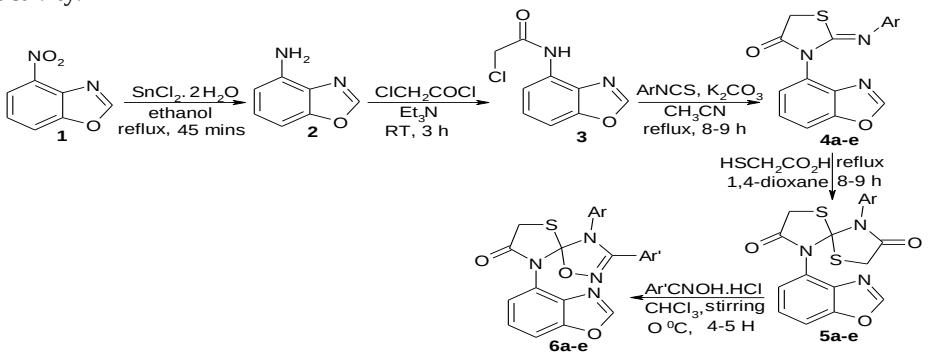
Brown solid; yield 71%; mp 158-160 °C; IR (KBr) 3042 (C-H, Ar), 2958 (C-H,CH_3), 1675 (C=O), 1582 (C=C, Ar), 1479 (C=N), 1185 (C-O), 688 (C-S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.74-7.35 (m, 3H, Ar-H), 7.42 (d, 2H, J = 7.4 Hz, Ar-H), 7.40 (d, 2H, J = 7.0 Hz, Ar-H), 7.39 (d, 2H, J = 7.4 Hz, Ar-H), 7.26 (d, 2H, J = 7.0 Hz, Ar-H), 5.65 (s, 1H, CH), 4.74 (s, 2H, CH_2), 3.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 165.2, 162.3, 153.2, 151.4, 142.5, 134.6, 133.6, 131.0, 130.2 (2), 126.3 (2), 125.6, 121.0, 118.4, 116.3 (2), 114.5 (2), 106.7, 103.5, 53.6, 28.4; MS: 492 *m/z* (M^+); Anal. Calcd. for $C_{24}H_{17}ClN_4O_4S$: C-58.48, H-3.48, Cl-7.19, N-11.37, O-12.98, S-6.50. Found: C-56.98, H-3.12, Cl-6.86, N-10.85, O-11.78, S-5.98.

2.6.17 9-Benzooxazol-4-yl-4-(4-nitro-phenyl)-3-(4-methyl-phenyl)-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-one (6e)

Yellow solid; yield 72%; mp 1152-154°C; IR (KBr) 3040 (C-H, Ar), 2962 (C-H,CH_3), 1671 (C=O), 1578 (C=C, Ar), 1475 (C=N), 1173 (C-O), 682 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70-7.31 (m, 3H, Ar-H), 7.48 (d, 2H, J = 7.1 Hz, Ar-H), 7.38 (d, 2H, J = 7.3 Hz, Ar-H), 7.36 (d, 2H, J = 7.1 Hz, Ar-H), 7.30 (d, 2H, J = 7.3 Hz, Ar-H), 5.54 (s, 1H, CH), 4.82 (s, 2H, CH_2), 3.76 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 165.2, 153.2, 151.4, 142.5, 139.5, 136.3, 134.6, 133.6, 131.0, 129.3 (2), 126.3 (2), 125.6, 123.5 (2), 118.4, 114.5 (2), 106.7, 103.5, 28.4, 223; MS: 487 *m/z* (M^+); Anal. Calcd. for $C_{24}H_{17}N_5O_5S$: C-59.13, H-3.51, N-14.37, O-16.41, S-6.58. Found: C-58.12, H-3.08, N-13.68, O-15.47, S-5.98.

3. Results and discussion

Based on these observations, inspired by the biological profile of benzooxazoles and thiazanones, their increasing importance in pharmaceutical and biological fields and in continuation of our research on biologically active heterocycles, we have introduced thiazanone moiety into the benzoxazole ring which leads to both active pharmacophores in a single molecular frame work for the intensified biological activities. Thus we have designed and synthesized a series of novel 9-benzooxazol-4-yl-3,4-diphenyl-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-ones **(6a-e)**. The synthetic route leading to the title compounds is summarized in scheme 1. The synthesis of the target compounds commenced from commercially available 4-nitro-1*H*-benzooxazole **(1)**. The initial intermediate, 4-amino-1*H*-benzooxazole **(2)** has been synthesized through reduction from compound **1** with thionyl chloride in presence of ethanol solvent on constant stirring at reflux temperature for 45 mins. The key intermediate, *N*-benzooxazol-4-yl-2-chloro-acetamide **(3)** was prepared at room temperature with constant stirring for 3 h of a mixture of compound **2** and chloro acetylchloride in tri ethyl amine solvent. 3-Benzooxazol-4-yl-2-phenylimino-thiazolidin-4-ones **(4a-e)** have been prepared by the cyclization of compound **3** with phenyl isothiocyanate in the presence of K_2CO_3 in acetonitrile at room temperature followed by reflection of reaction mixture for 8-9 h. Compounds **4a-e** were reacted with mercapto acetic acid in dioxane at reflux for 8-9 h to achieve 4-benzooxazol-4-yl-9-phenyl-1,6-dithia-4,9-diaza-spiro[4.4]nonane-3,8-diones **(5a-e)**. Further, the compounds **5a-e** when reacted with benzhydroxamoyl chloride in chloroform at room temperature at $0C^0$ for 4-5 h afforded the title compounds 9-benzooxazol-4-yl-3,4-diphenyl-1-oxa-6-thia-2,4,9-triaza-spiro[4.4]non-2-en-8-ones **(6a-e)**. The chemical structures of all the newly synthesized compounds were confirmed by their IR, ^{1}H & ^{13}C NMR, mass spectral data and elemental analysis. Further the target compounds **5a-e** and **6a-e** were used to evaluate their antimicrobial and anti-inflammatory activity.



4/5 Ar = (a) C_6H_5, (b) 4-OCH_3-C_6H_4, (c) 4-Cl-C_6H_4, (d) 4-Br-C_6H_4, (e) 4-NO_2-C_6H_4. **6** Ar = (a) C_6H_5, (b) C_6H_5, (c) 4-OCH_3-C_6H_4, (d) 4-Cl-C_6H_4, (e) 4-NO_2-C_6H_4. **6** Ar'= (a) C_6H_5, (b) 4-Cl-C_6H_4, (c) C_6H_5, (d) 4-OCH_3-C_6H_4, (e) 4-CH_3-C_6H_4.

3.1 Antimicrobial activity

The disc diffusion method[19] was used for the screening of anti microbial activity. The *in vitro* antibacterial activity of the synthesized compounds **5a-e** and **6a-e** was tested against three gram-positive bacteria *i.e. Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus faecalis* and against three gram-negative bacteria such as *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi* using a nutrient agar medium. The antifungal activity of the compounds was screened against two representative fungal organisms namely *Candia albicans* and *Aspergillus fumigatus* using Sabouraded dextrose agar medium. Amicacin (300 µg/ml) and Fluconazole (300 µg/ml) were used as reference compounds for the study of antibacterial and antifungal activity respectively. The lowest concentration (highest dilution) of the compounds at which, there was no visually detectable bacterial growth was taken as minimum inhibitory concentration (MIC). The zone of inhibition in mm caused by the various compounds on the microorganisms was measured.

Table 1 Antimicrobial activity of compounds 5a-e and 6a-e. Zone of inhibition in mm (activity index)*

Compound	Antibacterial activity					Antifungal activity		
	S. au.	***S. a***	***S. f.***	***E. c.***	***P. m.***	***S. t.***	***C. a.***	***A. f.***
5a	11 (0.46)	13 (0.59)	13 (0.50)	12 (0.60)	00 (0.00)	10 (0.55)	10 (0.43)	12 (0.48)
5b	13 (0.54)	11 (0.50)	12 (0.46)	13 (0.65)	13 (0.62)	11 (0.61)	12 (0.52)	13 (0.52)
5c	06 (0.25)	07 (0.31)	19 (0.73)	02 (0.01)	12 (0.57)	12 (0.66)	11 (0.48)	11 (0.44)
5d	08 (0.33)	09 (0.75)	10 (0.38)	11 (0.55)	11 (0.52)	10 (0.55)	12 (0.52)	10 (0.40)
5e	13 (0.54)	10 (0.45)	15 (0.57)	10 (0.50)	10 (0.47)	10 (0.55)	13 (0.56)	13 (0.52)
6a	21 (0.87)	19 (0.86)	18 (0.69)	16 (0.80)	17 (0.81)	15 (0.83)	16 (0.69)	16 (0.64)
6b	18 (0.75)	19 (0.86)	20 (0.77)	14 (0.70)	15 (0.71)	15 (0.83)	21 (0.91)	23 (0.92)
6c	20 (0.83)	17 (0.77)	22 (0.84)	17 (0.85)	18 (0.85)	17 (0.94)	16 (0.69)	21 (0.84)
6d	22 (0.91)	20 (0.90)	24 (0.92)	14 (0.70)	14 (0.66)	15 (0.83)	21 (0.91)	23 (0.92)
6e	19 (0.79)	19 (0.86)	21 (0.80)	15 (0.75)	16 (0.76)	14 (0.77)	13 (0.56)	16 (0.64)
Am.	24	22	26	20	21	18	—	—
Flu.	—	—	—	—	—	—	23	25

* Activity index = Inhibition area of the sample/inhibition area of the standard; Diameter of disc is 5 mm
S. au = S. aureus, S. a. = S. albus; S. f. = S. faecalis; E. c. = E. coli; P. m. = P. mirabilis; S. T. = S. Typhi; C. a. = C. albicans; A. f. = A. fumigatus; Am. = Amicacin; Flu. = Fluconazole

The results (Table 1) of the antimicrobial screening of the tested compounds revealed that, all the tested compounds exhibited moderate to significant antimicrobial activity against both bacteria and fungi comparable with that of reference compounds. Compound **5a** compare with other molecules was found to be totally inactive against *P. mirabilis.* Compound **5c** was good active only against *S. faecalis* and almost inactive towards *E. coli*. This compound exhibited moderate activity against the rest of organisms. Highest antibacterial activity was observed in the product **6d** against *S. aureus*, *S. albus* and *S. faecalis*, but shows only moderate activity against *E. coli* and *P. mirabilis*. This compound also performed high activity against two fungal organisms with marked activity index. Both compounds **6b** and **6c** exhibited highest antifungal activity against *C. albicans* and *A. fumigatus*. The remaining compounds exhibit moderate to good activity against all organisms employed. It can be concluded that the antimicrobial activity of such compounds may change by introduction or elimination of a specific group.

Table 7 *In-vivo* anti-inflammatory activity of 5a-e and 6a-e

Compound	Mean swelling volume ± S.E.M[a] (% inhibition of oedema)					
	0.5 h	1 h	2 h	3 h	4 h	Potency[b]
5a	0.762 ± 0.047 (00.0)	0.750 ± 0.057 (1.60)	0.712 ± 0.062 (8.10)	0.637 ± 0.047 (16.4)	0.612 ± 0.062 (19.7)	0.08
5b	0.733 ± 0.076 (3.80)	0.650 ± 0.070(14.7)	0.650 ± 0.040 (16.1)	0.587 ± 0.025 (23.0)	0.625 ± 0.050 (18.0)	0.72
5c	0.762 ± 0.047(0.00)	0.750 ± 0.057 (1.60)	0.750 ± 0.057 (3.20)	0.725 ± 0.050 (4.80)	0.725 ± 0.050 (4.80)	0.08
5d	0.737 ± 0.025 (3.28)	0.662 ± 0.047(13.1)	0.625 ± 0.028 (19.3)	0.575 ± 0.028 (24.5)	0.537 ± 0.047 (29.5)	0.64
5e	0.750 ± 0.057 (1.600)	0.737 ± 0.047 (3.30)	0.737 ± 0.047 (4.90)	0.725 ± 0.028 (4.80)	0.737 ± 0.025 (3.30)	0.16
6a	0.762 ± 0.047(00.0)	0.750 ± 0.040 (1.60)	0.750 ± 0.040 (3.20)	0.712 ± 0.047 (6.50)	0.700 ± 0.070 (8.10)	0.08
6b	0.700 ± 0.040 (8.10)	0.650 ± 0.040(14.7)	0.625 ± 0.028 (19.3)	0.587 ± 0.025 (23.0)	0.537 ± 0.047(29.5)	0.72
6c	0.750± 0.057 (1.60)	0.737± 0.047 (3.30)	0.619± 0.026 (19.1)	0.762± 0.047 (00.0)	0.634± 0.052 (18.3)	0.16
6d	0.762± 0.047 (00.0)	0.675± 0.064 (11.4)	0.620± 0.61 (19.1)	0.637± 0.047 (16.4)	0.541± 0.048 (29.4)	0.56
6e	0.760 ± 0.046(0.00)	0.749 ± 0.056 (1.50)	0.748 ± 0.058 (3.10)	0.727 ± 0.052 (4.70)	0.723 ± 0.051 (4.70)	0.08
IM	0.669 ± 0.148 (11.6)	0.605 ± 0.019 (20.2)	0.532 ± 0.012 (33.3)	0.429 ± 0.092 (44.5)	0.365 ± 0.042 (52.8)	1.00

[a] S.E.M. = Standard error mean, and all showed at least significant difference at $p < 0.05$ in comparison with control group;
[b] Potency is expressed as percentage oedema inhibition of the tested compounds relative to percentage oedema inhibition of Indomethacin "reference standard" at 1 h effect; IM = Indomethacin

3.2 Anti-inflammatory activity

The anti-inflammatory activity screening for the prepared compounds **5a-e** and **6a-e** (at a dose of 10 mg/kg body weight) was determined *in vivo* by the acute carrageenan-induced paw oedema standard method.[20] The anti-inflammatory properties were recorded at successive time intervals 0.5, 1, 2, 3, and 4 h and compared with reference standard, Indomethacin (at a dose of 10 mg/kg body weight). From the obtained results (Table 2), it was noticed that after 1 h, compounds **5b**, **5d**, **6b** and **6d** exhibited considerable anti-inflammatory properties with potency 0.72, 0.64, 0.72 and 0.56 respectively. The remaining compounds showed lower to moderate activity.

4. Conclusion

The outstanding properties of this new class of antibacterial and antifungal substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed. More extensive study is also warranted to determine additional physicochemical and biological parameters to have a deeper insight into structure-activity relationship and to optimize the effectiveness of this series of molecules.

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