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1,5-BENZODIAZEPINES AS CCK-B ANTAGONISTS. EFFECT OF HALOGEN SUBSTITUTION AT THE BENZO-FUSED RING ON POTENCY AND SELECTIVITY.

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Abstract. Some 1,5-benzodiazepines substituted with halogen atoms in the benzo-fused ring were synthesized and evaluated as CCK antagonists at both CCK-A and CCK-B receptors. Details of the binding of these agents to both receptors are reported and discussed briefly.

Introduction. Polypeptide cholecystokinin (CCK) is found widely in the human body. It has been shown to act at the so called peripheral receptor (CCK-A) controlling the pancreatic and biliary secretion, gallbladder contraction and gut motility.[1] Interaction with the central CCK receptor (CCK-B) results in neuromodulation and neurotransmission.[2] In the light of such a variety of effects, the development of compounds able to interact selectively with the two CCK receptors is highly desirable for the treatment, *inter alia*, of anxiety,[3] memory disorders,[4] Parkinson's disease,[5] obesity and pancreatic conditions.[6]

A considerable amount of work has been carried out on the synthesis and evaluation of 1,4-benzodiazepines acting as CCK-A[7] or CCK-B antagonists.[8] More recently, a novel class of compounds, namely the 1,5-benzodiazepines of type **1** has been found to act as potent CCK-B antagonists.[9] (***Fig. 1***):

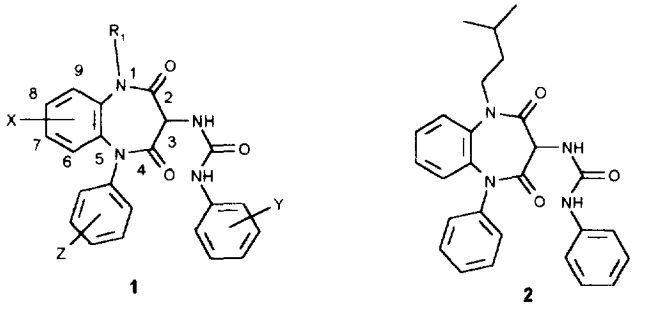


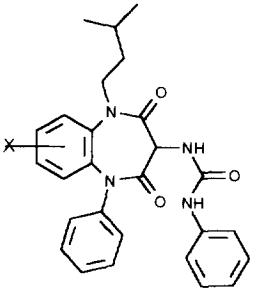
Fig. 1

In particular, compound **2** showed good potency and selectivity (pKi-A=6.49, pKi-B=8.81, selectivity (B/A)=209).

Among the many possible modifications of the benzodiazepine ring we were interested in the substitution at the benzo-fused ring with one or two halogen atoms.

It has been known for a long time the importance of 7-halogen substitutions for the activity of "classical" 1,4-benzodiazepines.[10] On the other hand, such a substitution does not seem to be of particular importance for the CCK antagonist activity of 1,4-benzodiazepines.[7]

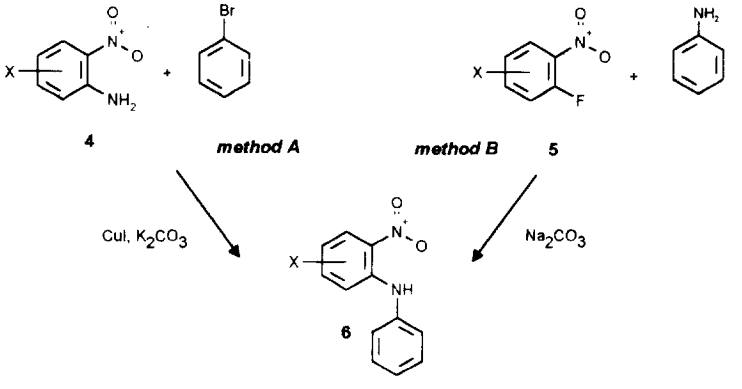
In order to assess the effect of a similar substitution pattern in the 1,5-benzodiazepines series, compounds **3a-e**, shown in ***Fig. 2***, were synthesized[11] and tested as CCK antagonists at both the central and peripheral receptors.



3a: X=8-Cl
3b: X=7-Cl
3c: X=7,8-diCl
3d: X=8-F
3e: X=7-F

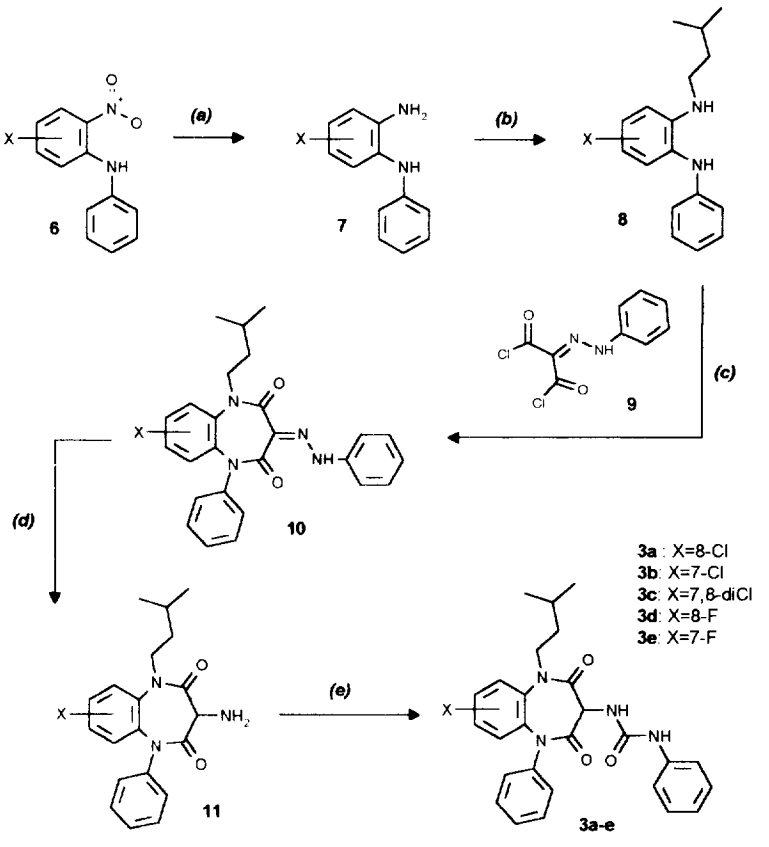
Fig. 2

Chemistry. The preparation of the above compounds was easily accomplished using existing procedures. The key intermediates of type **6** could be easily obtained according to the two methods depicted in ***Scheme 1***.[12]



Scheme 1

Once the halo-2-nitro-N-phenylanilines of type **6** were obtained, they were reduced with sodium dithionite to give the diamines **7**. Alkylation with isopentylbromide afforded compounds **8** which were cyclized by reaction with the phenylhydrazonomalonyl dichloride, **9**,[13] to the benzodiazepines **10**. These compounds were reduced to the amines **11** by means of powdered zinc in acetic acid. Reaction with phenylisocyanate yielded the final compounds **3a-e** (***Scheme 2***).



(a) $Na_2S_2O_4$, K_2CO_3, $EtOH/H_2O$; *(b)* isopentyl bromide, NaI, DMF; *(c)* **9**, THF; *(d)* Zn, AcOH, *(e)* PhNCO, CH_3CN

Scheme 2

A typical synthetic protocol is given below:

Intermediates **6**

method A

The halo-2-nitroaniline of type **4** (1 equivalent), was dissolved in bromobenzene (6.5 equivalents). Then potassium carbonate (0.34 equivalent) and CuI (0.1 equivalent) were added. After stirring at 150 °C for 36

hours, the crude material was purified by flash-chromatography eluting with mixtures cyclohexane/ethyl acetate yielding the corresponding compound of type **6** (32-63 % yields).

method B

A mixture of the halo-2-fluoronitrobenzene of type **5** (1 equivalent), aniline (3 equivalents) and sodium carbonate (1 equivalent) was stirred at 180 °C for 3 h. The reaction mixture was cooled to room temperature, then diluted with dichloromethane, washed with water, dried and evaporated under vacuum to give the crude compound which was purified by flash chromatography eluting with mixtures cyclohexane/ethyl acetate yielding the corresponding compound of type **6** (98% yield).

Intermediates 7

To a suspension of the halo-nitroaniline of type **6** in 95% ethanol, a solution of potassium carbonate (4 equivalents) and sodium hydrosulfite (3 equivalents) in water was added (the ethanol/water ratio is 3:2 in volume). The mixture was stirred at 23 °C for 2 hours, then it was acidified to pH=3.5 with concentrated hydrochloric acid and concentrated *in vacuo*. A 10% solution of sodium hydroxide was added until pH=10 and the solution was extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated *in vacuo* to give the crude compound which was purified by flash chromatography using mixtures cyclohexane/ethyl acetate as eluent to give the desired diamine of type **7** (62-70% yields)

Intermediates **8**

1-Bromo-3-methylbutane (1 equivalent) was added to a solution of the halo-diamine of type 7 and sodium iodide (1 equivalent) in dimethylformamide under a nitrogen atmosphere. The solution was stirred at 120 °C for 10 h, then cooled to room temperature, diluted with water and extracted with ethyl ether. The organic layer was washed with brine, dried and concentrated *in vacuo* to give a the crude material which was purified by flash chromatography eluting with mixtures cyclohexane/ethyl acetate to give the alkylated corresponding diamine **8** (49-62% yields)

Intermediates **10**

The halo-N-phenyl-N'-alkyl dianiline of type **8** (1 equivalent) and the phenylhydrazonomalonyldichloride, **9**, (1.17 equivalents) were each taken up in THF and dropped in a flask containing THF maintained under a nitrogen atmosphere. After complete addition the solution was heated to 70 °C for 1 h. The solution was diluted with ethyl acetate, washed with 5% sodium hydrogen carbonate solution and brine, dried and concentrated *in vacuo* to give an oil, which was purified by flash chromatography eluting with mixtures cyclohexane/ethyl acetate to give the desired cyclized compound **10** (61-83% yields).

Intermediates **11**

Zinc dust (10 equivalents) was added to a solution of the hydrazone of type **10** (1 equivalent) in glacial acetic acid. The mixture was stirred at 23 °C for 2 h, then diluted with 10% solution of sodium hydroxide until pH=9 and the mixture extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated *in vacuo* to give the crude material which was purified by flash chromatography eluting with mixtures cyclohexane/ethyl acetate to give the corresponding amine of type **11** (54-73% yields).

Final Compounds **3a-e**

Phenyl isocyanate (1.5 equivalents) was added to a solution of the intermediate **11** (1 equivalent) in dry acetonitrile under a nitrogen atmosphere. The mixture was stirred at 23 °C for 30 min, diluted with dichloromethane, washed with brine, dried and evaporated to give the crude compound which was triturated with ethyl ether to give the desired corresponding urea **3** (45%-94% yields).

Biology. Compounds **2** and **3a-e** were tested as displacers in the binding of [^{3}H]-CCK$_{8S}$ on guinea-pig brain CCK-B receptors[14] and on rat pancreas CCK-A receptors[15] in order to evaluate their potencies and selectivities. The results are summarized in ***Table 1***.

Table 1

compound	X	pKi-A(a)	pKi-B(a)	selectivity B/A
2	H	6.49	8.81	209
3a	8-Cl	6.02	7.38	23
3b	7-Cl	6.02	8.31	195
3c	7,8-diCl	5.80	8.01	162
3d	8-F	<6.00	8.05	>110
3e	7-F	6.65	8.34	49

(a) mean value of two experiments

Considering the "chloro" series, the binding affinities are always lower than those of the unsubstituted compound **2** at both receptors. In the "fluoro" series this is true only for the 8-fluoro derivative (**3d**) whereas the 7-fluoro compound (**3e**) shows a contrasting effect (increased pKi-A value and decreased pKi-B). In both series the substitution of position 7 seems to cause higher pKi-B values than the 8-substituted analogues. No trend can be assessed for the binding affinity *vs.* the electronegativity of the halogen. The overall effects results in both series in a decreased B/A selectivity with respect to the unsubstituted **2**.

Conclusion. A series of variously substituted 7 and/or 8 halo-1,5-benzodiazepines have been synthesized and evaluated *in vitro* as CCK-A and CCK-B antagonists. From the data shown in ***Table 1*** it is difficult to draw any clear conclusion or general rules as to the effect of the nature, position or number of halogen substitutents. Although none of the compounds showed an improved profile with respect to **2**, in one case (compound **3b**) the selectivity was very close (195 *vs.* 209).[16]

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16. In the continuation of our studies directed towards the identification of compounds endowed with higher CCK-B affinity and selectivity, the reported substitutions with halogens at the benzo-fused ring were more successfully applied to a different series of 1,5-benzodiazepines. This work will be reported in due course.

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