Bioorganic & Medicinal Chemistry Letters 21 (2011) 186–190

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

N-(Pyridin-3-yl)benzamides as selective inhibitors of human aldosterone synthase (CYP11B2)

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ARTICLE INFO

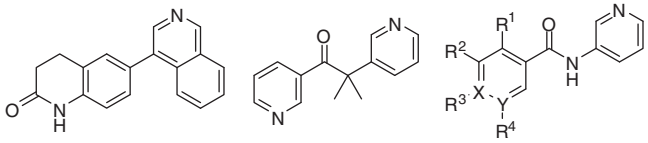
Article history:
Received 3 November 2010
Accepted 4 November 2010
Available online 12 November 2010

Keywords:
CYP11B2
Aldosterone synthase
Selective inhibition
Metyrapone
Aldosterone
Congestive heart failure
Myocardial fibrosis

ABSTRACT

A series of 23 *N*-(Pyridin-3-yl)benzamides was synthesized and evaluated for their potential to inhibit human steroid-11β-hydroxylase (CYP11B1) and human aldosterone synthase (CYP11B2). The most potent and selective CYP11B2 inhibitors (IC_{50} values 53–166 nM) were further evaluated for their potential to inhibit human CYP17 and CYP19, and no inhibition was observed. Clear evidence was shown for *N*-(Pyridin-3-yl)benzamides to be a highly selective class of CYP11B2 inhibitors in vitro.

Aldosterone synthase (CYP11B2) is the key enzyme in the human biosynthesis of mineralocorticoids, catalysing the three-step interconversion of 11-deoxycorticosterone to aldosterone via corticosterone and 18-hydroxycorticosterone.[1] Due to its pivotal role, CYP11B2 is claimed as an useful target for the treatment of hyperaldosteronism, myocardial fibrosis and congestive heart failure.[2] After having successfully developed selective inhibitors of crucial steroidogenic enzymes involved in the endocrine (CYP19[3–5] and CYP17[6–8]) as well as intracrine (5α-reductase[9–11] and 17β-HSD1[12–14]) stimulation of hormone dependent diseases, we more recently have been concerned with the development of selective CYP11B2 inhibitors.[2,15–21] Thus, we have discovered compound **1** to be a highly potent and selective inhibitor of CYP11B2.[15] We were able to verify the lowering of plasma aldosterone levels by **1** in rats, which can be considered as validation of the target and *proof-of-principle*.[22]



1 **2** Metyrapone **3**

In an effort to develop and evaluate new classes of compounds as CYP inhibitors, we based our investigations on the structure of the well-known CYP inhibitor Metyrapone **2**, which has been used for the treatment of hypercortisolism and *Cushing's* syndrome for several decades.[23–27] We decided to substitute the bridging 2,2-dimethylethanone moiety in **2** by a much easier accessible amide linker to generate *N*-(Pyridin-3-yl)benzamides **3**. In addition—and with respect to activity studies carried out with the bovine CYP11B enzyme by Hays et al.[28] resp. Tobes et al.[29]—we also investigated the exchange of the keto-substituted pyridine system of Metyrapone **2** against substituted phenyl moieties in order to improve the potency of the compounds. We were very pleased by the activity and astonishingly high CYP11B2 selectivity of these small and structurally quite simple compounds in vitro, and we would like to communicate our detailed observations in this Letter.

Compounds of type **3** were prepared by a routine amide coupling procedure using commercially available acid chlorides **4** and 3-aminopyridine **5** in pyridine at room temperature or with application of gentle heating (Scheme 1). The products were obtained as solids in moderate to good yields.

Routinely, all synthesized compounds **3** were evaluated for their potential to inhibit human CYP11B2 and CYP11B1 at an inhibitor concentration of c = 500 nM. Tests were carried out using our established comparative *in-house* test system (V79 Chinese hamster cells stably transfected with either human CYP11B2 or CYP11B1; substrate: 11-deoxycorticosterone for both enzymes; substrate concentration: c = 100 nM).[30] The results are shown in Table 1. As can be seen from data, none of compounds **3** showed significant inhibition

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URL: http://www.pharmmedchem.de.

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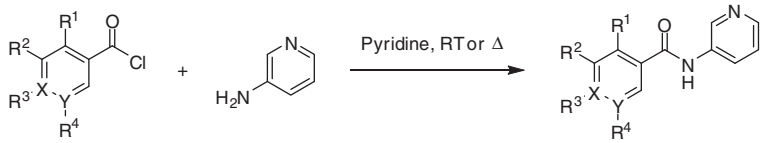
doi:10.1016/j.bmcl.2010.11.040



4 5 3

Scheme 1. Synthesis of *N*-(Pyridin-3-yl)benzamides **3**.

Table 1
Inhibition of human CYP11B2 and CYP11B1 enzymes by compounds **3**

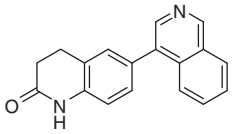
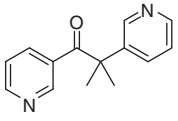
No.[a]	R^1	R^2	R^3	R^4	X	Y	CYP11B2 inhibition @ 500 nM[b] (%)	CYP11B1 inhibition @ 500 nM[b] (%)	log *P* (calcd)[c]
1	—	—	—	—	—	—	94 ± 2	91 ± 4	3.03
2	—	—	—	—	—	—	79 ± 4	94 ± 1	1.78
3a	H	H	H	—	C	N	8 ± 9	3 ± 2	0.81
3b	H	H	—	H	N	C	39 ± 7	4 ± 5	0.81
3c	H	Br	H	—	C	N	29 ± 3	0.4 ± 0.5	1.70
3d	H	H	H	H	C	C	30 ± 2	2 ± 2	1.98
3e	H	H	F	H	C	C	86 ± 3	7 ± 5	2.19
3f	H	H	Cl	H	C	C	88 ± 4	5 ± 7	2.76
3g	H	H	Br	H	C	C	64 ± 8	0	2.91
3h	H	H	OMe	H	C	C	19 ± 3	0	2.10
3i	H	H	OCF_3	H	C	C	3 ± 4	3 ± 3	3.21
3j	H	H	CF_3	H	C	C	1 ± 1	1 ± 1	2.99
3k	H	H	Me	H	C	C	25 ± 4	0	1.80
3l	H	H	Ph	H	C	C	6 ± 2	0	3.87
3m	H	H	CN	H	C	C	81 ± 5	9 ± 4	1.58
3n	H	H	NO_2	H	C	C	64 ± 3	0	1.88
3o	H	F	H	H	C	C	47 ± 11	1 ± 2	2.19
3p	H	Cl	H	H	C	C	26 ± 7	0	2.76
3q	F	H	H	H	C	C	34 ± 6	1 ± 2	1.78
3r	H	F	F	H	C	C	83 ± 2	6 ± 6	2.29
3s	H	OMe	OMe	H	C	C	2 ± 1	0.1 ± 0.1	1.75
3t	Cl	Cl	H	H	C	C	51 ± 3	4 ± 5	2.55
3u	F	H	F	F	C	C	70 ± 2	2 ± 2	2.03

[a] The purity of all tested compounds was ⩾95%.
[b] Test system: V79 Chinese hamster cells stably transfected with either human CYP11B2 or CYP11B1 enzyme; substrate: 11-deoxycorticosterone for both enzymes; substrate concentration: *c* = 100 nM.
[c] log *P* values were calculated using the add-on *ChemProp* implemented in *ChemDraw Ultra, Version 10.0*, CambridgeSoft.

of human CYP11B1 at *c* = 500 nM, while a number of derivatives strongly inhibit human CYP11B2. Concerning the two direct analogues **3a** and **3b** of Metyrapone **2**, isonicotinamide **3b** shows weak inhibition of CYP11B2, whereas nicotinamide **3a** is not active. Addition of a bromo substituent in *meta*-position of the nicotinamide moiety leads to the weakly active CYP11B2 inhibitor **3c**, but no improvement is achieved in comparison with **3b**. The unsubstituted benzamide **3d** is similarly active as **3b**. Introduction of halogeno substituents in *para*-position within the benzamide moiety (**3e**, **3f**, **3g**) leads to strong inhibitors of CYP11B2. Interestingly, similar activities could be observed with strongly electron-withdrawing substituents (-*M*-effect) like cyano derivative **3m** and nitro compound **3n**, whereas trifluoromethoxy-substituted **3i** and trifluoromethyl-substituted **3j** (-*I*-effect) showed no activity at all. Introduction of halogeno substituents in *meta*- (**3o**, **3p**) and *ortho*-position (**3q**) of the benzamide leads to selective CYP11B2 inhibitors with reduced activity in comparison with the *para*-substituted derivatives. The difluoro derivative **3r** shows similar activity to the monohalogenated compounds **3e**, **3f** and **3g**, whereas a slight drop of activity is observed for the trifluoro derivative **3u**. Compound **3t** bearing chloro substituents in *ortho*- and *meta*-position of the benzamide moiety is less active compared with **3u**.

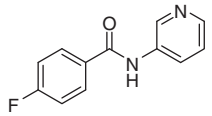
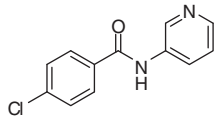
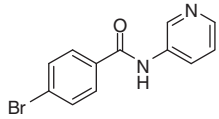
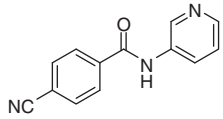
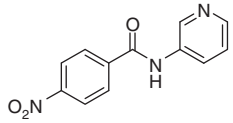
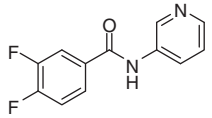
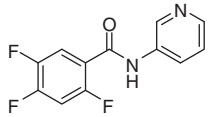
For the seven most active inhibitors of CYP11B2 (**3e**, **3f**, **3g**, **3m**, **3n**, **3r**, **3u**) IC_{50} values were determined in order to quantify the

Table 2
Inhibition of human CYP11B2 and CYP11B1 enzymes (IC_{50} values) for selected compounds of type **3**

No.[a]	Structure	CYP11B2 IC_{50}[b] (nM)	CYP11B1 IC_{50}[b]	Selectivity factor IC_{50} (CYP11B1)/IC_{50} (CYP11B2)
1		0.20[15]	33 nM[15]	187[15]
2		72 ± 17	14.6 ± 2.1 nM	0.23

(*continued on next page*)

Table 2 (*continued*)

No.[a]	Structure	CYP11B2 IC_{50}[b] (nM)	CYP11B1 IC_{50}[b]	Selectivity factor IC_{50} (CYP11B1)/IC_{50} (CYP11B2)
3e		82 ± 11	14.3 ± 3.3 μM	174
3f		65 ± 12	10.2 ± 2.2 μM	157
3g		104 ± 20	16.7 ± 2.8 μM	161
3m		78 ± 20	7.5 ± 1.1 μM	96
3n		145 ± 24	24.8 ± 3.4 μM	171
3r		53.5 ± 2.0	5.92 ± 0.89 μM	111
3u		166 ± 23	16.1 ± 2.8 μM	97

[a] The purity of all tested compounds was ⩾ 95%.
[b] Test system: V79 Chinese hamster cells stably transfected with either human CYP11B2 or CYP11B1 enzyme; substrate: 11-deoxycorticosterone for both enzymes; substrate concentration: *c* = 100 nM.

selectivity toward CYP11B1 (Table 2). As can be seen, all compounds are highly selective CYP11B2 inhibitors, the most selective one being **3e** with a selectivity factor of 174. Concerning this parameter, the compounds are comparable with reference compound **1** being developed in our group before.[15,22] However, with regard to activity, the *N*-(Pyridin-3-yl)benzamides **3** turned out to be far less potent inhibitors of CYP11B2. For the most active compound **3r**, an IC_{50} value of 53.5 nM has been determined, which means an approximately 250-fold lower potency in comparison with **1**. However, some of the compounds are exhibiting similar (**3e**, **3m**) or slightly higher (**3f**, **3r**) CYP11B2 inhibitory activity compared to Metyrapone **2**.

For all synthesized compounds **3** and the references **1** and **2**, log *P* values were calculated (Table 1).[31] There is no obvious correlation between the potency as CYP11B2 inhibitors and the lipophilicity of the compounds, which might have been expected due to the location of both CYP11B1 and CYP11B2 enzymes on the inner side of the mitochondrial membrane of adrenal cortex cells. For example, compounds **3e** and **3m** are equally potent inhibitors of CYP11B2, but differ by approximately 0.5 units in log *P*. However, for almost all compounds log *P* values of 1.5–3.0 have been calculated, showing them to be equally or more lipophilic than Metyrapone **2**, but less lipophilic than reference compound **1**.

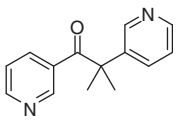
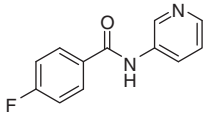
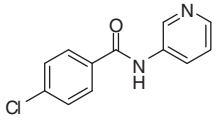
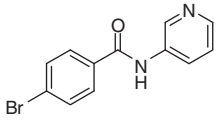
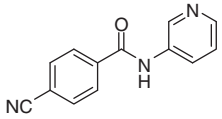
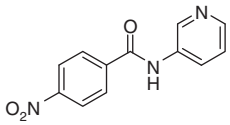
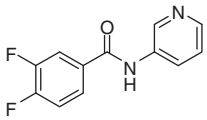
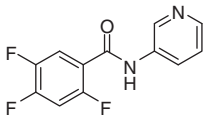
In order to obtain a more detailed selectivity profile for compounds **3** toward steroidogenic CYP enzymes, we additionally determined the ability of the seven most active inhibitors of CYP11B2 (**3e**, **3f**, **3g**, **3m**, **3n**, **3r**, **3u**) to inhibit CYP17[32,33] and CYP19[34] (Table 3). None of the compounds showed any significant inhibitory effect on these enzymes. Regarding the inhibition of CYP17 and CYP19 as potential unwanted side effect for the application of CYP11B2 inhibitors as drugs, the structural motifs of all seven compounds **3e**, **3f**, **3g**, **3m**, **3n**, **3r** and **3u** can therefore deliver useful information to overcome residual CYP17 and CYP19 activity, as observed for the highly potent inhibitor FAD286A.[35,36] Interestingly, the latter compound has shown activity in several animal models relevant for heart failure and organ damage caused by elevated plasma aldosterone level, thus demonstrating the importance of the drug target CYP11B2.[37–40]

In summary, we synthesized a panel of 23 *N*-(Pyridin-3-yl)benzamides **3** and evaluated them for their activity as inhibitors of CYP11B1, CYP11B2, CYP17 and CYP19. All compounds did not show any significant inhibition of CYP11B1, and several derivatives (**3e**, **3f**, **3g**, **3m**, **3n**, **3r**, **3u**) turned out to be potent inhibitors of CYP11B2. Those compounds exhibited no inhibitory activity on CYP17 and CYP19. The highest selectivity was observed for **3e** containing a fluoro substituent in *para*-position of the benzamide moiety, whereas the most active compound was the difluoro compound **3r**. Although the CYP11B2 potency of the compounds is by far lower in comparison to reference **1**, compounds **3** offer a highly interesting selectivity profile. Further investigations concerning metabolic stability and in vivo activity are pending.

Acknowledgements

We are indebted to Jeannine Jung, Gertrud Schmitt and Jannine Ludwig for performing the bioassays. Furthermore, we would like

Table 3
Inhibition of human CYP17 and CYP19 enzymes for selected compounds of type **3**

No.	Structure	CYP17 inhibition @ 2000 nM[a]	CYP19 inhibition @ 500 nM[b]
2		0%	0%
3e		0.3 ± 0.2%	1.1 ± 1.3%
3f		1.3 ± 2.3%	0%
3g		4.3 ± 2.7%	0.1 ± 0.1%
3m		0.2 ± 0.3%	0.2 ± 0.3%
3n		1.0 ± 0.9%	0%
3r		1.0 ± 1.7%	0.5 ± 0.8%
3u		0.6 ± 1.1%	0%

[a] CYP17 test system: 50,000 g sediment of a homogenate of *E. coli* recombinantly expressing human CYP17; substrate: progesterone (c = 25 μM); inhibitor concentration: c = 2.0 μM; Ketoconazole, IC_{50} = 2780 nM.
[b] CYP19 test system: human placental CYP19; substrate: androstenedione (c = 500 nM); inhibitor concentration: c = 500 nM; Fadrozole, IC_{50} = 30 nM.

to thank Martina Jankowski and Dr. Stefan Boettcher for the HPLC-MS measurements. We would like to thank Professor Dr. J. J. Rob Hermans, University of Maastricht, The Netherlands, for generous supply of the V79 CYP11B1 cells and Professor Dr. Rita Bernhardt, Saarland University, for the V79 CYP11B2 cells.

Supplementary data

Supplementary data (experimental details as well as spectral and HPLC-MS data for all compounds **3**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.040.

References and notes

1. Kawamoto, T.; Mitsuuchi, Y.; Toda, K.; Yokoyama, Y.; Miyahara, K.; Miura, S.; Ohnishi, T.; Ichikawa, Y.; Nakao, K.; Imura, H.; Ulick, S.; Shizuta, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1458.
2. Hartmann, R. W.; Müller, U.; Ehmer, P. B. *Eur. J. Med. Chem.* **2003**, *38*, 363.
3. Le Borgne, M.; Marchand, P.; Duflos, M.; Delevoye-Seiller, B.; Piessard-Robert, S.; Le Baut, G.; Hartmann, R. W.; Palzer, M. *Arch. Pharm.* **1997**, *330*, 141.
4. Leonetti, F.; Favia, A.; Rao, A.; Aliano, R.; Paluszcak, A.; Hartmann, R. W.; Carotti, A. *J. Med. Chem.* **2004**, *47*, 6792.
5. Gobbi, S.; Cavalli, A.; Rampa, A.; Belluti, F.; Piazzi, L.; Paluszcak, A.; Hartmann, R. W.; Recanatini, M.; Bisi, A. *J. Med. Chem.* **2006**, *49*, 4777.
6. Pinto-Bazurco Mendieta, M. A.; Negri, M.; Jagusch, C.; Müller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. *J. Med. Chem.* **2008**, *51*, 5009.
7. Hu, Q.; Negri, M.; Jahn-Hoffmann, K.; Zhuang, Y.; Olgen, S.; Bartels, M.; Müller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. *Bioorg. Med. Chem.* **2008**, *16*, 7715.
8. Hille, U. E.; Hu, Q.; Vock, C.; Negri, M.; Bartels, M.; Müller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. *Eur. J. Med. Chem.* **2009**, *44*, 2765.
9. Baston, E.; Hartmann, R. W. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1601.
10. Baston, E.; Palusczak, A.; Hartmann, R. W. *Eur. J. Med. Chem.* **2000**, *35*, 931.
11. Picard, F.; Schulz, T.; Hartmann, R. W. *Bioorg. Med. Chem.* **2002**, *10*, 437.
12. Frotscher, M.; Ziegler, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Neugebauer, A.; Fetzer, L.; Scherer, C.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. *J. Med. Chem.* **2008**, *51*, 2158.
13. Bey, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Werth, R.; Oster, A.; Algül, O.; Neugebauer, A.; Hartmann, R. W. *Bioorg. Med. Chem.* **2008**, *16*, 6423.
14. Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Ziegler, E.; Neugebauer, A.; Bhoga, U.; Bey, E.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. *J. Med. Chem.* **2008**, *51*, 4685.
15. Lucas, S.; Heim, R.; Ries, C.; Schewe, K. E.; Birk, B.; Hartmann, R. W. *J. Med. Chem.* **2008**, *51*, 8077.
16. Lucas, S.; Heim, R.; Negri, M.; Antes, I.; Ries, C.; Schewe, K. E.; Bisi, A.; Gobbi, S.; Hartmann, R. W. *J. Med. Chem.* **2008**, *51*, 6138.
17. Heim, R.; Lucas, S.; Grombein, C. M.; Ries, C.; Schewe, K. E.; Negri, M.; Müller-Vieira, U.; Birk, B.; Hartmann, R. W. *J. Med. Chem.* **2008**, *51*, 5064.
18. Voets, M.; Antes, I.; Scherer, C.; Müller-Vieira, U.; Biemel, K.; Marchais-Oberwinkler, S.; Hartmann, R. W. *J. Med. Chem.* **2006**, *49*, 2222.

19. Voets, M.; Antes, I.; Scherer, C.; Müller-Vieira, U.; Biemel, K.; Barassin, C.; Marchais-Ober-winkler, S.; Hartmann, R. W. *J. Med. Chem.* **2005**, *48*, 6632.
20. Ulmschneider, S.; Muller-Vieira, U.; Mitrenga, M.; Hartmann, R. W.; Oberwinkler-Marchais, S.; Klein, C. D.; Bureik, M.; Bernhardt, R.; Antes, I.; Lengauer, T. *J. Med. Chem.* **2005**, *48*, 1796.
21. Ulmschneider, S.; Muller-Vieira, U.; Klein, C. D.; Antes, I.; Lengauer, T.; Hartmann, R. W. *J. Med. Chem.* **2005**, *48*, 1563.
22. Ries, C.; Lucas, S.; Heim, R.; Birk, B.; Hartmann, R. W. *J. Steroid Biochem. Mol. Biol.* **2009**, *116*, 121.
23. Diez, J. J.; Iglesias, P. *Mini-Rev. Med. Chem.* **2007**, *7*, 467.
24. Nieman, L. K. *Pituitary* **2002**, *5*, 77.
25. Miller, J. W.; Crapo, L. *Endocr. Rev.* **1993**, *14*, 443.
26. Gower, D. B. *J. Steroid Biochem.* **1974**, *5*, 501.
27. Daniels, H.; van Amstel, W. J.; Schopman, W.; van Dommelen, C. *Acta Endocrinol. (Copenhagen)* **1963**, *44*, 346.
28. Hays, S. J.; Tobes, M. C.; Gildersleeve, D. L.; Wieland, D. M.; Beierwaltes, W. H. *J. Med. Chem.* **1984**, *27*, 15.
29. Tobes, M. C.; Hays, S. J.; Gildersleeve, D. L.; Wieland, D. M.; Beierwaltes, W. H. *J. Steroid Biochem.* **1985**, *22*, 103.
30. Ehmer, P. B.; Bureik, M.; Bernhardt, R.; Müller, U.; Hartmann, R. W. *J. Steroid Biochem. Mol. Biol.* **2002**, *81*, 173.
31. log *P* values were calculated using the add-on *ChemProp* implemented in *ChemDraw Ultra, Version 10.0*, CambridgeSoft.
32. Ehmer, P. B.; Jose, J.; Hartmann, R. W. *J. Steroid Biochem. Mol. Biol.* **2000**, *75*, 57.
33. Hutschenreuter, T. U.; Ehmer, P. B.; Hartmann, R. W. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 17.
34. Hartmann, R. W.; Batzl, C. *J. Med. Chem.* **1986**, *29*, 1362.
35. Schieweck, K.; Bhatnagar, A. S.; Matter, A. *Cancer Res.* **1988**, *48*, 834.
36. Lamberts, S. W.; Bruining, H. A.; Marzouk, H.; Zuiderwijk, J.; Uitterlinden, P.; Blijd, J. J.; Hackeng, W. H.; de Jong, F. H. *J. Clin. Endocrinol. Metab.* **1989**, *69*, 896.
37. Fiebeler, A.; Nussberger, J.; Shagdarsuren, E.; Rong, S.; Hilfenhaus, G.; Al-Saadi, N.; Dechend, R.; Wellner, M.; Meiners, S.; Maser-Gluth, C.; Jeng, A. Y.; Webb, R. L.; Luft, F. C.; Muller, D. N. *Circulation* **2005**, *111*, 3087.
38. Menard, J.; Gonzalez, M. F.; Guyene, T. T.; Bissery, A. *J. Hypertens.* **2006**, *24*, 1147.
39. Minnaard-Huiban, M.; Emmen, J. M. A.; Roumen, L.; Beugels, I. P. E.; Cohuet, G. M. S.; van Essen, H.; Ruijters, E.; Pieterse, K.; Hilbers, P. A. J.; Ottenheijm, H. C. J.; Plate, R.; de Gooyer, M. E.; Smits, J. F. M.; Hermans, J. J. R. *Endocrinology* **2008**, *149*, 28.
40. Mulder, P.; Mellin, V.; Favre, J.; Vercauteren, M.; Remy-Jouet, I.; Monteil, C.; Richard, V.; Renet, S.; Henry, J. P.; Jeng, A. Y.; Webb, R. L.; Thuillez, C. *Eur. Heart J.* **2008**, *29*, 2171.