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Synthesis of 2-Aryl-4*H*-3,1-Benzoxazin-4-ones: A Class of α-Chymotrypsin Inhibitors

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Twenty one derivatives of 2-aryl-4*H*-3,1-benzoxazin-4-one were synthesized and their potential therapeutically significance and structure-activity relationship were tested against α-chymotrypsin. Majority of synthesized compounds showed significant *in vitro* α-chymotrypsin inhibitory properties having IC_{50} values in the range of 5.42 ± 1.66 – 41.27 ± 1.33 μM, whereas standard inhibitor chymostatin have IC_{50} value 7.13 ± 1.06 μM. In the present series compounds 2-(2-fluorophenyl)-4*H*-3,1-benzoxazin-4-one (**3h**), 2-(2-bromophenyl)-4*H*-3,1-benzoxazin-4-one (**3n**) and 2-(1-naphthyl)-4*H*-3, 1-benzoxazin-4-one (**3t**) with IC_{50} values 7.22 ± 0.75, 6.99 ± 0.29 and 5.42 ± 1.66 μM, respectively were found to be most active members of series, even better than standard inhibitor α-chymostatin.

Keywords: 2-Aryl-4*H*-3,1-benzoxazin-4-ones, *in vitro* α-chymotrypsin inhibitory activity, Structure-activity relationship.

INTRODUCTION

Enzyme inhibition is an essential area of chemical biology since studies in this field have already led to the discovery of ample variety of drugs helpful in curing a number of diseases. Specific inhibitors act together with enzymes and block their activity towards their subsequent natural substrates. The treatment of a number of physiological conditions by enzyme inhibitor molecules reflects their importance as drugs[1]. Although the physiological role of serine proteases inhibitors have clearly been established. It has been considered that they are ingredient of plant's natural defence system against insect predation and function by inhibiting insect proteinases[2-5]. Hence these inhibitors have gained concern as apparent sources of engineered resistance against pests and pathogens for transgenic plants expressing heterologous inhibitors[3-6]. Tobacco plants transformed with gene coding serine protease inhibitor has been revealed to posses' insect pest resistance[6]. Bowman-Birk inhibitors have also been shown to be competent tumor suppressor agents *in vivo* and *in vitro*[7]. Serine proteases such as chymotrypsin and trypsin are involved in the devastation of certain fibrous proteins[8]. Chronic infection by hepatitis C virus can lead to the progressive liver injury, cirrhosis and liver cancer. A chymotrypsin like serine protease known NS3 protease is known to be essential for viral replication has become target for anti-HCV drugs[9]. So search for new effective inhibitors of serine proteases is an urgent need for the drug development. In the recent past we explored many classes of compounds for their potential use in medicinal chemistry as well as their new synthetic methodology developments[10-16].

During our preliminary studies on benzoxazinones, it has been observed that they have property to inhibit chymotrypsin[17,18]. A variety of biological activities has been associated with 2-substituted benzoxazin-4-ones such as antifungal, antibacterial[19-24] and antielastase properties[25]. They can be used for the treatment of obesity[26] and also found to be novel specific puromycin-sensitive aminopeptidase inhibitors[27]. Recently this class of compounds reported to show a strong dual inhibitory effect on human neutrophil elastase release[28].

The promising therapeutic potential of this class of compounds prompted us to devise, synthesize and biologically screen a series of aryl structural variants of 2-substituted-4*H*-3,1-benzoxazin-4-one against α-chymotrypsin.

EXPERIMENTAL

IR spectra (KBr discs or MeOH) were recorded on a Bruker FT-IR IFS48 spectrophotometer. EI mass spectra data were recorded with various MAT 711 (70 eV) spectrophotometers

and data are tabulated as *m/z*. ^{1}H NMR spectra were recorded in $CDCl_3$ using Bruker AC400 (300 MHz and 400 MHz) spectrophotometer, respectively. Chemical shifts are reported in δ (ppm) and coupling constants are given in Hz. Melting points were determined on a Büchi 434 melting point apparatus and were uncorrected. The progress of all reactions was monitored by TLC, which was performed on 2 × 5 cm aluminum sheets precoated with silica gel $60F_{254}$ to a thickness of 0.25 mm (Merck). The chromatograms were visualized under ultraviolet light (254-366 nm) or iodine vapours. All the carboxylic acids are commercially available (Flulka Aldrich).

General procedure for synthesis of 2-substituted-4*H*-3,1-benzoxzin-4-one (3a-3u)[29]: To a 100 mL round bottom flask containing the solution of anthranilic acid (1.37 g, 0.01 mol) in pyridine (30 mL) was cooled to 0 °C in an ice bath followed by the careful addition of acid chloride (0.02 mol). An exothermic reaction occurred. The reaction mixture was stirred for 5 min at 0 °C. The ice bath was removed and the reaction mixture was allowed to warm slowly to room temperature. The reaction mixture was further stirred for 0.5 h at room temperature. The reaction mixture was poured into ice cold water (200 mL) and precipitates were filtered off. The residue was washed free of pyridine with cold water (3 × 60 mL) and dried. Benzoxazin-4-one crystallized from ethanol as white prismatic needles. 4-Methyl-N-[2-(4-oxo-4*H*-3,1-benzoxazin-2-yl)phenyl]-benzenesulfonamide (**3u**) was also synthesized with similar procedure in which *p*-toluene sulphonylchloride was used instead of acid chloride.

2-Phenyl-4*H*-3,1-benzoxazin-4-one (3a): Yield: 90 % (2 g, 8.96 mmol); m.p.: 111 °C (lit.[29] m.p. = 123 °C); IR (KBr, ν_{max}, cm^{-1}): 1764.5, 1616, 1572, 1472; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, 1H, *J* = 7.1 Hz, H-5), 7.94 (t, 1H, *J* = 7.1 Hz, H-7), 7.72 (1H, d, *J* = 7.1 Hz, H-8), 7.64 (t, 1H, *J* = 7.1 Hz, H-6), 8.15 (d, 2H, *J* = 7.8 Hz, H-2´,6´), 7.57-7.61 (m, 3H, H-3´/4´/5´); EIMS: *m/z* (%) 223 (M^+, 100), 146 (16), 105 (52), 76 (5 %); Anal. calcd. for $C_{14}H_9NO_2$ (223.227): C, 75.33; H, 4.06; N, 6.27. Found: C, 75.30; H, 4.07; N, 6.30.

2-(2-Methylphenyl)-4*H*-3,1-benzoxazin-4-one (3b): Yield: 68 % (1.61 g, 6.79 mmol), m.p.: 112 °C (lit.[29] m.p. = 114 °C); IR (KBr, ν_{max}, cm^{-1}): 2960, 1758, 1611, 1470, 1218; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (1H, d, *J* = 7.8 Hz, H-5), 7.97-7.90 (m, 2H, H-4´/6´), 7.70 (d, 1H, *J* = 7.8, H-8), 7.64 (t, 1H, *J* = 7.8 Hz, H-6), 7.50 (t, 1H, *J* = 7.8 Hz, H-7), 7.32-7.38 (m, 2H, H-3´/5´), 2.44 (3H, s, CH_3); EIMS: *m/z* 237 (M^+, 100), 146 (7), 119 (58), 118 (30), 91 (33 %). Anal. calcd. for $C_{15}H_{11}NO_2$ (237.253): C, 75.94; H, 4.67; N, 5.90. Found: C, 75.95; H, 4.66; N, 5.88.

2-(3-Methylphenyl)-4*H*-3,1-benzoxazin-4-one (3c): Yield: 65 % (1.54 g, 6.5 mmol); m.p.: 104-106 °C (lit.[30] m.p. = 112-115 °C); IR (KBr, ν_{max}, cm^{-1}): 2957, 1757, 1602, 1474, 1321, 1260; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, 1H, *J* = 7.8 Hz, H-5), 8.10 (d, 1H, *J* = 7.5 Hz, H-6´), 7.81 (t, 1H, *J* = 7.8 Hz, H-6), 7.68 (d, 1H, *J* = 7.8 Hz, H-8), 7.49 (t, 2H, *J* = 8.1 Hz, H-7/5´), 7.39 (d, 1H, *J* = 7.3 Hz, H-4´), 7.37 (s, 1H, H-2´), 2.43 (s, 3H, CH_3); EIMS: *m/z* (%) 237 (M^+, 90), 193 (25), 146 (10), 119 (100), 91 (35), 76 (5%). Anal. calcd. for $C_{15}H_{11}NO_2$ (237.253): C, 75.94; H, 4.67; N, 5.90. Found: C, 75.92; H, 4.66; N, 5.88.

2-(4-Methylphenyl)-4*H*-3,1-benzoxazin-4-one (3d): Yield: 86 % (2.04 g, 8.6 mmol); m.p.: 149 °C (lit.[29] m.p. = 155 °C); IR (KBr, ν_{max}, cm^{-1}): 2963, 1757, 1608, 1473, 1315; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (d, 1H, *J* = 7.9 Hz, H-5), 8.2 (d, 2H, *J* = 8.2, H-2´/6´), 7.79 (t, 1H, *J* = 7.9 Hz, H-6), 7.64 (d, 1H, *J* = 7.9 Hz, H-8), 7.47 (t, 1H, *J* = 7.9 Hz, H-7), 7.29 (d, 2H, *J* = 8.2 Hz, H-3´/5´), 2.44 (s, 3H, CH_3); EIMS: *m/z* (%) 237 (95, M^+), 193 (31), 146 (10), 119 (100), 91 (42), 76 (3 %). Anal. calcd. for $C_{15}H_{11}NO_2$ (237.253): C, 75.94; H, 4.67; N, 5.90. Found: C, 75.95; H, 4.65; N, 5.88.

2-(2-Nitrophenyl)-4*H*-3,1-benzoxazin-4-one (3e): Yield: 70 % (1.87 g, 7 mmol); m.p.: 191 °C (lit.[29] m.p. = 193 °C); IR (KBr, ν_{max}, cm^{-1}): 1768, 1636, 1531, 1346, 1225; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, 1H, *J* = 7.8, H-5), 8.14 (d, 1H, *J* = 7.5 Hz, H-6´), 8.08 (d, 1H, *J* = 7.5, H-3´), 7.99 (t, 1H, *J* = 7.8 Hz, H-6), 7.93 (t, 1H, *J* = 7.5 Hz, H-5´), 7.88 (t, 1H, *J* = 7.8 Hz, H-7), 7.72 (d, 1H, *J* = 7.8, H-8), 7.69 (t, 1H, *J* = 7.5 Hz, H-4´); EIMS: *m/z* (%) 268 (M^+, 62), 146 (29), 133 (100), 118 (2), 76 (15). Anal. calcd. for $C_{14}H_8N_2O_4$ (268.224): C, 62.69 %; H, 3.01 %; N, 10.44 %. Found: 62.71; 3.02; N, 10.46.

2-(3-Nitrophenyl)-4*H*-3,1-benzoxazin-4-one (3f): Yield: 82 % (2.2 g, 8.2 mmol), m.p.: 165 °C (lit.[29] m.p. = 166 °C); IR (KBr, ν_{max}, cm^{-1}): 1756, 1626, 1530, 1474, 1346, 1220, 1036; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85 (s, 1H, H-2´), 8.56 (d, 1H, *J* = 7.6 Hz, H-4´), 8.47 (d, 1H, *J* = 7.6 Hz, H-6´), 8.17 (d, 1H, *J* = 8.0 Hz, H-5), 7.98 (t, 1H, *J* = 8.0 Hz, H-6), 7.89 (t, 1H, *J* = 8.0 Hz , H-7), 7.80 (d, 1H, *J* = 8.0 Hz , H-8), 7.66 (t, 1H, *J* = 7.6 Hz , H-5´); EIMS: *m/z* (%) 268 (M^+, 100), 222 (13), 150 (34), 146 (64), 104 (28), 76 (26); Anal. calcd. for $C_{14}H_8N_2O_4$ (268.224): C, 62.69 %; H, 3.01 %; N, 10.44. Found: C, 62.67, H, 3.03, N, 10.46.

2-(4-Nitrophenyl)-4*H*-3,1-benzoxazin-4-one (3g): Yield: 78 % (2.01 g, 7.8 mmol); m.p.: 198 °C (lit.[29] m.p. = 203 °C[29]); IR (KBr, ν_{max}, cm^{-1}): 1768, 1596, 1519, 1437, 1354, 1254; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.41 (d, 2H, *J* = 8.9 Hz, H-3´/5´), 8.30 (1H, d, *J* = 8.2Hz, H-5), 8.14 (2H, d, *J* = 8.9 Hz, H-2´/6´), 7.98 (1H, t, *J* = 8.2 Hz, H-6), 7.77 (d, 1H, *J* = 8.2 Hz, H-8), 7.67 (t, 1H, *J* = 8.2 Hz, H-7); EIMS: *m/z* (%) 268 (M+, 100), 222 (4), 146 (74), 104 (58), 76 (86 %). Anal. calcd. for $C_{14}H_8N_2O_4$ (268.224): C, 62.69 %; H, 3.01 %; N, 10.44 %. Found: C, 62.68; H, 3.02; 10.45.

2-(2-Fluorophenyl)-4*H*-3,1-benzoxazin-4-one (3h): Yield: 53 % (1.27 g, 5.3 mmol); m.p.: 110 °C (lit.[29] m.p. = 115 °C); IR (KBr, ν_{max}, cm^{-1}): 1772, 1625, 1606, 1477, 1320, 1278; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, 1H, *J* = 7.8 Hz, H-5), 8.11 (t, 1H, *J* = 7.6 Hz, H-4´), 7.83 (t, 1H, *J* = 7.8 Hz, H-6), 7.7 (d, 1H, J = 7.8 Hz, H-8), 7.51-7.56 (m, 2H, H-6´/7), 7.27 (t, 1H, *J* = 7.6 Hz, H-3´), 7.20 (1H, t, *J* = 7.6 Hz, H-5´); EIMS: 241(M^+, 100), 213 (9), 197 (98), 146 (85), 95 (70), 76 (15 %). Anal. calcd. for $C_{14}H_8NO_2F$ (241.217): C, 69.71 %; H, 3.34 %; N, 5.81 %. Found: C, 69.69; H, 3.36; N, 5.81.

2-(3-Fluorophenyl)-4*H*-3,1-benzoxazin-4-one (3i): Yield: 69 % (1.66 g, 6.9 mmol), m.p.: 133 °C; IR (KBr, ν_{max}, cm^{-1}): 1766, 1615, 1477, 1445, 1323, 1260; ^{1}H NMR (400 MHz, $CDCl_3$): 8.23 (d, 1H, *J* = 7.8 Hz, H-5), 8.08 (d, 1H, *J* = 7.8 Hz, H-8), 7.97-8.012 (m, 1H, H-2´), 7.82 (t, 1H, *J* = 7.8 Hz, H-7), 7.68 (d, 1H, *J* = 7.6 Hz, H-6´), 7.51(t, 1H, *J* = 7.95 Hz, H-5´), 7.46 (t, 1H, *J* = 8 Hz, H-6), 7.25-7.27 (m, 1H, H-4´); EIMS: *m/z* (%) 241 (100 %, M^+), 213 (5 %), 197 (76 %), 146 (36 %), 95 (38 %), 76 (5 %). Anal. calcd. for $C_{14}H_8NO_2F$ (241.217): C, 69.71 %; H, 3.34 %; N, 5.81 %. Found: C, 69.72; H, 3.36; N, 5.83.

2-(4-Fluorophenyl)-4*H*-3,1-benzoxazin-4-one (3j): Yield: 97 % (2.34 g, 9.7 mmol); m.p.: 168 °C (lit.[29] m.p. = 170 °C); IR (KBr, ν_{max}, cm^{-1}): 1764, 1601, 1508, 1472, 1321, 1231; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, 1H, *J* = 6.5 Hz, H-5), 8.21 (1H, d, *J* = 6.5 Hz, H-8), 7.81 (t, 1H, *J* = 6.5 Hz, H-6), 7.66 (d, 2H, *J* = 5.9 Hz, H-2´/6´), 7.54 (t, 1H, *J* = 6.5 Hz, H-7), 7.14-7.17 (m, 2H, H-3´/ 5´); EIMS: *m/z* (%) 241 (M^+, 31), 146 (7), 123 (100), 95 (40), 76 (4 %). Analysis calcd. for $C_{14}H_8NO_2F$ (241.217): C, 69.71 %; H, 3.34 %; N, 5.81 %. Found: C, 69.69; H, 3.36; N, 5.82.

2-(2-Chlorophenyl)-4*H*-3,1-benzoxazin-4-one (3k): Yield: 64 % (1.64 g, 6.4 mmol); m.p.: 136 °C (lit.[29] m.p. = 138 °C); IR (KBr, ν_{max}, cm^{-1}): 1769, 1622, 1474, 1316, 1272, 1225; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (d, 1H, *J* = 6.1 Hz, H-5), 7.87 (d, 1H, *J* = 6.8 Hz, H-6´), 7.84 (t, 1H, *J* = 6.1 Hz, H-7), 7.7 (d, 1H, *J* = 6.1 Hz, H-8), 7.57 (t, 1H, *J* = 6.1 Hz , H-6), 7.51 (d, 1H , J = 6.8 Hz, H-3´), 7.45 (t, 1H , *J* = 6.8 Hz, H-5´), 7.38 (t, 1H , *J* = 6.8 Hz, H-4´); EIMS: *m/z* (%) 259 (M^+, 15), 257 (44), 213 (31), 215 (11), 146 (21), 139 (47), 141 (19), 113 (6), 146 (21), 76 (5); Anal. calcd. for $C_{14}H_8NO_2Cl$ (257.672): C, 65.26 %); H, 3.13 %; N, 5.44 %. Found: C, 65.24; H, 3.15; N, 5.46.

2-(3-Chlorophenyl)-4*H*-3,1-benzoxazin-4-one (3l): Yield: 87 % (2.19 g, 8.5 mmol); m.p.: 153 °C (lit.[30] m.p. = 156-157 °C); IR (KBr, ν_{max}, cm^{-1}): 1761, 1601, 1566, 1473, 1416; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H, H-2´), 8.23 (1H, d, *J* = 7.8 Hz, H-5), 8.17 (d, 1H, *J* = 8.1 Hz, H-6´), 7.81 (t, 1H, *J* = 7.8 Hz, H-7), 7.68 (d, 1H, *J* = 7.8 Hz, H-8), 7.50-7.55 (m, 2H, H-4´/5´), 7.42 (t, 1H, *J* = 7.8 Hz, H-6); EIMS: *m/z* (%) 259 (M^+, 36), 257 (100), 213 (61), 215 (21), 146 (41), 139 (67), 141 (21), 113 (14), 111 (43), 76 (10 %); Analysis calcd. for $C_{14}H_8NO_2Cl$ (257.672): C, 65.26 %; H, 3.13 %; N, 5.44 %. Found: C, 65.28; H, 3.12; N, 5.44.

2-(4-Chlorophenyl)-4*H*-3,1-benzoxazin-4-one (3m): Yield: 85 % (2.18 g, 8.49 mmol); m.p.: 192 °C (lit.[29] m.p. = 190 °C); IR (KBr, ν_{max}, cm^{-1}): 1770, 1633, 1484, 1316, 1255; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (d, 1H, *J* = 7.9 Hz, H-5), 7.83 (2H, d, *J* = 7.4 Hz, H-2´/6´), 7.46 (t, 1H, *J* = 7.9, H-6), 7.34 (d, 1H, *J* = 7.9 Hz, H-8), 7.27(d, 2H, *J* = 7.4 Hz, H-3´/5´), 7.01 (t, 1H, *J* = 7.9 Hz, H-7); EIMS: *m/z* (%) 259 (M^+, 27), 257 (81), 213 (37), 215 (13), 146 (14), 139 (100), 141 (32), 113 (12), 111 (37), 76 (8 %). Anal. calcd. for $C_{14}H_8NO_2Cl$ (257.672): C, 65.26 %; H, 3.13 %; N, 5.44 %. Found: C, 65.26; H, 3.14; N, 5.43.

2-(2-Bromophenyl)-4*H*-3,1-benzoxazin-4-one (3n): Yield: 87 % (2.63 g, 8.7 mmol); m.p.: 115 °C (lit.[29] m.p. = 118 °C); IR (KBr, ν_{max}, cm^{-1}): 1767, 1626, 1474, 1315, 1270, 1224; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, 1H, *J* = 8 Hz, H-5), 7.82-7.86 (m, 2H, H-3´/5´), 7.71 (d, 2H, *J* = 7.9 Hz, H-8/6´), 7.56 (t, 1H, *J* = 8 Hz, H-7), 7.42 (t, 1H, *J* = 7.4, H-4´), 7.35 (t, 1H, *J* = 8 Hz, H-6); EIMS: *m/z* (%) 303 (M^+, 97), 301 (100), 257 (33), 259 (32), 222 (4), 183 (47), 185 (45), 146 (25), 76 (8 %). Anal. calcd. for $C_{14}H_8NO_2Br$ (302.123): C, 55.66 %; H, 2.67 %; N, 4.64 %. Found: C, 55.68; H, 2.66; N, 4.63.

2-(3-Bromophenyl)-4*H*-3,1-benzoxazin-4-one (3o): Yield: 68 % (2.05 g, 6.8 mmol); m.p.: 165 °C (lit.[30] m.p. = 160-161 °C); IR (KBr, ν_{max}, cm^{-1}): 1765, 1625, 1475, 1317, 1270, 1224; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.46 (s, 1H, H-2´), 8.22 (d, 1H, *J* = 7.9 Hz, H-5), 7.85 (t, 1H, *J* = 7.9, H-7), 7.69 (d, 2H, *J* = 7.9 Hz, H-8/6´), 7.52 (t, 1H, *J* = 8 Hz, H-6), 7.34 (t, 1H, *J* = 7.6 Hz, H-5´), 7.32 (d, 1H, *J* = 7.6 Hz, H-4´). EIMS: *m/z* (%) 303 (M^+, 97), 301 (100), 257 (29), 259 (38), 222 (6), 183 (47), 185 (48), 146 (21), 76 (11 %). Anal. calcd. for $C_{14}H_8NO_2Br$ (302.123): C, 55.66 %; H, 2.67 %; N, 4.64 %. Found: C, 55.68; H, 2.66; N, 4.63.

2-(4-Bromophenyl)-4*H*-3,1-benzoxazin-4-one (3p): Yield: 80 % (2.41 g, 8.0 mmol); m.p.: 182 °C (lit.[29] m.p. = 184 °C); IR (KBr, ν_{max}, cm^{-1}): 1764, 1619, 1480, 1320, 1256; 1H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, 1H, *J* = 7.8 Hz, H-5), 8.16 (d, 2H, *J* = 8.6 Hz, H-2´/ 6´), 7.81 (t, 1H, *J* = 7.8, H-7), 7.67 (d, 1H, *J* = 7.8 Hz, H-8), 7.63 (d, 2H, *J* = 8.6 Hz, H-3´5´), 7.51 (t, 1H, *J* = 7.8 Hz, H-6); EIMS: *m/z* (%) 303 (M^+, 98), 301 (100), 155 (46), 157 (45), 146 (38) 118 (2), 104 (5), 102 (5), 76 (42), 64 (5); Anal. calcd. for $C_{14}H_8NO_2Br$ (302.123): C, 55.66; H, 2.67; N, 4.64. Found: C, 55.65; H, 2.68; N, 4.65.

2-(2-Methoxyphenyl)-4*H*-3,1-benzoxazin-4-one (3q): Yield: 75 % (1.9 g, 7.5 mmol); m.p.: 129 °C (lit.[30] m.p. = 132-133 °C); IR (KBr, ν_{max}, cm^{-1}): 1769, 1607, 1490, 1467, 1255; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, 1H, *J* = 7.9 Hz, H-5), 7.84 (d, 1H, *J* = 7.6 Hz, H-6´), 7.79 (d, 1H, *J* = 7.6 Hz, H-4´), 7.70 (d, 1H, *J* = 7.9 Hz, H-8), 7.51 (t, 1H, *J* = 7.9 Hz, H-7), 7.48 (t, 1H, *J* = 7.9 Hz, H-6), 7.0-7.07 (m, 2H, H-3´/5´), 3.93 (s, 3H, -OCH_3); EIMS: 253 (M^+, 64,), 224 (26), 209 (3), 146 (8), 135 (63), 133 (23), 119 (100), 77 (25), 76 (4 %); Anal. calcd. for $C_{15}H_{11}NO_3$ (253.253): C, 71.14; H, 4.38; N, 5.53. Found: C, 71.12; H, 4.40; N, 5.54.

2-(4-Methoxyphenyl)-4*H*-3,1-benzoxazin-4-one (3r): Yield: 94 % (2.38 g, 9.4 mmol), m.p.: 150 °C (lit.[29] m.p. = 148 °C); IR (KBr, ν_{max}, cm^{-1}): 1757, 1606, 1568, 1472, 1259, 1168; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, 1H, *J* = 8.5 Hz, H-5), 8.2 (d, 1H, *J* = 8.5 Hz, H-8), 7.82 (t, 1H, *J* = 8.5 Hz, H-7), 7.63 (d, 2H, *J* = 8 Hz, H-2´/6´), 7.45 (t, 1H, *J* = 8.5 Hz, H-6), 6.99 (d, 1H, *J* = 8 Hz, H-3´/5´), 3.88 (s, 3H, OCH_3); EIMS: *m/z* (%) 253 (M^+, 23), 209 (5), 146 (3), 135 (100), 107 (8), 76(3), 64 (9 %). Anal. calcd. for $C_{15}H_{11}NO_3$ (253.253): C, 71.14, H, 4.38, N, 5.53. Found: C, 71.14; H, 4.39; N, 5.51.

2-(3, 4, 5-Trimethoxyphenyl)-4*H*-3,1-benzoxazin-4-one (3s). Yield: 40 % (1.25 g, 4 mmol); m.p.: 181 °C (lit.[30] m.p. = 185 °C); IR (KBr, ν_{max}, cm^{-1}): 1754, 1607, 1504, 1349, 1292, 1131; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20 (1H, d, *J* = 8.1 Hz, H-5), 7.80 (t,1H, *J* = 8.1 Hz, H-7), 7.66 (d, 1H, *J* = 8.1, H-8), 7.53 (s, 2H, H-2´/6´), 7.48 (t, 1H, *J* = 8.1 Hz, H-6), 3.92 (s, 9H, -OCH_3); EIMS: *m/z* (%) 313 (M^+, 100), 268 (5), 223 (2), 195 (28), 193 (3), 167 (6), 146 (30), 76 (2). Anal. calcd. for $C_{17}H_{15}NO_5$ (313.305): C, 65.17; H, 4.83; N, 4.47; found: C, 65.18; H, 4.82; N, 4.47.

2-(1-Naphthyl)-4*H*-3,1-benzoxazin-4-one (3t): Yield: 95 % (2.59 g, 9.5 mmol); m.p.: 136-137 °C (lit.[30] m.p. = 138-140 °C); IR (KBr, ν_{max}, cm^{-1}): 1747, 1596, 1510, 1471, 1320, 1223; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (d, 1H, *J* = 8.6 Hz, H-8´), 8.93 (d, 1H, *J* = 8.6 Hz, H-2´), 8.37 (d, 1H, *J* = 7.3 Hz, H-4´), 8.20 (d, 1H, *J* = 7.6 Hz, H-5), 8.13 (d, 1H, *J* = 7.6 Hz, H-8), 8.07(d, 1H, *J* = 8.6 Hz, H-5´), 7.99 (t, 1H, *J* = 8.6 Hz, H-7´), 7.79 (t, 1H, *J* = 7.6 Hz, H-7), 7.73-7.65 (m, 3H, H-6/6´/3´); EIMS: *m/z* (%) 273 (M^+, 20), 153 (4), 146 (7), 155 (100), 127 (67), 76 (4); Anal. calcd. for $C_{18}H_{11}NO_2$ (273.285): C, 79.11; H, 4.06; N, 5.13. Found: C, 79.11; H, 4.08; N, 5.11.

4-Methyl-N-[2-(4-oxo-4*H*-3,1-benzoxazin-2-yl)phenyl]-benzenesulfonamide (3u): Yield: 55 % (1.8 g, 4.59 mmol); m.p.: 212 °C (lit.[30] m.p. = 216 °C); IR (KBr, ν_{max}, cm^{-1}): 1778, 1704, 1604, 3446, 1570, 1474; ^1H NMR (400 MHz, $CDCl_3$): δ 11.92 (s, 1H, NH), 8.17 (d, 1H, *J* = 8.5 Hz, H-6´), 7.97 (d,1H, *J* = 7.7 Hz, H-5) 7.69 (t, 1H, *J* = 7.7 Hz, H-7), 7.56 (d, 1H, *J* = 7.7 Hz, H-8), 7.52 (d, 2H, *J* = 8 Hz, H-2´´/6´´), 7.44 (t, 1H, *J* = 7.7 Hz, H-6´), 7.25 (d, 2H, *J* = 8 Hz, H-3´´/H-5´´), 7.21 (t, 1H, *J* = 8.5 Hz, H-4´) 7.11 (d, 2H, *J* = 8.5 Hz, H-5´/3´), 2.27 (s, 3H, CH_3); EIMS: 392.2 (M^+, 100), 377 (3), 237 (66), 146 (4), 91 (20 %). CHN analysis calcd. for $C_{21}H_{16}N_2O_4$ (392.429): C, 64.27; H, 4.11; N, 7.14. Found: C, 64.28; H, 4.13; N, 7.12.

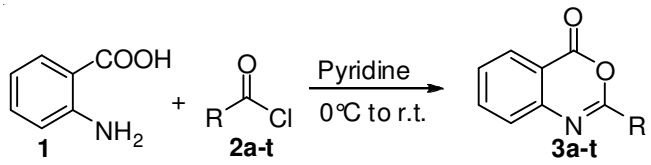
Chymotrypsin inhibition assay: The α-chymotrypsin inhibitory activity of compounds was performed by method of Cannel *et al.*[2]. Chymotrypsin (9 units/mL of 50 mM *Tris*-HCl buffer pH 7.6; Sigma Chemical Co. USA) was pre-incubated with the compounds for 20 min at 25 °C. The substrate solution of 100 µL volume (N-succinyl-phenylalanine-*p*-nitroanilide, 1 mg/mL of 50 mM *Tris*-HCl buffer pH 7.6) was added to start the enzyme reaction. The absorbance of released *p*-nitroaniline was continuously monitored at 410 nm until a significant color change had achieved. The final DMSO concentration in the reaction mixture was 7 %. All the experiments were carried out at least in triplicates and the results represent the mean ± S.E.M. (standard error of the mean). Chymostatin was used as a standard inhibitor for α-chymotrypsin inhibition.

RESULTS AND DISCUSSION

Anthranilic acid (**1**) was treated with aryl chlorides (**2a-2t**) in the presence of excess of pyridine to give 2-substituted benzoxazin-4-one (**3a-3t**) in moderate to excellent yields according to the literature protocol[29] (**Scheme-I**). The acid chlorides, where not commercially available, were prepared by the action of thionyl chloride on the appropriate carboxylic acid. The failure of aliphatic acyl halides to produce benzoxazinones is most likely due to ready hydrolysis of initially formed 2-alkyl-4*H*-3,1-benzoxazin-4-one[31].

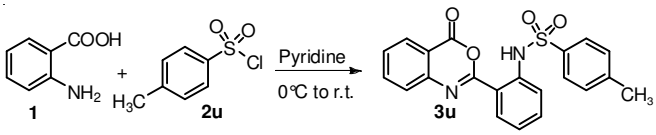
Similarly, anthranilic acid (**1**) reacts with *p*-tolylsulphonyl chloride (**2u**) in the presence of excess of pyridine to give 2-substituted benzoxazin-4-one (**3u**) having sulfonamide moiety in good yield (**Scheme-II**). This reaction probably proceeds *via* a mixed carboxylic acid-sulphonic acid anhydride intermediate[29].

***in vitro* α-Chymotrypsin inhibitory activity:** A small library of 2-substituted-4*H*-3,1-benzoxazin-4-one (**3a-3u**) were synthesized and their potential biological significance were investigated as α-chymotrypsin inhibitors. Most of them showed significant activity against α-chymotrypsin. *in vitro* α-Chymotrypsin inhibiton assay of benzoxazinone derivatives were performed according to liteature protocol[2] and a list of inhibitory activities results in term of IC_{50} values are summarized in Table-1. α-Chymostatin was used as standard inhibitor with IC_{50} value 7.13 ± 1.06 µM (Table-1). Further standard inhibitor (α-Chymostatin) is a tetra-peptide analogue and a potent inhibitor of a number of proteinases including the serine proteinases, chymotrypsin and streptomyces griseus proteinase A and several cysteine proteinases[32].



Compound	R	Compound	R
3a	C_6H_5-	**3k**	*o*-ClC_6H_4-
3b	*o*-$CH_3C_6H_4$-	**3l**	*m*-ClC_6H_4-
3c	*m*-$CH_3C_6H_4$-	**3m**	*p*-ClC_6H_4-
3d	*p*-$CH_3C_6H_4$-	**3n**	*o*-BrC_6H_4-
3e	*o*-$NO_2C_6H_4$-	**3o**	*m*-BrC_6H_4-
3f	*m*-$NO_2C_6H_4$-	**3p**	*p*-BrC_6H_4-
3g	*p*-$NO_2C_6H_4$-	**3q**	*o*-$OCH_3C_6H_4$-
3h	*o*-FC_6H_4-	**3r**	*p*-$OCH_3C_6H_4$-
3i	*m*-FC_6H_4-	**3s**	3,4,5-$(OCH_3)_3C_6H_2$-
3j	*p*-FC_6H_4-	**3t**	1-Naphthyl

Scheme-I: Synthesis of 2-aryl-4*H*-3,1-benzoxazin-4-ones



Scheme-II: Synthesis of 4-Methyl-N-[2-(4-oxo-4*H*-3,1-benzoxazin-2-yl)phenyl]-benzenesulfonamide

TABLE-1
α-CHYMOTRYPSIN INHIBITORY ACTIVITY (IC_{50})
OF 2-ARYL-4*H*-3,1-BENZOXAZIN-4-ONES (**3a-3u**)

Entry	Compound No.	R	IC_{50} ± SEM[a] (µM)
1	**3a**	C_6H_5-	11.54 ± 1.73
2	**3b**	*o*-$CH_3C_6H_4$-	41.27 ± 1.33
3	**3c**	*m*-$CH_3C_6H_4$-	35.0 ± 1.86
4	**3d**	*p*-$CH_3C_6H_4$-	13.26 ± 0.79
5	**3e**	*o*-$NO_2C_6H_4$-	N.A.[b]
6	**3f**	*m*-$NO_2C_6H_4$-	19.66 ± 1.31
7	**3g**	*p*-$NO_2C_6H_4$-	N.A.[b]
8	**3h**	*o*-FC_6H_4-	7.22 ± 0.75
9	**3i**	*m*-FC_6H_4-	14.92 ± 0.33
10	**3j**	*p*-FC_6H_4-	39.21 ± 0.34
11	**3k**	*o*-ClC_6H_4-	21.01 ± 0.13
12	**3l**	*m*-ClC_6H_4-	39.69 ± 0.52
13	**3m**	*p*-ClC_6H_4-	14.53 ± 0.35
14	**3n**	*o*-BrC_6H_4-	6.99 ± 0.29
15	**3o**	*m*-BrC_6H_4-	38.06 ± 1.93
16	**3p**	*p*-BrC_6H_4-	31.06 ± 0.96
17	**3q**	*o*-$OCH_3C_6H_4$-	12.62 ± 1.09
18	**3r**	*p*-$OCH_3C_6H_4$-	35.01 ± 2.33
19	**3s**	3,4,5-$(OCH_3)_3C_6H_2$-	N.A.[b]
20	**3t**	1-Naphthyl	5.42 ± 1.66
21	**3u**	Sulfonamide	16.23 ± 1.99
	Chymostatin[c]	Standard	7.13 ± 1.06

Notes: [a]SEM standard error of the mean; [b]N.A. Not active against enzyme α-chymotrypsin; [c]Standard inhibitors of enzyme α-chymotrypsin

Among these 21 compounds investigated for α-chymotrypsin inhibition, 6 compounds **3t**, **3n**, **3h**, **3a**, **3q** and **3d** showed promising results in comparison to standard inhibitor (Table-1). Any how certain variations were noted in results for example compounds **3e** and **3g** were found to be inactive having polar nitro group at *ortho*- and *para*-positions but

compound **3f** having nitro group at *meta*-position showed moderate activity with IC_{50} = 19.66 ± 1.31 μM (Table-1). Whereas compound **3s** was also found to be inactive with three adjacent methoxy groups (Table-1).

Compounds 2-naphthyl-4*H*-3,1-benzoxazin-4-one (**3t**) and 2-(2-bromophenyl)-4*H*-3,1-benzoxazin-4-one (**3n**) showed exciting α-chymotrypsin enzyme inhibitory activity with IC_{50} values 5.42 ± 1.66 μM and 6.99 ± 0.29 μM respectively, whereas the standard inhibitor has IC_{50} = 7.13 ± 1.06 μM. The 2-phenyl-4*H*-3,1-benzoxazin-4-one (**3a**) showed good inhibitory activity with IC_{50} = 11.54 ± 1.73 μM (Table-1). When phenyl group **3a** was replaced by naphthyl group in compound 3t (IC_{50} = 5.42 ± 1.66 μM), activity was almost doubled. The enhancement in activity may be attributed to steric bulk and prolongation of conjugation by naphthyl moiety. The α-chymotrypsin enzyme inhibitory activity in compounds **3b**, **3c** and **3d** having methyl moiety was gradually enhanced from *ortho*- to *para*-positions (Table-1). This is might be due to positive inductive effect of methyl substituent at *ortho* and *para* position. The complete reverse order of α-chymotrypsin enzyme inhibitory activity was observed in case of highly electronegative fluoro-substituent in compounds **3h**, **3i** and **3j** with negative inductive effect (Table-1). The 2-(2-fluorophenyl)-4*H*-1,3-benzoxazin-4-one (**3h**) was found to be most active among the fluoro-substituents with IC_{50} = 7.22 ± 0.75 μM and its *para*-analogue **3j** was found to be least active with IC_{50} = 39.21 ± 0.34 μM while *meta* analogue **3i** showed medium inhibitory activity (Table-1). Among the compounds **3k**, **3l** and **3m** with chloro-substituent at *ortho*-, *meta*- and *para*-positions, respectively, its *para*-analogue **3m** was found to be most active member with IC_{50} = 14.53 ± 0.35 μM whereas its *meta*-analogue **3l** was least active (IC_{50} = 39.69 ± 0.52 μM). This trend of activity may be explained on the basis of linear geometry of *para*-substituted phenyl ring. The 2-(2-bromophenyl)-4*H*-3,1-benzoxazin-4-one (**3n**) was found to be most active (IC_{50} = 6.99 ± 0.29 μM) among bromo-substituents (Table-1). Compound **3q** having postive mesomeric effect of methoxy at *ortho*-position showed very similar inhibitory activity to compound **3d** having methyl group at *para*-position. A marked decrease in activity was observed in case of **3r** having methoxy group at *para*-position. The compound **4u** having *p*-tolylsulfonyl group with sulfonamide moiety (IC_{50} = 16.23 ± 1.99 μM) showed similar activity as **3i** (IC_{50} = 14.92 ± 0.33 μM) (Table-1).

Comparing the activities with structure of compounds, it turns out that α-Chymotrypsin inhibitory activity is dependent on the electronic effects of substituent's present at *ortho*/*para* positions of aryl ring of investigated compounds.

Conclusion

There is growing trend in medicinal chemistry to treat the most fatal diseases by retarding the function of one or number of parameters related to the proliferation of diseased cells. In this regard, in our developed compounds library few members have shown excellent enzyme inhibitory function. The size, polarity, position of substituents, conjugation, inductive and mesomeric effects of the substituents influenced the activity. Our results show that it is feasible to develop small non-peptide molecule inhibitors which are effective and specific for α-chymotrypsin inhibition. Of this series, the most potent compounds 2-(2-fluorophenyl)-4*H*-3,1-benzoxazin-4-one (**3h**), 2-(2-bromophenyl)-4*H*-3,1-benzoxazin-4-one (**3n**) and 2-(1-naphthyl)-4*H*-3,1-benzoxazin-4-one (**3t**) may act as a potential lead molecules for the future research in the field of α-chymotrypsin inhibition.

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