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## Sulfonamide Derivatives of Benzylamine Block Cholesterol Biosynthesis in HepG<sub>2</sub> Cells : A New Type of Potent Squalene Epoxidase Inhibitors

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Abstract: Sulfonamide derivatives of ene-yne benzylamine 1 have been prepared and identified as a new class of potent SE inhibitors having demonstrated activity in HepG<sub>2</sub> cells as cholesterol biosynthesis inhibitors. © 1998 Elsevier Science Ltd. All rights reserved.

Mammalian squalene epoxidase (SE) (EC 1.14.99.7) participates to the regulation of cholesterol homeostasis *in vitro* and *in vivo*<sup>1</sup> and has a strategic position in the cholesterol biosynthetic pathway after the farnesyl pyrophosphate. This enzyme thus represents an interesting target since inhibition of SE should allow to block cholesterol biosynthesis without interfering with all other prenylation processes involving farnesyl pyrophosphate.<sup>2</sup>

Several companies have focused their efforts<sup>3</sup> on the discovery of potent SE inhibitors that could compete with HMG-CoA reductase inhibitors in lowering plasma cholesterol.<sup>4</sup> The strategy to combine an ene-yne benzylamine moiety with at least one thiophene ring into the same molecule turned out to be very powerful to reach high affinity inhibitors of mammalian SE.<sup>2,3</sup> In our laboratories, we have shown that structurally unique aryloxymethylsilanes could be used as powerful squalene epoxidase inhibitors which regulate cholesterol biosynthesis both *in vivo* and *in vitro*.<sup>5</sup> We now wish to report a new series of sulfonamide of type 1 as potent SE inhibitors in HepG<sub>2</sub> cells.



The sulfonamide derivatives of ene-yne benzylamine 1 were all prepared from the key phenol intermediate  $2.^{6}$  Our initial approach to the desired compound 1a ( $R^{1} = H$ , n = 1) is shown in Scheme 1. This strategy relied on the two carbon homologation of phenol 2 followed by reduction of the nitrile 3 and alkylation with the desired sulfonyl chloride.

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a. BrCH<sub>2</sub>CN, K<sub>2</sub>CO<sub>3</sub>, DMF, 70°C, 16 h, 60% b. LiAlH<sub>4</sub>, Et<sub>2</sub>O, 0°C, 2h, 75% c. 2-thienyl-SO<sub>2</sub>Cl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, 1h, 60 %

We found more convenient to use the route described in Scheme 2 for the synthesis of compounds **1b-1o**  $(\mathbb{R}^1 \neq \mathbb{H}, n = 1)$ . The key step in the preparation of the tertiary sulfonamides was the synthesis of alcohol **4** obtained by alkylation of phenol **2** with ethylene carbonate. Mesylation and treatment with an excess of desired amine in ethanol afforded very efficiently the secondary amine derivative which upon sulfonylation with a chosen sulfonyl chloride led to the corresponding compounds **1b-1o** in good overall yield (Table 1).<sup>7</sup>



a. (OCH<sub>2</sub>CH<sub>2</sub>O)CO, K<sub>2</sub>CO<sub>3</sub>, DMF, 80°C, 16 h, 85 % b. CH<sub>3</sub>SO<sub>2</sub>Cl, Et<sub>3</sub>N CH<sub>2</sub>Cl<sub>2</sub>, rt, 1h, 100 % c. RNH<sub>2</sub> (R = Me, Et, Pr, cyclopropyl), EtOH, rt, 20h, 70-85% d. ArSO<sub>2</sub>Cl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, 18h, 30-70%

A Suzuki coupling reaction<sup>8</sup> between thienyl bromide 1k or 1l and their counterparts 2- or 3-thienylboronic acids allowed the elaboration of the bis(thiophene) unit of 1p-1r in different substitution patterns (Scheme 3).



a. 2- or 3-thienylboronic acid, Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub> aq., DME, 120°C, 8h, 55-80%

The synthesis of compound 1s ( $R^1 = Me$ , n = 2) was accomplished in a very straightforward manner according to Scheme 4. Michael addition of phenol 2 on acrylonitrile catalyzed by Triton<sup>®</sup>B<sup>9</sup> followed by reduction of the nitrile function with lithium aluminium hydride afforded amine 5. The preparation of 1s was then readily achieved by N-methylation of 5 using ethyl chloroformate followed by reduction of the so-formed carbamate and, alkylation with 2-thienylsulfonyl chloride.

## Scheme 4



a. Acrylonitrile, Triton<sup>®</sup>B, MeCN, reflux, 36h, 80% b. LiAlH<sub>4</sub>, Et<sub>2</sub>O, O°C, 2h, 70% c. i. ClCO<sub>2</sub>Et, THF, rt, 1h ii. LiAlH<sub>4</sub>, Et<sub>2</sub>O, reflux, 5h, 80% d. 2-thienyl-SO<sub>2</sub>Cl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, 1h, 90 %

Sulfone 7, an analogue of sulfonamide derivative 1b was prepared according to Scheme 5. Alkylation of 3-thienyl sulfide with 1,3-bromochloropropane using DBU followed by oxidation to the sulfone moiety by m-CPBA afforded intermediate 6 which upon condensation with phenol 2 led to compound 7.

Scheme 5



a. 1,3-bromochloropropane, DBU, i-PrOH, 100°C, 1h, 95% b. m-CPBA (70%), CH<sub>2</sub>Cl<sub>2</sub>, 0°C, 2h, 96% c. 6, K<sub>2</sub>CO<sub>3</sub>, KI (cat.), DMF, 80°C, 60H, 30%

The potential SE inhibitors **1a-1s** and **7** have been evaluated as cholesterol biosynthesis inhibitors in human HepG<sub>2</sub> cells by measuring the extend of cholesterol radiolabelling after incubation with <sup>14</sup>C-mevalonate<sup>10</sup> according to a method previously described.<sup>5</sup> The results obtained are summarized in Table 1.

Cpd n°	n	R <sup>i</sup>	Ar	IC <sub>50</sub> , nM
1a	1	Н	2-thienyl	450
1b	1	Me	2-thienyl	60
<u>1c</u>	1	Me	3-thienyl	250
1d	1	Me	Ph-(4-F)	255
1e	1	Me	Ph-(4-Br)	330
1f	1	Ме	Ph-(4-CN)	200
1g	1	Ме	1-thiazolyl	170
1h	1	Me	3-pyridyl	150
<b>1i</b>	1	Me	2-thienyl methyl	200
1j	1	Me	benzothienyl	1800
1k	1	Me	2-thienyl-(5-Br)	390
11	1	Me	2-thienyl-(4-Br)	300
1m	1	Et	2-thienyl	90
1n	1	Pr	2-thienyl	170
10	1	cyclopropyl	2-thienyl	230
1p	1	Me	2-[4-(3-thienyl)thienyl]	90
1q	1	Me	2-[5-(3-thienyl)thienyl]	210
<u>1r</u>	1	Ме	2-[5-(2-thienyl)thienyl]	10000
1s	2	Me	2-thienyl	6400
7				290

Table 1 : Inhibition of Cholesterol Biosynthesis in HepG<sub>2</sub> Cells

The 2-thienyl derivative 1b is a potent SE and cholesterol biosynthesis inhibitor in HepG<sub>2</sub> cells (IC<sub>50</sub>=60nM). A linear increase in IC<sub>50</sub> values was observed when the methyl group in 1b was replaced by longer or bulkier alkyl substituents such as in 1m (R<sup>1</sup>=Et, IC<sub>50</sub>=90nM), 1n (R<sup>1</sup>=Pr, IC<sub>50</sub>=170nM) or 1o (R<sup>1</sup>=cyclopropyl, IC<sub>50</sub>=230nM). Extending the chain length between the 2-thiophene ring and the sulfamido group was dramatically disadvantageous to SE activity as demonstrated by compound 1s (IC<sub>50</sub>=6400nM). A large discrepancy was observed in SE inhibitory activity between the secondary sulfonamide 1a (IC<sub>50</sub>=450nM)

and the tertiary sulfonamide 1b. This comparison suggests that the presence of an hydrogen bond donor functionality (as found in 1a and not in 1b) is deleterious for activity. The affinity of sulfone 7 ( $IC_{50}=290nM$ ), just in between those of 1a and 1b showed that a very fine tuning has to be found in order to determine highly potent SE inhibitors within this series of derivatives.

Replacement of the thiophene ring in 1b by several substituted phenyl moieties such as 1d-1f did not result in an improvement of cholesterol biosynthesis inhibition. Moving to the isomeric 3-thienyl analogue 1c ( $IC_{50}=250nM$ ) or replacing the thiophene ring by the 1-thiazolyl unit (1g,  $IC_{50}=170nM$ ) gave a significant decrease in affinity over 1b while any other heterocycle such as pyridine (1h,  $IC_{50}=150nM$ ) or benzothienyl (1j,  $IC_{50}=1800nM$ ) led to a loss of affinity (from 2.5 fold to 75 fold) showing that optimal affinity should be reached using a 2-thiophene unit.

We have already demonstrated,<sup>11</sup> based on the structure of NB-598 (8), the importance of thienyl residue(s) for the design of potent squalene epoxidase inhibitors and especially whether this binding region has to be occupied by one or two thiophene rings. This led us to synthesize bis(thiophene) derivatives 1p-1r (Scheme 3). Position of the second thienyl ring is detrimental for activity as shown in table 1 (compare 1b to 1q or 1r) but even though the best compound 1p (IC<sub>50</sub>=90nM) as the same substitution pattern as in NB-598, the second ring does not seem useful in regard of its affinity to the one of 1b (IC<sub>50</sub>=60nM).



The most potent cholesterol biosynthesis inhibitor **1b** in HepG<sub>2</sub> cells has also been investigated as an SE inhibitor in an *in vitro* assay using pig, rat and dog enzymes as described earlier.<sup>12</sup> While inhibitory activity with pig (IC<sub>50</sub>=50nM) and dog (IC<sub>50</sub>=80nM) liver enzymes confirms the good ability of compound **1b** to inhibit mammalian SE in HepG<sub>2</sub> cells, the result obtained with the rat enzyme (IC<sub>50</sub>=6000nM) differs considerably. This observation was confirmed when **1b** was tested *in vivo* in rats and found almost inactive (ED<sub>50</sub>>>40 mg/kg) while under the same experimental conditions NB-598 (**8**) was an excellent inhibitor (ED<sub>50</sub>=2.5 mg/kg). These results are in agreement with what we have already observed<sup>11</sup> for different thienyl derivatives as inhibitors of mammalian SE and confirm observations previously made by Prestwich<sup>13</sup> concerning discrepancies between rat and pig enzymes. Thus, for what drug design is concerned, rat models (*in vitro* and *in vivo*) should be used with caution when screening for new SE inhibitors.

In conclusion, results disclosed herein demonstrate that sulfonamide derivatives of ene-yne benzylamine **1** represent a new class of potent SE inhibitors having demonstrated activity in HepG<sub>2</sub> cells as cholesterol biosynthesis inhibitors. Preliminary SAR studies show like in other type of SE inhibitors,<sup>11</sup> that the presence of a thienyl residue is an important structural feature for SE inhibition.

## **References and Notes**

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- All new compounds have been fully caracterized by <sup>1</sup>H NMR, elemental analysis and mass spectra. For example 1c: <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400MHz) δ ppm 7.96 (dd, J = 3.1, 1.3 Hz, 1H), 7.44 (dd, J = 5.0, 3.1 Hz, 1H), 7.33 (dd, J = 5.0, 1.3 Hz, 1H), 7.22 (t, J = 8.6 Hz, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.86 (s, 1H), 6.72 (brd, J = 8.6 Hz, 1H), 6.10 (td, J = 16.1, 6.2 Hz, 1H), 5.65 (d, J = 16.1 Hz, 1H), 4.12 (t, J = 5.6 Hz, 2H), 3.52-3.44 (m, 4H), 3.11 (d, J = 6.2 Hz, 2H), 2.99 (s, 3H), 2.60 (q, J = 7 Hz, 2H), 1.24 (s, 9H), 1.05 (t, J = 7 Hz, 3H). Calculated for C<sub>25</sub>H<sub>34</sub>N<sub>2</sub>O<sub>3</sub>S<sub>2</sub>: C, 63.26; H, 7.22; N, 5.90 Found: C, 63.02; H, 7.41; N, 5.84. LRMS (DCI) for C<sub>25</sub>H<sub>34</sub>N<sub>2</sub>O<sub>3</sub>S<sub>2</sub> 475 (M+1, 100), 355 (14), 329 (18), 209 (3), 166 (7).
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