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# 1,2,3,4,5,6-Hexahydrobenzo[*h*][1,6]naphthyridin-5-ones: 5-$HT_7$ Receptor Affinity

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**Abstract**

Synthesis of new analogues of DR 4004, derived from 1,2,3,4,5,6,-hexahydrobenzo-[*h*][1,6]naphthyridin-5-ones is described. Their central pharmacological effects on the 5-$HT_7$ receptor have been studied in-vitro.

A research programme has been developed to design and synthesize new compounds with therapeutic potential via the 5-HTergic system against CNS disorders, and to evaluate their biological properties. In particular, we have recently used the novel techniques of drug design and scaffolding in association with studies of quantitative structure-activity relationships (QSAR). Biological results (binding with subtypes of 5-hydroxytryptamine (5-HT) receptors) are obtained rapidly because of the development of an appropriate structure (ATBI) that contains the chemical structures and chemical and pharmacological properties of more than 400 compounds with 5-HTergic activity.

In this paper we describe the synthesis and reactivity of 1,2,3,4,5,6-hexahydrobenzo[*h*][1,6]-naphthyridines that have been recently reported (Rault et al 1995; Gillard et al 1997a, b; Bureau et al 1999). The structures of these compounds were similar to that of DR 4004 ($pK_i$ 8·67; Kikuchi et al 1999; Figure 1), which has high affinity and selectivity for the 5-$HT_7$ receptor.

## Materials and Methods

*Chemical procedures*

Melting points were determined on a Köfler block and are uncorrected. IR spectra were recorded on a Genesis Series FTIR spectrometer. $^1$H NMR spectra were recorded on a Jeol JNM-LA 400 spectrometer (400 MHz) employing $d_6$-DMSO as solvent. Chemical shifts ($\delta$ ppm) refer to tetramethylsilane, which was used as internal reference. NH signals appeared as broad singlets exchangeable with $D_2O$. In the data below, s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were recorded on a Jeol JMS GCmate spectrometer. Elemental analysis (C, H, N) was performed by INSA, Rouen, France and agreed with the proposed structures within $\pm$0·3% of the theoretical values.

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*Representative preparative procedures*

*5-Methoxy-1,2,3,4-tetrahydrobenzo[h][1,6]naphthyridine (***2a***).* Sodium methoxide (2 g, 36·6 mmol) was added to a solution of 5-chloro-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine **1a** (2 g, 9·2 mmol) in dry *N*, *N*-dimethylformamide (15 mL) and the solution was heated under reflux for 3 h. The solvent was removed under reduced pressure and the oily residue dissolved in water (60 mL) and extracted with diethyl ether (2 × 70 mL). The organic layer was washed with water (2 × 90 mL), dried with magnesium sulphate, treated with charcoal and evaporated to dryness to give an oil that crystallized from petroleum ether to give **2a** as a yellow powder (1·3 g, 72%); mp 147°C. IR (KBr) *v*: 3250, 1600, 1540, 1370, 1130 $cm^{-1}$; $^1$H NMR ($d_6$-DMSO) $\delta$: 7·91 (1H, d, $H_{10}$), 7·55 (1H, d, $H_7$), 7·46 (1H, t, $H_8$), 7·23 (1H, t, $H_9$), 7·06 (1H, s, NH), 3·90 (3H, s, $CH_3$), 3·34 (2H, t, $H_2$), 2·61 (2H, t, $H_4$), 1·85 (2H, m, $H_3$); $^{13}$C NMR ($d_6$-DMSO) $\delta$: 169·7, 148·9, 136·3, 131·9, 129·6, 122·1, 116·7, 113·8, 112·9, 47·8, 37·1, 36·6, 13·3. Calculated

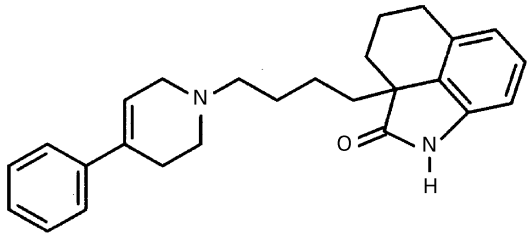


Figure 1. The structure of DR 4004.

for $C_{13}H_{14}N_2O$: C, 72·82; H, 6·54; N, 13·07; found: C, 73·08; H, 6·25; N, 12·82%.

*1,2,3,4,5,6-Hexahydrobenzo[h][1,6]naphthyridin-5-one (***4a***).* Potassium iodide (0·90 g, 5·60 mmol) was added to a solution of 5-methoxy-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine **2a** (1 g, 4·67 mmol) in glacial acetic acid (35 mL) and the mixture was heated at 110°C for 3 h. After evaporation of the solvent the residue was dissolved in water and extracted with chloroform (2 × 60 mL). The organic layer was washed with aqueous sodium hydrogen carbonate (2 × 100 mL) and iodine was removed with an aqueous solution of sodium thiosulphate (3 × 100 mL). The organic layer was then evaporated in-vacuo and the solid residue was recrystallized from ether to give **4a** as a white powder (0·75 g, 80·3%); mp > 260°C. IR (KBr) *v*: 3285, 2953, 1640, 1600, 1541, 1472, 1413, 1207 $cm^{-1}$; $^1H$ NMR ($d_6$-DMSO) $\delta$: 10·80 (1H, s, $H_6$, NH), 7·78 (1H, d, $H_{10}$), 7·37 (1H, t, $H_8$), 7·19 (1H, d, $H_7$), 7·07 (1H, t, $H_9$), 6·92 (1H, s, $H_1$, NH), 3·40 (2H, t, $H_2$), 2·45 (2H, t, $H_4$), 1·79 (2H, m, $H_3$); $^{13}C$ NMR ($d_6$-DMSO) $\delta$: 164·6, 147·4, 132·5, 128·0, 118·2, 117·6, 113·8, 112·9, 107·2, 37·0, 36·8, 13·2; MS m/z 200 ($M^+$), 181, 162. Calculated for $C_{12}H_{12}N_2O$: C, 71·98; H, 6·04; N, 13·99; found: C, 72·13; H, 5·76; N, 13·96%.

*5-Trifluoromethylsulphonyl-1,2,3,4-tetrahydrobenzo-[h][1,6]naphthyridine (***5a***).* Trifluoromethanesulphonyl anhydride (1·5 mL, 9 mmol) was added dropwise to a suspension of 1,2,3,4,5,6-hexahydrobenzo[*h*][1,6]naphthyridin-5-one **4a** (1 g, 5 mmol) cooled to 0°C in dry pyridine (12 mL). The mixture was stirred at room temperature overnight under argon. Water (50 mL) was added and the solution was extracted with dichloromethane (3 × 40 mL), washed with water (3 × 50 mL), and the organic layer was dried over magnesium sulphate, treated with charcoal and filtered through celite. Evaporation of the solvents furnished **5a** as an oil that was triturated in petroleum ether until crystallization (1 g, 60%); mp 80°C. IR (KBr) *v*: 3447, 2942, 1609, 1578, 1535, 1400, 1333, 1211, 1146, 1018, 903, 755 $cm^{-1}$; $^1H$ NMR ($d_6$-DMSO) $\delta$: 8·12 (1H, d, $H_{10}$), 8·06 (1H, s, NH), 7·66 (2H, m, $H_7$ and $H_8$), 7·49 (1H, m, $H_9$), 3·44 (2H, m, $H_2$), 2·72 (2H, t, $H_4$), 1·91 (2H, m, $H_3$); MS m/z 332 ($M^+$), 199, 183, 171. Calculated for $C_{13}H_{11}N_2O_3SF_3$: C, 46·99; H, 3·34; N, 8·43; found: C, 46·95; H, 3·48; N, 8·45%.

*5-N-Benzylpiperazino-1,2,3,4-tetrahydrobenzo[h]-[1,6]naphthyridine difumarate (***7a***).* *N*-Benzylpiperazine (0·18 mL, 1·08 mmol) was added to a solution of 5-trifluoromethylsulphonyl-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine **5a** (0·3 g, 0·9 mmol) and potassium carbonate (0·25 g, 1·8 mmol) in *N*,*N*-dimethylformamide (5 mL). After stirring for 3 h under reflux, the solvent was removed. The residue was dissolved in ether (100 mL) and washed with water (2 × 70 mL). The organic layer was dried over magnesium sulphate, treated with charcoal and evaporated to dryness to give an oil that was dissolved in isopropanol (3 mL). This solution was heated under reflux with fumaric acid (1·17 mmol) for 3 min. After cooling, the precipitate was filtered and dried to give **7a** as a yellow powder (0·1 g, 20%); mp 210°C. IR (KBr) *v*: 3258, 2995, 2862, 1702, 1640, 1591, 1556, 1349, 1279, 1184, 1165, 983, 965 $cm^{-1}$; $^1H$ NMR ($d_6$-DMSO) $\delta$: 7·93 (1H, d, $H_{10}$), 7·58 (1H, d, $H_7$), 7·48 (1H, t, $H_8$), 7·35 (5H, m, $H_{phenyl}$), 7·26 (2H, m, $N_H$ and $H_9$), 3·59 (2H, s, $CH_{2benzyl}$), 3·39 (2H, t, $H_2$), 3·18 (4H, m, $H_{2'}$ and $H_{6'}$), 2·67 (2H, t, $H_4$), 2·57 (4H, m, $H_{3'}$ and $H_{5'}$), 1·76 (2H, m, $H_3$); MS m/z 358 ($M^+$), 212, 199, 183, 146. Calculated for $C_{31}H_{35}N_4O_{8.5}$: C, 62·04; H, 5·83; N, 9·33; found: C, 62·07; H, 5·78; N, 9·46%.

*6-(4-Bromobutyl)-2,3,4,5-tetrahydro-1*H*-benzo[h]-[1,6]naphthyridin-5-one (***6a***).* Sodium hydride (0·45 g, 15 mmol) was added to a suspension of 1,2,3,4,5,6-hexahydrobenzo[*h*][1,6]naphthyridin-5-one **4a** (2 g, 10 mmol) in dry *N*,*N*-dimethylformamide (40 mL). The mixture was stirred for 15 min (approx.) at room temperature before addition of 1,4-dibromobutane (1·3 mL, 11 mmol). Stirring was then continued for 19 h at room temperature. This mixture was poured into water (150 mL) and extracted with diethyl ether (2 × 150 mL). The organic layer was washed with water (2 × 100 mL), dried over magnesium sulphate and evaporated under reduced pressure to give **6a** as an oily residue which was purified by chromatography on silica gel with ethyl acetate–cyclohexane (50 : 50 v/v) as eluent to give solid **6a** (0·73 g, 22%); mp 122°C. IR (KBr) *v*: 3308, 2929, 2844,

1624, 1599, 1534, 1453, 1325, 1260, 1190, 749 $cm^{-1}$; $^1H$ NMR ($d_6$-DMSO) $\delta$: 7·87 (1H, d, $H_{10}$), 7·52 (1H, t, $H_8$), 7·45 (1H, d, $H_7$), 7·17 (1H, t, $H_9$), 6·95 (1H, s, NH), 4·20 (2H, t, $H_{4'}$), 3·59 (2H, t, $H_{1'}$), 3·30 (2H, t, $H_2$), 2·48 (2H, t, $H_4$), 1·80 (6H, m, $H_{2'/3'}$ and $H_3$); MS m/z 335 ($M^+$), 255, 200, 174. Calculated for $C_{16}H_{19}N_2OBr$: C, 57·32; H, 5·71; N, 8·36; found: C, 57·67; H, 5·89; N, 8·68%.

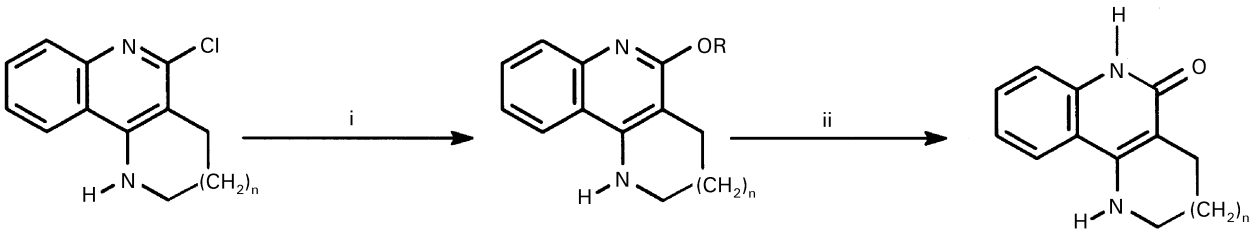
*6-[4-(4-Phenylpiperazin-1-yl)-butyl]-2,3,4,5-tetrahydro-1*H*-benzo[h][1,6]naphthyridin-5-one fumarate (**8a**)*. 6-(4-Bromobutyl)-2,3,4,5-tetrahydro-1*H*-benzo[*h*][1,6]naphthyridin-5-one **6a** (0.6 g, 1.79 mmol) was dissolved in *N*,*N*-dimethylformamide (10 mL) in the presence of potassium carbonate (0·29 g, 2·15 mmol) and *N*-phenylpiperazine (0·3 mL, 1·79 mmol) and the mixture was maintained under reflux for 45 min. After cooling, water (100 mL) was added and the mixture was extracted with chloroform (150 mL). The organic layer was washed with water (3 × 100 mL), dried over magnesium sulphate and calcium chloride, treated with charcoal, and concentrated to dryness to give an oily residue that was triturated in diethyl ether until crystallization. The resulting solid was dissolved in isopropanol (11 mL). Fumaric acid (2·76 mmol) was added and the mixture was warmed under reflux for 1 h. After cooling, a white powder was obtained; this was collected by filtration to give **8a** (0·5 g, 52%); mp 188°C. IR (KBr) *v*: 3308, 2932, 2842, 1702, 1625, 1599, 1542, 1455, 1362, 1213, 748 $cm^{-1}$; $^1H$ NMR ($d_6$-DMSO) $\delta$: 7·86 (1H, d, $H_{10}$), 7·50 (2H, m, $H_7$ and $H_{phenyl}$), 7·18 (3H, m, $H_8$ and $H_{phenyl}$), 6·93 (3H, m, NH, and $H_{phenyl}$), 6·78 (1H, t, $H_9$), 4·19 (2H, t, $H_{1'}$), 3·29 (2H, t, $H_2$), 3·16 (4H, m, $H_{7'}$ and $H_{9'}$), 2·61 (4H, m, $H_{6'}$ and $H_{10'}$), 2·50 (4H, m, $H_4$ and $H_{4'}$), 1·80 (2H, m, $H_3$), 1·59 (4H, m, $H_{2'}$ and $H_{3'}$); MS m/z 416 ($M^+$), 284, 255, 207, 161, 111, 84. Calculated for $C_{30}H_{36}N_4O_{5.5}$: C, 66·46; H, 6·83; N, 10·34; found: C, 66·59; H, 6·78; N, 10·32%.

## Results and Discussion

*Chemistry*

5- Chloro-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine **1a** and 6-chloro-2,3,4,5-tetrahydro-1*H*-azepino[3,2-*c*]quinoline **1b**, respectively, were obtained by rearrangement of pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5,11-dione and pyrido[2,1-*c*]-[1,4]benzodiazepin-6,12-dione by treatment with phosphorus oxychloride under microwave heating conditions (Rault et al 1995; Bureau et al 1999).

1,2,3,4,5,6 - Hexahydrobenzo [*h*][1,6] naphthyridin-5-one **4a** and its homologue 2,3,4,5,6,7-hexahydro-1*H*-azepino[3,2-*c*]quinoline **4b** were easily obtained by treating the chloroimidates **1a** and **1b** with sodium methoxide or ethoxide in *N*,*N*-dimethylformamide to give the corresponding alkyliminoethers **2a**, **2b** and **3a**, **3b** (Figure 2) which were then converted to the lactams **4a**, **4b** in good yield, by the action of potassium iodide (1·5 eq) in acetic acid at 100°C (Hoffman et al 1993). The tautomeric equilibrium lactam–lactim enabled reactivity of the $N_6$ amine group (lactam) or hydroxyl group (lactim). On the other hand, the lack of reactivity of nitrogen $N_1$ was because of the tautomeric equilibrium encountered in the 4-aminoquinoline series (Renault & Cartron 1966). Thus, the trifluoromethanesulfonate (triflate) **5a** was obtained by treatment of the chloroimidate **4a** with trifluoromethanesulphonyl anhydride (1·8 eq) in dry pyridine at room temperature for 12 h. This triflate reacted with *N*-benzylpiperazine (1·2 eq) in *N*,*N*-dimethylformamide under reflux for 3 h to give the amidine **7a** (Figure 3).




**1a** n = 1
**1b** n = 2

**2a** n = 1; R = $CH_3$
**2b** n = 2; R = $CH_3$
**3a** n = 1; R = $C_2H_5$
**3b** n = 2; R = $C_2H_5$

**4a** n = 1
**4b** n = 2

Figure 2. Preparation of 1,2,3,4,5,6-hexahydrobenzo[*h*][1,6]naphthyridin-5-one (**4a**) and 2,3,4,5,6,7-hexahydro-1*H*-azepino[3,2-*c*]quinoline (**4b**). Reagents: i. RONa, dimethylformamide; ii. KI, $CH_3COOH$.

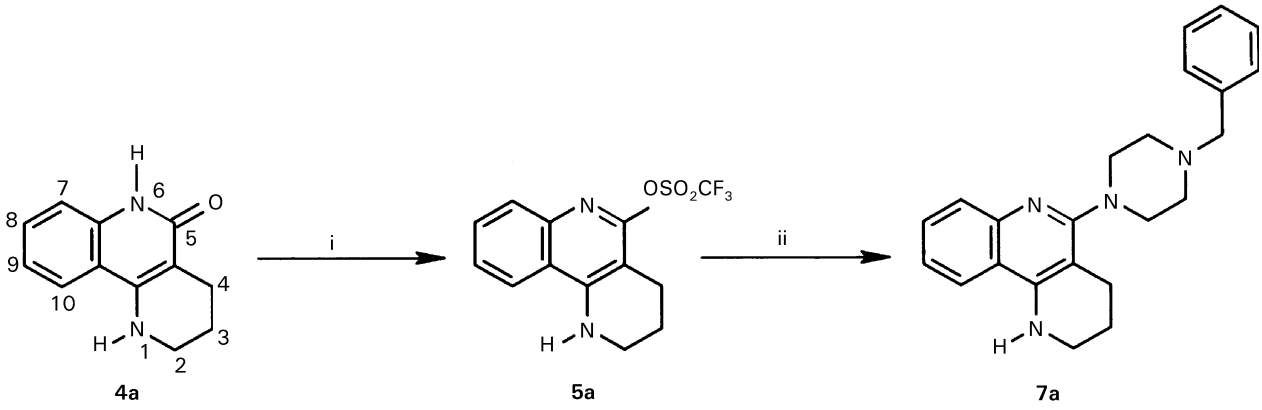



Figure 3. Preparation of 5-*N*-benzylpiperazino-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine difumarate (**7a**). Reagents: i. $Tf_2O$ (trifluoromethane sulphonic acid anhydride), pyridine; ii. *N*-benzylpiperazine, $K_2CO_3$, dimethylformamide.

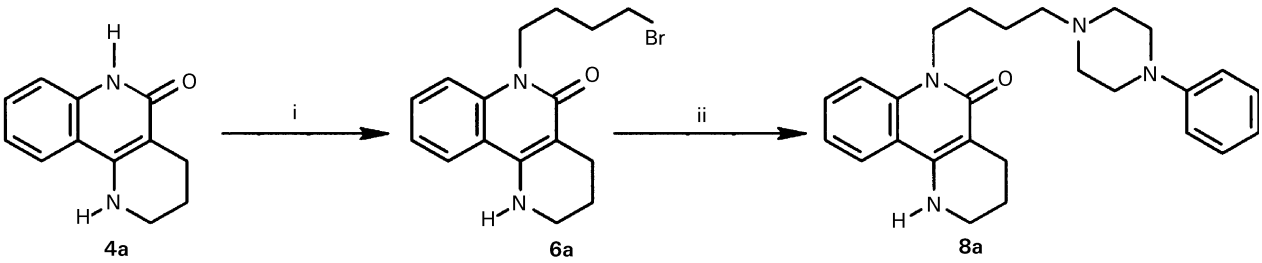



Figure 4. Preparation of 6-[4-(4-Phenyl-piperazin-1-yl)-butyl]-2,3,4,5-tetrahydro-1*H*-benzo[*h*][1,6]naphthyridin-5-one fumarate. Reagents: i. $Br(CH_2)_4Br$, HNa, dimethylformamide; ii. *N*-phenylpiperazine, $K_2CO_3$, dimethylformamide.

The direct substitution of chloroimidate **1a** was difficult and required high temperature and pressure—reaction in a sealed tube with microwave-heating.

The *N*-bromobutyllactam **6a** was prepared by the action of 1,4-dibromobutane in the presence of sodium hydride (1·5 eq) in *N*,*N*-dimethylformamide for 19 h at room temperature. Treatment of this lactam **6a** with *N*-phenylpiperazine in boiling *N*,*N*-dimethylformamide, in the presence of potassium carbonate, gave, after 45 min, the *N*-phenylpiperazinobutyllactam **8a** in good yield (Figure 4).

*Biological results*

To assess the central pharmacological action of compounds **7a** and **8a** on the 5-$HT_7$ receptor, samples of cloned subtype 7 5-HTergic receptor from man (Nen, CRM-047; 38 $\mu$g mL$^{-1}$) were incubated in buffer (Tris-HCl 50 nM, $MgSO_4$ 10 nM, EDTA 0·5 nM, pH 7·4) for 60 min with different amounts (0·5–8 nM) of [$^3$H]LSD (Shen et al 1993). Protein-radioligand binding was terminated by rapid filtration through Whatman GF/A fibre filters. The filters were washed with Tris-HCl buffer (50 mM, pH 7·4; 5 × 1 mL). Non-specific binding of [$^3$H]LSD was quantified in the presence of 10 $\mu$M clozapine.

Table 1. Percentage inhibition of [$^3$H]LSD binding by $10^{-6}$ and $10^{-8}$ M 5-*N*-benzylpiperazino-1,2,3,4-tetrahydrobenzo[*h*]-[1,6]naphthyridine difumarate (**7a**) and 6-[4-(4-phenylpiperazin-1-yl)-butyl]-2,3,4,5-tetrahydro-1*H*-benzo[*h*][1,6]naphthyridin-5-one fumarate (**8a**).

| | Inhibition (%) | |
|---|---|---|
| Compound | $10^{-6}$ M | $10^{-8}$ M |
| **7a** | 58 | 27 |
| **8a** | 88 | 12 |

Compounds **7a** and **8a** were tested for their capacity to displace [$^3$H]LSD (2 nM) from its binding site in the guinea-pig. The percentage inhibition (I%) was determined at $10^{-6}$ or $10^{-8}$ M and values of $K_i$ (inhibition constants) or IC50 (dose resulting in inhibition) were evaluated (Table 1).

These preliminary results are encouraging because the compound **8a** has an affinity of 0·1 $\mu$M (approx.) for the 5-$HT_7$ receptor from man.

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