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Synthesis and biological analysis of benzazol-2-yl piperazine sulfonamides as 11β-hydroxysteroid dehydrogenase 1 inhibitors

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ABSTRACT

In the last decade the inhibition of the enzyme 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) emerged as a promising new strategy to treat diabetes and several metabolic syndrome phenotypes. Using a molecular modeling approach and classical bioisosteric studies, we discovered a new class of 11β-HSD1 inhibitors bearing an arylsulfonylpiperazine scaffold. Optimization of the initial lead resulted in compound **11** that selectively inhibits 11β-HSD1 (IC_{50} = 0.7 μM).

In humans both circulating and tissue-specific levels of glucocorticoid (GC) hormones (active cortisol and inactive cortisone) are highly regulated. On a tissue- and cell-specific level, the interconversion of cortisol and cortisone is catalyzed by two isoenzymes of 11β-hydroxysteroid dehydrogenase (11β-HSD). The NADPH-dependent 11β-HSD1 functions predominantly as a reductase enzyme that is highly expressed in metabolic tissues, including the adipose tissue and the liver.[1] The oxidase enzyme 11β-HSD2 is dependent on the cofactor NAD^+ and is mainly expressed in the kidney and the colon.[2] GC hormones are key metabolic regulators. High GC levels have been implicated in the pathogenesis of diabetes and several phenotypes of metabolic syndrome. It has been demonstrated using transgenic mice that specific overexpression of 11β-HSD1 in adipose tissue causes visceral obesity, insulin-resistant diabetes and hyperlipidemia.[3] On the other hand, 11β-HSD1 knockout mice were resistant to the development of high-fat diet induced diabetes. Furthermore, 11β-HSD1 knockout mice showed improved glucose tolerance and insulin sensitivity,[4] suggesting 11β-HSD1 inhibition as a potential approach to treat metabolic diseases. However, inhibition of renal 11β-HSD2 leads to cardiovascular complications such as hypertension. For this reason, therapeutic 11β-HSD1 inhibitors should be highly selective over this isoenzyme.[5] Several potent 11β-HSD1 inhibitors have been described in the literature, for example, the natural triterpenoid glycyrretinic acid **1**,[6] and, among other highly selective inhibitors,[7] the synthetic adamantyl triazole (Merck-544) **2**[8] and 2-aminothiazole sulfonamide **3** (Fig. 1).[9]

We applied a previously developed common feature pharmacophore model for 11β-HSD1 inhibitors[10] for a virtual screening of our in-house compound library. Compound **12** (2-[4-(4-*tert*-

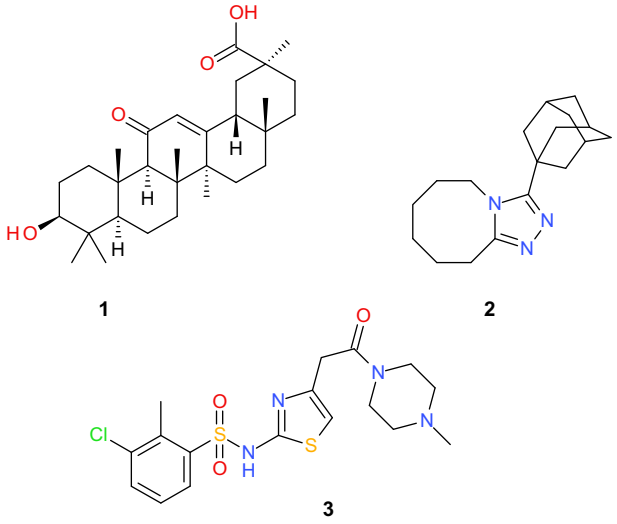

Figure 1. Exemplary 11β-HSD1 inhibitors.

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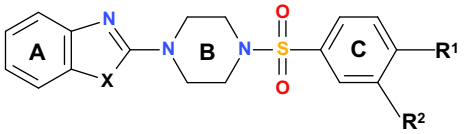
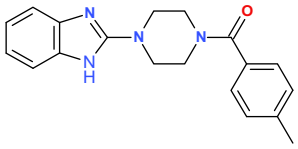
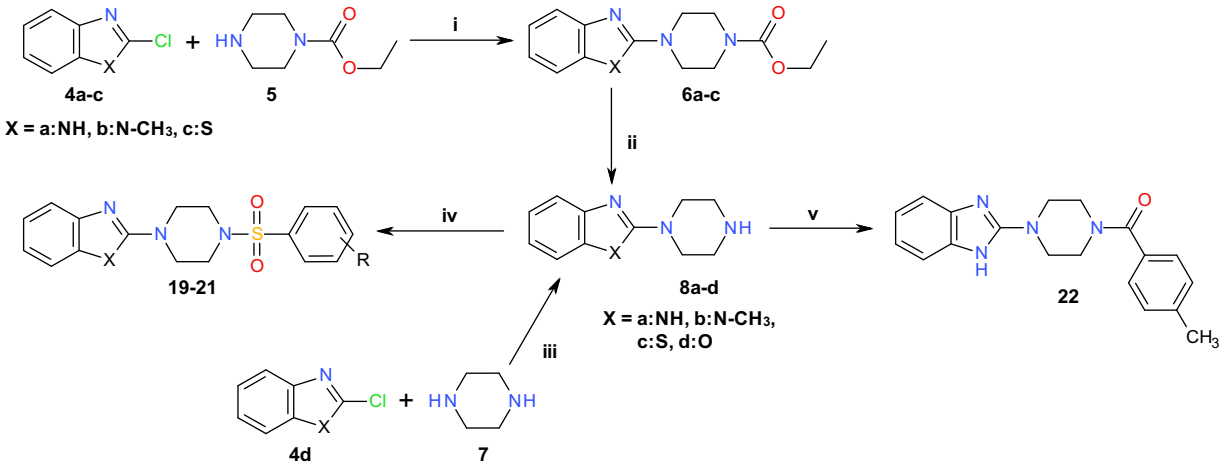
Table 1
Inhibition of 11β-HSD1-dependent conversion of cortisone to cortisol by the selected compounds



Compound	X	R^1	R^2	Residual activity at 20 μM (%)	IC_{50} (μM)
9	NH	$-CH_3$	–H	50	n.d.
10	N-CH_3	–H	–H	–	6.4 ± 0.6
11	N-CH_3	$-CH_3$	–H	–	0.7 ± 0.2
12	N-CH_3	–*t*-But	–H	–	4.8 ± 1.4
13	N-CH_3	$-OCH_3$	–H	–	4.2 ± 2.2
14	N-CH_3	$-OCH_3$	$-OCH_3$	–	18.5 ± 7.7
15	N-CH_3	$-OCH_3$	$-NO_2$	–	27.2 ± 11.0
16	N-CH_3	–COOH	–H	90	n.d.
17	N-CH_3	–H	–COOH	75	n.d.
18	N-CH_3	–CO(NHOH)	–H	90	n.d.
19	N-CH_3	–H	–CO(NHOH)	60	n.d.
20	O	–*t*-But	–H	18[b]	n.d.[a]
21	S	$-CH_3$	–H	89	n.d.
22				65	n.d.

[a] Insoluble in DMSO and DMSO/methanol (1:1).
[b] Unknown concentration because of the solubility; n.d.: not determined. Data represent mean ± SD from at least three independent experiments.



Scheme 1. Reagents and conditions: (i) 125 °C, neat, (81–97%); (ii) 48% HBr, 70 °C, (90%); (iii) DCM, TEA, 0 °C; (iv) DCM, TEA, substituted phenylsulfonyl chlorides, 0 °C (1 h), then rt; (v) DCM, TEA, **8a**, 4-methylbenzoyl chloride, 0 °C (1 h), then rt.

butylphenyl)sulfonylpiperazin-1-yl]-1-methyl-benzimidazole, Table 1) was recognized as a virtual hit. In the subsequent 11β-HSD1 enzyme inhibition assay performed as described earlier[11] it exhibited an IC_{50} of 4.8 μM (Table 1). Based on the result obtained we synthesized novel analogues of**12**, whereby the *tert*-butyl moiety was replaced by various electron donating or withdrawing functionalities. Furthermore, we investigated the effects of replacement of the 1-methyl benzimidazole ring by the isosteric heterocycles benzimidazole, benzoxazole and benzothiazole. Here, we report on the synthesis of these compounds and their inhibitory effects on 11β-HSD1 activity.

The general approach utilized for the synthesis of the target compounds is illustrated in Scheme 1. In analogy to Shafik et al.,[12] 2-chlorobenzazoles **4a–c** and N-ethoxycarbonylpiperazine (**5**) were heated together at 125 °C for 1–5 h. This nucleophilic N-substitution reaction provided**6a–c** in high yields. 1-Methyl-2-chlorobenzimidazole (**4b**) was synthesized according to a reported procedure.[13] Heating compounds **6a–c** at 70 °C with 48% aqueous HBr resulted in hydrolysis and decarboxylation to provide the piperazine derivatives **8a–c**. 2-Piperazin-1-yl-benzoxazole (**8d**) was synthesized by the treatment of 2-chlorobenzoxazole (**4d**) with piperazine hydrate (**7**) in CH_2Cl_2 at 0 °C.[14] The benzazolylpiperazine derivatives **8a–d**

afforded the target compounds **9**–**21** in high yields by reaction with the appropriate substituted phenylsulfonylchlorides. Compound **22** was obtained in high yield by reacting 4-methyl benzoylchloride with 2-piperazin-1-yl-benzimidazole.

All the compounds were characterized by IR and ^{1}H NMR spectroscopy. The purity of the compounds was determined by elemental analyses and the data for C, H, N are within ±0.4% of the calculated values.

11β-HSD1 inhibitory activities of the synthesized compounds were first evaluated at 20 μM in a cell-free assay (Table 1). At this concentration, compounds **16**, **18** and **21** did not reduce 11β-HSD1 activity, and compounds **9**, **17**, **19** and **22** showed 50% or less inhibition at 20 μM. Among compounds**10**–**15**, bearing a 1-methyl benzimidazol-2-yl moiety, **14** and **15** turned out to be weak inhibitors of the enzyme, whereas compounds **10**–**13** were potent inhibitors with IC_{50} of 0.7 ± 0.2–6.4 ± 0.6 μM. The nature of the substituent on the phenyl ring within this class of compounds revealed that the 4-methyl functionality (**11**) was the most favourable (IC_{50} = 0.7 ± 0.2 μM). Exchanging the methyl for a *tert*-butyl (**12**, IC_{50} = 4.8 ± 1.4 μM) or methoxy (**13**, IC_{50} = 4.2 ± 2.2 μM) in the *para*-position results in a reduction of activity by a factor of ~5, both of these compounds were found to be equipotent. Compared to the mono-methoxy compound **13**, a dimethoxy substitution (compound **14)** led to further reduction in 11β-HSD1 inhibition by a factor ~4 (Table 1). Compounds bearing an acidic (**16** and **17**) or highly polar (**18** and **19**) functionalities showed no or very low inhibitory effect at 20 μM. Another important observation from the biological data is that the replacement of the 1-methyl benzimidazol-2-yl by the isosteric benzimidazol-2-yl (**9**) or a benzothiazol-2-yl (**21**) ring, albeit they share the same substituent (methyl), results in compounds with weak or no inhibitory effect on 11β-HSD1 activity. Replacement by the benzoxazol-2-yl (**20**) resulted in a poorly soluble derivative that was not further investigated. Compound **22**, which bears a carbonyl functionality instead of a sulfone showed weak inhibitory activity. Assessment of 11β-HSD2 activity (performed as described earlier[11]) revealed that none of the compounds analysed in the present study inhibited this enzyme at a concentration of 20 μM.

The biological evaluation of the newly synthesized compounds revealed that electron donating substituents (e.g., methyl, methoxy or *tert*-butyl groups) in the benzene moiety are tolerated by the enzyme, whereas acidic or electron withdrawing groups are unfavourable for the inhibitory activity. To rationalize the biological data, we predicted binding modes for all compounds that were tested in the 11β-HSD1 assay using molecular docking. For the docking, an X-ray crystal structure, where the co-crystallized ligand belongs to the same chemical scaffold as our compounds was chosen[15] (PDB-code 3czr). The docking studies were performed using GOLD,[16,17] that uses a genetic algorithm for predicting the binding orientations. The binding site was defined as a 7 Å sphere, centered by the hydroxyl-oxygen of Tyr-177. To ensure the optimal ligand positioning, a hydrogen bond constraint between the ligand and backbone nitrogen of Ala-172 was set. GoldScore was used as a scoring function. The program was allowed to terminate the docking run in case the best ranked solutions were within 1 RMSD of each other, otherwise ten binding modes were predicted. With these settings, the program reproduced the binding orientation of the cocrystallized ligand, thus validating the docking settings. All of the active compounds **9**–**15** and **20** in their best-ranked poses aligned well with the cocrystallized ligand. In view of the predicted binding orientation, the hydrophobic substituents of the compounds occupied a hydrophobic pocket located next to the cofactor (Fig. 2A). Nonpolar substituents formed hydrophobic interactions with the enzyme (Fig. 2B and C). Therefore, the inactivity of the compounds with acidic or electron withdrawing substituents could be caused by a lack of favourable interaction

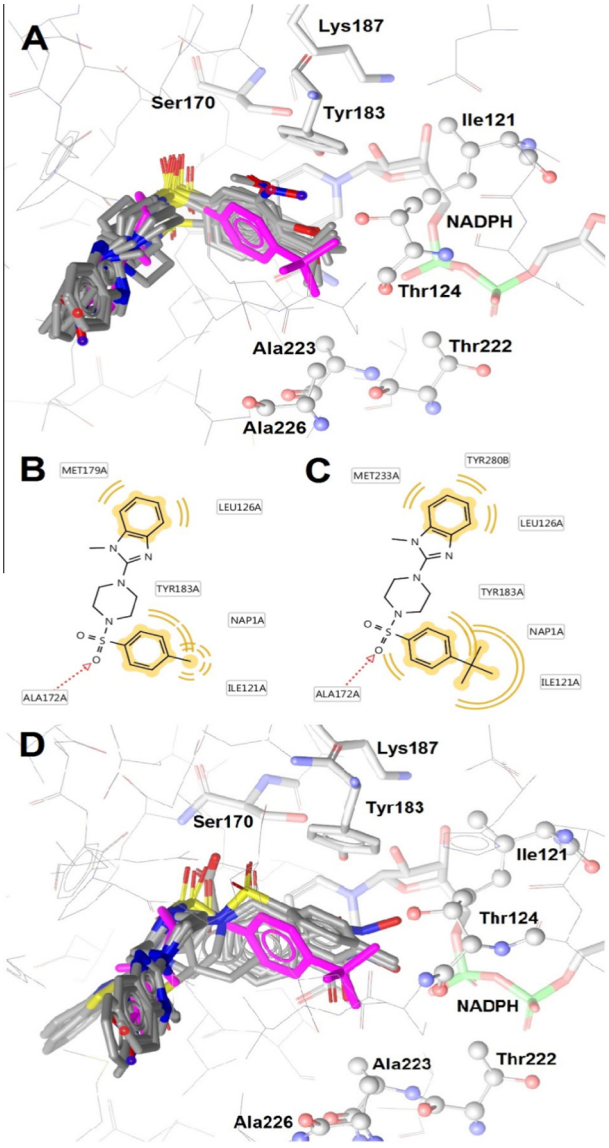


Figure 2. The predicted binding orientations of the active compounds (**9**–**15** and **20**, A), ligand–protein interactions of compounds **11** (B) and **12** (C), and binding orientations of the inactive compounds (D). The active compounds (grey) align with the cocrystallized ligand (magenta) and occupy a hydrophobic pocket formed by Ile-121, Thr-124, Thr-222, Ala-223 and Ala-226 (depicted in ball and stick style). Inactive compounds **21** and **22** do not align with the cocrystallized ligand and stick out of the binding pocket. Other inactive compounds have polar substituents in the hydrophobic pocket, suggesting unfavourable interactions. The catalytic triad Ser-170–Tyr-183–Lys-187 and the cofactor are highlighted in stick style. In the 2D-representations, the ligand–protein interactions are colour-coded as following: hydrophobic interaction—yellow, hydrogen bond acceptor—red arrow.

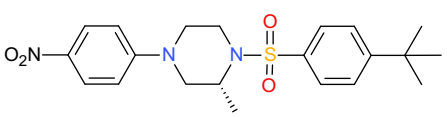


Figure 3. Compound **45** (2*R*-1-(4-*tert*-butylphenyl)sulfonyl-2-methyl-4-(4-nitrophenyl)piperazine).[15]

partners in this area. In addition, the inactive compounds with nonpolar substituents (compounds **21** and **22**) have a different binding orientation (Fig. 2D), and stick out of the binding pocket.

Previously, Sun et al.,[15] have described synthesis- and SAR-studies focusing on a similar 11β-HSD1 inhibitor scaffold.

They reported compounds where the A-part of the molecule (Table 1) bears either a 2-pyridyl or a substituted phenyl residue. In our studies, we replaced the A-part of the molecule with 2-benzazolyl rings. Although the compounds described in the present study were found to be 11β-HSD1 inhibitors, our best compound (**11**) was 300-fold less active than compound **45** (Fig. 3) reported by Sun et al.

Considering our results we can conclude that hydrophobic groups in the 4-position of the phenyl ring (C-part) was advantageous for 11β-HSD1 inhibition. Within this group the highest activity was gained by a 4-methyl substituent. In view of improving the activity of our compounds, it is planned to synthesize compounds where the A-part of compound **11** is substituted with various electron donating or electron withdrawing groups.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.07.047.

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