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Synthesis and evaluation of *N*-[(1*S*,2*S*)-3-(4-chlorophenyl)-2-(3-cyanophenyl)-1-methylpropyl]-2-methyl-2-aminopropanamide as human cannabinoid-1 receptor (CB1R) inverse agonists

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ABSTRACT

Obesity is a chronic medical condition that is affecting large population throughout the world. CB1 as a target for treatment of obesity has been under intensive studies. Taranabant was discovered and then developed by Merck as the 1st generation CB1R inverse agonist. Reported here is part of our effort on the 2nd generation of CB1R inverse agonist from the acyclic amide scaffold. We replaced the oxygen linker in taranabant with nitrogen and prepared a series of amino heterocyclic analogs through a divergent synthesis. Although in general, the amine linker gave reduced binding affinity, potent and selective CB1R inverse agonist was identified from the amino heterocycle series. Molecular modeling was applied to study the binding of the amino heterocycle series at CB1 binding site. The in vitro metabolism of representative members was studied and only trace glucuronidation was found. Thus, it suggests that the right hand side of the molecule may not be the appropriate site for glucuronidation.

Characterized by excessive body weight and fat, obesity is a chronic medical condition that is affecting people throughout the world. It is often the cause of further serious medical complications including insulin resistance, Type 2 diabetes, cardiovascular disease (CVD), stroke, hypertension, cancer and arthritis.[1] There are two FDA-approved drugs for chronic treatment of obesity with modest efficacy: the sympathomimetic agent, sibutramine, and the lipase inhibitor, orlistat. More efficacious and diverse treatments for obesity and weight maintenance are highly desired.

It has been established that the cannabinoid system plays a role in regulating feeding behavior and body weight.[2] There are two types of cannabinoid receptors: cannabinoid-1 receptor (CB1R) and cannabinoid-2 receptor (CB2R). Among the pharmaceutical activities of agonists of CB1R are enhanced food intake and increased body weight. Conversely, antagonists/inverse agonists suppress food intake and reduce body weight.[3] Thus the CB1R has been an intensively studied target for development of new anti-obesity agents. Rimonabant (SR141716, **1**)[4] was developed by Sanofi-Aventis and had been approved in Europe as a new treatment for obesity, but was withdrawn later due to psychiatric side effects. After extensive studies on various scaffolds as CB1R inverse agonists,[5] taranabant (MK-0364, **2,** Fig. 1)[6] was discovered by Merck scientists and was then advanced into development and completed Phase III clinic trials.[7]

Our efforts on second generation CB1R inverse agonists focused on discovering compounds that maintained a similar CB1R profile to MK-0364 but exhibited a more balanced metabolism. Thus, it would be less dependent on CYP3A4 mediated clearance than MK-0364. By introducing a suitable functional group for direct conjugation, for example, we could minimize the potential for DDI's.[7b,c] It has been shown that in addition to carboxylic acids, some heterocycles can also form glucuronide adducts.[8] Thus, one strategy was to replace the trifluoromethylpyridyloxy moiety in **2** with an amino heterocycle moiety as shown in structure **3**. Either the *exo*-cyclic nitrogen or the heterocyclic nitrogen may be the site for glucuronidation. This is depending on the steric and electronic factors specific to the heterocycle. The *exo*-cyclic nitrogen is sterically hindered due to the adjacent *gem*-dimethyl group and also less nucleophilic due to conjugation with the heterocycle. Thus, the heterocycle nitrogen may have better chance to be the site for glucuronidation.

To evaluate the amine linker versus an oxygen linker, compound **3a** was synthesized (Scheme 1) for direct comparison with compound **2**. Amino acid **4** was reacted with 2-chloro-5-trifluoromethylpyridine **5** in the presence of 2 equiv of DBU to give

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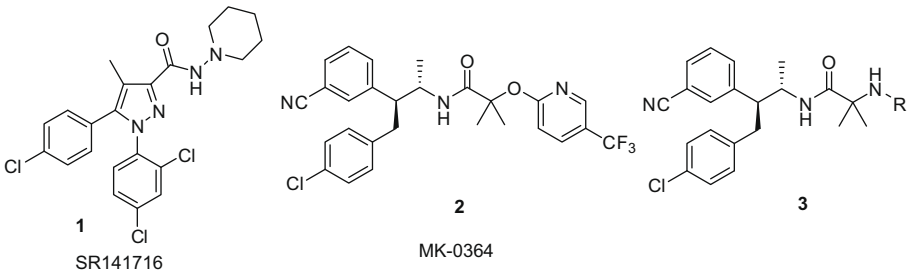
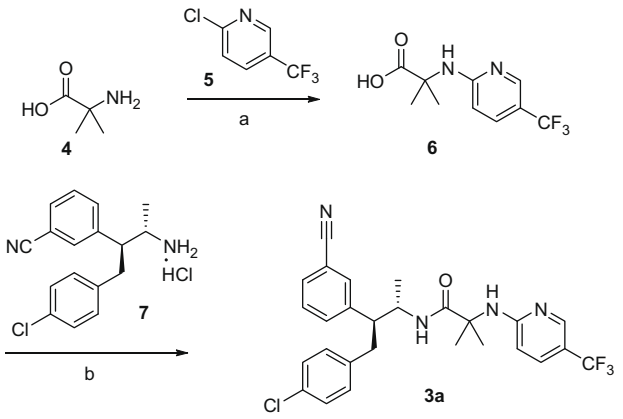


Figure 1.



Scheme 1. Reagents and conditions: (a) DBU, dimethylsulfoxide, 150 °C, 44%; (b) *N*-methylmorpholine, PyBop, 59%.

aminopyridine **6** in 44% yield. The acid was then coupled with amine **7** to give compound **3a**, which was less active than **2** but still quite potent with an IC_{50} of 7.5 nM against CB1R. Encouraged by this result, more compounds from the amino heterocycle series were explored.

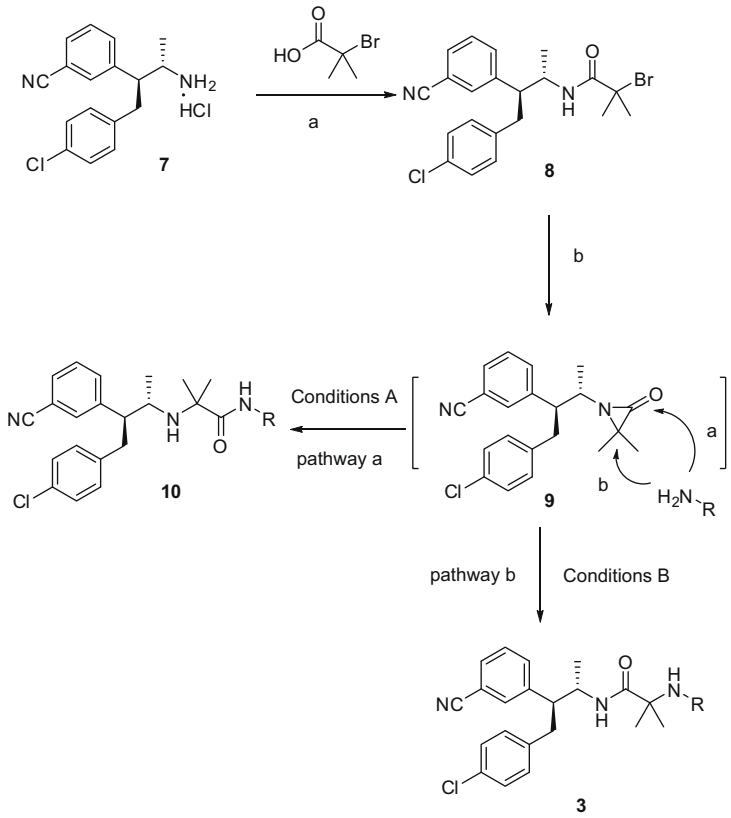
To prepare more amino heterocycle analogs, a more efficient divergent synthesis was then developed as shown in Scheme 2. Amine **7** was coupled with 2-bromo-2,2-dimethylacetic acid to yield 2-bromo acetamide **8**. This compound was used as a common intermediate for other amino heterocycle analogs. To validate this strategy, compound **3a** was synthesized by this method. 2-Amino-5-trifluoromethyl pyridine was first treated with NaH, followed by compound **8** (conditions A). The reaction, however, gave a reversed amide product **10a** in 26% yield. A mechanism for the formation of **10a** is outlined in Scheme 2. In the presence of a strong base, the amide N–H was first deprotonated. The subsequent intramolecular N-alkylation gave an aziridine lactam intermediate **9,** which can be opened by a nucleophile through either pathway *a* or *b*. Similar aziridine lactam formation has been reported in the literature.[9] Usually a 'hard' nucleophile takes pathway *a* to give the reversed amide **10** while a 'soft' nucleophile takes pathway *b* to give the desired product **3**. A solution of 2-amino-5-trifluoromethylpyridine and **8** in CH_2Cl_2 was treated with 50% aqueous NaOH (conditions B). Upon completion, the reaction afforded the desired isomer **3a** in 37% yield. These conditions B were then applied to other amino-heterocycles and yielded compounds **3b–3p** in 20–70% yields.

The inhibition of the human CB1 and CB2 receptors by compounds **2**, **3** and **10** are shown in Table 1. Compounds **3** are still selective for CB1 receptor with respect to CB2 receptor. However, in terms of CB1R binding affinity, compound **3a** (entry 2) is 25-fold less active than compound **2** with the simple change from oxygen to nitrogen. Meanwhile, such replacement resulted in reduced CB1/CB2 selectivity. CB2R is known to play a role in pain perception while its role in weight control is not well understood. It is desirable to maintain a high selectivity for CB1R when obesity treatment is concerned to avoid possible complication in future development. Chloropyridines **3b** and **3c** are slightly less active in binding than **3a**. Comparing **3a** with **3c** indicates that *o*-substitution may result in reduced binding affinity. Moving the CF_3 group from 5-position to 4-position resulted in fivefold improvement to give highly potent compound **3d** (entry 5) that showed an excellent IC_{50} of 1.6 nM. The CB1/CB2 selectivity is also significantly improved. Apparently, the lipophilic CF_3 is important for binding affinity: as shown in **3e**, changing CF_3 to a less lipophilic CH_3 resulted in an IC_{50} of 37.7 nM, which is 24-fold less active than **3d**. Further reduction of the local lipophilicity by removing the methyl group afforded even lower binding activity as shown by **3f**. Changing pyridine to pyrimidine gave no improvement for binding to CB1 receptor but the CB1/CB2 selectivity was significantly improved as showed by **3g**.

In addition to six-membered ring heterocycles, some five-membered heterocycles were also tested. As showed in entries 9 and 10, isoxazoles **3h** and **3i** gave similar IC_{50} of 46.1 nM and 47.7 nM, respectively. Following similar trends observed in the pyridine series, an additional methyl group in **3j** resulted in a twofold improvement in binding. Increasing local lipophilicity by introducing a *t*-butyl group resulted in further improved binding activity: 5-*t*-butyl oxazole **3k** showed an IC_{50} of 8.6 nM. 5-Phenylisoxazole **3l**, however, gave a much lower binding activity of 186 nM. This suggests that there may be some steric limitation in this part of binding pocket. 4-Methylpyrazole **3m** and *N*-methylpyrazole **3n** both gave reduced binding affinity. Two fused heterocycles were also tested and a similar trend was observed. *N*-Methylbenzimidazole **3o** (entry 16) gave an IC_{50} of 97 nM, twofold improvement compared to **3n** that has a similar *N*-methyl group. Isoquinoline **3p** (entry 17) gave an IC_{50} of 10 nM, more than threefold of improvement compared to methylpyridine **3e**. An amino alkyl group was tested to replace the heterocycle moiety. As shown in entry 18, compound **3r** that contains a dimethylaminopropyl group lost much of the CB1 activity.

Interestingly, the structurally distinct reversed amides **10a** and **10l** also showed binding activity for CB1 receptor and were selective for CB1 with respect to CB2. Compound **10a** gave an IC_{50} of 132 nM, 17-fold less active than **3a**. The isoxazole **10l** showed an IC_{50} of 136 nM that was slightly more active than its counterpart **3l**, and a higher CB1/CB2 selectivity than **3l**. On the other hand, the simple switch of the *gem*-dimethyl group and the carbonyl group had a big impact on CYP activity. Compound **3a** afforded an IC_{50} = 2.03 μM for CYP 3A4 while compound **10a** exhibited an IC_{50} > 35 μM for CYP 3A4. With the advantage of significantly improved CYP activity, the reversed amide **10** may be a lead for the future development of new CB1R agents.

Some of the compounds that showed good binding affinity were tested for their functional activity by measuring the forskolin induced intra-cellular cAMP level. The results are shown in Table 2.



Scheme 2. Reagents and conditions: (a) Et_3N, EDCI, 94%; (b) conditions A: NaH, THF; conditions B: 50% aqueous NaOH, Bu_4NBr, CH_2Cl_2, 20–70%.

These four compounds from the amino heterocycle series also behaved as inverse agonists and showed functional activities that were better or comparable to that of taranabant **2.** Compound **3a** showed an EC_{50} of 3.2 nM. Compound **3d** that showed the best binding affinity also gave the best functional activity: an EC_{50} of 0.9 nM, 2.5-fold better than that of **2**. Compounds **3k** and **3p** gave EC_{50} of 3.5 nM and 1.9 nM, respectively. Thus, the amino heterocycle compounds are still potent CB1R inverse agonists.

For further understanding of the binding of the aminoheterocycle series, a modeling study of **3a** was pursued. A bound conformation of **2** was established by overlaying representative conformers of **2** with rimonabant. The active conformation of compound **3a** overlays well with that of **2**. However, **3a** requires more energy (0.54 kcal/mol) than **2** to convert the lowest energy conformation to active conformation. This is probably because that the NH moiety in **3a** tends to be coplanar with the pyridine moiety. Thus, it requires extra energy to break this conjugation to form the active conformation in which the NH moiety is not coplanar with the pyridine moiety. The computational and docking studies of **2** (MK-0364) with a CB1R homology model was recently reported.[11] Same homology model was applied here and binding mode is shown in Figure 2. It was found that amino acid residue Lys192 is close to the NH moiety of compound **3a**. This may destabilize the binding of **3a** due to disfavored positive–positive electrostatic interaction between the two amine moieties. That the amino moiety of **3a** may have disfavorable interaction on the binding site is also supported by that the calculated binding energy for compound **3a** is 5.9 kcal/mol higher than that of MK-0364. Thus, the decreased binding affinity of **3a** may be due to a less favored effect of the amino moiety on conformation and with the receptor binding site.

To determine whether compounds from the amino heterocycles series are capable of forming glucuronide adducts, several compounds were incubated with rat and human liver microsomes[12] in the presence of UDPGA and analyzed by LC–MS/MS for the corresponding glucuronide.[13] The results are shown in Table 3. The aminopyridine series compounds afforded only trace amount of glucuronide in human liver microsomes. The aminoxazole compound formed slightly more glucuronide in both rat and human liver microsomes but the amount was still minor based on LC–MS. Compounds from the pyrimidine series were not tested in the liver microsomal studies due to their low CB1R binding affinity. In general, two criteria have to be met in order for a molecule to form a glucuronide adduct. First, it must have a functional group that is chemically capable of forming a glucuronide that is reasonably stable. Second, this functional group must be in an appropriate position in the molecule where allows the formation of the glucuronide. The latter criteria relates to the compound's affinity for the enzymes which catalyze glucuronidation (UGT's). Pyridine is known to form glucuronides, but compound **3f** and other heterocycles failed to form glucuronides in a meaningful amount. This suggests that the right hand side of **3** may not be a favorable region for glucuronidation.

The metabolism of compound **3a** was also studied in both rat and human cryopreserved hepatocytes. Similar to compound **2**, the aminopyridine **3a** is still metabolized through oxidative pathway by formation of metabolite with molecular weight of M+16, which then forms the glucuronide adduct. The direct glucuronide adduct of **3a** was not observed in either study.

In conclusion, we developed a divergent synthesis of acyclic 2-*N*-heterocyclicamino acetamide as human cannabinoid-1 receptor (CB1R) inverse agonists. This method provided an efficient synthesis of a variety of aminoheterocycle analogs of this family. Although in general, replacing the oxygen in taranabant (**2**) with nitrogen resulted in lower binding affinity, potent CB1R inverse agonists were still identified from the aminoheterocycle series. The most active compound from this series is more potent than **2**

Table 1
The CB1 and CB2 binding affinity of compounds **2, 3** and **10**[10]

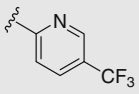
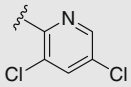
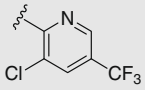
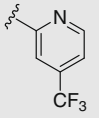
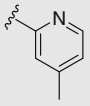
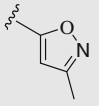
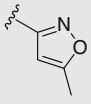
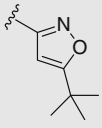
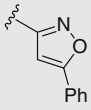
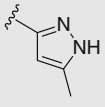
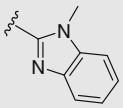
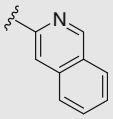
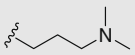
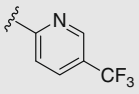
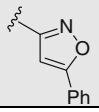
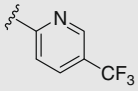
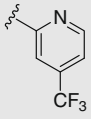
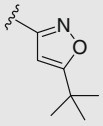
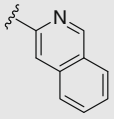
Entry	Compound	R	CB1 IC_{50}[a] (nM)	CB2 IC_{50}[a] (nM)	Selectivity CB2/CB1
1	**2**		0.3	285	950
2	**3a**		7.5	322	43
3	**3b**		12.2	699	57
4	**3c**		17.9	419	23
5	**3d**		1.6	353	221
6	**3e**		37.7	547	15
7	**3f**		49	1830	37
8	**3g**		49.2	14020	286
9	**3h**		46.1	3863	84
10	**3i**		47.7	3213	67
11	**3j**		19.5	5158	265
12	**3k**		8.6	254	30
13	**3l**		186	234	1.3
14	**3m**		301	6113	20
15	**3n**		203	13290	65

Table 1 (*continued*)

Entry	Compound	R	CB1 IC_{50}[a] (nM)	CB2 IC_{50}[a] (nM)	Selectivity CB2/CB1
16	**3o**		97	1414	15
17	**3p**		10.1	117	12
18	**3r**		2982	25890	8.7
19	**10a**		132	1721	13
20	**10l**		136	2312	17

[a] Binding affinity determined by inhibition of binding of [^{3}H]CP-55940 to recombinant human CB1 or CB2 receptors expressed on Chinese Hasmster Ovary (CHO) cells. ($n = 2$).

Table 2
The CB1 functional activity of **2** and selected compounds **3**[10]

Entry	Compound	R	CB1 EC_{50}[a] (nM)
1	**2**		2.4
2	**3a**		3.2
3	**3d**		0.9
4	**3k**		3.5
5	**3p**		1.9

[a] Functional activity was determined by measuring the cAMP level using adenylyl cyclase activation flash plate assay following the procedure recommended by the manufacture ($n = 2$).

in functional activity assay. Computational studies of **3a** provided some explanations for the reduced binding affinity caused by the simple change from oxygen to nitrogen. Some representative aminoheterocycle compounds were tested in rat and human liver microsomal incubation, where it was found that glucuronides were only formed in trace amounts. Thus it suggested that the right hand side of the molecule may not be an appropriate site to introduce phase II metabolism. Further studies on the 2nd generation of

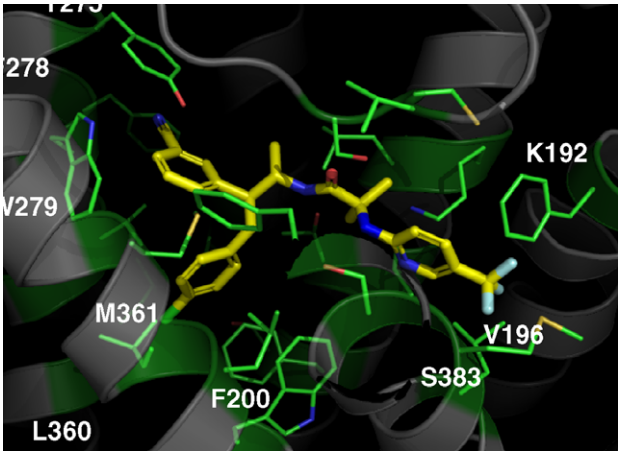


Figure 2. The binding mode of **3a** in the CB1 homology model.

Table 3
Glucuronide formation studies of **3d, 3f, 3g** in rat and human liver microsomes

Entry	Compounds	In rat liver microsome	In human liver microsome
1	**3d**	Not observed	Trace
2	**3f**	Not observed	Trace
3	**3g**	Trace	Trace

CB1R inverse agonist from the acyclic amide scaffold will be reported in due courses.

Acknowledgment

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References and notes

1. Olshansky, S. J.; Passaro, D. J.; Hershow, R. C.; Layden, J.; Carnes, B. A.; Brody, J.; Hayflick, L.; Butler, R. N.; Allison, D. B.; Ludwig, D. S. *N. Engl. J. Med.* **2005**, *352*, 1138.
2. Vickers, S. P.; Kennett, G. A. *Curr. Drug Target* **2005**, *6*, 215.
3. Cota, D. *Diabetes Metab. Res. Rev.* **2007**, *23*, 507.
4. Xie, S.; Furjanic, M. A.; Ferrara, J. J.; McAndrew, N. R.; Ardino, E. L.; Ngondara, A.; Bernstein, Y.; Thomas, K. J.; Kim, E.; Walker, J. M.; Nagar, S.; Ward, S. J.; Raffa, R. B. *J. Clin. Pharm. Ther.* **2007**, *32*, 209.
5. (a) Armstrong, H. E.; Galka, A.; Lin, L. S.; Lanza, T. J.; Jewell, J. P.; Shah, S. K.; Guthikonda, R.; Truong, Q.; Chang, L. L.; Quaker, G.; Colandrea, V. J.; Tong, X.; Wang, J.; Xu, S.; Fong, T. M.; Shen, C.-P.; Lao, J.; Chen, J.; Shearman, L. P.; Stribling, D. S.; Rosko, K.; Strack, A.; Ha, S.; Van der Ploeg, L.; Goulet, M. T.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2184; (b) Debenham, J. S.; Madsen-Duggan, C. B.; Walsh, T. F.; Wang, J.; Tong, X.; Doss, G. A.; Lao, J.; Fong, T. M.; Schaeffer, M.-T.; Xiao, J. C.; Huang, C. R.; Shen, C.-P.; Feng, Y.; Marsh, D. J.; Stribling, D. S.; Shearman, L. P.; Strack, A. M.; MacIntyre, D. E.; Van der Ploeg, L. H. T.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 681; (c) Plummer, C. W.; Finke, P. E.; Mills, S. G.; Wang, J.; Tong, X.; Doss, G. A.; Fong, T. M.; Lao, J. Z.; Schaeffer, M.-T.; Chen, J.; Shen, C.-P.; Stribling, D. S.; Shearman, L. P.; Strack, A. M.; Van der Ploeg, L. H. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1441.
6. (a) Hagmann, W. K. *Arch. Pharm.* **2008**, *341*, 405; (b) Lin, L. S.; Lanza, T. J.; Jewell, J. P.; Liu, P.; Shah, S. K.; Qi, H.; Tong, X.; Wang, J.; Xu, S. S.; Fong, T. M.; Shen, C.-P.; Lao, J.; Xiao, J. C.; Shearman, L. P.; Stribling, D. S.; Rosko, K.; Strack, A.; Marsh, D. J.; Feng, Y.; Kumar, S.; Samuel, K.; Yin, W.; Van der Ploeg, L. H. T.; Goulet, M. T.; Hagmann, W. K. *J. Med. Chem.* **2006**, *49*, 7584; (c) Liu, P.; Lin, L. S.; Hamill, T. G.; Jewell, J. P.; Lanza, T. J.; Gibson, R. E.; Krause, S. M.; Ryan, C.; Eng, W.; Sanabria, S.; Tong, X.; Wang, J.; Levorse, D. A.; Owens, K. A.; Fong, T. M.; Shen, C.-P.; Lao, J.; Kumar, S.; Yin, W.; Payack, J. F.; Springfield, S. A.; Hargreaves, R.; Burns, H. D.; Goulet, M. T.; Hagmann, W. K. *J. Med. Chem.* **2007**, *50*, 3427.
7. (a) Fremming, B. A.; Boyd, S. T. *Curr. Opin. Invest. Drugs* **2008**, *9*, 1116; (b) Addy, C.; Rothenberg, P.; Li, S.; Majumdar, A.; Agrawal, N.; Li, H.; Zhong, L.; Yuan, J.; Maes, A.; Dunbar, S.; Cote, J.; Rosko, K.; Van Dyck, K.; De Lepeleire, I.; de Hoon, J.; Van Hecken, A.; Depre, M.; Knops, A.; Gottesdiener, K.; Stoch, A.; Wagner, J. *J. Clin. Pharmacol.* **2008**, *48*, 734; (c) Addy, C.; Li, S.; Agrawal, N.; Stone, J.; Majumdar, A.; Zhong, L.; Li, H.; Yuan, J.; Maes, A.; Rothenberg, P.; Cote, J.; Rosko, K.; Cummings, C.; Warrington, S.; Boyce, M.; Gottesdiener, K.; Stoch, A.; Wagner, J. *J. Clin. Pharmacol.* **2008**, *48*, 418; (d) Addy, C.; Wright, H.; Van Laere, K.; Gantz, I.; Erondu, N.; Musser, B. J.; Lu, K.; Yuan, J.; Sanabria-Bohorquez, S. M.; Stoch, A.; Stevens, C.; Fong, T. M.; De Lepeleire, I.; Cilissen, C.; Cote, J.; Rosko, K.; Gendrano, I. N., III; Nguyen, A. M.; Gumbiner, B.; Rothenberg, P.; de Hoon, J.; Bormans, G.; Depre, M.; Eng, W.; Ravussin, E.; Klein, S.; Blundell, J.; Herman, G. A.; Burns, H. D.; Hargreaves, R. J.; Wagner, J.; Gottesdiener, K.; Amatruda, J. M.; Heymsfield, S. B. *Cell Metab.* **2008**, *7*, 68; (e) Cole, P.; Serradell, N.; Rosa, E.; Bolos, J. *Drugs Future* **2008**, *33*, 206; (f) Merck discontinued development of Taranabant as announced on October 2nd, 2008.
8. (a) Chen, G.; Dellinger, R. W.; Sun, D.; Spratt, T. E.; Lazarus, P. *Drug Metab. Dispos.* **2008**, *36*, 824; (b) Klieber, S.; Hugla, S.; Ngo, R.; Arabeyre-Fabre, C.; Meunier, V.; Sadoun, F.; Fedeli, O.; Rival, M.; Bourrie, M.; Guillou, F.; Maurel, P.; Fabre, G. *Drug Metab. Disp.* **2008**, *36*, 851; (c) Yan, Z.; Caldwell, G. W.; Gauthier, D.; Leo, G. C.; Mei, J.; Ho, C. Y.; Jones, W. J.; Masucci, J. A.; Tuman, R. W.; Galemmo, R. A., Jr.; Johnson, D. L. *Drug Metab. Disp.* **2006**, *34*, 748.
9. Guziec, F. S. J.; Torres, F. F. *J. Org. Chem.* **1993**, *58*, 1604.
10. Shen, C.-P.; Xiao, C. J.; Amstrong, H.; Hagmann, W. K.; Fong, T. M. *Eur. J. Pharmacol* **2006**, *531*, 41.
11. Lin, L. S.; Ha, S.; Ball, R. G.; Tsou, N. N.; Castonguay, L. A.; Doss, G. A.; Fong, T. M.; Shen, C.-P.; Xiao, J. C.; Goulet, M. T.; Hagmann, W. K. *J. Med. Chem.* **2008**, *51*, 2108.
12. Rat and human liver microsomes were prepared in house as outlined in the literature: Lu, A. Y.; Levin, W. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 1334.
13. Incubation of the compound with hepatic microsomes: compound (10 μM) was incubated with rat or human liver microsomes (1 mg/mL protein) in potassium phosphate buffer (100 mM, pH 7.4) containing $MgCl_2$ (10 mM), alamethicine (10 μg/mg protein, Sigma), D-saccharic acid 1,4-lactone (5 mM, Sigma) and uridine 5′-diphosphoglucuronic acid trisodium salt (UDPGA, 3 mM, sigma). The incubation was carried out at 37 °C in a shaking water bath for 90 min, terminated using two volumes cold acetonitrile, vortexed, and centrifuged at 10,000 g for 10 min. The supernatant was analyzed via LC–MS/MS.