Synthesis and Anticonvulsant Activities of 5-(2-Chlorophenyl)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepines

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A group of 5-(2-chlorophenyl)-10-(substituted)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepines **7a-c** were synthesized by the acid catalyzed reaction of 5-(2-chlorophenyl)-2-hydrazino-3*H*-pyrido[3,4-*e*]-[1,4]diazepine (**6**) with either trimethyl orthoformate, triethyl orthoacetate or triethyl orthobenzoate, respectively. 5-(2-Chlorophenyl)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (**7a**) and 5-(2-chlorophenyl)-10-methyl-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (**7b**) exhibited good anticonvulsant activity in the subcutaneous metrazol anticonvulsant screen which serves as a model for absence (petit mal) epilepsy.

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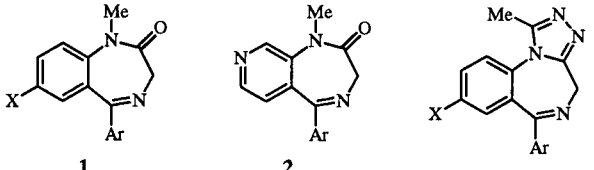
Introduction.

The [1,4]benzodiazepin-2-one class of compounds **1**, (X = Cl, NO_2) exhibit a broad spectrum of useful central nervous system effects including anticonvulsant (particularly against metrazol induced seizures), sedative, hypnotic and muscle relaxant activities [1-3]. In an earlier study we developed an efficient synthetic methodology involving *ortho*-directed lithiation [4], for the preparation of 5-aryl-1,3-dihydro-1-methyl-2*H*-pyrido[3,4-*e*][1,4]diazepin-2-ones **2**, which are electronically analogous to [1,4]benzodiazepin-2-ones **1**, that exhibit anticonvulsant activity [5]. A structurally related class of triazolo[1,4]benzodiazepines **3**, which in many cases are more potent than the corresponding [1,4]benzodiazepin-2-one analogs [3], were found to have potent tranquilizing, muscle relaxant and anticonvulsant effects [4]. It was therefore of interest to prepare tricyclic triazolo derivatives of 5-aryl-1,3-dihydro-1-methyl-2*H*-pyrido[3,4-*e*][1,4]diazepin-2-ones **2**. Accordingly, we now describe the synthesis and anticonvulsant activities of 5-(2-chlorophenyl)-7*H*-pyrido[4,3-*f*]-[1,2,4]triazolo[4,3-*a*][1,4]diazepines **7a-c**.

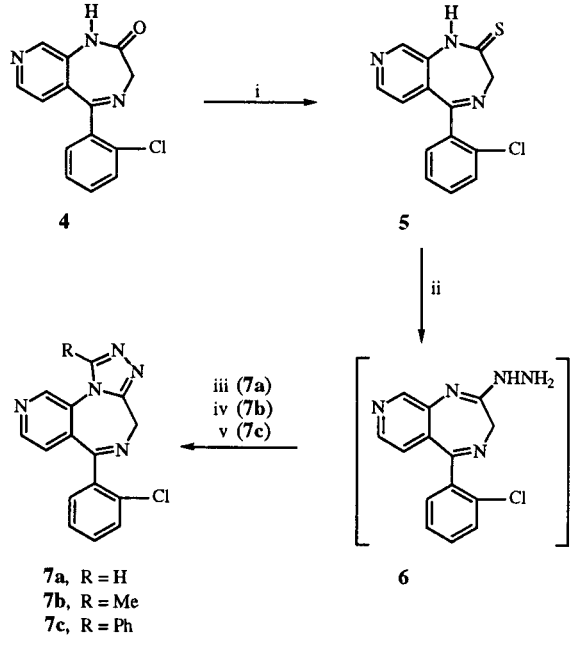


1 **2** **3**

Chemistry.

Thionation of 5-(2-chlorophenyl)-1,3-dihydro-2*H*-pyrido[3,4-*e*][1,4]diazepin-2-one (**4**) by reaction with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent) afforded 5-(2-chlorophenyl)-1,3-dihydro-2*H*-pyrido[3,4-*e*][1,4]diazepine-2-thione (**5**) in 81% yield, which was subsequently elaborated to 5-(2-chlorophenyl)-2-hydrazino-3*H*-pyrido[3,4-*e*]-[1,4]diazepine (**6**) upon reaction with hydrazine (see Scheme 1). Due to its chemical instability, 5-(2-chlorophenyl)-2-hydrazino-3*H*-pyrido[3,4-*e*][1,4]diazepine (**6**) was immediately cyclized in the presence of concentrated sulfuric acid to the target products 5-(2-chlorophenyl)-

Scheme 1 [a]



4 **5** **6**

7a, R = H
7b, R = Me
7c, R = Ph

[a] Reagents and conditions: i, 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent), toluene, reflux, 1.5 hours; ii, hydrazine, MeOH, 25°, 2.5 hours; iii, trimethyl orthoformate, ethanol, concentrated sulfuric acid, 0° → 25°, 2 hours (**7a**); iv, triethyl orthoacetate, ethanol, concentrated sulfuric acid, 0° → 25°, 2 hours (**7b**); v, triethyl orthobenzoate, ethanol, concentrated sulfuric acid, 0° → 25°, 2 hours (**7c**).

7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (**7a**, 78%), 5-(2-chlorophenyl)-10-methyl-7*H*-pyrido[4,3-*f*]-[1,2,4]triazolo[4,3-*a*][1,4]diazepine (**7b**, 62%), or 5-(2-chlorophenyl)-10-phenyl-7*H*-pyrido[4,3-*f*][1,2,4]triazolo-[4,3-*a*][1,4]diazepine (**7c**, 67%) by reaction with trimethyl orthoformate, triethyl orthoacetate or triethyl orthobenzoate, respectively.

Biological Results.

The anticonvulsant activities of the 5-(2-chlorophenyl)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepines **7a-c** were determined by the Antiepileptic Drug Development Program (Administered by the Section on Epilepsy, National Institutes of Health, Bethesda, MD). Anticonvulsant activities against subcutaneous metrazol and maximal electroshock induced seizures, which are models for absence (petit mal) and generalized tonic clonic (grand mal) seizures, respectively were measured in mice and/or rats at fifteen minutes, thirty minutes or four hours after intraparietoneal or oral administration of the test compound using the procedures previously reported [6]. All of the 5-(2-chlorophenyl)-10-substituted-7*H*-pyrido-[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepines (**7a**, 10-hydrogen; **7b**, 10-methyl; **7c**, 10-phenyl) were inactive, or weakly active, in the maximal electroshock screen (ED_{50} is defined as the effective dose of the test compound that protects 50% of the animals from seizures; **7a**, ED_{50} >300 mg/kg for an intraparietoneal dose in mice; **7b**, a 300 mg/kg intraparietoneal dose protected 1/1 (one-out-of-one) mice at thirty minutes and 0/1 mice at four hours, and a 50 mg/kg oral dose protected 3/4 rats at four hours; **7c**, a 300 mg/kg intraparietoneal dose protected 1/1 mice at thirty minutes and 0/1 mice at four hours). In contrast, the 10-hydrogen compound **7a** (ED_{50} = 0.069 mg/kg for an intraparietoneal dose in mice at fifteen minutes and a 50 mg/kg oral dose protected 4/4 and 2/4 rats at two and four hours, respectively) and the 10-methyl compound **7b** (a 1 mg/kg intraparietoneal dose protected 4/4 mice at thirty minutes; ED_{50} = 0.87 mg/kg for an oral dose in rats at fifteen minutes) in the subcutaneous metrazol screen. Although the 10-phenyl compound **7c** showed activity in the subcutaneous metrazol screen where a thirty mg/kg intraparietoneal dose protected 4/4 mice at thirty minutes, a 50 mg/kg oral dose in rats was inactive at either thirty minutes or four hours. The ED_{50} values for the standard reference drugs in these screens were clonazepam (subcutaneous metrazol, ED_{50} = 0.02 mg/kg; maximal electroshock, ED_{50} = 86.6 mg/kg) and valproic acid (subcutaneous metrazol, ED_{50} = 148.6 mg/kg; maximal electroshock, ED_{50} = 271.7 mg/kg). The 10-hydrogen (TD_{50} is defined as the dose of the test compound that is toxic to 50% of the mice; **7a**, TD_{50} = 232 mg/kg for an intraparietoneal dose in mice) and 10-methyl (**7b**, TD_{50} >500 mg/kg for an oral dose in rats) analogs of 5-(2-chlorophenyl)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine were non-toxic agents in these anticonvulsant screens.

The ability of the 10-hydrogen (**7a**) and 10-phenyl (**7c**) analogs of 5-(2-chlorophenyl)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine to prevent convulsions induced by other convulsion-inducing agents was also investigated in mice at fifteen minutes following an intraparietoneal dose of the test compound [(subcutaneous bicuculline test: **7a**, ED_{50} = 0.79 mg/kg; **7c**, ED_{50} = 17 mg/kg); (subcutaneous picrotoxin test: **7a**, ED_{50} >20 mg/kg; **7c**, ED_{50} = 25 mg/kg); subcutaneous strychnine test: **7a**, ED_{50} >20 mg/kg; **7c**; ED_{50} >50 mg/kg)]. Subcutaneous bicuculline blocks gamma-aminobutryric acid receptors and this interferes with gamma-aminobutyric acid inhibitory function thereby inducing both clonic and tonic extensor convulsions. Subcutaneous picrotoxin interferes with chloride channels that are regulated by gamma-aminobutyric acid receptors thereby inducing minimal threshold seizures. The ability of 5-(2-chlorophenyl)-10-phenyl-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (**7c**) to displace 50% of 10 n*M* [^{3}H]flunitrazepam from mouse whole brain P_2 pellets (IC_{50} = 2.59 μ*M*) indicates that it has moderate affinity for the benzodiazepine receptor site(s) as compared with the reference drug clonazepam [5-(2-chlorophenyl)-7-nitrobenzo[1,4]diazepin-2-one, IC_{50} = 0.003 μ*M*].

The results of this study indicate that the 10-hydrogen (**7a**) and 10-methyl (**7b**) analogs of 5-(2-chlorophenyl)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine exhibit anticonvulsant activity intermediate between that of clonazepam and valproic acid in the subcutaneous metrazol screen which is a model for absence (petit mal) epilepsy.

EXPERIMENTAL

Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H nmr) were recorded on a Bruker AM-300 spectrometer. The assignment of exchangeable protons (N*H*) was confirmed by the addition of deuterium oxide. Infrared spectra (ir) were recorded on a Nicolet 5DX FT spectrometer. All of the products gave rise to a single spot using Whatman MK6F silica gel microslides (250 μ*M* thickness) with a solvent of high, medium and low polarity. Silica gel column chromatography was carried out using Merck 7734 (70-230 mesh) silica gel. 5-(2-Chlorophenyl)-1,3-dihydro-2*H*-pyrido[3,4-*e*][1,4]diazepin-2-one (**4**) was prepared according to the literature procedure [5].

5-(2-Chlorophenyl)-1,3-dihydro-2*H*-pyrido[3,4-*e*][1,4]diazepine-2-thione (**5**).

A mixture of 5-(2-chlorophenyl)-1,3-dihydro-2*H*-pyrido-[3,4-*e*][1,4]diazepin-2-one (6.2 g, 22.8 mmoles) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent, 5.54 g, 14 mmoles) in toluene

(150 ml) was refluxed for ninety minutes. The solvent was removed *in vacuo*, ethyl acetate (100 ml) and aqueous sodium hydroxide (80 ml of 10% w/v) were added to the residue and the organic fraction was separated. The aqueous fraction was extracted with ethyl acetate (2 x 80 ml) and the combined organic extracts were washed with water (60 ml) and then brine (60 ml). Drying the organic fraction (sodium sulfate), and removal of the solvent *in vacuo* gave a residue which was purified by silica gel column chromatography using ethyl acetate-hexane (6:1, v/v) as eluant. Recrystallization of the product from methanol-benzene afforded 5-(2-chlorophenyl)-1,3-dihydro-2*H*-pyrido[3,4-*e*][1,4]diazepine-2-thione as yellow crystals (5.31 g, 81%), mp 221-222°; ir (potassium bromide): ν 3460, 3181, 3122, 3099, 3050, 2976, 2910, 2861, 1630, 1614, 1565, 1516 cm^{-1}; ^1H nmr (deuteriodimethyl sulfoxide): δ 4.75 (s, 2H, CH_2), 6.95 (d, $J_{6,7}$ = 5.8 Hz, 1H, H-6), 7.53-7.57 (m, 4H, phenyl hydrogens), 8.35 (d, $J_{6,7}$ = 5.8 Hz, 1H, H-7), 8.70 (s, 1H, H-9), 12.50 (s, 1H, NH).

Anal. Calcd. for $C_{14}H_{10}ClN_3S$: C, 58.43; H, 3.50; N, 14.60. Found: C, 58.53; H, 3.50; N, 14.67.

5-(2-Chlorophenyl)-2-hydrazino-3*H*-pyrido[3,4-*e*][1,4]diazepine (**6**).

Hydrazine (5.93 g, 104 mmoles) was added dropwise with stirring to a solution of 5-(2-chlorophenyl)-1,3-dihydro-2*H*-pyrido[3,4-*e*][1,4]diazepine-2-thione (1.55 g, 5.4 mmoles) in methanol (40 ml) with stirring at 25°. The reaction was allowed to proceed at 25° with stirring for two and one-half hours prior to pouring onto ice-water (100 ml). Extraction with chloroform (3 x 60 ml), washing the chloroform extracts with water (40 ml) and then brine (40 ml), drying the chloroform fraction (sodium sulfate) and removal of the solvent *in vacuo* yielded 5-(2-chlorophenyl)-2-hydrazino-3*H*-pyrido[3,4-*e*][1,4]diazepine as a solid (1.25 g, 81%) that was used immediately in subsequent reactions without further purification; ^1H nmr (deuteriochloroform): δ 3.65-5.20 (br m, 5H, N*H*NH_2, CH_2), 6.85 (d, $J_{6,7}$ = 5.8 Hz, 1H, H-6), 7.48-7.52 (m, 4H, phenyl hydrogens), 8.20 (d, $J_{6,7}$ = 5.8 Hz, 1H, H-7), 8.60 (s, 1H, H-9).

5-(2-Chlorophenyl)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]-diazepine (**7a**).

Concentrated sulfuric acid (0.74 ml) was added dropwise to a mixture of 5-(2-chlorophenyl)-2-hydrazino-3*H*-pyrido[3,4-*e*]-[1,4]diazepine (2.27 g, 7.9 mmoles), trimethyl orthoformate (4.23 g, 39.8 mmoles) and ethanol (75 ml) at 0° with stirring. The reaction was allowed to proceed at 25° with stirring for two hours. The reaction mixture was neutralized with a saturated solution of aqueous sodium bicarbonate and this mixture was concentrated *in vacuo*. Dissolution of the residue in chloroform (100 ml), washing the chloroform extract with water (40 ml) and then brine (40 ml), drying the chloroform fraction (sodium sulfate) and removal of the solvent *in vacuo* gave a residue that was purified by silica gel column chromatography. Elution with ethyl acetate-methanol (9:1, v/v) afforded 5-(2-chlorophenyl)-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine which was recrystallized from acetone-hexane (1.83 g, 78%), mp 184-185°; ir (potassium bromide): ν 3107, 2984, 1622, 1589, 1532 cm^{-1}; ^1H nmr (deuteriochloroform): 5.04 (br s, 2H, H-7), 7.16 (d, $J_{3,4}$ = 5.8 Hz, 1H, H-4), 7.32-7.52 and 7.56-7.66 (two m, 4H total, phenyl hydrogens), 8.70 (d, $J_{3,4}$ = 5.8 Hz, 1H, H-3), 8.76 (s, 1H, H-10), 9.00 (s, 1H, H-1).

Anal. Calcd. for $C_{15}H_{10}ClN_5$: C, 60.92; H, 3.41; N, 23.68. Found: C, 60.59; H, 3.49; N, 23.31.

5-(2-Chlorophenyl)-10-methyl-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (**7b**).

Concentrated sulfuric acid (0.72 ml) was added dropwise to a mixture of 5-(2-chlorophenyl)-2-hydrazino-3*H*-pyrido[3,4-*e*]-[1,4]diazepine (2.22 g, 7.8 mmoles), triethyl orthoacetate (6.32 g, 38.9 mmoles) and ethanol (72 ml) at 0° with stirring. The reaction was allowed to proceed at 25° with stirring for two hours. The reaction mixture was neutralized with a saturated solution of aqueous sodium bicarbonate and this mixture was concentrated *in vacuo*. Dissolution of the residue in chloroform (100 ml), washing the chloroform extract with water (40 ml) and then brine (40 ml), drying the chloroform fraction (sodium sulfate) and removal of the solvent *in vacuo* gave a residue that was purified by silica gel column chromatography. Elution with ethyl acetate-methanol (9:1, v/v) afforded 5-(2-chlorophenyl)-10-methyl-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine which was recrystallized from methanol (1.49 g, 62%), mp 242-243°; ir (potassium bromide): ν 3059, 2992, 1622, 1589, 1548, 1524 cm^{-1}; ^1H nmr (deuteriochloroform): δ 2.70 (s, 3H, CH_3), 4.20 (d, J_{gem} = 12 Hz, 1H, H-7'), 5.63 (d, J_{gem} = 12 Hz, 1H, H-7"), 7.14 (d, $J_{3,4}$ = 5.8 Hz, 1H, H-4), 7.30-7.48 and 7.58-7.68 (two m, 4H total, phenyl hydrogens), 8.68 (d, $J_{3,4}$ = 5.8 Hz, 1H, H-3), 8.88 (s, 1H, H-1).

Anal. Calcd. for $C_{16}H_{12}ClN_5$: C, 62.04; H, 3.90; N, 22.61. Found: C, 62.39; H, 3.90; N, 22.53.

5-(2-Chlorophenyl)-10-phenyl-7*H*-pyrido[4,3-*f*][1,2,4]triazolo-[4,3-*a*][1,4]diazepine (**7c**).

Concentrated sulfuric acid (0.75 ml) was added dropwise to a mixture of 5-(2-chlorophenyl)-2-hydrazino-3*H*-pyrido[3,4-*e*]-[1,4]diazepine (2.58 g, 9 mmoles), triethyl orthobenzoate (10.15 g, 45 mmoles) and ethanol (75 ml) at 0° with stirring. The reaction was allowed to proceed at 25° with stirring for two hours. The reaction mixture was neutralized with a saturated solution of aqueous sodium bicarbonate and this mixture was concentrated *in vacuo*. Dissolution of the residue in chloroform (100 ml), washing the chloroform extract with water (40 ml) and then brine (40 ml), drying the chloroform fraction (sodium sulfate) and removal of the solvent *in vacuo* gave a residue that was purified by silica gel column chromatography. Elution with ethyl acetate-methanol (9:1, v/v) afforded 5-(2-chlorophenyl)-10-phenyl-7*H*-pyrido[4,3-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine which was recrystallized from methanol (2.5 g, 67%), mp 287-288° dec; ir (potassium bromide): ν 3058, 2967, 2918, 2861, 1614, 1589, 1532 cm^{-1}; ^1H nmr (deuteriochloroform): δ 4.20 (d, J_{gem} = 12 Hz, 1H, H-7'), 5.60 (d, J_{gem} = 12 Hz, 1H, H-7"), 7.15 (d, $J_{3,4}$ = 5.8 Hz, 1H, H-4), 7.30-7.75 (m, 9H total, phenyl hydrogens), 8.30 (s, 1H, H-1), 8.55 (d, $J_{3,4}$ = 5.8 Hz, 1H, H-3).

Anal. Calcd. for $C_{21}H_{14}ClN_5$: C, 67.83; H, 3.79; N, 18.83. Found: C, 67.69; H, 3.70; N, 18.84.

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