Synthesis, Structure−Activity Relationship, and Evaluation of SR141716 Analogues: Development of Central Cannabinoid Receptor Ligands with Lower Lipophilicity

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Exploration of the central CB_1 cannabinoid receptors using positron emission tomography (PET) will allow for an understanding of the pharmacological and physiological role played by these receptors in the CNS. Current tracers are highly lipophilic compounds that exhibit very high nonspecific to specific binding ratios and as a result are inapt for use in humans. We have synthesized a series of less lipophilic analogues of SR141716 to serve as potential radioligands. Binding affinities of the series and a functional electrophysiological assay of three of our compounds have been presented.

Introduction

Lipophilicity is an important parameter in developing in vivo imaging agents[1] for positron emission tomography (PET) and single photon emission computed tomography (SPECT). The existing CB_1 radiotracers,[2−6] mainly analogues of the selective CB_1 antagonist SR141716, exhibit high nonspecific binding and modest affinity, which limits their application in imaging. This paper describes our effort to develop novel SR141716 analogues, as potential PET tracers, that display lower lipophilicity than existing CB_1 radiotracers while still maintaining high affinity. The limiting factor for the design of our target compounds was the necessity to introduce either a fluoro/bromo substituent, which would to be suitable for radiolabeling with positron-emitting halogens (^{18}F, ^{76}Br), or a methyl-containing substituent that could be radiolabeled with [^{11}C]methyl iodide. Additionally, since antagonists serve as superior radioligands than agonists for in vivo use because they do not impose the potential psychoactive effects, functional assays were carried out on a few of the compounds to confirm their antagonistic property.

Results and Discussion

Chemistry. The general strategy for preparation of SR141716 analogues with modified substituents on the phenyl rings is presented in Scheme 1. Some of the intermediate compounds were prepared according to known methods.[7] Condensation of the diketone ester lithium enolate obtained from the requisite propiophenone **4**, with substituted phenylhydrazine hydrochloride, followed by refluxing in acetic acid resulted in the formation of the core pyrazole structure **5**. Conversion of the ethyl ester to the desired hydrazide **7** was accomplished via basic hydrolysis to give the corresponding carboxylic acid **6** and subsequent treatment with thionyl chloride, followed by acylation with the desired amine. The formation of 4′-hydroxy analogue **7b** was carried out through **6b**, prepared by demethylation of the corresponding 4′-methoxy carboxylic acid **6a** (Scheme 1). Mitsunobu coupling of **7b** with (±) *N*-*t*-BOC-prolinol followed by deprotection with TFA gave **7k**.

The depicted method in Scheme 1 proved unsuccessful for the preparation of 4-bromopyrazole derivatives and led us to explore an alternate pathway (Scheme 2) which was an improvement over previously reported procedures.[8] To insert a fluoro group in position 4, the synthesis was carried out from 2-fluoroacetophenone **11** which was synthesized from **10** via a modification of an earlier procedure.[9] The desired fluorinated ketone **11** was then converted to the ethyl ester **5i** (Scheme 3, pathway I). To ensure that 1,5-diarylpyrazole derivative was obtained via pathway I, as opposed to the undesirable 1,3-diaryl product, an alternate synthesis of the fluoro ester **5i** was carried out using pathway II by fluorination of **8** with Selectfluor. To our knowledge, this is the first reported method for inserting a fluoro group in the C-4 position of pyrazoles using an N−F reagent. Such a method is more practical for a conventional chemistry lab than the fluorination of pyrazole derivatives with F_2.[10]

Log D_{oct} Determination. Octanol−water partition coefficient at pH 7.4 ($ElogD_{oct}$) was determined utilizing the rp-HPLC protocol[11] as well as using software (clogD).[12]

In Vitro Binding Assay. The binding affinities of all compounds were determined by using a competition assay with rat cerebellum homogenate against [^{125} I]-AM251.[13]

Structure−Activity Relationships (SAR). The first stage of exploring the SAR of pyrazole analogues was to modify the R_1 substituent in the C-5 benzene ring (Table 1). Substitution of *p*-chloro with a *p*-methoxy group (**7a**) resulted in lower lipophilicity and binding affinity compared to SR141716 (3). The methylenedioxy group in **7c** also decreased lipophilicity and resulted in

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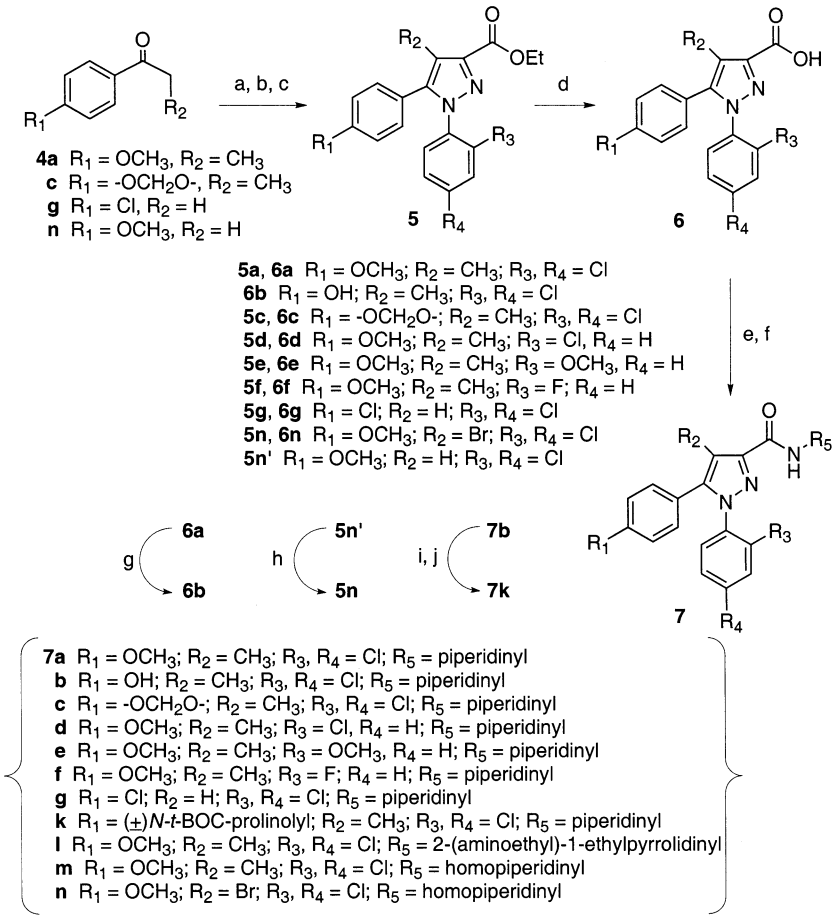
Scheme 1[a]



[a] Reagents: (a) LiHMDS, Et_2O, $(CO_2Et)_2$, −78 → 0 °C; (b) For **5a**,**b**,**g**,**n** and **8**: 2,4-dichlorophenylhydrazine hydrochloride; **5d**: 2-chlorophenylhydrazine hydrochloride; **5e**: 2-methoxyphenylhydrazine hydrochloride; **5f**: 2-fluorophenylhydrazine hydrochloride, EtOH, rt; (c) AcOH, reflux; (d) KOH, MeOH, reflux; (e) $SOCl_2$, $PhCH_3$, reflux; (f) For **7a**−**g**,**k**: 1-aminopiperidine; **7l**: 2-(aminoethyl)-1-ethylpyrrolidine; **7m**,**n**: 1-aminohomopiperidine, Et_3N, CH_2Cl_2; (g) HBr, gl. CH_3COOH, reflux; (h) Br_2, CH_2Cl_2, −5 °C; (i) (±)N-*t*-BOC-prolinol, DEAD, Ph_3P, THF; (j) CF_3COOH, CH_2Cl_2, rt.

Table 1.

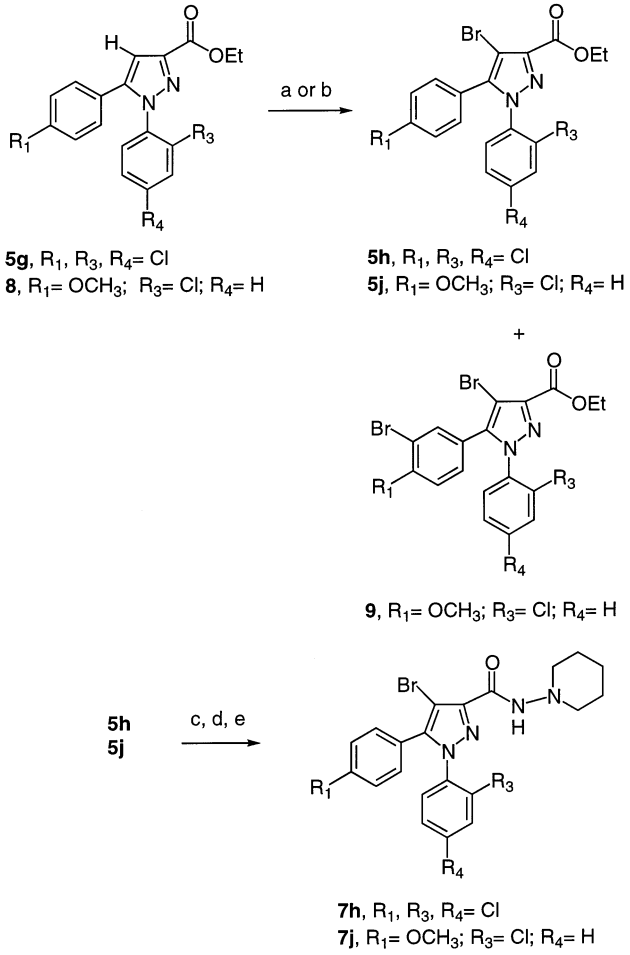
compd	name	R_1	R_2	R_3	R_4	R_5	$K_i \pm$ SEM (nM)	clogD	$ElogD_{oct}$
1	AM251	I	CH_3	Cl	Cl	piperidinyl	0.6 ± 0.04	5.25	5.46
2	AM281	I	CH_3	Cl	Cl	piperidinyl	4.5 ± 0.1	3.71	-
3	SR141716	Cl	CH_3	Cl	Cl	piperidinyl	1.8 ± 0.2	4.81	5.36
7a	NIDA-41020	OCH_3	CH_3	Cl	Cl	piperidinyl	4.1 ± 0.1	4.06	4.78
7b	NIDA-41057	OH	CH_3	Cl	Cl	piperidinyl	104 ± 10	3.46	4.20
7c	NIDA-41075	$-OCH_2O-$	CH_3	Cl	Cl	piperidinyl	7.1 ± 0.2	4.11	4.69
7d	NIDA-41087	OCH_3	CH_3	Cl	H	piperidinyl	8.0 ± 0.1	3.45	3.72
7e	NIDA-41093	OCH_3	CH_3	OCH_3	H	piperidinyl	440 ± 24	2.86	3.89
7f	NIDA-41127	OCH_3	CH_3	F	H	piperidinyl	37.8 ± 1.7	2.91	4.03
7g	NIDA-41119	Cl	H	Cl	Cl	piperidinyl	9.0 ± 1	4.35	5.14
7h	NIDA-41109	Cl	Br	Cl	Cl	piperidinyl	1.4 ± 0.2	4.94	5.38
7i	NIDA-42033	OCH_3	F	Cl	H	piperidinyl	18.2 ± 1.4	3.43	4.01
7j	NIDA-42055	OCH_3	Br	Cl	H	piperidinyl	6.2 ± 1	3.58	4.09
7k	NIDA-42071	prolinolyl	CH_3	Cl	Cl	piperidinyl	390 ± 55	1.23	2.09
7l	NIDA-42087	OCH_3	CH_3	Cl	Cl	2-aminoethyl-1-ethyl pyrrolidinyl	380 ± 15	1.61	3.15
7m	NIDA-42077	OCH_3	CH_3	Cl	Cl	homopiperidinyl	7.0 ± 0.3	4.62	5.34
7n	NIDA-42093	OCH_3	Br	Cl	Cl	homopiperidinyl	5.2 ± 0.5	4.75	5.17

slightly decreased binding. Incorporation of a *p*-hydroxy (**7b**) or a pyrrolidinyl moiety (**7k**) in the para-position showed desirably low lipophilicity, but the compounds exhibited low binding affinity. Remarkably, the binding affinities of compounds of R_1-subseries (**1**, **3**, **7a**, **7b**, **7k**) display a linear correlation with clogD values according to eq 1. Correlation of the binding affinities ($pK_i = -\log K_i$) of another SR141716 R_1-subseries[7] with the calculated[12] clogD values gave a similar linear trend line following eq 2.[16]

$$pK_i = 0.6987 \times \text{clogD} + 5.3167 \ (R^2 = 0.862, n = 6) \quad (1)$$

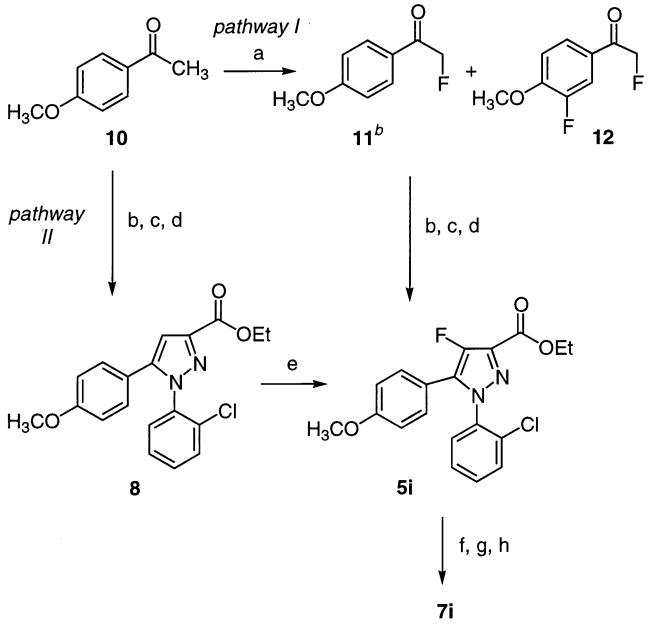
$$pK_i = 0.4939 \times \text{clogD} + 5.3621 \ (R^2 = 0.663, n = 6) \quad (2)$$

Scheme 2[a]



[a] Reagents: (a) Br_2, CH_2Cl_2, −5 °C; (b) NBS, CH_3CN, rt; (c) KOH, MeOH, reflux; (d) $SOCl_2$, $PhCH_3$, reflux; (e) 1-aminopiperidine, Et_3N, CH_2Cl_2.

Scheme 3[a,b]



[a] Reagents: (a) Selectfluor, MeOH, reflux; (b) LiHMDS, Et_2O, $(CO_2Et)_2$, −78 °C → 0 °C; (c) 2-chlorophenylhydrazine hydrochloride, EtOH, rt; (d) AcOH, reflux; (e) Selectfluor, CH_3CN, reflux; (f) KOH, MeOH, reflux; (g) $SOCl_2$, $PhCH_3$, reflux; (h) 1-aminopiperidine, Et_3N, CH_2Cl_2. [b]Compound **11** has been synthesized previously[18] but was prepared according to our protocol for this publication. All analytical data correlated with that found in the literature.

This finding suggests the possibility that analogues of SR141716 with more hydrophilic R_1 substituents than chlorine are likely to display reduced binding affinity. It is also in agreement with the previously proposed pharmacophoric alignment of C-5 benzene ring in SR141716 with the hydrophobic pentyl side-chain of Δ^9-THC.[19]

The lower lipophilicity of **7a**, combined with its similar binding to the **3**, led us to investigate substitutions on the N-1 aryl ring of the pyrazole ring (R_3 and R_4) while keeping the methoxy group intact. Elimination of the R_4 *p*-chloro group resulted in a lower binding analogue **7d**. However, its lower clogD value led us to explore different substituents at the ortho-position (R_3). The replacement of the *o*-chloro group with an *o*-fluoro **7f** and *o*-methoxy **7e** produced low affinity analogues. Comparison of the R_3 subseries of compounds **7d**, **7e**, and **7f** demonstrates the extreme sensitivity of R_3 substituent toward receptor recognition.

Substitution of the C-4 methyl of the pyrazole ring with a bromo substituent (**7h**) led to a more lipophilic compound possessing enhanced binding affinity. However, replacement of the C-4 methyl in SR141716 with hydrogen yielded a less lipophilic compound **7g**, with lower binding affinity. The presence of a fluoro group in the position C-4 lowered the affinity and lipophilicity (compare **7i** with **7d** and **7j**). Thus, it seems reasonable to conclude that within the synthesized series, more lipophilic R_2 substituents (Br > CH_3 > F > H) enhance binding affinity. These observations are consistent with other studies,[14] indicating that replacement of a methyl group with more lipophilic bromine and iodine leads to better binding. Modification of the hydrazide portion of the lead molecule was also carried out. Substitution with a pyrrolidinyl group (**7l**) and homopiperidinyl group(**7m** and **7n**), did not exhibit improved affinities. This discrepancy may be attributed to the specific role of *p*-OMe group in the C-5 phenyl ring.

Functional Assay. To assess the functional properties of these compounds, we performed extracellular electrophysiological recordings of field potentials in the nucleus accumbens of rat brain slices, where CB agonists have been previously shown to exhibit glutamate release.[15] Pretreatment of the slices with **7a**, **7c**, or **7d** blocked the ability of the CB agonist WIN 55,212-2 to reduce the field potentials, but had no effect on the baseline (pretreatment) synaptic responses (data not shown). When these compounds were applied subsequently to WIN 55,212-2, the inhibitory effect of the agonist was rapidly and completely reversed.[16] This reversal was due to antagonism, rather than "wash-out" of WIN 55,212-2, since previous data have shown that this lipophilic agonist does not typically wash out of brain slices.[15]

Conclusion

We have synthesized a series of SR141716 (**3**) analogues as potential ligands for PET studies of cannabinoid receptors targeting high affinity ligands with low lipophilicity. Some of these compounds possess lower lipophilicity values and comparable binding affinity to

3. A facile method for the synthesis of C-4 fluoro analogues of **3** has been developed. Moreover, using a functional electrophysiological assay, we have demonstrated that several compounds of the series (**7a**, **7c**, and **7d**) possess antagonistic properties and are able to rapidly and completely reverse the effects of WIN 55,221-2.

Currently, **7a**,[17] the methoxy analogue of **3**, has been selected as a potential target for the development of corresponding $^{11}CH_3$-labeled derivatives.

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Supporting Information Available: Experimental data. Correlation of binding affinity versus lipophilicity (Figure 1). Functional reversal of CB agonist mediated inhibition of potentials in brain slices by SR141716 analogues (Figure 2). This material is available free of charge via the Internet at http://pubs.acs.org.

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