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Synthesis and biological evaluation of sulfonamidooxazoles and β-keto sulfones: selective inhibitors of 11β-hydroxysteroid dehydrogenase type I

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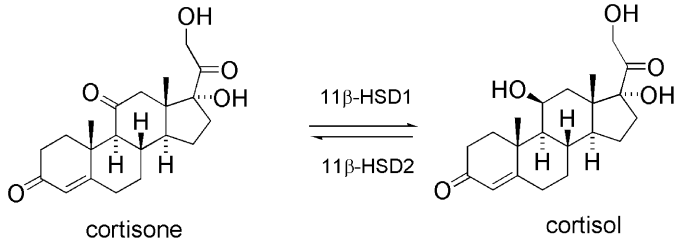
Abstract—The design, synthesis, and biological evaluation of arylsulfonamidooxazoles as 11β-HSD1 inhibitors and the serendipitous discovery of β-keto sulfones as potent 11β-HSD1 inhibitors are described here. These two classes of compounds are not active against 11β-HSD2 and therefore may have significant therapeutic potential for metabolic syndrome, type 2 diabetes and related metabolic dysfunctions.

Metabolic disorders, such as obesity and type 2 diabetes, have assumed epidemic proportions and present major challenges for healthcare systems.[1–3] The dramatic increase in the prevalence of obesity in recent years has led to an increased recognition of 'metabolic syndrome', a collection of metabolic and cardiovascular abnormalities, that is a precursor to type 2 diabetes. Metabolic syndrome is characterized by abdominal obesity, impaired glucose tolerance, dyslipidemia, low high density lipoprotein (HDL) cholesterol, and hypertension.[4,5] Several recent investigations have implicated aberrant glucocorticoid receptor (GR) signaling in the development of several phenotypes associated with metabolic syndrome. Glucocorticoid hormones are key metabolic regulators. The major activator of the GR in humans is cortisol and the adrenal cortex is the major source of circulating cortisol. Recent evidence suggests that GR signaling depends not only on the circulating cortisol levels, but also on the intracellular generation of cortisol through reduction of the inactive glucocorticoid, cortisone. The reduction reaction is catalyzed by 11β-hydroxysteroid dehydrogenase type I (11β-HSD1) with the concomitant oxidation of NADPH, while cortisone itself is generated by the action of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) on cortisol using NAD as a cofactor (Scheme 1).[6]



Scheme 1. Interconversion of cortisone and cortisol by 11β-HSD types 1 and 2 enzymes.

Keywords: Inhibitor; 11β-HSD1; Sulfonamidooxazole; β-Keto sulfone.
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These two enzymes have very different expression profiles: 11β-HSD1 is highest in liver, adipose, and the central nervous system, whereas 11β-HSD2 is expressed in kidney, colon, and other tissues where mineralocorticoid receptor (MR) signaling is important. The MR is activated by aldosterone but it can also bind cortisol with similar affinity. 11β-HSD2 functions to reduce local cortisol concentrations, thereby preventing illicit activation of MR by cortisol. Disruption or mutations in the 11β-HSD2 gene result in sodium retention, hypokalemia, and hypertension because of inappropriate glucocorticoid occupation of the mineralocorticoid receptor in the kidney.[7]

A potential role for 11β-HSD1 inhibitors in metabolic disease in vivo has been demonstrated using a transgenic mouse approach. Over expression of 11β-HSD1 predominantly in adipose tissue shows several features similar to those observed in patients with metabolic syndrome. Transgenic mice over expressing 11β-HSD1 in the liver have also been generated and while they are not obese they do show several features of metabolic syndrome.[8] Inactivation of the 11β-HSD1 gene in mice confers resistance to diet-induced obesity and improves both insulin sensitivity and lipid profiles.[8] Administration of specific 11β-HSD1 inhibitors in mouse models of insulin resistance led to improved hyperglycemia and insulin sensitivity.[9,10] Recent studies with the non-specific 11β-HSD1 inhibitor carbenoxolone also show improved hepatic insulin sensitivity and decreased glucose production in humans.[11] However, the 11β-HSD2 inhibitory activity of carbenoxolone was a limiting factor because it induces renal mineralocorticoid excess at higher doses. Thus, while inhibition of 11β-HSD1 is an attractive strategy for the design of therapeutics for metabolic syndrome and type 2 diabetes, it is obvious that an inhibitor against 11β-HSD1 must not affect 11β-HSD2.

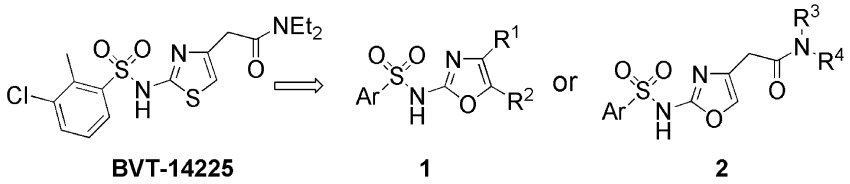
Our goal is to develop novel, selective, potent, and orally bioavailable 11β-HSD1 inhibitors. Presented herein is the design, synthesis, and biological evaluation of arylsulfonamidooxazoles as 11β-HSD1 inhibitors and the serendipitous discovery of β-keto sulfones as potent 11β-HSD1 inhibitors. These two classes of compounds are not active against 11β-HSD2 and therefore may have significant therapeutic potential in metabolic syndrome, type 2 diabetes and related metabolic dysfunctions.

Arylsulfonamidothiazole compounds from Biovitrum[9] have been shown to be selective human 11β-HSD1 inhibitors. For example, **BVT-14225** (Scheme 2) was reported to have IC_{50} of 52 nM against 11β-HSD1 in their microsomal enzyme assay and to be 1000-fold less potent against 11β-HSD2. Since then, many patents and presentations related to selective 11β-HSD1 inhibition have become public.[12a–d] Our screening approach was to use a cell-based assay to identify active hits, this ensures that active compounds will also be cell permeable and potentially more drug-like. Detailed examination of the initial Biovitrum patents indicated that their claims were very specific to the thiazole core template. We targeted sulfonamidoxazoles of the general structures **1** or **2** hoping to maintain potency while potentially improving the physicochemical properties such as solubility. Previous experience suggested that oxazole analogs have better water solubility than their thiazole counterparts.

It is easy to envisage that a common intermediate **3** would be beneficial because it could be used to build a library of analogs useful for an initial SAR investigation on both sides of the oxazole ring (Scheme 2). Interestingly, while the synthesis of simple 2-aminooxazoles is well established via the condensation between urea and 2-bromo-1-substituted ethanone[13] or through condensation of a cyanamide and a 2-hydroxy ketone,[14] there are very few publications describing the direct synthesis of 4-[2-aminooxazole]acetic acid **3**.[15]

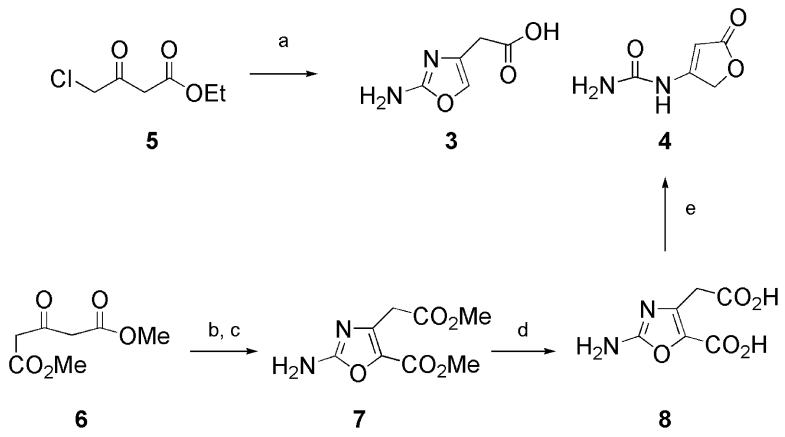
Direct preparation of intermediate **3** through the condensation of urea and ethyl 4-chloroacetoacetate was low yielding (~5%) due to the formation of undesired furanone **4** (1-(5-oxo-2,5-dihydrofuran-3-yl)urea) in 60% yield (Scheme 3). Our efforts to prepare intermediate **3** based on the procedures published by Tanabe and co-workers[15] were also accompanied by rearrangement to form the furanone **4** as the major product in 60% yield in addition to small amount (~5% yield) of desired **3** (Scheme 3). Compounds **3** and **4** have the same molecular formula, therefore unamibiguous structure assignment of furanone **4** was made using NMR techniques such as gHMBC, gCOSY, and gHSQC.[16]

Since a quick synthesis of desired common intermediate **3** turned out to be more complicated, we decided to prepare simple 2-aminooxazoles such as **10** and use them to prepare sulfonamidooxazoles for a SAR study (Scheme 4). Several 4-substituted 2-aminooxazoles have been prepared through the condensation of urea and 2-bromo-1-substituted ethanones in good to moderate yield. Subsequent sulfonylation in pyridine or with Hunig's base did not proceed at all due to the weak nucleophilicity of the amino group. Schotten–Baumann conditions did not improve the yield either. The desired sulfonamidooxazoles were finally obtained through

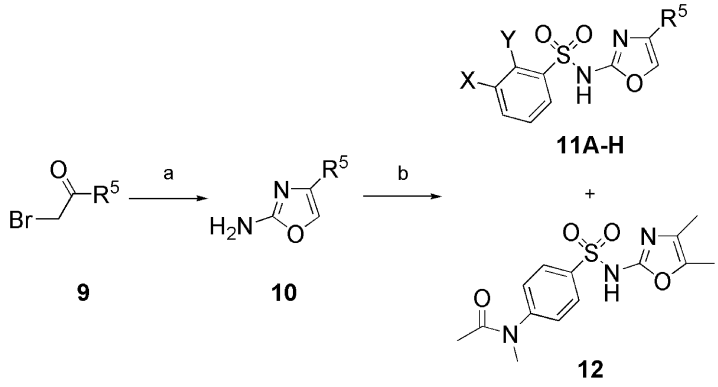


BVT-14225 1 2

Scheme 2. Proposed sulfonamidooxazole analogs.



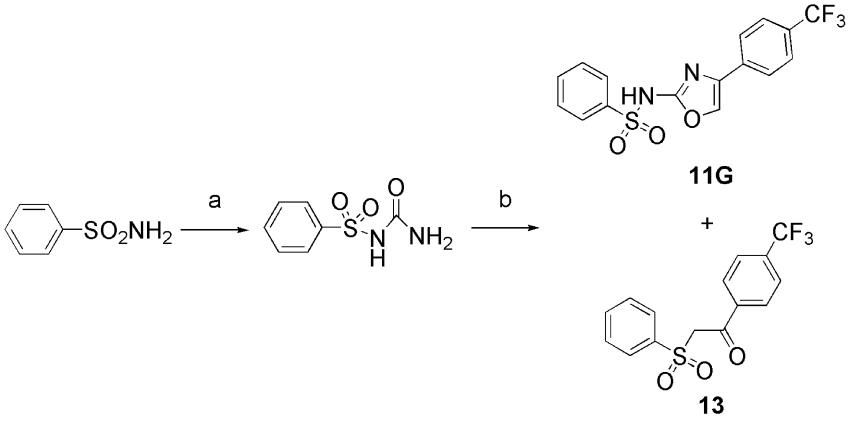
Scheme 3. Reagents and conditions: (a) urea, toluene reflux, DMF reflux, EtOH reflux, or NaBr/EtOH reflux; (b) Br_2, CH_2Cl_2, 0 °C (76%); (c) urea, EtOH reflux, 5 h (85%); (d) 1 N NaOH, 80 °C, 30 min (56%); (e) 1 N NaOH, 100 °C, 5 h (60%).



Scheme 4. Reagents and conditions: (a) urea, ethanol, reflux (65–75%); (b) NaH, $ArSO_2Cl$, THF, 0 °C (65–80%).

deprotonation of the amino proton with sodium hydride followed by sulfonylation with various arylsulfonyl chlorides (Scheme 4). Analogs such as **11A–H** and **12** were isolated in moderate to good yield.[17]

Analog **11G** was also prepared through an alternate route outlined in Scheme 5. Benzenesulfonyl urea was prepared in good yield by heating benzene sulfonamide and potassium isocyanate. The sulfonyl urea thus obtained was used directly in the following condensation with 4-(trifluoromethyl)phenacyl bromide. Analog **11G** was isolated in ~20% yield. The yield was quite poor so this was the only analog synthesized with this approach. However, an interesting difference in activity was observed when testing **11G** prepared from Schemes 4 and 5. Sulfonamidooxazole **11G** from the route shown



Scheme 5. Reagents and conditions: (a) KOCN, ethanol, 100 °C, 20 min irradiation in microwave; (b) 4-(trifluoromethyl)phenacyl bromide, 1:1 DMF/ethanol, 140 °C, 20 min irradiation in microwave.

Table 1. Biological activities of sulfonamidooxazoles **11A**–**H**, **12**, sulfonamidothiazole **BVT-14225**, and β-keto sulfone **13**

Compds	X	Y	R^5	11β-HSD1, IC_{50} (μM)[a,18]	11β-HSD2, IC_{50} (μM)[a,18]
11A	Cl	Me	Me	58.7	N/A
11B	Cl	CH_2CO_2Me	Me	>100	N/A
11C	Cl	CH_2CO_2H	Me	>100	N/A
11D	Cl	Me	Ph	17.3	>200
11E	Cl	Me	4-(CN)phenyl	48	>200
11F	H	H	Ph	23	>200
11G	H	H	4-(CF_3)phenyl	9	>200
11H	H	H	Biphenyl	2.3	>200
12[b]	N/A	N/A	N/A	100	>200
BVT-14225[c]	N/A	N/A	N/A	1.5	>200
13	N/A	N/A	N/A	0.19	>200

[a] Assay run in duplicate.
[b] Structure of **12** in Scheme 4.
[c] Structure of **BVT-14225** in Scheme 2.

in Scheme 5 had a 9 μM IC_{50} against 11β-HSD1, while the product prepared using Scheme 5 showed an IC_{50} of 1 μM. Careful examination of HPLC traces using different gradient and pH conditions revealed an additional small peak (10% based on UV) in the product from Scheme 5. Suspecting that this minor impurity might be a potent inhibitor and therefore responsible for skewing the true IC_{50} of analog **11G**, we set out to isolate this side product. A small amount of this minor product was finally isolated and the structure was determined to be β-keto sulfone **13** using NMR, HRMS, and IR. One possible explanation is that the technical grade of benzene sulfonamide employed contains a small amount of benzenesulfinic acid ammonium salt. Upon heating, benzenesulfinic acid ammonium salt might react with 4-(trifluoromethyl)phenacyl bromide to give β-keto sulfone **13**. Pure β-keto sulfone **13** was sent for biological testing and this hypothesis was proven correct.

The sulfonamidooxazole **11A** had only modest activity,[18] see Table 1. Placing polar groups such as ester and amide at left side benzene ring led to inactive compounds (**11B**,**C**, and **12**), suggesting that polar groups are not preferred at this site. However, variations to oxazole substituent R_5 revealed a site for activity enhancement. Simply replacing methyl group (**11A**) with a phenyl group, **11D**, gave a threefold increase in activity against 11β-HSD1. Placing a polar group such as cyano at this phenyl group decreased the activity, **11E**. The chloro and methyl substitution on the left side benzene ring was shown to not have a significant effect on potency, **11F**. Substituting a *para*-trifluoromethylphenyl at R_5 further improved the activity threefold, **11G**. The most potent compound from this series is **11H**, with biphenyl substitution at R_5, which showed an IC_{50} of 2.3 μM suggesting a lipophilic group in this position is strongly preferred.

Of equal importance, none of these arylsulfonamidooxazole analogs inhibit 11β-HSD2 at concentrations as high as 200 μM.[18] For analog **11H**, this translates to over 100-fold selectivity in cell based assays. As a starting point for further optimization, **11H** possesses low solubility, but good permeability and achieves reasonable levels of exposure after oral dosing (50 mg/kg) in mice, AUC of 6.23 μg h/mL.[19] This compound is currently being evaluated in various in vivo diabetes models, including the KKAy mouse and ob/ob mouse.

β-Keto sulfone **13**, isolated as minor side product from the preparation of sulfonamidooxazole **11G** when synthesized via Scheme 5, was also tested in our cell-based assay. As anticipated, this compound was a very potent inhibitor of human 11β-HSD1 (Table 1). This compound shows no activity against 11β-HSD2 at concentration as high as 200 μM, suggesting more than 1000-fold selectivity over the 11β-HSD2 isoform. A detailed SAR study of the β-keto sulfone series accompanied by pharmacological testing of analogs from this series is currently on-going. A similar structure, 2-oxo-ethanesulfonamide derivatives, was reported as 11β-HSD1 inhibitor recently.[12]

We are currently investigating the mechanism by which sulfonamidooxazoles and β-keto sulfones inhibit 11β-HSD1 activity. These studies may be especially interesting for the β-keto sulfone series as the ketone functionality in these compounds may mimic the role of the ketone moiety in the endogenous 11β-HSD1 substrate cortisone.

In summary, we described herein the design, synthesis, and biological evaluation of sulfonamidooxazoles as a novel class of selective and orally available 11β-HSD1 inhibitors. The design was based on a known 11β-HSD1 inhibitor pharmacophore. Limited SAR study suggested that lipophilic and bulky substituents on the oxazole ring can improve the activity. Compounds such as **11H** show activity in the μM range in a cell-based assay and >100-fold selectivity over 11β-HSD2. We also disclosed our serendipitous discovery of the β-keto sulfone, which is a very potent 11β-HSD1 inhibitor with an IC_{50} of 190 nM. β-Keto sulfone **13** has more than 1000-fold selectivity over 11β-HSD2.

Acknowledgements

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16. Structure determination of (1-(5-oxo-2,5-dihydrofuran-3-yl)urea) **4**: ^{1}H NMR (400 MHz, DMSO-d_6): 5.10 (s, 2H), 5.33 (s, 1H), 6.51 (br s, 2H), 9.60 (s, 1H). ^{13}C NMR (DMSO-d_6): 69.4, 91.3, 154.8, 163.6, 174.8. The number of carbons (5) and carbon types were confirmed through direct carbon observation and DEPT analysis. The chemical shifts of both urea nitrogens could be observed as ^{15}N HSQC correlations to their associated protons at 60 °C, and were consistent with this moiety. The correct furanone connectivities were observed in the ^{13}C HMBC, as were the connectivities between the NH proton of the urea and the methylene and methane carbons of the furanone. Finally, the NOE pattern as measured with GOESY was also consistent with the structure as shown.
17. All final compounds were characterized by ^{1}H NMR and either HRMS, LC/MS, or CHN.
18. CHO cells were stably transformed with plasmid containing the full-length sequence of human 11β-HSD1 (Locuslink i.d. 3290) to generate the CHO-HSD1 cell line.
CHO cells were stably transformed with plasmid containing the full-length sequence of human 11β-HSD2 (Locuslink i.d. 3291) to generate the CHO-HSD2 cell line. Non-transformed CHO cells do not have detectable 11β-HSD1 or 11β-HSD2 activity. Cell-based assays for 11β-HSD1 were performed using the CHO-HSD1 cell line. The effect of test article on 11β-HSD1 activity was assayed by determination of the conversion of radioactive cortisone to cortisol. In this assay, 50,000 CHO-HSD1 cells were plated in 24 well-plates and incubated overnight. The cells were washed once with ethylene glycol dimethyl ether and 197.5 μL ethylene glycol dimethyl ether was added to each well. Appropriate drug concentrations (2.5 μL) were added to each well and the cells were incubated for 30 min at 37 °C/5% CO_2. [1,2-^{3}H]-cortisone was diluted to a concentration of 200 nM in ethylene glycol dimethyl ether and 50 μL of the 200 nM solution was added to each well. Final cortisone concentration in the assay was 40 nM. Cells were incubated for 2 h at 37 °C/5% CO_2. At the end of the incubation, steroids in media were extracted with 3 vol of ethyl acetate. The organic phase was transferred to a fresh tube, dried, resuspended in 5 μL methanol, and spotted on a 60 Å silica TLC plate. The plate was run in 92% chloroform/8% ethanol. The plate was dried and scanned using an AR-2000 TLC Imaging Scanner (BioScan Inc., Washington DC). IC_{50} values were determined by plotting cortisol formed against inhibitor concentration and fitting the data to using Origin 7.0 software (Origin Lab Corporation, Northampton, MA).
Inhibition of 11β-HSD2 was determined using the same assay, except that the CHO-HSD2 line was used and [1,2-^{3}H]-hydrocortisone was used as the substrate.
19. Male C57 mice were orally dosed with the testing compound at a single dose of 50 mg/kg in 2% Tween 80 and 0.5% methyl cellulose. Plasma concentrations of the testing compound were measured by LC–MS/MS. The area under curve (AUC) was calculated by a non-compartmental method with WinNonlin Software (version 4.1).