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Hepatoselectivity of statins: Design and synthesis of 4-sulfamoyl pyrroles as HMG-CoA reductase inhibitors

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Abstract—4-Sulfamoyl pyrroles were designed as novel hepatoselective HMG-CoA reductase inhibitors (statins) to reduce myalgia, a statin-induced adverse effect. The compounds were prepared via a [3 + 2] cycloaddition of a Münchnone with a sulfonamide-substituted alkyne. We identified compounds with greater selectivity for hepatocytes compared to L6-myocytes than rosuvastatin and atorvastatin. There was an inverse correlation of myocyte potencies and *C*log *P* values. A number of analogs were effective at reducing cholesterol in acute and chronic in vivo models but they lacked sufficient chronic in vivo activity to warrant further development.

It is well documented that coronary heart disease is a leading cause of death in the US and is positively correlated with a high plasma LDL-C level.[1,2] 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are the treatment of choice for lowering plasma LDL-C. Since lovastatin was first introduced in the 1980s, other statins have made their way to the market with improved efficacy: for example, pravastatin, simvastatin, atorvastatin, and the latest addition, rosuvastatin.

The most common adverse effect associated with the statins is myalgia, manifested by muscle stiffness, muscle weakness, fatigue, and cramps.[3] It is thought that the cause of this side effect is inhibition of myocytic HMG-CoA reductase.[4] It has also been suggested that statins with greater hepatoselectivity may help reduce the observed side effect as they are less available to muscle tissues.[5,6] One way to obtain hepatoselective statins is to utilize organic anion transporting polypeptides (OATPs) since these uptake transporters are expressed on hepatocytes but not on myocytes, and statins are known to be viable substrates.[17] By making statins more hydrophilic, one could anticipate reducing non-selective passive diffusion into all cells and increasing selectivity for cells capable of internalizing statins through active transport. However, at the design stage it is challenging to determine the optimal hydrophilicity of novel statins. Thus, we elected to use atorvastatin **1** as a starting point and to evaluate the impact on tissue selectivity of altering its lipophilicity. In order to rapidly assess tissue selectivity, we used hepatocytes and L6-myocytes as primary assays.

Statins have been successfully co-crystallized with HMG-CoA reductase.[18] As shown in Figure 1, salient features of the binding of atorvastatin to the enzyme include (i) strong hydrogen bonding interactions provided by the 3,5-dihydroxy heptanoic acid fragment of **1**; (ii) the isopropyl group fitting into a small lipophilic pocket; and (iii) a hydrogen bonding interaction between the 4-carboxamide oxygen and the hydroxyl group of Ser 565.

Similarly, it is known that rosuvastatin **2**, one of the most efficacious statins, forms a strong hydrogen bond with Ser 565 through a sulfonamide oxygen, and it

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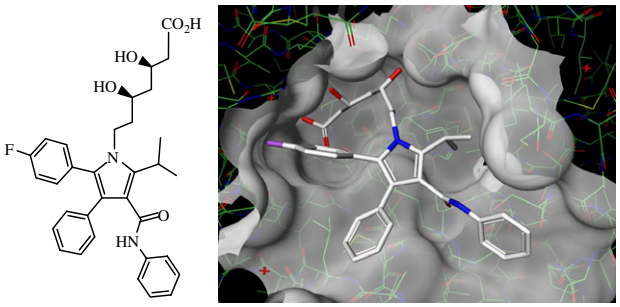
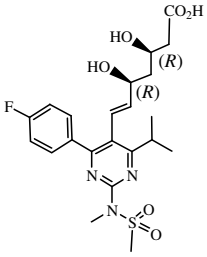


Figure 1. The structure of atorvastatin **1** and its key interactions with the HMG CoA reductase (resolution of the X-ray co-crystal structure, 2.22 Å, RCSB PDB ID: 1 hwk).

was of interest to us to see what impact this would have on the activity and hepatoselectivity of pyrrole-based statins.

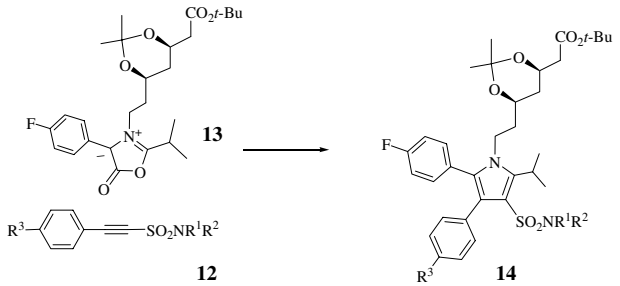


Rosuvastatin **2**

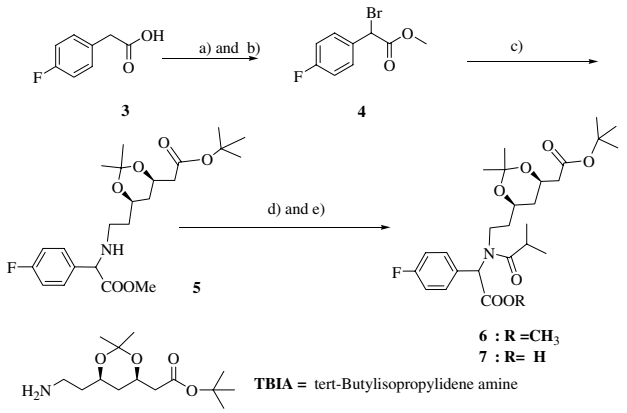
As a general chemistry strategy we retained the 5-member heterocyclic core of atorvastatin as a key scaffold as well as the essential interactions within the active site of the enzyme, and elected to investigate replacement of the carboxamide moiety with a sulfonamide.

We envisioned that a [3 + 2] cycloaddition of two components, a Münchnone **13** and an alkyne substituted with sulfonamide **12** (Scheme 1), might achieve efficient construction of a penta-substituted 4-sulfamoyl pyrrole.[14] Formation of pyrroles from the reactions of alkynes with Münchnones has been reported with sulfonyl alkynes.[8,9]

The synthesis of the Münchnone precursor **7** is shown in Scheme 2. 4-Fluorophenylacetic acid **3** was esterified to produce the corresponding ester followed by bromination using NBS under acidic condition to afford the α-bromo ester **4**. A subsequent SN2 reaction with TBIA[15]



Scheme 1. A [3 + 2] cycloaddition approach for preparing 4-sulfamoyl pyrroles.



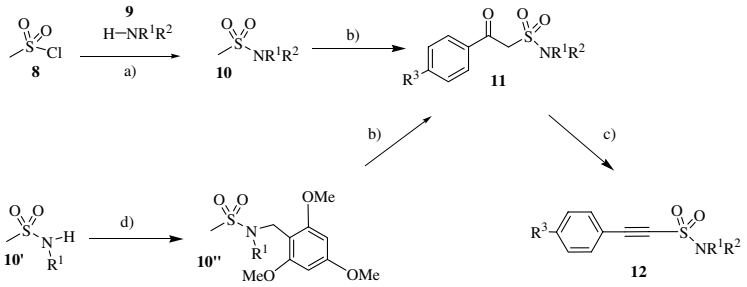
Scheme 2. Reagents and conditions: (a) MeOH, cat. TsOH 100%; (b) NBS/HBr in CCl_4, 65 °C for 5 h, 85%; (c) TBIA 1.1 equiv, triethylamine 1.5 equiv, acetonitrile, room temperature, 89%; (d) isobutyryl chloride 1.1 equiv, triethylamine 1.25 equiv, DCM, 90%; (e) LiOH 1.5 equiv/MeOH, room temperature, 16 h, 99% (Ref. 21).

provided a diastereomeric mixture of 4-fluorophenylglycine derivative **5**.[11–13] Acylation of this amine with isobutyryl chloride proceeded smoothly to generate the methyl ester **6**, which was hydrolyzed with methanolic LiOH to give the Münchnone precursor **7**.

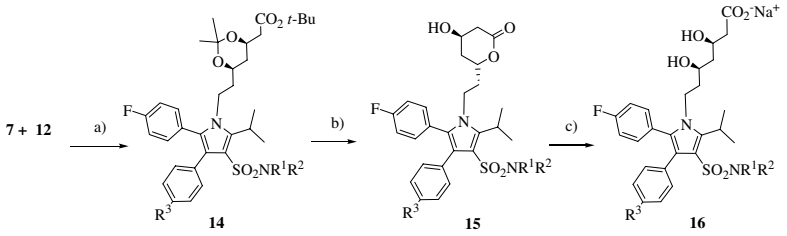
The synthesis of the alkynyl tertiary sulfonamides is shown in Scheme 3.[7] Reaction of methane sulfonyl chloride with the appropriate amine gave the sulfonamide **10** in high yields. To prepare alkynyl secondary sulfonamides, a 2,4,6-trimethoxy benzyl protecting group for the NH was utilized, which was installed via reductive amination of sulfonamides **10** (Scheme 3). Proton abstraction from **10** with *n*-BuLi followed by reaction with the appropriate ester gave good yields of the β-keto sulfonamides **11**. The alkynyl sulfonamides **12** were formed in excellent yields by reaction of **11** with 2-chloro-*N*-methyl pyridinium iodide under basic conditions.[7]

As shown in Scheme 4, heating of **7** with acetic anhydride in toluene formed the Münchnone **13** in situ, which reacted with the alkynes **12** to afford the pyrroles **14** in good yields. Theoretically, two sulfamoyl pyrrole regioisomers are possible during the cycloaddition. Only the desired regioisomer, with the sulfonamide at the 4-position, was formed.[20] As evidenced in an X-ray crystal structure of **16a** (Fig. 2), the observed regioselectivity can be rationalized by the preferred orientation of the alkyne sulfonamide **12** in relation to the Münchnone **7** prior to the cycloaddition. Treatment of **14** with trifluoroacetic acid removed the trimethoxybenzyl group from the sulfonamide nitrogen, cleaved the *tert*-butyl esters and acetonide, affording the lactones **15**. Finally, the lactone was treated with one equivalent of 1 N NaOH and lyophilized to yield the pyrrole sodium salts **16**.

As shown in Figure 2, an X-ray co-crystal structure of **16a** and the enzyme confirmed the desired regioisomer. It is noteworthy that the compound uses a water molecule as a hydrogen bonding bridge to Ser 565. The bridging water molecule appears to be a general feature



Scheme 3. Reagents and conditions: (a) triethylamine/DCM, 0 °C, 4 h, 88%; (b) 1.1 equiv *n*-butyl lithium/THF, −78 °C, 2 h, followed by p-substituted benzoic acid methyl ester, 78%; (c) 2-chloro-*N*-methylpyridinium iodide 1.5 equiv, triethylamine 2.5 equiv/DCM, room temperature, 16 h, 75%; (d) 2,4,6-trimethoxybenzaldehyde 1.1 equiv, $NaBH_4$, THF at room temperature, 4 h, 89% (Ref. 21).



Scheme 4. Reagents and conditions: (a) acetic anhydride, toluene at 60 °C, 5 h, 60–80%; (b) 30 (v/v) % TFA/DCM, room temperature, 2 h, quantitative; (c) 1 equiv of 1 N NaOH/THF, room temperature, 4 h, quantitative (Ref. 21).

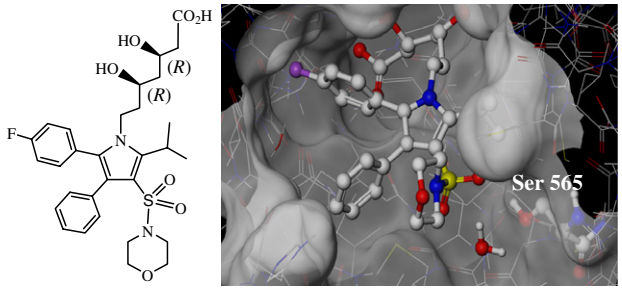


Figure 2. The X-ray crystal structure of **16a** bound to HMG-CoA reductase (PDB ID code 3BGL).

of binding between the sulfonamides and the enzyme, which differs from both atorvastatin and rosuvastatin.

Table 1 contains selectivity data of some marketed statins. The highest hepatoselectivity is found in rosuvastatin (926) and the lowest selectivity in cerivastatin (4.1). The percent myalgia incidence correlates with the hepatoselectivity with the exception of rosuvastatin. The ratio of C_{max}/L6 value is more consistent with the incidence of myalgia.

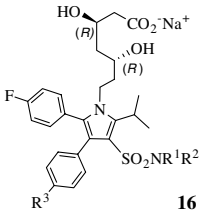
Examples of the 4-sulfamoyl pyrrole analogs are shown in an order of hepatoselectivity in Table 2. In general, most of the analogs exhibited excellent potency in vitro and selectivity toward hepatocyte over myocyte, particularly, the *N*-cycloalkyl sulfonamides, that is, **16a**, **16b**, **16c**, and **16d**. The ring size seemed to have little effect on the selectivity until the azetidine sulfonamide, **16k**. Interestingly, the rotation-restricted analog **16q** showed no markedly better in vitro potency than the *N*-benzyl sulfonamide analog, **16h**. In addition, it was observed that the secondary sulfonamide **16h** was more hepatoselective than **16q** or the *N*-methyl benzyl sulfonamide analogs, **16r** and **16s**. The meta-substituted phenyl sulfonamides, that is, **16e**, **16f**, and **16g** were more hepatoselective than the para-substituted analogs, that is, **16o** and **16p**. It was interesting to see that the phenyl sulfonamide analogs **16t**, **16u**, and **16v** showed the poorest hepatoselectivity as these analogs are structurally the closest to atorvastatin **1**. The observed low selectivity was consistent with the higher *C*log *P* values (5.95,

Table 1. A correlation of myalgia frequency and the ratio of C_{max}/ L6 myocyte

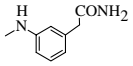
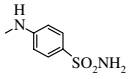
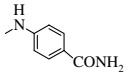
Statin	Hep (nM)	L6-Myo (nM)	Selectivity	C_{max}[a]	C_{max}/L6	Myalgia incidence
Cerivastatin	1.7	7	4.1	25	3.6	Withdrawn
Simvastatin	1.3	150	115	120	0.80	18.2%[b]
Rosuvastatin	0.27	250	926	75	0.30	High dose limited
Pravastatin	17	7550	444	100	0.01	10.9%[b]

[a] Human C_{max} at highest available dose (nM).
[b] Percent values are quoted from the PRIMO study results.[10]

Table 2. Biological activity of 3-sulfamoylpyrroles **16** in order of hepatoselectivity



16

16	–NR^1R^2	R^3	In vitro[a] IC_{50} (nM)	Hep[b] IC_{50} (nM)	L6 Myo[c] IC_{50} (nM)	Selectivity[d]	MAICS[e] (%)	*C*log *P*[f]
16a		H	4.5	0.80	10,000	12,469	−55.6	4.00
16b		H	2.9	0.67	7000	10,479	−56.0	5.24
16c		H	2.6	0.5	5200	10,400	−75.9	4.69
16d		H	2.0	2.4	22,000	9167	−61.3	5.24
16e		F	18	5	36,000	7340	ND	4.96
16f		F	1.0	1.4	10,000	7299	ND	3.51
16g		F	9.4	0.4	2500	6250	−44.2	4.18
16h		F	13	0.37	2200	5945	−58.6	5.86
16i		H	2.7	3.0	10,500	3559	ND	2.17
16j	–NMe_2	H	6.5	0.45	1380	3066	ND	4.05
16k		H	1.8	1.1	2150	1954	−34.8	4.12
16l	–NMe_2	F	6.1	0.75	1400	1866	−58.8	4.19
16m	–NHMe	F	27	0.22	ND	ND	−33.0	4.10
16n		F	9.7	3.3	5700	1748	−15.0	4.88
16o		F	4.9	2.7	4380	1622	−15.0	3.99
16p		F	5.8	1.8	2340	1300	−34.4	4.33
16q		F	8.4	2.5	2500	984	ND	6.25
Rosuvastatin		3.7	0.27	250	926	−82.4	1.90	
16r		F	8.0	0.69	550	797	ND	5.97
Atorvastatin		3.8	0.99	142	144	−75.2	4.46	
16s		H	8.5	0.25	140	566	ND	5.82
16t		F	15	0.47	60	127	−51.8	5.95
16u		F	2.0	0.96	45	45	ND	5.76

(*continued on next page*)

Table 2 (*continued*)

16	$-NR^1R^2$	R^3	In vitro[a] IC_{50} (nM)	Hep[b] IC_{50} (nM)	L6 Myo[c] IC_{50} (nM)	Selectivity[d]	MAICS[e] (%)	*C*log *P*[f]
16v		H	9.0	0.57	20	34	−49.2	5.32

[a] Rat microsomal HMGCoA reductase inhibition.
[b] Cholesterol-synthesis inhibition in hepatocyte.
[c] Cholesterol-synthesis inhibition in L6 myocyte.
[d] Ratio of hepatocyte IC_{50}/L6 IC_{50}.
[e] Acute % cholesterol-synthesis inhibition at 1 mpk in mouse; for in vitro assay procedures, see Ref. 16.
[f] *C*log *P* is a calculated hydrophilicity of a compound. It is defined as the logarithm of its partition coefficient between *n*-octanol and water $\log(C_{octanol}/C_{water})$. The *C*log *P* values were calculated based on the carboxylic acid form instead of the sodium salt for a comparison purpose to other statins.

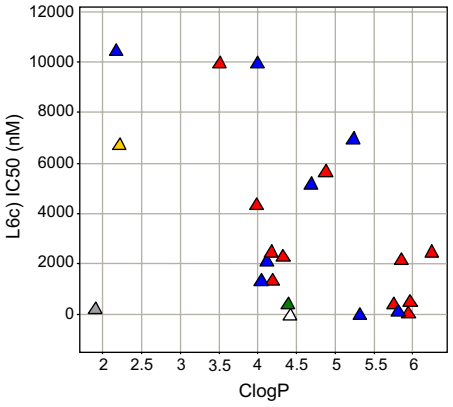


Figure 3. A plot of L6-myocyte potency versus *C*log *P* of 4-sulfamoyl pyrrole analogs. The red filled triangles represent the analogs of R^3 = F, and the blue triangles represent the analogs R^3 = H. The commercially available statins are also shown: gray (rosuvastatin), green (simvastatin), yellow (pravastatin), and transparent (atorvastatin).

5.76, and 5.32, respectively) compared to that of atorvastatin (4.46) as shown in Table 2.

A plot of L6 myocyte IC_{50} vs *C*log *P* is presented in Figure 3. In general, the L6-myocyte potency showed an inverse correlation to the *C*log *P* values. Interestingly, the majority of the 4-sulfamoyl pyrrole analogs possess higher myocyte IC_{50}s than rosuvastatin, despite having higher *C*log *P*s and similar hepatocyte IC_{50}s. This suggests that the observed inverse correlation of L6 IC_{50} and *C*log *P* likely holds only within a specific structural class. Based on the data presented in Table 1 for marketed statins, we would expect these new sulfamoylpyrroles to have reduced potential for myalgia relative to current therapies.

In general, cycloalkyl sulfonamide analogs (e.g., **16a**, **16b**, **16c**, and **16d**) showed high in vivo acute cholesterol-synthesis inhibition (MAICS). Incidentally, these analogs were also highly hepatoselective (ca. > 10,000). Several analogs exceeded 50% reduction in MAICS as seen in column (e) in Table 2.

The best analogs were run in the chronic in vivo hamster efficacy model.[19] Unfortunately, the best compounds were less efficacious in reducing LDL-C (**16a** −11%, **16c** −38%, **16l** −30%) than rosuvastatin (−62%).

In conclusion, we have designed, prepared, and tested 4-sulfamoyl pyrrole analogs as novel inhibitors of HMG-CoA reductase. Some of the most potent analogs showed excellent selectivity (superior to rosuvastatin) toward hepatocytes over myocytes. It was observed that the L6-myocytic potency is inversely correlated with the *C*log *P* of the analog. Although some analogs effectively inhibited cholesterol synthesis acutely in a mouse model and lowered LDL-C chronically, none of the compounds had efficacy greater than or equal to that of atorvastatin or rosuvastatin.

Acknowledgments

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20. A typical example of the regioselectivity is shown in the X-ray co-crystal structure of **16a** and the HMG CoA reductase in Figure 2.
21. Experimental and corresponding data are placed in supporting information section as well as in US Patent No. 7250444 B2.