Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 645–651
Bioorganic & Medicinal Chemistry Letters

Synthesis and SAR of 5,6-diarylpyridines as human CB1 inverse agonists

Laura C. Meurer,[a,*] Paul E. Finke,[a] Sander G. Mills,[a] Thomas F. Walsh,[a] Richard B. Toupence,[a] Mark T. Goulet,[a] Junying Wang,[a] Xinchun Tong,[a] Tung M. Fong,[b] Julie Lao,[b] Marie-Therese Schaeffer,[b] Jing Chen,[b] Chun-Pyn Shen,[b] D. Sloan Stribling,[c] Lauren P. Shearman,[c] Alison M. Strack[c] and Lex H. T. Van der Ploeg[b]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Metabolic Disorders, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[c]*Department of Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*

Received 8 October 2004; revised 10 November 2004; accepted 15 November 2004
Available online 2 December 2004

Abstract—Structure–activity relationship studies for two series of 2-benzyloxy-5-(4-chlorophenyl)-6-(2,4-dichlorophenyl)pyridines having either a 3-cyano or 3-carboxamide moiety resulted in the preparation of the 2-(3,4-difluorobenzyloxy)-3-nitrile analog **10d** and the 2-(3,4-difluorobenzyloxy)-3-(*N*-propylcarboxamide) analog **16c**, (hCB1 IC_{50} = 1.3 and 1.7 nM, respectively) as potent and selective hCB1 inverse agonists. Their synthesis and biological activities are described herein.

1. Introduction

Preparations of *Cannabis sativa*, such as marijuana and hashish, have been used for medicinal and recreational purposes for centuries. Recently, the cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) has received renewed interest as a potential treatment for analgesia, nausea, and appetite stimulation.[1] Several related cannabinoid compounds can serve as ligands for and activate the G-protein coupled cannabinoid receptor type 1 (CB1), located predominantly in the central nervous system (CNS) but also in the peripheral nervous system, and/or type 2 (CB2), expressed mostly by immune cells. CB1 agonists, such as Δ^9-THC, are known to stimulate food intake, whereas CB1 receptor knockout mice are lean and resistant to diet-induced obesity.[2] Initially, the search for CB1 antagonists/inverse agonists was based on the structure of known agonists such as Δ^9-THC.[1,3] The first potent and selective hCB1 inverse agonist **1** (SR141716, Fig. 1) was reported in 1994 by researchers at Sanofi-Synthelabo and belonged to a new family of CB1 ligands based on a 1,5-diphenylpyrazole structure.[3,4] In a 2-week rat feeding study, **1** was found to cause an initial decrease in food intake and then a sustained slower weight gain over the 14-day dosing period.[5] In addition, a more recent study showed that **1** produced a marked and sustained reduction of adiposity in diet-induced obese mice, which was more than expected from the reduction of food intake.[6] These studies suggested that CB1 and its endogenous ligands modulate energy balance via a dual mechanism of intake reduction and increased energy expenditure. The CB1 agonist WIN-55212-2 at 1 μM was found to increase lipoprotein lipase activity of mouse primary adipocytes, which could be blocked by **1**, although it was not clear

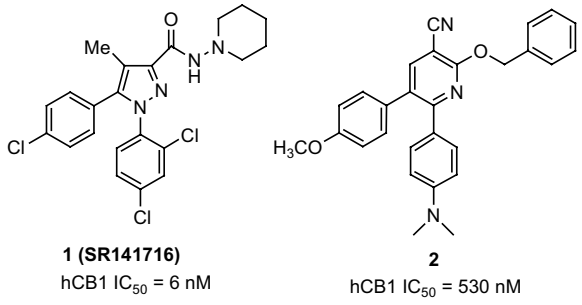


Figure 1. Structure of SR141716 and the Merck lead structure.

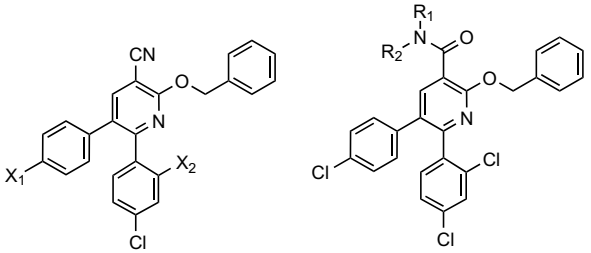
Keywords: Human CB1 receptor; Inverse agonist; Pyridine.
* Corresponding author. Tel.: +1 732 594 4291; fax: +1 732 594 5790; e-mail: laura_meurer@merck.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.11.031

whether this was mediated by the CB1 receptor.[7] CB1 receptors are coupled to the $G_{i/o}$ class of G-proteins, which inhibit adenylate cyclase activity.[8] CB1 agonists such as CP-55940[9] and WIN-55,212-2[10] decrease forskolin-induced cAMP accumulation in tissue from rodent and mammalian brain in a concentration-dependent manner. However, **1** produced a dose-dependent increase in forskolin-induced cAMP,[11] thus suggesting inverse agonism of the CB1 receptor.[12]

Our interest in discovering novel anti-obesity agents based on the CB1 hypothesis was initially pursued with a high throughput screening of the Merck sample collection to identify potential leads for an hCB1 antagonist program. The 5,6-diarylpyridine **2** (Fig. 1) was found to have moderate affinity for the hCB1 receptor (IC_{50} = 530 nM) and became a lead structure in this program. Based on reported structure–activity relationship (SAR)[13] and modeling[14] studies of **1**, the pyridine ring of **2** was viewed as being a possible alternative scaffold to the pyrazole of the Sanofi compound with the pyridine nitrogen being equivalent to N-2 of the pyrazole. Other interesting features of **2** relative to the structure of **1** were the possible overlap of the lipophilic benzyloxy group with the *N*-piperidine amide and the role of the 3-cyano as a surrogate for the proposed amide carbonyl interaction at the binding site. The moderate affinity of **2** was attributed to the inappropriate substitution on the phenyls.[13]

Our initial efforts examined the more closely related 6-(4-chlorophenyl) substituted pyridine **3a** (Fig. 2). The surprisingly poor binding on the hCB1 receptor (**3a**, IC_{50} = 2800 nM)[15] with this substitution suggested that the chlorination pattern on the two phenyl moieties would be critical. Therefore, the substitution pattern corresponding to **1** having a 5-(4-chlorophenyl) and a 6-(2,4-dichlorophenyl) was subsequently examined as in **3b**. Interestingly, a greater than 200-fold increase in hCB1 binding was observed (IC_{50} = 11 nM), confirming this rationale. A subsequent functional activity analysis of **3b** indicated that it was acting as an inverse agonist having an EC_{50} = 70 nM with a 52% increase in cAMP.[15] The role of the nitrile was also investigated by replacement with an amide as shown in structure **4** and an extensive investigation of 5-(4-chlorophenyl)-6-(2,4-dichlorophenyl)pyridines in both series was undertaken. In addition, two des-benzyloxy series having just a 2- or 3-amido functionality as is present in **1** were also prepared as described herein.



3a $X_1 = X_2 = H$; hCB1 IC_{50} = 2800 nM
3b $X_1 = X_2 = Cl$: hCB1 IC_{50} = 11 nM

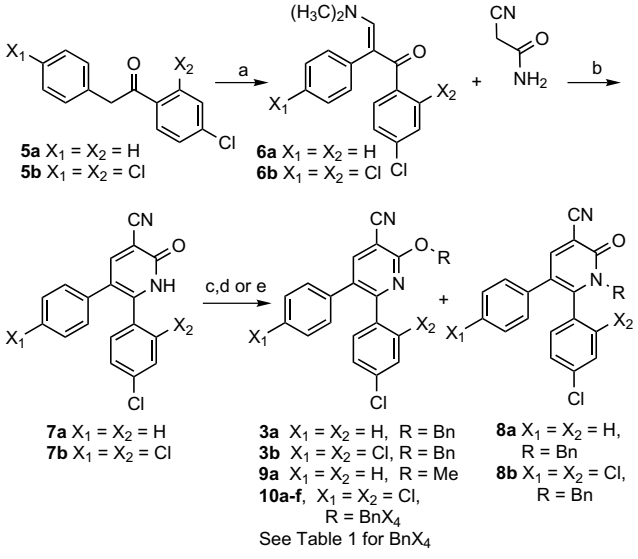
4

Figure 2. Basic structures of two initial SAR series. See Tables 1 and 2.

2. Chemistry[16]

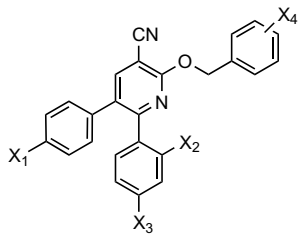
The preparation of **3a** and **3b** employed the analogous chemistry that had provided the original lead **2** (Scheme 1). Thus, commercially available benzyl 4-chlorophenyl ketone (**5a**) or 4-chlorobenzyl 2,4-dichlorophenyl ketone[17] (**5b**) were reacted with DMF dimethyl acetal to afford **6a**,**b**. Treatment with cyanoacetamide and sodium hydride in DMF effected ring closure to the 5,6-diaryl-3-cyanopyridin-2-ones **7a**,**b**. Alkylation with benzyl bromide in the presence of cesium carbonate afforded predominantly the *O*-benzyl pyridines **3a** (^{1}H NMR ($CDCl_3$): δ 5.64 (s, 2H, O–CH_2Ph)) and **3b** along with the *N*-benzyl pyridin-2-ones **8a** (^{1}H NMR ($CDCl_3$): δ 5.23 (s, 2H, N–CH_2Ph)), and **8b**, which were readily separated by silica gel chromatography (ratio of **3** to **8** = 1.5–3:1).[16] The methyl ether **9a** was obtained from **7a** via alkylation with methyl iodide. Based on the initial binding results for **3a** and **3b**, the Sanofi type trichloro series was selected for further SAR studies. The effect of substitution on the benzyl ether was first explored as indicated in Scheme 1 (**10a–f**; X_{1-2} = Cl, R = substituted benzyl) and listed in Table 1. Subsequent to these benzyloxy substitution studies, several alternative 5- and 6-phenyl substitution patterns were prepared in the 3,4-difluorobenzyloxy series (see Table 1, **10g–k**) starting with the appropriate benzyl phenyl ketone in Scheme 1.

The effect of converting the 3-cyano to an amide moiety as in **1** was then investigated in the trichloro series (Scheme 2). Hydrolysis of **7b** in sulfuric acid provided the acid **11**, which was subsequently converted to the methyl ester **12** by treatment with HCl/MeOH. Alkylation of **12** with benzyl bromide and cesium



Scheme 1. Reagents and conditions: (a) $Me_2NCH(OMe)_2$, DMF, 75 °C (100% crude); (b) MeOH, NaH, DMF (60–75% from **5**); (c) BnBr, Cs_2CO_3, DMF (50–65% **3**, 18–35% **8**); (d) MeI, Cs_2CO_3, DMF (30%); (e) X_4BnBr or X_4BnI, Cs_2CO_3, DMF (40–50% **10a–f**).

Table 1. Structures and hCB1 activities for 2-benzyloxy-3-cyano derivatives **3** and **10**

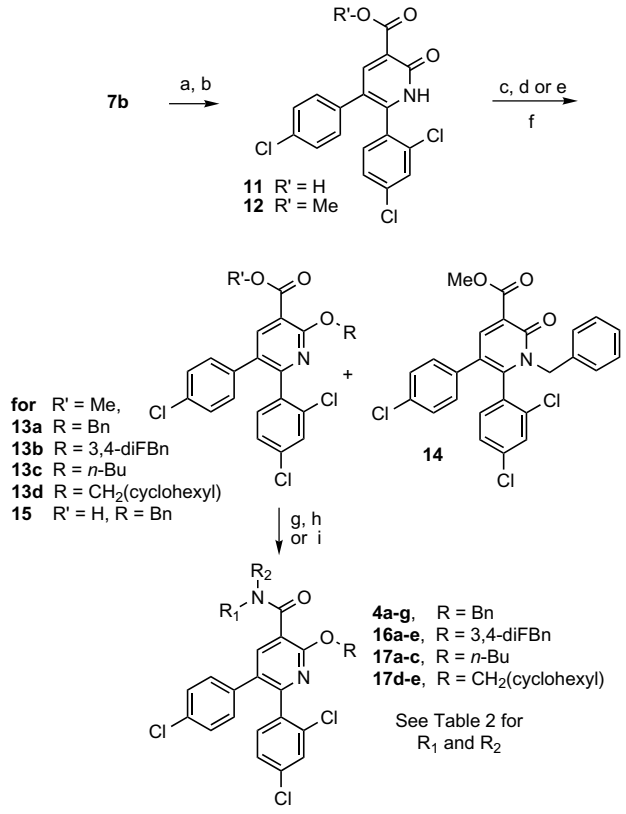


Compound No.	X_1	X_2	X_3	X_4	hCB1 IC_{50} (nM)[a]	SEM[b]
1					6.2	
3a	H	H	Cl	H	2800	340
3b	Cl	Cl	Cl	H	11	2
10a	Cl	Cl	Cl	4-F	3.1	1.1[c]
10b	Cl	Cl	Cl	3,5-diF	1.8	0.8
10c	Cl	Cl	Cl	2,4-diF	2.7	0.9[c]
10d	Cl	Cl	Cl	3,4-diF	1.3	0.8[c]
10e	Cl	Cl	Cl	3-Cl	5.8	2.2
10f	Cl	Cl	Cl	4-CF_3	3.4	0.5
10g	Cl	H	Cl	3,4-diF	18	4[c]
10h	Cl	Cl	H	3,4-diF	8	3
10i	F	Cl	Cl	3,4-diF	7.2	1.0[c]
10j	Me	Cl	Cl	3,4-diF	6.3	1.0
10k	F	F	F	3,4-diF	66	18[c]

[a] See Ref. 15 for assay details.
[b] $n = 2$, except as noted.
[c] $n = 4$.

carbonate afforded equal amounts of the *O*-benzyl **13** and the *N*-benzyl **14** derivatives.[16] Hydrolysis of the methyl ester **13** gave the acid **15**. Reaction with oxalyl chloride followed by addition of various amines or simultaneously with Py–BOP activation provided the amides **4a**–**g** (Table 2). As a result of the potent affinity obtained with the 3,4-difluorobenzyl ether **10d** (Table 1), a hybrid series of simple alkyl amides **16a**–**e** was examined as listed in Table 2. Also, an investigation of replacements for the benzyl ether was initiated with the preparation of a series of 2-alkoxyl analogs **17a**–**e** (Table 2) via alkylation of **12** and subsequent conversion to the amides as above.

In an attempt to shorten the synthesis of the amides in Scheme 2, the hydrolysis of the cyano group of **3b** was attempted to prepare the acid intermediate **15**. However, the vigorous conditions needed to hydrolyze the cyano group also lead to cleavage of the benzyl moiety to give back the prior acid **11**. However, it was discovered that reaction of **11** with oxalyl chloride provided the intermediate 2-chloropyridine acid chloride **18a**, which on subsequent treatment with several amines afforded a series of 2-chloro-3-amidopyridines **19a**–**d** (Scheme 3, Table 3). It was then envisioned that selective hydrogenation of **18b** might afford a synthesis of simpler amide analogs more closely related to **1**. Thus, methyl ester **12** was treated with oxalyl chloride to give the 2-chloropyridine **18b**. Mild hydrogenation at atmospheric pressure afforded a 26% yield of desired des-chloro **18c** along with unreacted starting material and a mixture of dechlorination by-products. Fortunately, **18c** was readily isolated by silica gel chromatography and was subsequently converted as in Scheme 2 to the amides **20a**–**d** (Table 3).



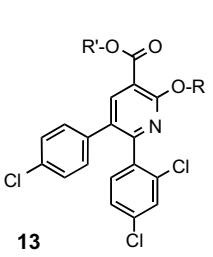
Scheme 2. Reagents and conditions: (a) aq H_2SO_4, 140 °C; (b) HCl, MeOH (69% from **7b**); (c) BnBr, Cs_2CO_3, DMF (47% **13a**, 48% **14**); (d) 3,4-diFBnBr, Cs_2CO_3, DMF (40% **13b**); (e) RI or RBr, Cs_2CO_3, DMF (67% **13c**; 29% **13d**); (f) NaOH, MeOH (75–85%); (g) oxalyl chloride, DMF (cat), CH_2Cl_2; (h) HNR_1R_2, Et_3N, CH_2Cl_2 (55–90%); (i) HNR_1R_2, Py–BOP, DIPEA, CH_2Cl_2 (40–85%).

Based on analogy to the Sanofi pyrazole and our pyridine scaffold, it was also of interest to prepare a series of 2-amido-5-(4-chlorophenyl)-6-(2,4-dichlorophenyl) pyridines (**29a**–**i**, Table 4) to see if the 2-substitution improved binding compared to the 3-amido series **20a**–**d**.[18] In an independent synthesis (Scheme 4), 4-chlorocinnamic acid (**21**) was reduced to the alcohol **22** via formation of the mixed anhydride and subsequent reduction with sodium borohydride.[19] Swern oxidation of **22** afforded the aldehyde **23**, which was treated with ethyl azido acetate **24** to provide the ethyl 2-azido-5-phenylpenta-2,4-dienoate **25**.[20] Conversion to the pyridine ring was accomplished by stirring **25** overnight with triphenylphosphene to provide phosphazene **26**, which was subsequently reacted with 2,4-dichlorobenzaldehyde (**27**) at 60 °C to provide the desired 2-substituted pyridine scaffold as the methyl ester intermediate **28**.[21] Utilizing previously described chemistry, a series of amides **29a**–**i** were similarly prepared (Table 4).

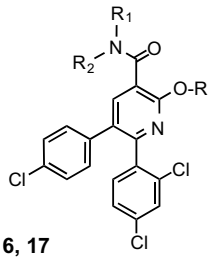
3. Results and discussion

Binding affinities on recombinant human CB1 receptor expressed in Chinese Hamster Ovary (CHO) cells for compounds in Tables 1–4 were determined with a standard binding assay using [^{3}H]CP-55940 as the radioligand.[15] Interesting compounds were further evaluated

Table 2. Structures and hCB1 activities of 2-benzyloxy- and 2-alkoxy-3-ester derivatives **13** and 3-amido derivatives **4**, **16**, and **17**



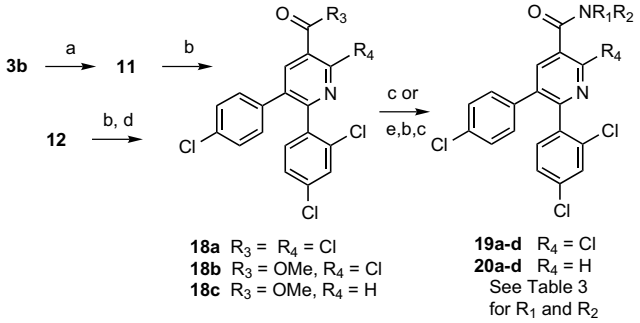
13



4, 16, 17

Compound No.	R	**13** R′	**4**, **16**, **17** NR_1R_2	hCB1 IC_{50} (nM)[a]	SEM[b]
4a	Bn	—	NH_2	18	4
4b	Bn	—	NHMe	7.0	2[c]
4c	Bn	—	NMe_2	22	5[c]
4d	Bn	—	NHEt	1.4	0.5
4e	Bn	—	NH(*n*-Pr)	6	1.3
4f	Bn	—	NH(cyclopentyl)	18	4
4g	Bn	—	NH(piperidin-1-yl)	41	18
13a	Bn	Me	—	3.5	1.5
13b	3,4-diFBn	Me	—	1.3	0.2[c]
13c	*n*-butyl	Me	—	1.9	0.9
13d	CH_2(cyclohexyl)	Me	—	2.9	0.1
16a	3,4-diFBn	—	NHMe	3.1	1.0
16b	3,4-diFBn	—	NHEt	1.9	1.0
16c	3,4-diFBn	—	NH(*n*-Pr)	1.7	0.4[c]
16d	3,4-diFBn	—	$NH(CH_2)_2F$	1.5	0.1
16e	3,4-diFBn	—	NH(*i*-Pr)	1.8	0.2
17a	*n*-butyl	—	NH_2	32	16
17b	*n*-butyl	—	NHMe	17	7
17c	*n*-butyl	—	NH(*n*-Pr)	21	2
17d	CH_2(cyclohexyl)	—	NHMe	3.4	1.5
17e	CH_2(cyclohexyl)	—	NH(*n*-Pr)	5.2	1.1

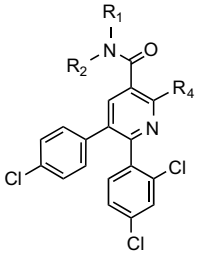
[a] See Ref. 15 for assay details.
[b] $n = 2$, except as noted.
[c] $n = 4$.



Scheme 3. Reagents and conditions: (a) 50% H_2SO_4, 140 °C (quant.); (b) oxalyl chloride, CH_2Cl_2 (82% for **18b**); (c) HNR_1R_2, Et_3N, CH_2Cl_2 (52% **19a** from **3b**); (d) 5% Pd/C, H_2 (26%); (e) NaOH, MeOH (90%).

in a functional assay also using recombinant human CB1 receptors expressed in CHO cells.[15] Binding affinities were also routinely measured for the CB2 receptor expressed in CHO cells and using [^{3}H]WIN-55,212-2 as radioligand.[15,22] While the initial result with the 6-(4-chlorophenyl) derivative **3a** was disappointing (IC_{50} = 2800 nM), the 200-fold improvement in the binding affinity of **3b** to an IC_{50} = 11 nM validated the hypothesis that a pyridine could replace the pyrazole of **1**. In addition, **3b** was found to be >200-fold selective for hCB1 over hCB2 (IC_{50} > 2000 nM (34%)) in the binding assay. Also encouraging was the finding that

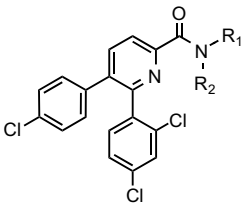
Table 3. Structures and hCB1 activities of 3-amido derivatives **19** and **20**



Compound No.	R_4	R_3	hCB1 IC_{50} (nM)[a]	SEM[b]
19a	Cl	NMe_2	1300	400
19b	Cl	NH(*n*-Pr)	52	4
19c	Cl	NH(*n*-hexyl)	58	6
19d	Cl	NH(piperidin-1-yl)	400	250
20a	H	piperidin-1-yl	460	50
20b	H	NH(*n*-hexyl)	65	6
20c	H	NH(cyclohexyl)	210	80
20d	H	NH(piperidin-1-yl)	480	170

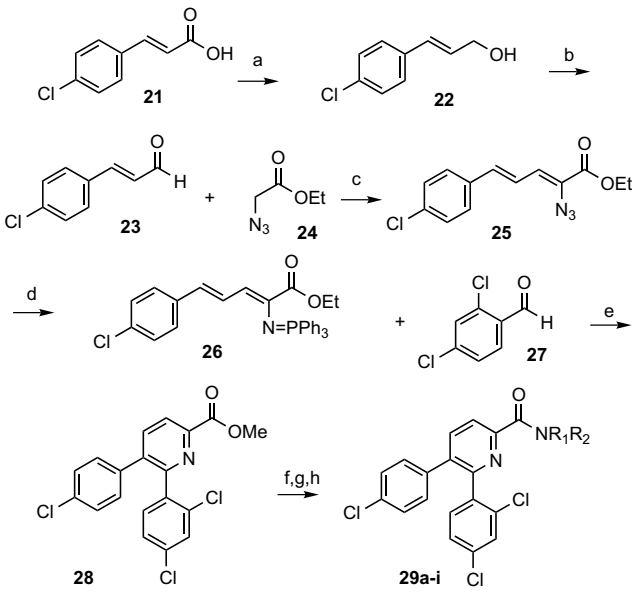
[a] See Ref. 15 for assay details.
[b] $n = 2$.

Table 4. Structures and hCB1 activities of 2-amido derivatives **29**



Compound No.	$-NR_1R_2$	hCB1 IC_{50} (nM)[a]	SEM[b]
29a	piperidin-1-yl	340	13
29b	NH(*n*-hexyl)	43	11
29c	NH(4-heptyl)	32	11
29d	NH(cyclopentyl)	53	13[c]
29e	NH(cyclohexyl)	95	38
29f	NH(cycloheptyl)	35	10
29g	NH(piperidin-1-yl)	56	18
29h	NHPh	210	40
29i	NHBn	31	2

[a] See Ref. 15 for assay details.
[b] $n = 2$.
[c] $n = 3$.



Scheme 4. Reagents and conditions: (a) $ClCO_2Et$, Et_3N, THF, then $NaBH_4$, H_2O (79%); (b) oxalyl chloride, DMSO, then DIPEA, CH_2Cl_2 (88%); (c) $NaOCH_3$, NaOH (100%); (d) PPh_3, Et_2O, 0 °C; (e) CH_3CN, 60 °C (22% from **23**); (f) NaOH, MeOH (96%); (g) oxalyl chloride, DMF (cat), CH_2Cl_2; (h) HNR_1R_2, Et_3N, CH_2Cl_2 (35–75%).

the hCB1 functional assay indicated both **3a** (up to −67% @ 3000 nM) and **3b** (−52% maximal response with an $EC_{50} = 28 \pm 20$ nM, $n = 3$) were interacting with the hCB1 receptor as inverse agonists. The diminished binding for the methyl ether **9a** was also in agreement with the benzyl taking the place of the piperidine amide. The *N*-benzyl pyridin-2-one by-products **8a** and **8b** (Scheme 1, IC_{50} = 6000 and 2000 nM, respectively) were relatively inactive, as were all other *N*-substituted analogs in both the 3-cyano and 3-amido series and all intermediates, and will not be further mentioned.

The affinity of **3b** was further explored with a benzyl substitution SAR study (Table 1). The 4-fluoro and 4-trifluoromethyl derivatives **10a** and **10f** and the 3-chloro analog **10e** showed improved binding (IC_{50} = 3.1, 3.4, and 5.8 nM). 3,5-Disubstitution as in the 3,5-difluoro **10b** (IC_{50} = 1.8 nM) was also beneficial while the 2-substitution of the 2,4-difluoro **10c** (IC_{50} = 2.7 nM) did not afford any apparent improvement over the 4-fluoro **10a**. The optimum substitution appeared to be obtained with the 3,4-difluoro compound **10d** (IC_{50} = 1.3 nM), which also had potent inverse agonist functional activity ($EC_{50} = 8 \pm 3$ nM with an average −110% maximal response, $n = 4$) and was now 400-fold selective for hCB1 over hCB2 (IC_{50} = 1.3 vs 520 nM) in the binding assays.

As already seen with **3a**, the 4-chloro substitution on the 5-phenyl was critical and even replacement of the 5-(4-chlorophenyl) with a 4-fluoro- or 4-methylphenyl as in **10i** and **10j** reduced affinity 5-fold (IC_{50} = 7.2 and 6.3 nM). The effect of substitution on the 6-phenyl ring was also examined in the 3,4-difluorobenzyloxy series with the preparation of the 6-(4-chlorophenyl) **10g** and 6-(2-chlorophenyl) **10h** analogs which, as expected,[13a] afforded reduced binding compared to **10d** (IC_{50} = 18, 8.0, and 1.3 nM, respectively). Since the trifluoro analog **10k** resulted in a 50-fold loss of affinity (IC_{50} = 66 nM), the trichloro substitution pattern of **1** was determined to be optimal for binding in our pyridine series.

Replacement of the 3-cyano of **3b** with an amide moiety as in **1** was then investigated, first in the unsubstituted benzyl ether series with **4a**–**g** and subsequently, after the discovery of **10d**, in the 3,4-difluorobenzyl series **16a**–**e** (Table 2). The SAR indicated that a secondary amide was preferred as seen with the series of primary **4a**, secondary **4b**, and tertiary **4c** amides (IC_{50} = 18, 7, and 22 nM, respectively). The observation that the methyl esters **13a**–**d** were equipotent or even better than the corresponding NHMe amides **4b**, **16a**, **17b**, and **17d**, respectively, implied that there is not a direct N–H interaction, only steric and/or lipophilic effects of the *N*-alkyl moiety. These effects were apparently optimum with the *N*-ethyl derivative **4d** (hCB1 IC_{50} = 1.4 nM; functional activity $EC_{50} = 21 \pm 2$ nM with an average −60% agonist response, $n = 3$), while *n*-propyl **4e** or larger again gave poorer binding. Incorporating the piperidine moiety of **1** into **4g** or the comparably sized cyclopentyl of **4f** also reduced affinity (IC_{50} = 18 and 41 nM) implying that the benzyloxy was in fact occupying the same area of the receptor where the piperidine of **1** binds. Analogs with the potency enhancing 3,4-difluorobenzyl were subsequently prepared (**16a**–**e**, Table 2). While some improvement was observed for **16a** and **16c** compared to **4b** and **4e**, this change did not afford an additional 10-fold improvement as had been obtained in the 3-cyano series (**16a**–**c**; IC_{50} = 3.1, 1.9, and 1.7 nM). Both **16b** and **16c** were shown to be inverse agonists ($EC_{50} = 13 \pm 2$ and 50 ± 20 nM with an average −60 and −73% maximal response, respectively, $n = 2$) and were both >400-fold selective for hCB1 (hCB2 IC_{50} = 820 and 815 nM) in the binding assays. Addition of a fluorine as in **16d** or branching of the alkyl as in **16e**,

did not further improve binding (IC_{50} = 1.5 and 1.8 nM). In the hope of improving solubility, the 2-benzyloxy group was also replaced with two alkoxy moieties as shown in Table 2. Interestingly, the smaller *n*-butyl group (**17a**–**c**) gave only moderately diminished results and the isosteric cyclohexylmethyl showed comparable or better results than the benzyl compound (**17b**,**d**, and **4b**; IC_{50} = 17, 3.4, and 7 nM). This implied that the receptor interaction in this area was simply binding to a lipophilic pocket without a direct aromatic interaction.

Removal of the 2-benzyloxy moiety was studied in the 2-chloro (**19a**–**d**, Table 3) and des-chloro-3-amido (**20a**–**d**, Table 3) series and resulted in overall decreased affinity. The 3-propyl and 3-hexyl amides **19b**,**c**, and **20b** were moderately active (IC_{50} = 52, 58, and 65 nM, respectively); however, the *N*-piperidinyl amides **19d** and **20d** and the isosteric cyclohexyl amide **20c** (IC_{50} = 400, 480, and 210 nM, respectively) were surprisingly inactive compared to **1** and the benzyloxy derivatives discussed above. The secondary amides **19a** and **20a** were also comparatively inactive.

The poor binding of the 3-amido compounds led to the investigation of the 2-amido series where somewhat more encouraging results were obtained (Table 4). The *N*-piperidinyl amide **29g** analogous to **1** was found to be modestly active (IC_{50} = 56 nM). The corresponding cyclohexyl derivative **29e**, as well as the cyclopentyl **29d** and cycloheptyl **29f**, were about the same, as were the noncyclic *n*-hexyl **29b** and 4-heptyl **29c** and the benzyl amide **29i**. The secondary piperidine amide **29a** and aniline derivative **29h** had very weak binding. Although these amides were not particularly potent, their overall functional data appeared to indicate the desired inverse agonist activity at high concentrations (data not shown). There are several possible reasons for the relatively poor binding of these 2- and 3-amido pyridine derivatives compared to **1**. The divergent affinity might be related to the ring size giving a slightly different relative orientation of the three pyridine substituents, and/or the basicity difference of the nitrogen, and/or possibly the effect of a lack of a neighboring methyl, which would force the amide moiety of **1** to be perpendicular to the pyrazole ring, although in the 3-carboxamide series the neighboring chloro in **19d** did not have an effect compared to **20d**.

4. In vivo studies

Based on their favorable hCB1 binding and functional assay results, **10d** and **16c** were selected for further in vivo evaluation. Preliminary pharmacokinetic (PK) studies in male Sprague–Dawley rats (1 mg/kg i.v., 2 mg/kg p.o.) with **10d** indicated good i.v. pharmacokinetic properties (AUC_{nom} = 9.3 μM h kg/mg; Cl_p = 3.6 mL/min/kg; Vd_{ss} = 0.8 L/kg; $t_{1/2}$ = 3.6 h) and moderate oral absorption (F = 27%), but slow brain penetration and a low brain-to-plasma ratio (B/P ratio = 0.03–0.26 at 0.25–4 h).[23] In a food intake and body weight loss study using diet-induced obese rats fed ad libitum, **10d** at 1, 3, and 10 mg/kg p.o. did not show an immediate reduction in food intake at any of the doses as seen with **1**. However, by 18 h post-dosing, the 10 mg/kg dose afforded a cumulative, but non-significant ($p > 0.05$), 22% food intake reduction and a dose-dependent weight loss of 1 g compared to an 8 g gain for vehicle treated animals ($p < 0.05$). These results suggested that the slow CNS exposure, as evident from the B/P ratio experiment, prevented an immediate food intake effect. A similar feeding study with the slightly less potent **16c** afforded a cumulative 16% reduction in food intake at 10 mg/kg ($p > 0.05$), but did not give a net weight loss (5 g gain vs an 11 g gain for vehicle). The results with **16c** were consistent with its poorer PK profile (AUC_{nom} = 5.2 μM h kg/mg; Cl_p = 5.8 mL/min/kg, Vd_{ss} = 0.4 L/kg; $t_{1/2}$ = 2.5 h), lower oral absorption (F = 12%), and slower brain penetration (B/P ratio = 0.01–0.06 at 0.25–4 h).

5. Conclusions

The finding that both the 2- and 3-amido pyridine analogs were only moderately potent hCB1 receptor inverse agonists was disappointing based on the similarity to the Sanofi compound **1**. This might be a result of the additional ring atom slightly changing the relative orientation of the three pyridine substituents relative to the proposed nitrogen interaction, and/or the pyridine nitrogen did not have an equivalently effective receptor interaction as the pyrazole nitrogen due to electronic differences, and/or the effect of the neighboring methyl of **1**. However, the excellent binding affinity of the six-membered ring 3-cyano-2-(3,4-difluorobenzyloxy) pyridine derivative **10d** and the equivalent 3-(*N*-propylcarboxamido) derivative **16c** demonstrated the possibility that the amide moiety of the five-membered pyrazole **1** could be split into a lipophilic portion and a polar functionality. This finding might allow more flexibility in selection of derivatives in the future with enhanced PK and CNS exposure profiles and improved physical and off-target properties than could be achieved with a single amide functionality. Further SAR studies will be published elsewhere in the near future.

References and notes

1. For general reviews of cannabinoid receptor ligands and potential therapeutic applications, see: (a) Di Carlo, G.; Izzo, A. A. *Expert Opin. Invest. Drugs* **2003**, *12*, 39; (b) Pertwee, R. G. *Expert Opin. Invest. Drugs* **2000**, *9*, 1553; (c) Xiang, J.-N.; Lee, J. C. *Ann. Rep. Med. Chem.* **1999**, *34*, 199.
2. (a) Trillou, C. R.; Delgorge, C.; Menet, C.; Arnone, M.; Soubrie, P. *Int. J. Obesity* **2004**, *28*, 640; (b) Zimmer, A.; Zimmer, A. M.; Hohmann, A. G.; Herkenham, M.; Bonner, T. I. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5780.
3. Barth, F.; Rinaldi-Carmona, M. *Curr. Med. Chem.* **1999**, *6*, 745.
4. Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D.; Ferrarra, P.; Soubrie, P.; Breliere, J. C.; Le Fur, G. *FEBS Lett.* **1994**, *350*, 240.

5. Colombo, G.; Agabio, R.; Diaz, G.; Lobina, C.; Reali, R.; Gessa, G. L. *Life Sci.* **1998**, *8*, 113.
6. Trillou, C. R.; Arnone, C.; Gonalons, N.; Keane, P.; Maffrand, J. P.; Soubrie, P. *J. Physiol. Regul. Integr. Comp. Physiol.* **2003**, *284*, 345.
7. Cota, D.; Marsicano, G.; Tschop, M.; Grubler, Y.; Flachskamm, C.; Schubert, M.; Auer, D.; Yassouridis, A.; Thone-Reineke, C.; Ortmann, S.; Tomassoni, F.; Cervino, C.; Nisoli, E.; Linthorst, A. C. E.; Pasquali, R.; Lutz, B.; Stalla, G. K.; Pagotto, U. *J. Clin. Invest.* **2003**, *112*, 423.
8. Pertwee, R. G. *Pharmacol. Ther.* **1997**, *74*, 129.
9. Koe, B. K.; Milne, G. M.; Weissman, A.; Johnson, M. R.; Melvin, L. S. *Eur. J. Pharmacol.* **1985**, *109*, 201.
10. D'Ambra, T. E.; Estep, K. G.; Bell, M. R.; Eissenstat, M. R.; Josef, K. A.; Ward, S. J.; Haycock, D. A.; Baizman, E. R.; Casiano, F. M.; Beglin, N. C. *J. Med. Chem.* **1992**, *35*, 124.
11. Mato, S.; Pazos, A.; Valdizan, E. M. *Eur. J. Pharmacol.* **2002**, *443*, 43.
12. (a) Landsman, R. S.; Burkey, T. H.; Consroe, P.; Roeske, W. R.; Yamamura, H. I. *Eur. J. Pharmacol.* **1997**, 334R1–334R2; (b) Bouaboula, M.; Perrachon, S.; Milligan, L.; Canat, X.; Rinaldi-Carmona, M.; Portier, M.; Barth, F.; Calandra, B.; Pecceu, F.; Lupker, J.; Maffrand, J. P.; Le Fur, G.; Casellas, P. *J. Biol. Chem.* **1997**, *272*, 22330.
13. (a) Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 769; (b) Wiley, J. L.; Jefferson, R. G.; Grier, M. C.; Mahadevan, A.; Razdan, R. K.; Martin, B. R. *J. Pharm. Exp. Ther.* **2001**, *296*, 1013.
14. Shim, J.-Y.; Welsh, W. J.; Cartier, E.; Edwards, J. L.; Howlett, A. C. *J. Med. Chem.* **2002**, *45*, 1447.
15. The reported binding results (±SEM) were an average of 2–4 independent determinations, which were normally within 50% of the average value based on a standard sample. For the assay conditions for both the binding and functional assays, see: Felder, C. C.; Joyce, K. E.; Briley, E. M.; Mansouri, J.; Mackie, K.; Blond, O.; Lai, Y.; Ma, A. L.; Mitchell, R. L. *Mol. Pharmacol.* **1995**, *48*, 443.
16. ^{1}H NMR and/or LC–MS data were in agreement with the assigned structures. For full experimental and spectral details, see: Finke, P. E.; Meurer, L. C.; Debenham, J. S.; Toupence, R. B.; Walsh, T. F. WO Patent 03/082191 A2, 2003; *Chem. Abstr*. **2003**, *139*, 307686f.
17. Newman, M. S.; Reid, D. F. *J. Org. Chem.* **1958**, *23*, 665.
18. An EPO patent from Sanofi-Synthelabo related to this same 2-amido pyridine structure has recently been published, see: Barth, F.; Martinez, S.; Rinaldi-Carmona, M. WO Patent 03/084930 A1, 2003; *Chem. Abstr*. **2003**, *139*, 307689j.
19. Ishizumi, K.; Koga, K.; Yamada, S. *Chem. Pharm. Bull.* **1968**, *16*, 492.
20. Boukou-Poba, J. P.; Farnier, M.; Guilard, R. *Tetrahedron Lett.* **1979**, *19*, 1717.
21. Barluenga, J.; Ferrero, M.; Palacios, F. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2193.
22. CB2 binding affinities were routinely measured for all compounds[15] and, generally, were less sensitive to these SAR changes than were the CB1 affinities. Higher CB2 to CB1 IC_{50} ratios were observed for the more active benzyloxy compounds of Tables 1 and 2 (50–500) than for the less active amides of Table 3 (0.2–10) and Table 4 (5–30).
23. Ritschel, W. A.; Kearns, G. L. In *Handbook of Basic Pharmacokinetics Including Clinical Applications*; Graubart, J. I., Ed., 5th ed.; American Pharmaceutical Association, 1999.