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Tetrazole-biarylpyrazole derivatives as cannabinoid CB1 receptor antagonists

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Abstract—Cannabinoid CB1 receptors have been the focus of extensive studies since the first clinical results of rimonabant (SR141716) for the treatment of obesity and related metabolic disorders were reported in 2001. To further evaluate the properties of CB receptors, we have designed a new series of tetrazole-biarylpyrazoles. The various analogues were efficiently prepared and bio-assayed for binding to cannabinoid CB1 receptor. Six of the new compounds which displayed high in vitro CB1 binding affinities were assayed for binding to CB2 receptor. Noticeably, cyclopentyl-tetrazole (**9a**) demonstrated good binding affinity and selectivity for CB1 receptor (IC_{50} = 11.6 nM and CB2/CB1 = 366).

The World Health Organization (WHO) recently declared that obesity has become a global epidemic, posing a serious threat to public health because of the increased risk of associated health problems.[1,2] There are growing evidences that obesity as a chronic disease cannot be cured only by short-term diet or exercise. Reducing weight by caloric restriction generally fail as most obese patients readily regain their lost weight thereafter. Therefore, medical treatment of obesity became a necessity.[3–5]

Recent development of obesity drugs reveals that it is possible to control appetite and reduce weight by blocking cannabinoid receptors in the brain, liver or muscle, via cannabinoid (CB1) receptor antagonists or CB1 receptor inverse agonists.[6,7] A cannabinoid CB1 receptor antagonist is designed to block the effects of endogenous cannabinoids. This type of drug is particularly interesting since it not only causes weight loss but also reverses the metabolic effects of obesity such as insulin resistance and hyperlipidemia.[8] The other cannabinoid receptor, CB2, is related to immune regulation and neurodegeneration.[9] Therefore, the CB1/CB2 selectivity should be taken into consideration for new drug development of anti-obesity agent.

The first specific cannabinoid CB1 receptor antagonist, rimonabant, was discovered in a high throughput screening programme at Sanofi-Synthélabo in 1994.[10] Several CB1 receptor antagonists including rimonabant, SLV319,[11] CP-945,598 and MK-0364[12] have been reported to be in various phase of clinical trials.[1,6,13,14] A pharmacophore model for the binding of a low energy conformation of rimonabant in the CB1 receptor has been well-documented.[14,15] The key receptor–ligand interaction is known to be a hydrogen bond between the carbonyl group of rimonabant and the Lys192–Asp366 residue of the CB1 receptor, thereby exerting a

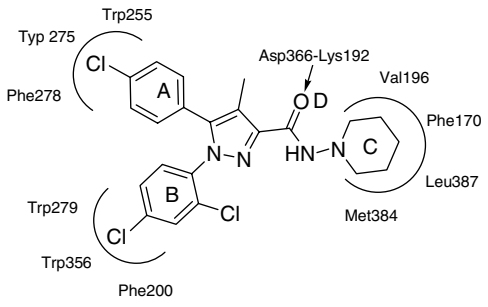

Figure 1. Rimonabant and its receptor–ligand interaction.

Keywords: Rimonabant; Anti-obesity; Cannabinoid receptor antagonist; Tetrazole.
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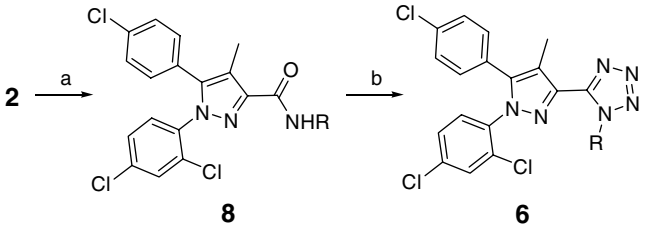
stabilizing effect on the Lys192–Asp366 salt bridge as shown in Figure 1.[6]

So far, various analogues of rimonabant by using strategy such as conformational constraints or scaffold hopping have been designed for the purpose of enhancement of binding affinity and selectivity for CB1 receptor.[6] For the most part, replacements of pyrazole core to 5- or 6-membered ring scaffolds were actively studied. We envisioned that the key carbonyl group of rimonabant might be replaced to the corresponding imine-type functionality or 'imine'-containing heterocycles. Similar approaches were already demonstrated successfully with imidazole[16a,b] or oxadiazole[16c] scaffolds.

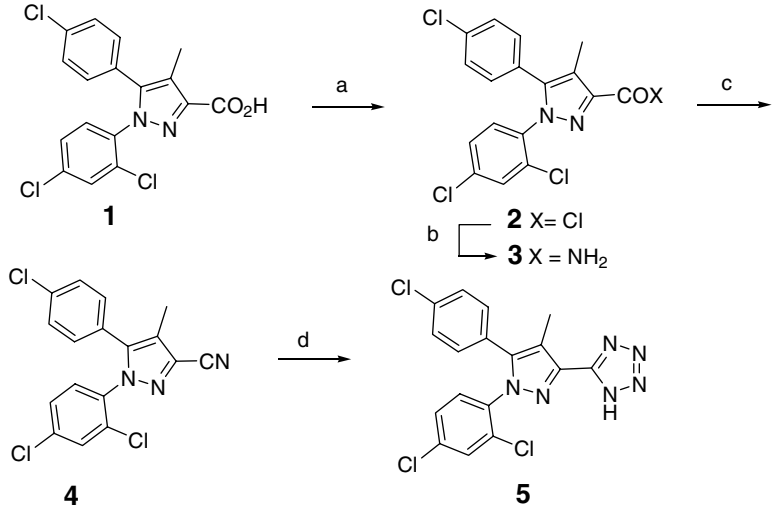
Among many heterocycles involving 'imine-type' functionality, we were particularly interested in tetrazole as a viable surrogate of amide, since modification of the amide moiety into tetrazole could impart a favourable balance of potency and physicochemical properties to allow for further in vivo efficacy evaluation. Herein, we wish to describe the design, synthesis and biological evaluation of tetrazole-biarylpyrazole analogues as novel CB1 receptor antagonists.

Synthesis of tetrazole-biarylpyrazoles commenced with the generic acid **1**.[17] Acid **1** was converted to the acyl chloride **2** with thionyl chloride, and this intermediate was then treated with ammonium hydroxide solution in methylene chloride to afford the corresponding amide **3**. Subsequently, nitrile **4** was prepared by condensation of amide **3** with phosphoryl chloride in a high yield. Treatment of nitrile **4** with sodium azide efficiently gave rise to tetrazole **5** in a [2+3]-cycloaddition fashion (Scheme 1).[18,19]

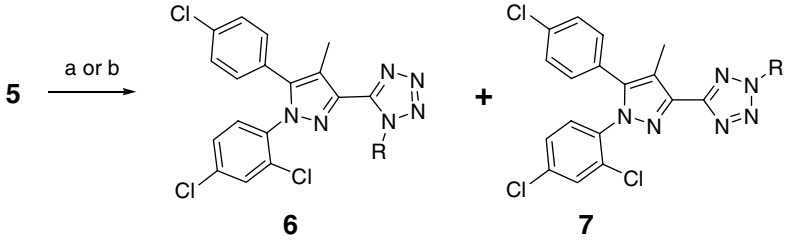
With requisite tetrazole **5** in hand, the synthesis of 2- and 3-tetrazole was studied (see Scheme 2). Tetrazole **5** reacted with alkyl bromide or iodide in the presence of potassium carbonate in DMF to yield alkyl tetrazole **6** and **7**, respectively. Tetrazoles **6** and **7** were separated by silica-gel column chromatography. Alternatively, Mitsunobu reaction conditions were utilized for alkylation of tetrazole **5** with aliphatic alcohols to produce tetrazoles **6** and **7**, respectively, as shown in Scheme 2.



Scheme 3. Reagents and conditions: (a) NH_2R, Et_3N, DMAP, CH_2Cl_2; (b) PCl_5, $PhCH_3$, 10 min; then HN_3, rt 1 day then reflux.



Scheme 1. Reagents and conditions: (a) $SOCl_2$, $PhCH_3$, reflux; (b) ammonium hydroxide solution, CH_2Cl_2, rt; (c) $POCl_3$, DMF; (d) NaN_3, NH_4Cl, DMF, microwave (180 °C, 20 min), 96% overall yield (4 steps).



Scheme 2. Reagents and conditions: (a) RX, K_2CO_3, DMF, rt or 80 °C; (b) ROH, PPh_3, DIAD, THF, 0 °C to rt.

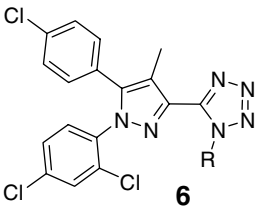
As another method, synthesis of alkyl tetrazole **6** progressed via different pathway as shown in Scheme 3.[20] Thus, treatment of acyl chloride **2** with primary, secondary and aryl amines in the presence of triethylamine in methylene chloride generated the corresponding amides **8**. Preparation of tetrazole **6** from amide **8** was accomplished efficiently by the action of hydrazoic acid and phosphorus pentachloride as shown in Scheme 3.

As depicted in Scheme 4, each regioisomer of tetrazole **6s** or **7s** was unmistakably assigned by preparation of tetrazoles **6s** with different reaction pathways as previously described, and subsequent comparison of ^{1}H NMR spectra of each sample. All other tetrazole compounds shown in Table 1 were also identified in an analogous fashion.

The inhibitions data of several key tetrazole-biarylpyrazoles for the CB1 receptor are shown in Table 1.[21] Basically, methyl tetrazole **6a** and **7a** had poor in vitro activity for CB1 receptor. But when methyl was superseded with ethyl **6b** or *n*-propyl **6c**, it showed more than fivefold increase in binding potency compared to **6a**. 1-Ethyl-1*H*-tetrazole **6b** showed approximately fivefold more potent than 2-ethyl-2*H*-tetrazole **7b**. But the relationship between relative orientation of substituents of tetrazole and in vitro binding activity became less obvious when the alkyl chain became elongated (see Table 1: **6c**, **6e**, **6g** and **6h**, or **7c**, **7e**, **7g** and **7h**). Better in vitro inhibition of binding displayed when straight alkyl chains on tetrazole than branched alkyl chains if the same number of carbons was counted (see Table 1: **6c** vs **6d** and **6e** vs **6f**, or **7c** vs **7d** and **7e** vs **7f**). Among aliphatic alkyl chains tested, the best result for alkyl substituted tetrazole was obtained when *n*-octyl was alkylated to 1*H*-tetrazole (**6h**) or 2*H*-tetrazole (**7h**). They showed good binding affinity for rat CB1R ($IC_{50} \sim 50$ nM), indicating the importance of non-polar moiety in order to optimally bind to a hydrophobic area of CB1 receptor. Interestingly, 1-cyclohexyl-1*H*-tetrazole **6s** could not be observed because of poor solubility in DMSO.

The 1-phenyl-1H-tetrazole **6i** displayed an improvement in inhibition of binding compared to the simple aliphatic chains (IC_{50} = 33.8 nM). Heteroaryl groups such as methylpyridines (**6k**, **6l**, **6m**) seemed to be tolerated for the replacement to a degree ($IC_{50} \sim 100$ nM), but none appeared more potent than the simple phenyl group. However, hydrophobic methylthiophene (**6p**) appears to restore similar level of inhibition of phenyl group for the region. The carbocycle derivatives (**6r**, **6s**, **6u**) displayed good binding inhibitions (IC_{50} = 42.3, 26.5, 26.7 nM, respectively), indicating that there might be some SAR potential for other carbocycles. The surprisingly poor result for cyclopropyl (**6q**, IC_{50} = 181 nM)

Table 1. Inhibitions of rCB1 receptor of alkyl substituted tetrazole[a,b]



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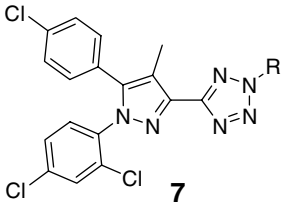

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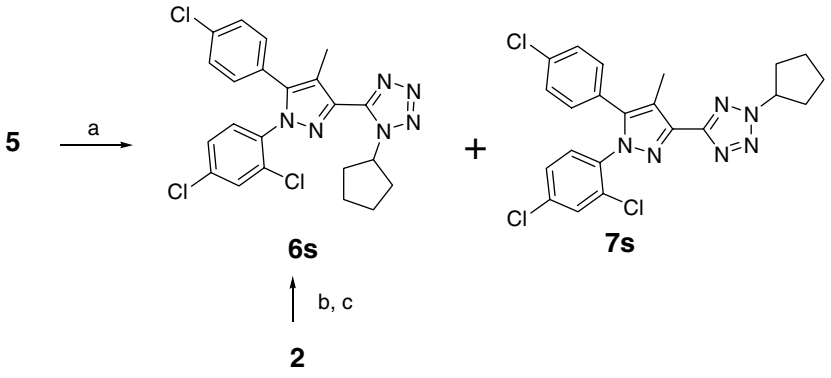
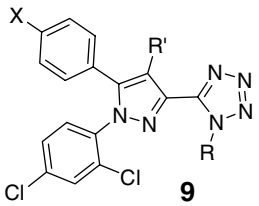
R	Compound	IC_{50}[b]	Compound	IC_{50}[b]
Me	**6a**	1060	**7a**	>1000
Et	**6b**	202	**7b**	>1000
n-Pr	**6c**	68.9	**7c**	62.1
i-Pr	**6d**	166	**7d**	101
n-Bu	**6e**	87.8	**7e**	87.7
^{t}Bu	**6f**	361		
n-Pentyl	**6g**	184	**7g**	122
n-Octyl	**6h**	47.3	**7h**	50.6
Phenyl	**6i**	33.8		
Benzyl	**6j**	94.3	**7j**	105
2-Methylpyridine	**6k**	105	**7k**	93
3-Methylpyridine	**6l**	103	**7l**	100
4-Methylpyridine	**6m**	88.2	**7m**	135
2-Methylfuran	**6n**	53.8	**7n**	93.8
3-Methylfuran	**6o**	105	**7o**	195
3-Methylthiophene	**6p**	39.8	**7p**	77.6
Cyclopropyl	**6q**	181		
Cyclobutyl	**6r**	42.3		
Cyclopentyl	**6s**	26.5	**7s**	67.9
Cyclohexyl	**6t**	Insoluble	**7t**	75.8
Cycloheptyl	**6u**	26.7		
Cyclohexylmethyl			**7v**	85.3

[a] CB1 receptor was collected from brain tissue of SD rat.
[b] These data were obtained by single determinations.



Scheme 4. Reagents and conditions: (a) cyclohexylbromide, K_2CO_3, DMF, 16% (**6s**) and 61% (**7s**); (b) cyclohexylamine, Et_3N, CH_2Cl_2, rt; (c) PCl_5, $PhCH_3$, 10 min; then HN_3, rt 1 day then reflux, 74% overall yield.

Table 2. Inhibitions of CB1 and CB2 receptors of cycloalkyl substituted tetrazole[a,b]



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R	R′	X	Compound	rCB1 IC_{50}[c]	hCB2 IC_{50}[c]
Rimonabant				4.5[d]	1760[d]
Cyclopentyl	Me	Cl	**6s**	26.5	>10,000
Cycloheptyl	Me	Cl	**6u**	26.7	818
Cyclopentyl	Me	Br	**9a**	11.6	4240
Cycloheptyl	Me	Br	**9b**	16.5	>10,000
Cyclopentyl	Et	Cl	**9c**	22.6	840
Cyclopentyl	Et	Br	**9d**	14.2	1930

[a] CB1 receptor was collected from brain tissue of SD rat.
[b] CB2 receptor was recombinant human receptor expressed in CHO cell.
[c] These data were obtained by single determinations.
[d] These data were obtained by in-house assay.

suggested that there might be a size requirement for the tetrazole alkyl region to attain good binding to the CB1 receptor. As shown in the case of cyclopentyl (**6s**, **7s**), 1-substituted 1H-tetrazoles often turned out to be more potent than 2-substituted 2H-tetrazoles.

In order to improve inhibition levels to the CB1 receptor, a structural replacement unit A to 4-bromophenyl was undertaken. In addition, extension of methyl to ethyl on pyrazole core was also studied. A structurally related series of tetrazole-biarylpyrazole derivatives was prepared in an analogous fashion previously described in Scheme 3. The inhibitions of binding of these tetrazole analogues are shown in Table 2.

With observation of inhibition activity of CB2 receptor, interesting compounds were further evaluated. The IC_{50} values were measured for the recombinant human CB2 receptor expressed in CHO cells and employing [3H]WIN-55,212-2 as a radio-ligand.[22] Replacement of 5-(4-chlorophenyl) **6s** (IC_{50} = 26.5 nM) with 5-(4-bromophenyl) **9a** (IC_{50} = 11.6 nM) improved CB1 receptor binding affinity in more than twofold. This phenomenon is clearly demonstrated by comparing **6u** (IC_{50} = 26.7 nM) versus **9b** (IC_{50} = 16.5 nM) and **9c** (IC = 22.6 nM) versus **9d** (IC_{50} = 14.2 nM), indicating the importance of bromine on the phenyl ring for the improvement of activity of CB1 receptor inhibition. Regarding CB2 receptor activity, there appeared to be no improvement as chlorines were switched to the corresponding bromides. On the other hand, extension of methyl to ethyl on pyrazole ring in this series was not as sensitive as the halogen substitution of phenyl ring for the inhibition of CB1 receptor, as exemplified by two pairs of compounds involving **6s** (IC_{50} = 26.5 nM) to **9c** (IC_{50} = 22.6 nM) and **9a** (IC_{50} = 11.6 nM) to **9d** (IC_{50} = 14.2 nM).

In conclusion, we investigated a series of tetrazole-biarylpyrazole derivatives for their inhibition of binding for cannabinoid CB1 and CB2 receptors. We have identified a novel tetrazole-based series of small molecule cannabinoid CB1 antagonists that show potency comparable to that of known CB1 antagonists. Several compounds in this series exhibited potent CB1 receptor binding affinities, validating the hypothesis that tetrazole could replace amide functionality to act as a bioisostere of amide moiety of rimonabant.

Tetrazoles substituted with alkyl chains, aryl or heteroaryl showed moderate potency for CB1R binding. So far, the best results in this series were obtained when cycloalkyl tetrazoles were attempted. Thus, cyclopentyl-tetrazole **9a** exhibited high level activity as well as good selectivity of CB1R over CB2R. Additional PK and in vivo efficacy studies in addition to further SAR studies of the novel tetrazole-based series will be the subject of future publications.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.02.061.

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