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2-Imino-1,3,4-thiadiazole Derivatives of GABA as $GABA_A$ Antagonists

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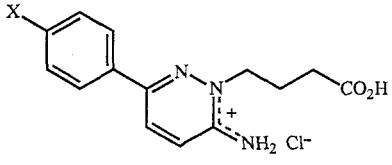
Abstract

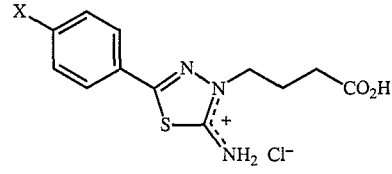
New heterocyclic derivatives of GABA, with a GABA moiety on the 3 position of a 2-imino-1,3,4-thiadiazole ring, have been readily prepared by alkylation of 2-amino-5-aryl-1,3,4-thiadiazoles in dimethylformamide. They are active as $GABA_A$ antagonists, inhibiting the action of GABA on the guinea-pig ileum about 5 times less potently than bicuculline.

Aminopyridazine derivatives of γ-aminobutyric acid (GABA), such as SR 42641 (1a) and SR 95531 (1b), have been shown to act as selective and competitive $GABA_A$ antagonists in the mammalian central nervous system.[1,2] These synthetic derivatives were more potent than the classical antagonist bicuculline.[3,4]

The pyridazine derivatives contained an endo–exocyclic amidinic system, also found in the potent steroidal GABA antagonist RU 5135,[5] and structure–activity relationships showed that maximal activity was observed with a butyric acid side chain on N 2 of the pyridazine ring. Extended conjugation of the π electron system of the pyridazine ring by an aromatic substituent at C 6, resulted in enhanced potency particularly with the 4-chlorophenyl and 4-methoxyphenyl derivatives (1a) and (1b) respectively.



(1a) X = Cl
(1b) X = OMe



(2a) X = Cl
(2b) X = OMe

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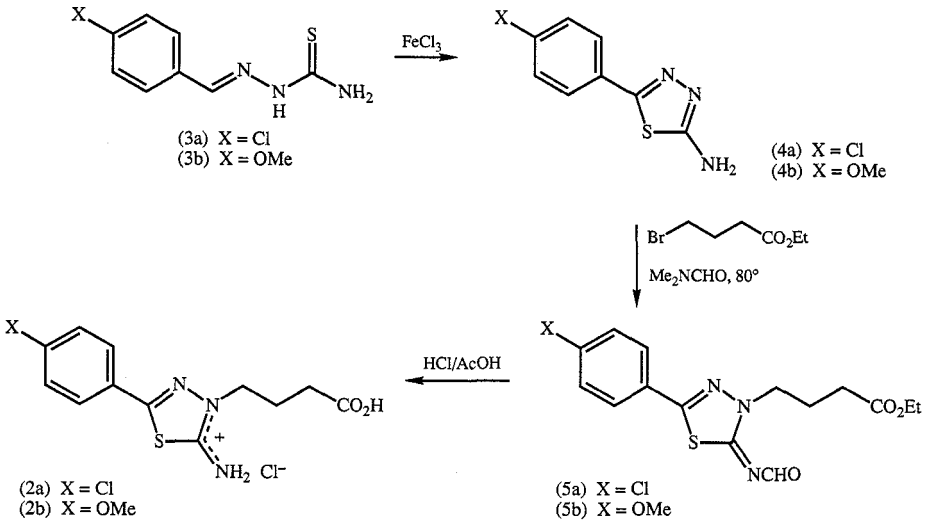
We report here a study of 1,3,4-thiadiazoles related to (1a,b), in which a sulfur atom has been used as a bioisosteric replacement for the carbon–carbon double bond.[6] We reasoned that these compounds should be available by a short and easy synthetic route involving alkylation of a 2-amino-1,3,4-thiadiazole. A survey of the literature revealed that simple alkylation with methyl iodide[7] occurred on the more basic N3.[8] If the 2-amino-1,3,4-thiadiazoles[8,9] are compared with the 3-aminopyridazines, substitution on N3 of the thiadiazole ring or on N2 of the pyridazine ring should result in a similar charge delocalization across the endo–exo amidinic group. Investigation of pK_a values of thiadiazoles indicated that 2-amino-5-phenyl-1,3,4-thiadiazole is a weak base (pK_a 2·9), whereas when the heterocyclic ring is held in the tautomeric imino form with a 3-ethyl substituent, the pK_a increases to 7·9.[10] This 3-alkyl-2-imino-1,3,4-thiadiazole system would therefore be sufficiently basic under physiological pH for a substantial proportion to be in the protonated form, as are the imino pyridazines (1a,b). The 1,3,4-thiadiazole analogues synthesized, (2a,b), incorporate the same aromatic substituents as the most potent pyridazines.



Scheme 1

As shown in Scheme 1, oxidative cyclization with ferric chloride of the thiosemicarbazones (3a,b) prepared from thiosemicarbazide and the appropriate benzaldehyde, afforded the parent 1,3,4-thiadiazoles (4a,b). Alkylation of the thiadiazole with ethyl 4-bromobutyrate in dimethylformamide resulted in the

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incorporation of the ethyl butyrate side chain. Infrared spectroscopy of the esters showed no N–H stretch, and ^{1}H n.m.r. spectroscopy showed a sharp singlet at δ 8·9–9·0 indicative of a formyl proton rather than the expected broad imino signal. Formylation on the exo-imino position was also evident from ^{13}C n.m.r. data and confirmed by mass spectroscopy. Acid hydrolysis of the formyl 1,3,4-thiadiazole esters afforded the required thiadiazole acids (2a,b).

Alkylation of 2-amino-1,3,4-thiadiazoles with methyl iodide is known to occur primarily at position N3,[7] and ^{1}H n.m.r. data of methyl substituted derivatives shows the 3-methyl resonance to be about δ 0·6 downfield from the corresponding exocyclic methyl derivative.[11] Although (5a,b) were the only compounds that could be purified by crystallization of the alkylation products, a series of more polar by-products was observed. A fraction from the chromatography of (5a) indicated two major by-products with no formyl groups and with NCH_2 ^{1}H n.m.r. signals at δ 4·04 and 4·46. On comparison with our isolated alkylation product (5a) (δ 4·51 for NCH_2), the signal at δ 4·04 may indicate the presence of some exocyclic nitrogen alkylation product, and acid hydrolysis of the by-product mixture on a t.l.c. scale indicated a mixture of (2a) and a second product of slightly higher R_F. Further evidence for the reactions giving N3 alkylated derivatives (2a,b) comes from the pK_a values for the basic endo–exo amidinic system (7·4 and 7·7 respectively).

These compounds were tested as $GABA_A$ antagonists on the GABA-induced transient contraction of the guinea-pig isolated ileum preparation.[12] All compounds were screened at 10 and 100 μM [some were not completely dissolved at this concentration (see Experimental section)] on the contraction produced by 10 μM GABA. The parent thiadiazoles (4a,b) antagonized at 100 μM but were inactive at 10 μM, while the activity of (5a,b) was complicated by ready hydrolysis to (2a,b). The thiadiazole acids (2a,b) proved to be the most active GABA antagonists, and concentrations that were of similar potency to 5 μM bicuculline were investigated. The presence of 5 μM bicuculline changed the dose–response curve for GABA so that the ED_{50} (concentration of GABA to produce 50% of maximal contraction) shifted from 5 to about 45 μM. To obtain similar shifts in the GABA dose–response curve, the presence of 30 μM (2a,b) was needed (changing the GABA ED_{50} to about 50 and 55 μM respectively). Analysis of the results by calculating 'apparent pA_2' values (where pA_2 is the measure of potency of a competitive antagonist[13]) indicated values of 5·45 (30 μM) for (2a) and 5·48 (30 μM) for (2b), while the pA_2 for bicuculline was calculated to be 6·23 (5 μM) (comparable to the pA_2 value previously reported[14]). The antagonists (2a) and (2b) are therefore about 7 and 5 times less potent than bicuculline, respectively.

The thiadiazole acids (2a,b) are crystalline, water soluble derivatives with good 'wash-out' properties in isolated tissue experiments. Despite the fact that the lower activity compared with bicuculline will preclude them from being useful pharmacological agents, this work shows that bioisosteric replacement

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of a 3-imino-2-substituted pyridazine with the easily synthesized 2-imino-3-substituted-1,3,4-thiadiazole results in compounds that are also active as $GABA_A$ antagonists.

Experimental

Melting points (uncorrected) were measured on a Reichert hot stage apparatus. Microanalyses were determined by the Australian Microanalytical Service, Melbourne. Infrared spectra were recorded on a Perkin–Elmer 177 spectrophotometer and refer to Nujol mulls of the solids. pK_a values ($pK_a \pm$s.e.m.) were obtained as described by Albert *et al.*[15] by using ultraviolet spectra recorded on a Perkin–Elmer 124 spectrophotometer at 25° in 0·01 M potassium dihydrogen phosphate buffer or 0·01 M Tris buffer in a pH range of 4·59–8·83. ^1H and ^{13}C n.m.r. spectra were recorded by using a JEOL FX-90Q instrument at 89·6 and 22·5 MHz respectively in either $CDCl_3$ or $(CD_3)_2SO$ with tetramethylsilane as internal standard. ^{13}C n.m.r. resonances were assigned with the aid of off-resonance spectra. Low-resolution mass spectra data refers to chemical ionization by using methane as reagent gas on a TSQ46 Finnigan/MAT mass spectrometer. R_F values refer to thin-layer chromatography (t.l.c.) run on aluminium-backed Merck Kieselgel 60 F_{254} plates, and were determined by using the following solvent systems: (A) ethyl acetate/light petroleum (b.p. 60–80°) (3:7); (B) ethyl acetate/light petroleum (b.p. 60–80°) (1:1); and (C) butanol/acetic acid/water (4:1:1). The plates were visualized by exposure to short wavelength ultraviolet light. Chromatographic separations were performed by short-column vacuum chromatography[16] with Merck Kieselgel 60 H (t.l.c. grade). Dimethylformamide was predried overnight with phosphorus pentoxide and filtered and distilled under reduced pressure (b.p. 59°/30 mm) prior to use. Ethyl 4-bromobutyrate was purchased from Aldrich and distilled (b.p. 80–82°/10 mm) prior to use. Bicuculline was purchased from Sigma.

Thiosemicarbazones (3a,b)

The starting thiosemicarbazones were prepared[17] by warming thiosemicarbazide and the substituted benzaldehyde in ethanol, filtering off the product and recrystallizing from 50% aqueous ethanol to give 4-chlorobenzaldehyde thiosemicarbazone (3a) as white prisms, R_F 0·7 (solvent B), m.p. 213–214° (lit.[17] 220°), 92% yield, and 4-methoxybenzaldehyde thiosemicarbazone (3b) as white plates, R_F 0·55 (solvent B), m.p. 176–177° (lit.[17] 177–178°), 91% yield.

2-Amino-5-(4-substituted phenyl)-1,3,4-thiadiazoles (4a,b)

The substituted thiadiazoles were prepared by ferric chloride oxidative cyclization of the corresponding thiosemicarbazones by using a procedure similar to that of Zubets *et al.*,[18] except that the workup and purification involved neutralization with 2 M ammonium hydroxide and dissolution of the product in hot ethanol. Recrystallization from ethanol gave 2-amino-5-(4-chlorophenyl)-1,3,4-thiadiazole (4a) as thick white needles, R_F 0·2 (solvent B), m.p. 229–230° (lit.[19] 229°), 59% yield, and 2-amino-5-(4-methoxyphenyl)-1,3,4-thiadiazole (4b) as straw coloured prisms, R_F 0·15 (solvent B), m.p. 192–194° (lit.[19] 190°), 67% yield.

Ethyl 5-(4-Chlorophenyl)-2-formylimino-1,3,4-thiadiazole-3(2H)-butanoate (5a)

The following procedure is a variation of that described by Wermuth *et al.*[1] To a solution of 2-amino-5-(4-chlorophenyl)-1,3,4-thiadiazole (2·8 g, 13 mmol) in dimethylformamide (50 ml)

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was added ethyl 4-bromobutyrate (4 g, 20 mmol), and the solution heated at 80° for 16 h. The mixture was neutralized by using 5% potassium carbonate solution and the product taken up into ethyl acetate, washed with water and brine, and dried (Na_2SO_4). The evaporated extract was then purified by short-column vacuum chromatography with ethyl acetate/light petroleum (3 : 7) as the eluent. The fractions containing product were combined and the solvents evaporated to leave a white powder, which was recrystallized from ethyl acetate/light petroleum (1 : 4) to give *ethyl 5-(4-chlorophenyl)-2-formylimino-1,3,4-thiadiazole-3(2H)-butanoate* (5a) (1·5 g, 33%) as white needles, R_F 0·24 (solvent A), R_F 0·4 (solvent B), m.p. 66–67° (Found: C, 50·7; H, 4·5; N, 11·6. $C_{15}H_{16}ClN_3O_3S$ requires C, 50·9; H, 4·5; N, 11·9%). ν_{max} (Nujol) 1728s, 1615s, 832s cm^{-1}. ^1H n.m.r. δ ($CDCl_3$) 1·25, t, J 7·3 Hz, CO_2Et; 2·01–2·56, m, $NCH_2CH_2CH_2CO_2Et$; 4·13, q, J 7·3 Hz, CO_2Et; 4·51, t, J 6·5 Hz, $NCH_2CH_2CH_2CO_2Et$; 7·41, 7·50, 7·73, 7·83, aromatic AA′BB′ system; 8·96, s, =NCHO. ^{13}C n.m.r. δ ($CDCl_3$) 14·2, CO_2CH_2Me; 23·7, $NCH_2CH_2CH_2CO_2Et$; 31·1, $NCH_2CH_2CH_2CO_2Et$; 50·2, NCH_2; 60·6, CO_2CH_2Me; 127·7, Ar-C3; 128·1, Ar-C1; 129·5, Ar-C2; 137·4, Ar-C4; 155·2, 164·1, C2 or C5; 170·7, CHO; 172·3, C=O. Mass spectrum *m/z* 356, 354 (MH, 35, 100%), 328, 326 (MH−CO, 9, 25), 140, 138 (12, 32).

Two by-products from this reaction with lower R_F values (0·13, 0·11 in solvent A), were isolated as a mixture from the chromatography column and crystallized from ethyl acetate/light petroleum (1 : 4) to yield the mixture as a white powder (170 mg). ^1H n.m.r. δ ($CDCl_3$) 4·04, t, J 6·4 Hz, $=NCH_2$; 4·46, t, J 6·1 Hz, NCH_2; with no formyl singlet present, but two sets of aromatic protons and two ethyl ester signals present. Acid hydrolysis of a small amount of this mixture (see procedure below) yielded products with R_F values of 0·53 and 0·50 (solvent C), the latter R_F corresponding to that of (2a).

Ethyl 2-Formylimino-5-(4-methoxyphenyl)-1,3,4-thiadiazole-3(2H)-butanoate (5b)

This product was prepared as above from 2-amino-5-(4-methoxyphenyl)-1,3,4-thiadiazole (4b) to give *ethyl 2-formylimino-5-(4-methoxyphenyl)-1,3,4-thiadiazole-3(2H)-butanoate* (5b) (20%) as pale yellow plates, R_F 0·2 (solvent A), R_F 0·35 (solvent B), m.p. 64° (Found: C, 55·2; H, 5·5; N, 11·7. $C_{16}H_{19}N_3O_4S$ requires C, 55·0; H, 5·4; N, 12·0%). ν_{max} (Nujol) 1725s, 1600s, 830s, cm^{-1}. ^1H n.m.r. δ ($CDCl_3$) 1·25, t, J 7·2 Hz, CO_2Et; 2·15–2·50, m, $NCH_2CH_2CH_2CO_2Et$; 3·88, s, MeO; 4·13, q, J 7·2 Hz, CO_2Et; 4·49, t, J 6·5 Hz, $NCH_2CH_2CH_2CO_2Et$; 6·93, 7·03, 7·72, 7·82, aromatic AA′BB′ system; 8·93, s, =NCHO. ^{13}C n.m.r. δ ($CDCl_3$) 14·3, CO_2CH_2Me; 23·7, $NCH_2CH_2CH_2CO_2Et$; 31·2, $NCH_2CH_2CH_2CO_2Et$; 50·0, NCH_2; 55·5, MeO; 60·7, CO_2CH_2Me; 114·6, Ar-C3; 122·2, Ar-C1; 128·2, Ar-C2; 156·3, 164·1, C2 or C5; 162·1, Ar-C4; 170·7, CHO; 172·4; C=O. Mass spectrum *m/z* 350 (MH, 100%), 322 (MH−CO, 3), 134 (15).

5-(4-Chlorophenyl)-2-imino-1,3,4-thiadiazole-3(2H)-butanoic Acid, Hydrochloride Salt (2a)

The ethyl ester (4a) (70 mg, 0·2 mmol) was dissolved in a mixture of concentrated HCl (32%) and glacial acetic acid (10 ml) (1 : 1), and the solution heated at 100° for 16 h. The solvents were removed under reduced pressure and the product crystallized from ethanol/ether (2 : 3) to yield *5-(4-chlorophenyl)-2-imino-1,3,4-thiadiazole-3(2H)-butanoic acid, hydrochloride salt* (2a) (45 mg, 67%), as white needles, R_F 0·5 (solvent C), m.p. 206–207° (Found: C, 42·9; H, 4·0; N, 12·4. $C_{12}H_{13}Cl_2N_3O_2S$ requires C, 43·1; H, 3·9; N, 12·6%). ν_{max} (Nujol) 3305–3040br, 1725sh, 1700s, 1640s, 830s, cm^{-1}. ^1H n.m.r. δ [$(CD_3)_2SO$] 1·83–2·21, m, $NCH_2CH_2CH_2CO_2H$; 2·25–2·58, m, $NCH_2CH_2CH_2CO_2H$; 4·27, t, J 6·6 Hz, NCH_2; 7·60, 7·70, 7·82, 7·92, aromatic AA′BB′ system; 10·89, br s, $=NH_2^+$. Mass spectrum *m/z* 300, 298 (MH, 33, 100%), 140, 138 (9, 33). pK_a 7·36±0·11.

2-Imino-5-(4-methoxyphenyl)-1,3,4-thiadiazole-3(2H)-butanoic Acid, Hydrochloride Salt (2b)

The ethyl ester (4b) (350 mg, 1·0 mmol) was hydrolysed as above and the product crystallized from ethanol/ether (2 : 3) to give *2-imino-5-(4-methoxyphenyl)-1,3,4-thiadiazole-3(2H)-butanoic acid, hydrochloride salt* (2b) (302 mg, 92%), as white rods, R_F 0·5 (solvent C), m.p. 206–207° (Found: C, 47·4; H, 4·8; N, 12·5. $C_{13}H_{16}ClN_3O_3S$ requires C, 47·3; H, 4·9; N, 12·7%). ν_{max} (Nujol) 3280–3000br, 1740s, 1725sh, 1635sh, 1605s, 1565s, 1495s, 825s cm^{-1}.

^{1}H n.m.r. δ [$(CD_3)_2SO$] 1·80–2·18, m, $NCH_2\mathbf{CH_2}CH_2CO_2H$; 2·26–2·58, m, $NCH_2CH_2\mathbf{CH_2}CO_2H$; 3·84, s, MeO; 4·28, t, J 6·5 Hz, NCH_2; 7·06, 7·16, 7·73, 7·83, aromatic AA′BB′ system; 10·99, br s, $=NH_2^+$. Mass spectrum m/z 294 (MH, 100%), 134 (33). pK_a 7·72±0·05.

Guinea-Pig Isolated Ileum Preparation[12]

Male guinea-pigs weighing 250–300 g were killed by cervical dislocation. Segments of distal ileum 3–4 cm in length, taken at least 5 cm from the ileo-caecal valve were removed, emptied of their contents and mounted vertically in a 20 ml organ bath. The bath contained modified Krebs-bicarbonate solution of composition (mM): Na^+, 151·0; K^+, 4·6; Mg^{2+}, 0·6; Ca^{2+}, 2·5; Cl^-, 134·9; HCO_3^-; 24·9; $H_2PO_4^-$, 1·3; SO_4^{2-}, 0·6; glucose, 7·7; (pH 7·4 at 35°). The Krebs solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2.

The effects of drug treatment were examined on resting tissue. Isometric contractions of the longitudinal muscle were recorded by using a Grass polygraph recorder. Tissues were placed under a resting tension of 1 g and left to equilibrate for 30–40 min before the agonist was applied. Added drug volumes never exceeded 1·2% of the bath volume. Stock solutions of bicuculline and the compounds (2a,b)–(5a,b) were made as hydrochloride salts dissolved in distilled water or 1 M HCl, and the stock solutions diluted with distilled water. Compounds (3a,b) and (5a,b) did not completely dissolve at 100 μM in 1M HCl, and were also tested after dissolving in methanol (0·001% final concentration).

Initial screening for $GABA_A$ antagonist activity was attempted at 100 μM. (2a,b) and (4a,b) dissolved readily at this concentration and all of these completely antagonized responses to 10 μM GABA except (4b) which gave 30% of maximum contraction. (5a,b) also blocked reponses to 10 μM GABA but hydrolysis to (2a) and (2b) could be demonstrated by t.l.c. When screened at 10 μM, (3a,b) and (4a,b) showed no significant inhibition, while (5a,b) readily hydrolysed to give responses similar to (2a,b). Dose–response curves where then determined for GABA by using antagonist concentrations of both 30 and 50 μM for the most active compounds (2a,b), and these gave dose–response curves comparable to 5 μM bicuculline . 'Apparent pA_2' values were determined by using ED_{50} values obtained from the dose–response curves.[13]

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