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Structure–activity relationships of *trans*-substituted-propenoic acid derivatives on the nicotinic acid receptor HCA2 (GPR109A)

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ABSTRACT

Nicotinic acid (niacin) has been used for decades as an antidyslipidemic drug in man. Its main target is the hydroxy-carboxylic acid receptor HCA2 (GPR109A), a G protein-coupled receptor. Other acids and esters such as methyl fumarate also interact with the receptor, which constituted the basis for the current study. We synthesized a novel series of substituted propenoic acids, such as fumaric acid esters, fumaric acid amides and cinnamic acid derivatives, and determined their affinities for the HCA2 receptor. We observed a rather restricted binding pocket on the receptor with *trans*-cinnamic acid being the largest planar ligand in our series with appreciable affinity for the receptor. Molecular modeling and analysis of the structure–activity relationships in the series suggest a planar *trans*-propenoic acid pharmacophore with a maximum length of 8 Å and out-of-plane orientation of the larger substituents.

Since the 1950's nicotinic acid (niacin) has been used as an antidyslipidemic drug in man. Even today nicotinic acid is the most efficacious drug to raise the levels of HDL, the 'good' cholesterol.[1] In 2003 different groups identified that the lipid-lowering actions of nicotinic acid are mediated by the G protein-coupled receptor HCA2. HCA2 is also known as GPR109A, HM74A, NIACR1 or, in mice, as PUMA-G. It is a member of a G protein-coupled receptor subfamily involved in metabolism, with HCA3 (GPR109B) and HCA1 (GPR81) as closely related members.[2–5] The HCA2 receptor is primarily expressed in adipocytes, spleen tissue, retinal pigment epithelium,[6] intestinal epithelium[7] and various immune cells such as monocytes and macrophages.[8] Unfortunately, the HCA2 expression in a type of epidermal macrophages known as Langerhans cells is the cause of flushing of the skin, a harmless but unpleasant side effect of nicotinic acid which undermines treatment compliance.[3,9]

Due to the discovery of the HCA2 receptor, industrial and academic groups have now started or intensified synthetic research lines to improve on the poor safety and pharmacokinetic properties of nicotinic acid. The majority of promising novel agonists, such as derivatives of acifran, anthranilic acids, anthranilic acid bioisosteres, xanthines, barbituric acid and pyrazole-3-carboxylic acids, was published and/or patented by GSK, Merck, Arena, Schering-Plough, Roche, Incyte, and our group.[10] Recently, 'simple' acids such as *trans*-cinnamic acid and 4-hydroxy-cinnamic acid have been described as modestly active HCA2 receptor agonists with potencies in the higher micromolar range. Cinnamic acid derivatives had been described before as anti-inflammatory compounds and as suppressors of elevated blood lipid levels in atherosclerosis.[11–13]

Some other simple acid derivatives, that is, methyl fumarate and ethyl fumarate, were also reported as potent agonists for the HCA2 receptor.[14] These fumarates have long been known as antipsoriasis compounds.[15] They are micromolar affinity agonists for the HCA2 receptor, but have not been extensively explored in a synthetic structure–activity approach. Therefore, we decided to investigate the medicinal chemistry of such fumaric and cinnamic acid derivatives in more detail. These constrained propenoic acid derivatives appeared also useful in a pharmacophore analysis that we performed.

The fumaric acid esters **2** and **3** (Table 1) were commercially available. Compounds **4**–**24** (Table 1 and Supplementary data) were synthesized according to two methods; (A) starting from fumaric acid (**1**), the appropriate alcohol and EDC dissolved in DMF.[16] (B) starting from a mixture of fumaric acid (**1**) and the suitable alcohol dissolved in DMF under microwave conditions.[17] Method A resulted in a mixture of both *trans* (**4**, **9–11**, **13**) and *cis* isomers of the desired esters, even if the reaction was carried out at 0 °C. Due to the difficult separation of the two isomers this method was eventually not preferred. According to method B, described by Aver'yanov,[17] an equimolar mixture of **1** and the appropriate alcohol in DMF was heated in a sealed tube in the microwave at 180 °C. This method resulted solely in the desired *trans* substituted fumarates (**5–8**, **12**, **14–24**). With both methods also a substantial amount of the disubstituted fumarates was formed.

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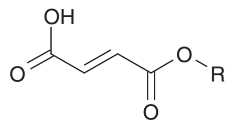
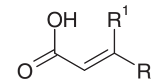
Table 1
Affinities of substituted fumaric acid esters **1**–**24** in radioligand binding assays of the human HCA2 receptor



Compound	R	K_i (μM) or % disp.[a]
1	H	10%
2	Me	0.18 ± 0.03
3	Et	0.41 ± 0.02
4	Pr	1.0 ± 0.1
5	*i*Pr	4.2 ± 0.9
6	Bu	0.76 ± 0.19
7	Pe	0.70 ± 0.05
8	Hex	2.5 ± 0.03
9	cHex	17%
10	Phenyl	10%
11	Benzyl	3.5 ± 0.2
12	Benzyl-α-methyl rac.	5.7 ± 0.1
13	Phenyl ethyl	10 ± 1
14	Phenyl propyl	26%
15	2-Br benzyl	0%
16	2-OMe benzyl	5%
17	3-Br benzyl	9.8 ± 0.6
18	3-Cl benzyl	8.9 ± 1.5
19	3-F benzyl	2.4 ± 0.6
20	3-OMe benzyl	4%
21	4-Br benzyl	21%
22	4-Cl benzyl	14%
23	4-Me benzyl	30%
24	4-OMe benzyl	7%

[a] K_i ± SEM (n = 3), % displacement at 10 μM (average of n = 2, with less than 10% difference between the two values). K_i values were determined in full displacement studies on membranes from HEK293T cells stably expressing HCA2 (GPR109A), using [^{3}H]-nicotinic acid as the radioligand. Single point displacement assays were carried out using 10 μM of the test compound and 20 nM [^{3}H]-nicotinic acid.

Table 2
Affinities of *trans*-substituted-propenoic acids **25**–**58** in radioligand binding assays on the human HCA2 receptor



Compound	R	R[1]	Ki (μM) or % disp.[a]
25	Me	H	6%
26	Et	H	19%
27	Me	Me	0%
28	Phenyl	H	4.9 ± 1.8
29	2-OH phenyl	H	4%
30	2-Me phenyl	H	0%
31	3-OH phenyl	H	0%
32	3-Me phenyl	H	0%
33	3-Cl phenyl	H	6%
34	3-NO_2 phenyl	H	0%
35	4-OH phenyl	H	14 ± 2
36	4-Me phenyl	H	2%
37	4-Cl phenyl	H	17%
38	4-OMe phenyl	H	0%
39	4-NH_2 phenyl	H	2%
40	4-$N(CH_3)_2$ phenyl	H	0%
41	3,4-di-OH phenyl	H	4%
42	3-OMe, 4-OH phenyl	H	0%
43	3,4-OCH_2O– phenyl	H	7%
44	Phenyl	Me	1%
45	Phenyl	Phenyl	0%
46	Phenyl	NHCOMe	0%
48	Pyridin-3-yl	H	3%
49	Pyrrol-2-yl	H	7%
50	Furan-3-yl	H	14 ± 2.5
51	Furan-2-yl	H	8.1 ± 0.8
52	5-Br-furan-2-yl	H	9%
53	5-Me-furan-2-yl	H	7%
54	5-Et-furan-2-yl	H	14%
55	5-(4-Cl-Ph)-furan-2-yl	H	0%
56	Thiophen-2-yl	H	5.5 ± 0.3
57	3-Br-thiophen-2-yl	H	6%
58	4-Br-thiophen-2-yl	H	6%

[a] See footnote Table 1.

The propenoic acids with aromatic rings (**49, 52–54, 57** and **58**) that were not commercially available were prepared in a 32–74% yield (Table 2 and Supplementary data), catalyzed by piperidine via the Knoevenagel condensation of the commercially available aromatic aldehydes and malonic acid (**47**).[18]

All compounds listed in Tables 1 and 2 were tested at 10 μM in radioligand binding assays for displacement of [^{3}H]-nicotinic acid (20 nM) from the human HCA2 receptor stably expressed in HEK293 cells. Homologous displacement with unlabeled nicotinic acid yielded a K_i value of 64 nM for nicotinic acid (data not shown). Both the methyl and ethyl fumarates (**2** and **3**) also displayed submicromolar affinities for the HCA2 receptor (Table 1), comparable to data reported by Tang.[14] In comparison with the reference agonist nicotinic acid, only a three-fold (methyl derivative) or seven-fold (ethyl derivative) lower affinity was obtained. The unsubstituted fumaric acid (**1**) did not display any appreciable affinity towards the receptor, suggesting that the intact ester is crucial for receptor activity. In a series of aliphatic fumarate esters, increasing size did not substantially affect the receptor affinity. The propyl, butyl and pentyl substituents (**4, 6** and **7**) showed affinities between 0.7 and 1.0 μM, which is in the same range as the ethyl derivative. The larger hexyl substituent (**8**) resulted in a slightly poorer K_i value of 2.5 μM. Branched aliphatic compounds were also less tolerated, for example, derivatives **5** and **9**. A phenyl substituent (**10**) was not well tolerated either, but introduction of a spacer between the fumarate moiety and the aromatic system resulted in a gain of affinity. The methylene spacer, as in **11** (K_i = 3.5 μM), appeared to be optimal since α-methylbenzyl (**12**), phenylethyl (**13**) and phenylpropyl (**14**) substituents resulted in K_i values of 5.7 μM and 10 μM, and 26% of radioligand displacement at 10 μM, respectively. Subsequently, various additional substitutions of the benzylic ring system were explored. The binding pocket of the HCA2 receptor was not able to accommodate the *ortho* substituted compounds **15** and **16** at a concentration of 10 μM. *meta* Substitution, on the other hand, was better tolerated (**17–20**). The 3-bromo- and 3-chloro-benzyl derivatives (**17** and **18**) showed a slight decrease in affinity and the smaller 3-fluoro-benzyl compound (**19**) a slight increase in affinity with respect to the unsubstituted benzyl fumarate. On the contrary the 3-methoxy-benzyl derivative **20** showed no affinity for the receptor. Furthermore, introduction of *para* substituents such as halogen, methyl or methoxy (**21–24**) resulted in a reduced affinity.

Since the ester moiety in methyl and ethyl fumarate can be hydrolyzed in vivo,[19] we investigated the non-hydrolysable amide linker as an alternative. However, these *trans* amide isosteres of compounds **2, 3, 10** and **11** were not able to bind to the receptor at 10 μM (data not shown).

To further explore the SAR, a number of *cis* analogs of the active *trans* fumaric acid derivatives were synthesized and tested, namely the maleic acid esters and maleic acid amides. None of these *cis* compounds interacted with the receptor (data not shown).

Next, a series of *trans*-substituted-propenoic acids (**25–46, 48–58**) were tested for their affinities (Table 2). The small aliphatic compounds **25–27** were without effect, while the phenyl derivative **28** (cinnamic acid) showed micromolar affinity. In our assay, compound **28** showed a higher affinity (K_i = 4.9 μM) compared to the K_i value of 36 μM reported by Ren et al.[20] To explore this lead,

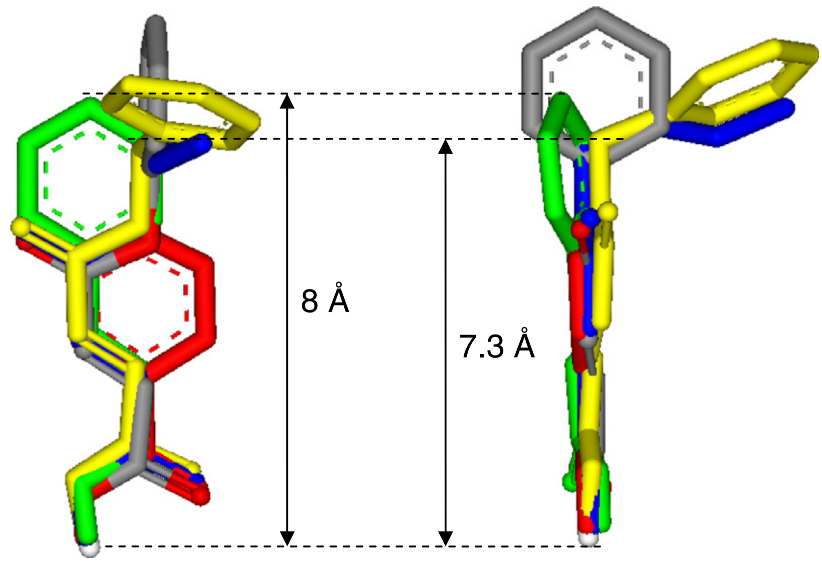


Figure 1. Aligned pharmacophore model (left—nicotinic acid in plane, right—nicotinic acid 90° rotated) constructed of the active compounds nicotinic acid, **6**, **11** and **28** and the inactive compound **10**. Red: nicotinic acid, green: cinnamic acid (**28**), gray: phenyl fumarate (**10**), blue: butyl fumarate (**6**), yellow: benzyl fumarate (**11**).

commercially available aromatic substituted *trans* propenoic acids were tested for their affinities (**29–43**). Only the 4-hydroxy derivative **35** was able to bind with an affinity of 14 μM. Related substituents such as 4-methoxy (**38**) and 4-amino (**39**) decreased the affinity dramatically. In general, except for the 4-hydroxy group, aromatic substitution is not tolerated on the *ortho*, *meta* or *para* position. Also substituents at the β-position of cinnamic acid (**44–46**) resulted in a dramatic decrease in binding. Replacement of the phenyl moiety in cinnamic acid by aromatic isosteres such as 3-pyridinyl (**48**) and 2-pyrrole (**49**) resulted in a significant loss of affinity. On the contrary, 2-furanyl (**51**) and 2-thiophenyl (**56**) were accommodated like the phenyl compound. 3-Furanyl substitution (**50**) resulted in a two-fold decrease compared to the 2-furanyl derivative **51**. As in the cinnamic acid series, the 5-substituted 2-furanyl derivatives (**52–55**) and both the 3-bromo and 4-bromo-substituted 2-thiophenyl derivatives (**57, 58**) were devoid of affinity for the receptor.

To visualize the SAR, a pharmacophore model was generated by manually superimposing the minimized structures of: nicotinic acid, cinnamic acid **28**, fumaric acid esters **6** and **11**, and the inactive phenylfumaric acid ester **10** (Fig. 1). The alignment of the two sp^2 carbons of the propenoic fragment, which all the compounds have in common, resulted in a planar and constrained pharmacophore. The carbonyl oxygen of the ester function of compounds **6, 10** and **11** and the nitrogen of nicotinic acid overlay smoothly as a hydrogen acceptor region. This might explain the improved binding characteristics of the fumaric acid esters compared to cinnamic acid and also why the *trans* configuration is superior over the *cis* substituted propenoic acids. Molecular modeling and analysis of the structure–activity relationships in the series suggest a planar *trans*-propenoic acid pharmacophore with a maximum length of 8 Å, because this is the size of the largest planar ligand (**28**) in our series with appreciable affinity for the receptor. Larger compounds need an out-of-plane orientation as in the case of the fumaric acid ester series (**2–24**).

Molecular modeling studies of the Merck Research group based on anthranilic acid derivatives confirmed the importance of the planar orientation of the carboxylic acid function and the nearby α,β sp^2 carbon atoms.[21,22] Full saturation of the phenyl ring in anthranilic acid resulted in inactive compounds. If the double bond in the α,β-position was maintained, as in tetrahydro-anthranilic acids, the planar orientation and also the affinity was regained however.[22]

In conclusion, methyl fumarate, ethyl fumarate and cinnamic acid have been published as agonists for the HCA2 receptor.[14,20] Our synthetic program confirmed the affinity of these compounds for the HCA2 receptor and further explored the structure–activity relationships for a series of derivatives. Molecular modeling studies and the analysis of the structure–activity relationships in the series suggest a planar *trans*-propenoic acid pharmacophore with a maximum length of 8 Å and out-of-plane orientation of the larger substituents

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Supplementary data

Supplementary data (reaction schemes and experimental data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.091.

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