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# Synthesis and biodistribution of 8-iodo-11-(4-methyl-piperazino)-5H-dibenzo[b,e][1,4]-diazepine: Iozapine

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**Abstract**—8-Iodo-11-(4-methylpiperazino)-5H-dibenzo[b,e][1,4]-diazepine: Iozapine, a potential $D_4$-receptor ligand was synthesized using oxidative iodo-destannylation reaction. The preliminary biodistribution studies of radioiodinated iozapine have shown that the compound is taken up in the brains of mice and rabbits.


The neurotransmitter dopamine plays an important role in the development of several neurological and psychiatric disorders such as schizophrenia,[1,2] Huntington's disease, and Parkinson's disease.[3] Schizophrenia is a mental disorder characterized by chaotic jumbling and fragmentation of sensation and thought processes. The Dopamine hypothesis is one of the several proposed in the last 50 years to understand the biology of schizophrenia, which links the positive psychotic symptoms with hyperactivity of dopaminergic neurons in the mesolombic region of the brain.[4] There are five different subtypes of dopamine receptors, which have been characterized ($D_1$–$D_5$). These are further subdivided into two main families, $D_1$ (including $D_1$ and $D_5$) and $D_2$ (including $D_2$, $D_3$, and $D_4$).[5,6] Amongst the many themes of antipsychotic drug action,[4] a high affinity for $D_4$ receptors relative to $D_2$ receptors has been proposed to rationalize efficacy against positive symptoms and low potential for extrapyramidal symptoms. Clozapine (**1**) (8-chloro-11-(4-methylpiperazino)-5H-dibenzo[b,e][1,4]-diazepine) is an effective antipsychotic agent and is devoid of extrapyramidal side effects.[7] Although it has considerable value in the treatment of schizophrenia, its therapeutic use has been restricted by the relative high incidence of agranulocytosis.[7]

From receptor binding studies it has been shown that clozapine, in addition to blocking the $D_2$ receptor, also blocks other dopamine and serotonin receptors in the human brain[8] and exerts potent antagonist effects on the adrenergic, cholinergic, and histaminergic receptors.[9] The atypical antipsychotic profile of clozapine has been suggested to arise from its preferential blockade of the dopamine $D_1$ or $D_4$ receptors.[8,10] Clozapine showed 10-fold higher selectivity for $D_4$ over $D_2$ receptors.[11,12] Although $D_4$ receptors represent a relatively minor proportion of the total dopamine receptor population in the basal ganglia of the normal human brain, Seeman et al. found that there is a sixfold increase in the population of these receptors in the brain of schizophrenics.[13] To overcome the side effects of clozapine lots of efforts have been made in the past, which centered on the modification of ring B, C or D, but not much work has been done on ring A.[14] No efforts in changing chlorine were ever made to see the improvement in the biological properties of the parent molecule, we decided to take ring A of clozapine and modify the chlorine in the ring A, that can also give a molecule which can be easily

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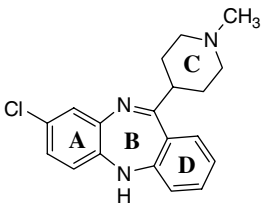
radiolabeled with minimum structural or chemical change Figure 1.

Scheme 1 depicts the synthesis of iozapine **2**. The tricyclic lactam **7** was synthesized by a modification of the reported (Hunziker) procedure by coupling anthranilic acid **4** with 2,5-dibromonitrobenzene **3** under Ullman conditions which gave the nitro acid **5**. Subsequent reduction using sodium dithionate afforded the amino compound **6**. Cyclization of the amino compound **6** was achieved by heating in methanol in the presence of a catalytic amount of sulfuric acid. This gave the desired cyclized product **7** and also the amino ester **8** which was in turn converted to **7** by treatment with sodamide in dioxane. Transformation of the bromo lactam **7** to compound **9** was carried out by treatment with phosphorus oxychloride and a catalytic amount of dimethyl formamide in dichloromethane to form the imido-chloride and subsequent reaction with *N*-methylpiperazine in dioxane. Reaction of **9** with bis(tributyltin) in dioxane in the presence of a catalytic amount of tetra-kis-(triphenyl phosphino)palladium gave the tributyltin precursor **10**. Finally oxidative iodo-destannylation of **10** gave the target compound **2** in 81% yield.[15]



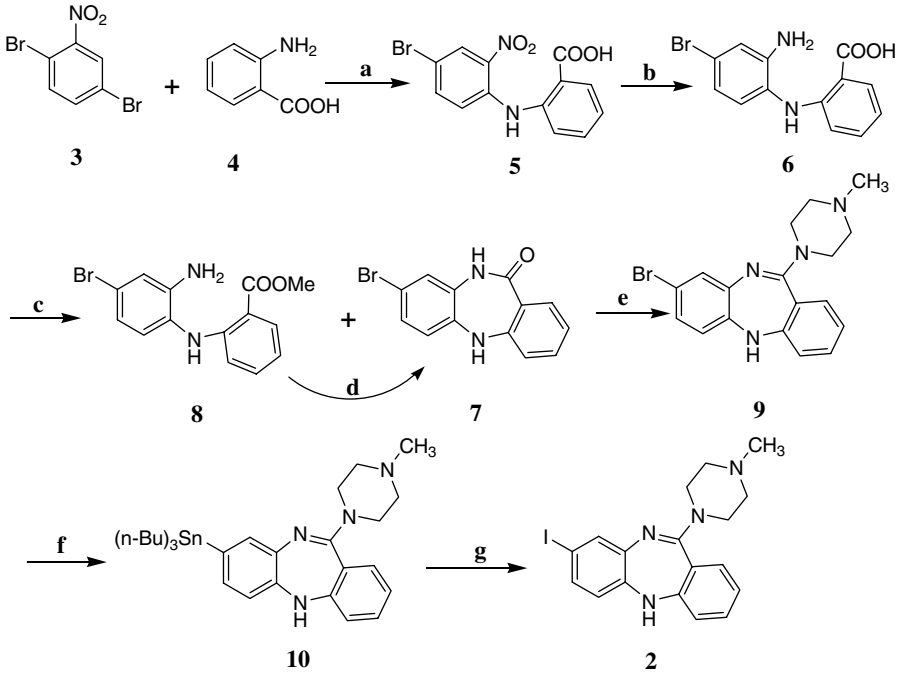

**Clozapine (1)**

**Figure 1.** Structure of Clozapine.

The preparation of radioiodinated iozapine was carried out by oxidative iodo-destannylation of the tin derivative **10** using radioiodide and hydrogen peroxide. The labeling was more than 90% complete in 15 min. at room temperature. Purification of the product was done by HPLC. The radiochemical purity of the material after HPLC was >99% as determined by TLC with radiochemical yields ranging from 58 to 80%.

Biodistribution studies were carried out in male Balb/c mice (23–26 g). Five mice were used for each time period. Each mouse was injected with 0.1 mL of [$^{125}$I]Iozapine (7–8 KBq) via the lateral tail vein. At different time periods up to 2 h after the injection, the animals were sacrificed by $CO_2$ asphyxiation. The organs of interest were excised, weighed, and counted in a dual channel automatic gamma counter. The mouse organ distribution results from 5 to 120 min after injection are shown in Table 1.

Hepatobiliary clearance is relatively rapid with approximately 35% of the injected activity in the intestine at 1–2 h. There appears to be some in vivo deiodination with thyroid and stomach radioactivity tending to increase with time. The blood levels did not decrease as rapidly as most other tissues. This may be due to deiodination of the compound resulting in relatively high levels of radioiodide in the blood. However, the $^{125}$I species in the blood was not determined. There is good initial total brain uptake of approximately 3.5%. However this rapidly dropped to less than 0.1% of the injected activity by




**Scheme 1.** Reagents and conditions: (a) Cu Powder, $K_2CO_3$, amyl alcohol, 135–140 °C, 20 h, 76%; (b) 2 N $NH_4OH$, $Na_2S_2O_4$, 80 °C, 30 min, 88%; (c) MeOH, $H_2SO_4$, reflux, 48 h, 68%; (d) $NaNH_2$, dioxane, reflux, 20 h, 86%; (e) $POCl_3$, DMF, $CH_2Cl_2$, 2-methylpiperazine, dioxane, 20 h, reflux, 62%; (f) $[(nBu)_3Sn]_2$, $[P(Ph)_3]_4Pd(O)$, dioxane, 100 °C, 20 h, 66%; (g) NaI, $H_2O_2$, HCl, ethanol, 2 h, 81%.

**Table 1.** Percent of injected dose per organ[a]

| Organ | 5 min | 15 min | 60 min | 120 min |
|---|---|---|---|---|
| Blood | 4.17 ± 0.25 | 3.31 ± 0.13 | 3.49 ± 0.17 | 1.77 ± 0.60 |
| Spleen | 0.37 ± 0.05 | 0.31 ± 0.07 | 0.13 ± 0.32 | 0.04 ± 0.01 |
| Pancreas | 1.41 ± 0.23 | 1.02 ± 0.10 | 0.34 ± 0.02 | 0.16 ± 0.08 |
| Stomach | 1.77 ± 0.83 | 3.56 ± 0.63 | 7.35 ± 0.88 | 4.61 ± 1.31 |
| Intestine | 9.16 ± 1.77 | 17.73 ± 1.55 | 35.19 ± 2.14 | 34.13 ± 3.07 |
| Liver | 11.12 ± 1.20 | 10.38 ± 0.75 | 12.03 ± 0.63 | 9.80 ± 0.58 |
| Kidney | 5.42 ± 0.36 | 4.41 ± 0.39 | 2.15 ± 0.21 | 1.19 ± 0.16 |
| Lung | 4.01 ± 0.43 | 1.60 ± 0.16 | 0.50 ± 0.07 | 0.17 ± 0.05 |
| Heart | 0.60 ± 0.04 | 0.28 ± 0.02 | 0.13 ± 0.01 | 0.05 ± 0.02 |
| Thyroid | 0.0 ± 0.01 | 0.13 ± 0.06 | 0.68 ± 0.17 | 1.30 ± 0.49 |
| Brain | 3.49 ± 0.41 | 2.79 ± 0.42 | 0.80 ± 0.16 | 0.10 ± 0.02 |
| Upper body | 23.30 ± 1.51 | 17.41 ± 1.37 | 11.54 ± 0.51 | 5.72 ± 1.52 |
| Lower body | 18.94 ± 1.82 | 15.02 ± 1.81 | 10.24 ± 0.95 | 4.97 ± 0.48 |

Tissue distribution of [$^{125}$I]Iozapine.
[a] Mean of five mice at each time.

**Table 2.** Percent of injected dose per organ[a]

| Region | 5 min | 15 min | 60 min | 120 min |
|---|---|---|---|---|
| Cortex | 7.22 ± 1.03 | 6.65 ± 1.21 | 2.05 ± 0.33 | 0.22 ± 0.07 |
| Striatum | 7.28 ± 0.78 | 6.76 ± 1.31 | 2.11 ± 0.24 | 0.20 ± 0.06 |
| Hippocampus | 7.07 ± 0.78 | 6.64 ± 1.25 | 2.52 ± 0.84 | 0.27 ± 0.09 |
| Thalamus | 7.82 ± 0.85 | 6.15 ± 0.99 | 1.56 ± 0.35 | 0.20 ± 0.06 |
| Cerebellum | 6.16 ± 0.65 | 4.71 ± 0.73 | 0.94 ± 0.28 | 0.12 ± 0.04 |
| Medulla | 6.78 ± 1.08 | 5.23 ± 0.99 | 1.20 ± 0.20 | 0.16 ± 0.04 |

Regional brain distribution of [$^{125}$I]Iozapine in mice.
[a] Mean of five mice at each time.

**Table 3.** Percent of injected dose per region[a]

| Region | 0 h | 0.5 h | 1 h | 1.5 h | 2.0 h | 2.5 h | 3.0 h | 20.0 h |
|---|---|---|---|---|---|---|---|---|
| Whole body | 100.0 | 98.7 | 95.8 | 97.7 | 97.4 | 99.4 | 96.9 | 54.6 |
| Lung | 14.7 | 10.7 | 10.4 | 8.1 | 7.6 | 6.0 | 6.4 | 2.3 |
| Thyroid | 1.5 | 1.4 | 1.4 | 1.3 | 1.4 | 1.2 | 1.4 | 1.9 |
| Brain | 2.2 | 2.2 | 1.8 | 1.6 | 1.5 | 1.5 | 1.4 | 0.7 |

Tissue distribution of [$^{123}$I]Iozapine.
[a] Mean of two rabbits at each time.

two hours. This is indicative of the material crossing the blood brain barrier but then diffusing out rapidly with no binding. The clearance rate from the brain was in fact considerably faster than from the blood. The whole brains were incubated for 24 h in a 9:1 mixture of sodium phosphate buffer (5 mM, pH 7) and formalin (37%) and then dissected into cortex, striatum, hippocampus, thalamus, cerebellum, and medulla, weighed, and counted. The results are shown in Table 2. The results showed that there is no preferential uptake (on a per gram basis) in any of the regions of the brain investigated. As well, the clearance from each of the regions was similar.

The species difference studies were carried out in rabbits using [$^{123}$I]Iozapine by injecting 20 MBq of the drug and imaging by gamma camera. The geometric means of anterior and posterior counts of the regions of interest at various time periods were determined, corrected for decay, and expressed as a percentage of the initial whole body mean counts as in Table 3. Brain uptake in rabbits, while somewhat lower initially, had a considerably longer retention than seen in mice. Whereas the mice brain levels decreased to 0.1% by 2 h after injection, the rabbits had an initial uptake slightly over 2% and this remained at approximately 1.5% at 3 h.

Species difference indicated by the considerably prolonged retention in brains of rabbits than mice has suggested that iozapine (**2**) might have the potential as brain imaging agent in the study of schizophrenia and as an effective antipsychotic agent. Receptor binding studies are still in progress in our group and will be reported soon.

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15. Compound **10** (298 mg, 0.51 mmol) was suspended in dry ethanol (5 mL) at 0 °C and to it, potassium iodide (93 mg, 0.56 mmol), hydrochloric acid (1 N, 4 mL), and hydrogen peroxide (30%, 1 mL) were added portionwise with constant stirring. The reaction mixture was brought to room temperature and stirred for another 2 h. The excess of $H_2O_2$ was removed by adding dropwise saturated solution of sodium disulfite (7 mL) with stirring. The resulting reaction mixture was neutralized cautiously with solid sodium bicarbonate and extracted with dichloromethane. The organic layer was separated and dried over $Na_2SO_4$. After evaporation of the solvent in vacuum, the residue was taken up in benzene (20 mL) and washed with cold hydrochloric acid (0.25 N, 25 mL). The aqueous layer was separated and made alkaline with concentrated ammonium hydroxide, extracted with dichloromethane ($3 \times 10$ mL). The organic layer was separated, dried over $Na_2SO_4$, and evaporation of the solvent in vacuum gave a yellow colored solid, which was crystallized from benzene–hexane to give 172 mg (yield 81%) of the final product **2**, mp 181–182 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ in ppm: 2.48 (s, 3H, C$H_3$), 2.57 (br s, 4H, $2\times -CH_2-$, $J = 5.0$ Hz), 3.50 (br s, 4H, $2\times -CH_2-$, $J = 5.0$ Hz), 4.95 (s, 1H, N*H*), 6.45 (d, 1H, Ar-*H*), 6.85 (d, 1H, Ar-*H*), 7.15 (t, 1H, Ar-*H*), 7.20 (dd, 1H, Ar-*H*), 7.45 (m, 2H, Ar-*H*), 7.50 (d, 1H, Ar-*H*). $^{13}$C NMR (500 MHz, $CDCl_3$) $\delta$ in ppm: 162.72, 152.614, 142.362, 141.754, 135.630, 132.028, 131.898, 130.290, 128.341, 123.064, 120.892, 120.138, 87.035, 55.024, 47.270, 46.158. HRMS (EI): $C_{18}H_{19}IN_4$ calcd: 418.07 found 418.065. Elemental Anal. calcd for $C_{18}H_{19}IN_4$ were C, 51.69; H, 4.58; N, 13.39 found C, 52.02, H, 4.62; N, 13.32.