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A novel $GABA_A$ alpha 5 receptor inhibitor with therapeutic potential ☆

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ABSTRACT

Novel 2,3-benzodiazepine and related isoquinoline derivatives, substituted at position 1 with a 2-benzothiophenyl moiety, were synthesized to produce compounds that potently inhibited the action of GABA on heterologously expressed $GABA_A$ receptors containing the alpha 5 subunit ($GABA_A$ α_5), with no apparent affinity for the benzodiazepine site. Substitutions of the benzothiophene moiety at position 4 led to compounds with drug-like properties that were putative inhibitors of extra-synaptic $GABA_A$ α_5 receptors and had substantial blood-brain barrier permeability. Initial characterization in vivo showed that 8-methyl-5-[4-(trifluoromethyl)-1-benzothiophen-2-yl]-1,9-dihydro-2*H*-[1,3]oxazolo[4,5-*h*][2,3] benzodiazepin-2-one was devoid of sedative, pro-convulsive or motor side-effects, and enhanced the performance of rats in the object recognition test. In summary, we have discovered a first-in-class GABA-site inhibitor of extra-synaptic $GABA_A$ α_5 receptors that has promising drug-like properties and warrants further development.

1. Introduction

Gamma-aminobutyric acid (GABA) is an important neurotransmitter in the CNS the biological actions of which are mediated by ionotropic ($GABA_A$) and metabotropic ($GABA_B$) cell-surface receptors. The $GABA_A$ receptor is a Cys-loop ligand-gated ion-channel that consists of five membrane-spanning protein subunits lining a central pore that is highly selective for Cl^- (Barnard et al., 1988; Miller and Smart, 2010; Olsen and Sieghart, 2008; Rudolph and Möhler, 2014). The predominant subunit configuration of the neuronal $GABA_A$ receptor is $2\alpha+2\beta+1\gamma$. Whilst all three subunits are encoded by distinct gene subfamilies, it is thought that the functional receptor isoforms consist of identical α and β subunits (McKernan and Whiting, 1996). The $GABA_A$ receptor is the target of several drugs of clinical significance, most notably hypnotic, sedative, anesthetic, anti-convulsive and anxiolytic agents. The best-known anxiolytic agent is diazepam and its more modern versions all targeting the allosteric benzodiazepine (BDZ) modulatory site of the receptor (Miller and Smart, 2010; Olsen and Sieghart, 2008). It is the widespread use and abuse of diazepam-like anxiolytics that prompted intensive research on their target of action (Atack, 2011). The distribution of $GABA_A$ receptor isoforms in the brain indicated non-redundant physiological roles (Pirker et al., 2000; Wisden et al., 1992). Indeed, by systematic analysis of the effects of diazepam in strains of mice in which one of the 4 diazepam sensitive genes (α1-3, 5) was rendered unresponsive to the drug, (Low et al., 2000; McKernan et al., 2000) it was possible to dissect the relative contributions of $GABA_A$ receptor isoforms to the pharmacologic profile

Abbreviations:: BDZ, benzodiazepine; BW, body weight; HEK293, human embryonic kidney cells; SPRD, Sprague-Dawley

☆We would like to dedicate this paper to the memory of our friend and colleague, Dr Péter Kiricsi.

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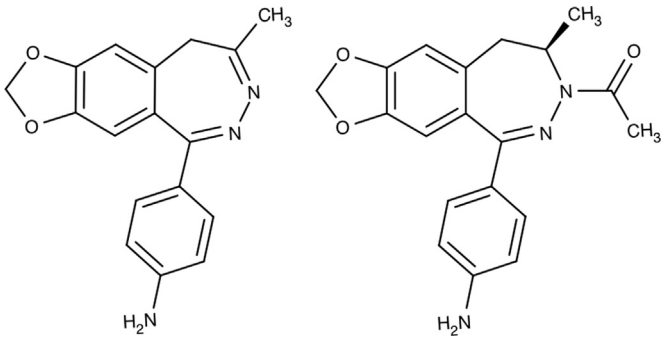

Fig. 1. The 2,3-benzodiazepine based structures of the non-competitive AMPA antagonists GYKI 52466 (left) and talampanel (GYKI-53773) (Ábrahám et al., 2000).

of diazepam (Rudolph and Möhler, 2014). Thus, the case for producing isoform-selective $GABA_A$-ergic drugs devoid of unwanted side-effects such as sedation, pharmaco-dependence, muscle weakness, ataxia, and amnesia in the case of agonists, anxiety, convulsions and insomnia in the case of inhibitors had a sound scientific rationale (Atack, 2011; Skolnick, 2012). However, more than two decades of effort to produce selective and clinically viable BDZ-site ligands has yielded a single compound, RG1662 (Knust et al., 2009) that is still in clinical trials, while all others have failed. The reasons for failure were diverse and have been reviewed extensively (Atack, 2010, 2011; Skolnick, 2012).

Our previous efforts included the synthesis of tricyclic 2,3-benzodiazepine inhibitors of AMPA receptors (Fig. 1) (Gigler et al., 2007; Kapus et al., 2008). In order to obtain structures of enhanced metabolic stability and efficacy with reduced toxicity (Kumagai et al., 1994), the methylenedioxy substituent was modified into an oxazolone moiety. Moreover, the 4-aminophenyl substituent at position 1 of the 2,3-BDZ scaffold was replaced by a benzothiophen-2-yl group. Surprisingly, the novel compounds had weak anxiolytic activity in rats and were found to modulate $GABA_A$ receptors recombinantly expressed in *Xenopus* oocytes (not shown). This prompted the screening of a small, focused library of *cca.* 400 compounds (Ling et al., 2012) by the voltage-sensitive FLIPR-dye assay, which eventually led to the discovery of an inhibitor of $GABA_A$ α_5 receptors that has promising drug-like properties and is described in the present paper.

2. Materials and methods

2.1. Chemistry

All melting points were determined on a Leica Gallen microscopic hot stage melting point apparatus and are uncorrected. IR spectra were obtained on a Bruker Vector 22FT-IR spectrometer in KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker Avance III 400 (400 and 100 MHz for ^{1}H and ^{13}C NMR spectra, respectively) or Varian Inova 500 (500 and 125 MHz for ^{1}H and ^{13}C NMR spectra, respectively) or Varian Mercury Plus 200 (200 and 50 MHz for ^{1}H and ^{13}C NMR spectra, respectively) spectrometer using TMS as internal standard. Chemical shifts (δ) and coupling constants (J) are given in ppm and in Hz, respectively. Elemental analysis was performed on a Perkin-Elmer 2400 analyzer. All reactions were followed by analytical thin layer chromatography (TLC) on silica gel 60 F_{254}.

5-(2-Oxopropyl)-1,3-benzoxazol-2(3*H*)-one (5). To a solution of 1-(3-amino-4-hydroxyphenyl)propan-2-one (**4**) hydrochloride (50 g, 250 mmol) in tetrahydrofuran (500 ml) was added 1,1′-carbonyldiimidazole (CDI, 48.25 g, 290 mmol) and the mixture was refluxed for 2 h. After cooling to room temperature the precipitate was filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in EtOAc (400 ml), the solution was washed with a 5 w/w% HCl aq. solution (2 × 200 ml) and brine (2 × 200 ml), the organic layer was concentrated in vacuo until crystallization started. The crystals were separated by filtration to give the title compound (29.1 g, 63%) as a colorless solid, mp 115–116 °C. IR (KBr): 3175, 1769, 1703, 1470, 1267, 936 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.55 (br s, 1H), 7.19 (d, J=8.1 Hz, 1H), 6.91 (d, J=1.4 Hz, 1H), 6.88 (dd, J=1.7, 8.2 Hz, 1H), 3.78 (s, 2H), 2.13 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 206.08, 154.66, 142.26, 130.82, 130.44, 123.16, 111.03, 109.27, 49.31, 29.55. $C_{10}H_9NO_3$ (191.19): calcd. C 62.82, H 4.74, N 7.33; found C 63.14, H 4.79, N 7.22.

5-(2-Hydroxypropyl)-1,3-benzoxazol-2(3*H*)-one (6). To a solution of 5-(2-oxopropyl)-1,3-benzoxazol-2(3*H*)-one (**5**, 35.2 g, 0.18 mol) in EtOAc (350 ml) and water (120 ml) was added sodium borohydride (21 g, 0.55 mol) in portions over a period of 20 min with ice cooling, and the reaction mixture was stirred for 1 h. Then the mixture was acidified to pH=2 with a 10 w/w% HCl aq. solution and after separation of the layers, the aqueous layer was further extracted with EtOAc (3 × 90 ml). The organic layers were combined, dried and concentrated to a dense suspension. Diisopropyl ether was added to the suspension, and the crystals were separated by filtration, followed by recrystallization from water–MeOH (7:3, 160 ml) to give the title compound as a white solid (25.8 g, 73%), mp 133–134 °C. IR (KBr): 3251, 1784, 1740, 1499, 1260 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.13 (d, J=8.1 Hz, 1H), 6.92 (d, J=1.4 Hz, 1H), 6.87 (dd, J=1.6, 8.1 Hz, 1H), 3.79 (m, 1H), 2.66 (m, 1H), 2.59 (m, 1H), 1.03 (d, J=6.1Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 154.75, 141.79, 135.57, 130.16, 122.79, 110.67, 108.95, 67.40, 45.13, 23.24. $C_{10}H_{11}NO_3$ (193.20): calcd. C 62.17, H 5.74, N 7.25; found C 62.29, H 5.87, N 7.10.

5-(1-Benzothiophen-2-yl)-7-methyl-2-oxo-1,2-dihydroisochromeno[6,7-*d*][1,3]oxazol-6-ium perchlorate (3a). To a suspension of 5-(2-hydroxypropyl)-1,3-benzoxazol-2(3*H*)-one (**6**, 3.08 g, 15.9 mmol) and 1-benzothiophen-2-carbaldehyde (**8a**, 2.58 g, 15.9 mmol) in EtOAc (30 ml) was added a HCl solution in EtOAc (15%, 15 ml) and the reaction mixture was stirred for 20 h at room temperature. After ca. 1.5 h the solid materials were dissolved and after ca. 3 h crystals precipitated. The white crystals were collected by filtration, washed with EtOAc and dried to give a mixture of *cis*- and *trans*-isochromane **7a**. To a solution of this product in acetone (77 ml) was added dropwise Jones reagent [12.38 ml, 33.43 mmol of chromium (VI) oxide] at 0–10 °C over a period of 40 min. The mixture was stirred at ambient temperature until the reaction was complete (followed by TLC) and it was then poured into ice-cold water (200 ml). The precipitate was filtered off, washed with water (5 × 15 ml) and dried. The crude diketone **9a** thus obtained was suspended in EtOAc (58 ml) and 70 w/w% aqueous perchloric acid solution (0.82 ml, 9.47 mmol) was added under reflux. The reflux was maintained for further 10 min under vigorous stirring. After cooling to ambient temperature, the crude product was isolated by filtration, to give **3a** (2.75 g, 40%) as deep-orange crystals, mp 289–291 °C. IR (KBr): 3059, 1817, 1588, 1493, 1464, 1437, 1109, 1053 cm^{-1}. ^{1}H NMR ($CDCl_3$+TFA, 500 MHz) δ: 10.44 (br s, 1H), 8.70 (s, 1H), 8.64 (s, 1H), 8.16 (m, 1H), 8.07 (m, 1H), 7.82 (s, 1H), 7.79 (s, 1H), 7.75 (m, 1H), 7.65 (m, 1H), 2.94 (s, 3H). ^{13}C NMR ($CDCl_3$+TFA, 125 MHz) δ: 171.62, 154.66, 147.48, 145.09, 143.72, 143.17, 139.21, 138.04, 131.18, 130.21, 127.57, 127.27, 122.95, 118.47, 115.99, 109.55, 107.16, 19.43. $C_{19}H_{12}ClNO_7S$ (433.82): calcd. C 52.60, H 2.79, N 3.23, S 7.39, Cl 8.17; found C 52.61, H 2.75, N 3.23, S 7.27. Cl 8.11.

7-Methyl-2-oxo-5-[4-(trifluoromethyl)-1-benzothiophen-2-yl]-1,2-dihydroisochromeno[6,7-*d*][1,3]oxazol-6-ium perchlorate (3b). This compound was prepared according to the procedure described for compound **3a**, starting from compound **6** (1.0 g, 5.17 mmol) and 4-(trifluoromethyl)-1-benzothiophen-2-carbaldehyde (**8b**, 1.19 g, 5.17 mmol) to give the title compound **3b** (1.06 g, 41%) as yellow crystals, mp 296–298 °C. IR (KBr): 3082, 1819, 1588, 1449, 1122, 921 cm^{-1}. ^{1}H NMR ($CDCl_3$+TFA, 400 MHz) δ: 8.70 (s, 1H), 8.64 (s, 1H), 8.28 (m, 1H), 8.01 (s, 1H), 7.97 (s, 1H), 7.95 (m, 1H),