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Synthesis and biological evaluation of 1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives as new ligands of cannabinoid receptors

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Abstract—Cannabinoid receptors have been studied extensively in view of their potential functional role in several physiological and pathological processes. For this reason, the search for new potent, selective ligands for subtype CB receptors, CB_1 and CB_2, is still of great importance, in order to investigate their role in various physiological functions. The present study describes the synthesis and the biological properties of a series of 1,8-naphthyridine derivatives, characterised by the presence of some important structural requirements exhibited by other classes of cannabinoid ligands, such as an aliphatic or aromatic carboxamide group in position 3, and an alkyl or arylalkyl substituent in position 1. These compounds were assayed for binding both to the brain and to peripheral cannabinoid receptors (CB_1 and CB_2). The results obtained indicate that the naphthyridine derivatives examined possess a greater affinity for the CB_2 receptor than for the CB_1 receptor. In particular, derivatives **6a** and **7a** possess an appreciable affinity for the CB_2 receptor, with K_i values of 5.5 and 8.0 nM respectively; also compounds **4a**, **5a** and **8a** exhibit a good CB_2 affinity, with K_i values in the range of 10–44 nM. Furthermore, compounds **3g–i** and **18** revealed a good CB_2 selectivity, with a CB_1/CB_2 ratio >20.

1. Introduction

The use of Cannabis preparations for medicinal purposes has been known about for a thousand years; however, its utility in recent years has been limited because of its psychoactive properties. Following the identification of Δ^9-tetrahydrocannabinol[1] (Δ^9-THC, Fig. 1) as the principal psychoactive constituent of marijuana, other cannabimimetic compounds were discovered, including endogenous arachidonyl-ethanolamide (anandamide)[2] (Fig. 1) and compounds with structures differing quite dramatically from that of the 'classic' cannabinoid compounds, such as bicyclic cannabinols[3,4] typified by CP55940 (Fig. 1), cannabimimetic indoles, pyrroles and indenes,[5] of which WIN-55,212-2 (Fig. 1) is the prototypical example. These compounds led to the identification and characterization of two subtypes of G-protein-coupled membrane cannabinoid receptors, termed CB_1,[3,6,7] and CB_2,[8] primarily present in the nervous system and in the immune system respectively. These receptors exert an action either in the central nervous system, by the control of cognition, memory and motor function and perception of pain, or in peripheral systems, in particular in the urogenital, gastrointestinal and cardiovascular ones. Furthermore, the CB_2 receptors are widely expressed in immune cells, B cells and natural killer cells.

Recently, there has been renewed interest in cannabinoids as anticancer agents. In particular Ajulemic acid (AJA, dimethylheptyl-THC-11-oic acid) (Fig. 1), a synthetic analogue of THC has proved to be equipotent or more potent than THC in several anti-inflammatory bioassays, presenting significant antitumor effects.[9,10]

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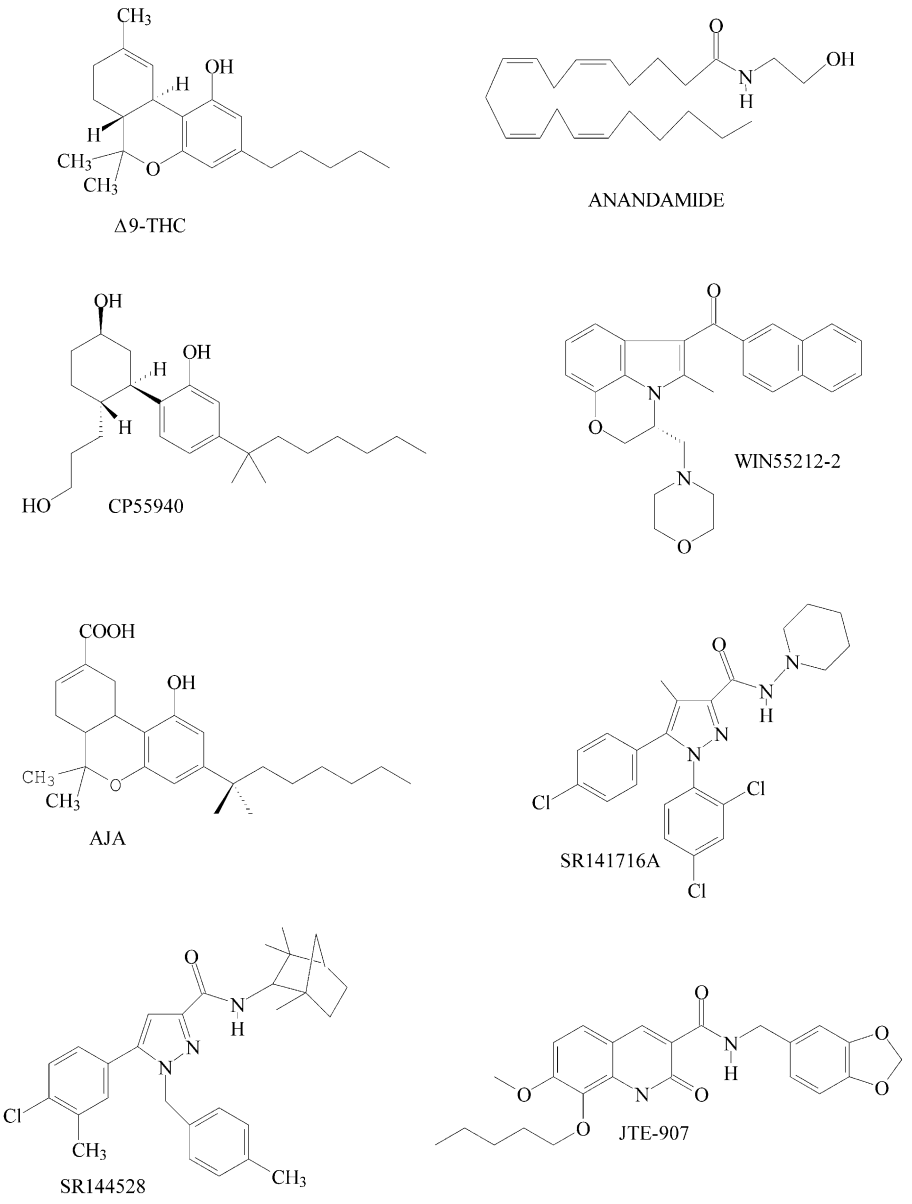


Figure 1. Molecular structures of cannabinoid CB_1 and CB_2 receptor ligands.

Moreover AJA is without any psychoactive effects and has a favourable toxicity profile. It has been reported that its antineoplastic effects are mediated primarily through actions on CB_2 receptors.[10] As regards selective ligands to cannabinoid subtype receptors, the arylpyrazole derivatives SR141716[11] and SR144528[12] (Fig. 1) have been reported to be CB_1- and CB_2-selective ligands, respectively, with an antagonist activity. Furthermore, a novel 1,2-dihydroquinoline-3-carboxamide (JTE-907)[13] (Fig. 1), a selective ligand for the CB_2 receptor, has recently been synthesized. At present, the research and development of new potent, selective ligands for CB_1 and/or CB_2 is still of great importance for the investigation the role of the two subtypes of cannabinoid receptors in various physiological functions. In particular, new potent, selective CB_2 receptor ligands are important to understand certain effects of cannabinoids, such as their immunosuppressant and anti-inflammatory activities.[14,15]

On the basis of the above considerations, the present paper describes the synthesis and the biological properties of a series of 1,8-naphthyridine derivatives with a general structure **A** (Fig. 2), variously substituted on the heterocyclic nucleus. These compounds are characterized by the presence of certain important structural requirements shown by other classes of cannabinoid ligands, such as an aliphatic or aromatic carboxamide group in position 3, present in quinolone derivatives like JTE-907[13] (Fig. 1) and in arylpyrazole derivatives

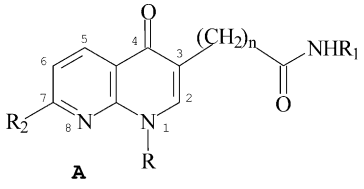


Figure 2. General structure of 1,8-naphthyridine derivatives.

like SR-144528[12] (Fig. 1) and an alkyl or arylalkyl substituent in position 1, present in aminoalkylindole derivatives like WIN-55,212-2[5] (Fig. 1) and in SR-144528[12] (Fig. 1).

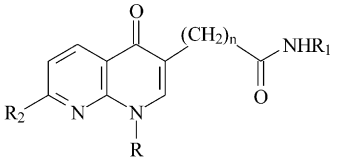
2. Chemistry

The compounds described in this study are shown in Table 1, and their synthetic methods are outlined in Schemes 1–5. 1,8-Naphthyridin-3-carboxamide derivatives with a general structure A (Fig. 2) were prepared from 7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxylic acid ethyl ester (**1**) which was obtained following the synthethic route described in literature.[16] The heating of ester **1** with the appropiate amine in a sealed tube provided the carboxamide derivatives **2a–l** (Scheme 1). The 1-ethylmorpholine derivatives **3a–l** were obtained by treatment of **2a–l** in anhydrous DMF with NaH for 1 h and then with 4-(2-chloroethyl)morpholine hydrochloride (Scheme 1).

Analogously, the 3-carboxycyclohexylamide derivatives **4a–8a** and the 3-carboxybenzylamide derivatives **4e–8e** and **4l** were prepared from the 1,8-naphthyridine derivatives **2a**, **23** or **2l**, respectively, with the appropiate benzylchloride or alkylchloride (Scheme 2).

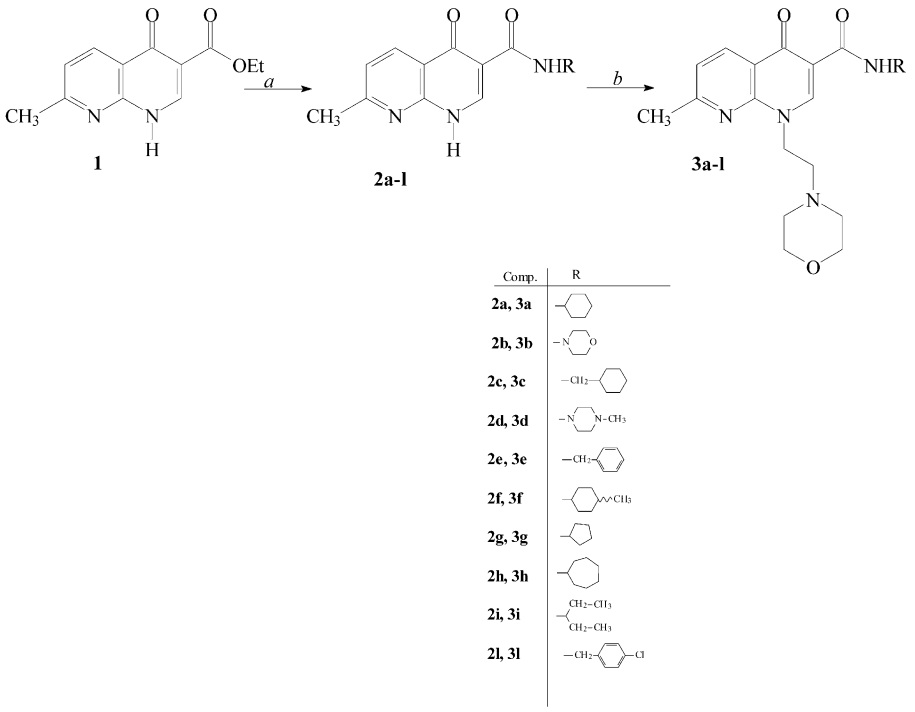
To confirm that the alkylation reaction leads to the 1-position substituted derivatives, the *N*-cyclohexyl-1-benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxyamide (**4a**) was also prepared following a different synthetic route, as described in Scheme 3. The ethyl ester **1** was firstly *N*-alkylated, as reported above, to obtain the 1-benzyl derivative **9** and was then transformed into the expected cyclohexylamide derivative **4a**. The reaction of the 1-benzyl-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxylic acid ethyl ester (**9**) with aqueous 10% sodium

Table 1. Radioligand binding data of 1,8-naphthyridine derivatives **2a**, **2e**, **3a–l**, **4a**,**e–8a**,**e**, **4l**, **15**, **16**, **18**, **25a**,**b** and **26a–d**

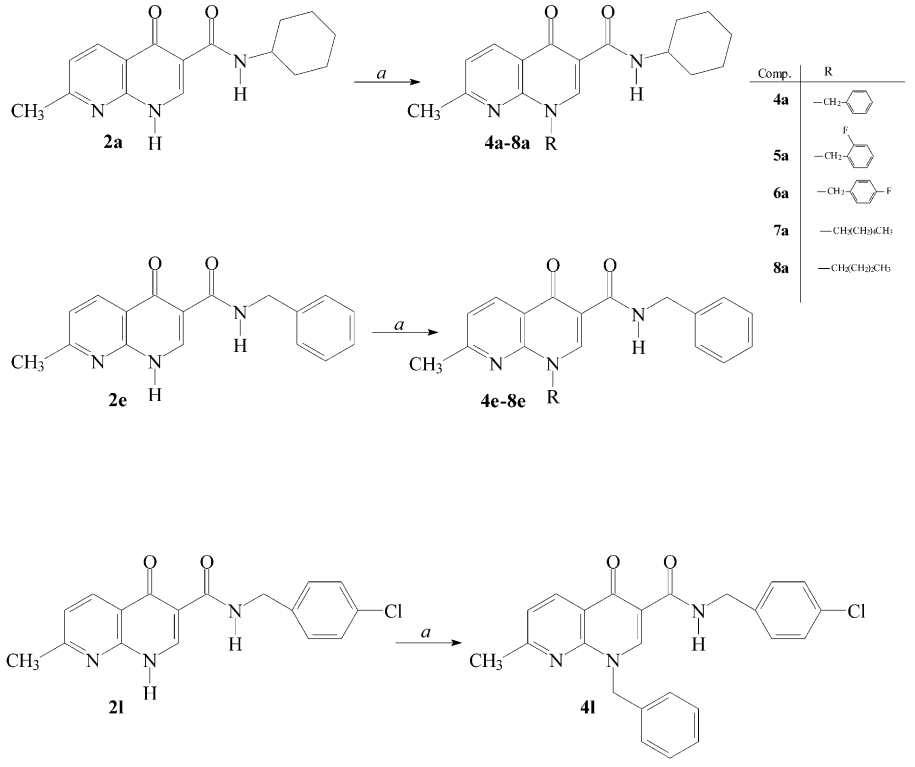


Compd	R	R_1	R_2	n	Receptor affinity(nM) K_iCB_1[a]	Selectivity ratio K_iCB_2[b]	K_iCB_1/K_iCB_2
2a	H	cyclohexyl	CH_3	0	1000	1000	
2e	H	benzyl	CH_3	0	1000	1000	
3a	Ethylmorph	cyclohexyl	CH_3	0	1000	100±8	10
3b	Ethylmorph	morph	CH_3	0	1000	1000	
3c	Ethylmorph	CH_2cyclohexyl	CH_3	0	1000	117±15	9
3d	Ethylmorph	N-CH_3pipz	CH_3	0	1000	1000	
3e	Ethylmorph	benzyl	CH_3	0	1000	475±25	2
3f	Ethylmorph	4-CH_3-cyclohexyl	CH_3	0	537±24	30±2	17.9
3g	Ethylmorph	cyclopentyl	CH_3	0	1000	50±4	20
3h	Ethylmorph	cycloheptyl	CH_3	0	560±33	22±2	25.5
3i	Ethylmorph	isopentyl	CH_3	0	1000	50±3	20
3l	Ethylmorph	*p*-Cl-benzyl	CH_3	0	1000	1000	
4a	benzyl	cyclohexyl	CH_3	0	127±13	10±0.5	13
4e	benzyl	benzyl	CH_3	0	1000	1000	
4l	benzyl	*p*-Cl-benzyl	CH_3	0	1000	1000	
5a	*o*-F-benzyl	cyclohexyl	CH_3	0	208±17	44±2	4.7
5e	*o*-F-benzyl	benzyl	CH_3	0	1000	600±60	2
6a	*p*-F-benzyl	cyclohexyl	CH_3	0	15±1.8	5.5±0.4	2.7
6e	*p*-F-benzyl	benzyl	CH_3	0	457±40	65.3±6	7
7a	*n*-hexyl	cyclohexyl	CH_3	0	95±3	8.0±0.2	11.9
7e	*n*-hexyl	benzyl	CH_3	0	1000	325±25	3
8a	*n*-butyl	cyclohexyl	CH_3	0	262±10.4	17.5±1	15
8e	*n*-butyl	benzyl	CH_3	0	1000	1000	
15	Ethylmorph	benzyl	NH_2	0	1000	1000	
16	Ethylmorph	cyclohexyl	NH_2	0	1000	1000	
18	Ethylmorph	cyclohexyl	Cl	0	1000	25±1.8	40
25a	Ethylmorph	benzyl	CH_3	1	1000	1000	
25b	benzyl	benzyl	CH_3	1	1000	729±82	1.4
26a	Ethylmorph	cyclohexyl	CH_3	1	1000	1000	
26b	benzyl	cyclohexyl	CH_3	1	1000	530±50	2
26c	n-hexyl	cyclohexyl	CH_3	1	1000	1000	
26d	n-butyl	cyclohexyl	CH_3	1	1000	1000	
SR141716A					1.8±00.75	514±30	0.0035
SR144528					70±10	0.28btc00.4	250

[a] Affinity of compounds for CB_1 receptor was evaluated using mouse cerebellum membranes and [^{3}H]-CP 55,940.
[b] Affinity of compounds for CB_2 receptor was assayed using mouse spleen homogenate and [^{3}H]-CP 55,940. Ki values were obtained from five indipendent experiments carry out in triplicate and are expressed as the mean±standard error.



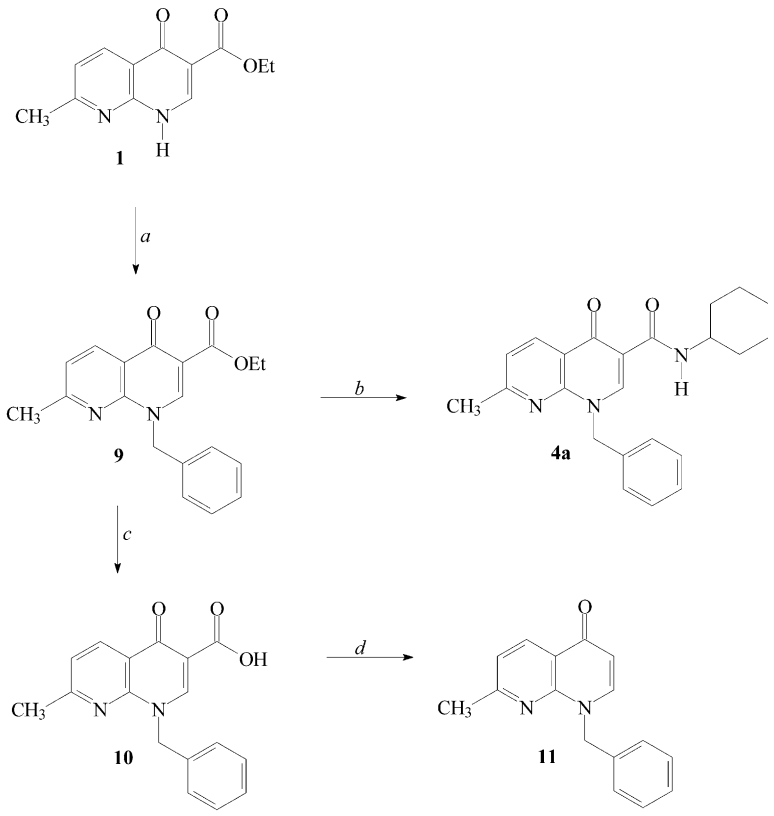
Scheme 1. (a) RNH_2; (b) DMF, NaH, 4-(2-chloroethyl)morpholine hydrochloride.



Scheme 2. (a) DMF, NaH, RCl.

hydroxide at reflux gave the 3-carboxylic acid derivative **10**. This compound was refluxed in Dowtherm A in the presence of copper chromite as a catalyst to afford 1-benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (**11**), which had previously been synthesised by following a different method.[17]

As described in Scheme 4, starting from the 7-acetamido-1,8-naphthyridin-4(1H)-on-3-carboxylic acid ethyl ester **12**,[18] analogously to the procedure described above, was transformed into the 3-carboxyamide derivatives **13** and **14** by reaction in a sealed tube with the appropriate amine. Under these conditions, also the



Scheme 3. (a) DMF, NaH, $C_6H_5CH_2Cl$. (b) cyclohexylamine. (c) 10% NaOH (d) Dowtherm A, copper chromite.

hydrolysis of the acetamido group takes place. The reaction of **13** and **14** in anhydrous DMF and NaH with 4-(2-chloroethyl)morpholine hydrochloride afforded the 1,8-naphthyridine derivatives **15** and **16** (Scheme 4). Diazotization of *N*-cyclohexyl-7-amino-1,8-naphthyridin-4(1H)-on-3-carboxyamide (**14**), carried out in aqueous 37% hydrochloric acid at −5 °C during the addition of the $NaNO_2$, and then at 40 °C for 3 h, gave the corresponding 7-chloro derivative **17**. This last compound was then converted into the corresponding 1-ethylmorpholine derivative **18** by reaction with 4-(2-chloroethyl)morpholine hydrochloride under the same conditions described above. The reaction of 7-methyl-2,3-dihydro-1,8-naphthyridine-4(1*H*)-one (**19**)[19] with glyoxylic acid at 100 °C for 1 h afforded the 3-methylcarboxylic acid **20** (Scheme 5) which, by reaction with anhydrous EtOH and 96% sulphuric acid at 80 °C, was converted into the ethylester **21**, which had previously been synthesised by following a different method.[20] The heating of ester **21** with benzylamine or cyclohexylamine in a sealed tube provided the corresponding 3-methylcarboxamide derivatives **22** and **23** (Scheme 5). Lastly **22** and **23**, by reaction with the appropriate benzylchloride or alkylchloride in anhydrous DMF and NaH, afforded the desired 1,8-naphthyridine derivatives **25a**,**b** and **26a**–**d**.

3. Biology

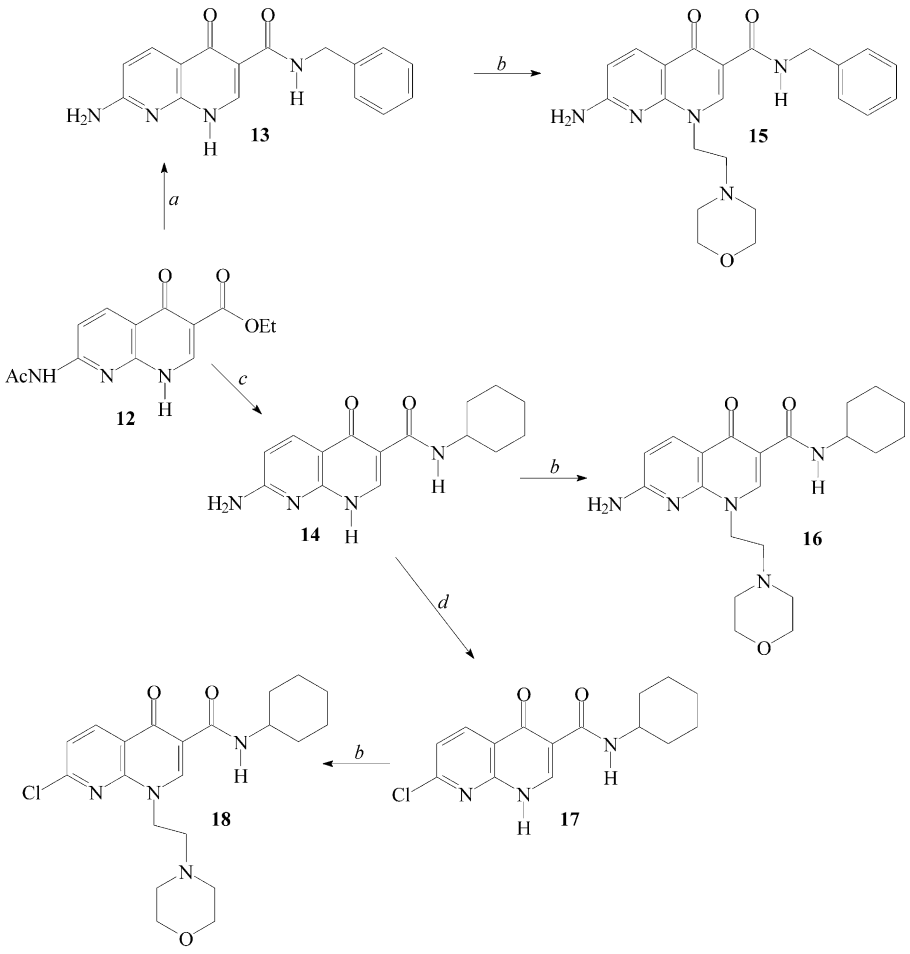
Affinities of the 1,8-naphthyridine derivatives **2a**,**e**, **3a**–**l**, **4a**,**e**–**8a**,**e**, **4l**, **15**, **16**, **18**, **25a**,**b** and **26a**–**d** for CB_1 and CB_2 receptors were assessed by competition experiments with [^{3}H]-CP 55,940 in mouse cerebral membranes and in mouse spleen homogenate, respectively. For the purposes of comparison, and as references values, we have also included results for the two prototypical cannabinoid ligands, SR141716A and SR144528.[21]

4. Results and discussion

The 1,8-naphthyridine derivatives examined have in common the presence in the heterocyclic nucleus of a substituent of various kinds on the nitrogen in position 1, an amido substituent in position 3 and a substituent in position 7.

4.1. CB_1 Receptor affinities

An examination of the results shown in Table 1 reveals that for the 7-methyl-3-carboxyamido compounds (**2a**,**e**, **3a**–**l**, **4a**,**e**,**l** and **5a**,**e**–**8a**,**e**), the absence of a substituent on the nitrogen in position 1 determined a poor affinity (**2a** and **2e** $K_i > 1000$). Likewise, the compounds with an ethylmorpholino group in position 1, regardless of the nature of the carboxyamido substituent in position 3, exhibited a poor affinity, with K_i values >1000. The only exceptions were the **3f** and **3h** derivatives, with a modest K_i value ($K_i = 537$ and 560 nM respectively). The presence of an n-alkyl substituent (*n*-butyl or *n*-hexyl), with the simultaneous presence of an aliphatic carboxyamide, such as cyclohexylamide, in position 3, determined a fair affinity, which proved to be higher for the *n*-hexyl-substituted compound (**7a** $K_i = 95$ nM and **8a** $K_i = 262$ nM). Analogously, for 3-carboxycyclohexylamide derivatives, the presence in position 1 of a benzyl



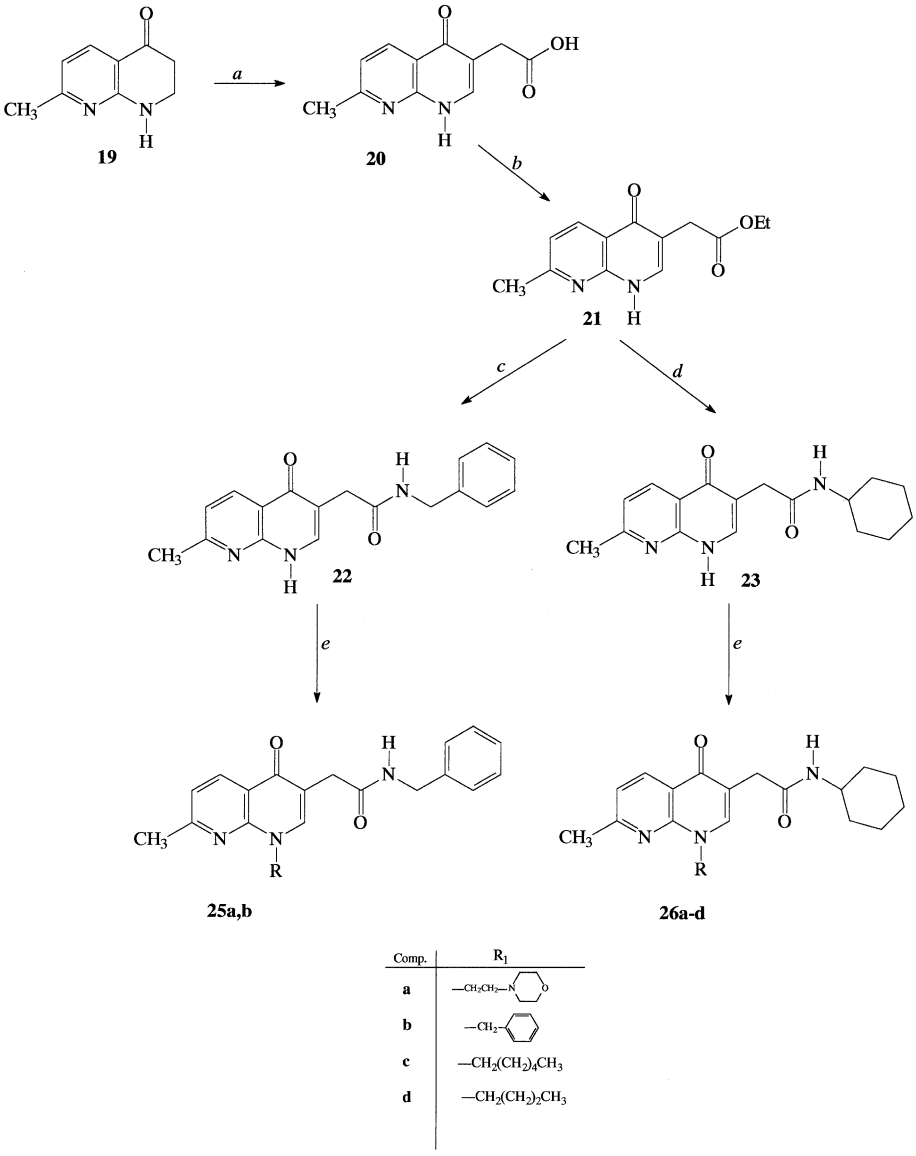
Scheme 4. (a) $C_6H_5CH_2NH_2$. (b) DMF, NaH, 4-(2-chloroethyl)morpholine hydrochloride. (c) cyclohexylamine. (d) $NaNO_2$, HCl.

group, whether substituted or not, led to compounds with a fair affinity. In particular, the *p*-fluoro benzyl derivative (**6a**) proved to be the compound with the highest affinity in this series towards the CB_1 receptor, with a K_i value of 15 nM. Furthermore, it appeared to be clear that 3-carboxycyclohexylamide derivatives generally possess a higher affinity towards CB_1 compounds than 3-benzyl carboxyamide compounds. The substitution of the methyl in position 7 with an amino group or an atom of chlorine did not lead to an improvement in the affinity. Lastly, the presence of a methylene spacer between the heterocyclic nucleus and the carboxyamidic group in position 3, produced a markedly negative effect on the affinity of these compounds for the CB_1 receptor, as confirmed by a comparison of compounds **26b–d** with **4a**, **7a** and **8a**.

4.2. CB_2 Receptor affinities

The results obtained indicate that, as had previously been found for the CB_1 receptor, the naphthyridine derivatives without any substituents on the nitrogen in position 1 exhibit a poor affinity towards the CB_2 receptor (**2a** and **2e** $K_i > 1000$). Furthermore, an important result to be underlined is that 3-carboxycyclohexylamide derivatives possess a higher affinity than 3-carboxybenzylamide compounds, analogously to results for the CB_1 receptor. In particular, the **6a** and **7a** derivatives possess a significant affinity, with K_i values of 5.5 and 8.0 nM, respectively; also compounds **4a**, **5a** and **8a** possess a good affinity, with values of K_i in the range of 10-44 nM. Among the 3-carboxycyclohexylamide compounds, the *N*-ethylmorpholino derivatives possess a lower affinity than the *N*-benzyl or *N*-alkyl ones. For the *N*-alkyl derivatives, the n-hexyl group proved to be more effective than the *n*-butyl. For the *N*-benzyl derivatives, the presence of an atom of fluorine on the benzyl increases the affinity, above all of the substitution is the para position. Unlike findings for the CB_1 receptor, the substitution of the methyl in position 7 of the naphthyridine nucleus with the NH_2 group determines a decrease in the affinity, as confirmed by a comparison of **16** with **3a**, and of **15** with **3e**, while the presence in this position of an atom of chlorine determines an approximately 4-fold increase in the affinity, as can be seen from a comparison of **18** with **3a**. Lastly, in line with findings for the CB_1 receptor, the presence of a methylene spacer between the naphthyridine nucleus and the carboxyamido group leads to a marked decrease in the affinity.

In conclusion, the results obtained show that the naphthyridine derivatives examined generally exhibit a higher affinity for the CB_2 receptor than for the CB_1 receptor. Furthermore, some of these compounds showed a good CB_2 selectivity, such as **3g–i** and **18**, for which the CB_1/CB_2 ratio is >20.



Scheme 5. (a) glyoxylic acid. (b) EtOH, H_2SO_4. (c) $C_6H_5CH_2NH_2$. (d) cyclohexylamine. (e) DMF, NaH, RCl.

It is also possible to hypothesise that in order to obtain a good affinity on the CB_2 receptor, the presence is necessary in position 1 of a *n*-alkylic or benzylic substituent, which may be substituted, and in position 3, of a carboxyamide of an aliphatic nature, such as the cyclohexylamide directly linked to the 1,8-naphthyridine nucleus (Fig. 2).

In the light of these results, it is clear that this series of naphthyridine derivatives represents a new class of heterocyclic derivatives, acting as ligands of cannabinoid receptors. The study of these compounds deserves to be developed in order to be optimise their affinity and selectivity towards the cannabinoid receptor subtypes.

5. Experimental

5.1. Chemistry

5.1.1. General information. All melting points were taken on a Kofler hot stage apparatus and are uncorrected. IR spectra in Nujol mulls were determined on an ATI Mattson Genesis Series FTIR spectrometer. ^{1}H NMR spectra were recorded with a Bruker AC-200 spectrometer in δ units from TMS as an internal standard. Analytical TLC was carried out on Merck 0.2 mm precoated silica-gel glass plates (60 F-254) and location of spots was detected by illumination with a UV lamp. Elemental analyses (C, H, N) were within ±0.4% of the theoretical values and were performed on a Carlo Erba elemental analyzer model 1106 apparatus.

5.1.2. General procedure for the synthesis of 7-methyl-1,8-naphthyridin-3-carboxyamide derivatives (2a–l). A mixture of 1 mmol of 7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxylic acid ethyl ester (**1**) and 10 mmol of the appropriate amine was heated in a sealed tube at 120 °C (**2a**, **c**, **e**–**h**, **l**) or at 160 °C (**2b**, **d**, **i**) for 24 h. After cooling, the reaction mixture was treated with ethyl ether to give a solid residue which was collected by filtration and purified by crystallization (**2a**, **c**–**l**) or by flash-

chromatography, eluting with ethyl acetate/methanol, 10:1 (**2b**) to obtain the title compounds.

5.1.3. *N*-Cyclohexyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2a). Yield 85%; mp 302–304 °C (ethyl acetate).^{1}H NMR (DMSO) δ 10.31 (d, 1H, NH), 8.74 (s, 1H, H_2), 8.43 (d, 1H, H_5), 7.25 (d, 1H, H_6), 3.90 (brs, 1H, CH), 2.57 (s, 3H, CH_3), 1.81–1.06 (m, 10H, cyclohexyl). Anal. $C_{16}H_{19}N_3O_2$ (MW 285.34): C, 67.35; H, 6.71; N, 14.74%; found: C, 67.01; H, 6.32; N, 14.50%.

5.1.4. 7-Methyl-*N*-(morpholin-4-yl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2b). Yield 29%; mp 243–245 °C (ethyl acetate).^{1}H NMR ($CDCl_3$) δ 10.75 (brs, 1H, NH), 8.61 (s, 1H, H_2), 8.48 (d, 1H, H_5), 7.43 (d, 1H, H_6), 3.68 (m, 4H, morpholine), 2.87 (m, 4H, morpholine), 2.63 (s, 3H, CH_3). Anal. $C_{14}H_{16}N_4O_3$ (MW 288.30): C, 58.33; H, 5.59; N, 19.44%; found: C, 58.42; H, 5.76; N, 19.53%.

5.1.5. *N*-(Cyclohexylmethyl)-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2c). Yield 82%; mp 262–264 °C (cyclohexane). ^{1}H NMR (DMSO) δ 9.89 (t, 1H, NH), 8.62 (s, 1H, H_2), 8.47 (d, 1H, H_5), 7.43 (d, 1H, H_6), 3.40 (m, 1H, CH,), 3.18 (m, 2H, NCH_2), 2.62 (s, 3H, CH_3), 1.88–1.34 (m, 10H, cyclohexyl). Anal. $C_{17}H_{21}N_3O_2$ (MW 299.36): C, 68.23; H, 7.07; N, 14.05%; found: C, 68.50; H, 7.05; N, 14.11%.

5.1.6. 7-Methyl-*N*-(4-methylpiperazin-1-yl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2d). Yield 64%; mp 235–237 °C (ethyl acetate).^{1}H NMR (DMSO) δ 10.73 (brs, 1H, NH), 8.61 (s, 1H, H_2), 8.48 (d, 1H, H_5), 7.42 (d, 1H, H_6), 2.85 (m, 4H, piperazine), 2.62 (s, 3H, CH_3), 2.50 (m, 4H, piperazine), 2.19 (s, 3H, NCH_3). Anal. $C_{15}H_{19}N_5O_2$ (MW 301.34): C, 59.80H, 6.36; N, 23.24%; found: C, 60.11; H, 6.45; N, 23.38%.

5.1.7. *N*-Benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2e). Yield 70%; mp 278–280 °C (ethyl acetate).^{1}H NMR (DMSO) δ 10.21 (t, 1H, NH), 8.66 (s, 1H, H_2), 8.47 (d, 1H, H_5), 7.41 (d, 1H, H_6), 7.30 (brs, 5H, Ar), 4.55 (d, 2H, CH_2), 2.62 (s, 3H, CH_3). Anal. $C_{17}H_{15}N_3O_2$ (MW 293.30): C, 69.62; H, 5.13; N, 14.33%; found: C, 68.02; H, 5.05; N, 14.04%.

5.1.8. 7-Methyl-*N*-(4-methylcyclohexyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2f). Yield 56%; mp 260–270 °C (cyclohexane). ^{1}H NMR (DMSO) δ 10.55 and 10.16 (2d, 1H, NH), 8.75 and 8.74 (2s, 1H, H_2), 8.48 and 8.43 (2d, 1H, H_5), 7.29 and 7.25 (2d, 1H, H_6), 4.10 (m, 1H, $H_{1'}$), 3.70 (m, 1H, $H_{4'}$), 2.62 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 1.87–1.68 (m, 8H, cyclohexyl). Anal. $C_{17}H_{21}N_3O_2$ (MW 299.37): C, 68.20; H, 7.07; N, 14.05%; found: C, 68.40; H, 7.15; N, 14.01%.

5.1.9. *N*-Cyclopentyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2g). Yield 68%; mp 280–283 °C (ethyl acetate). ^{1}H NMR (DMSO) δ 9.88 (d, 1H, NH), 8.61 (s, 1H, H_2), 8.48 (d, 1H, H_5), 7.42 (d, 1H, H_6), 4.23 (m, 1H, CH), 2.62 (s, 3H, CH_3), 1.98–1.44 (m, 8H, cyclopentyl). Anal. $C_{15}H_{17}N_3O_2$ (MW 271.31): C, 66.40; H, 6.32; N, 15.49%; found: C, 66.59; H, 6.55; N, 15.51%.

5.1.10. *N*-Cycloheptyl-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxamide (2h). Yield 70%; mp 270–272 °C (ethyl acetate). ^{1}H NMR (DMSO) δ: 9.93 (d, 1H, NH), 8.61 (s, 1H, H_2), 8.49 (d, 1H, H_5), 7.42 (d, 1H, H_6), 4.15 (m, 1H, CH), 2.62 (s, 3H, CH_3), 1.85–1.56 (m, 12H, cycloheptyl). Anal. $C_{17}H_{21}N_3O_2$ (MW 299.36): C, 68.20; H, 7.07; N, 14.05%; found: C, 68.02; H, 7.05; N, 14.04%.

5.1.11. *N*-(1-Ethylpropyl)-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2i). Yield 81%; mp 213–215 °C (toluene). ^{1}H NMR (DMSO) δ: 9.73 (d, 1H, NH), 8.63 (s, 1H, H_2), 8.49 (d, 1H, H_5), 7.43 (d, 1H, H_6), 3.83 (m, 1H, CH), 2.62 (s, 3H, CH_3), 1.48 (m, 4H, CH_2), 0.85 (m, 6H, CH_3). Anal. $C_{15}H_{19}N_3O_2$ (MW 277,33): C, 65.91; H, 7.01; N, 15.38%; found: C, 65.67; H, 6.99; N, 15.32%.

5.1.12. *N*-(*p*-Chlorbenzyl)-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (2l). Yield 61%; mp 280–283 °C (ethyl acetate).^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1H, H_2), 8.59 (d, 1H, H_5), 7.30 (m, 5H, Ar + H_6), 4.66 (d, 2H, CH_2), 2.68 (s, 3H, CH_3). Anal. $C_{17}H_{14}ClN_3O_2$ (MW 327.76): C, 62.30; H, 4.31; N, 12.82%; found: C, 62.15; H, 4.11; N, 12.50%.

5.1.13. General procedure for the synthesis of N_1-substituted 1,8-naphthyridine derivatives (3a–l, 4a–8a, 4e–8e, 4l). 1.25 mmol of NaH was added to a solution of 1 mmol of 7-methyl-1,8-naphthyridine-3-carboxamide derivatives **2a–l** in 10 mL of dry *N*,*N*-dimethylformamide. After 1 h the appropriate chloride (1 mmol) was added and the mixture was stirred for 24 h at room temperature for compounds **3c**, **d**, **f**, **g**, **i**, **4e–8e** and **4l** or at 50 °C for compounds **3a**, **b**, **e**, **h**, **l** and **4a–8a**. The products were then obtained by the following work-up: in the case of **3a–l**, the solvent was evaporated in vacuo and the solid obtained was treated with water and collected by filtration (**3c, d, f, g, i**) or extracted with chloroform (**3a, b, e, h, l**) and then the combinated extracts were washed with water, dried (magnesium sulphate) and evaporated to dryness in vacuo. For compounds **4a–8a**, **4e–8e** and **4l**, the reaction mixture was treated with water and then the precipitate formed was collected by filtration.

5.1.14. *N*-Cyclohexyl-7-methyl-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3a). Yield 30%; mp 191–195 °C (trituration with ethyl ether). ^{1}H NMR ($CDCl_3$) δ 10.15 (t, 1H, NH), 8.91 (s, 1H, H_2), 8.65 (d, 1H, H_5), 7.30 (d, 1H, H_6), 4.61 (t, 2H, CH_2N), 4.15 (brs, 1H, CH) 3.67 (m, 4H, morph), 2.78 (t, 2H, NCH_2), 2.68 (s, 3H, CH_3), 2.54 (m, 4H, morph), 2,00–1.26 (m, 10H, cyclohexyl). Anal. ($C_{22}H_{30}N_4O_3$) (MW 398.49): C, 66.31; H, 7.59; N, 14.06%; found: C, 66.51; H, 7.83; N, 14.42%.

5.1.15. 7-Methyl-*N*-morpholin-4-yl-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3b). Yield 25%; mp 146–148 °C [flash chromatography elut-

ing with ethyl acetate/toluene/diethylamine (10:4:1)]. ^{1}H NMR ($CDCl_3$) δ 10.82 (s, 1H, NH), 8.92 (s, 1H, H_2), 8.64 (d, 1H, H_5), 7.32 (d, 1H, H_6), 4.62 (t, 2H, NCH_2), 3.89 (m, 4H, morph), 3.66 (m, 4H, morph), 3.00 (m, 4H, morph), 2.77 (t, 2H, NCH_2), 2.69 (s, 3H, CH_3), 2,53 (m, 4H, morph). Anal. ($C_{20}H_{27}N_5O_4$) (MW 401.46): C, 59.84; H, 6.78; N, 17.44%; found: C, 59.51; H, 7.02; N, 17.62%.

5.1.16. *N*-(Cyclohexylmethyl)-7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3c). Yield 85%; mp 161–162 °C (ethyl acetate). ^{1}H NMR (DMSO) δ 9.95 (t, 1H, NH), 8.93 (s, 1H, H_2), 8.55 (d, 1H, H_5), 7.47 (d, 1H, H_6), 4.66 (t, 2H, CH_2N), 3.49 (m, 4H, morph), 3.40 (m, 1H, CH), 3.20 (t, 2H, CH_2N), 2.64 (s, 3H, CH_3), 2.62 (t, 2H, CH_2N), 2.45 (m, 4H, morph), 1.90–1.68 (m, 10H, cyclohexyl). Anal. ($C_{23}H_{32}N_4O_3$) (MW 412.52): C, 66.99; H, 7.77; N, 13.59%; found: C, 66.72; H, 7.79; N, 13.64%.

5.1.17. 7-Methyl-*N*-(4-methylpiperazin-1-yl)-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3d). Yield 51%; mp 182–185 °C (cyclohexane). ^{1}H NMR ($CDCl_3$) δ 10.78 (t, 1H, NH), 8.92 (s, 1H, H_2), 8.64 (d, 1H, H_5), 7.31 (d, 1H, H_6), 4.61 (t, 2H, NCH_2), 3.66 (m, 4H, morph), 3.04 (m, 4H, piperazine), 2.78 (t, 2H, NCH_2), 2.68 (s, 3H, CH_3), 2.53 (m, 4H, morph), 2,50 (m, 4H, piperazine), 2.35 (s, 3H, NCH_3). Anal. ($C_{21}H_{30}N_6O_3$) (MW 414.50): C, 60.84; H, 7.30; N, 20.28%; found: C, 61.05; H, 7.45; N, 20.53%.

5.1.18. *N*-Benzyl-7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3e). Yield 61%; mp 154–157 °C (trituration with ethyl ether). ^{1}H NMR ($CDCl_3$) δ 10.25 (t, 1H, NH), 8.94 (s, 1H, H_2), 8.64 (d, 1H, H_5), 7.34 (m, 6H, Ar + H_6), 4.60 (m, 4H, $CH_2N + CH_2Ar$), 3.67 (m, 4H, morph), 2.79 (t, 2H, NCH_2), 2.68 (s, 3H, CH_3), 2.55 (m, 4H, morph). Anal. ($C_{23}H_{26}N_4O_3$) (MW 406.47): C, 67.96; H, 6.45; N, 13.78%; found: C, 68.12; H, 6.54; N, 13.58%.

5.1.19. 7-Methyl-*N*-(4-methylcyclohexyl)-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3f). Yield 79%; ^{1}H NMR (DMSO) δ 10.18 and 9.78 (2d, 1H, NH), 8.94 and 8.92 (2s, 1H, H_2), 8.58 and 8.53 (2d, 1H, H_5), 7.65 (d, 1H, H_6), 4.66 (t, 2H, CH_2N), 4.15 (m, 1H, CH), 3.49 (m, 4H, morph), 2.64 (s, 3H, CH_3), 2.49 (t, 2H, CH_2N), 2.44 (t, 4H, morph), 1.68–1.87 (m, 12H, cyclohexyl + CH_3). Anal. ($C_{23}H_{32}N_4O_3$) (MW 412.52): C, 66.99; H, 7.77; N, 13.59%; found: C, 67.26; H, 7.80; N, 13.54%.

5.1.20. *N*-Cyclopentyl-7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3g). Yield 63%; mp 208–210 °C (ethyl acetate). ^{1}H NMR (DMSO) δ 9.85 (d, 1H, NH), 8.93 (s, 1H, H_2), 8.55 (d, 1H, H_5), 7.47 (d, 1H, H_6), 4.66 (t, 2H, CH_2N), 4.25 (m, 1H, CH), 3.48 (m, 4H, morph), 2.67 (t, 2H, CH_2N), 2.64 (s, 3H, CH_3), 2.48 (m, 4H, morph), 1.88–1.22 (m, 8H, cyclopentyl). Anal. ($C_{21}H_{28}N_4O_3$) (MW 384.47): C, 65.62; H, 7.29; N, 14.58%; found: C, 65.39; H, 7.32; N, 14.52%.

5.1.21. *N*-Cycloheptyl-7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3h). Yield 67%; mp 174–177 °C (ethyl acetate). ^{1}H NMR (DMSO) δ 9.93 (d, 1H, NH), 8.92 (s, 1H, H_2), 8.55 (d, 1H, H_5), 7.45 (d, 1H, H_6), 4.79 (t, 2H, CH_2N), 4.15 (m, 1H, CH), 3.48 (m, 4H, morph), 2.68 (t, 2H, CH_2N), 2.64 (s, 3H, CH_3), 2.50 (m, 4H, morph), 1.87–1.57 (m, 12H, cycloheptyl). Anal. ($C_{23}H_{32}N_4O_3$) (MW 412,50): C, 66.96; H, 7.82; N, 13.58%; found: C, 66.72; H, 7.80; N, 13.54%.

5.1.22. *N*-(1-Ethylpropyl)-7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3i). Yield 86%; mp 140–142 °C (cyclohexane). ^{1}H NMR (DMSO) δ 9.65 (d, 1H, NH), 8.92 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.46 (d, 1H, H_6), 4.66 (t, 2H, CH_2N), 3.85 (m, 1H, CH), 3.48 (m, 4H, morph), 2.66 (t, 2H, CH_2N), 2.64 (s, 3H, CH_3), 2.49 (m, 4H, morph), 1.53 (m, 4H, CH_2), 0.87 (m, 6H, CH_3). Anal. ($C_{21}H_{30}N_4O_3$) (MW 386.48): C, 65.28; H, 7.77; N, 14.50%; found: C, 65.11; H, 7.80; N, 14.44%.

5.1.23. *N*-(*p*-Chlorbenzyl)-7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (3l). Yield 61%; mp 132–134 °C (trituration with ethyl ether). ^{1}H NMR ($CDCl_3$) δ 10.27 (t, 1H, NH), 8.92 (s, 1H, H_2), 6.64 (d, 1H, H_5), 7.30 (m, 5H, Ar + H_6), 4.62 (m, 4H, $CH_2N + CH_2Ar$), 3.67 (m, 4H, morph), 2.79 (t, 2H, NCH_2), 2.68 (s, 3H, CH_3), 2.54 (m, 4H, morph). Anal. ($C_{23}H_{25}N_4O_3Cl$) (MW 440.92): C, 62.65; H, 5.71; N, 12.71%; found: C, 62.43; H, 5.93; N, 12.52%.

5.1.24. 1-Benzyl-*N*-cyclohexyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (4a). Yield 57%; mp 194–196 °C (cyclohexane). ^{1}H NMR (DMSO) δ 9.85 (d, 1H, NH), 9.07 (s, 1H, H_2), 8.55 (d, 1H, H_5), 7.48 (d, 1H, H_6), 7.32 (m, 5H, Ph), 5.80 (s, 2H, CH_2Ph), 3.85 (m, 1H, CH), 2.64 (s, 3H, CH_3), 1.85–1.31 (m, 10H, cyclohexyl). Anal. ($C_{23}H_{25}N_3O_2$) (MW 375,46): C, 73.57; H, 6.71; N, 11.19%; found: C, 73.65; H, 6.69; N, 11.15%.

5.1.25. *N*-Cyclohexyl-1-(*o*-fluorobenzyl)-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (5a). Yield 63%; mp 205–208 °C (cyclohexane). ^{1}H NMR (DMSO) δ 9.85 (d, 1H, NH), 9.08 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.46 (d, 1H, H_6), 7.42 (m, 4H, Ph), 5.81 (s, 2H, CH_2Ph), 3.85 (m, 1H, CH), 2.60 (s, 3H, CH_3), 1.87–1.23 (m, 10H, cyclohexyl). Anal. ($C_{23}H_{24}FN_3O_2$) (MW 393,45): C, 70.21; H, 6.15; N, 10.68%; found: C, 69.94; H, 6.35; N, 10.64%.

5.1.26. *N*-Cyclohexyl-1-(*p*-fluorobenzyl)-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (6a). Yield 64%; mp 222–224 °C (ethyl acetate). ^{1}H NMR (DMSO) δ 9.85 (d, 1H, NH), 9.10 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.48 (d, 1H, H_6), 7.18 (m, 4H, Ph), 5.76 (s, 2H, CH_2Ph), 3.82 (m, 1H, CH), 2.65 (s, 3H, CH_3), 1.86-1.23 (m, 10H, cyclohexyl). Anal. ($C_{23}H_{24}FN_3O_2$) (MW 393,45): C, 70.21; H, 6.15; N, 10.68%; found: C, 69.94; H, 6.12; N, 10.64%.

5.1.27. *N*-Cyclohexyl-1-hexyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (7a). Yield 47%; m.p 110–111 °C (petroleum ether 60–80 °C). ^{1}H NMR (DMSO) δ

9.85 (d, 1H, NH), 8.93 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.47 (d, 1H, H_6), 4.53 (t, 2H, CH_2N), 3.85 (m, 1H, CH), 2.65 (s, 3H, CH_3), 1.81–1.28 (m, 18H, cyclohexyl + hexyl) 0.85 (m, 3H, CH_3). Anal. ($C_{22}H_{31}N_3O_2$) (MW 369.50): C, 71.51; H, 8.48; N, 11.37%; found: C, 71.51; H, 8.44; N, 11.33%.

5.1.28. 1-Butyl-*N*-cyclohexyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (8a). Yield 44%; mp 156–158 °C (cyclohexane). ^{1}H NMR (DMSO) δ 9.90 (d, 1H, NH), 8.93 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.47 (d, 1H, H_6), 4.54 (t, 2H, CH_2N), 3.85 (m, 1H, CH), 2.66 (s, 3H, CH_3), 1.81–0.87 (m, 14H, cyclohexyl + butyl), 0.90 (t, 3H, CH_3). Anal. ($C_{20}H_{27}N_3O_2$) (MW 341.44): C, 70.35; H, 7.97; N, 12.31%; found: C, 70.51; H, 7.39; N, 12.33%.

5.1.29. 1-Benzyl-*N*-benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (4e). Yield 56%; mp 174–176 °C (cyclohexane). ^{1}H NMR (DMSO) 10.18 (t, 1H, NH), 9.14 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.47 (d, 1H, H_6), 7.30 (m, 10H, Ar), 5.80 (s, 2H, NCH_2) 4.56 (d, 2H, NCH_2), 2.64 (s, 3H, CH_3), Anal. ($C_{24}H_{21}N_3O_2$) (MW 383.44): C, 75.18; H, 5.52; N, 10.96%; found: C, 74.87; H, 5.33; N, 10.60%.

5.1.30. *N*-Benzyl-1-(*o*-fluorobenzyl)-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (5e). Yield 60%; mp 193–195 °C (cyclohexane). ^{1}H NMR (DMSO) 10.25 (t, 1H, NH), 9.08 (s, 1H, H_2), 8.61 (d, 1H, H_5), 7.29 (m, 8H, Ar + H_6), 7.08 (m, 2H, Ar), 5.74 (s, 2H, NCH_2) 4.68 (d, 2H, NCH_2), 2.69 (s, 3H, CH_3), Anal. ($C_{24}H_{20}FN_3O_2$) (MW 401.44): C, 71.81; H, 5.02; N, 10.47%; found: C, 71.57; H, 5.36; N, 10.10%.

5.1.31. *N*-Benzyl-1-(*p*-fluorobenzyl)-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (6e). Yield 77%; mp 206–209 °C (cyclohexane). ^{1}H NMR (DMSO) 10.20 (t, 1H, NH), 9.03 (s, 1H, H_2), 8.62 (d, 1H, H_5), 7.32 (m, 8H, Ar + H_6), 7.01 (m, 2H, Ar), 5.65 (s, 2H, NCH_2) 4.67 (d, 2H, NCH_2), 2.70 (s, 3H, CH_3). Anal. ($C_{24}H_{20}FN_3O_2$) (MW 401.44): C, 71.81; H, 5.02; N, 10.47%; found: C, 71.77; H, 5.26; N, 10.25%.

5.1.32. *N*-Benzyl-1-hexyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (7e). Yield 70%; mp 140–142 °C (cyclohexane). ^{1}H NMR (DMSO) δ 10.22 (t, 1H, NH), 8.99 (s, 1H, H_2), 8.53 (d, 1H, H_5), 7.47 (d, 1H, H_6), 7.33 (m, 5H, Ar) 4.57 (m, 4H, $2CH_2N$), 2.64 (s, 3H, CH_3), 1.79 (m, 2H, hexyl), 1.28 (brs, 6H, hexyl), 0.83 (m, 3H, CH_3). Anal. ($C_{23}H_{27}N_3O_2$) (MW 377.48): C, 73.18; H, 7.21; N, 11.13%; found: C, 73.08; H, 7.15; N, 10.89%.

5.1.33. *N*-Benzyl-1-butyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (8e). Yield 44%; mp 140–143 °C (cyclohexane). ^{1}H NMR (DMSO) δ 10.22 (t, 1H, NH), 8.98 (s, 1H, H_2), 8.53 (d, 1H, H_5), 7.47 (d, 1H, H_6), 7.33 (m, 5H, Ar) 4.55 (m, 4H, $2CH_2N$), 2.65 (s, 3H, CH_3), 1.78 (m, 2H, butyl), 1.28 (m, 2H, butyl), 0.91 (t, 3H, CH_3). Anal. ($C_{21}H_{23}N_3O_2$) (MW 349.43): C, 72.18; H, 6.63; N, 12.03%; found: C, 71.95; H, 6.99; N, 11.95%.

5.1.34. *N*-(*p*-Chlorbenzyl)-1-benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (4l). Yield 71%; m.p 177–178 °C (cyclohexane). ^{1}H NMR (DMSO) 10.10 (t, 1H, NH), 9.12 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.48 (d, 1H, H_6), 7.37 (m, 9H, Ar), 5.80 (s, 2H, NCH_2) 4.56 (d, 2H, NCH_2), 2.64 (s, 3H, CH_3). Anal. ($C_{24}H_{20}ClN_3O_2$) (MW 417.89): C, 68.98; H, 4.82; N, 10.06%; found: C, 68.83; H, 5.12; N, 10.43%.

5.1.35. Ethyl 1-benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxylate (9). NaH (0.0870 g, 1.81 mmol, 50% in mineral oil) was added to a solution of 7-methyl-1,8-naphthyridine **1** (0.350 g, 1.5 mmol) in 10 mL of dry DMF. After 1 h benzyl chloride (0.13 mL, 1 mmol) was added and the mixture was stirred for 24 h at rt The solution was evaporated in vacuo and the addition of ethyl ether caused the precipitation of the title compound as a pure solid: 0.396 g (yield 81%); mp 140–141 °C. ^{1}H NMR (DMSO) δ 8.96 (s, 1H, H_2), 8.44 (d, 1H, H_5), 7.41 (d, 1H, H_6), 7.36 (m, 5H, Ph), 5.69 (s, 2H, CH_2Ph), 4.23 (q, 2H, CH_2), 2.59 (s, 3H, CH_3), 1.27 (t, 3H,CH_3). Anal. $C_{19}H_{18}N_2O_3$ (MW 322.35): C, 70.79; H, 5.63; N, 8.69%; found: C, 70.50; H, 5.60; N, 8.70%.

5.1.36. 1-Benzyl-*N*-cyclohexyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (4a). A mixture of the 7-methyl-1,8-naphthyridine derivative **9** (0.130 g, 0.40 mmol) and cyclohexylamine (0.50 mL, 4.0 mmol) was heated in a sealed tube at 120 °C for 24 h. After cooling the reaction mixture was treated with ethyl ether to give **4a** as a pure solid: 0.115 g (yield 76.6%).

5.1.37. 1-Benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxylic acid (10). A mixture of ethyl 1-benzyl-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxylate (0.300 g, 0.93 mmol) in 4 mL of 10% sodium hydroxide solution was refluxed for 1.5 h. After cooling the solid was filtered and the pH of the solution was adjusted to 4 with aqueous 37% hydrochloric acid to obtain the title compound as a pure solid: 0.167 g (yield 63%); mp 235–237 °C. ^{1}H NMR (DMSO) δ: 9.34 (s, 1H, H_2), 8.62 (d, 1H, H_5), 7.59 (d, 1H, H_6), 7.32 (m, 5H, Ph), 5.85 (s, 2H, CH_2Ph), 2.68 (s, 3H, CH_3). Anal. $C_{17}H_{14}N_2O_3$ (MW 294.30): C, 69.38; H, 4.79; N, 9.52%; found: C, 69.00; H, 4.45; N, 9.50%.

5.1.38. 1-Benzyl-7-methyl-1,8-naphthyridin-4-(1*H*)-one (11). A mixture of 1-benzyl-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxylic acid (0.108 g, 0.35 mmol) and 10 mg of copper chromite in 4 mL of Dowtherm A was refluxed for 2 h. Filtration while hot and addition of hexane caused the precipitation of the pure product: 0.030 g (yield 33%); mp 120–123 °C. ^{1}H NMR (DMSO) δ: 8.38 (d, 1H, H_5), 8.26 (s, 1H, H_2), 8.32 (m, 6H, H_{6+}Ph), 6.15 (d, 1H, H_3), 5.57 (s, 2H, CH_2Ph), 2.60 (s, 3H, CH_3). Anal. $C_{16}H_{14}N_2O$ (MW 250.29): C, 76.78; H, 5.64; N, 11.19%; found: C, 76.50; H, 5.60; N, 11.30%.

5.1.39. 7-Amino-*N*-benzyl (13) and 7-Amino-*N*-cyclohexyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (14). A mixture of ethyl 7-amino-1,8-naphthyridin-4(1*H*)-on-3-carboxylate (0.276 g, 1 mmol) and the appropriate

amine (10 mmol) was heated in a sealed tube at 120 °C for 24 h. After cooling the reaction mixture was treated with ethyl ether to give a solid residue which was collected by filtration and purified by crystallization. **13:** 0.190 g (yield 57%); mp 260–263 °C (ethyl acetate). ^{1}H NMR (DMSO) δ: 11.80 (brs, 1H, OH), 10.48 (d, 1H, NH), 8.38 (s, 1H, H_2), 8.09 (d, 1H, H_5), 7.32 (m, 5H,Ar), 7.12 (brs, 2H, NH_2), 6.56 (d, 1H, H_6), 4.52 (d, 2H, CH_2). Anal. $C_{16}H_{14}N_4O_2$ (MW 294.31): C, 65.31; H, 4.76; N, 19.05%; found: C, 63.43; H, 5.02; N, 19.23%. **14**: 0.260 g (yield 79%); mp 275–278 °C (ethyl acetate). ^{1}H NMR (DMSO) δ: 11.80 (brs, 1H, OH), 10.14 (d, 1H, NH), 8.33 (s, 1H, H_2), 8.10 (d, 1H, H_5), 7.10 (s, 2H, NH_2), 6.55 (d, 1H, H_6), 3.90 (brs, 1H, CH), 1.84–1.29 (m, 10H, cyclohexyl). Anal. $C_{15}H_{18}N_4O_2$ (MW 286.33): C, 62.92; H, 6.34; N, 19.57%; found: C, 63.15; H, 6.32; N, 19.86%.

5.1.40. 7-Amino-*N*-benzyl (15) and 7-amino-*N*-cyclohexyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (16). NaH (0.090 g 1.82 mmol, 50% in mineral oil) was added to a solution of 7-amino-1,8-naphthyridine derivative **13** or **14** (0.91 mmol) in 10 mL of dry DMF. After 1 h, 4-(2-chloroethyl)-morpholine hydrochloride (0.170 g, 0.91 mmol) was added and the mixture was stirred for 24 h at 50 °C. The solvent was evaporated in vacuo and the solid was dissolved in chloroform; the solution was washed with water and evaporated under reduced pressure. Addition of ethyl ether caused the precipitation of the title compounds as pure solids. **15**: 0.156 g (yield 42%); mp 192–195 °C. ^{1}H NMR (DMSO) δ 10.44 (brs, 1H, NH), 8.68 (s, 1H, H_2), 8.14 (d, 1H, H_5), 7.33 (m,5H Ar), 7.21 (brs, 2H, NH_2), 6.58 (d, 1H, H_6), 4.53 (m, 4H, 2 NCH_2), 3.51 (m, 4H, morph), 2.63 (m, 2H, NCH_2), 2.42 (m, 4H, morph). Anal. $C_{22}H_{25}N_5O_3$ (MW 407.46): C, 64.86; H, 6.14; N, 17.20%; found: C, 65.02; H, 6.43; N, 17.53%. **16**: 0.142 g (yield 39%); mp 171–174 °C. ^{1}H NMR ($CDCl_3$) δ 10.15 (brs, 1H, NH), 8.62 (s, 1H, H_2), 8.14 (d, 1H, H_5), 7.18 (brs, 2H, NH_2), 6.58 (d, 1H, H_6), 4.50 (m, 2H, NCH_2), 3.90 (brs, 1H, CH), 3.50 (m, 4H, morph), 2.62 (m, 2H, NCH_2), 2.48 (m, 4H, morph), 1.95–1.20 (m, 10H, cyclohexyl). Anal. $C_{21}H_{29}N_5O_3$ (MW 399.48): C, 63.14; H, 7.32; N, 17.53%; found: C, 63.34; H, 7.53; N, 17.78%.

5.1.41. 7-Chloro-*N*-cyclohexyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (17). Sodium nitrite (1.14 g, 16.6 mmol) was added portionwise to a cooled solution (−5 °C) of 7-amino-*N*-cyclohexyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (0.952 g, 3.33 mmol) in 115 mL of concentrated hydrochloric acid. After standing for 3 h at 40 °C, the mixture was poured over crushed ice and the pH was adjusted to 4–5 with aqueous concentrated ammonium hydroxide. The solid was collected by filtration, washed with water and purified by flash chromatography, using ethyl acetate as the eluant to obtain the title compound: 0.54 g (yield 52%); mp 266–268 °C. ^{1}H NMR (DMSO) δ 9.80 (d, 1H, NH), 8.65 (s, 1H, H_2), 8.59 (d, 1H, H_5), 7.60 (d, 1H, H_6), 3.85 (m, 1H, CH), 1.95–1.31 (m, 10H, cyclohexyl). Anal. $C_{15}H_{16}ClN_3O_2$ (MW 305.76): C, 58.92; H, 5.27; N, 13.74%; found: C, 58.61; H, 5.25; N, 13.71%.

5.1.42. 7-Chloro-*N*-cyclohexyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide (18). NaH (0.053 g, 1.10 mmol, 50% in mineral oil) was added to a solution of the 7-chloro-1,8-naphthyridine derivative **17** (0.167 g, 0.55 mmol) in 10 mL of dry DMF. After 1 h, the 4-(2-chloroethyl)-morpholine hydrochloride (0.102 g, 0.55 mmol) was added and the mixture was stirred for 24 h at 50 °C. The solvent was evaporated in vacuo and the solid obtained was washed with water, collected by filtration and purified by crystallization from cyclohexane: 0.162 g (yield 70%); mp 188–190 °C. ^{1}H NMR (DMSO) δ: 9.80 (d, 1H, NH), 8.96 (s, 1H, H_2), 8.66 (d, 1H, H_5), 7.66 (d, 1H, H_6), 4.62 (t, 2H, CH_2N), 3.87 (m, 1H, CH), 3.47 (m, 4H, morph), 2.66 (t, 2H, CH_2N), 2.46 (m, 4H, morph), 1.88–1.23 (m, 10H, cyclohexyl). Anal. $C_{21}H_{27}N_4O_3Cl$ (MW 418.92): C, 60.21; H, 6.50; N, 13.37%; found: C, 60.53; H, 6.49; N, 13.34%.

5.1.43. (4-Hydroxy-7-methyl-1,8-naphthyridin-3-yl)acetic acid (20). A mixture of 7-methyl-2,3-dihydro-1,8-naphthyridine-4(1*H*)-one **19** (0.500 g, 3,09 mmol) and glyoxylic acid (0.830g, 9,02 mmol) in 10 mL of anhydrous ethanol and KOH (0.700 g, 12,47 mmol) was refluxed for 1 h. After cooling, the suspension was filtered and the pH of the filtrate was adjusted to 3 with aqueous 37% hydrochloric acid, to obtain a solid which was collected by filtration, washed with H_2O and crystallized from ethanol: (0.31 g, yield 46%); mp dec 315 °C. ^{1}H NMR (DMSO) δ 12.00 (brs, 1H, OH), 8.33 (d, 1H, H_5), 7.92 (s, 1H, H_2), 7.25 (d, 1H, H_6), 3.37 (s, 2H, CH_2), 2.57 (s, 3H, CH_3). Anal. $C_{11}H_{10}N_2O_3$ (MW 218.21): C, 60.55; H, 4.62; N, 12.84%; found: C, 60.11; H, 5.00; N, 12.60%.

5.1.44. Ethyl (4-hydroxy-7-methyl-1,8-naphthyridin-3-yl)acetate (21). A solution of (4-hydroxy-7-methyl-1,8-naphthyridin-3-yl)acetic acid **20** (2,94 mmol) in 50 mL of anhydrous ethanol and 1 mL of 98% sulphuric acid was heated at 80 °C for 4 h. After cooling, the pH was adjusted to 8 with a saturated solution of $NaHCO_3$, and the solution was evaporated in vacuo to obtain a solid residue which was washed with H_2O, collected by filtration and crystallized from ethyl acetate: 0.48 g, yield 67%; mp 228–230 °C. ^{1}H NMR (DMSO) δ 10.30 (brs, 1H, OH), 8.61 (d, 1H, H_5), 7.83 (s, 1H, H_2), 7.19 (d, 1H, H_6), 4.20 (q, 2H, CH_2), 3.59 (s, 2H, CH_2), 2.67 (s, 3H, CH_3), 1.28 (t, 3H, CH_3). Anal. $C_{13}H_{14}N_2O_3$ (MW 246.26): C, 63.40; H, 5.73; N, 11.38%; found: C, 63.29; H, 6.01; N, 11.35%.

5.1.45. *N*-Benzyl-2-(4-hydroxy-7-methyl-1,8-naphthyridin-3-yl)acetamide (22) and *N*-cyclohexyl-2-(4-hydroxy-7-methyl-1,8-naphthyridin-3-yl)acetamide (23). A mixture of ethyl ester **21** (0.300 g, 1.20 mmol) and 12 mmol of the appropriate amine in a sealed tube was heated at 120 °C for 24 h. After cooling, the reaction mixture was treated with ethyl ether to give a residue which was collected by filtration and purified by crystallization. **22**: 0.320 g, yield 87%; mp dec 314–316 °C (ethanol). ^{1}H NMR δ 12.0 (br, 1H, OH), 1.87 (d, 2H, H_5 + NH), 7.90 (s, 1H, H_2), 7.25 (m, 6H, H_6 + Ar), 4.24 (d, 2H, CH_2), 3.32 (s, 2H, CH_2), 2.56 (s, 3H, CH_3). Anal. $C_{18}H_{17}N_3O_2$ (MW 307.34): C, 70.34; H, 5.58; N, 13.67%; found: C, 70.00; H, 5.68; N, 13.70%. **23**: 0.250g, yield 70%; mp

dec 320 °C (ethanol). ^{1}H NMR δ 12.0 (br, 1H, OH), 8.34 (d, 1H, H_5), 7.79 (brs, 2H, H_2 + NH), 7.25 (d, 1H, H_6), 3.46 (m, 1H, CH), 3.23 (s, 2H, CH_2), 2.57 (s, 3H, CH_3), 1.80–1.10 (m, 10H, cyclohexyl). Anal. $C_{17}H_{21}N_3O_2$ (MW 299.37): C, 68.20; H, 7.07; N, 14.04%; found: C, 68.50; H, 7.10; N, 14.16%.

5.1.46. General procedure for the synthesis of N1-substituted-2-(4-hydroxy-7-methyl-1,8-naphthyridin-3-yl)acetamide derivatives (25a,b and 26a–d). 1.2 mmol of NaH was added to a stirred solution of 1 mmol of N-substituted-2-(4-hydroxy-7-methyl-1,8-naphthyridin-3-yl)-acetamide derivatives **22** or **23** in 10 mL of dry *N*,*N*-dimethylformamide at 50 °C for compounds **25a** and **26a** or at room temperature for compounds **25b** and **26b–d**. After 1 h, the appropriate chloride or bromide (1 mmol) was added, and the mixture was stirred for 24 h at 50 °C for compounds **25a** and **26a** or at room temperature for compounds **25b** and **26b–d**. The products were then obtained by the following work-up: in the case of **26a**, the solvent was evaporated in vacuo and the solid was washed with water and collected by filtration, whereas for the other compounds (**25b** and **26a–d**), the reaction mixture was treated with water and the precipitate formed was collected by filtration.

5.1.47. *N*-Benzyl-2-[7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-yl]acetamide (25a). Yield 25%; mp 60–63 °C (cyclohexane). ^{1}H NMR δ 8.40 (d, 1H, H_5), 8.12 (s, 1H, H_2), 7.29 (m, 6H, H_6 + Ar), 4.47 (m, 2H, NCH_2), 4.27 (d, 2H, CH_2), 3.55 (m, 4H, morph), 3.30 (s, 2H, CH_2), 2.61 (m, 2H, NCH_2), 2.58 (s, 3H, CH_3). Anal. $C_{24}H_{28}N_4O_3$ (MW 420.50): C, 68.50; H, 6.71; N, 13.32%; found: C, 68.30; H, 7.00; N, 13.29%.

5.1.48. *N*-Benzyl-2-(1-benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-yl)acetamide (25b). Yield 86%; mp 172–174 °C (cyclohexane). ^{1}H NMR δ 8.58 (d, 1H, H_5), 7.92 (s, 1H, H_2), 7.80 (br, 1H, NH), 7.27 (m, 6H, H_6 + Ar), 5.63 (s, 2H, NCH_2), 4.39 (d, 2H, NCH_2), 3.50 (s, 2H, CH_2), 2.67 (s, 3H, CH_3). Anal. $C_{25}H_{23}N_3O_2$ (MW 397.47): C, 75.56; H, 5.83; N, 10.57%; found: C, 75.21; H, 5.92; N, 10.19%.

5.1.49. *N*-Cyclohexyl-2-[7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-yl] acetamide (26a). Yield 50%; mp 95–98 °C (cyclohexane). ^{1}H NMR δ 8.39 (d, 1H, H_5), 8.08 (s, 1H, H_2), 7.79 (d, 1H, NH), 7.29 (d, 1H, H_6), 4.46 (m, 2H, NCH_2), 3.49 (m, 5H, CH + morph), 3.23 (s, 2H, CH_2), 2.61 (m, 2H, NCH_2), 2.59 (s, 3H, CH_3), 2.40 (m, 4H, morph), 1.83–1.05 (m, 10H, cyclohexyl). Anal. $C_{23}H_{32}N_4O_3$ (MW 412.52): C, 66.96; H, 7.82; N, 13.58%; found: C, 66.70; H, 8.10; N, 13.29%.

5.1.50. *N*-Cyclohexyl-2-(1-benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-yl)acetamide (26b). Yield 76%; mp 207–209 °C (cyclohexane). ^{1}H NMR δ 8.61 (d, 1H, H_5), 7.92 (s, 1H, H_2), 7.33 (brs, 5H, Ar), 7.23 (d, 1H, H_6), 5.61 (s, 2H, NCH_2), 3.70 (m, 1H, CH), 3.40 (s, 2H, CH_2), 2.67 (s, 3H, CH_3), 1.91–1.12 (m, 10H, cyclohexyl). Anal. $C_{24}H_{27}N_3O_2$ (MW 389.49): C, 74.03; H, 6.99; N, 10.79%; found: C, 73.61; H, 7.11; N, 10.35%.

5.1.51. *N*-Cyclohexyl-2-(1-hexyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-yl)acetamide (26c). Yield 27%; mp 123–125 °C (cyclohexane). ^{1}H NMR δ 8.59 (d, 1H, H_5), 7.84 (s, 1H, H_2), 7.20 (brs, 1H, NH), 7.22 (d, 1H, H_6), 4.38 (t, 2H, CH_2), 3.77 (m, 1H, CH), 3.43 (s, 2H, CH_2), 2.66 (s, 3H, CH_3), 1.95–1.10 (m, 18H, cyclohexyl + $(CH_2)_4$), 0.98 (t, 3H, CH_3). Anal. $C_{23}H_{33}N_3O_2$ (MW 383.53): C, 72.06; H, 8.67; N, 10.97%; found: C, 71.79; H, 8.65; N, 10.80%.

5.1.52. *N*-Cyclohexyl-2-(1-butyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-yl)acetamide (26d). Yield 65%; mp 177–179 °C (cyclohexane). ^{1}H NMR δ 8.60 (d, 1H, H_5), 7.85 (s, 1H, H_2), 7.20 (brs, 1H, NH), 7.23 (d, 1H, H_6), 4.37 (t, 2H, CH_2), 3.75 (m, 1H, CH), 3.43 (s, 2H, CH_2), 2.66 (s, 3H, CH_3), 1.93–1.05 (m, 14H, cyclohexyl + $(CH_2)_2$), 0.89 (t, 3H, CH_3). Anal. $C_{21}H_{29}N_3O_2$ (MW 355.47): C, 70.95; H, 8.22; N, 11.83%; found: C, 70.64; H, 8.55; N, 11.56%.

5.2. Biology

5.2.1. General information. Male CD1 mice weighing 20–25 g, (Charles River, Calco, LC, Italy) were housed in animal care quarters maintained at 22 ± 2 °C on a 12-h light/dark cycle, and food and water were available ad libitum. All experimental protocols were accepted by the Ethical Committee at the University of Cagliari and performed in strict accordance with the E.C. regulation for care and use of experimental animals (EEC N°86/609).

[^{3}H]-CP-55,940 (specific activity 180 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). CP 55,940 was obtained from Tocris Cookson Ltd (Bristol, UK). For biochemical experiments, drugs were dissolved in dimethyl-sulphoxide, (DMSO). DMSO concentration in the different assays never exceeded 0.1% (v/v) and was without any effects on radioligand binding.

5.2.2. Tissue preparation. Mice were killed by cervical dislocation and the brain (minus cerebellum) and spleen were rapidly removed and placed on an ice-cold plate. After thawing, tissues were homogenized in 20 vol. (w/v) of ice-cold TME buffer (50 mM Tris–HCl, 1 mM EDTA and 3.0 mM $MgCl_2$, pH 7.4).The homogenates were centrifuged at 1,086×*g* for 10 min at 4 °C, and the resulting supernatants were centrifuged at 45,000×*g* for 30 min in a Beckman SW41 swing-out rotor, at 4 °C.

5.2.3. Binding study at CB_1 and CB_2 receptors. [^{3}H]-CP-55,940 binding was performed by a modification of the method previously described.[22] Briefly, the membranes (30–80 μg of protein) were incubated with 0.5–1 nM of [^{3}H]-CP55940 for 1 h at 30 °C in a final volume of 0.5 mL of TME buffer containing 5 mg/mL of fatty acid-free bovine serum albumin (BSA). Non-specific binding was estimated in the presence of 10 μM of CP55940. All binding studies were performed in disposable glass tubes pre-treated with Sigma-Cote (Sigma Chemical Co. Ltd., Poole, UK), in order to reduce non-specific binding. The reaction was terminated by rapid filtration through

Whatman GF/C filters presoaked in 0.5% polyethyleneimine (PEI) using a Brandell 96-sample harvester (Gaithersburg, MD, USA). Filters were washed five times with 4 mL aliquots of ice cold Tris HCl buffer (pH 7.4) containing 1 mg/mL BSA The filter-bound radioactivity was measured in a liquid scintillation counter (Tricarb 2100, Packard, Meridien, USA) with 4 mL of scintillation fluid (Ultima Gold MV, Packard). Protein determination was performed by means of the Bradford[23] protein assay, using BSA as a standard in accordance with the protocol of the supplier (Bio-Rad, Milan, Italy).

5.2.4. Data analysis. All experiments were performed in triplicate and results were confirmed in at least five independent experiments. Data from radioligand inhibition experiments were analyzed by non-linear regression analysis of a Sigmoid Curve using the Graph Pad Prism program. IC_{50} values were derived from the curves calculated and converted to K_i values as described previously.[24]

References and notes

1. Gaoni, Y.; Mechoulam, R. *J. Am. Chem. Soc.* **1964**, *86*, 1648.
2. Devane, W. A.; Hanus, L.; Breuer, A.; Perwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946.
3. Devane, W. A.; Dysarz, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. *Mol. Pharmacol.* **1988**, *34*, 605.
4. Melvin, L. S.; Milne, G. M.; Johnson, M. R.; Wilken, G. H.; Howlett, A. C. *Drug Des. Disc.* **1995**, *13*, 155.
5. Huffman, J. W. *Curr. Med. Chem.* **1999**, *6*, 705.
6. Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; deCosta, B. R.; Rice, K. C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1932.
7. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. *Nature* **1990**, *346*, 561.
8. Munro, S.; Thomas, K. L.; Abu-shaar, M. *Nature* **1993**, *365*, 61.
9. Zurier, R.; Rosetti, R.; Lane, J. H.; Goldberg, J. M.; Hunter, S. A.; Burstein, S. H. *Arthritis Rheum.* **1998**, *41*, 163.
10. Recht, L. D.; Salmonsen, R.; Rosetti, R.; Jang, T.; Pipia, G.; Kubiatowski, T.; Karim, P.; Ross, A. H.; Zurier, R.; Litofsky, N. S.; Burstein, S. *Biochemical Pharmacol.* **2001**, *62*, 755.
11. Barth, F.; Rinaldi-Carmona, M. *Curr. Med. Chem.* **1999**, *6*, 745.
12. Rinaldi-Carmona, M.; Barth, F.; Millan, J., *et al. J. Pharmacol. Exp. Ther.* **1998**, *284*, 644.
13. Iwamura, H.; Suzuki, H.; Ueda, Y.; Kaya, T.; Inaba, T. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 420.
14. Pertwee, R. G. *Curr. Med. Chem.* **1999**, *6*, 635.
15. Pertwee, R. G.; Fernando, S. R. *Br. J. Pharmacol.* **1996**, *118*, 2199.
16. Lappin, G. R. *J. Am. Chem. Soc.* **1948**, *70*, 3348.
17. Carboni, S.; Da Settimo, A; Bertini, D; Ferrarini, P. L.; Livi, O.; Tonetti, I. *Il Farmaco* **1973**, *9*, 722.
18. Carboni, S; Da Settimo, A; Bertini, D; Ferrarini, P. L.; Tonetti, I. *Gazzetta chimica italiana* **1971**, *101*, 129.
19. Carboni, S; Da Settimo, A; Bertini, D; Ferrarini, P. L.; Livi, O.; Tonetti, I. *Il Farmaco* **1975**, *30*, 237.
20. Vasvári-Debreczy, L.; Hermecz, I.; Mészáros, Z.; Dvortsák, P.; Tóth, G. J. C. S. *Perkin I* **1980**, 227.
21. Mussinu, J. M.; Ruiu, S.; Mulè, A. C.; Pau, A.; Carai, M. A. M.; Loriga, G.; Marineddu, G.; Pinna, G. A. *Biorg. Med. Chem.* **2003**, *11*, 251.
22. Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D.; Ferrara, P.; Soubriè, P.; Brelière, J. C.; Le Fur, G. *FEBS Lett.* **1994**, *350*, 240.
23. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
24. Cheng, X.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.