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Sulfonamide Derivatives of Benzylamine Block Cholesterol Biosynthesis in $HepG_2$ Cells : A New Type of Potent Squalene Epoxidase Inhibitors

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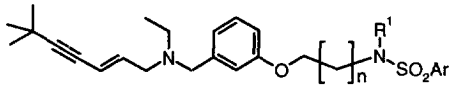
Abstract: Sulfonamide derivatives of ene-yne benzylamine **1** have been prepared and identified as a new class of potent SE inhibitors having demonstrated activity in $HepG_2$ cells as cholesterol biosynthesis inhibitors.

Mammalian squalene epoxidase (SE) (EC 1.14.99.7) participates to the regulation of cholesterol homeostasis *in vitro* and *in vivo*[1] 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.[2]

Several companies have focused their efforts[3] on the discovery of potent SE inhibitors that could compete with HMG-CoA reductase inhibitors in lowering plasma cholesterol.[4] 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.[2,3] 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*.[5] We now wish to report a new series of sulfonamide of type **1** as potent SE inhibitors in $HepG_2$ cells.



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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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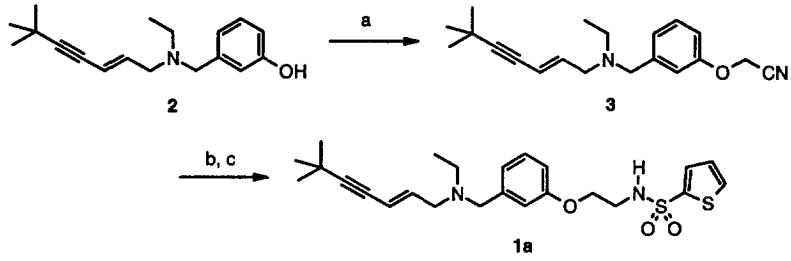
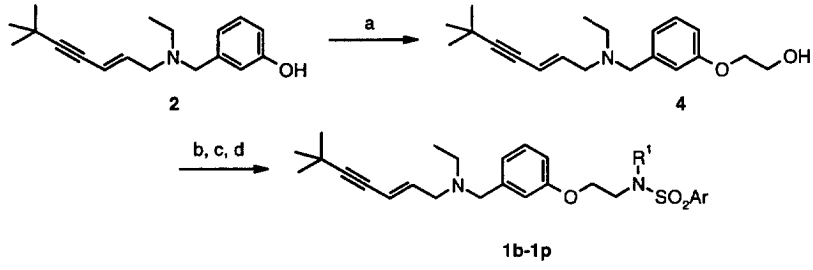
Scheme 1



a. $BrCH_2CN$, K_2CO_3, DMF, 70°C, 16 h, 60% b. $LiAlH_4$, Et_2O, 0°C, 2h, 75%
c. 2-thienyl-SO_2Cl, Et_3N, CH_2Cl_2, rt, 1h, 60 %

We found more convenient to use the route described in Scheme 2 for the synthesis of compounds **1b-1o** ($R^1 \neq H$, 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).[7]

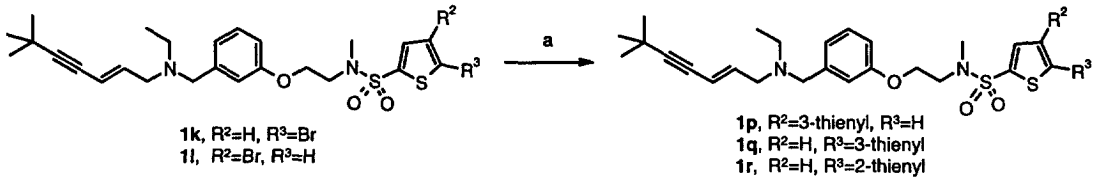
Scheme 2



a. $(OCH_2CH_2O)CO$, K_2CO_3, DMF, 80°C, 16 h, 85 % b. CH_3SO_2Cl, Et_3N CH_2Cl_2, rt, 1h, 100 % c. RNH_2 (R = Me, Et, Pr, cyclopropyl), EtOH, rt, 20h, 70-85% d. $ArSO_2Cl$, Et_3N, CH_2Cl_2, rt, 18h, 30-70%

A Suzuki coupling reaction[8] 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).

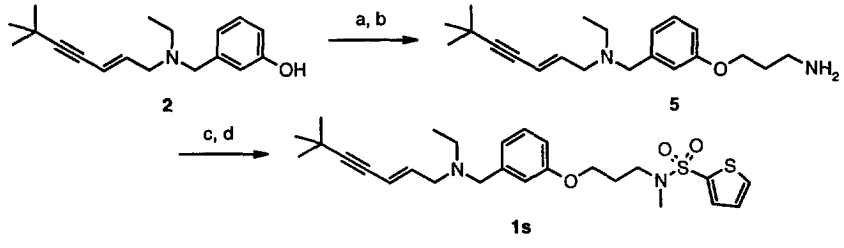
Scheme 3



a. *2- or 3-thienylboronic acid*, $Pd(PPh_3)_4$, Na_2CO_3 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®B[9] 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.

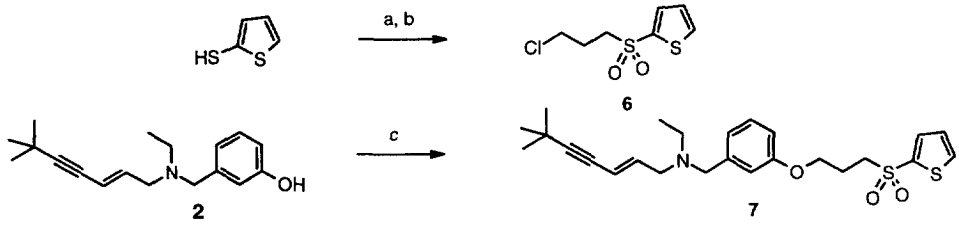
Scheme 4



a. *Acrylonitrile*, Triton®B, MeCN, reflux, 36h, 80% b. $LiAlH_4$, Et_2O, O°C, 2h, 70% c. i. $ClCO_2Et$, THF, rt, 1h ii. $LiAlH_4$, Et_2O, reflux, 5h, 80% d. 2-thienyl-SO_2Cl, Et_3N, CH_2Cl_2, 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_2Cl_2, 0°C, 2h, 96% c. **6**, K_2CO_3, KI (cat.), DMF, 80°C, 60H, 30%

The potential SE inhibitors **1a-1s** and **7** have been evaluated as cholesterol biosynthesis inhibitors in human $HepG_2$ cells by measuring the extend of cholesterol radiolabelling after incubation with ^{14}C-mevalonate[10] according to a method previously described.[5] The results obtained are summarized in Table 1.

Table 1 : Inhibition of Cholesterol Biosynthesis in $HepG_2$ Cells

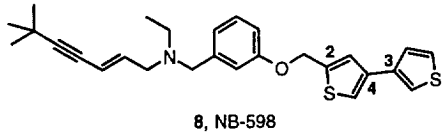
Cpd n°	n	R^1	Ar	IC_{50}, nM
1a	1	H	2-thienyl	450
1b	1	Me	2-thienyl	60
1c	1	Me	3-thienyl	250
1d	1	Me	Ph-(4-F)	255
1e	1	Me	Ph-(4-Br)	330
1f	1	Me	Ph-(4-CN)	200
1g	1	Me	1-thiazolyl	170
1h	1	Me	3-pyridyl	150
1i	1	Me	2-thienyl methyl	200
1j	1	Me	benzothienyl	1800
1k	1	Me	2-thienyl-(5-Br)	390
1l	1	Me	2-thienyl-(4-Br)	300
1m	1	Et	2-thienyl	90
1n	1	Pr	2-thienyl	170
1o	1	cyclopropyl	2-thienyl	230
1p	1	Me	2-[4-(3-thienyl)thienyl]	90
1q	1	Me	2-[5-(3-thienyl)thienyl]	210
1r	1	Me	2-[5-(2-thienyl)thienyl]	10000
1s	2	Me	2-thienyl	6400
7				290

The 2-thienyl derivative **1b** is a potent SE and cholesterol biosynthesis inhibitor in $HepG_2$ cells (IC_{50}=60nM). A linear increase in IC_{50} values was observed when the methyl group in **1b** was replaced by longer or bulkier alkyl substituents such as in **1m** (R^1=Et, IC_{50}=90nM), **1n** (R^1=Pr, IC_{50}=170nM) or **1o** (R^1=cyclopropyl, IC_{50}=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_{50}=6400nM). A large discrepancy was observed in SE inhibitory activity between the secondary sulfonamide **1a** (IC_{50}=450nM)

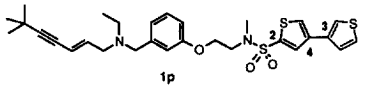
and the tertiary sulfonamide **1b**. This comparison suggests that the presence of an hydrogen bond donor functionality (as found in **1a** and not in **1b**) is deleterious for activity. The affinity of sulfone **7** (IC_{50}=290nM), just in between those of **1a** and **1b** showed that a very fine 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.

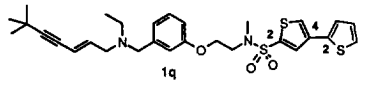
We have already demonstrated,[11] based on the structure of NB-598 (**8**), the importance of thienyl residue(s) for the design of potent squalene epoxidase inhibitors and especially whether this binding region has to be occupied by one or two thiophene rings. This led us to synthesize bis(thiophene) 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_{50}=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_{50}=60nM).



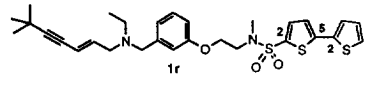
8, NB-598



1p



1q



1r

The most potent cholesterol biosynthesis inhibitor **1b** in $HepG_2$ cells has also been investigated as an SE inhibitor in an *in vitro* assay using pig, rat and dog enzymes as described earlier.[12] While inhibitory activity with pig (IC_{50}=50nM) and dog (IC_{50}=80nM) liver enzymes confirms the good ability of compound **1b** to inhibit mammalian SE in $HepG_2$ cells, the result obtained with the rat enzyme (IC_{50}=6000nM) differs considerably. This observation was confirmed when **1b** was tested *in vivo* in rats and found almost inactive (ED_{50}>>40 mg/kg) while under the same experimental conditions NB-598 (**8**) was an excellent inhibitor (ED_{50}=2.5 mg/kg). These results are in agreement with what we have already observed[11] for different thienyl derivatives as inhibitors of mammalian SE and confirm observations previously made by Prestwich[13] 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 $HepG_2$ cells as cholesterol biosynthesis inhibitors. Preliminary SAR studies show like in other type of SE inhibitors,[11] that the presence of a thienyl residue is an important structural feature for SE inhibition.

References and Notes

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7. All new compounds have been fully caracterized by ^1H NMR, elemental analysis and mass spectra. For example **1c**: ^1H-NMR ($CDCl_3$, 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 $C_{25}H_{34}N_2O_3S_2$: C, 63.26; H, 7.22; N, 5.90 Found: C, 63.02; H, 7.41; N, 5.84. LRMS (DCI) for $C_{25}H_{34}N_2O_3S_2$ 475 (M+1, 100), 355 (14), 329 (18), 209 (3), 166 (7).

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10. This cellular model is a powerful and reliable tool for screening SE inhibitors. This model is doubly informative since it gives both quantitative evaluation of the potency of the inhibitors (determination of IC_{50} values), but also a qualitative measure of the mechanism of action.

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