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## THE SYNTHESIS OF A RADIOLIGAND WITH HIGH POTENCY AND SELECTIVITY FOR CCKB/ GASTRIN RECEPTORS

S. Barret Kalindjian\*, Elaine A. Harper, and Michael J. Pether

James Black Foundation, 68 Half Moon Lane, London SE24 9JE, UK

Abstract: The synthesis and characterisation of a non-peptide, tritiated radioligand, [<sup>3</sup>H] JB93182, which has high affinity and selectivity for CCKB/gastrin receptors, is described. Copyright © 1996 Elsevier Science Ltd

Gastrin and cholecystokinin are structurally related peptide hormones which are found both in the CNS and in gastrointestinal tissue.<sup>1</sup> Two classes of receptors for these hormones have been classified, namely CCKA and CCKB/gastrin, and selective non-peptide antagonists for each have been described.<sup>2</sup> We have recently reported on two new series of selective CCKB/gastrin receptor antagonists, <sup>3-5</sup> which culminated in the identification of the indole derivative, JB93182, compound 1,<sup>6</sup> as a ligand of particular interest. Some of the binding properties of this compound have already been disclosed.<sup>5,7</sup>

In order to help elucidate the possible plurality in CCKB/gastrin binding sites as has been suggested,<sup>8-10</sup> we felt that it was important to examine various receptor containing tissues with a radiolabelled antagonist. The most commonly used radioligand for the CCKB/gastrin receptor system is the agonist [<sup>125</sup>I] Bolton Hunter CCK8S and, indeed, assays using this material had helped to identify JB93182.<sup>5</sup> Only a few examples of radiolabelled antagonists at CCKB/gastrin receptors are available. [<sup>3</sup>H]-L-365,260 has been reported by Chang *et al.* <sup>11</sup> but while this material has an adequate selectivity profile with respect to CCKA receptors,<sup>12</sup> it has been shown to have a relatively poor specific to non-specific binding ratio.<sup>13</sup> Both a tritiated<sup>13</sup> version of the Parke-Davis ligand PD 140,376 (**2**) and an iodinated<sup>14</sup> analogue have also been described. However, in some assays PD compounds from this series have been shown to behave as partial agonists<sup>15</sup> at CCKB/gastrin receptors. Our observations using the cold ligand suggest that this is a feature shared by PD 140,376,<sup>16</sup> which in some cases might lead to a lack of clarity in interpreting radioligand binding data.

This communication will describe the preparation and radiochemical characterisation of  $[^{3}H]$ -JB93182 (compound 8).









Reagents: a) 3,5-bis(benzyloxycarbonyl)aniline,  $iPr_2NEt$ ,  $PyBrOP^{\textcircled{R}}$ , DCM; b) (i) TFA, aq K<sub>2</sub>CO<sub>3</sub>; (ii) Indole-5,6-dicarboxylic acid anhydride, CH<sub>3</sub>CN,  $\Delta$ ; c) 1-Adamantanemethylamine,  $iPr_2NEt$ ,  $PyBOP^{\textcircled{R}}$ , DCM; d) LiOH, THF, H<sub>2</sub>O, MeOH,  $\Delta$ ; e) T<sub>2</sub>, 10% Pd on C,  $iPr_2NEt$ , EtOH.



The synthesis was carried out in five steps as shown in Scheme 1. The initial target, the iodo derivative 7, was chosen as it allowed the possibility of introducing either an iodinated or a tritiated label in a final step. Thus the BOC derivative of 4-iodo-L-phenylalanine, 3, was coupled with 3,5-bis(benzyloxycarbonyl)aniline<sup>17</sup> in dichloromethane (DCM) solution in the presence of the coupling reagent PyBrOP<sup>®</sup> [CAS reg. No. 132705-51-2, Novabiochem] and diisopropylethylamine. The reaction produced a 64% yield of the condensed product 4. Removal of the BOC protection from this intermediate using TFA, revealed an amine which was reacted with indole-5,6-dicarboxylic acid anhydride,<sup>5</sup> in refluxing acetonitrile for 30 min. A mixture of compound 5 and the regioisomer with side-chains reversed was obtained in an overall yield of 40% and in an approximately 4:1 ratio. No attempt was made to separate the regioisomers at this stage, instead the mixture was reacted with 1-adamantanemethylamine in dichloromethane solution in the presence of the coupling reagent PyBOP® [CAS reg. No. 128625-52-5, Novabiochem] and diisopropylethylamine. After stirring overnight at room temperature, the desired less polar regionsomer 6 was isolated by column chromatography in 35% yield. This material was treated for about 1 min with a hot aqueous lithium hydroxide solution in a mixture of methanol-THF to give the crude diacid 7 which was purified by reverse phase HPLC.<sup>18</sup> In order to confirm that this key intermediate corresponded to the correct regioisomer of JB93182, a small amount was treated with hydrogen over 10% palladium on charcoal to remove the iodine. This gave a material that co-eluted on HPLC with an authentic sample of JB93182.

With compound 7 in hand, attempts were made to introduce a radiolabel. An iodine exchange reaction was carried out, but the radiolabelled material isolated proved to be too unstable for use. Instead tritiation was performed using tritium gas over 10% palladium on charcoal in a mixture of diisopropylethylamine and ethanol. This procedure incorporated a single tritium atom into the compound to leave the desired radiolabelled material, compound 8, with a specific activity of 28 Ci/mmol and a radiochemical purity of at least 97% by HPLC. Saturation analysis indicated that compound 8 labelled a homogenous population of sites in rat cerebral cortical membranes. The B<sub>max</sub> was  $3.61\pm 0.65$  pmol g<sup>-1</sup> (original wet weight of tissue), and the pK<sub>D</sub> was  $9.48\pm0.08$  (n=5 ± s.e. mean). A range of CCKB/gastrin receptor ligands such as L-365,260 (pK<sub>i</sub> 7.63± 0.07, Hill slope  $1.17\pm0.13$ , n=8) competed with the binding of compound 8 to rat cortical membranes. A more

detailed analysis of the pharmacological behaviour of this labelled ligand and the significance of these results are presented elsewhere.19,20

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