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A convenient transesterification method for synthesis of AT2 receptor ligands with improved stability in human liver microsomes

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ABSTRACT

A series of AT_2R ligands have been synthesized applying a quick, simple, and safe transesterification-type reaction whereby the sulfonyl carbamate alkyl tail of the selective AT_2R antagonist C38 was varied. Furthermore, a limited number of compounds where acyl sulfonamides and sulfonyl ureas served as carboxylic acid bioisosteres were synthesized and evaluated. By reducing the size of the alkyl chain of the sulfonyl carbamates, ligands **7a** and **7b** were identified with significantly improved *in vitro* metabolic stability in both human and mouse liver microsomes as compared to C38 while retaining the AT_2R binding affinity and AT_2R/AT_1R selectivity. Eight of the compounds synthesized exhibit an improved stability in human microsomes as compared to C38.

The octapeptide hormone angiotensin II (Ang II) is the main effector of the Renin-Angiotensin-Aldosterone system (RAAS). Ang II mediates its effects through activation of two G-protein coupled receptors (GPCRs), the angiotensin II type 1 (AT_1R) and the angiotensin II type 2 (AT_2R) receptors. AT_1R is involved in regulation of blood pressure and electrolyte balance and is a well-established drug target for the treatment of hypertension and heart failure (angiotensin receptor blockers, ARBs). The first ARB, losartan, was introduced into the market in 1995.[1] The antihypertensive angiotensin converting enzyme inhibitors (ACE inhibitors, e.g. captopril introduced into the market 1978) act by suppressing the formation of Ang II.[2] In contrast to the well-investigated AT_1R, less is known about the role of the AT_2R. It is abundant during fetal development but only very low levels of AT_2R can be detected in healthy adults. However, in certain pathological conditions e.g. myocardial infarction, heart and renal failure, and some brain injuries, a pronounced upregulation of AT_2R is frequently observed. Thus, the receptor is upregulated in areas of tissue damage and it is postulated that AT_2R is important in tissue repair. The physiological actions mediated by AT_2R have been reviewed.[3–10]

The use of AT_2R as a potential drug target has recently seen two different approaches and produced compounds that have reached clinical trials. The selective AT_2R agonist C21/M024 (Vicore Pharma) discovered by Anders Hallberg's group at our laboratory[11] has entered Phase I clinical trials for the indication idiopathic pulmonary fibrosis. The malonic acid sulfonamide derivative MP-157, a selective AT_2R agonist from Mitsubishi Tanabe Pharma, is also in Phase I clinical trials in Europe and aimed for the cardiovascular system.[12] The AT_2R antagonist EMA401 (Spinifex/Novartis) has completed a phase II clinical trial with positive results in patients with postherpetic neuralgia,[13] a form of chronic neuropathic pain.[14,15] AT_2R antagonists as potential new chemical agents for the treatment of peripheral neuropathic pain is based on the findings that AT_2R exhibits a higher expression in damaged nerve tissue e.g. in the dorsal root ganglia (DRG). Furthermore, activation of these AT_2Rs by the endogenous ligand Ang II potentiates pain signaling by increasing neurite length and density, and by nociceptor sensitization by phosphorylation of nociceptor ion channels on the DRG via AT_2R secondary messenger pathways.[16–20]

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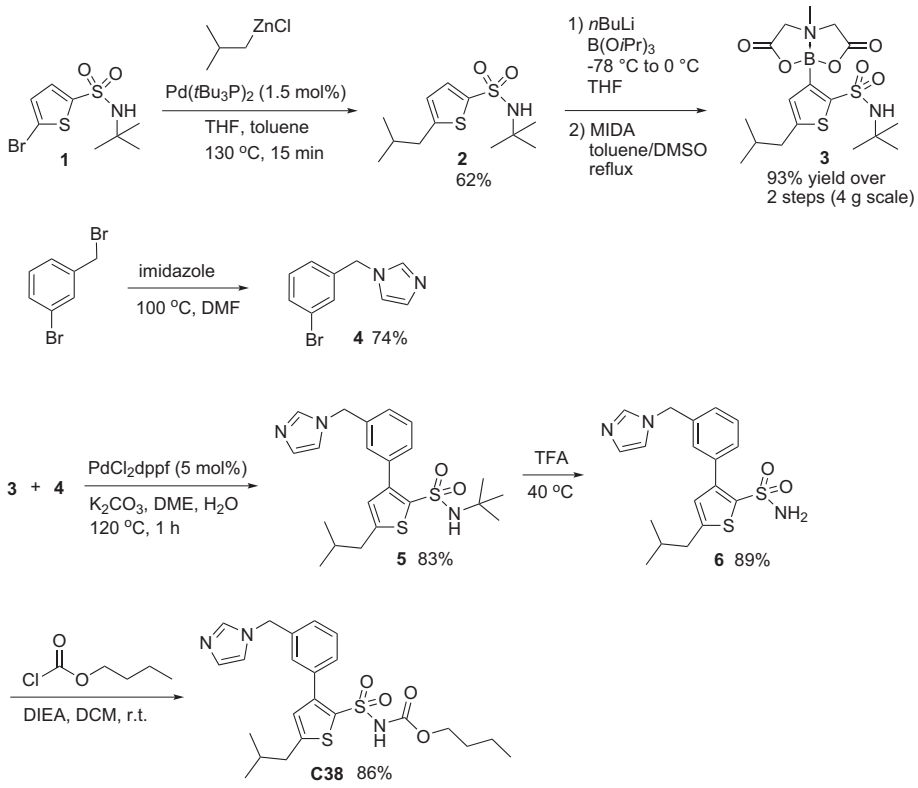


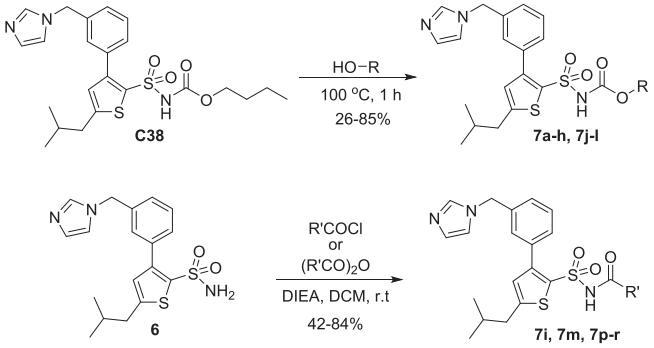
Scheme 1. Improved synthesis of the AT_2R antagonist **C38**.

We published the first selective drug-like AT_2R antagonists in 2012, among them compound **C38** which in structure closely relates to the AT_2R agonist C21.[21–23] Profiling **C38** in various ADME *in vitro* assays revealed a relatively short half-life of **C38** in human liver microsomes indicating poor metabolic stability.

A large number of structural modifications at several different sites of the AT_2R antagonist **C38** were explored but no efforts to alter the butylsulfonyl carbamate moiety were conducted. We had previously in the AT_2R agonist project found the *n*-butyl chain superior in producing potent compounds in all series studied[11,24] and thus the *n*-butyl chain was initially kept intact.[25]

The recent discovery that alkylsulfonyl carbamates can be interconverted to alternative alkylsulfonyl carbamates by a transesterification-type reaction by simply heating in an alkyl alcohol,[26,27] gave us the incentive to explore this part of the **C38** scaffold. In addition, we were encouraged to explore the impact of using acyl sulfonamides and sulfonyl ureas as conceivable replacements for the sulfonyl carbamate group.

During the efforts of profiling the properties of **C38**, a larger batch of the compound was required. This was achieved through a modified version of the previously published procedure (Scheme 1). First, a Negishi coupling of 5-bromo-*N*-(*tert*-butyl) thiophene-2-sulfonamide **1** with isobutylzinc under microwave heating[28] provided *N*-(*tert*-butyl)-5-isobutylthiophene-2-sulfonamide **2** in reasonable yield. This intermediate **2** was then converted to the MIDA boronate **3** in excellent yield. Compared to the corresponding boronic acid (semi-solid, stored in freezer) the MIDA boronate **3** is much easier to handle and store (solid, stable at ambient temperature under air).[29] The MIDA boronate **3** was subjected to a Suzuki coupling with 1-(3-bromobenzyl)-1*H*-imidazole **4** producing **5** in very good yield.[30] Deprotection of the *tert*-butyl sulfonamide **5** was performed in neat TFA to give the primary sulfonamide **6** in quantitative yield and finally the primary sulfonamide was coupled with butyl chloroformate to give the desired



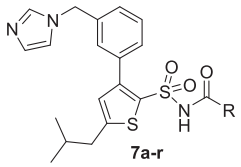
Scheme 2. Synthesis of new AT_2R ligands.

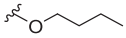
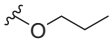
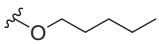
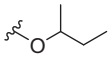
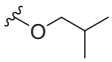
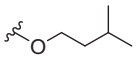
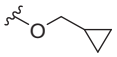
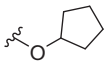
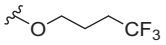
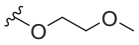
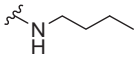
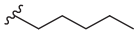
C38 in enough quantity to allow compound profiling as well as use as starting material for variations of the alkylsulfonyl carbamate motif (Scheme 1).

Essentially employing our previously developed transesterification/transcarbamoylation method, **C38** was heated in various alkyl alcohols (straight and branched) of various sizes at 100 °C for 60 min (Scheme 2).[26] The resulting products **7a–l** (Table 1) were successfully isolated in 26–85% yield,[33] except for the reaction with *t*-BuOH where only primary sulfonamide was isolated. The *tert*-butylsulfonyl carbamate **7i** was instead isolated by reacting the primary sulfonamide with Boc anhydride. Also 2-methoxyethanol requires a special permit for use and handling in Sweden and as a consequence 2-methoxyethyl chloroformate was coupled with **6** to give **7m** (Scheme 2, Table 1).

Heating **C38** in primary or secondary alkylamines at 120–150 °C allowed for the formation of sulfonyl ureas **7n** (18%)[33] and **7o** (59%) by aminolysis of the sulfonyl carbamate (Table 1). Acylsulfonamides **7p–7r** were synthesized from primary sulfonamide **6** by

Table 1
Receptor binding results and *in vitro* metabolic stability.



Compound	R	AT_2R K_i (nM)[a]	AT_1R (%inh@10 μM)[a]	HLM t½ (min)[b]	MLM t½ (min)[b]
C38		270	7	12	70
7a		400	8	77	220
7b		300	5	61	180
7c		290	10	11	82
7d		390	−3	6.5	17
7e		160	18	17	79
7f		440	16	43	47
7g		130	14	7.0	38
7h		230	8	4.9	13
7i		270	17	18	28
7j		140	13	9.4	59
7k		180	7	5.6	31
7l		730	3	6.6	27
7m		480	10	27	120
7n		400	15	20	56
7o		2300	13	nd[c]	nd[c]
7p		340	18	20	66
7q		300	13	4.8	6.9
7r		280	3	7.0	8.9

[a] Radioligand binding assays performed by Eurofins Cerep SA, France.
[b] *In vitro* half-life ($t_{1/2}$) in human (HLM) and mouse liver microsomes (MLM) was determined as described in the Supplementary Information.
[c] Not determined.

coupling with either acid chlorides or anhydrides (Scheme 2, Table 1).

The AT_2R binding assay previously used by our group (membrane preparations from pig uterus myometrium)[31] is no longer available in house.[21,24,25] We therefore needed to search for an alternative source of binding data. The decision was made to employ membranes from HEK-293 cells expressing human AT_2R (HEK293-hAT_2R) with [^{125}I][Sar^1,Ile^8]-angiotensin II as the radioligand as assay using a seven-point dose–response curve in duplicate measurements at each concentration (Eurofins Cerep SA, France). Upon re-testing the compound **C38** in the new assay, a 10–15-fold drop in potency (K_i) was observed as compared to what had been found with the previous binding assay which was based on pig membrane preparations (270 nM vs 19 nM). The reason for drop in potency when changing assay is not clear but it has been noticed and discussed previously.[32]

Testing of the alkyl sulfonylcarbamate series for AT_2R binding revealed a relatively flat structure–activity relationship. The alkyl chain could be shortened and branched without any significant change in AT_2R binding affinity. Importantly, a good selectivity compared to AT_1R was maintained as all compounds showed less than 20% inhibition of [^{125}I][Sar^1,Ile^8]-angiotensin II binding to AT_1R at 10 μM. The *in vitro* metabolic stability of the compounds were subsequently determined using human liver microsomes

(HLM). From the obtained data we found a clear relationship between size and lipophilicity of the carbamate chain and the HLM stability of the compounds. Reducing log P/log D clearly, and perhaps not surprisingly, increased the metabolic stability. The stability of the compounds were also assessed in mouse liver microsomes (MLM) to get an estimate on the likelihood of exposure after administration of the compounds in murine models of neuropathic pain. As seen in Table 1, this series of derivatives are in general less prone to undergo metabolism in MLM than in HLM. Notably though, the methyl (**7a**) and ethyl (**7b**) carbamates show good metabolic stability in both human and mouse liver microsomes. This observation combined with the fact that **7a** and **7b** are more or less equipotent to **C38** with regard to AT_2R binding affinity and AT_2R/AT_1R selectivity make them valuable tools for *in vivo* models of neuropathic pain in mouse.

Having evaluated the alkyl sulfonylcarbamates we turned our focus to alternative, but related carboxylic acid bioisosteres with the aim of finding more potent but still metabolically stable compounds. Using **C38** as starting material, aminolysis of the carbamate with different amines produced sulfonyl ureas **7n**, **7o**. Unfortunately, no trends towards improved properties were noted. Moreover, a series of three acyl sulfonamides were synthesized from the primary sulfonamide **5** and the corresponding acyl chlorides or anhydrides (Table 1, entries **7p**–**7r**). The potencies of these compounds were in the same range as **7a** and **7b** but could not match their metabolic stability.

In conclusion, we have prepared and evaluated a series of AT_2R ligands by using a quick, simple, and safe method developed in our laboratory[26,27] whereby the sulfonyl carbamate alkyl tail was varied by a transesterification-type reaction. In addition, a limited number of compounds where acyl sulfonamides and sulfonyl ureas were assessed as carboxylic acid bioisosteres were synthesized. The key finding was that reduced size of the alkyl chain of the sulfonyl carbamates, compounds (**7a** and **7b**) were identified with significantly improved *in vitro* metabolic stability as compared to **C38** while retaining the AT_2R binding affinity and high AT_2R/AT_1R selectivity. The relative stability in mouse liver microsomes suggests that the compounds could serve as suitable research tools in experimental models of neuropathic pain in rodents. Planning of *in vivo* mouse studies is ongoing.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, analytical data) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2017.11.042.

References

1. Wexler RR, Greenlee WJ, Irvin JD, et al. *J Med Chem*. 1996;39:625–656.
2. Ondetti MA, Rubin B, Cushman DW. *Science (80-)*. 1977;196:441–444.
3. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. *Pharmacol Rev*. 2000;52:415–472.
4. Horiuchi M, Akishita M, Dzau VJ. *Hypertension*. 1999;33:613–621.
5. Steckelings UM, Unger T. *Expert Opin Invest Drugs*. 2012;21:763–766.
6. McCarthy CA, Widdop RE, Denton KM, Jones ES. *Curr Hypertens Rep*. 2013;15:25–30.
7. Danyel LA, Schmerler P, Paulis L, Unger T, Steckelings UM. *Integr Blood Press Control*. 2013;6:153–161.
8. Foulquier S, Steckelings UM, Unger T. *Nature*. 2013;493. S9–S9.
9. Larhed M, Hallberg M, Hallberg A. *Med Chem Rev*. 2016;51:69–82.
10. Carey RM. *Am J Hypertens*. 2017;30:339–347.
11. Wan Y, Wallinder C, Plouffe B, et al. *J Med Chem*. 2004;47:5995–6008.
12. Yoshida T, Sakashita H, Numata A, Tahara S, Kawasumi H. Preparation of novel malonic acid sulfonamide derivatives as angiotensin AT2 receptor agonists 2008, WO2008156142.
13. Rice ASC, Dworkin RH, McCarthy TD, et al. *Lancet*. 2014;383:1637–1647.
14. Campbell JN, Meyer RA. *Neuron*. 2006;52:77–92.
15. Basbaum AI, Bautista DM, Scherrer G, Julius D. *Cell*. 2009;139:267–284.
16. Gallinat S, Yu M, Dorst A, Unger T, Herdegen T. *Mol Brain Res*. 1998;57:111–122.
17. Patil J, Schwab A, Nussberger J, Schaffner T, Saavedra JM, Imboden H. *Regul Pept*. 2010;162:90–98.
18. Anand U, Facer P, Yiangou Y, et al. *Eur J Pain*. 2013;17:1012–1026.
19. Smith MT, Woodruff TM, Wyse BD, Muralidharan A, Walther T. *Pain Med*. 2013;14:1557–1568.
20. Anand U, Yiangou Y, Sinisi M, et al. *Mol Pain*. 2015;11:38.
21. Murugaiah AMS, Wu X, Wallinder C, et al. *J Med Chem*. 2012;55:2265–2278.
22. Guimond M-O, Wallinder C, Alterman M, Hallberg A, Gallo-Payet N. *Eur J Pharmacol*. 2013;699:160–171.
23. Wallinder C, Sköld C, Botros M, et al. *ACS Med Chem Lett*. 2015;6:178–182.
24. Wu X, Wan Y, et al. *J Med Chem*. 2006;49:7160–7168.
25. Murugaiah AMS, Wallinder C, Mahalingam AK, et al. *Bioorg Med Chem*. 2007;15:7166–7183.
26. Isaksson R, Kumpiņa I, Larhed M, Wannberg J. *Tetrahedron Lett*. 2016;57:1476–1478.
27. Kumpiņa I, Isaksson R, Sävmarker J, Wannberg J, Larhed M. *Org Process Res Dev*. 2016;20:440–445.
28. Nöteberg D, Schaal W, Hamelink E, Vrang L, Larhed M. *J Comb Chem*. 2003;5:456–464.
29. Gillis EP, Burke MD. *J Am Chem Soc*. 2007;129:6716–6717.
30. Wannberg J, Sabnis YA, Vrang L, et al. *Bioorg Med Chem*. 2006;14:5303–5315.
31. Nielsen AH, Schauser K, Winther H, Dantzer V, Poulsen K. *Clin Exp Pharmacol Physiol*. 1997;24:309–314.
32. Behrends M, Wallinder C, Wieckowska A, et al. *ChemistryOpen*. 2014;3:65–75.
33. In cases where lower yields were observed (e.g. **7f** and **7n**) the main component of the reaction mixture was primary sulfonamide formed by hydrolysis of the butyl carbamate starting material.