Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 21, pp. 2501-2506, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00435-1

SYNTHESIS OF NOVEL IODINATED RADIOLIGANDS WITH HIGH AFFINITY AND SELECTIVITY FOR CCK-B/GASTRIN RECEPTORS

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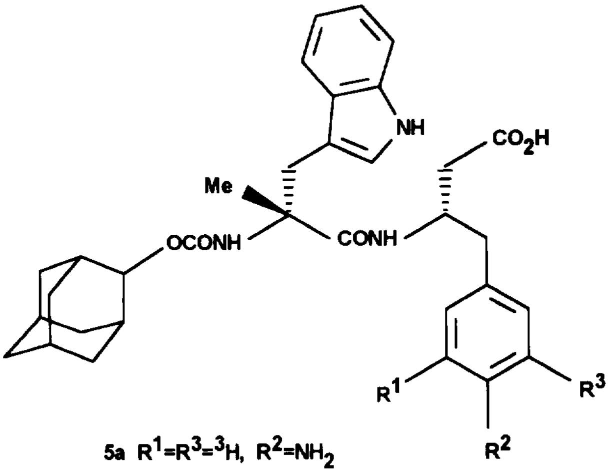
Abstract: The syntheses of two non-peptide radio-iodinated ligands [^{125}I]-PD142308 and [^{125}I]-PD142251 with high affinity and selectivity for the CCK-B/gastrin receptor are described via a route which introduces the radiolabel at the final or penultimate step. The CCK-A and CCK-B binding affinities of both ligands are described.

Recent interest in the neuropeptide cholecystokinin has resulted in the development of potent and selective antagonists for both the CCK-A and CCK-B/gastrin receptor subtypes[1,2]. Human CCK-B and gastrin receptors have recently been cloned and shown to be identical [3,4]. Potential therapeutic uses of CCK-B antagonists include the treatment of gastro-intestinal disorders e.g. gastric ulcers and gastrin dependant tumours and CNS disorders e.g. anxiety, pain and panic[1]. We have recently described a series of chemically novel, potent and selective CCK-B receptor antagonists[5] and reported the synthesis of an example of this series in tritiated form, [^{3}H]-PD 140376[6] (5a). This ligand has been shown to have advantages over the only current alternative radioligand [^{3}H]-L365260, reported by Chang et al.[7], in that it has greater affinity and selectivity for the CCK-B/gastrin receptor than [^{3}H]-L365260 and has a higher ratio of specific to non-specific binding in both cortex and gastric mucosa[8]. [^{3}H]-PD 140376 also has the potential for cross-linking to the receptor via the arylamino moiety[9]. We wished to synthesise radio-iodinated analogues of this general series in order to obtain ligands with higher specific activity. In addition, the synthetic route allows for incorporation of the radiolabel at the final or penultimate step, and could be adapted to give ligands suitable for imaging techniques such as SPECT (single photon emission computed tomography)[10], or PET (positron emission tomography)[10,11]. Traditionally radio-iodinations have utilised aqueous media but these methods proved unsuitable in this case due to the insolubility of the ligands and consequently alternative methods were investigated. The syntheses of two radio-iodinated ligands is described together with an account of their CCK receptor binding profiles.

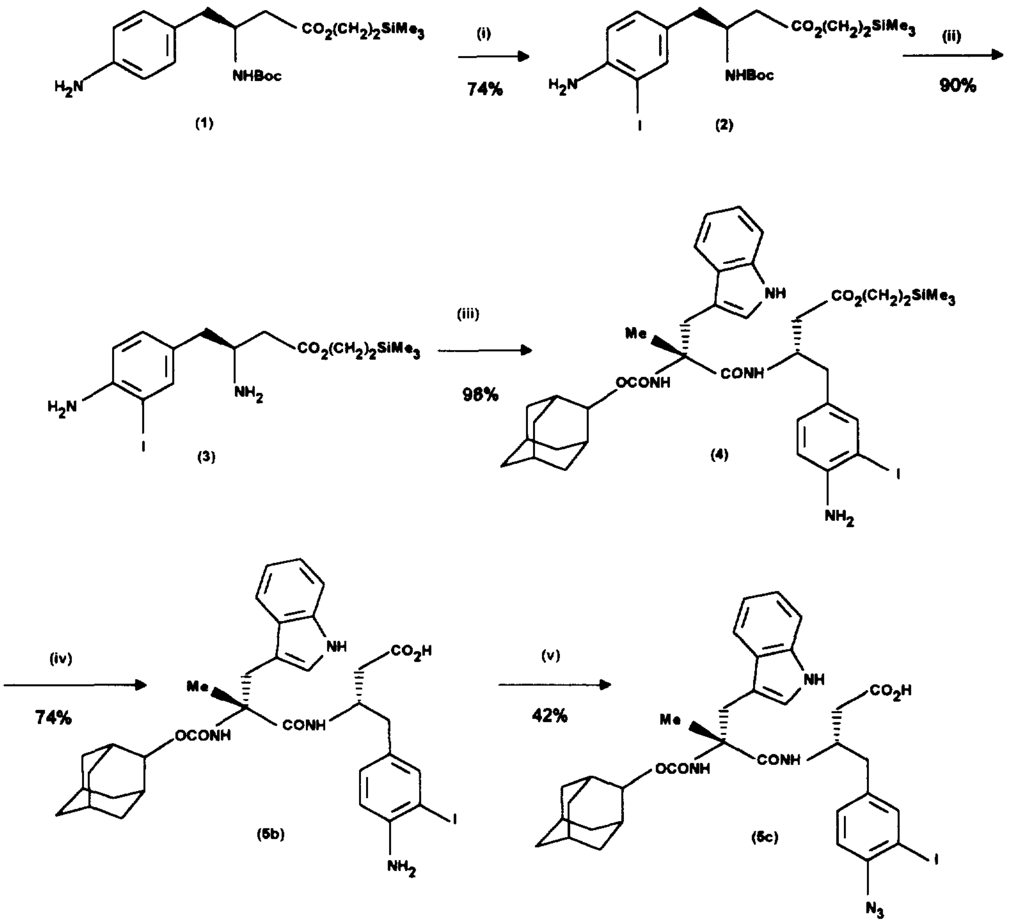


5a $R^1=R^3={}^3H$, $R^2=NH_2$

5b $R^1=H$, $R^2=NH_2$, $R^3=I$

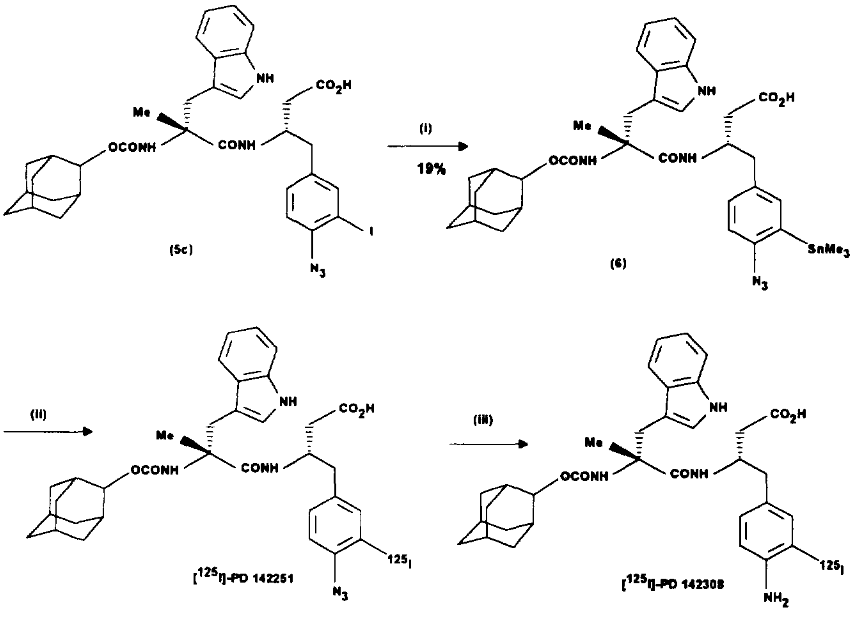
5c $R^1=H$, $R^2=N_3$, $R^3=I$

Scheme 1



Reagents: i) NaI, chloramine T, AcOH; ii) p-toluenesulphonic acid, CH_2Cl_2;
iii) 2-Adamantyloxycarbonyl-α-Me-Trp-OH(ref. 6), DCC, HOBt, EtOAc; iv) TBAF, THF; v) NaN_3, NaONO, AcOH,H_2O.

Both target compounds (5b)[12] and (5c)[13] exhibit high potency and selectivity for the CCK-B/gastrin receptor and have the potential for crosslinking to the receptor via chemical or photochemical means[9]. The [^{125}I]-radioligands were synthesised from a common intermediate introducing the radiolabel as the final or penultimate step. The key intermediate PD 160197 (6)[14] in the synthesis of both radioligands was obtained from the di-protected intermediate (1)[6] in six steps (Scheme 1). The procedure for synthesising the 3-iodo-4-aminophenyl intermediate (5b) has been described for the related di-iodo analogue[6,12], and can be adapted for the synthesis of (6) by using only one equivalent of sodium iodide and chloramine T in the iodination step. Conversion of the amine to an azide was achieved by treatment of (5b) with excess sodium nitrite and sodium azide in aqueous acetic acid, and the compound obtained was converted to the corresponding trimethyltin derivative (6) by treatment with hexamethyl di-tin in the prescence of tetrakis(triphenylphoshine)palladium(0), following the procedure of Blaszczak et al.[16] This key intermediate was purified prior to the radio-iodination by reverse phase chromatography.



Reagents: (i) $Pd(PPh_3)_4$, Sn_2Me_6, dioxane (ii) NaI, H_2O_2, MeOH (iii) NEt3, propane-1,3-dithiol, MeOH

Scheme 2

Iodination of the purified intermediate with sodium iodide and hydrogen peroxide gave [^{125}I]-PD 142251 which was converted to [^{125}I]-PD 142308 by reduction of the azide with propane-1,3-dithiol[17] in the presence of triethylamine and the product purified by HPLC.

In homogenate binding studies, using [^{125}I]-Bolton and Hunter labelled CCK-8S and mouse cerebral cortex (CCK-B) and rat pancreas (CCK-A) membranes, prepared as described elsewhere[18], both PD 142308 (CCK-B K_i 0.52 nM ; CCK-A K_i 158 nM) and PD 142251 (CCK-B K_i 0.88 nM ; CCK-A K_i 392 nM) were found to have high affinity and selectivity for the CCK-B receptor. The specific binding of [^{125}I]-PD 142308 and [^{125}I]-PD 142251 to guinea-pig[8] and pig[19] cerebral cortex membranes, respectively, was saturable with both radioligands binding with high affinity to an apparent single population of binding sites. The equilibrium binding constant (K_D) and maximal binding capacity (B_{max}) were, respectively, 0.25 nM and 200 fmol/mg protein for [^{125}I]-PD 142308 and 8.1 nM and 5.8 pmol/mg protein for [^{125}I]-PD 142251. For comparison, the K_D and B_{max} values for [^{3}H]-PD 140376 were 0.11nM and 119 fmol/mg protein[8]. The specific binding of [^{125}I]-PD 142308 was inhibited by CCK-related peptide agonist ligands including CCK-8S (K_i 0.53nM) and pentagastrin (K_i 14.5 nM) and also by the CCK-B receptor selective antagonists CI-988[18] (K_i 0.97 nM) and unlabelled PD 142308 (K_i 0.40 nM). Similarly for [^{125}I]-PD 142251 the rank order of agonists was CCK-8S (K_i 1.3nM) > pentagastrin (K_i 52 nM) and for antagonists the unlabelled form of PD 142251 (K_i 7.6 nM) > CI-988 (K_i 12 nM).

Acknowledgement: We are grateful to Amersham International for performing the radiolabelling experiments[20].

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12. Compound 9b. mp 117-122°c; $[\alpha]_D^{20}$=+14° (c=1,MeOH); ^{1}H nmr (400MHz, d_6-DMSO) δ 1.17 (s, 3H, CH_3), 1.40-1.50 (m, 2H) and 1.60-1.80 (m, 8H) and 1.85-1.95 (m, 4H, 5xCH_2 and 4xCH of adamantyl), 2.21 (dd, J=15.9,6.6Hz, 1H, $CH\underline{H}CO_2$), 2.29 (dd, J=15.6,5.9Hz, 1H, $CH\underline{H}CO_2$), 2.55 (dd, obscured by DMSO, 1H, $CH\underline{H}Ph$), 2.55 (dd, J=13.4,7.3Hz, 1H, $C\underline{H}HPh$), 3.08 (d, J=14.4Hz, 1H, $C\underline{H}H$-indole), 3.3 (d, obscured by water, 1H, $C\underline{H}H$-indole), 4.00-4.10 (m, 1H, CH), 4.62 (s, 1H, $C\underline{H}OCONH$), 4.97 (s, 2H, NH_2), 6.61 (d, J=8.3Hz, 1H, CH) 6.65 (bs, 1H, OCONH), 6.80-6.85 (m, 3H, 3xCH), 6.97 (t, J=7.8Hz, 1H, CH), 7.25 (d, J=7.8Hz, 1H, CH), 7.32 (s, 1H, CH), 7.40 (d, J=7.6Hz, 1H, CH), 7.57 (d, J=7.8Hz, 1H, CONH), 10.78 (s, 1H, NH), 12.2 (bs, CO_2H). Anal. Calc'd for $C_{33}H_{39}IN_4O_5.0.5H_2O$: C, 56.01; H, 5.70; N, 7.92. Found: C, 55.96; H, 5.73; N, 7.85.

13. Compound 9c. mp 111-6°c; $[\alpha]_D^{20}$ =0° (c=1,MeOH); ^{1}H nmr (300 MHz, d_6-DMSO) δ 1.22 (s, 3H, CH_3), 1.40-1.60 (m, 2H) and 1.70-1.90 (m, 8H) and 1.90-2.00 (m, 4H, 5xCH_2 and 4xCH of adamantyl), 2.30-2.50 (m, 2H, CH_2CO_2), 2.70-2.80 (m, 2H, CH_2Ph), 3.13 (d, J=14.6Hz, 1H, $C\underline{H}H$-indole), 3.38 (d, 1H, obscured by water, $CH\underline{H}$-indole), 4.15-4.30 (m, 1H, CH), 4.70 (s, 1H, $C\underline{H}OCONH$), 6.74 (bs, 1H, OCONH), 6.90-7.00 (m, 2H, 2xCH), 7.05 (t, J=7.2Hz, 1H, CH), 7.20-7.40 (m, 3H, 3xCH), 7.46 (d, J=8.2Hz, 1H, CH), 7.69 (s, 2H, CH and CONH), 10.87 (s, 1H, NH), 12.3 (bs, CO_2H). Anal. Calc'd for $C_{33}H_{37}IN_6O_5.0.5H_2O$: C, 54.03; H, 5.08; N, 11.46. Found: C, 54.06; H, 5.12; N, 11.27.

14. Compound 10. ^{1}H nmr (300MHz, d_6-DMSO) δ 0.25 (s, 9H, $Sn\{CH_3\}_3$), 1.17 (s, 3H, CH_3), 1.40-1.50 (m, 2H), and 1.65-2.00 (m, 12H, 5xCH_2 and 4xCH of adamantyl), 2.30-2.40 (m, 2H, CH_2CO_2), 2.70-2.80 (m, 2H, CH_2Ph), 3.10 (d, J=15.0Hz, 1H, $CH\underline{H}$-indole), 3.3 (obscured by water, $CH\underline{H}$-indole), 4.15-4.25 (m, 1H, CH), 4.64 (s, 1H, $C\underline{H}OCONH$), 6.66 (bs, 1H, OCONH), 6.80-6.90 (m, 2H, 2xCH), 7.00 (t, J=7.4Hz, 1H, CH), 7.10-7.25 (m, 3H, 3xCH), 7.29 (d, J=8.3Hz, 1H, CH), 7.41 (d, J=7.8Hz, 1H, CH), 7.61 (d, J=8.8Hz, 1H, CONH), 10.8 (s, 1H, NH).

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20. [^{125}I]-PD 142308 will be commercially available from Amersham International.

(*Received in Belgium* 20 *July* 1995; *accepted* 21 *September* 1995)