Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 4035–4037

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

5-Amino-1-(2,6-dichloro-4-trifluoromethyl-phenyl)-3-[$^3H_3$]-methylsulfanyl-1*H*-pyrazole-4-carbonitrile (*CTOM*): Synthesis and Characterization of a Novel and Selective Insect GABA Receptor Radioligand

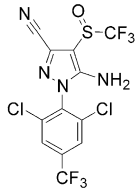
Sanath K. Meegalla,[a,*] Dario Doller,[a] Gary M. Silver,[b] Nancy Wisnewski,[b] Richard M. Soll[a] and Dale Dhanoa[a]

[a]*3-Dimensional Pharmaceuticals, Inc., 665 Stockton Drive, Suite 104, Exton, PA 19341, USA*
[b]*Heska Corporation, 1613 Prospect Parkway, Fort Collins, CO 80525, USA*

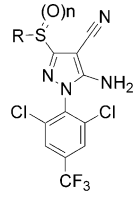
Received 6 January 2003; revised 1 May 2003; accepted 22 August 2003

Abstract—Pyrazole **2a** is a novel, potent ligand for insect GABA receptors obtained from housefly head membrane preparations ($K_i = 8$ nM). It is 500-fold selective against the mammalian receptor (mouse brain preparations). Its specifically tritiated version (**2b**) was synthesized by reduction of disulfide **10** with $NaBH_4$ followed by alkylation with [$^3H_3$]-CH_3I.

γ-Aminobutyric acid (GABA) receptors are membrane-bound glycoproteins that belong to the ligand-gated ion channel receptor family. These receptors play a major role in the inhibition of central nervous system (CNS) neuronal activity in vertebrates and invertebrates. GABA receptors are also the target for a relatively new class of highly effective insecticides, the arylpyrazoles, which are used for the chemical control of economically important pests and insects such as fleas, ticks, houseflies, grasshoppers and cockroaches.[1] Arguably the most important member of this family, fipronil (**1**), has attained the position of world leader in sales for this market. An important characteristic of the arylpyrazoles as a class is their high selectivity, which provides them with a favorable toxicity profile for insects relative to their safety against vertebrates. At the molecular level, the selectivity of the arylpyrazoles is a reflection of the target-site specificity between the GABA receptors of insects and vertebrates. Within the family of different insect GABA receptors three distinct binding sites have been identified: a GABA agonist binding site, a benzodiazepine binding site and a convulsant binding site. The convulsant binding site of GABA receptors in insects is the major target site for many of the drugs and insecticides currently on the market. Mechanism of action studies demonstrated that **1** is a non-competitive GABA antagonist in insects, acting either with an allosteric binding site or by irreversible binding.[2]



1 (fipronil)
5-Amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethanesulfinyl-1*H*-pyrazole-3-carbonitrile



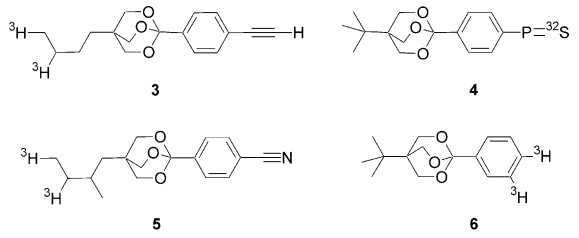
2a: R = CH_3, n = 0
5-Amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-methylsulfanyl-1*H*-pyrazole-4-carbonitrile
2b: R = C^3H_3 n = 0 (CTOM)
2c: R = CH_3, n = 1

The development of suitable radioligands constitutes a major step in furthering the understanding of GABA receptor pharmacology. An effective insect GABA radioligand should satisfy several important requirements. It should have high affinity for the insect GABA receptors, with high species selectivity, preferably high insecticidal activity, high specific activity upon chemical labeling and chemical stability upon standard storage conditions [e.g., (−18 °C) dimethyl sulfoxide solutions in freezer]. Radiolabeled ligands **3**–**6**, among others,

*Corresponding author. Tel.: +1-610-458-8959; fax: +1-610-458-8249; e-mail: meegalla@3dp.com

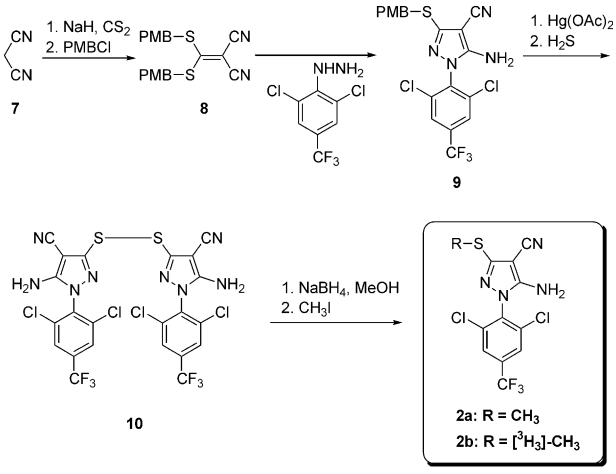
0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.042

have proven to be only partially satisfactory tools in insect receptor studies, probably due in part to their low to moderate insecticidal activity. Radioligand **3** ([^{3}H]-EBOB), which is currently widely used, also has the drawback of being fairly chemically unstable, presumably due to the hydrolytic nature of its orthoester functionality, and therefore requiring periodical purifications prior to being used.



Our structure-insecticidal activity relationship studies among arylpyrazoles yielded compound **2a** [5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-methylsulfanyl-1*H*-pyrazole-4-carbonitrile, CTOM in its radiolabeled version **2b**] as a potent (housefly $K_i = 8$ nM) and selective (500-fold selectivity vs mouse receptor) GABA receptor ligand. Since this compound satisfied the requirements of high affinity and insect selectivity, we envisioned a synthetic route to introduce a tritiated thiomethyl group at the 3-position of the pyrazole ring, which would enable us to investigate the additional requirements for a new, stable, high specific activity radioligand.

The synthetic methodology employed in our investigations is shown in Scheme 1. In order to introduce a tritiated thiomethyl group at C-3 we contemplated the use of a reduction/alkylation sequence with [$^3H_3$]-methyl iodide on disulfide **10**. Precursor disulfide **10** was prepared starting with commercially available malononitrile **7**. The anion of malononitrile was reacted with carbon disulfide, and the resulting ketenedithioenolate intermediate was alkylated in situ by quenching with *p*-methoxybenzylchloride (PMBCl) to obtain bisalkylated ketenedithioacetal **8**.[3] Cyclocondensation of **8** with 2,6-dichloro-4-trifluoromethylphenylhydrazine afforded arylpyrazole **9**. Removal of the protecting PMB group from **9** was carried out using $Hg(OAc)_2$ to obtain disulfide **10**, arising from air-oxidation of the intermediate sulfide. Reduction of disulfide **10** with $NaBH_4$ followed by in situ alkylation with CH_3I furnished unlabeled **2a** in good yields (27% overall yield).[5]



Scheme 1.

When the final step was carried out with radiolabeled [$^3H_3$]-CH_3I we were able to obtain labeled compound **2b** with 82.5 Ci/mmol specific activity. HPLC analysis showed greater than 99.5% radiochemical purity. Continuous use of **2b** has demonstrated its chemical stability.

Unlabeled compound **2a** exhibited 8 nM binding affinity for GABA receptors in house fly head preparations and 4 μM binding affinity in mouse brain preparations, resulting in a 500-fold selectivity for insect GABA receptors.[4] Despite its high in vitro binding affinity and selectivity, this compound did not show significant in vivo insecticidal activity when tested in either flea or tick contact assays or when fed to fleas in a blood meal using an artificial feeding apparatus. We currently have a limited amount of experimental information to explain this fact. One possibility may be rapid metabolic inactivation upon absorption, as the corresponding sulfoxide **2c**, which was isolated from flea feces after blood feeding and showed no activity in flea contact assays. Other possible causes that could contribute to the lack of in vivo activity include binding to a non-insecticidal site of GABA receptors, high non-specific protein binding and poor bioavailability of this compound in fleas and ticks. One further possibility is that compound **2a** is acting as an antagonist of compound **1**, binding at the same allosteric site without having insecticidal properties. To address this potential scenario, compound **1** and **2a** were co-administered in a flea contact assay to assess the blocking potential of **2a**. Compound **2a**, which alone showed no insecticidal activity, administered at 10-, 20-, and 100- fold excess of three concentration titration of compound **1**, which alone showed 50–100% flea mortality, exhibited no effect on the insecticidal properties of compound **1**. Thus, it is unlikely that compound 2a is acting as a conventional antagonist of compound **1**.

In summary, we have developed a new, highly-selective and stable GABA receptor radioligand, CTOM. This was prepared in high specific activity and radiochemical purity from readily available commercial materials in four steps. We believe compound **2b** will be a useful tool in studying in vitro insect GABA receptor biology.

Acknowledgements

We wish to thank Mike Kolpak, Stephen Eisnnagel, Heidi Ott, Joely Maddux, Victor Ozols, and Scott Walmsley for analytical and technical support.

References and Notes

1. Hainzl, D.; Cole, L. M.; Casida, J. E. *Chem. Res. Toxicol.* **1998**, *11*, 1529.

2. Bloomquist, J. R. *Annu. Rev. Entomol.* **1996**, *41*, 163.
3. Villemin, D.; Ben Alloum, A. *Synthesis* **1991**, 301.
4. Dhanoa, D. S.; Meegalla, S.; Doller, D.; Soli, R. M. US Patent 6,409,988.
5. ^{1}H NMR spectra were recorded on a Bruker AC 300 NMR spectrometer, in $CDCl_3$ as solvent. Chemical shifts are reported in ppm relative to tetramethylsilane as an internal standard. Mass spectra were recorded on a LCQ Finnigan mass spectrometer. Commercially available solvents and reagents were used as received. Flash chromatography was carried out using Merck Kieselgel 60 silica gel.

{Bis[(4 - methoxyphenyl)methylthio]methylene}methane - 1,1 - dicarbonitrile (8). Anhydrous dimethyl formamide (40 mL) was slowly added to a rapidly stirred mixture of malononitrile (13.2 g, 0.2 mol), NaH (9.6 g, 0.4 mol) and carbon disulfide (22.8 g, 0.3 mol) in anhydrous benzene (200 mL) at room temperature. The reaction mixture was stirred for 30 min and 4-methoxybenzyl chloride (93.6 g, 0.6 mol) was added. The resulting mixture was stirred for 12 h, and benzene (50 mL) and ice-water (200 mL) were added. The organic layer was separated, dried, and concentrated under vacuum. The product was purified by flash chromato-graphy on silica gel, eluting with ethyl acetate/hexanes (15:85). Yield: 45.8 g (0.12 mol, 60%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.22 (4H, d, $J = 8.6$ Hz), 6.85 (4H, d, $J = 8.6$ Hz), 4.33 (4H, s); 3.79 (6H, s).

5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-3-[(4-methoxyphenyl)-methylthio]pyrazole-4-carbonitrile (9). A solution of 2,6-dichloro-4-trifluoromethyl phenyl hydrazine (245 mg, 1.0 mmol) and {bis[(4-methoxyphenyl)methylthio] methylene}-methane-1,1-dicarbonitrile (382 mg, 1.0 mmol) in isopropyl alcohol (15 mL) was heated at reflux temperature for 16 h. The solvent was removed under reduced pressure and the desired product obtained after chromatographic separation (silica gel) using ethyl acetate–hexanes (331 mg, yield: 70%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.7 (2H, s), 7.2 (2H, d, $J = 8.7$ Hz), 6.8 (2H, d, $J = 8.7$ Hz), 4.41 (2H, broad), 4.23 (2H, s). MS: $M + 1 = 473$ (calcd 473).

5-Amino-3-({5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-cyano-pyrazol-3-yl}disulfanyl)-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]pyrazole-4-carbo-nitrile (10). Compound **9** (473 mg, 1.0 mmol) was dissolved in trifluoroacetic acid (6.5 mL) containing anisole (0.2 mL) at 0 °C, and $Hg(OAc)_2$ (383 mg, 1.2 mmol) was added. The resulting mixture was stirred at 0 °C for 30 min, and the volatiles evaporated under reduced pressure. The residue was dried under high-vacuum and dissolved in ethanol. H_2S (g) was bubbled through the solution for 30 min. The black precipitate was filtered through a Celite pad. The filtrate was concentrated, dissolved in methylene chloride, and thoroughly washed with $NaHCO_3$ (satd soln, 30 mL). The residue was dissolved in methanol (20 mL) and stirred open to the air for 24 h. Methanol was removed under reduced pressure, and the residue purified by flash chromatography (25% ethyl acetate–hexanes) to yield the desired disulfide 10 (229 mg, 65% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.75 (4H, s), 4.7, (4H, broad). MS: $M + 1 = 703$ (calcd 703).

5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-3-[$^3H_3$]-methylthio-pyrazole-4-carbonitrile (2b). A solution of disulfide **9** (20 mg, 29 μmol) in ethanol (5 mL) was treated with excess $NaBH_4$ (15 mg) at room temperature. After 10 min excess [$^3H_3$]-methyl iodide (50 μL) was added and the mixture stirred at room temperature for 2 h. The reaction mixture was cooled down to 0 °C (ice-water bath) and unreacted $NaBH_4$ was destroyed by dropwise addition of aqueous HCl (10%, 1 mL). The mixture was neutralized and extracted with ethyl ether (3×10 mL). The organic fractions were combined and washed with brine, dried, and the solvent removed under reduced pressure. Column chromatography yielded pure compound **2b** (0.77 mCi, 82.5 Ci/mmol). MS: 367.2 and 369.2 (calcd 367). ^{1}H NMR (for (**2a**), $CDCl_3$ 300 MHz): δ 7.77 (2H, s), 4.55, (2H, broad), 2.54 (3H, s).