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SYNTHESIS AND EVALUATION OF NOVEL 1,5-BENZODIAZEPINES AS POTENT AND SELECTIVE CCK-B LIGANDS. EFFECT OF THE SUBSTITUTION OF THE N-5 PHENYL WITH ALKYL GROUPS.

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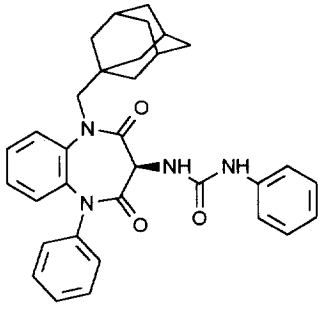
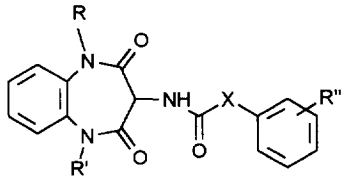
Abstract: The synthesis and biological evaluation of both 3-ureido and 3-carbamate derivatives of 1,5-benzodiazepines bearing bulky alkyl substituents at N-1 and N-5 positions is reported. Their activity as CCK-B receptor antagonists is discussed and compared with the related N-5-phenyl derivatives.

Introduction. The action of Cholecystokinin (CCK) on the central CCK-B receptor is responsible for the modulation of anxiogenic responses and pain.[1,2] Recently, we have found that 3-ureido[3] and -carbamate[4] 1,5-benzodiazepines bearing branched and/or bulky alkyl groups at the N-1 position and an N-5 aryl substituent are potent CCK-B antagonists. Our investigations in this field led to the discovery of GV150013X (**Fig 1, I**) as a very potent, selective and long lasting compound, active *in vitro* and *in vivo*, which has been selected for exploratory development in the treatment of panic attacks and anxiety.[5]



I

GV150013X



II

Fig. 1

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During our studies, we investigated a variety of possible modifications on the benzodiazepine nucleus which could improve the potency and selectivity parameters.[6] Among these, the type of substituent at N-1 was particularly significant in determining the overall activity of the molecule. Also the modulation of the substitution at the ureido and/or the N-5 phenyl rings or even on the benzofused ring[7,8] led to further refinements of the pharmacological properties of the class. Finally, a dramatic increase in B receptor selectivity was achieved when the resolution of the stereogenic center at C-3 was performed on the more active racemic compounds.[9,10]

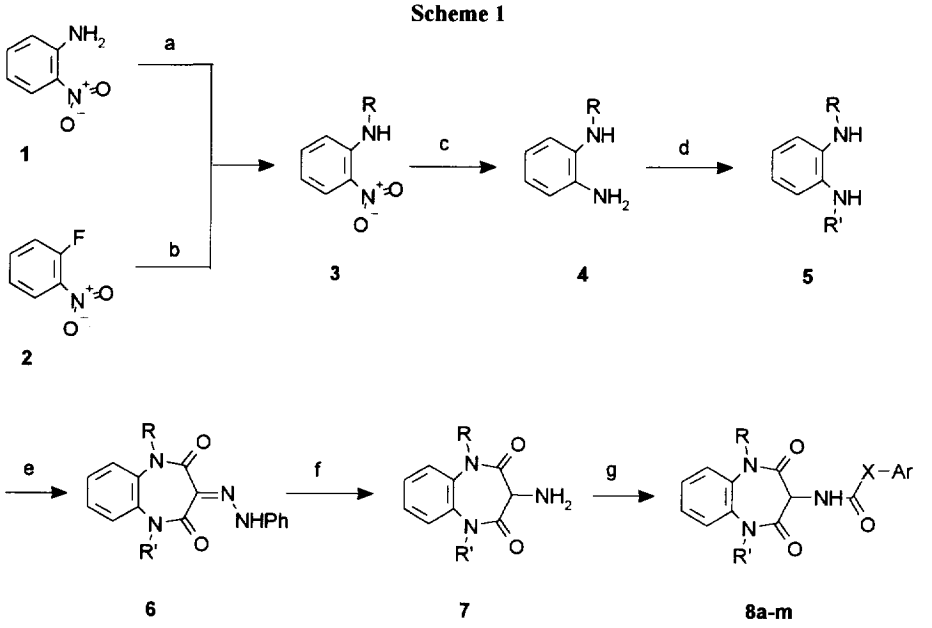
Then, in order to understand the importance of the substituent at N-5 with regards to both potency and selectivity, we turned our attention to the replacement of the phenyl ring with substituted alkyl or cycloalkyl groups. At the same time, we wished to evaluate the importance of the stereogenic center at C-3. Therefore, we undertook the synthesis of both symmetrical and asymmetrical 1,5-benzodiazepines (**Fig. 1, II**), and prepared both the 3-ureido and 3-carbamate derivatives. As a first approach, we maintained highly branched and bulky groups at N-1, such as 1-adamantylmethyl and 3-methylbutyl which had already been evaluated in the N-5-phenyl series. So, the first issue to be established was whether the replacement of the N-5 phenyl ring by an alkyl or cycloalkyl group could still afford active compounds. The replacement by a cyclohexyl group was deemed to be a determining example; the other alkyl groups selected as N-5 substituents were 1-adamantylmethyl, 3-methylbutyl, 2-cyclopentylpropyl and benzyl. In this paper we wish to report the preliminary results of these investigations.

Chemistry. The preparation of the appropriate disubstituted phenylene diamine **5** (**Scheme 1**) had to be optimized for each case. The starting material could either be o-nitroaniline **1** or o-nitrofluorobenzene **2**, which, by alkylation or aromatic nucleophilic substitution, afforded the nitroanilines **3**. Reduction of the nitro group followed by alkylation or reductive amination yielded the key intermediates **5**. Alternatively, o-phenylene diamine **9** (**Scheme 2**) could also be used as the starting material, to give both symmetrical and asymmetrical disubstituted diamines **5**. Hence, the synthetic pathway proceeded as in the parent N-5 phenyl series: condensation with phenylhydrazonomalonyl dichloride, reduction, then urea or carbamate formation, to give the final compounds **8a-m**.[3]

The asymmetrical compounds were synthesized as racemic mixtures at C-3. A number of chemical and analytical methods for the resolution into enantiomers of the more selective compound in this class, namely, N-1-(1-Adamantylmethyl)-N-5-cyclohexyl benzodiazepine derivative **8a**, were attempted but met with little success.

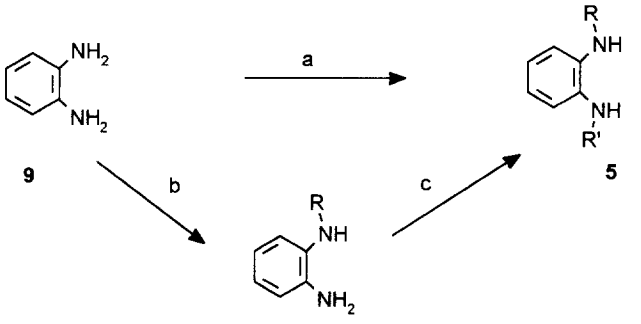
All compounds were evaluated *in vitro* for their CCK-A and -B receptor affinity and selectivity, by measuring their ability to displace tritiated CCK-8S bound on guinea pig brain CCK-B receptors[11] and on rat pancreas CCK-A receptors.[12] Affinity is reported as pKi values.

Scheme 1



a) RBr, K2CO3/CuI, toluene, reflux, 8h, 20-40%; b) RNH2, toluene, 0°C, quant.; c) Na2S2O4, K2CO3, EtOH/H2O, 23°C, 24h, or Fe/HCl, EtOH/H2O, reflux, 30min, 45-90%; d) NaI, R'X, DMF, 140°C, or R'CHO, NaBH4, AcOH/AcONa, EtOH/H2O, 35-50%; e) $PhNHN{=}C(COCl)_2$, THF, 50°C, 50-90%; f) Zn/AcOH, 23°C, 70-80%; g) ArNCO, AcCN or ArOCOCl/Py, CH_2Cl_2, 23°C, 50-85%.

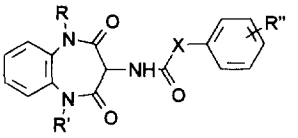
Scheme 2



a) RBr, K2CO3, DMF, 23°C or reflux, 60%; b) RCHO, NaBH4, AcOH/AcONa, EtOH/H2O, 23°C, 20-40%; c) R'X, NaI, DMF, 23°C or reflux, 25-55%.

Discussion. The affinity values for CCK-A and CCK-B receptors of the 1,5-benzodiazepines synthesized are shown in **Table 1** and are compared to GV150013X[5,6] and to its racemic precursor.

TABLE 1



Entry	R	R'	X	R''	CCK-B	CCK-A	B/A
GV150013X I (+ isomer)	CH2-1-Adamantyl	Phenyl	NH	H	9.02	5.95	1175
I (racemic mixture)	CH2-1-Adamantyl	Phenyl	NH	H	8.64	6.15	309
8a	CH2-1-Adamantyl	Cyclohexyl	NH	H	8.62	6.25	234
8b	CH2-1-Adamantyl	Cyclohexyl	NH	Cl	7.97	6.62	22
8c	CH2-1-Adamantyl	Cyclohexyl	O	H	7.37	5.96	26
8d	CH2-1-Adamantyl	3-Methylbutyl	NH	H	8.86	6.54	209
8e	CH2-1-Adamantyl	3-Methylbutyl	NH	m-NMe2	8.25	6.02	170
8f	CH2-1-Adamantyl	3-Methylbutyl	O	H	7.47	5.85	42
8g	CH2-1-Adamantyl	3-Methylbutyl	O	m-NMe2	8.12	5.80	209
8h	3-Methylbutyl	Benzyl	NH	H	8.41	7.18	17
	R = R'						
8i	3-Methylbutyl		NH	H	8.62	7.21	26
8j	3-Methylbutyl		NH	m-NMe2	8.59	7.08	32
8k	3-Methylbutyl		O	m-NMe2	7.54	6.10	28
8l	CH2-1-Adamantyl		NH	H	7.46	5.70	58
8m	CH(CH3)CH2-cyclopentyl		NH	H	7.77	6.64	11

As can be seen in **Table 1**, the asymmetrically substituted derivatives gave good results in terms of B/A selectivity, especially in the case of the N-1-(1-Adamantylmethyl)-N-5-cyclohexyl derivative **8a**. This result made it clear that the N-5-phenyl ring was not essential for the activity on CCK receptors and that its replacement with appropriate alkyl groups would give active compounds. This was further shown by compounds **8d**, **8e** and the carbamate **8g**, the latter having a significantly high selectivity for a carbamate derivative not substituted on the benzofused ring.[7]

Generally speaking, the asymmetric derivatives here reported (entries **8a-h**) show a slightly lower affinity for the B receptor compared to that of the corresponding N-5-phenyl compounds,[6-8] while maintaining the affinity for the A receptor. In addition, it is shown that those bulky alkyl groups that gave the better pharmacological profiles in the N-5-phenyl series, still give good results also in the N-5-alkyl series.

As in the N-5-phenyl series, an electronwithdrawing substituent such as chlorine on the ureido phenyl ring lowers the overall activity (**8b**). The benzyl group (**8h**) as a replacement for phenyl has a dramatic detrimental effect on the selectivity, while the corresponding N-1-(3-Methylbutyl)-N-5-phenyl derivative showed a B/A selectivity value of 209.

As far as the symmetrical derivatives are concerned, a strong decrease in affinity for both receptors is observed for entries **8k-m**, although the selectivity value for entry **8l** is maintained to some extent. Better affinity for the CCK-B receptor is shown by the compounds in entries **8i-j**; but unfortunately, the affinity for the A receptor is too high.

These results clearly indicated that a strong feature for a benzodiazepine as a selective CCK-B ligand was the permanence of the chirality at the C-3 position. Conversely, it appears obvious from the data above that N-5-alkyl benzodiazepines were a promising class of compounds active at the CCK-B receptor, giving derivatives potentially as active as the parent N-5-phenyl counterparts, and that there was space for further modulation of their pharmacological profile by carefully selecting the type of alkyl substituents to be introduced at the N-1 and N-5 positions. Highly selective CCK-B ligands belonging to this class were thus at hand and the results of these further investigations will be reported in due course.

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