Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVIII, No. 12

Synthesis of Iodine-123 Labelled Analogues of Imidazenil and Ethyl-Imidazenil for Studying Benzodiazepine Receptors Using SPECT

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Summary

The [^{123}I]iodinated analogues of the benzodiazepine receptor partial agonist imidazenil and N-ethyl imidazenil have been synthesised for the study of the central benzodiazepine receptor using SPECT. [^{123}I]Iodoimidazenil and [^{123}I]N-ethyliodoimidazenil were prepared by nucleophilic bromine-iodine exchange in acetic acid at 150°. The products were purified by semi-preparative reverse-phase HPLC with average radiochemical yields of 80% in a total synthesis time of 80 minutes. The specific activity was determined to be greater than 2500 Ci/mmol. The radiochemical and chemical purity assessed by radio-TLC and HPLC were found to be 98%. Alternatively, iododestannylation reactions via the trimethyltin precursors with Na[^{123}I] in the presence of Chloramine-T or peracetic acid resulted in yields of only 20-25% with the bulk of activity being lost as volatile methyl [^{123}I]iodide.

Key Words: Imidazenil, iodine-123, benzodiazepine receptor, partial agonist, SPECT.

Introduction

Imidazenil, 6-(2'-bromophenyl)-8-fluoro-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxamide **1** is a novel, low efficacy, [1,4]imidazobenzodiazepine which exhibits positive allosteric modulation of $GABA_A$ with specific high affinity binding to the benzodiazepine sites of the $GABA_A$ receptor complex (Ki = 0.5 nM).[1] It has been described as a new benzodiazepine recognition site ligand that induces selective pharmacological effects and behavioural characteristics qualitatively different from those of classical benzodiazepines such as diazepam, and alprazolam.[2] In addition, the pharmacological efficacy of this compound is associated with lower liabilities for sedation, muscle

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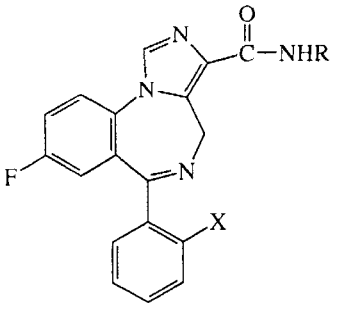
CCC 0362–4803/96/121121–12

Received 11 June 1996
Revised 6 September 1996

relaxation, tolerance and dependence and is comparable to the characteristics of the partial agonists Bretazenil.[2,3] Chronic administration of this compound in pharmacologically active doses in mice failed to induce tolerance to the effects of this drug on $GABA_A$ receptor function.[3] Although the detailed molecular interactions of partial allosteric modulators such as Imidazenil and Bretazenil have not been established, it has been suggested that the selective pharmacological efficacy may be attributed to preferential affinity or specific activation of subpopulations of $GABA_A$ receptors in distinct brain regions.[4-7]



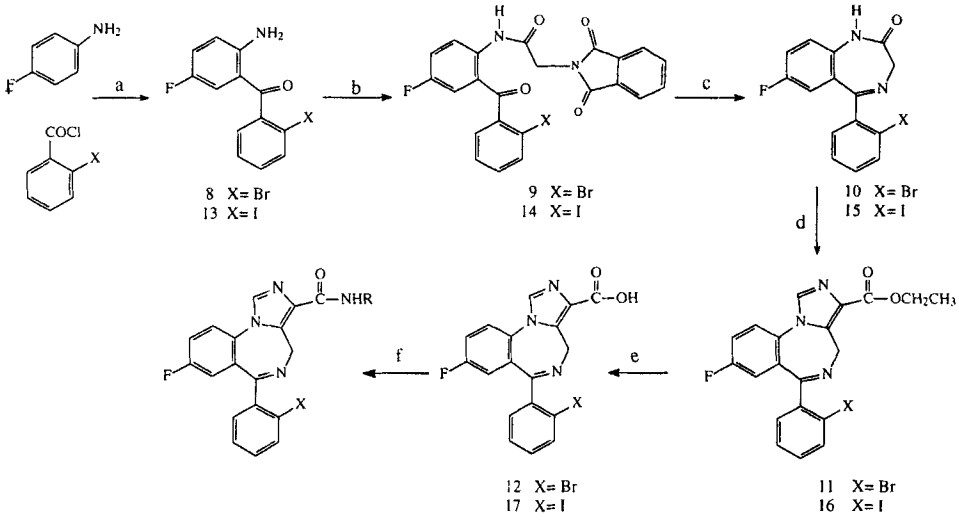
1	R = H,	X = Br
2	R = H,	X = I
3	R = Ethyl,	X = Br
4	R= Ethyl,	X= I
5	R = H,	X = ^{123}I
6	R= Ethyl,	X= ^{123}I
18	R = H,	X = $Sn(CH_3)_3$

Figure 1

The identification of structurally different and neuron specific $GABA_A$ receptor subtypes suggests that a selective pharmacological profile could be obtained by the availability of ligands with selective affinity for receptor subtypes.[8] As a consequence there has been a substantial effort into the design and synthesis of ligands with distinct chemical profiles and pharmacological efficacy.[9,10] Likewise there has been a considerable effort towards the synthesis of radioligands for the *in vitro* and *in vivo* study of $GABA_A$ receptors in neurodegenerative diseases using PET and SPECT.[11-13] The presence of the bromine atom in the 2' position of imidazenil lends itself to the development of other halogenated derivatives with retention of biological and pharmacological activity. Initial screening of the iodinated analogues **2** and **4** indicated potent *in vitro* binding to the central benzodiazepine receptor (BZR) of 1.1 nM and 0.006 nM respectively, while the intermediate esters **11** and **16** revealed only a moderate affinity of 7.4 nM and 178 nM ([^{3}H]flunitrazepam) respectively (unpublished data). Based on these results and the need to develop suitable radiotracers for probing BZR's using SPECT, we chose to label compounds **2** and **4** with iodine-123. Herein we report the synthesis and radiolabelling of the partial agonists imidazenil and N-ethyl imidazenil with iodine-123.

Results and Discussion

Imidazenil **1**, N-ethylimidazenil **3** and the iodinated derivatives **2** and **4** were synthesised according to modified methods described in the literature[14-16] (Scheme 1). The 2-amino-5-fluorophenyl-(2'-bromophenyl)methanone **8** and 2-amino-5-fluorophenyl-(2'-iodophenyl)methanone **13** were prepared by the condensation of the respective 2-halobenzoyl chlorides with 4-fluoroaniline in the presence of



Scheme 1

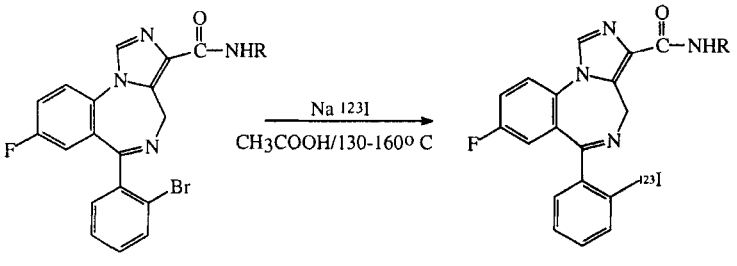
Reagents a: Anhydrous $ZnCl_2$, H_2SO_4; b: N-phthaloyl glycine acid chloride THF; c: NH_2NH_2/ methanol; d: Potassium *tert*-butoxide, diethyl chlorophosphate, ethyl isocyano-acetate/ potassium *tert*-butoxide -20°C, THF; e: 5M HCl; f: PCl_5, dry NH_3 or ethyl amine.

anhydrous zinc chloride followed by acid hydrolysis.[15,16] Reaction of the amino benzophenones with N-phthaloyl glycine acid chloride gave the amides **9** and **14** which upon hydrolysis of the N-phthaloyl protecting group with hydrazine in methanol gave the respective 1,4-benzodiazepines **10** and **15**. Addition of the anion of ethyl isocyanoacetate in THF at -20° to a solution of the iminophosphonate, prepared by the successive addition of potassium *tert*-butoxide and diethyl chlorophosphate to either **10** or **15** in THF at -20°, gave the ethyl imidazole esters **11** and **16**. Acid hydrolysis of the ethyl esters with 5N hydrochloric acid at 90° gave the corresponding acids **12** and **17**. Activation of the carboxylic acids with PCl_5 in dichloromethane followed by the addition of excess dry ammonia gave imidazenil **1** and iodoimidazenil **2**. The addition of ethylamine in place of ammonia to the above acid chlorides gave the corresponding N-ethyl derivatives **3** and **4**. The preparation of the corresponding tributyl stannane from imidazenil using bistributyltin and palladium tetrakistriphenylphosphine in refluxing toluene was sluggish even after 4 days of heating.[17] This was attributed to the low solubility of the bromo-derivative in toluene and possibly steric interactions between the 1,4-benzodiazepine ring and the ortho substituents of the phenyl ring. Heating the iodine analogue **2** in toluene containing 10 % DMF for 3-4 h resulted in complete solubilisation and the deposition of palladium(0) from the reaction mixture. Although TLC indicated the formation of a less polar compound suggestive of crude tributyl stannane, attempted isolation of this compound by column chromatography was unsuccessful. Preparation of the less bulky trimethyl stananne

derivative **18** was achieved by heating a mixture of iodoimidazenil **2**, bistrimethyltin and palladium tetrakistriphenylphosphine in refluxing toluene.[17] The reaction was complete in 4 hours, as evident from the formation of the black precipitate of palladium(0). The trimethyl stannane was obtained as an off-white crystalline solid after purification by flash chromatography using ethyl acetate-petroleum spirit followed by recrystallisation from ethyl acetate-hexane.

Synthesis and Purification of [^{123}I]Iodoimidazenil and [^{123}I]N-ethyliodoimidazenil

i) Nucleophilic halogen exchange. [^{123}I]Iodoimidazenil **5** and [^{123}I]N-ethyl-iodoimidazenil **6** were prepared by nucleophilic substitution of the 2'-bromine of imidazenil and N-ethylimidazenil respectively with Na^{123}I in acetic acid[18] (Scheme 2).



1 R = H
3 R= Ethyl

5 R = H
6 R = Ethyl

Scheme 2

The reaction was carried out by the addition of the bromo precursor in glacial acetic acid to the Na^{123}I which had been previously dried under a stream of nitrogen at 100°C. The radiochemical yield of the reaction was temperature and time dependant (Figure 2). At a concentration of 0.5 mg/ml, the optimum conditions for both compounds were 150° for 30-40 min. Prolonged heating at elevated temperatures (>160°) led to a significant amount of decomposition, reduced overall yields and complex mixtures of products as determined by HPLC and radio-TLC. Purification and isolation of the labelled product by semi-preparative reversed phase (RP) HPLC gave products with radiochemical yield of 80 ± 5% (n=24) with radiochemical and chemical purity ≥98% as assessed by HPLC and radio-TLC. Low concentrations of the bromoprecursor and long HPLC retention times were required to ensure its absence in the final solution (Figure 3). The specific activity of the final product was >2500Ci/mmol.

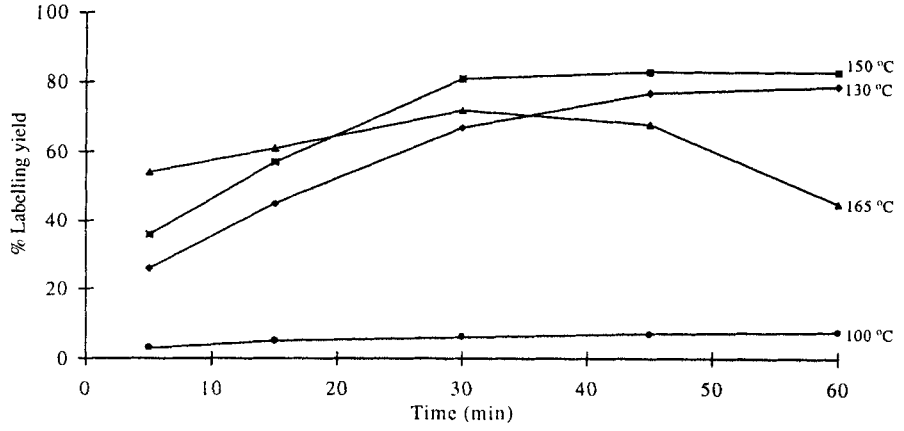


Figure 2. Radiochemical yields and time course of 2'-bromine-[^{123}I]iodine nucleophilic exchange for imidazenil in acetic acid at different temperatures as determined by radio-TLC.

The facile nucleophilic iodine-bromine exchange reaction of these systems in the absence of activating copper salts may be attributed to the activating effects of the adjacent imine which may be protonated when the reaction is carried out in acetic acid. No reaction was observed when the above reaction was carried out in aprotic solvents such as dioxane, DMF or dimethylacetamide at a range of temperatures.

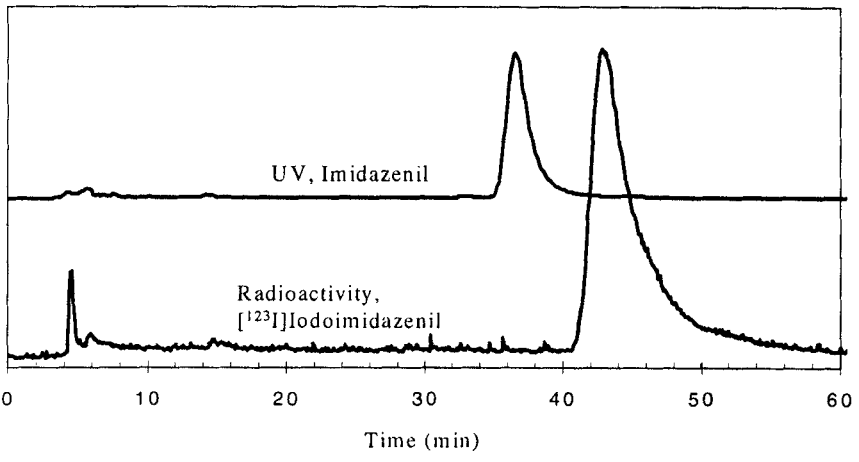
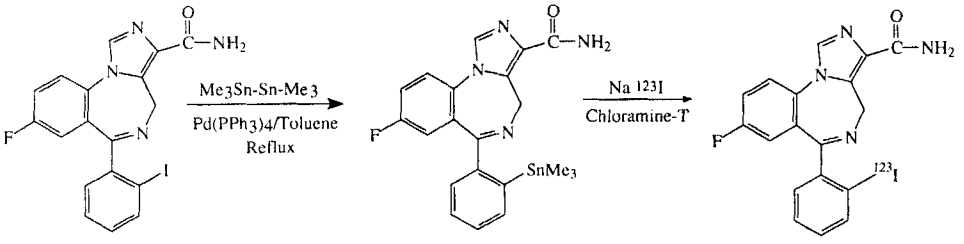


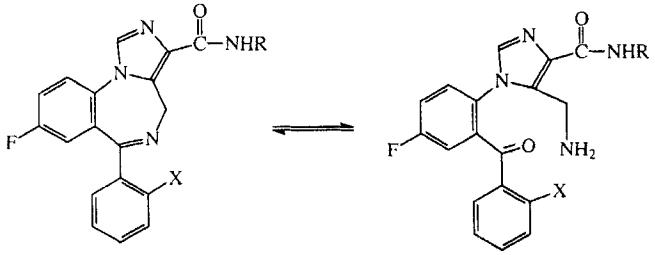
Figure 3. U.V. and Radio-chromatograms of crude reaction mixtures of Imidazenil and [^{123}I]iodoimidazenil after nucleophilic bromine-[^{123}I]iodine exchange in acetic acid at 150°.

b) Iododestannylation. [^{123}I]Iodoimidazenil was also prepared by iododestannylation in ethanol or methanol using no carrier added Na^{123}I (Scheme 3). Using 10^{-3} M Chloramine-T in 1M HCl or 3.5% peracetic acid in acetic acid resulted in only 25-30% product.



Scheme 3

The bulk of the activity was in the form of the volatile byproduct $CH_3[^{123}I]I$ which was rapidly lost by evaporation during the course of the reaction. The reaction was dependant on pH; at pH above 1 (0.1M HCl) product yield decreased and the amount of $CH_3[^{123}I]I$ increased. At neutral pH no product was evident by HPLC or radio-TLC. The volatile product was determined to be $CH_3[^{123}I]I$ by co-injection with cold iodomethane and follows from competitive electrophilic reaction at one of the aliphatic methyl groups of the stannane compared to the aryl carbon.[19,20,21]



I II

Figure 4

It has been reported that imidazo [1,4]benzodiazepines such as triazolam, diazepam, flunitrazepam and others, undergo a pH dependent hydrolysis of the azomethine bond in aqueous acidic solutions to give the corresponding aminobenzophenone (Figure 4). These exist as a pH dependent equilibrium mixture of the ring closed **I** and ring opened forms **II** which reversibly cyclize into the original form.[22] Electron withdrawing groups such as that contributed by the carbonyl group from structure **II** ortho to the stannane have been known to favour competing electrophilic reactions at the alky-tin site in comparison to the aryl-tin site.[19,20,21] Likewise, activating effects in the above nucleophilic substitution reaction contributed by the ortho carbonyl of the ring opened form **II** in acetic acid which may contain traces of water cannot be excluded.

Conclusion

[^{123}I]Iodoimidazenil and [^{123}I]N-ethyliodoimidazenil were prepared by nucleophilic bromine-[^{123}I]iodine exchange in acetic acid at 150° C for 30 minutes. The [^{123}I]labelled products were purified by C-18 RP HPLC with radiochemical yields exceeding 80% after chromatography and specific activity of 2500 Ci/mmol. Preparation of [^{123}I]Iodoimidazenil by iododestannylation reactions in the presence of chloramine-T or peracetic acid resulted in only 25-30% radiochemical yields with a significant component of the activity lost as CH_3[^{123}I]I.

Materials and Methods

Imidazenil, iodoimidazenil and the N-ethyl analogues were synthesised according to literature methods.[10,11] Hexamethylditin and tetrakistriphenylphosphine palladium were purchased from Aldrich. Ethyl isocyanoacetate, potassium *tert*-butoxide and diethyl chlorophosphate were purchased from Fluka. ^{1}H-NMR spectra were obtained on a Joel FX400 NMR spectrometer. Mass spectra were performed on a VG Quattro Triple Quadrupole in Electrospray mode in acetonitrile. Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses were carried out by the Microanalytical Unit, A.N.U., Canberra, Australia. Chromatographic separations were carried out on an Alltech semipreparative RP C-18 column (10μm, 10 x 250 mm) using a Waters 510 pump, a Spectrophysics-Linear UV detector set at 254 nm and a modified on line NaI -Berthold radioactivity detector. Specific activity was determined by taking aliquot's of the final solution of known volume and radioactivity and applied to an analytical Goldpak Excil C-18 RP HPLC column (10 μm, 250 mm x 4.6 mm). The area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured and compared to a standard curve relating mass to UV absorbance. Central benzodiazepine receptor binding assays were performed by NovaScreen (Hanover, MD) under the NIH Drug Discovery and Development Program using guinea pig cortical membranes with [^{3}H]flunitrazepam as the competing ligand. No carrier added Na^{123}I was produced by the National Medical Cyclotron, Sydney, Australia using the ^{124}Xe(p,2n) reaction.

Chemical Synthesis

5-(2-Bromophenyl)-7-fluoro-1,3-dihydro-1,4-benzodiazepin3-2(2H)-one (10). A solution of 2-amino-5-fluorophenyl-(2'-bromophenyl)methanone **8** (32.2 g, 0.11 mol) in dry THF (400 ml) was treated with phthaloylglycine acid chloride (24.4 g, 0.11 mol) and the reaction mixture stirred under nitrogen for 24 h. Evaporation of the solvent left a yellow solid which was triturated with warm HCl (6M, 100 ml) and filtered. The yellow solid was washed with diethyl ether and dried to give the N-phthaloyl acetamide **9** (46g, 88%) m.p.179-180°C. ^{1}H NMR δ ($CDCl_3$) 4.64 (s, 2H, CH_2), 6.91 (s,

1H, NH), 7.01 (m, Ar), 7.24-7.33 (m, 2H, Ar), 7.36-7.50 (m, 3H, Ar), 7.63-7.68 (m, 1H, Ar), 7.73-7.8 (m, 2H, Ar), 8.86-8.96 (m, 2H, Ar), 8.74-8.80 (m, 1H, Ar). This was immediately dissolved in methanol (600 ml), treated with hydrazine hydrate (45 ml) and stirred for 24 h. The resultant suspension was filtered and the filtrate was evaporated to give a pale yellow solid. Recrystallisation from ethyl acetate gave **10** (22.6 g, 71%) as a pale yellow solid m.p. 192-194°C (lit[14] 194-196). ^{1}H NMR δ ($CDCl_3$) 4.39 (s, 2H, CH_2), 7.79 (dd, J 8.8, 2.8 Hz, 1H, Ar), 7.12-7.28 (m, 2H, Ar), 7.30-7.39 (m, 1H, Ar), 7.40-7.48 (m, 1H, Ar), 7.51-7.60 (m, 2H, Ar), 9.24 (s, 1H, NH).

6-(2-Bromophenyl-8-fluoro-4H-imidazo[1-5-a][1,4]benzodiazepine-3-carboxylic acid ethyl ester (11) A solution of **10** (4.6 g, 13.8 mmol) in dry THF (50 ml) at -20° C under nitrogen was treated with potassium *tert*-butoxide (1.8 g, 16 mmol) followed by diethyl chlorophosphate (2.4 ml, 16 mmol). Stirring was continued until the temperature reached 10° C. The reaction mixture was cooled again and treated successively with ethyl isocyanoacetate (1.87 g, 16.5 mmol) and potassium *tert*-butoxide (1.7 g, 15 mmol). After stirring for an additional 1.5 hours with warming to room temperature the mixture was quenched with aqueous ammonium chloride and extracted with chloroform (3 x 80 ml). The organic phase was washed with water, dried (Na_2SO_4) and evaporated to give a pale yellow oil. Purification by flash chromatography using ethyl acetate-hexane 40:60 followed by recrystallisation from ethyl acetate gave **11** (2.7 g, 46%) as a white solid m.p. 208-209° C (lit[14] 209-210°C). ^{1}H NMR δ ($CDCl_3$) 1.42 (t, J 7.0 Hz, 3H, CH_3), 4.15 (bm, 1H, H4), 4.43 (m, 2H, CH_2), 6.10 (bm, 1H, H4), 6.90 (dd, J 8.5, 2.8 Hz, 1H, Ar), 7.28-7.33 (m, 1H, Ar), 7.34-7.39 (m, 1H, Ar), 7.42-7.47 (m, 1H, Ar), 7.49-7.54 (m, 1H, Ar), 7.55-7.58 (m, 1H, Ar), 7.60-7.66 (m, 1H, Ar) 7.97 (s, 1H, H1). MS (ES) m/z: 453(55), 452(55), 430(M^{+2}, 50), 428(M^+, 45), 384(M^{+2} - OCH_2CH_3,, 100), 382(M^+- OCH_2CH_3, 88).

6-Bromophenyl-8-fluoro-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylic acid (12). A suspension of the ethyl ester **11** (0.8 g, 1.87 mmol) in hydrochloric acid (6N, 20 ml) was heated to 90° for 24 h. The solution was evaporated to dryness and the residue dissolved in hot water (5 ml). Sodium acetate (0.8 g) was added with heating until crystallisation was induced. After cooling the product was filtered, washed with water and dried to give the corresponding acid **12** (0.7 g, 90%). Recrystallisation from ethanol-water gave **12** as a white solid m.p. 276-278° C (lit[14] 280-285° C). ^{1}H NMR δ (DMSO) 4.15 (bm, 1H, H4), 5.85 (bm, 1H, H4), 6.88 (dd, J 8.8, 2.8 Hz, 1H, Ar), 7.37-7.44 (m, 1H, Ar), 7.51-7.56 (m, 1H, Ar), 7.57-7.63 (m, 2H, Ar), 7.64-7.70 (m, 1H, Ar), 7.94-7.99 (m, 1H, Ar) 8.35 (s, 1H, H1). MS (ES) m/z: 403(45), 402(M^{+2}, 70), 400(M^+, 100), 384(10), 382(10), 272(50), 271(22).

6-(2-Bromophenyl)-8-fluoro-4H-imidazo[1,5-a]1,4]benzodiazepine-3-carboxamide 1. A mixture of the acid **12** (0.64 g, 1.6 mmol) and phosphorus pentachloride (0.54 g, 2.6 mmol) in dry dichloromethane (40 ml) was stirred at room temperature for 2 h. The solution was saturated with

dry ammonia gas followed by the addition of 20 ml of ammonium hydroxide solution. After 15 minutes of stirring the organic phase was washed with water dried and evaporated to give crude imidazenil. Recrystallisation from ethanol-water gave **1** (0.38 g, 59%) as colourless crystals m.p. 290-292°C (lit[14] 298-299). ^{1}H NMR (DMSO) δ 4.15 (bm, 1H, H4), 5.95 (bm, 1H, H4), 6.87 (dd, J 9.0, 2.9 Hz, 1H, Ar), 7.3 (s, 1H, NH), 7.37-7.44 (m, 1H, Ar), 7.50 (s, 1H, NH), 7.51-7.56 (m, 1H, Ar), 7.58-7.62 (m, 2H, Ar), 7.63-7.69 (m, 1H, Ar), 7.93-7.98 (m, 1H, Ar) 8.33 (s, 1H, H1). MS (ES) m/z: 423(47), 421(55), 401(M^{+2}, 100), 399(M^+, 65), 384(56), 382(54).

6-(2-Bromophenyl)-N-ethyl-8-fluoro-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxamide (3) A mixture of the acid **12** (0.5 g, 1.25 mmol) was treated with phosphorus pentachloride (0.5 g, 2.5 mmol) in dichloromethane (40 ml) followed by ethyl amine as above. Recrystallisation from ethyl acetate-hexane gave N-ethylimidazenil **3** (0.32 g, 60%) as colourless crystals m.p. 216-217°C (lit[14] 218-220). ^{1}H NMR δ ($CDCl_3$) 1.24 (t, J 7.3 Hz, 3H, CH_3), 3.46 (dq, 2H, CH_2), 4.15 (bm, 1H, H4), 5.65 (m, 1H, NH), 6.30 (bm, 1H, H4), 6.89 (dd, J 8.4, 2.7 Hz, 1H, Ar), 7.26-7.38 (m, 2H, Ar), 7.42-7.53 (m, 2H, Ar), 7.56-7.66 (m, 2H, Ar), 8.09 (s, 1H, H1). MS (ES) m/z: 430(26), 429(M^{+2}, 8), 428(M^{+1}, 24), 385(20), 384(80), 383(18), 382(50), 303(70).

5-(2-Iodophenyl)-7-fluoro-1,3-dihydro-1,4-benzodiazepin3-2(2H)-one (15). Treatment of a solution of 2-amino-5-fluorophenyl-(2'-iodophenyl)methanone **13** (14 g, 0.041 mol) and phthaloylglycine acid chloride (9.1 g, 0.041 mol) in THF (250 ml) as above gave a yellow solid of the N-phthaloyl acetamide **14** (17.7 g, 82%) m.p. 197-199°C. ^{1}H NMR δ ($CDCl_3$) 4.64 (s, 2H, CH_2), 6.95-7.04 (dd, J 9.0, 3.0 Hz, 1H, Ar), 7.18-7.35 (m, 3H, Ar), 7.43-7.51 (m, 1H, Ar), 7.72-7.80 (m, 2H, Ar), 7.89-7.97 (m, 2H, Ar), 8.72-8.82 (m, 1H, Ar). The acetamide **14** (10g, 0.19 mol) was dissolved in ethanol (250 ml), treated with hydrazine hydrate (95%, 8 ml) and stirred for 24 h. The resultant suspension was filtered and the filtrate evaporated to give a brown yellow solid (6.1 g, 85%). Recrystallisation from ethyl acetate gave **15** as a pale yellow solid m.p. 182-184°C. ^{1}H NMR δ ($CDCl_3$) 4.39 (s, 2H, CH_2), 6.75 (dd, J 8.7, 2.8 Hz, 1H, Ar), 7.13-7.18 (m, 2H, Ar), 7.21-7.26 (m, 1H, Ar), 7.46-7.49 (m, 2H, Ar), 7.85-7.88 (d, J 8.4 Hz, 1H, Ar), 8.95 (bs, 1H, NH). MS (ES) m/z: 382 (M^{+2}, 8%), 381(M^{+1}, 100), 255(M^{+2}-I, 18), 253(M^+-I, 8), 226(3), 197(3).

6-(2-Iodophenyl-8-fluoro-4H-imidazo[1-5-a][1,4]benzodiazepine-3-carboxylic acid ethyl ester (16) A solution of **15** (2.5 g, 6.57 mmol) in THF (40 ml) at -20° C under nitrogen was treated successively with potassium *tert*-butoxide (0.88 g, 7.8 mmol), diethyl chlorophosphate (1.36 g, 7.8 mmol), ethyl isocyanoacetate (0.89 g, 7.8 mmol) and another equivalent of potassium *tert*-butoxide (0.88 g, 7.8 mmol) as above. Recrystallisation of the crude product from ethyl acetate gave the iodo-ethyl ester **16** as pale yellow-white solid m.p. 238-240°C (1.5 g, 48%). ^{1}H NMR δ ($CDCl_3$) 1.43 (t, J 7.0 Hz, 3H, CH_3), 4.15 (bm, 1H, H4), 4.41 (m, 2H, CH_2), 6.20 (bm, 1H, H4), 6.87 (dd, J 8.4, 2.7 Hz, 1H, Ar), 7.10-7.16 (m, 1H, Ar), 7.35-7.42 (m, 1H, Ar), 7.45-7.55 (m, 2H, Ar), 7.59-7.66 (m,

1H, Ar), 7.78 (d, J 7.9 Hz, 1H, Ar), 7.99 (s, 1H, H1). MS (ES) m/z: 499(8), 498(33), 477(20), 476(M^{+1}, 100), 431(M^{+1} -OCH_2CH_3,, 13), 430(M^+- OCH_2CH_3, 100), 413(8), 288(22). Anal. calc'd for $C_{20}H_{15}FIN_3O_2$: C, 50.54; H, 3.18; N, 8.84 % Found: C, 50.62; H, 2.98; N, 8.80 %

6-Iodophenyl-8-fluoro-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylic acid (17). Hydrolysis of the ethyl ester **16** (0.8 g, 1.68 mmol) in hydrochloric acid (6N, 50 ml) at 90° C for 24 h as above gave a pale yellow solid **17** (0.55 g, 70%) m.p. 270-272°C. ^{1}H NMR δ (DMSO) 4.15 (bm, 1H, H4), 5.8 (bm, 1H, H4), 6.83 (dd, J 9.0, 2.9 Hz, 1H, Ar), 7.18-7.24 (m, 1H, Ar), 7.50-7.58 (m, 2H, Ar), 7.65-7.72 (m, 1H, Ar), 7.82-7.86 (m, 1H, Ar), 7.96-8.02(m, 1H, Ar), 8.31 (s, 1H, H1). MS (ES) m/z: 466(100), 448(M^{+1}, 100), 271(13).

6-(2-Iodophenyl)-8-fluoro-4H-imidazo[1,5-a]1,4]benzodiazepine-3-carboxamide (2). The acid **17** (0.5 g, 1.1 mmol) was treated with phosphorus pentachloride (0.5 g, 2.4 mmol) in dichloromethane (40 ml) followed by dry ammonia gas as above. Recrystallisation from ethanol-water gave iodoimidazenil **2** (0.33 g, 66%) as a white solid m.p. 276-278°C. ^{1}H NMR (DMSO) δ 4.15 (bm, 1H, H4), 5.6 (bs, 1H, NH), 6.25 (bm, 1H, H4), 6.87 (dd, J 8.6, 2.8 Hz, 1H, Ar), 7.10-7.15 (m, 1H, Ar), 7.2 (bs, 1H, NH), 7.35-7.40 (m, 1H, Ar), 7.45-7.56 (m, 2H, Ar), 7.58-7.63 (m, 1H, Ar), 7.78 (d, J 7.8 Hz, 1H, Ar), 8.03 (s, 1H, H1). MS (ES) m/z: 470(8), 469(50), 448(M^{+2}, 12), 447(M^{+1}, 100), 430(42), 288(10). Anal. calc'd for $C_{18}H_{12}FIN_4O$: C, 48.45; H, 2.71; N, 12.56 % Found: C, 47.97; H, 2.45; N, 12.43 %

6-(2'-Iodophenyl)-N-ethyl-8-fluoro-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxamide (4). The acid **17** (0.4 g, 0.89 mmol) was treated with phosphorus pentachloride (0.4 g, 1.9 mmol) in dichloromethane (20 ml) followed by excess dry ethyl amine gas as above. Recrystallisation from ethyl acetate-hexane gave N-ethyliodoimidazenil **4** as a white solid (0.35 g, 83%), m.p. 228-230°C. ^{1}H NMR ($CDCl_3$) δ 1.24 (t, J 7.3 Hz, 3H, CH_3), 3.47 (dq, 2H, CH_2), 4.15 (bm, 1H, H4), 6.30 (bm, 1H, H4), 6.86 (dd, J 8.5, 2.9 Hz, 1H, Ar), 7.10-7.16 (m, 1H, Ar), 7.35-7.56 (m, 3H, Ar), 7.3-7.5 (m, 1H, NH), 7.60-7.65 (m, 1H, Ar), 7.75-7.80 (d, J 8.1 Hz, 1H, Ar), 8.1 (s, 1H, H1). MS (Fab) m/z: 476(M^{+2}, 15) 430(M^+-$NHCH_2CH_3$, 10), 338(25). Anal. calc'd for $C_{20}H_{16}FIN_4O$: C, 50.65; H, 3.40; N, 11.81 % Found: C, 50.44; H, 3.11; N, 11.57 %

6-(2'-(trimethylstannyl)phenyl)-N-ethyl-8-fluoro-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxamide (18). To a suspension of iodoimidazenil **2** (0.20 g, 0.45 mmol) in dry toluene (5 ml) was added hexamethylditin (0.3 ml) followed by a catalytic amount of $Pd(PPh_3)_3$. The reaction mixture was heated to reflux for 4 h after which the initial solid dissolved and a black residue was deposited. The toluene was evaporated and the crude residue purified by flash chromatography using ethyl acetate-petroleum spirit (40:60) to give the trimethyl stannane **18** (0.13 g, 59%) m.p. 138-140°C. ^{1}H NMR (DMSO) δ 0.17 (s, 9H, $Sn(CH_3)_3$), 4.03 (d, J 12.5 Hz, 1H, H4), 5.90 (d, J12.4 Hz, 1H, H4), 7.08-7.19 (m, 1H, Ar), 7.23 (s, 1H, NH), 7.34-7.39 (m, 2H, Ar), 7.45 (s, 1H, NH),

7.45-7.50 (m, 1H, Ar), 7.64-7.67 (m, 1H, Ar), 7.69-7.76 (m, 1H, Ar), 7.92-7.98 (m, 1H, Ar), 8.3 (s, 1H, H1). MS (ES) m/z: 484(M^+, 100) 469(M^+-CH_3, 50), 338(12), 339(11), 321(16), 320(14).

[^{123}I]6-(2-Iodophenyl)-8-fluoro-4H-imidazo[1,5-a]1,4]benzodiazepine-3-carboxamide (5).

a) Nucleophilic halogen exchange. [^{123}I]Sodium iodide (1-50 mCi, 10-200 µl in 0.1M NaOH) was evaporated to dryness under a stream of nitrogen at 90-100°C. The bromo precursor **1** (50 µg) in glacial acetic acid 100 µl was added to the activity and the reaction mixture heated at 150°C in a sealed vial for 30 minutes. The reaction mixture was cooled, diluted with mobile phase and injected onto a C-18 RP semi-preparative column. The product was eluted with a mobile phase consisting of acetonitrile and 0.02M H_3PO_4 (32:68) which had been adjusted to pH 7.1 with triethylamine at a flow rate of 3.0 ml/min. The retention times of imidazenil **1** and [^{123}I]iodoimidazenil **5** were 37 and 43 min respectively. The radiochemical purity as assessed by analytical HPLC and radio-TLC was ≥98% with radiochemical yield of 80 ± 5% (n=24) at 150°C for 30-40 min.

b) Iododestannylation. To the trimethyl stannane **18** (50-200 µg) in ethanol (300 µl) was added Na^{123}I (1-5 mCi, 10-500 µl in 0.1M NaOH). Chloramine-T (50 µg) in 1M HCl was added and the mixture left to stand for 10 minutes with intermittent shaking and regular measurement of activity. After 10 minutes the solution was quenched with sodium bisulfate (10%, 100 µl) and injected onto the above HPLC system. The product corresponding to [^{123}I]Iodoimidazenil was recovered in 25-30% yield with a second major peak at 50 minutes (20%). The remainder of the activity was lost by rapid evaporation during the course of the reaction.

[^{123}I] 6-(2-Iodophenyl)-N-ethyl-8-fluoro-4H-imidazo[1,5-a]1,4]benzodiazepine-3-carboxamide (5). To dried Na^{123}I (1-20 mCi, 10-100 µl in 0.1M NaOH) was added the bromo precursor **3** (50 µg) in glacial acetic acid 100 µl and the reaction mixture heated at 150°C in a sealed vial for 30 minutes as above. The reaction mixture was purified as above with a mobile phase consisting of acetonitrile and 0.02M H_3PO_4 (35:65) pH 7.1 with triethylamine at a flow rate of 4.0 ml/min. The retention times of N-ethyl imidazenil **3** and [^{123}I[N-ethyl iodoimidazenil **6** were 42 and 48 min respectively. The radiochemical purity as assessed by analytical HPLC and radio-TLC was 98% with radiochemical yield of 80 ± 5% (n=15) at 150°C for 30-40 min.

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