

SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF CERTAIN BACLOFEN ANALOGUES

MOHAMED I. ATTIA^{a,b*}, CLAUS HERDEIS^{b*}, HANS BRÄUNER-OSBORNE^c

^a*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

^b*Department of Pharmaceutical Chemistry, Institute of Pharmacy and Food Chemistry, Würzburg University, Am Hubland, 97074, Würzburg, Germany*

^c*Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark*

(*RS*)-4-Amino-3-(4-chlorophenyl)butanoic acid (baclofen, **2**) is the prototypic selective GABA_BR agonist and is used clinically for the treatment of spasticity associated with brain and spinal cord injuries. Synthesis and GABA_B receptor agonist activity of certain amino acids structurally related to baclofen (**2**) are reported. (*RS*)-4-amino-3-(4-ethynylphenyl)butanoic acid hydrochloride (**1b**) showed GABA_B receptor agonist activity with EC₅₀ = 240 μM whereas (*RS*)-4-amino-3-(4-iodophenyl)butanoic acid hydrochloride (**1c**) emerged as the best GABA_B receptor agonist congener in the synthesized compounds with ED₅₀ = 32 μM.

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1. Introduction

The principle inhibitory neurotransmitter in the central nervous system, γ -aminobutyric acid (GABA, **1**, Figure 1), exerts its effect through three different receptor subtypes: ligand-gated chloride channels GABA_A and GABA_C receptors, and G-protein-coupled metabotropic (GPCRs) GABA_B receptors [1]. GABA_B receptors were discovered in 1980 by Norman G. Bowery [2] and act post- and pre-synaptically to inhibit neuronal excitability and neurotransmitter release, respectively. The molecular structure of GABA_B receptors has remained elusive for many years, until a cDNA encoding a putative GABA_B receptor was cloned in 1997 [3]. GABA_B receptor is composed of two subunits, GABA_{B1} and GABA_{B2}, to form a heterodimer which is necessary for GABA_B receptors to be functionally active [4]. The former subunit contains the GABA binding domain, while GABA_{B2} subunit provides the G-protein-coupling mechanism and also incorporates an allosteric modulatory site within its heptahelical structure [5].

(*RS*)-4-Amino-3-(4-chlorophenyl)butanoic acid (baclofen, **2**, Figure 1), the aromatic analogue of GABA, introduced as racemate in 1973 in the therapy of muscle spasticity, is still the classical prototype of the selective GABA_BR agonists [6]. The most predominant pharmacological effects of baclofen are the muscle relaxant, anti-nociceptive and anti-drug craving effects, as well as the reduction in cognitive behavior [7]. (*RS*)-Baclofen (**2**) is used clinically for the treatment of spasticity associated with brain and spinal cord injuries [8], drug addiction and alcoholism [9], gastroesophageal reflux disease (GERD) [10], cancer pain [11] and overactive bladder [12]. The GABA_B activity of racemic baclofen is known to reside primarily in the *R*-(-)-enantiomer [13].

*Corresponding author: mattia@ksu.edu.sa

portions over a period of 5 min to a cold (0-5 °C) stirred solution of cuprous chloride (5.60 g, 57 mmol) in concentrated hydrochloric acid (60 ml). After complete addition, the mixture was heated to 80 °C under stirring for 20 min. An equal volume of water was then added and the mixture was cooled in an ice bath. After 2 h, the white crystals were filtered, washed with water

and dried under vacuum to give 4.54 g (82 %) of **8a** as a white crystals, m.p. 278-280 °C (lit.[16] 277 °C). This material was pure enough to be used in the next step without further purification.

IR (neat, cm^{-1}) exhibited bands at 3982-2523, 1693, 1605, 1574, 1400, 1271. ^1H NMR (DMSO- d_6): δ (ppm) = 8.06 (d, J = 1.53 Hz, 2H, $\text{H}_{\text{arom.}}$), 8.35 (t, J = 1.53 Hz, 1H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (DMSO- d_6): δ (ppm) = 129.2, 133.6, 134.1, 134.6 ($\text{C}_{\text{arom.}}$), 166.2 (carbonyl acid).

2.1.2. Synthesis of 5-chloroisophthalic acid dimethyl ester (7a)

A mixture of 5-chloroisophthalic acid (**8a**) (2.05 g, 10 mmol) and concentrated sulfuric acid (0.52 ml) in methanol (40 ml) was refluxed with stirring for 24 h. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in dichloromethane (25 ml) and washed with saturated sodium bicarbonate solution (2 x 15 ml). The organic layer was dried (Na_2SO_4), evaporated under vacuum to give 2.15 g (92 %) of **7a** [17] as a white powder m.p. 76-78 °C which was used in the next step without further purification. IR (neat, cm^{-1}) exhibited bands at 1731, 1582, 1431, 1312, 1248. ^1H NMR (DMSO- d_6): δ (ppm) = 3.90 (s, 6H, 2 x OCH_3), 8.04 (d, J = 1.23 Hz, 2H, $\text{H}_{\text{arom.}}$), 8.24 (t, J = 1.23 Hz, 1H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (DMSO- d_6): δ (ppm) = 53.3 (OCH_3), 128.7, 132.8, 133.7, 134.9 ($\text{C}_{\text{arom.}}$), 164.9 (ester carbonyl).

2.1.3. Synthesis of 5-chloroisophthalic acid monomethyl ester (6a)

1 N NaOH (8.00 ml, 8 mmol) was added to a solution of 5-chloroisophthalic acid dimethyl ester (**7a**) (2.06 g, 9 mmol) in methanol (75 ml). The reaction mixture was stirred at ambient temperature for 18 h. The solvent was removed under reduced pressure, the residue was dissolved in saturated sodium bicarbonate solution (25 ml) and extracted with dichloromethane (2 x 10 ml). The aqueous layer was cooled (5-10 °C), acidified using concentrated hydrochloric acid and extracted with dichloromethane (3 x 20 ml). The combined organic layers were dried (Na_2SO_4), evaporated under vacuum to give 1.49 g (77 %) of **6a** [17] as a white powder m.p. 172-174 °C which was pure enough to be used in the next step. IR (neat, cm^{-1}) exhibited bands at 3085-2539, 1736, 1703, 1602, 1580, 1309, 1262. ^1H NMR (DMSO- d_6): δ (ppm) = 3.91 (s, 3H, OCH_3), 8.04-8.09 (m, 2H, $\text{H}_{\text{arom.}}$), 8.32 (t, J = 1.23 Hz, 1H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (DMSO- d_6): δ (ppm) = 53.6 (OCH_3), 128.9, 132.8, 133.3, 133.9, 134.2, 134.8 ($\text{C}_{\text{arom.}}$), 165.1 (ester carbonyl), 165.9 (carboxylic acid).

2.1.4. Synthesis of 3-chloro-5-hydroxymethylbenzoic acid methyl ester (5a) To a solution of 5-chloroisophthalic acid monomethyl ester (**6a**) (1.07 g, 5 mmol) in tetrahydrofuran (15 ml) was added dropwise borane-dimethyl sulfide complex (1.01 ml, 10.0 mmol). The reaction mixture was stirred at ambient temperature for 30 h. Methanol (20 ml) was carefully added dropwise to the reaction mixture and stirring was continued for further 30 min. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in dichloromethane (15 ml) and washed with saturated sodium bicarbonate solution (2 x 10 ml). The organic layer was dried (Na_2SO_4), evaporated under vacuum to afford 0.87g (87 %) of **5a** [17] as a pale yellow oil which was used in the next step without further purification. IR (neat, cm^{-1}) exhibited bands at 3600-3150, 1719, 1582, 1433, 1285. ^1H NMR (CDCl_3): δ (ppm) = 2.88 (br. s, 1H, OH), 3.69 (s, 3H, OCH_3), 4.46 (s, 2H, CH_2OH), 7.29 (s, 1H, $\text{H}_{\text{arom.}}$), 7.61 (s, 1H, $\text{H}_{\text{arom.}}$), 7.64 (t, J = 1.53 Hz, 1H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (CDCl_3): δ (ppm) = 52.9 (OCH_3), 64.2 (CH_2OH), 126.2, 128.9, 131.5, 132.1, 135.0, 143.7 ($\text{C}_{\text{arom.}}$), 166.4 (ester carbonyl).

2.1.5. Synthesis of 3-bromomethyl-5-chlorobenzoic acid methyl ester (4a)

Phosphorous tribromide (0.87 ml, 9 mmol) was added dropwise to a cold (-30 °C), stirred 3-chloro-5-hydroxymethylbenzoic acid methyl ester (**5a**) (0.60 g, 3 mmol). The reaction mixture was allowed to warm to room temperature and stirred for further 30 min at ambient temperature. The reaction mixture was carefully poured into ice-cold water (20 ml) and extracted with diethyl

ether (3 x 15 ml). The combined ether extracts were dried (Na_2SO_4), evaporated under reduced pressure and the residue was purified by column chromatography using petroleum ether (40-60 °C): diethyl ether (8 : 2) to give 0.47 g (60 %) of **4a** [17] as a white powder m.p. 56-58 °C. IR (neat, cm^{-1}) exhibited bands at 1717, 1578, 1448, 1429, 1291. ^1H NMR (CDCl_3) δ (ppm) = 3.96 (s, 3H, OCH_3), 4.48 (s, 2H, CH_2Br), 7.59 (t, $J = 1.83$ Hz, 1H, $\text{H}_{\text{arom.}}$), 7.96 (d, $J = 1.83$ Hz, 2H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (CDCl_3): δ (ppm) = 31.6 (CH_2Br), 52.9 (OCH_3), 128.7, 129.9, 132.7, 133.7, 135.2, 140.4 ($\text{C}_{\text{arom.}}$), 165.7 (ester carbonyl).

2.1.6. Synthesis of 3-azidomethyl-5-chlorobenzoic acid methyl ester (**3a**)

Sodium azide (1.30 g, 20 mmol) was added to a solution of 3-bromomethyl-5-chlorobenzoic acid methyl ester (**4a**) (0.79 g, 3 mmol) in acetone (15 ml) and water (4 ml). The reaction mixture was refluxed for 18 h. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane (20 ml) and washed with water (3 x 10 ml). The organic layer was dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by column chromatography using petroleum ether (40-60 °C): diethyl ether (8 : 2) to afford 0.58 g (86%) of **3a** as a pale yellow oil. IR (neat, cm^{-1}) exhibited bands at 2098, 1723, 1582, 1433, 1283. ^1H NMR (CDCl_3): δ (ppm) = 3.96 (s, 3H, OCH_3), 4.43 (s, 2H, $-\text{CH}_2-\text{N}=\text{N}$), 7.53 (t, $J = 1.53$ Hz, 1H, $\text{H}_{\text{arom.}}$), 7.89 (s, 1H, $\text{H}_{\text{arom.}}$), 7.99 (t, $J = 1.53$ Hz, 1H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (CDCl_3): δ (ppm) = 52.9 (OCH_3), 54.1 ($-\text{CH}_2-\text{N}=\text{N}$), 127.6, 129.8, 132.6, 132.7, 135.4, 138.3 ($\text{C}_{\text{arom.}}$), 165.8 (ester carbonyl).

2.1.7. Synthesis of 3-azidomethyl-5-chlorobenzoic acid (**2a**)

1 N NaOH (2.00 ml, 2 mmol) was added to a solution of 3-azidomethyl-5-chlorobenzoic acid methyl ester (**3a**) (0.23 g, 1 mmol) in methanol (15 ml). The reaction mixture was refluxed for 2.5 h. The solvent was removed under reduced pressure, the residue was dissolved in saturated sodium bicarbonate solution (10 ml) and extracted with dichloromethane (2 x 5 ml). The aqueous layer was cooled (0-5 °C), acidified with concentrated hydrochloric acid and extracted with dichloromethane (3 x 10 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated under vacuum to give 0.18 g (83%) of **2a** as a pale yellow powder m.p. 92-94 °C and was used in the next step without further purification. IR (neat, cm^{-1}) exhibited bands at 3093-2406, 2097, 1687, 1603, 1583, 1410, 1295. ^1H NMR (CDCl_3): δ (ppm) = 4.47 (s, 2H, $-\text{CH}_2-\text{N}=\text{N}$), 7.59 (t, $J = 1.83$ Hz, 1H, $\text{H}_{\text{arom.}}$), 7.96 (s, 1H, $\text{H}_{\text{arom.}}$), 8.06 (t, $J = 1.83$ Hz, 1H, $\text{H}_{\text{arom.}}$), 11.8 (br.s, 1H, COOH). ^{13}C NMR (CDCl_3): δ (ppm) = 54.0 ($-\text{CH}_2-\text{N}=\text{N}$), 128.1, 130.4, 131.8, 133.6, 135.6, 138.5 ($\text{C}_{\text{arom.}}$), 171.2 (carboxylic acid). $\text{C}_8\text{H}_6\text{ClN}_3\text{O}_2$: calcd. C 45.41, H 2.86, N 19.86; found C 45.33, H 2.95, N 19.46.

2.1.8. Synthesis of 3-aminomethyl-5-chlorobenzoic acid hydrochloride (**1a**)

To a solution of 3-azidomethyl-5-chlorobenzoic acid (**2a**) (0.21 g, 1 mmol) in 95% 2-propanol (10 ml) and concentrated hydrochloric acid (0.50 ml) was added PtO_2 (0.04 g). The mixture was hydrogenated on a Parr shaker apparatus under 4 bar pressure of hydrogen gas for 18 h at ambient temperature. The catalyst was removed by filtration and the solvent was evaporated under vacuum. The residue was dissolved in water (10 ml) and extracted with dichloromethane (2 x 25 ml). The aqueous layer was evaporated under reduced pressure and the residue was recrystallized (2-propanol /diethyl ether) to afford 0.18 g (82 %) of **1a** as a white hygroscopic powder m.p. 260-263 °C. IR (neat, cm^{-1}) exhibited bands at 3137-2887, 1709, 1607, 1581, 1395, 1207. ^1H NMR (D_2O): δ (ppm) = 4.13 (s, 2H, $-\text{CH}_2-\text{NH}_2$), 7.59 (t, $J = 1.83$ Hz, 1H, $\text{H}_{\text{arom.}}$), 7.81 (t, $J = 1.83$ Hz, 2H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (D_2O): δ (ppm) = 42.6 ($-\text{CH}_2-\text{NH}_2$), 128.6, 130.5, 132.9, 133.9, 135.0, 135.2 ($\text{C}_{\text{arom.}}$), 168.9 (carboxylic acid). $\text{C}_8\text{H}_9\text{Cl}_2\text{NO}_2$: calcd. C 43.27, H 4.09, N 6.31; found C 43.49, H 4.23, N 6.28.

2.1.9. Synthesis of (*E*)-3-(4-iodophenyl)acrylic acid methyl ester (**7b**)

To a solution of (methoxycarbonylmethylene)triphenylphosphorane [18] (3.35 g, 10 mmol) in dry dichloromethane (10 ml) was added dropwise a solution of 4-iodobenzaldehyde (**8b**) (2.32 g, 10 mmol) in dichloromethane (5 ml). The resulting pale yellow solution was stirred at room temperature for one hour (NMR monitoring). The reaction mixture was evaporated under vacuum and the residue was purified by column chromatography using petroleum ether (40-60

°C): diethyl ether (9 : 1) to yield 2.68 g (93%) of **7b** [18] as a pale yellow powder m.p. 128-130 °C. IR (neat, cm^{-1}) exhibited bands at 1711, 1636, 1437, 1310, 1189. ^1H NMR (CDCl_3): δ (ppm) = 3.83 (s, 3H, OCH_3), 6.74 (d, $J = 16.18$ Hz, 1H, 2-H), 7.27 (d, $J_{\text{AB}} = 8.53$ Hz, 2H, $\text{H}_{\text{arom.}}$), 7.63 (d, $J = 16.15$ Hz, 1H, 3-H), 7.75 (d, $J_{\text{AB}} = 8.55$ Hz, 2H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (CDCl_3): δ (ppm) = 52.2 (OCH_3), 96.9 ($\text{C}_{\text{arom.}}$), 118.9 (C-2), 129.9, 134.3, 138.5 ($\text{C}_{\text{arom.}}$), 144.0 (C-3), 167.5 (C-1). $\text{C}_{10}\text{H}_9\text{IO}_2$: calcd. C 41.69, H 3.15; found C 41.29, H 3.15.

2.1.10. Synthesis of (RS)-3-(4-iodophenyl)-4-nitrobutanoic acid methyl ester (**6b**)

To a stirred solution of (*E*)-3-(4-iodophenyl)acrylic acid methyl ester (**7b**) (2 g, 7 mmol) in nitromethane (14 ml) was added Triton B (0.6 ml). The resulting pale yellow solution was heated at 85 °C for two hours. The reaction mixture was cooled (0-5 °C), acidified using 1 N hydrochloric acid and evaporated under reduced pressure. The residue was dissolved in diethyl ether (20 ml) and washed with water (2 x 10 ml). The organic layer was dried (Na_2SO_4), evaporated under vacuum and the residue was purified by column chromatography using petroleum ether (40-60 °C): diethyl ether (1 : 1) to give 2.18 g (89%) of **6b** as a white powder m.p. 58-60 °C. IR (neat, cm^{-1}) exhibited bands at 1730, 1721, 1561, 1536, 1374. ^1H NMR (CDCl_3): δ (ppm) = 2.77-2.85 (m, 2H, 2-H), 3.66 (s, 3H, OCH_3), 3.96-4.02 (m, 1H, 3-H), 4.63 (dd, $J_{4a-3} = 8.25$ Hz, $J_{\text{gem}} = 12.83$ Hz, 1H, 4- H_a), 4.74 (dd, $J_{4b-3} = 6.7$ Hz, $J_{\text{gem}} = 12.83$ Hz, 1H, 4- H_b), 7.01 (d, $J_{\text{AB}} = 8.25$ Hz, 2H, $\text{H}_{\text{arom.}}$), 7.69 (d, $J_{\text{AB}} = 8.53$ Hz, 2H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (CDCl_3): δ (ppm) = 37.7 (C-2), 40.1 (C-3), 52.5 (OCH_3), 79.4 (C-4), 94.1, 129.7, 138.4, 138.6 ($\text{C}_{\text{arom.}}$), 171.2 (C-1). $\text{C}_{11}\text{H}_{12}\text{INO}_4$: calcd. C 37.84, H 3.46, N 4.01; found C 37.80, H 3.38, N 3.71.

2.1.11. Synthesis of (RS)-4-(4-iodophenyl)-2-pyrrolidinone (**5b**)

To a cold (0 °C) mixture of concentrated hydrochloric acid (1.5 ml) and methanol (1.5 ml) was added simultaneously in portions both zinc dust (0.46 g, 7 mmol) and a solution of (*RS*)-3-(4-iodophenyl)-4-nitrobutanoic acid methyl ester (**6b**) (0.25 g, 0.7 mmol) in methanol (3 ml) through 30 min. The reaction mixture was further stirred for 30 min at 0 °C. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in water (10 ml), extracted with diethyl ether (3 x 5 ml). The aqueous layer was basified with 6 N sodium hydroxide solution (15 ml), extracted with diethyl ether (3 x 20 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated under vacuum to afford 0.13 g (65%) of **5b** as a white powder m.p. 144-145 °C (lit. [20] 143-145 °C) which was pure enough to be used in the next step without further purification. IR (neat, cm^{-1}) exhibited bands at 3181, 1703, 1477, 1285, 1267. ^1H NMR (CDCl_3): δ (ppm) = 2.28 (dd, $J_{3a-4} = 8.55$ Hz, $J_{\text{gem}} = 16.78$ Hz, 1H, 3- H_a), 2.77 (dd, $J_{3b-4} = 8.85$ Hz, $J_{\text{gem}} = 16.78$ Hz, 1H, 3- H_b), 3.41 (dd, $J_{5a-4} = 7$ Hz, $J_{\text{gem}} = 9.15$ Hz, 1H, 5- H_a), 3.66 (quin, $J_{4-3a} = J_{4-3b} = J_{4-5a} = J_{4-5b} = 8.23$ Hz, 1H, 4-H), 3.82 (m, 1H, 5- H_b), 7.03 (d, $J_{\text{AB}} = 8.23$ Hz, 2H, $\text{H}_{\text{arom.}}$), 7.09 (br.s, 1H, N-H), 7.68 (d, $J_{\text{AB}} = 8.23$ Hz, 2H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (CDCl_3): δ (ppm) = 38.3 (C-3), 40.2 (C-4), 49.9 (C-5), 92.7, 129.2, 138.3, 142.2 ($\text{C}_{\text{arom.}}$), 178.1 (lactam).

2.1.12. Synthesis of (RS)-1-tert-butylloxycarbonyl-4-(4-iodophenyl)-2-pyrrolidinone (**4b**)

To a stirred solution of (*RS*)-4-(4-iodophenyl)-2-pyrrolidinone (**5b**) (0.23 g, 0.8 mmol), *tert*-butyl dicarbonate (0.35 g, 1.6 mmol) and 4-(dimethylamino)pyridine (0.1 g, 0.8 mmol) in dichloromethane (15 ml) was added triethylamine (0.08 g, 0.11 ml, 0.8 mmol) under nitrogen atmosphere. The resulting solution was stirred for 30 h at ambient temperature. The volatiles were removed under reduced pressure, the residue was dissolved in diethyl ether (15 ml), washed with 1 N hydrochloric acid (2 x 5 ml), washed with water (2 x 5 ml), dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by column chromatography using petroleum ether (40-60 °C): diethyl ether (1 : 1) to yield 0.26 g (84%) of **4b** as a white powder m.p. 100-102 °C. IR (neat, cm^{-1}) exhibited bands at 1774, 1696, 1369, 1343, 1288. ^1H NMR (CDCl_3): δ (ppm) = 1.31 (s, 9H, *t*-Bu.), 2.43 (dd, $J_{3a-4} = 9.45$ Hz, $J_{\text{gem}} = 17.08$ Hz, 1H, 3- H_a), 2.67 (dd, $J_{3b-4} = 8.25$ Hz, $J_{\text{gem}} = 17.08$ Hz, 1H, 3- H_b), 3.27 (quin, $J_{4-3a} = J_{4-3b} = J_{4-5a} = J_{4-5b} = 8.55$ Hz, 1H, 4-H), 3.43 (dd, $J_{5a-4} = 8.23$ Hz, $J_{\text{gem}} = 10.68$ Hz, 1H, 5- H_a), 3.93 (dd, $J_{5b-4} = 7.95$ Hz, $J_{\text{gem}} = 10.68$ Hz, 1H, 5- H_b), 6.78 (d, $J_{\text{AB}} = 8.25$ Hz, 2H, $\text{H}_{\text{arom.}}$), 7.46 (d, $J_{\text{AB}} = 8.25$ Hz, 2H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (CDCl_3): δ (ppm) = 28.4 ($\text{OC}(\text{CH}_3)_3$), 36.4 (C-4), 40.5 (C-3), 53.2 (C-5), 83.6 ($\text{OC}(\text{CH}_3)_3$), 93.1, 129.2, 138.4, 140.8

(C_{arom.}), 150.2 (urethane), 172.9 (lactam). C₁₅H₁₈INO₃: calcd. C 46.53, H 4.69, N 3.62; found C 46.72, H 4.69, N 3.24.

2.1.13. Synthesis of (RS)-1-tert-butyloxycarbonyl-4-(4-trimethylsilanylethynylphenyl)-2-pyrrolidinone (3b)

To a stirred suspension of (RS)-1-tert-butyloxycarbonyl-4-(4-iodophenyl)-2-pyrrolidinone (**4b**) (0.78 g, 2 mmol), bis (triphenylphosphine)palladium dichloride (0.014 g, 0.02 mmol) and cuprous iodide (0.008 g, 0.04 mmol) in triethylamine (20 ml) was added trimethylsilylacetylene (0.24 g, 0.34 ml, 2.4 mmol) under nitrogen atmosphere. The yellow reaction mixture was stirred at ambient temperature for 12 h. The black reaction mixture was filtered and washed with diethyl ether (10 ml). The filtrate was evaporated under reduced pressure, the residue was dissolved in diethyl ether (20 ml), washed with water (3 x 10 ml), dried (Na₂SO₄) and evaporated under reduced pressure. The residue was purified by column chromatography using petroleum ether (40-60 °C): diethyl ether (1 : 1) to afford 0.64 (90%) of **3b** as a pale yellow powder m.p. 118-120 °C. IR (neat, cm⁻¹) exhibited bands at 1790, 1690, 1352, 1293, 1156. ¹H NMR (CDCl₃): δ (ppm) = 0.01 (s, 9H, Si(CH₃)₃), 1.30 (s, 9H, *t*-Bu.), 2.44 (dd, *J*_{3a-4} = 9.45 Hz, *J*_{gem} = 17.38 Hz, 1H, 3-H_a), 2.66 (dd, *J*_{3b-4} = 8.25 Hz, *J*_{gem} = 17.38 Hz, 1H, 3-H_b), 3.29 (quin, *J*_{4-3a} = *J*_{4-3b} = *J*_{4-5a} = *J*_{4-5b} = 8.53 Hz, 1H, 4-H), 3.43 (dd, *J*_{5a-4} = 8.55 Hz, *J*_{gem} = 10.68 Hz, 1H, 5- H_a), 3.92 (dd, *J*_{5b-4} = 7.93 Hz, *J*_{gem} = 10.68 Hz, 1H, 5- H_b), 6.94 (d, *J*_{AB} = 8.23 Hz, 2H, H_{arom.}), 7.22 (d, *J*_{AB} = 8.25 Hz, 2H, H_{arom.}). ¹³C NMR (CDCl₃): δ (ppm) = 0.00 (Si(CH₃)₃), 28.1 (OC(CH₃)₃), 36.3 (C-4), 40.1 (C-3), 52.9 (C-5), 83.2 (OC(CH₃)₃), 94.8, 104.5 (C≡C), 122.4, 126.7, 132.6, 141.0 (C_{arom.}), 149.9 (urethane), 172.7 (lactam). C₂₀H₂₇SINO₃: calcd. C 67.19, H 7.61, N 3.92; found C 67.48, H 7.69, N 3.77.

2.1.14. Synthesis of (RS)-4-amino-3-(4-ethynylphenyl)butanoic acid hydrochloride (1b)

To a stirred solution of (RS)-1-tert-butyloxycarbonyl-4-(4-trimethylsilanylethynylphenyl)-2-pyrrolidinone (**3b**) (0.25 g, 0.7 mmol) in tetrahydrofuran (9 ml) was added 1 M lithium hydroxide (3 ml). The resulting reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure, the residue was diluted with water (10 ml) and extracted with diethyl ether (3 x 5 ml). The aqueous layer was acidified using 0.5 M potassium hydrogen sulfate solution and extracted with diethyl ether (3 x 15 ml). The organic extracts were dried (Na₂SO₄) and evaporated under vacuum to give 0.19 g (90%) of (RS)-4-tert-butyloxycarbonylamino-3-(4-ethynylphenyl)butanoic acid (**2b**) as a yellow powder m.p. 126-128 °C. The crude **2b** was dissolved in ~2.5 M dry hydrogen chloride/ethyl acetate solution (1.6 ml) and the reaction mixture was stirred at room temperature for one hour. The reaction mixture was filtered and washed with diethyl ether (5 ml). The precipitated solid was recrystallized from 2-propanol/diethyl ether to yield 0.13 g (87%) of **1b** as a white powder m.p. 209-211 °C. IR (neat, cm⁻¹) exhibited bands at 3244-2654, 1724, 1520, 1185, 823. ¹H NMR (D₂O): δ (ppm) = 2.66 (dd, *J*_{2a-3} = 8.53 Hz, *J*_{gem} = 16.18 Hz, 1H, 2-H_a), 2.79 (dd, *J*_{2b-3} = 5.80 Hz, *J*_{gem} = 16.18 Hz, 1H, 2-H_b), 3.12-3.41 (m, 3H, 3-H and 4-H), 3.45 (s, 1H, C≡CH), 7.28 (d, *J*_{AB} = 8.23 Hz, 2H, H_{arom.}), 7.48 (d, *J*_{AB} = 8.25 Hz, 2H, H_{arom.}). ¹³C NMR (D₂O): δ (ppm) = 38.8 (C-2), 40.2 (C-3), 43.9 (C-4), 79.1 (C≡CH), 83.8 (C≡CH), 121.7, 128.5, 133.3, 139.9 (C_{arom.}), 175.7 (C-1). C₁₂H₁₄ClNO₂: calcd. C 60.13, H 5.89, N 5.84; found C 59.78, H 5.90 N 5.55.

2.1.15. Synthesis of (RS)-4-amino-3-(4-iodophenyl)butanoic acid hydrochloride (1c)

To a stirred solution of (RS)-1-tert-butyloxycarbonyl-4-(4-iodophenyl)-2-pyrrolidinone (**4b**) (0.25 g, 0.7 mmol) in tetrahydrofuran (6 ml) was added 1 M lithium hydroxide (2 ml). The resulting reaction mixture was stirred at ambient temperature for two hours. The solvent was evaporated under reduced pressure, the residue was diluted with water (10 ml) and extracted with diethyl ether (3 x 5 ml). The aqueous layer was acidified using 0.5 M potassium hydrogen sulfate solution, extracted with diethyl ether (3 x 15 ml). The organic extracts were dried (Na₂SO₄), and evaporated under vacuum to yield 0.23 g (81%) of (RS)-4-tert-butyloxycarbonylamino-3-(4-iodophenyl)butanoic acid (**2c**) as a pale yellow powder m.p.146-148 °C. The crude **2c** was dissolved in ~2.5 M dry hydrogen chloride/ethyl acetate solution (1.5 ml) and the reaction mixture was stirred at ambient temperature for one hour. The reaction mixture was filtered, washed with diethyl ether (5 ml). The precipitated solid was recrystallized from 2-propanol/diethyl ether to give

0.16 g, (83%) of **1c** as a white powder m.p. 208-210 °C (lit. [20] 190-195 °C). IR (neat, cm^{-1}) exhibited bands at 3154-2720, 1719, 1587, 1520, 1414, 1194. ^1H NMR (D_2O): δ (ppm) = 2.63 (dd, $J_{2a-3} = 8.55$ Hz, $J_{\text{gem}} = 16.18$ Hz, 1H, 2- H_a), 2.76 (dd, $J_{2b-3} = 5.78$ Hz, $J_{\text{gem}} = 16.18$ Hz, 1H, 2- H_b), 3.08-3.18 (m, 1H, 4- H_a), 3.24-3.36 (m, 2H, 3-H and 4- H_b), 7.04 (d, $J_{\text{AB}} = 8.55$ Hz, 2H, $\text{H}_{\text{arom.}}$), 7.68 (d, $J_{\text{AB}} = 8.23$ Hz, 2H, $\text{H}_{\text{arom.}}$). ^{13}C NMR (D_2O): δ (ppm) = 38.4 (C-2), 39.9 (C-3), 43.9 (C-4), 93.7, 130.3, 138.5, 138.7 ($\text{C}_{\text{arom.}}$), 175.4 (C-1). $\text{C}_{10}\text{H}_{13}\text{ClINO}_2$: calcd. C 35.16, H 3.84, N 4.10; found C 35.52, H 3.79 N 4.00.

2.2. Pharmacological evaluation

2.2.1. Materials

GABA was obtained from Sigma (St. Louis, MO, USA). Culture media, serum and antibiotics were obtained from Invitrogen (Paisley, UK). The rat GABA_BR plasmids and the Gαq-z5 construct were generous gifts from Dr. Janet Clark (National Institute of Health, Bethesda, MD, USA) and Dr. Bruce Conklin (University of California, San Francisco, CA, USA). The tsA cells were a generous gift from Dr. Penelope S. V. Jones (University of California, San Diego, CA, USA).

2.2.2. Methods

TsA cells (a transformed human embryonic kidney (HEK) 293 cell line) [21] were maintained at 37 °C in a humidified 5% CO_2 incubator in Dulbecco's modified Eagle medium (DMEM) supplemented with penicillin (100U/ml), streptomycin (100mg/ml) and 10% fetal calf serum. One million cells were split into a 10 cm tissue culture plate and transfected the following day with 0.7 μg GABA_BR1b-pcDNA3.1, 3.5 μg GABA_BR2-pcDNA3.1 and 0.7 μg Gαq-z5-pcDNA using SuperFect as a DNA carrier according to the protocol by the manufacturer (Qiagen, Hilden, Germany). The day after transfection, cells were split into one poly-D-lysine coated 96-well black-walled-clear-bottomed tissue culture plates in the same medium as mentioned above and incubated overnight. The following day the measurement of intracellular calcium was performed as follows. The media was exchanged with Hanks balanced saline solution containing 1 mM CaCl_2 , 1 mM MgCl_2 , 20 mM HEPES, 2.5 mM probenidol and 4 μM Fluo-4AM (pH = 7.4). The cells were incubated for 1 h at 37 °C in a humidified 5% CO_2 incubator. Cells were then washed twice with the same buffer without Fluo-4AM and finally 100 μl of the buffer was left in the wells. The cell plate was then transferred to the NovoStar (BMG Labtechnologies, Offenburg, Germany) and the basal fluorescence level was adjusted to ~ 10000 fluorescence units (FU) using excitation/emission wavelengths of 485-520 nm, respectively. Fluorescence readings were measured for 45 s after addition of ligand and response was calculated as peak response minus basal level. Inactive compounds were also tested as antagonists. Twenty min after application of ligand, 10 μM GABA was added to the well and fluorescence was measured as above. All experiments were performed in triplicate and the results are given as mean \pm S.E.M of 3-4 experiments.

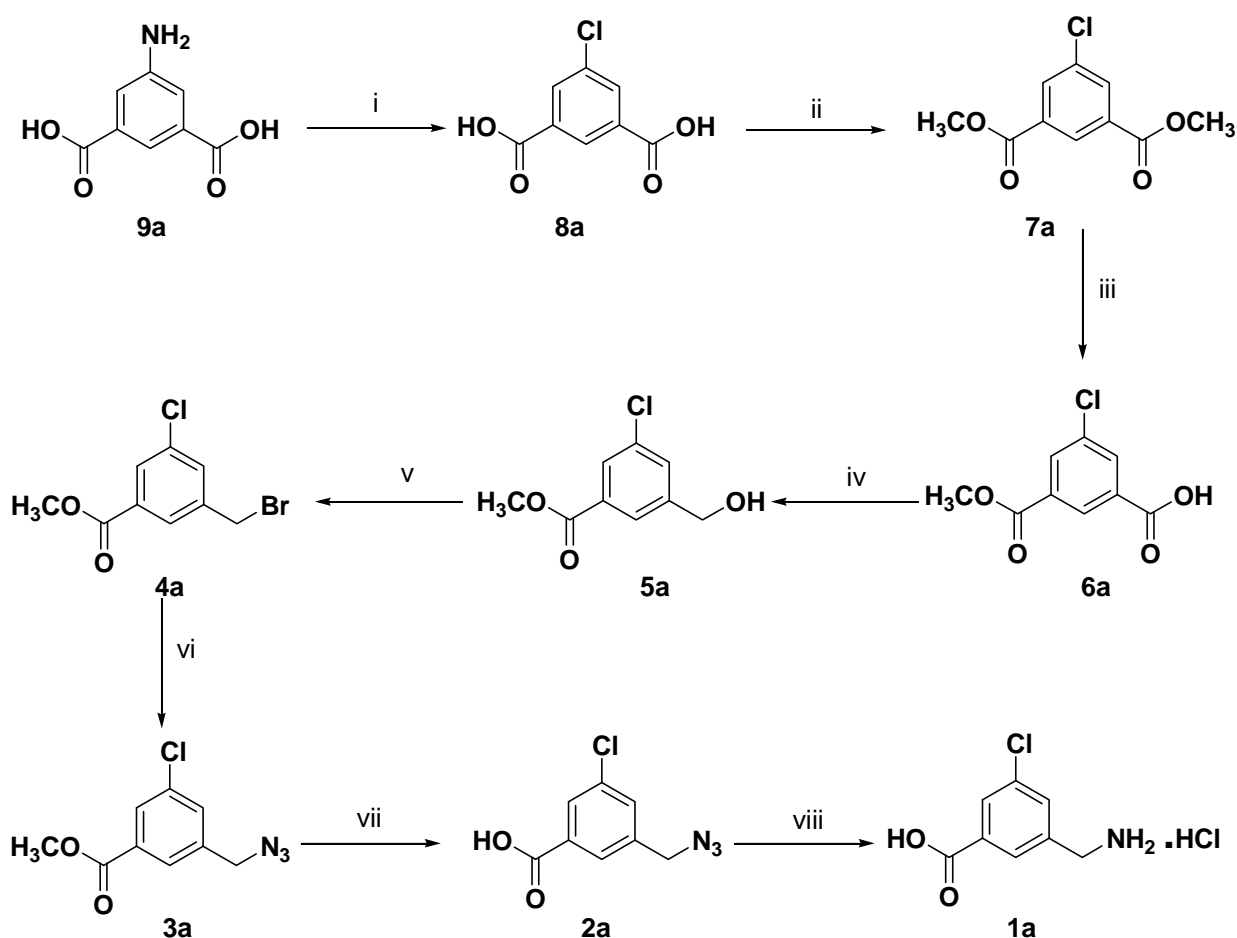
3. Results and discussion

3.1. Chemistry

The developed synthetic pathway for the preparation of the title compound **1a** is illustrated in Scheme 1. The pathway was commenced by diazodization of 5-aminoisophthalic acid (**9a**) via adopting the procedure mentioned by Gunstone and Tucker [22]. The produced crude 5-chloroisophthalic acid (**8a**) was subjected to esterification using the procedure described by Dimick *et al.* [23] in methanol and a catalytical amount of concentrated sulfuric acid to yield diester **7a**. Subsequent saponification of **7a** using one mole of 1 N sodium hydroxide solution

afforded monoester 5-chloroisophthalic acid monomethyl ester (**6a**) which was subjected to selective reduction of carboxylic acid functionality in the presence of ester functionality using borane-dimethyl sulfide complex to afford the alcohol **5a**. Elaboration of the alcohol functionality

of **5a** to the corresponding bromo derivative **4a** was accomplished by addition of phosphorous tribromide to **5a** in solvent-free medium according to the procedure mentioned by Amrollah-Madjdabadi *et al.* [24] Compound **4a** was allowed to react with sodium azide in refluxing acetone to give compound **3a** which yielded the benzoic acid derivative **2a** upon reflux in methanolic sodium hydroxide solution. Catalytic hydrogenation of the azide functionality of **2a** in 95% 2-propanol and concentrated hydrochloric acid in the presence of PtO₂ gave the target compound 3-aminomethyl-5-chlorobenzoic acid hydrochloride (**1a**). The crude **1a** was recrystallized from 2-propanol/diethyl ether mixture to afford **1a** in 82% yield as a white hygroscopic powder. The structure of **1a** has been established through, IR, ¹H NMR, ¹³C NMR, and microanalytical data.

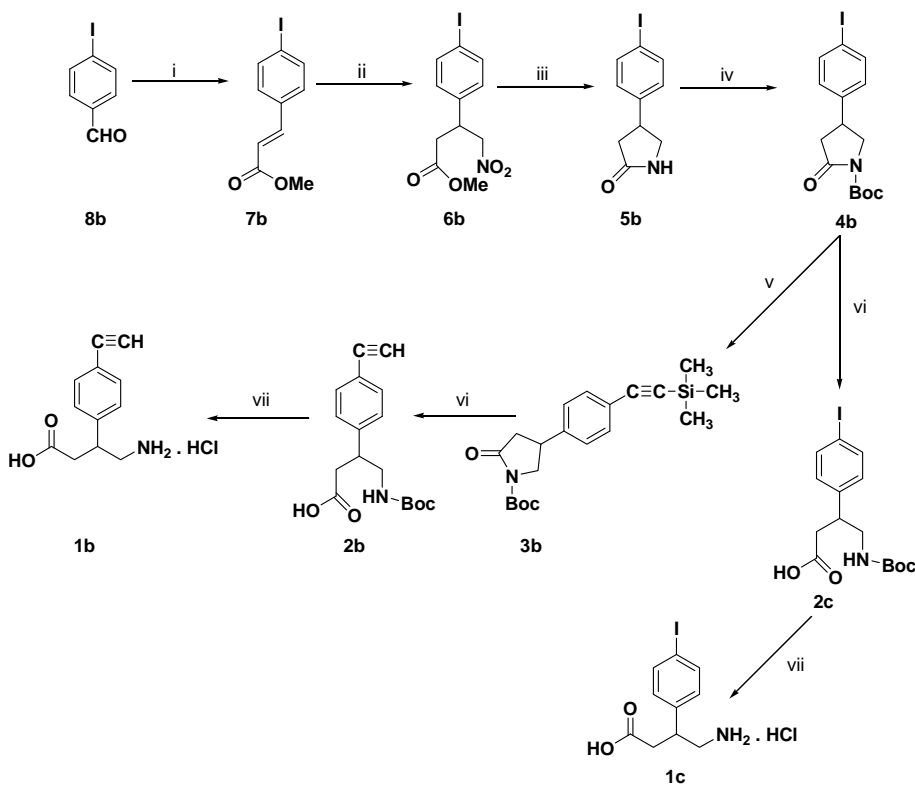


Scheme 1: Synthesis of the target compound **1a**.

Reagents and conditions: i) 1) NaNO₂ / H₂SO₄ / 70 °C 2) CH₃COOH / 40 °C / 0.5h 3) CuCl / conc. HCl / 80 °C / 20 min; ii) CH₃OH/H₂SO₄/reflux /24h; iii) 1 N NaOH/CH₃OH/25 °C/18h; iv) Borane-dimethyl sulfide complex/ THF/25 °C /30h; v) PBr₃/-30 ° /0.5h; vi) NaN₃/acetone/H₂O/reflux/18h; vii) 1N NaOH/CH₃OH/ reflux/2.5h; viii) H₂ / PtO₂ / 40 Psi / 95% 2-propanol /25 °C / 18h.

An examination of the literature exposed that the electronegativity of the ethynyl functionality is similar to that of the chloro functionality. In addition, the ethynyl group would be able to act as a hydrogen bond acceptor or donor in the receptor active site [25]. Accordingly, we are convinced to synthesize the *para* ethynyl analogue of baclofen, namely, (*RS*)-4-amino-3-(4-ethynylphenyl)butanoic acid hydrochloride (**1b**), as depicted in Scheme 2. Thus, (*E*)-3-(4-iodophenyl)acrylic acid methyl ester (**7b**) has been successfully achieved by adopting the general trivial procedure of Wittig reaction [26] using 4-iodobenzaldehyde (**8b**) and (methoxycarbonyl-

methylene)triphenylphosphorane [19]. Condensation of nitromethane with compound **7b** in the presence of benzyltrimethylammonium hydroxide (Triton B, ~40% solution in methanol) was done in methanol according to the procedure described by Berthelot and his co-workers [27] to furnish the nitro ester **6b**. Selective reduction of the nitro functionality of **6b** without dehalogenation of the aromatic halogen has been established by using zinc dust in methanol/concentrated hydrochloric acid mixture at 0°C for 30 min followed by treatment of the produced amino ester with 6 N NaOH solution to give the pivotal lactam (*RS*)-4-(4-iodophenyl)-2-pyrrolidinone (**5b**) in 65% yield. Compound **5b** is the key intermediate in the synthetic strategy employed to achieve our targets **1b** and **1c**. Protection of the amide nitrogen of **5b** with *tert*-butyloxycarbonyl group has been successfully achieved *via* reaction of **5b** with *tert*-butyldicarbonate in dichloromethane in the presence of triethylamine and 4-(dimethylamino)pyridine (DMAP) to give **4b**. Subsequently, Sonogashira-Hagihara coupling [28] was conducted *via* addition of trimethylsilylacetylene (TMSA) to a suspension of **4b** in triethylamine in the presence of a catalytical amount of bis (triphenylphosphine)palladium dichloride and cuprous iodide to produce (*RS*)-1-*tert*-butyloxycarbonyl-4-(4-trimethylsilylanylethynylphenyl)-2-pyrrolidinone (**3b**). Compound **3b** was subjected to cleavage of trimethylsilyl group and lactam ring opening under mild basic conditions. The presence of *tert*-butyloxycarbonyl as a lactam nitrogen protecting group facilitated the lactam ring opening under mild basic conditions. Thus, 1 M lithium hydroxide solution was added to a solution of **3b** in tetrahydrofuran and the reaction mixture was stirred at room temperature for 18h to afford (*RS*)-4-*tert*-butyloxycarbonylamino-3-(4-ethynylphenyl)butanoic acid (**2b**) in 90% yield. Finally, *N*-boc protecting group in compound **2b** has been cleanly removed by stirring a solution of **2b** in ~2.5 M dry hydrogen chloride/ethyl acetate at room temperature for one hour to yield crude (*RS*)-4-amino-3-(4-ethynylphenyl)butanoic acid hydrochloride (**1b**) which was recrystallized from 2-propanol/diethyl ether mixture to furnish the title compound **1b** as a colourless crystals in 87% yield. The structure of **1b** has been established through IR, ¹H NMR, ¹³C NMR, and microanalytical data.



Scheme 2: Synthesis of the target compounds **1b** and **1c**.

Reagents and conditions: i) $\text{Ph}_3\text{P}=\text{CHCO}_2\text{CH}_3/\text{dry CH}_2\text{Cl}_2/25\text{ }^\circ\text{C}/1\text{h}$; ii) $\text{CH}_3\text{NO}_2/\text{Triton B}/85\text{ }^\circ\text{C}/2\text{h}$; iii) 1) $\text{CH}_3\text{OH}/\text{HCl}/\text{Zn}/0\text{ }^\circ\text{C}/0.5\text{h}$ 2) 6N NaOH ; iv) $\text{Boc}_2\text{O}/\text{DMAP}/\text{TEA}/\text{CH}_2\text{Cl}_2/25\text{ }^\circ\text{C}/30\text{h}$; v) $\text{PdCl}_2(\text{PPh}_3)_2/\text{CuI}/\text{TMSA}/\text{TEA}/25\text{ }^\circ\text{C}/12\text{h}$; vi) $1\text{M LiOH}/25\text{ }^\circ\text{C}/18\text{h}$; vii) $2.5\text{M dry HCl}/\text{ethyl acetate}/25\text{ }^\circ\text{C}/1\text{h}$.

(*RS*)-4-Amino-3-(4-iodophenyl)butanoic acid hydrochloride (**1c**) is a baclofen analogue containing the iodo functionality as a bioisostere for the chloro functionality in the *para* position of baclofen. Compound **1c** has been successfully achieved from the lactam **4b** by lactam ring opening and deprotection of the *N*-*boc* functionality as illustrated in Scheme 2. The structure of **1c** has been established through IR, ^1H NMR, ^{13}C NMR, and microanalytical data.

3.2. GABA_B receptor agonistic activity

The synthesized compounds **1a-c** were evaluated for their GABA_BR agonist activity on tsA cells transfected with GABA_{B1b}/GABA_{B2}/Gαq-z5 based on Ca^{2+} measurement rather than measurement of inositol phosphate generation as previously published [29].

The GABA_B agonist activity of the synthesized compounds **1a-c** is summarized in Table 1. Compounds **1b** and **1c** are active as GABA_BR agonists (EC_{50} value = 240 and 32 μM , respectively, whereas compound **1a** ($\text{EC}_{50} > 300\text{ } \mu\text{M}$) is considered inactive as GABA_BR agonist in the GABA_BR subtype used in our assay. Compound **1a** was evaluated as GABA_BR antagonist at 1mM concentration against 10 μM GABA, however, it was not effective as GABA_BR antagonist.

Table 1: GABA_BR agonist of compounds 1a-c, GABA, and (*RS*)-baclofen

Compound No.	EC_{50} (μM)	$\text{pEC}_{50} \pm \text{S.E.M}$
1a	>300	<3.52
1b	240	3.62 ± 0.1
1c	32	4.49 ± 0.1
GABA	0.84	6.08 ± 0.1
(<i>RS</i>)-baclofen	5.8	5.24 ± 0.1

4. Conclusion

Synthesis and GABA_B receptor agonist activity of certain amino acids structurally related to the clinically used drug, baclofen (**2**), are reported. Inclusion of chlorophenyl moiety into DAVA backbone gave compound **1a** which was devoid of both agonist and antagonist activities on GABA_B receptors. Replacement of *para* chloro substituent of baclofen (**2**) with ethynyl group led to compound **1b** which exhibited GABA_B receptor agonist activity with $\text{EC}_{50} = 240\text{ } \mu\text{M}$. The iodo analogue of baclofen, compound **1c**, emerged as the best GABA_B receptor agonist congener in the synthesized compounds with $\text{ED}_{50} = 32\text{ } \mu\text{M}$.

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