### Title

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# Computer-Aided Selective Optimization of Side Activities of Talinolol

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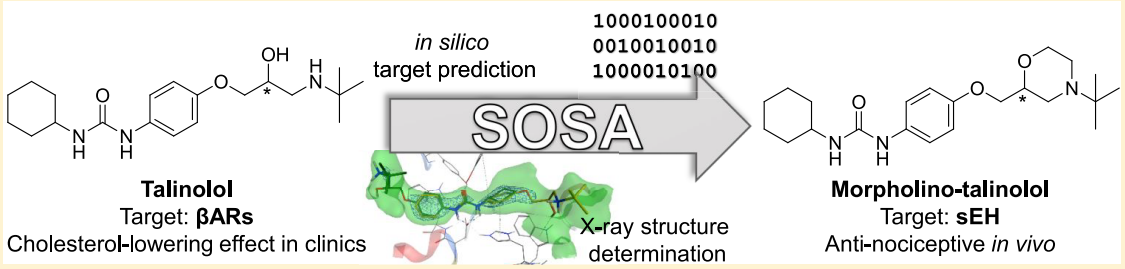
Supporting Information




**ABSTRACT:** Selective optimization of side activities is a valuable source of novel lead structures in drug discovery. In this study, a computer-aided approach was used to deorphanize the pleiotropic cholesterol-lowering effects of the beta-blocker talinolol, which result from the inhibition of the enzyme soluble epoxide hydrolase (sEH). X-ray structure analysis of the sEH in complex with talinolol enables a straightforward optimization of inhibitory potency. The resulting lead structure exhibited *in vivo* activity in a rat model of diabetic neuropatic pain.

**KEYWORDS:** *Selective optimization of side activities, soluble epoxide hydrolase, polypharmacology, computer-aided drug design, structure-based drug design*

Selective optimization of side activities, also known as the SOSA approach, has been proposed to be a very effective method of lead identification and optimization.[1] Following SOSA, a side activity of a drug, which is observed in clinics, is enhanced by subsequent introduction of structural changes. SOSA is an extremely promising strategy to identify novel lead structures exhibiting good bioavailability and low toxicity due to the fact that the starting point has been already approved in humans. However, in most cases the side activity of a drug is an adverse effect or quite weak and is only reported from clinical trials or clinical practice.

Talinolol (**1**) is an unselective antagonist of the beta adrenergic receptors (βAR), also referred to as a beta-blocker, and is used as an antihypertensive agent. Interestingly, in several small clinical trials talinolol was described to have beneficial effects on triglycerides and cholesterol levels compared to propranolol.[2−5] These results were investigated in the TALIP study, and it could be shown that talinolol treatment resulted in reduced LDL cholesterol levels in comparison to atenolol.[6] The reduction of the cholesterol levels could not be explained by the inhibition of the beta adrenergic receptors; thus, an off-target activity might be responsible for this pleiotropic effect. These observations make talinolol a valuable starting point for the SOSA approach. However, the optimization of the phenotypic effect is not always rational due to the multifactorial response to a drug.

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Thus, deorphanization of the target responsible for the side activity makes the optimization procedure straightforward.

Computer-aided target deorphanization, also referred to as polypharmacology detection or target identification, has become popular in the past few years.[7,8] In this study we used three different popular web-based target deorphanization tools to propose alternative targets of talinolol: HitPickV2,[9] SuperPred,[10] and SwissTargetPrediction[11] (see Supporting Information). All three tools independently predicted soluble epoxide hydrolase (sEH) as the most probable novel target of talinolol. Inhibition of sEH is related to cholesterol and lipid lowering effects.[12,13] For propranolol, sEH was absent in the list of the predicted targets produced by all three tools.

Based on the *in silico* target prediction, we investigated the inhibitory activity of talinolol toward sEH *in vitro* using the fluorogenic substrate PHOME.[14] Talinolol inhibited sEH with an $IC_{50}$ of 2.8 $\mu$M, while propranolol remained inactive (Table 1). In human patients, talinolol reaches a $C_{max}$ of 191 ng/mL (0.53 $\mu$M) after 12 weeks application of 100 mg per day.[6] Other studies[2−5] which describe the cholesterol and lipid lowering effects of talinolol were performed with even higher daily dosages of talinolol (200 mg −300 mg) suggesting that these effects could indeed be caused by inhibition of sEH.[16−18] However, although significant results were obtained in this study, the inhibitory potency of talinolol toward sEH is not sufficient for direct repurposing. Furthermore, the blood pressure lowering effects and adverse effects of beta-adrenergic antagonism activity (($K_i$ ($\beta$AR1) = 0.24 ± 0.05 $\mu$M; $K_i$ ($\beta$AR2) = 0.9 ± 0.1 $\mu$M) determined by radioligand binding assay of the Psychoactive Drug Screening Program (PDSP)[15]) also make the usage of high-dosed talinolol impossible. However, it makes talinolol an ideal candidate for the SOSA approach, which should ideally aim at enhancing the sEH inhibitory activity and simultaneous reduction of the $\beta$AR antagonism.

**Table 1. Activity and *in Vitro* Pharmacological Data of Propranolol, Talinolol, and Morpholino-Talinolol**

| Property | Propranolol | Talinolol (**1**) | Morpholino-talinolol (**2**) |
|---|---|---|---|
| *In vitro* inhibition of soluble epoxide hydrolase (sEH) | inactive | $IC_{50}$ = 2.8 ± 0.2 $\mu$M | $IC_{50}$ = 0.077 ± 0.004 $\mu$M |
| Metabolic stability in RLM after 1 h | 37 ± 2% | 86 ± 6% | 71 ± 1% |
| Permeability ($logP_e$) | −1.99 ± 0.04 | −5.2 ± 0.7 | −2.31 ± 0.004 |

In order to rationalize our SOSA approach, we cocrystallized talinolol with the C-terminal domain of sEH. The binding mode was used for structure-based optimization. We observed two possible orientations of talinolol in the active site of sEH (Figure 1A) with a number of different conformations. The presence of the inhibitor was verified by a polder map shown in green (Figure 1A). The urea moiety substituted by two lipophilic residues was identified as the key pharmacophore responsible for sEH binding, which correlates well with previous studies.[19] The ethanolamine moiety, which is a crucial part of the beta adrenergic receptor pharmacophore, does not form crucial directed interactions with the binding site residues, and the electron density for this moiety is not well-resolved. Thus, we decided to remove the hydrogen

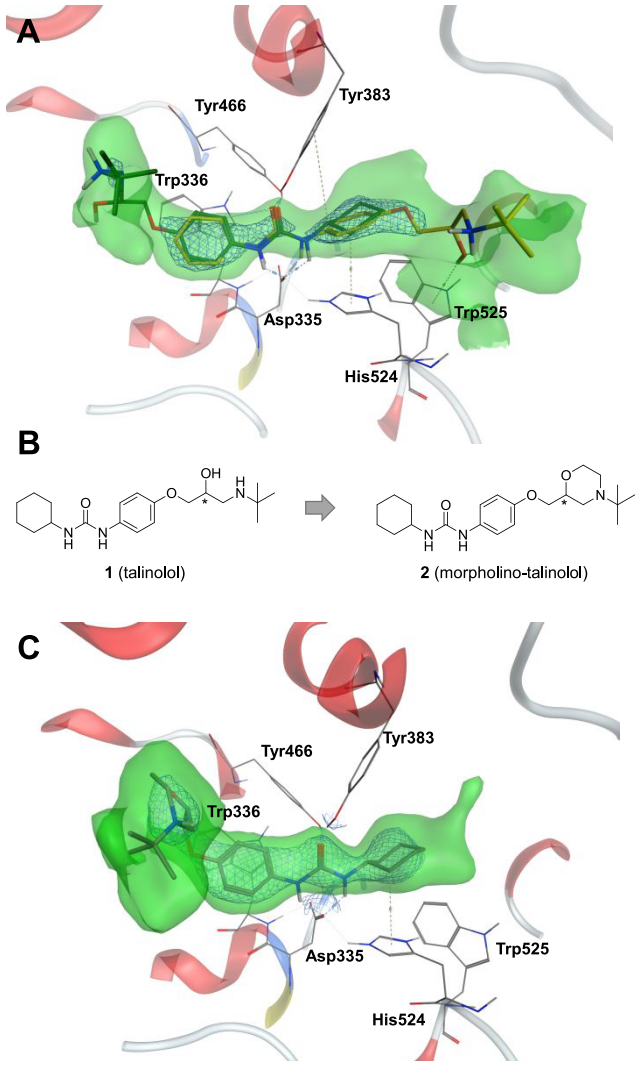
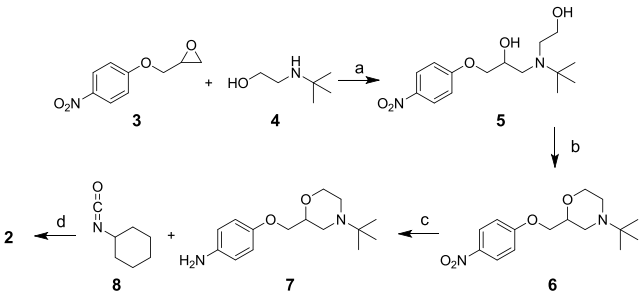



**Figure 1.** Design of morpholino-talinolol. A shows the X-ray crystallographic structure of the C-terminal domain of sEH in complex with talinolol (PDB record 6HGV). Two of the possible conformations of talinolol were modeled in the density of the polder map covering both orientations in the binding pocket. C shows the structure of the C-terminal domain in complex with morpholino-talinolol (PDB record 6HGX). In both structures, the mFo-DFc polder map around the ligand is shown in green (countered at 3$\sigma$), while the 2mFo-DFC map of the ligands is shown in blue (countred at 1$\sigma$). B shows the structure of talinolol (left) and morpholino-talinolol (right).

**Scheme 1. Synthesis of Morpholino-Talinolol. (a) EtOH, $\mu$W, 150 °C, 15 bar, 30 min, 20%; (b) 1. NaH, 2. p-TsCl, THF, 0 °C − rt, 72 h, 61%; (c) Pd/C, $H_2$, EtOH, rt, 6 h, 97%, (d) DIPEA, abs. DCM, rt, 20 h, 33%.**



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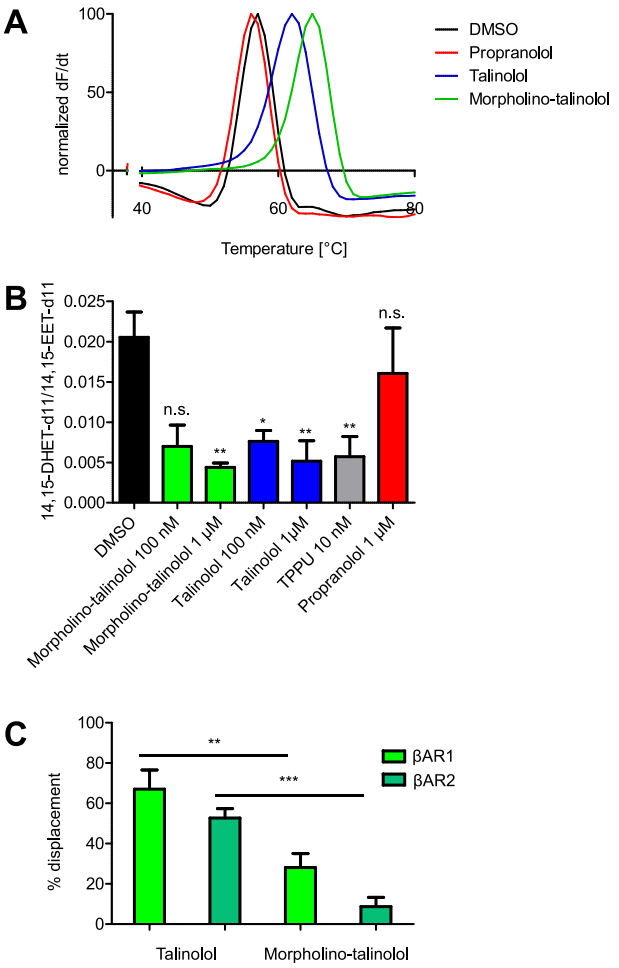


**Figure 2.** (A) Differential scanning fluorimetry assay demonstrates that talinolol and morpholino-talinolol, but not propranolol, stabilize sEH. The graph displays the normalized first derivation of a representative measurement. (B) sEH inhibition in HEP-G2 lysates. (±)14(15)-EET-d11 was used as sEH substrate and the EET/DHET ratios were measured via LC-MS/MS. Results are given as mean + SEM out of three independent experiments. (C) Radioligand displacement of talinolol and morpholino-talinolol from $\beta$AR1 and $\beta$AR2. The *t* test for B and C for two independent means was performed using a web server (https://www.socscistatistics.com/tests/studentttest/default2.aspx, 6th May 2019); *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

bonding functionalities of the ethanolamine moiety by ring closure (Figure 1B). The synthesis of the designed morpholino-talinolol (**2**) was accomplished through coupling of the secondary amine precursor **4** to the epoxide moiety of **3**. Subsequently, ring closure of the morpholine was mediated by *p*-tosyl chloride. The aromatic nitro group of **6** was reduced using palladium on charcoal and coupled to cyclohexyl isocyanate **8** to yield the desired product **2** (see Scheme 1).

Morpholino-talinolol was tested *in vitro*, and significant increase in potency toward sEH could be measured ($IC_{50}$ = 0.077 $\mu$M). The design hypothesis could be confirmed using X-ray crystallography. The co-crystal structure of morpholino-talinolol and the C-terminal domain of sEH revealed that the urea moiety interacts with the catalytic triad acting as an epoxide mimetic. The cyclohexyl moiety occupies the smaller hydrophobic pocket while the substituted phenyl part reaches into the larger hydrophobic tunnel (see Figure 1C).

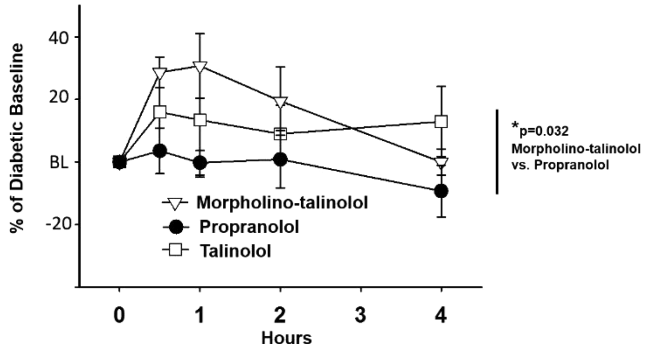


**Figure 3.** Effects of talinolol and derivatives on diabetic neuropathic pain. Pain (allodynia) was assessed in diabetic neuropathic rats ($n$ = 6/group) after single administration of 10 mg/kg each compound in PEG 300. Morpholino-talinolol was significant compared to the effects of propranolol but not talinolol ($p$ = 0.032). Two way analysis of variance, Holm-Sidak method post hoc. Naïve baseline averaged 84.6 gr versus diabetic baselines 41.0 gr (normalized above) for all groups.

Morpholino-talinolol displays one dominant orientation in the binding site of sEH.

Differential scanning fluorimetry (DSF) assay was used to confirm the direct binding of morpholino-talinolol to sEH (Figure 2A). Talinolol caused a pronounced shift of the melting point of sEH (62.5 ± 0.5 °C vs 57.0 ± 0.0 °C DMSO control) while propranolol did not affect the temperature-dependent denaturation of sEH significantly (56.3 ± 0.5 °C). Morpholino-talinolol caused a more pronounced thermal shift of 65.1 ± 0.3 °C which correlates nicely with the lower $IC_{50}$ value *in vitro*. We could also confirm the sEH inhibitory activity of talinolol and morpholino-talinolol in HEP-G2 cells by monitoring the conversion of 14,15-EET-d11. In this setting, both compounds reduced the conversion of the externally added EET like the most advanced sEH inhibitor TPPU, while propranolol did not show an effect (Figure 2B). As expected from the bridging of the ethanolamine moiety, the binding affinity of morpholino-talinolol toward $\beta$ARs was significantly reduced in comparison to talinolol (Figure 2C).

Preliminary evaluation of the *in vitro* metabolic stability and penetrability (Table 1) suggested that morpholino-talinolol should be orally available, which supports the intention of the SOSA approach to deliver lead structures with good initial pharmacokinetics. In order to accomplish the successful SOSA approach, we examined the *in vivo* activity of morpholino-talinolol in comparison to talinolol and propranolol in rats. sEH inhibitors exhibit a pronounced blood pressure lowering effect comparable to beta blockers.[20,21] Furthermore, sEH substrate EETs have pronounced antinociceptive effects, which are strongly enhanced by the application of sEH inhibitors.[22] Therefore, pain was modeled using diabetic neuropathy induced by streptozocin, which targets and kills the pancreatic beta islet cells rendering the rats with type I diabetes and neuropathic pain, in which different sEH inhibitors were found to be active.[23] Propranolol, a beta blocker without sEH inhibitory activity, which was confirmed in three orthogonal assays, was used as negative control in order to exclude the possibility that the antinociceptive effects originate from the $\beta$AR antagonism activity. After 5 days the allodynia of diabetic rats was confirmed, and a von Frey assay was performed.[24] Morpholino-talinolol blocked allodynia, and this antinociception was sustained up to 2 h before declining (Figure 3).



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This study demonstrates the unique opportunities of *in silico* polypharmacology prediction to deorphanize the side activities of a drug observed in clinical studies. Furthermore, once the target of interest has been discovered, the rational application of the SOSA approach is straightforward. The use of an approved drug warrantees an acceptable pharmacokinetic profile while potency optimization can be achieved with a low number of optimization steps.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00075.

Experimental data including synthesis, assay details, crystallization protocol, and animal experiments (PDF)

Target prediction results from HitPickV2, SuperPred, and SwissTargetPrediction (PDF)

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### Author Contributions

[▽]K.H. and J.S.K. contributed equally and are named in alphabetical order. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.


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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

LDL, low density lipoprotein; PDSP, Psychoactive Drug Screening Program; sEH, soluble epoxide hydrolase; SOSA, selective optimization of side activities

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