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Development and evaluation of a pharmacophore model for inhibitors of aldosterone synthase (CYP11B2)

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Abstract—Recently, we proposed inhibition of aldosterone synthase (CYP11B2) as a novel strategy for the treatment of congestive heart failure and myocardial fibrosis and synthesized a large number of inhibitors. In this work, a pharmacophore model for CYP11B2 inhibitors was developed by superimposition of active and non-active compounds. This model was confirmed by the synthesis of two pyridyl substituted acenaphthene derivatives (**A**,**B**). This new class of compounds as well as the pharmacophore could be helpful for the discovery of novel inhibitors.

Aldosterone synthase (CYP11B2) is the key enzyme of mineralocorticoid biosynthesis. It catalyzes the conversion of 11-deoxycorticosterone to the most potent mineralocorticoid, aldosterone.[1] Pathological elevations in plasma aldosterone levels increase blood pressure and play a detrimental role in cardiovascular diseases.[2] In two recent clinical studies, the aldosterone receptor antagonists spironolactone and eplerenone were found to reduce mortality in patients with chronic congestive heart failure and in patients after myocardial infarction, respectively.[3,4] The treatment with these antagonists, however, is accompanied with severe side effects like hyperkalemia.[5] A new pharmacological approach for the treatment of hyperaldosteronism, congestive heart failure, and myocardial fibrosis was recently suggested by us: inhibition of aldosterone formation with CYP11B2-inhibitors.[6,7] Non-steroidal, selective inhibitors are to be preferred, because they can be expected to have less side effects on the endocrine system. The inhibitors must not affect other P450 (CYP) enzymes. In the case of 11-β-hydroxylase (key enzyme of glucocorticoid biosynthesis, CYP11B1), this was very difficult to achieve, since it has a sequence homology of more than 93% compared to CYP11B2.[8] Therefore, we initiated a drug discovery program[6,7] which finally resulted in potent and selective inhibitors originating from three classes of compounds: *E*- and *Z*-heterocyclic substituted methylene-tetrahydronaphthalenes and -indanes and heterocyclic substituted naphthalene derivatives.[9–11]

Here, we describe a pharmacophore model to get insight into the 3-dimensional shape of the binding pocket. Compounds named 'inhibitors' were highly active in V79MZh11B2 cells,[12] expressing human CYP11B2 (IC_{50} values <100 nM).[9–11] 'Non-inhibitors' showed little to no activity (IC_{50} values >300 nM).[9–11]

These compounds are supposed to occupy the substrate binding site in the apoprotein moiety. They also complex with their aromatic nitrogen (pharmacophore point **N**, Fig. 1b) the iron ion of the heme, which is located in the active site as well. This complexation mechanism does not only increase binding affinity of the inhibitors but also prevents oxygen activation at the heme, which is required for the catalytic process. No X-ray structure of CYP11B2, which is located in the inner mitochondrial membrane, is available. Homology approach based protein models have been improved continuously,[9–11,13] but still are of limited predictive value.

The compounds shown in Table 1 were first built in Sybyl 7.0 (Tripos Associates; Inc., St. Louis, MO 2001, USA) and then optimized in conjugate-gradient modus. Subsequently all molecules underwent a conformational analysis with MacroModel V7.0 (1999 Schrödinger, Inc.) and were energy-minimized in MMFF94s[14] forcefield as implemented in Sybyl 7.0. A dielectric constant

Keywords: Pharmacophore model; Aldosterone synthase; CYP11B2 inhibitors.
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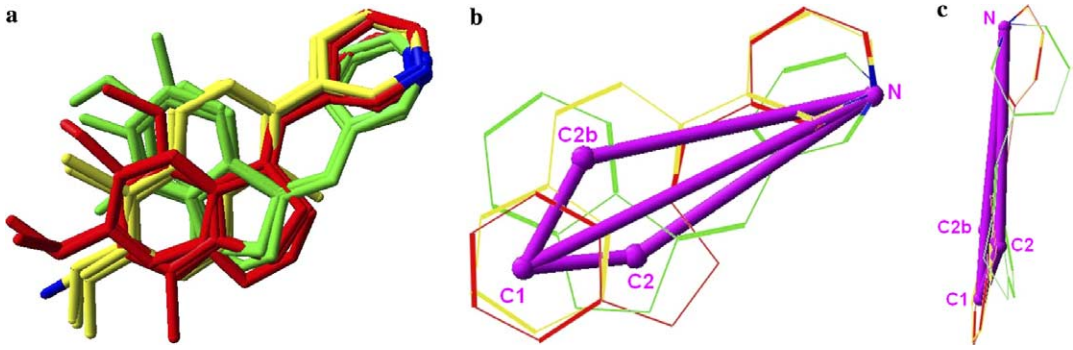


Figure 1. Superimposition of compounds: (a) alignment of inhibitors from **17** to **39** and **66** to **81** (see Table 1); (b) skeletal structure of the pharmacophore model: overlay of the unsubstituted 2-(3-pyridyl) naphthalene (**66** yellow) and the unsubstituted *E*- and *Z*-3-pyridylmethylene-indane (**17** and **18**; red and green); the pharmacophore (magenta) is built by connecting the ring centroids (**C1**, **C2**, and **C2b**) and the aromatic nitrogen **N**; (c) sideview of the pharmacophore.

of 1.0 has been used throughout and the optimizations have been terminated at a gradient of 0.001 kcal/mol. After defining the ring centroids for each compound of the three classes (**C1**, **C2**, and **C2b**, Fig. 1b), the compounds **17**–**39**—with the exception of the non-inhibitors—were first superimposed on compounds **20** and **21** with Sybyl by a three-point-fit strategy using the very same atoms for *E*-(red) and *Z*-(green) 3-pyridyl substituted tetrahydronaphthalenes and -indanes (Fig. 1a).[15]

The naphthalene inhibitors (**66**–**69**, **72**–**74**, and **76**–**78**; yellow, Fig. 1a) were aligned with both *E*- and *Z*-(3-pyridyl)methylene-indanes and -tetrahydronaphthalenes, respectively.[16,17] These naphthalenes as well as the *E*- and *Z*-imidazolyl substituted tetrahydronaphthalene and -indane inhibitors (not shown) fitted very well into the pharmacophore (Fig. 1b). The superimposition of non-inhibitors—using the same procedure as applied for the inhibitors—revealed that the corresponding substituents did not match with the model (Table 2).

The pharmacophore model (Fig. 1b) was built on four pharmacophore points, chosen on the basis of the global superimposition (Fig. 1):

- **N**: heterocyclic nitrogen (all inhibitors).
- **C1**: ring centroid (*E*-(3-pyridyl)methylene-tetrahydronaphthalenes and -indanes and naphthalene derivatives).
- **C2**: ring centroid (*E*- and *Z*-(3-pyridyl)-methylene-indanes and -tetrahydronaphthalenes).
- **C2b**: ring centroid (*Z*-(3-pyridyl)methylene-tetrahydronaphthalenes and -indanes and naphthalene derivatives).

Each compound fits with three of these points and has nitrogen **N** in common. The lone pair of the heterocyclic nitrogen of all compounds has to point in almost the same direction (Fig. 1a) for complexing the heme iron. The three ring centroids (**C1**, **C2**, and **C2b)** are located in one plane, **P0**, forming a planar three-ring-system (ace-naphthene or dihydro-phenalene), while the aromatic nitrogen **N** is located slightly above plane **P0** (see geometric parameters, Table 3). Actually, the whole pharmacophore seems to be quite planar.

The steric features of active and non-active CYP11B2-inhibitors were further explored by the 'steric inclusion area (SIA)' and the 'steric exclusion area (SEA),' respectively (Figs. 2a and b). The SIA is mainly located in the region of substituents R3, R4, and R8 (see Table 2). Larger groups as formic acid methyl ester can only be introduced in position R4 (**72**), whereas position R8 is of limited size. The SEA is located at the non-aromatic ring and the exocyclic double bond of the *Z*-isomer (substituents R1 and R2, Table 2). Additionally, large substituents as benzyloxy- or 3-pyridyl-groups in the region of R4 and R5 are not suitable for proper inhibitor binding (**32** and **79**). The SEA is also located close to R9, since methyl-groups lead to a strong decrease of activity (**34**).

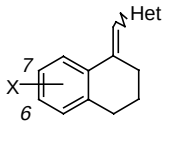
To validate the model, a compound was generated as a hybrid structure of the used *E*- and *Z*-isomers as well as the naphthalene compounds, the acenaphthene derivative (**A**). Compound **A** was synthesized in four steps: nitration of acenaphthene[18] and subsequently hydrogenation[19] leading to a mixture of two isomers: 3- and 5-aminoacenaphthene. In the following Sandmeyer reaction, the bromo derivatives were formed and used for Suzuki coupling with 3-pyridine boronic acid. The resulting mixture was subsequently chromatographed and the isomers **A** and **B** were isolated (Scheme 1).

Both isomers were tested for activity in V79 cells,[12] expressing human CYP11B2.[6] The IC_{50} values of the compounds are given in Table 4.

As expected compound **A** exhibited strong inhibitory activity (IC_{50} = 10 nM), thus confirming the validity of the pharmacophore model. Surprisingly the isomer **B** was also very potent. This can be explained by the alignment of the acenaphthene derivatives **A** and **B** (Fig. 3). The compounds display a very similar shape; they only differ in the position of the non-aromatic cyclopentene-ring.

After having discovered the acenaphthene isomers as highly potent lead compounds, we wanted to know about their selectivity toward CYP11B1 (Table 4).

Table 1. Inhibitors and non-inhibitors of aldosterone synthase (CYP11B2)



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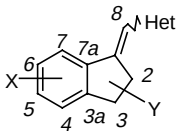
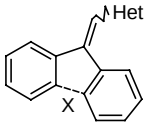


3, 4, 8, 9, 11-18, 20-26, 29, 31-49, 52, 53, 55-58, 63-65



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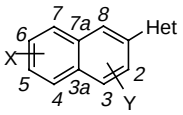


66-81

Compound	X,Y	Het[a]	Isomer	IC_{50}[b] [nM]
1	H	Im	*E*	25
2	H	Im	*Z*	10
3	H	Im	*E*	41
4	H	Im	*Z*	11
5	7-CN	Im	*E*	>500
6	7-CN	Im	*Z*	13
7	6-CN	Im	*Z*	23
8	5-CN	Im	*E*	36
9	5-CN	Im	*Z*	36
10	7-Cl	Im	*E*	47
11	5-F	Im	*E*	17
12	5-F	Im	*Z*	14
13	5-Cl	Im	*E*	89
14	5-Cl	Im	*Z*	4
15	5-Br	Im	*E*	93
16	5-Br	Im	*Z*	10
17	H	3-Py	*E*	11
18	H	3-Py	*Z*	92
19	H	3-Py	*E*	22
20	5-F	3-Py	*E*	7
21	5-F	3-Py	*Z*	11
22	5-Cl	3-Py	*E*	26
23	5-Cl	3-Py	*Z*	73
24	5-Br	3-Py	*E*	37
25	5-OMe	3-Py	*E*	34
26	5-OMe	3-Py	*Z*	26
27	6-OMe	3-Py	*E*	57
28	6-OMe	3-Py	*Z*	878
29	6-OMe	3-Py	*E*	>500
30	6,7-diOMe	3-Py	*E*	>500
31	5-OEt	3-Py	*E*	79
32	5-OBn	3-Py	*E*	>500
33	6-Me	3-Py	*E*	>500
34	6-Me	3-Py	*Z*	>500
35	4-Me	3-Py	*Z*	>500
36	4-F	3-Py	*E*	21
37	4-Cl	3-Py	*E*	9
38	4-Cl	3-Py	*Z*	31
39	7-OMe	3-Py	*E*	27
40	H	4-Py	*E*	>500
41	H	4-Py	*Z*	931
42	5-F	4-Py	*E*	1098
43	5-F	4-Py	*Z*	34
44	5-Cl	4-Py	*E*	1515
45	5-Cl	4-Py	*Z*	301
46	5-Br	4-Py	*E*	2640
47	5-Br	4-Py	*Z*	484
48	5-OMe	4-Py	*E*	>500
49	5-OMe	4-Py	*Z*	>500
50	6-OMe	4-Py	*E*	>500
51	6-OMe	4-Py	*Z*	>500
52	6-OMe	4-Py	*E*	>500
53	6-OMe	4-Py	*Z*	>500
54	6,7-diOMe	4-Py	*E*	>500
55	6-Me	4-Py	*E*	>500

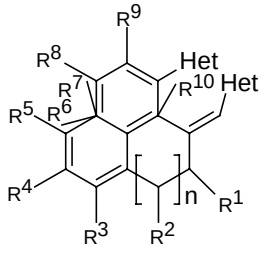
(*continued on next page*)

Table 1 (*continued*)

Compound	X,Y	Het[a]	Isomer	IC_{50}[b] [nM]
56	6-Me	4-Py	*Z*	>500
57	5-F	Pyrim	*E*	27
58	5-F	Pyrim	*Z*	>500
59	bond	3-Py	—	>500
60	no bond	3-Py	—	>500
61	bond	4-Py	—	>500
62	no bond	4-Py	—	>500
63	3-Me	3-Py	*E*	>500
64	3-Me	3-Py	*Z*	>500
65	3-Phenyl	3-Py	*E*	>500
66	H	3-Py	—	28
67	5-OH	3-Py	—	23
68	5-OMe	3-Py	—	6
69	5-OEt	3-Py	—	12
70	5-OPr	3-Py	—	>500
71	5-OBn	3-Py	—	>500
72	5-COOMe	3-Py	—	72
73	5-Br	3-Py	—	15
74	5-CN	3-Py	—	3
75	6-OMe	3-Py	—	>500
76	4-Cl, 5-OMe	3-Py	—	13
77	4-Cl, 5-OMe, 8-Cl	3-Py	—	28
78	4-Br, 5-OMe	3-Py	—	33
79	4-(3-Pyridyl)	3-Py	—	>500
80	2,3-Benzene-annelated	3-Py	—	>500
81	5-OMe	4-Py	—	>500

[a] Heterocycle: Im, imidazole; 3-Py, 3-pyridine; 4-Py, 4-pyridine; Pyrim, pyrimidine.
[b] Activity determined in V79MZh 11B2 cells as described.[9–11]

Table 2. Substitution patterns of inhibitors and non-inhibitors of CYP11B2



n=0,1

Inhibitors: Het= 3-Pyridine, Imidazole, Pyrimidine
Non-Inhibitors: Het= 4-Pyridine

R	Appropriate substituents	Inappropriate substituents
R^1	H, CH_3	Phenyl, anellated rings (R^1 to R^2)
R^2	—	—
R^3	H, F, Cl, CH_3, OMe	—
R^4	H, F, Cl, Br, CN, OH, OMe, OEt, COOMe	OPr, OBn
R^5	H, Cl, Br	Me, OMe, CN, 3-Pyridyl
R^6	H, Cl	—
R^7	H, OMe	—
R^8	H, F, Cl, Br, CN, OMe	Anellated rings (R^8 to R^9)
R^9	H, CN	Me
R^{10}	Cl	—

Both inhibitors showed little inhibition of CYP11B1, which makes them interesting candidates for further development.

Table 3. Geometric parameters of the pharmacophore

Distances (Å)		Angles (°)		Angles respect to the planes (°)	
C1–C2	2.0–2.3	**C1–N–C2b**	12.5–15	**P0–P1**	13.3
C1–C2b	2.2–2.4	**C1–N–C2b**	10–12	**P0–P2**	11.9
C2–C2b	2.1–2.3	**C2–N–C2b**	23.5–25	**P1–P2**	157.8
C1–N	7.1–8.1				
C2–N	5.2–6.0				
C2b–N	5.2–6.4				

where:

- **P0**: plane containing **C1**, **C2**, and **C2b**;
- **P1**: plane containing **C1**, **N**, and **C2**;
- **P2**: plane containing **C1**, **N**, and **C2b**;
- **P3**: plane containing **C2**, **N**, and **C2b**.

Summarizing, the first pharmacophore model for inhibitors of aldosterone synthase (CYP11B2) was built by superimposition of a series of compounds synthesized by our group. This pharmacophore could be confirmed by synthesis and biological evaluation of two hybrid compounds derived from the model.

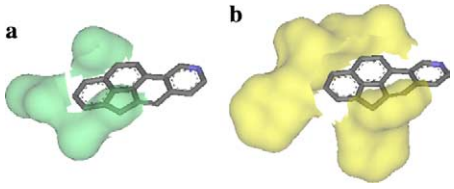
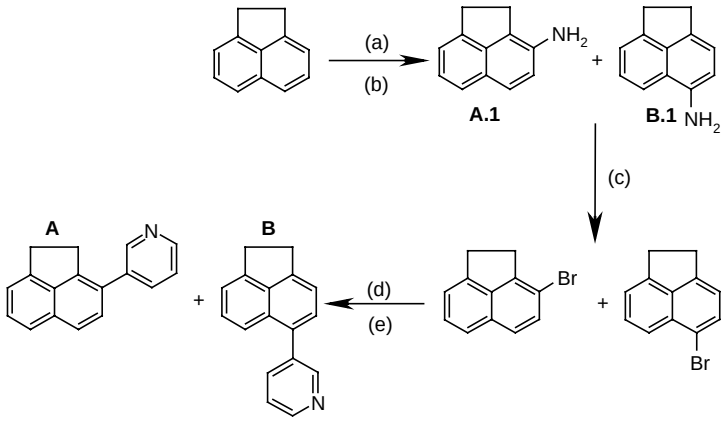


Figure 2. Unsubstituted hybrid-structure with: (a) the steric inclusion area (SIA); (b) the steric exclusion area (SEA).



Scheme 1. Reagents and conditions: (a) HNO_3, in acetic anhydride, 20 h at 10 °C; (b) H_2, Pt/C (5%), in THF; (c) 1—$NaNO_2$, in HBr, 0 °C, 2—CuBr, in HBr/toluene, 0 °C, addition of the diazonium salt, 10 min at 0 °C, 2 h at 100 °C; (d) Na_2CO_3-solution, 3-pyridine-boronic acid, in methanol, tetrakis(triphenyl-phosphin)palladium, N_2, reflux, 12 h; (e) separation of **A** and **B** using column chromatography.

Table 4. Biological evaluation of hybrid compounds **A** and **B**

Compound	IC_{50} value[a] (nM)		Selectivity[d]
	CYP11B1[b]	CYP11B2[c]	
A	2452	10	245
B	2896	14	207

[a] Mean value of four determinations, standard deviation less than 20%.
[b] Hamster fibroblasts (V79 cells) expressing human CYP11B1; substrate deoxycorticosterone, 100 nM.
[c] Hamster fibroblasts (V79 cells) expressing human CYP11B2; substrate deoxycorticosterone, 100 nM.
[d] IC_{50} CYP11B1/IC_{50} CYP11B2.

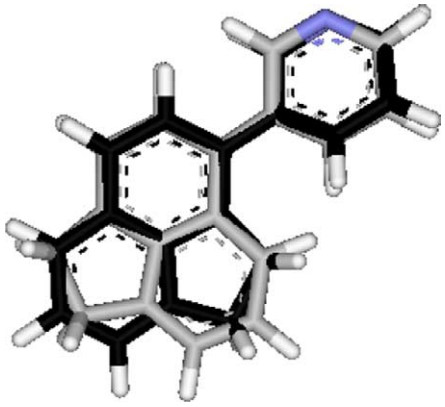


Figure 3. Alignment of hybrid compound **A** (black) and its isomer **B** (gray).

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.2005.09.059.

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15. Alignment was performed as follows: *E*-(3-pyridyl) methylene-indanes and -tetrahydronaphthalenes (aromatic

nitrogen **N**, centroid **C2**, C7a or C8a, respectively), Z-(3-pyridyl)methylene-indanes and -tetrahydronaphthalenes (aromatic nitrogen **N**, centroid **C2**, C3a or C4a, respectively).
16. Alignment was performed as follows: *E*-(3-pyridyl) methylene-indanes and -tetrahydronaphthalenes (aromatic nitrogen **N**, centroid **C1**, C7a or C8a, respectively), 3-pyridyl substituted naphthalenes (aromatic nitrogen **N**, centroid **C1**, C7a).
17. Alignment was performed as follows: *Z*-(3-pyridyl) methylene-indanes and -tetrahydronaphthalenes (aromatic nitrogen **N**, centroid **C2b**, C3a or C4a, respectively), 3-pyridyl substituted naphthalenes (aromatic nitrogen **N**, centroid **C2b**, C7a).
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