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LETTER

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Synthesis and Evaluation of the Metabolites of AMG 221, a Clinical Candidate for the Treatment of Type 2 Diabetes

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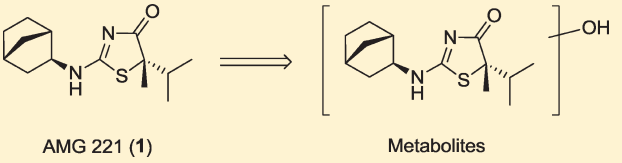
Supporting Information

ABSTRACT: All eight of the major active metabolites of (*S*)-2-((1*S*,2*S*,4*R*)-bicyclo[2.2.1]heptan-2-ylamino)-5-isopropyl-5-methylthiazol-4(5*H*)-one (AMG 221, compound **1**), an inhibitor of 11β-hydroxysteroid dehydrogenase type 1 that has entered the clinic for the treatment of type 2 diabetes, were synthetically prepared and confirmed by comparison with samples generated in liver microsomes. After further profiling, we determined that metabolite **2** was equipotent to **1** on human 11β-HSD1 and had lower in vivo clearance and higher bioavailability in rat and mouse. Compound **2** was advanced into a pharmacodynamic model in mouse where it inhibited adipose 11β-HSD1 activity.



KEYWORDS: AMG 221, 11β-HSD1 inhibitors, metabolites, clinical candidate

Since the publication of the Food and Drug Administration's Guidance for Industry: Safety Testing of Drug Metabolites in 2008 (FDA MIST guidelines),[1] there has been a renewed focus on studying the metabolites of clinical candidates during the drug development process throughout the pharmaceutical industry.[2] While the FDA MIST guidelines recommend that certain metabolites be subjected to additional safety studies, metabolites of clinical candidates are also needed for pharmacokinetic and pharmacological studies. In fact, there are examples where a metabolite, after being profiled further, replaced the parent compound as the drug candidate.[3,4] Therefore, the development of synthetic routes to supply sufficient amounts of metabolites for their complete characterization has become an important objective for many drug discovery and development programs.

Recently, we reported on the discovery of AMG 221 (**1**), an inhibitor of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) that has entered clinical trials for the treatment of type 2 diabetes.[5,6] During our development efforts, we discovered that **1** was metabolized into eight common oxidation products among rat, mice, dog, monkey, and human (**2**−**9**) (Chart 1). The norbornyl and isopropyl groups of **1** underwent metabolic oxidation to give hydroxylation products **2**, **3**, **5**−**8**, ketone **4**, and alkene **9**. For structure elucidation (see the Supporting Information for more details), these metabolites were isolated from in vitro dog/human liver microsomal incubations supplemented with NADPH or isolated from rat urine obtained following a single dose administration of AMG 221 (**1**). However, the bioactivity and pharmacokinetics of these metabolites could not be determined with the small amount of material generated from microsomes. Therefore, we set out to prepare these metabolites by synthetic methods so that we might more fully characterize these compounds and further confirm their structures.

Turning first to the synthesis of the metabolites formed on the norbornyl ring (**2**−**5**), we devised a route (Scheme 1) that allowed us to prepare three of these from a common intermediate **16**. We envisioned using chiral norbornyl diol **10**,[7] as an intermediate for the preparation of analogs **2**−**4**. Preparation of norbornyl diol **10** by Hiyashi's method[7] followed by monobenzylation delivered compound **11**. Compound **11** was treated with *tert*-butylchlorodiphenylsilane in DMF to give compound **12**, which was then debenzylated by hydrogenation to give compound **13**. The chirality of the hydroxyl group was inverted through a two step sequence by oxidation to the ketone, followed by reduction with L-selectride to give compound **14**. A Mitsunobu reaction with phthalimide followed by deprotection with hydrazine gave a primary amine.[8] Addition of benzoyl isothiocyanate to the amine followed by hydrolysis produced thiourea **15**. Heating thiourea **15** with ethyl 2-bromo-isovalerate in a microwave reactor delivered 5-isopropylthiazolone. Alkylation at the 5-position of the thiazolone was achieved with LDA and methyl iodide, and after removal of the TBDPS protecting group, the desired thiazolone **16** was obtained as a mixture of two diastereomers. The metabolite **2** was obtained through chiral supercritical fluid chromatography (SFC) separation of compound **16**. The *exo*-hydroxyl group of **16** was converted to the *endo*-hydroxyl group through oxidation and stereoselective reduction with L-selectride,[9] followed by chiral SFC separation to give

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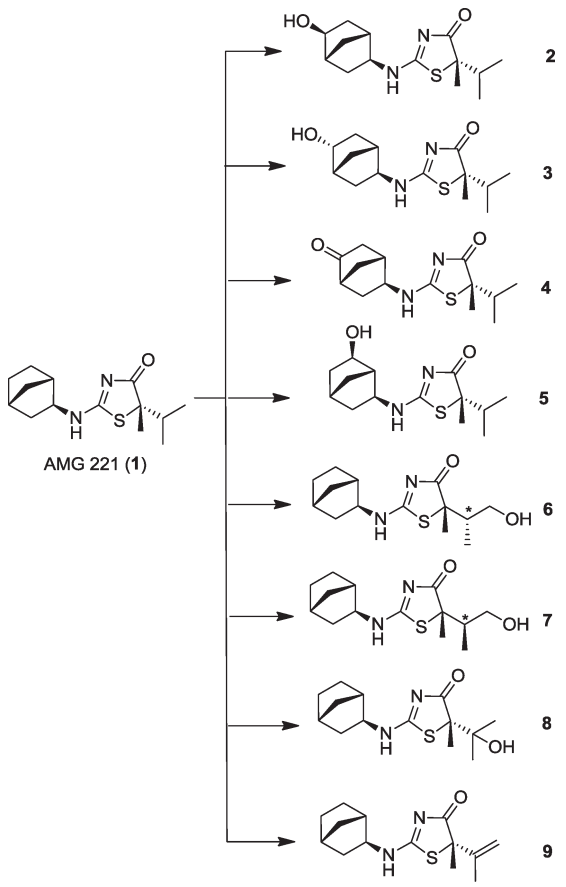
metabolite **3**. Metabolite **4** was isolated after the oxidation of **16** and chiral separation.

Chart 1. Structures of 1 (AMG 221) and Its Metabolites[a]



[a]*The stereochemistry is arbitrarily assigned.

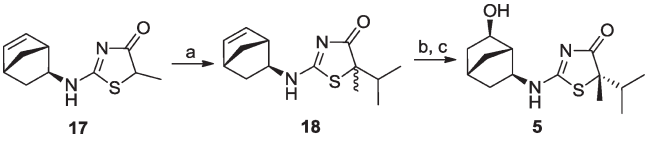
Metabolite **5** was prepared by the route shown in Scheme 2. Compound **17**[10] was converted to **18** through alkylation of the enolate with isopropyl iodide. Hydroboration and oxidation of compound **18** gave metabolite **5** after chiral SFC.

The metabolites **6** and **7** were obtained by the route shown in Scheme 3. The thiazolone **19**[10] was alkylated with LDA and 2-(2-bromopropoxy)tetrahydro-2*H*-pyran, and then, the THP group was removed to give compound **20** as a mixture of four diastereomers. After chiral SFC separation, **6** and **7** were obtained

Treating thiazolone **19** with LDA and acetone at low temperature gave compound **21** as a mixture of two diastereomers. Upon chiral separation, metabolite **8** was obtained. When compound **21** was treated with DAST at room temperature, the fluorinated product **22** was obtained as the major product[11] along with a small amount of the elimination product **23**, which was further purified by chiral chromatography to give metabolite **9**.

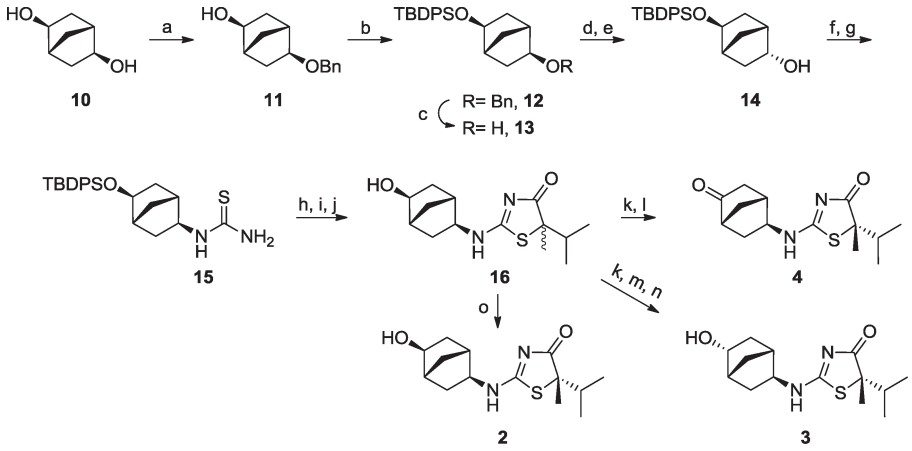
With enough quantities of all identified metabolites in hand, the in vitro potencies and preclinical pharmacokinetic properties were measured (see Tables 1 and 2). We were intrigued to find that all metabolites tested were active in the human 11β-HSD1 biochemical assay. The in vivo pharmacokinetics of some of the more potent metabolites (**2**, **3**, **4**, and **8**) were assessed in rats, with compound **2** having the lowest clearance and highest bioavailability of the metabolites tested. Compound **2** was also tested in mouse pharmacokinetic studies, and as was found in rat,

Scheme 2. Preparation of Compound 5[a]



[a] (a) $(i\text{-Pr})_2$NLi, 2-iodopropane, THF, −78 °C, 71%. (b) (1) BH_3-THF, THF, 0 °C; (2) H_2O_2, NaOH, 65 °C, 37% (2 steps). (c) Chiral chromatography.

Scheme 1. Preparation of Compounds 2−4[a]



[a] Reagents and conditions: (a) NaOH, BnBr, 15-Crown-5, THF, 10 °C, 43%. (b) TBDMSCl, imidazole, DMF, room temperature, 96%. (c) H_2, 10% Pd−C, CH_3OH, room temperature, quant. (d) PCC, CH_2Cl_2, 0 °C, 98% (crude). (e) L-Selectride, THF, −78 °C, 76%. (f) DEAD, Ph_3P, phthalimide, THF, room temperature, 68%. (g) (1) Hydrazine, ethanol, reflux; (2) benzoyl isothiocyanate, $CHCl_3$, room temperature; (3) K_2CO_3, CH_3OH, room temperature, 74% (3 steps). (h) Ethyl 2-bromoisovalerate, $(i\text{-Pr})_2$EtN, ethanol, microwave, 150 °C, 87%. (i) $(i\text{-Pr})_2$NLi, CH_3I, THF, −78 °C, 74%. (j) TBAF, THF, 0 °C, 98%. (k) PCC, CH_2Cl_2, 0 °C, 87%. (l) Chiral chromatography, 36%. (m) L-Selectride, THF, −78 °C, 87%. (n) Chiral chromatography, 41%. (o) Chiral chromatography, 48%.

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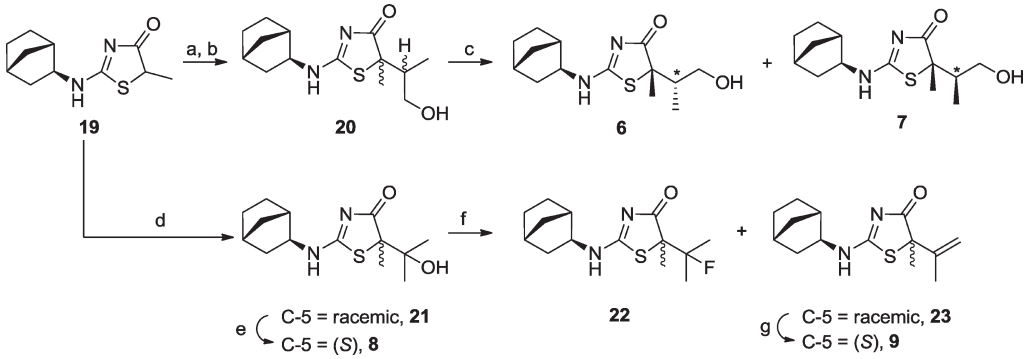
Scheme 3. Preparation of Compounds 6−9[a]



[a] (a) $(i\text{-Pr})_2$NLi, $CH_3CHBrCH_2OTHP$, THF, −78 °C. (b) *p*-TsOH, CH_3OH, room temperature, 13% (2 steps). (c) Chiral chromatography, 34% (**6**), 8% (**7**). (d) $(i\text{-Pr})_2$NLi, acetone, THF, −78 °C, 30%. (e) Chiral chromatography, 41%. (f) DAST, CH_2Cl_2, room temperature. (g) Chiral chromatography, 3.5% (two steps). *The stereochemistry is arbitrarily assigned.

Table 1. Potency of Compounds Against 11β-HSD1 of Different Species

	human		mouse
no.	SPA[a] K_i ± SEM[c] (nM)	cell[b] IC_{50} ± SEM[c] (nM)	SPA[a] K_i ± SEM[c] (nM)
1	12.8 ± 9.1	10.1 ± 6.2	70.8 ± 51.1
2	16.5 ± 4.8	12.7 ± 10.2	152 ± 80
3	17.9 ± 5.0	15.0 ± 12.6	181 ± 88
4	23.5 ± 11.8	10.2 ± 2.4	230 ± 20
5	449 ± 134	237 ± 181	
6	37.3 ± 9.6	71.6 ± 34.7	
7	27 ± 1.4	87	
8	17.5 ± 7.7	21.3 ± 20	
9	6.0 ± 1.4	16	

[a] K_i was derived from a SPA using the species 11β-HSD1 expressed in *E. coli* cells and ^{3}H-cortisone as the substrate. [b] IC_{50} was determined from a whole cell assay using CHO cells overexpressing human 11β-HSD1. [c] SEM for at least two independent determinations.

Table 2. Pharmacokinetics of Selected Compounds in Male Sprague−Dawley Rats[a]

compd	1	2	3	8
CL (L/h/kg)	1.16	0.44	0.74	1.92
Vss (L/kg)	1.53	0.97	1.43	0.53
$t_{1/2}$ (h)	12.18[b]	2.17	2.41	0.24
MRT	1.53	2.22	1.94	0.28
F (%)	34	58	NA	53

[a] iv dose and formulation: (2 mg/kg) in DMSO; oral dose and formulation: 10 mg/kg, 0.1% Tween-80, 0.5% CMC, and 99.4% water. [b] Terminal $t_{1/2}$.

it had a lower clearance and higher bioavailability than compound **1** (Table 3).

Compound **2** was then tested in a mouse pharmacodynamic (PD) model measuring the 11β-HSD1 activity in the adipose ex vivo (see Figure 1).[5] In a time−course study at 10 and 30 mg/kg (po), compound **2** significantly inhibited 11β-HSD1 at 2 and

Table 3. Pharmacokinetics of Compounds 1 and 2 in Mice[a]

	male CD1 mouse	
compd	1	2
CL (L/h/kg)	3.31	1.48
Vss (L/kg)	0.90	1.43
$t_{1/2}$ (h)	3.32	5.72
F (%)	31	>100

[a] iv dose and formulation: 2 mg/kg in 20% captisol, 80% water for **1**, and 10% dimethylacetamide, 23% water, 67% PEG 400 for **2**; oral dose and formulation: 10 mg/kg in 10% Pluronic F108 90% OraPlus for **1**, 0.1% Tween-80, and 0.5%CMC in water for **2**.

4 h for the 10 mg/kg group, and at 2, 4, 6, and 8 h for the 30 mg/kg group. The magnitude of inhibition for the 10 mg/kg group was 38% at 2 h and 31% at 4 h. For the 30 mg/kg group, the magnitude of inhibition was 51% for 2 h, 46% for 4 h, and ca. 36% for 6 and 8 h. The free plasma concentrations of **2** that achieved 46% inhibition of 11β-HSD1 in the adipose at 4 h was 2.1 μM (mouse plasma protein binding = 40.9%), a value 14-fold higher than the mouse 11β-HSD1 K_i. In contrast, compound **1** achieved 47% inhibition of 11β-HSD1 in the fat at 4 h[5] with a free plasma concentration of 0.24 μM (mouse plasma protein binding = 90.1%), a value just 3-fold higher than the mouse 11β-HSD1 K_i. One possible explanation for why metabolite **2** may require higher free plasma concentrations than compound **1** to achieve a similar pharmacodynamic is that **2** is less lipophilic and may not penetrate adipose tissue as well. Indeed, at the 4 h time point, the fat to plasma ratio was 0.27 for **2** (30 mg/kg), whereas the fat to plasma ratio was 1.34 for **1** (50 mg/kg). However, because metabolite **2** does show some inhibition of 11β-HSD1in the fat and because compound **2** has lower in vivo clearance in the mouse, these data suggest that **2** may contribute to pharmacodynamic effects observed with compound **1**.

In summary, we have successfully prepared and profiled all eight metabolites identified from compound **1**. These synthetic compounds were found to match the isolated metabolites in the coelution experiments, respectively. Therefore, the chemical structures of these metabolites were further confirmed (see the Supporting Information). These metabolites all showed activity in the 11β-HSD1 biochemical assay with metabolites **2**, **3**, and **8**

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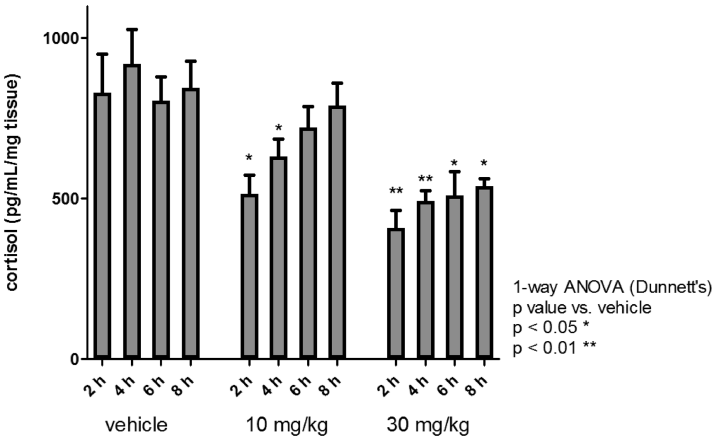

dose (mg/kg)	10				30			
time (h)	2	4	6	8	2	4	6	8
plasma (μM)	4.50 ± 0.58	1.05 ± 0.22	0.38 ± 0.12	0.19 ± 0.04	19.5 ± 4.7	3.59 ± 1.29	1.71 ± 0.44	0.39 ± 0.16
fat (μM)	0.98 ± 0.48	0.82 ± 0.90	0.22 ± 0.21	0.13 ± 0.17	4.67 ± 2.26	0.98 ± 0.78	2.02 ± 2.45	0.58 ± 1.07

Figure 1. Inhibition of 11β-HSD1 activity with **2** (dosed PO) in the mesenteric fat of mouse.

having biochemical potency similar to parent compound **1**. Four metabolites, **2**−**4** and **8**, were assessed in rat PK studies, and metabolites **2** and **3** had in vivo clearance less than parent compound **1**. Because compound **2** had the lowest in vivo clearance and was nearly equipotent to **1**, it was further evaluated in mouse studies. Metabolite **2** had superior mouse PK to **1** and inhibited adipose 11β-HSD1 in a mouse PD study, suggesting that metabolite **2** may contribute to PD effects seen with **1**. These results have compelled our clinical team to explore how the metabolites of **1** may contribute to human PD. These results will be reported in the near future.

■ ASSOCIATED CONTENT

Supporting Information. Details about the structural elucidation and experimentals for the preparation of all eight metabolites. This material is available free of charge via the Internet at http://pubs.acs.org.

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