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Short communication

Arylpiperidines as a new class of oxidosqualene cyclase inhibitors

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ABSTRACT

The cyclization of oxidosqualene to lanosterol, catalyzed by the enzyme oxidosqualene cyclase (OSC), goes through a number of carbocationic high energy intermediates (HEI), and mimicking these intermediates is a promising approach for the development of OSC inhibitors. 3-Arylpiperidines (or tetrahydropyridines) were designed as steroidomimetic rings A + C equivalents containing two protonable amino groups for mimicking both the pro-C4 HEI and the pro-C20 HEI of the OSC-mediated cyclization cascade. Inhibitory activity is strongly dependent on the nature of the lipophilic substituent representing an equivalent of the sterol side chain. Here aromatic residues (substituted benzyl, cinnamyl, naphthylmethyl) were found to be most suitable. Docking experiments on a first optimized 3-arylpiperidine compound led to an isomeric 4-arylpiperidine with submicromolar activity on human OSC. This inhibitor reduced total cholesterol biosynthesis in a cellular assay with an IC_{50} value of 0.26 μM.

1. Introduction

Sterol biosynthesis is a complex biosynthetic pathway in which the pre-squalene part is devoted to building the open triterpene 2,3-oxidosqualene starting from the common precursor acetyl-CoA, and in the "tailoring section" the sterol intermediates are remodelled to generate the species-specific stereols (cholesterol, ergosterol or phytosterols, in animal, fungal or plant cells, respectively). The two sections are connected by one of the most outstanding mono-enzymatic reactions in nature, the cyclization of 2,3-oxidosqualene to the tetracyclic steroids, catalyzed by the enzyme oxidosqualene cyclases (OSC; EC 5.4.99.7). This reaction produces lanosterol in non-photosynthetic organisms and, mostly, cycloartenol in photosynthetic organisms. This reaction has been studied mechanistically in detail over several decades, and is now understood quite well [1,2]. Two crystal structures of human OSC, one with lanosterol, the product of the enzymatic reaction, and one with the inhibitor Ro 48–8071, have been published [3]. They provide unique insights into the three-dimensional structure of the enzyme and the contribution of the amino acids in its active site to this fascinating cyclization.

The resulting sterols exhibit important physiological roles. Cholesterol in man is a precursor of sexual hormones, mineralocorticoids, and glucocorticoids, as well as a risk factor in cardiovascular diseases. Ergosterol is a crucial component of cell membranes in pathogenic fungi and protozoa. Therefore, oxidosqualene cyclases are attractive targets for designing cholesterol lowering [4], antifungal [5,6], and antiparasitic agents [7–9]. Very recently, anticancer effects of OSC inhibitors against glioblastoma [10] and breast cancer cells [11,12] have been demonstrated. Thus, novel potent, selective, and non-toxic OSC inhibitors are highly desirable.

OSC-catalyzed cyclization of 2,3-oxidosqualene is triggered by protonation of the epoxide ring of the substrate and proceeds through a number of carbocationic high energy intermediates (HEI). This occurs during both the formation of the new carbon–carbon bonds for the construction of the tetracyclic steroid scaffold (in the sequence pro-C4, C10, C8, C13 and C20; Fig. 1A) and the subsequent rearrangement of the protosteryl carbocation pro-C20 (in the sequence pro-C17, C13, C14, and C8 or C9 depending on the product) until the removal of a proton and formation of lanosterol or cycloartenol [1]. Since HEI generally interact very tightly with the enzyme, compounds mimicking the HEI have a much

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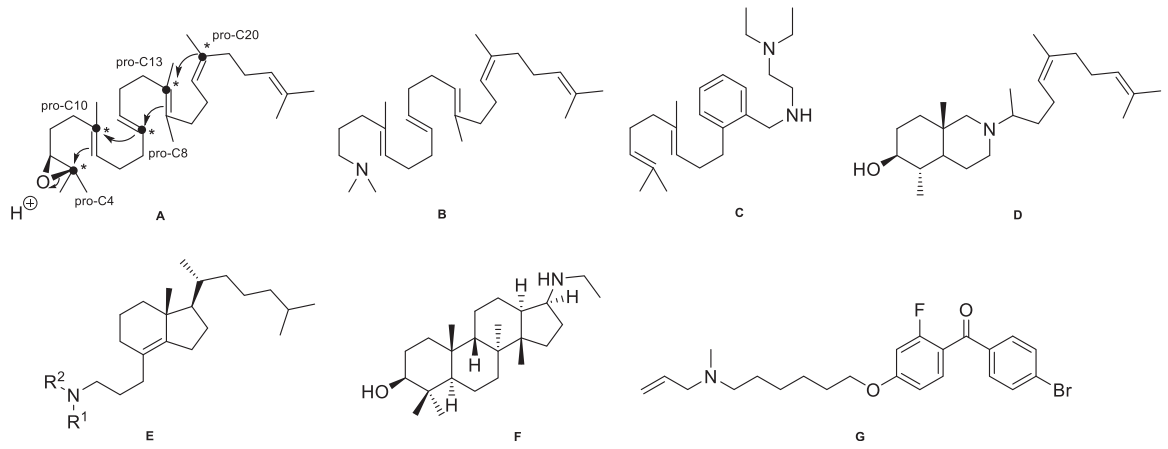


Fig. 1. Oxidosqualene cyclase (OSC)-mediated cyclization cascade starting with protonation of the epoxide, going through five carbocationic high energy intermediates (positions marked with an *) (A); Selected OSC inhibitors: acyclic azasqualene analogue of the pro-C4 HEI (B), monocyclic OSC inhibitor mimicking the pro-C20 HEI (C), bicyclic inhibitors mimicking the pro-C8 (D) and pro-C4 (or pro-C10) HEI (E), steroidal pro-C20 HEI analogue (F), and the non-terpenoic benzophenone-type OSC inhibitor Ro 48–8071 (G).

higher affinity (up to three orders of magnitude) for the enzyme than analogues of the substrate, and thus have the potential to be highly effective enzyme inhibitors [13]. Based on this rationale, a considerable number of OSC inhibitors mimicking individual cationic HEI of the cascade described above have been developed in the past (Fig. 1). These inhibitors contain aliphatic amino groups which are protonated under physiological conditions, and thus can mimic the cationic HEI.

Among already established inhibitors of OSC from various organisms are azasqualenes (**B**) [14,15], and various monocyclic (**C**) [5,16], bicyclic (**D**, **E**) [14,17], and tricyclic [18] steroidomimetic analogues of carbocationic HEI. Surprisingly, aminosteroids (e.g. **F**), which are structurally closely related to the protosteryl cation (pro-C20 HEI), were found to be poor OSC inhibitors [18,19], whereas flexible mimics of the pro-C20 HEI (**C** [5] and 19-azasqualenes [15]) are potent inhibitors. Furthermore, aminoalkyl benzophenones (**G**) [4,20,21] and related compounds [9,22–24], fulfilling concise structural requirements [25], are OSC inhibitors. The mode of binding of Ro 48–8071 (**G**) to human OSC has been elucidated by co-crystallization [3].

In continuation of our recent work on the development of inhibitors of oxidosqualene cyclases from various organisms [9,17,22,23] and other enzymes in ergosterol [26] and cholesterol biosynthesis [27–30] we describe here a novel chemotype of selective inhibitors of human oxidosqualene cyclase. Inspired by the fact that numerous cationic HEI occur in the course of the construction of the steroid core in this mono-enzymatic reaction, we intended to develop inhibitors which are able to mimic both the first occurring HEI (at pro-C4) and the one appearing furthest away from ring A, namely the pro-C20 HEI (protosteryl cation) (Fig. 2). As a central building block we selected a benzene ring resembling ring C of the sterol, to this we attached a piperidine (or tetrahydropyridine) ring as a ring A mimic containing the protonable nitrogen at a position fitting with C-4 of the sterol. The second protonable amino group was introduced in the para position in the form of a benzylic amino group. A methyl substituent at this amino group would resemble the C-21 methyl group of the sterols, whereas an additional substituent (SC) was to mimic the lipophilic side chain of the steroids. Since the arylpiperidines designed this way have structural similarity to "steroidomimetic" biphenyl derivatives, inhibitors of C17,12-lyase in estrogen biosynthesis [31,32], we expected them to have affinity for the steroid-producing enzyme OSC. On the other hand, the target compounds should have sufficient structural flexibility to avoid the affinity problems of fully steroidal mimics of the pro-C20 HEI described above (Fig. 1**F**).

The newly designed molecules were assayed in a whole-cell assay suitable for detection of target enzymes in the post-squalene part of cholesterol biosynthesis [27]. Compounds which turned out to be OSC inhibitors in this assay were further submitted to assays on inhibition of OSC from different organisms (*Saccharomyces cerevisiae; Homo sapiens* and *Trypanosoma cruzi*, both expressed in the OSC-defective yeast *S. cerevisiae*) [33], and for determination of their inhibition of total cholesterol biosynthesis in a human cell line [27].

2. Results and discussion

2.1. Chemistry

The target 3-arylpiperidines and 3-aryltetrahydropyridines

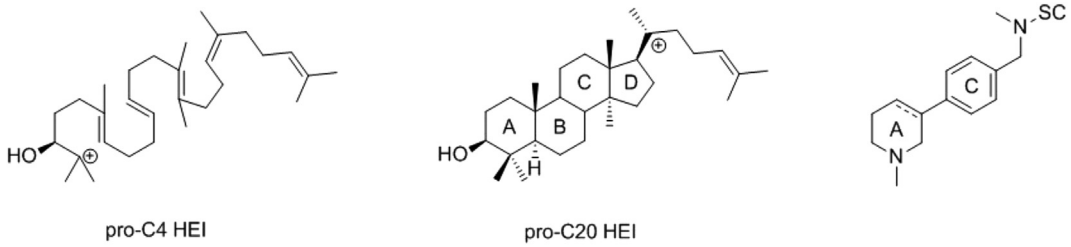


Fig. 2. The pro-C4 HEI and the pro-C20 HEI of the OSC-mediated cyclization cascade. 3-Arylpiperidines (or tetrahydropyridines) as steroidomimetic rings A + C equivalents (right). The two protonable amino groups are designed to mimic both the pro-C4 HEI and the pro-C20 HEI. (SC = lipophilic side chain equivalent).

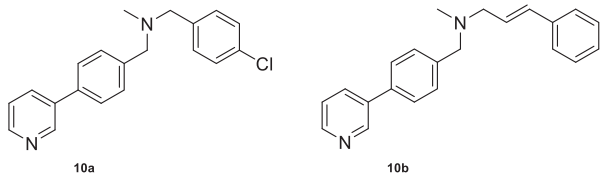
(Fig. 2) were obtained from the central building block **1** (Scheme 1), which is readily available through Suzuki cross-coupling [34] of 3-pyridylboroxine and 4-bromo-*N*-methylbenzylamine. After protection of the benzylic amino group as the Boc derivative **2**, the pyridine was converted to the *N*-methylpyridinium salt **3** by reaction with iodomethane. Subsequent reduction with sodium borohydride [35] gave the 1,2,5,6-tetrahydropyridine **4** in good yield. After deprotection with trifluoroacetic acid, the lipophilic side chain equivalents were attached to the resulting secondary amine **5** using two different protocols depending on the commercial availability of the building blocks. We selected branched alkyl chains, which mimic the sterol side chain very well, different benzyl residues of similar size (which can be found in a number of benzophenone-type OSC inhibitors [4,8,9,20,21]), as well as a cinnamyl group (inspired by the side chain of the squalene epoxidase inhibitor naftifine [36]), its dihydro analogue, and a naphthylmethyl group (inspired by the core element of the squalene epoxidase inhibitors naftifine and terbinafine). The tertiary amines **6a,b,d,e,g, j,l** were obtained by a one-pot protocol consisting of reaction with an appropriate aldehyde followed by reduction with sodium borohydride. The amines **6c,f,h,i,k** were readily obtained by direct N-alkylation of **5** with the respective alkyl bromides in DMF in the presence of triethylamine.

A series of analogous piperidine derivatives **9a,b,c,g,i,k,l** were prepared in the same manner from the secondary amine **8**. This amine was available from tetrahydropyridine **4** by catalytic hydrogenation of the olefinic group to give **7**, followed by removal of the Boc protecting group. In this series we deleted a number of side chain equivalents, which had shown no promise in a preliminary screening of tetrahydropyridines **6a-l**.

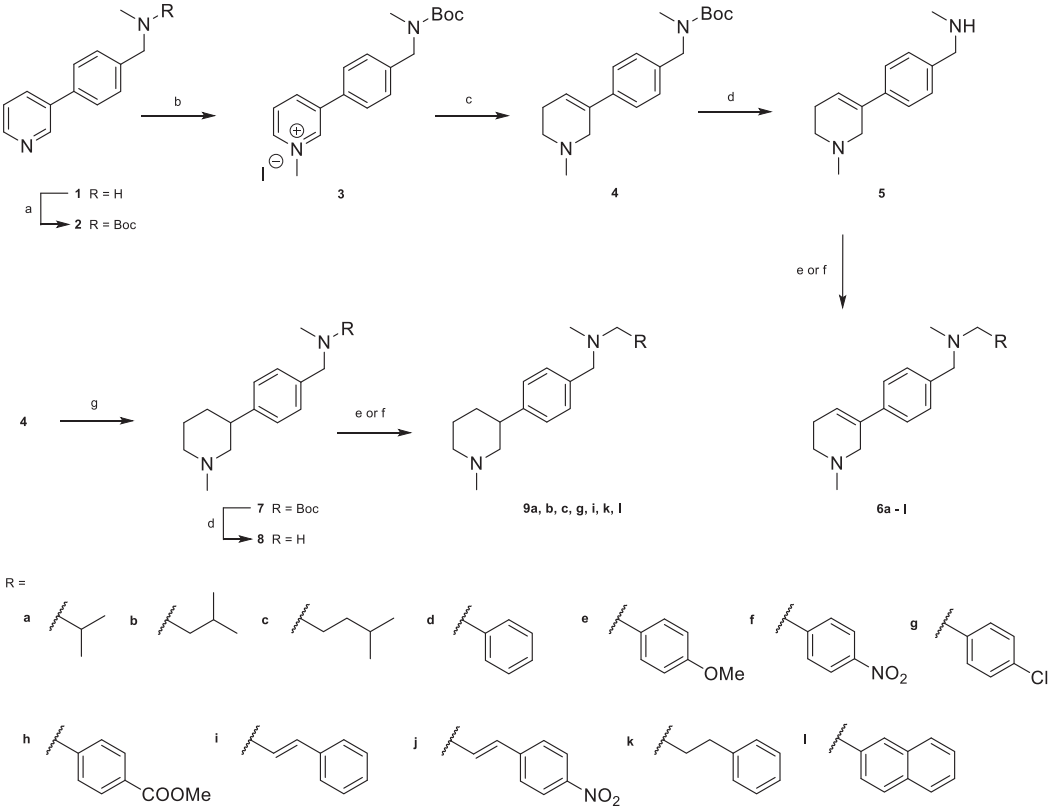
In order to confirm that a protonable nitrogen in the piperidine or tetrahydropyridine rings of compounds of type **6** and **9**, mimicking the pro-C4 HEI, contributes to OSC inhibition, we prepared pyridine congeners **10a** and **10b** bearing two of the most promising side chain equivalents. For this purpose, building block **1** was reacted in the manner described above with 4-chlorobenzaldehyde/sodium borohydride and cinnamyl bromide/triethylamine, respectively (Scheme 2).

The role of the central benzene ring (equivalent to ring C of the steroids) was investigated by replacing it with a 1,2,3-triazole ring, easily accessible via a modified Huisgen azide-alkyne cycloaddition [37]. The N-protected racemic 3-azidopiperidine **11** [38] and the propargylamine **12** [39] bearing the promising 4-chlorobenzyl residue were reacted under standard conditions (sodium ascorbate, copper sulfate) to give the 1,2,3-triazole **13** in good yield. After removal of the Boc protecting group the piperidine ring was *N*-methylated with formaldehyde/sodium cyanoborohydride (Scheme 3).

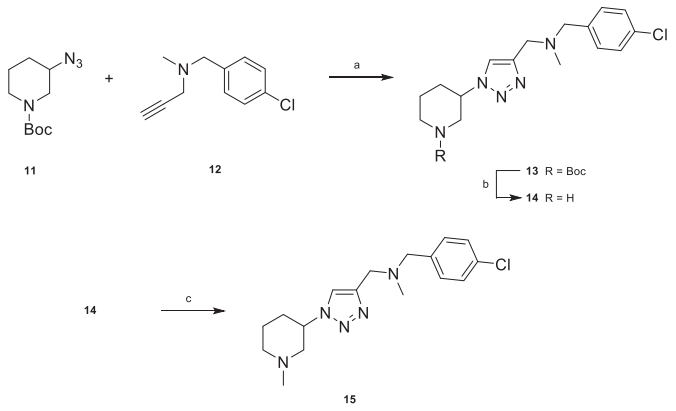
As the 4-chlorobenzyl compound **9g** had emerged as a very potent OSC inhibitor, we intended to investigate a considerably more steroid-like analogue. For this purpose, we introduced a ring



Scheme 2. Arylpyridines prepared from **1**: Reagents and conditions: For **10a**: 4-chlorobenzaldehyde, $NaBH_4$, MeOH, AcOH, r.t., 18 h (81%); (f) 3-bromo-1-phenyl-1-propene, Et_3N, DMF, r.t., 18 h (53%).



Scheme 1. Reagents and conditions: (a) Boc_2O, Et_3N, MeOH, r.t., 3 h (74%); (b) CH_3I, acetone, r.t., 18 h (65%); (c) $NaBH_4$, MeOH, r.t., 18 h (71%); (d) CF_3COOH, CH_2Cl_2, r.t., 1 h (84–92%); (e) R–CHO, $NaBH_4$, MeOH, AcOH, r.t., 18 h (45–96%); (f) R–CH_2Br, Et_3N, DMF, r.t., 18 h (20–65%); (g) H_2, Pd/C (cat.), MeOH, r.t., 20 h (77%).



Scheme 3. Reagents and conditions: (a) sodium ascorbate (cat.), $CuSO_4$ x $5H_2O$ (cat.), *n*-butanol/water, r.t., 14 h (58%); (b) CF_3COOH, CH_2Cl_2, r.t., 1 h (78%); (c) aqueous HCHO, $NaCNBH_3$, AcOH (cat.), MeOH, r.t., 3 h (78%).

D equivalent by replacing the central benzyl moiety of **9a** with an indane. This modification was inspired by reports on estrogenic [40] as well as antiestrogenic [41] compounds bearing arylperhydroindane scaffolds, and on inhibitors of the enzymes 17β-hydroxysteroid dehydrogenase [42] and 17α-hydroxylase-17,20-lyase [31] bearing arylindanes as rings A + CD equivalents.

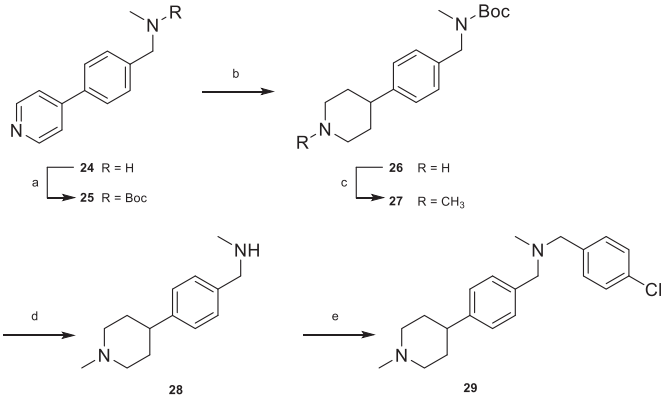
5-Bromoindan-1-one (**16**) was coupled with 3-pyridylboroxine under Suzuki conditions [34], accelerated by microwave irradiation [43], to give pyridylindanone **17** in high yield. Reductive amination with methylamine and sodium borohydride gave the methylaminoindane **18**. In order to avoid the presence of two competing secondary amino groups in the forthcoming reactions, the secondary amino group of **18** was protected as the Boc derivative (**19**). High pressure catalytic hydrogenation over palladium in methanol/acetic acid gave a moderate yield of the piperidine **20** as a racemic mixture of inseparable diastereomers. N-Methylation of the piperidine under standard conditions, followed by deprotection of the amino group at the indane moiety gave the secondary amine **22**, which was converted to the *N*-(4-chlorobenzyl) derivative **23** by reductive benzylation with 4-chlorobenzaldehyde and sodium cyanoborohydride (Scheme 4).

Docking experiments (see section below) suggested that the piperidin-4-yl regioisomer of the until then most attractive inhibitor **9g** might have even higher affinity for the active site of the human OSC. Known phenylpyridine **24**, readily available from 4-pyridineboronic acid and 4-bromobenzaldehyde via Suzuki cross-coupling, followed by reductive amination with methylamine [45], was protected with the Boc group and then hydrogenated in the same manner as described for **20** to give the 4-arylpiperidine **26**. N-Methylation of the piperidine ring, *N*-deprotection at the benzylic position, and *N*-benzylation with 4-chlorobenzaldehyde and sodium borohydride gave the target compound **29** (Scheme 5).

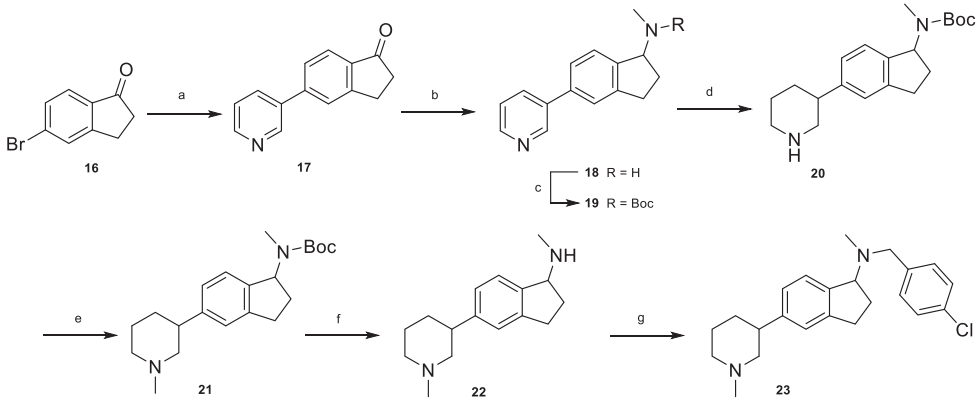
2.2. Screenings

2.2.1. Identification of inhibitors of enzymes in cholesterol biosynthesis

In a first step the compounds were subjected to an in-house qualitative screening system for the detection of target enzymes in the post-squalene part of cholesterol biosynthesis [27]. In this whole-cell assay human HL-60 cells were incubated with the test compounds. After cell lysis and extraction, the sterol pattern was analyzed by GC–MS. Inhibition of particular enzymes leads to typical changes in the sterol pattern. The results are presented in Table 1. In the tetrahydropyridine series containing 12 different side chain equivalents (see Scheme 1), compounds **6a-c** bear aliphatic chains, however, despite having the highest similarity to the native cholesterol side chain, these did not interfere with cholesterol biosynthesis. In contrast, most of the compounds bearing aromatic substituents lead to a significant accumulation of 2,3-oxidosqualene, clearly indicating an inhibition of the enzyme



Scheme 5. Reagents and conditions: (a) Boc_2O, Et_3N, MeOH, r.t., 3 h (88%); (b) H_2 (40 bar), Pd/C (cat.), MeOH-AcOH, r.t., 24 h (29%); (c) aqueous HCHO, $NaCNBH_3$, AcOH (cat.), MeOH, r.t., 3 h (70%); (d) CF_3COOH, CH_2Cl_2, r.t., 1 h (75%); (e) 4-chlorobenzaldehyde, $NaBH_4$, MeOH, AcOH, r.t., 18 h (74%).



Scheme 4. Reagents and conditions: (a) 3-pyridylboroxine, $Pd(Ph_3P)_4$ (cat.), Na_2CO_3, dioxane-water, microwave irradiation, 95 °C, 15 min (83%); (b) CH_3NH_2–HCl, Et_3N, $MgClO_4$ (cat.), 1,2-dichloroethane, r.t., 18 h (69%); (c) Boc_2O, Et_3N, MeOH, r.t., 3 h (87%); (d) H_2 (40 bar), Pd/C (cat.), MeOH-AcOH, r.t., 24 h (29%); (e) aqueous HCHO, $NaCNBH_3$, AcOH (cat.), MeOH, r.t., 3 h (78%); (f) CF_3COOH, CH_2Cl_2, r.t., 1 h (97%); (g) 4-chlorobenzaldehyde, $NaBH_4$, MeOH, AcOH, r.t., 18 h (71%).

Table 1
Target enzymes of the inhibitors in cholesterol biosynthesis and effect on total cholesterol biosynthesis of the OSC inhibitors in a whole-cell assay; general cytotoxicity determined in a MTT assay on a human cell line (HL-60).

Compound	Target enzyme in cholesterol biosynthesis	Inhibition of total cholesterol biosynthesis IC_{50} (μM)[a]	Cytotoxicity (MTT assay) IC_{50} (μM)[a]
6a	–[b]	n.d.[c]	>50
6b	–	n.d.	>50
6c	–	n.d.	>50
6d	–	n.d.	>50
6e	–	n.d.	>50
6f	OSC[d]	2.29	28
6g	OSC	2.14	>50
6h	–	n.d.	>50
6i	OSC	0.68	>50
6j	OSC	1.28	>50
6k	(OSC)[e]	n.d.	>50
6l	OSC	0.37	>50
9a	–	n.d.	>50
9b	–	n.d.	>50
9c	–	n.d.	>50
9g	OSC	1.29	>50
9i	OSC	1.10	>50
9k	(OSC)[e]	n.d.	>50
9l	OSC	1.32	40
10a	–	n.d.	21
10b	–	n.d.	44
15	7-DHCR[f]	n.d.	>50
23	OSC	0.14	>50
29	OSC	0.26	>50
BIBX 79	OSC	0.31	>50
Ro 48–8071	OSC	0.092	8
Cisplatin	n.d.	n.d.	5

[a] Experiments carried out in triplicate.
[b] No changes in the sterol pattern downstream squalene detectable.
[c] Not determined.
[d] Inhibition of oxidosqualene cyclase, detected by the accumulation of 2,3-oxidosqualene.
[e] Only very weak accumulation of 2,3-oxidosqualene.
[f] Weak inhibition of 7-dehydrocholesterol reductase, detected by accumulation of 7-dehydrocholesterol.

oxidosqualene cyclase. In the series of *N*-benzyl compounds the nature of the substituents had a strong influence on the biological activity. Compounds with unsubstituted (**6d**) or electron-rich (**6e**) benzyl residues were inactive, whereas those bearing small electron-releasing substituents (NO_2, Cl in **6f,g**) at the benzyl group showed significant activity. An ester substituent (**6h**), however, led to complete loss of activity. Larger aromatic substituents like styryl (**6i,j**) and naphthylmethyl (**6l**) were tolerated well. Virtually identical results were obtained in the piperidine series (**9g,i,l**). Replacing the piperidine and tetrahydropyridine rings with a pyridine ring (**10a,b**) led to a complete loss of activity. Compound **15**, bearing a 1,2,3-triazole ring instead of the central benzene ring led only to a very slight accumulation of dehydrocholesterol, indicating an inhibition of the enzyme 7-dehydrocholesterol reductase, the very last enzyme in cholesterol biosynthesis. But in comparison to the phenethyl tetrahydroisoquinoline-type 7-dehydrocholesterol reductase inhibitors described by us previously [29], the potency of **15** is very poor. The piperidin-4-yl regioisomer **29** of the inhibitor **9g** was found to be an inhibitor of OSC as well.

2.2.2. Assay for inhibition of total cholesterol biosynthesis in cells

Compounds which led to a significant accumulation of 2,3-oxidosqualene in the first, qualitative assay were further evaluated for their inhibition of total cholesterol biosynthesis in HL-60 cells [27]. In this assay the cells are incubated with the inhibitors in the presence of 2-^{13}C-acetate. This leads to an incorporation of ^{13}C atoms into the cholesterol molecules biosynthesized during the incubation period, and allows to distinguish newly synthesised cholesterol from unlabelled matrix cholesterol, present in the cells before incubation, by GC–MS. The IC_{50} values determined this way are presented in Table 1. A number of tetrahydropyridine- (**6f,g,i,j,l**) and piperidine-type (**9g,i,l**) compounds inhibited total cholesterol biosynthesis with IC_{50} values in the range of 0.4–2.3 μM. The indane congener **23** and the piperidin-4-yl regioisomer **29** were even more potent (0.14 and 0.26 μM), showing activities comparable to those of the prominent OSC inhibitors BIBX 79 [24] and Ro 48–8071 [4].

2.2.3. Assay for inhibition of ergosterol biosynthesis in fungi

In order to investigate the antifungal potential [5,6] and the species selectivity of the new OSC inhibitors, the active compounds were subjected to our recently developed assay for the identification of inhibitors of ergosterol biosynthesis in fungi [44]. This assay works in a similar mode as the one described above for cholesterol biosynthesis and was performed on the strains *S. cerevisiae*, *Yarrowia lipolytica*, and *Candida glabrata*. Neither of the highly active inhibitors of human OSC described above led to a recognizable accumulation of precursor sterols, nor did incubation with these compounds reduce the ergosterol content of the cells (data not shown here). This indicates that the inhibitors described here do not interfere to a mentionable extent with the biosynthesis of ergosterol in fungi.

2.2.4. Cytotoxic activity

For the detection of undesired cytotoxic properties, the compounds were screened in a MTT assay according to the method of Mosmann [46] on human leukaemia HL-60 cells with cisplatin (IC_{50} = 5 μM) used as reference. The results are shown in Table 1. Almost all of the compounds were free from cytotoxicity. However, among the potent OSC inhibitors, the nitrobenzyl compound **6f** showed slight cytotoxicity (IC_{50} = 28 μM), for this reason this compound was not investigated further. In contrast, reference OSC

inhibitor Ro 48–8071 [10] (Fig. 1**G**) showed noteworthy cytotoxicity (IC_{50} = 8 μM). BIBX 79 [22], another prominent OSC inhibitor, was inactive in this assay.

2.2.5. Effect of inhibitors on OSCs from different organisms

OSC inhibitors are potential cholesterol-lowering drugs, but also play a role in treatment of fungal and protozoal infections. In order to elucidate the respective potential and species-selectivities, we performed in vitro experiments with human, fungal, and protozoal OSC. Activity of the compounds as inhibitors of OSC was tested by incubating homogenates prepared from cell cultures of different yeast strains with radiolabelled 2,3-oxidosqualene in the presence of increasing inhibitor concentrations. The results are presented in Table 2. Only the most active substances from the abovementioned investigations were analyzed. Tetrahydropyridines **6g,i,l**, piperidine congeners **9g,i,l**, indane **23**, and **29**, the 4-piperidyl analogue of **9g**, showed very similar patterns of inhibition. None of these compounds inhibits OSC from *T. cruzi*, and *S. cerevisiae* OSC is only slightly inhibited by two of the compounds (**6g**, **29**). In contrast, most of the compounds were identified as moderate to strong inhibitors of human OSC. The IC_{50} values determined for human OSC were in good concordance with the values determined for inhibition of total cholesterol biosynthesis in the whole-cell assay.

In contrast, Ro 48-8071 is an unselective inhibitor of the three OSCs.

2.3. Docking experiments

In order to understand the requirements for binding of the most active OSC inhibitors, docking experiments were performed based on the crystal structure of human OSC co-crystallized with Ro 48–8071 [3]. The results shown in Fig. 3 demonstrate that compound **9g** (*S* enantiomer shown) has a binding pose very similar to the one of Ro 48–8071. Both molecules show edge-to-face stacking of the haloarene ring with Trp192 and a cation-π interaction with Trp581. The only difference is that the protonated piperidine nitrogen of **9g** appears to be too far away from the carboxylate group of Asp455 for building up the salt bridge seen with the side chain of Ro 48–801. The benzylic amino group of **9g** is sandwiched between His232 and Phe696, forming cation-π interactions. For the *R* enantiomer the docking experiments predicted comparable interactions (data not shown). This suggests that both enantiomers of **9g** contribute to the observed biological activity. The isomeric achiral piperidine **29** shows a binding pose very similar to the one of (*S*)-**9g**, but due the more distal position of the ring nitrogen this isomer is more likely to build up the salt bridge with Asp455, what can explain the observed increase in inhibitory activity.

3. Discussion

The results presented here demonstrate that the concept of mimicking two of the cationic high energy intermediates occurring in the course of the OSC-catalyzed cyclization cascade in one molecule is feasible. 3-Arylpiperidines and their 1,2,5,6-tetrahydopyridine congeners are virtually equipotent inhibitors of human OSC in the case they bear a suitable lipophilic sterol side chain equivalent. Surprisingly, branched aliphatic residues which are most similar to the native sterol side chain, proved to be ineffective, whereas substituted benzyl, cinnamyl and naphthylmethyl groups led to potent enzyme inhibitors. This confirms earlier observations from others [13,16] and our groups [30,47], that the shape of the group occupying the binding site of the inconspicuous sterol side chain is of enormous significance for the affinity of sterol biosynthesis inhibitors.

Docking experiments clearly indicate that the 4-chlorobenzyl compound **9g** occupies the substrate binding site of human OSC very well and its binding mode is very similar to the one demonstrated for the established inhibitor Ro 48–8071 in a crystal structure [3]. Both the piperidine residue designed to mimic the pro-C4 HEI, and the benzylic amino group designed to mimic the pro-C20 HEI, contribute significantly to the interaction with the active site of the enzyme.

In contrast to the unselective reference inhibitor Ro 48–8071 [9], the newly designed inhibitors did not show noteworthy inhibition of yeast and trypanosomal OSC, and were devoid of undesired cytotoxicity.

For most of the compounds the IC_{50} values obtained on human OSC correlated very well with the IC_{50} values for inhibition of total cholesterol biosynthesis in a whole-cell assay. From this fact we may draw the conclusion that for these compounds inhibition of OSC is the dominating, if not exclusive factor triggering the attenuation of cholesterol biosynthesis in cells. Surprisingly, the naphthylmethyl compounds (**6l**, **9l**; factors 6 to >25) and the rigid indane derivative **23** (factor 30) showed significantly higher activity in the cellular assay on cholesterol biosynthesis than in vitro on the human OSC. This suggests that other factors, probably the inhibition of an enzyme in the pre-squalene part of cholesterol biosynthesis (which cannot be detected by our assay [25]), contribute significantly to the overall effects of these compounds.

For this reason the 4-chlorobenzyl compound **9g** was selected as the lead structure for further optimization. Replacing the central phenyl ring with a 1,2,3-triazole ring completely eliminated OSC inhibitory activity. However, the achiral 4-piperidyl regioisomer **29**, which was considered on the basis of our docking experiments, turned out to be even more active (factor 4–5 in both assays) than

Table 2
Effect of inhibitors on oxidosqualene cyclase activity of homogenates prepared from yeast recombinant strains SMY8 expressing *H. sapiens* and *T. cruzi* OSC, as well as *S. cerevisiae* wild-type strain FL100. For comparison, IC_{50} values determined for inhibition of total cholesterol biosynthesis in a whole-cell assay (see Table 1).

Compound	IC_{50} (μM)			
	OSC from *H. sapiens*[a]	OSC from *T. cruzi*[a]	OSC from *S. cerevisiae*[a]	Cholesterol biosynthesis inhibition
6g	2.51	>10	6.35	2.14
6i	5.28	>10	>10	0.68
6l	>10	>10	>10	0.37
9g	2.06	>10	>10	1.29
9i	2.06	>10	>10	1.10
9l	8.51	>10	>10	1.32
23	4.22	>10	>10	0.14
29	0.51	>10	4.83	0.26
Ro 48–8071[b]	0.17	0.90	0.90[c]	0.092[c]

[a] Values are the means of two separate experiments, each one carried out in duplicate. The maximum deviations from the mean were less than 10%.
[b] From Ref. [9].
[c] Determined on *S. cerevisiae* OSC expressed in SMY8.

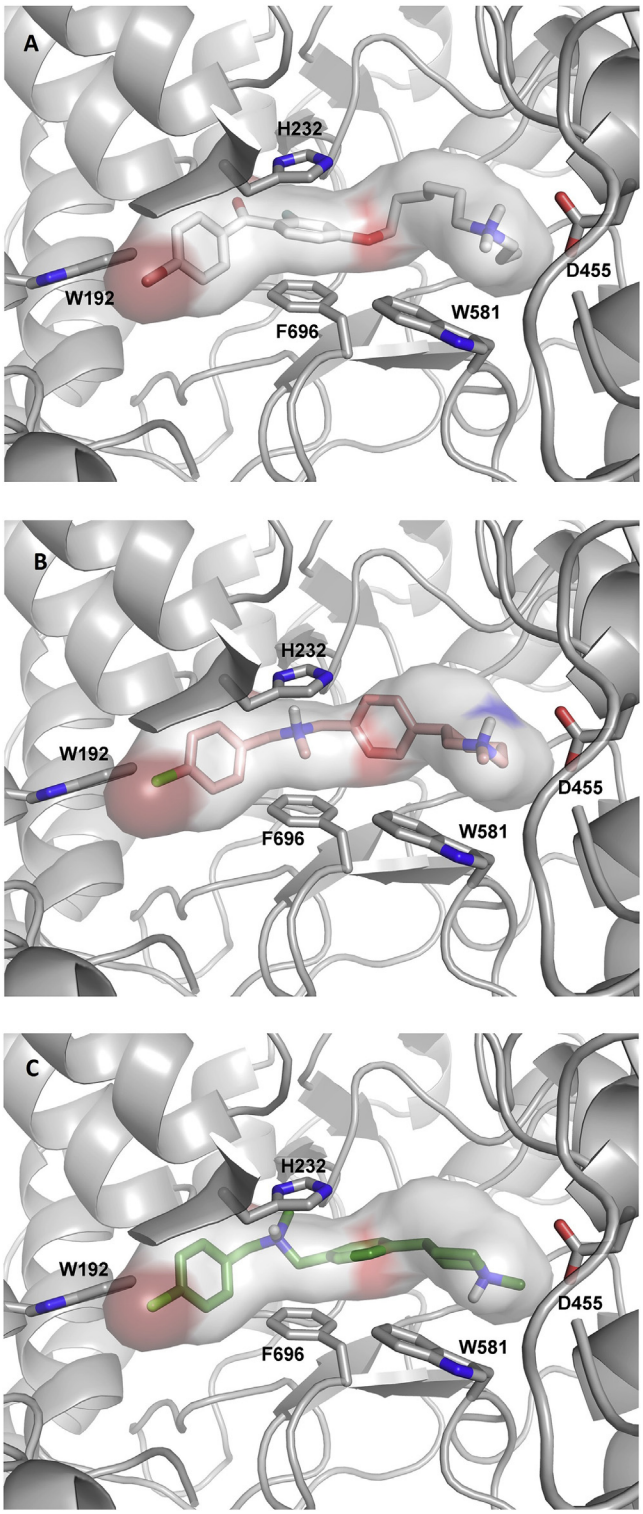


Fig. 3. A: Overview of Ro 48–871 (grey carbons) binding into the OSC active site (from PDB code 1W6J; dockings gave identical results). Key ligand-protein contacts include edge-to-face stacking of the bromobenzene moiety with Trp192 and π–π stacking of the central fluorobenzene moiety with Phe696. The protonated tertiary amine side-chain forms a cation-π contact with Trp581 as well as a salt bridge with Asp455. B: Docking pose of compound **9g** (pale red carbons; *S* isomer shown) docked into the OSC active site. **9g** shows a binding mode very similar to the one of Ro4877, and forms overall similar interactions. The most notable difference is that its central protonated amine moiety is sandwiched between His232 and Phe696, forming cation- π interactions, while its central benzene moiety does not engage in face-to-face π–π stacking interactions. C: The docking pose of compound **29** (pale green carbons) is very similar to the one of compound **9g**.

the 3-piperidyl compound **9g**, and favourably compares with the equipotent, but unselective OSC inhibitor Ro 48–8071. Compounds **9g** and **29** show acceptable lipophilicity (calculated logP values about 4.7), and fulfil Lipinski's rule of five [48], so they can be considered promising candidates for further development.

4. Experimental

4.1. General Procedure A: introduction of the N-Boc-protecting group in secondary amines

The amine (1 equiv) was dissolved in methanol (4 mL/mmol), triethylamine (2 equiv) and di-*tert*-butyl dicarbonate (2 equiv) were added, and the mixture was stirred for 3 h at room temperature. The solvent was evaporated, the residue suspended in satd. aqueous Na_2CO_3 solution and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated. Purification was done by flash column chromatography.

4.2. General Procedure B: reductive N-alkylation of secondary amines

To a solution of one equivalent of the amine (0.2–1.0 mmol), the appropriate aldehyde (1.5–2.5 equiv) and glacial acetic acid (0.5 equiv) in methanol (4 mL/mmol amine), sodium borohydride (1.85–2 equiv) was added in portions over a period of 10 min with stirring, and the reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated under reduced pressure, the residue taken up in CH_2Cl_2 (30 mL/mmol amine) and washed with concentrated sodium carbonate solution (30 mL/mmol amine). The aqueous solution was extracted with CH_2Cl_2 (3 × 20 mL/mmol amine) and the combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (CH_2Cl_2:CH_3OH 9:1 and 1% NEt_3).

5. 3.General Procedure C: N-alkylation of secondary amines

A solution of secondary amine (0.55 mmol), the appropriate alkyl bromide (0.36–0.46 mmol) and triethylamine (0.55 mmol) in *N*,*N*-dimethylformamide (3 mL) was stirred for 18 h at room temperature. The solvent was evaporated under reduced pressure and concentrated sodium carbonate solution (30 mL) was added to the crude residue. The aqueous solution was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic layers were dried over $MgSO_4$ und evaporated. The crude material was purified by flash column chromatography (CH_2Cl_2:CH_3OH, 9:1 and 1% NEt_3).

5.1. General Procedure D: reductive N-methylation of secondary amines

The *N*-Boc-amine (1 equiv) and was dissolved in methanol (1% acetic acid) (3 mL/mmol), formaldehyde (5 equiv, 37% in H_2O) and sodium cyanoborohydride (2.5 equiv) were added, and the mixture was stirred for 3 h at room temperature. The solvent was evaporated, the residue suspended in satd. Na_2CO_3 solution and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated. Purification was done by flash column chromatography.

5.2. General Procedure E: cleavage of the N-Boc-protecting group

The *N*-Boc-amine was dissolved in CH_2Cl_2 and trifluoracetic acid (1:1) (12 mL/mmol) and the mixture stirred for 1 h at room temperature. The mixture was alkalized with satd. Na_2CO_3 solution and extracted three times with CH_2Cl_2. The combined organic layers

were dried over Na_2SO_4 and evaporated.

5.3. 3-[4-(N-tert-butoxycarbonyl-N-methylaminomethyl)-phenyl]-1-methylpyridinium iodide (**3**)

To a solution of *tert*-butyl *N*-methyl-*N*-4-(pyridine-3-yl)-benzylcarbamate (**2**) (175 mg, 0.557 mmol) in acetone (5 mL) iodomethane (347 μL, 5.57 mmol) was added and the mixture was stirred for 18 h at room temperature. The precipitate was collected by filtration, washed with cold acetone (10 mL) and recrystallized from acetone to afford **3** as white solid (158 mg, 65%); ^{1}H NMR (500 MHz, CD_2Cl_2): δ = 9.31 ppm (s, 1H), 8.89–8.85 (m, 2H), 8.17–8.14 (m, 1H), 7.87–7.86 (m, 2H), 7.51–7.49 (m, 2H), 4.55 (s, 2H), 4.51 (s, 3H), 2.91 (s, 3H), 1.53–1.47 (m, 9H); ^{13}C NMR (125 MHz, CD_2Cl_2): δ = 157.6 ppm, 145.1, 144.9, 144.0, 142.3, 133.8, 129.7, 129.3, 129.0, 126.0, 81.7, 53.3, 49.3, 34.9, 28.8; IR (KBr): $\tilde{\nu}$ = 3447 cm^{-1}, 3022, 2974, 2951, 1675, 1496, 1479, 1421, 1390, 1369, 1325, 1307, 1232, 1200, 1158, 949, 885, 835, 796, 772, 674, 616, 570; MS (CI, CH_5^+): *m/z* (%) = 315 (6), 299 (84), 243 (100), 199 (36); MS (EI, 70 eV): *m/z* (%) = 314 (16), 242 (100), 197 (46), 168 (62), 142 (54); HR-MS (EI, 70 eV): *m/z* = 313.1910 $[M]^+$: calcd for $C_{19}H_{25}N_2O_2$: 313.1916.

5.4. tert-Butyl N-methyl-4-(1-methyl-1,2,5,6-tetrahydropyridine-3-yl)-benzylcarbamate (**4**)

Sodium borohydride (260 mg, 6.76 mmol) was added in portions to a solution of 3-[4-(*N-tert*-butoxycarbonyl-N-methylaminomethyl)-phenyl]-1-methylpyridinium iodide (**3**) (595 mg, 1.35 mmol) in methanol (20 mL) over a period of 10 min and the mixture was stirred for 18 h at room temperature. The reaction mixture was poured into a conc. aqueous Na_2CO_3 solution (20 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (CH_2Cl_2:CH_3OH 9:1) to afford **4** as colourless oil (303 mg, 71%): ^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.33–7.31 ppm (m, 2H), 7.18–7.16 (m, 2H), 6.14–6.12 (m, 1H), 4.38 (s, 2H), 3.26 (dd, *J* = 2.6 and 4.5 Hz, 2H), 2.78 (s, 3H), 2.55 (t, *J* = 5.8 Hz, 2H), 2.42 (s, 3H), 2.37–2.32 (m, 2H), 1.50–1.40 (m, 9H); ^{13}C NMR (100 MHz, CD_2Cl_2): δ = 156.0 ppm, 138.8, 137.2, 134.7, 127.1, 124.9, 122.1, 79.3, 56.2, 52.2, 51.4, 45.7 33.8, 28.2, 26.5; IR (NaCl): $\tilde{\nu}$ = 2973 cm^{-1}, 2934, 2839, 2781, 1903, 1694, 1513, 1480, 1455, 1425, 1391, 1365, 1289, 1247, 1174, 1142, 1047, 1001, 973, 953, 920, 880, 819, 798, 770, 666; MS (CI, CH_5^+): *m/z* (%) = 317 (36) $[M+H]^+$, 261 (100), 186 (16); MS (EI, 70 eV): *m/z* (%) = 316 (41) $[M]^+$, 260 (62), 216 (58), 186 (45), 172 (56), 142 (100), 130 (76), 115 (28); HR-MS (EI, 70 eV): *m/z* = 316.2146 $[M]^+$: calcd for $C_{19}H_{28}N_2O_2$: 316.2151.

5.5. (RS)-tert-butyl N-methyl-4-(1-methylpiperidine-3-yl)-benzylcarbamate (**7**)

A solution of **4** (255 mg, 1.18 mmol) and palladium on carbon (10%, 20 mg) in methanol (5 mL) was hydrogenated for 20 h at room temperature. The mixture was filtered through celite, evaporated, purified by flash column chromatography (CH_2Cl_2:CH_3OH 9:1) to afford **7** as orange oil (290 mg, 77%): ^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.21–7.19 ppm (m, 2H), 7.16–7.14 (m, 2H), 4.36 (s, 2H), 2.91–2.81 (m, 3H), 2.77 (s, 3H), 2.25 (s, 3H), 1.96–1.85 (m, 3H), 1.79–1.64 (m, 2H), 1.45 (s, 9H), 1.42–1.34 (m, 1H); ^{13}C NMR (125 MHz, CD_2Cl_2): δ = 155.6 ppm, 144.2, 136.7, 127.8), 79.6, 63.5, 56.2, 52.6, 51.9, 46.7, 42.9, 34.0, 31.5, 28.6, 26.1; IR (NaCl): $\tilde{\nu}$ = 2971 cm^{-1}, 2932, 2854, 2778, 1698, 1514, 1479, 1463, 1450, 1419, 1391, 1365, 1306, 1285, 1244, 1174, 1144, 1091, 1061, 1047, 1026, 986, 957, 880, 814, 789, 771; MS (CI, CH_5^+): *m/z* (%) = 319 (30) $[M+H]^+$, 263 (100); MS (EI, 70 eV): *m/z* (%) = 318 (100) $[M]^+$, 262 (25), 217 (53); HR-MS (EI, 70 eV): *m/z* = 318.2289 $[M]^+$: calcd for $C_{19}H_{30}N_2O_2$: 318.2307.

5.6. (RS)-(N-Methyl-4-(1-methylpiperidine-3-yl)-benzylamine (**8**)

Prepared following General Procedure E using **7** (200 mg, 0.628 mmol) to yield 115 mg (84%) of **8** as yellow oil (flash column chromatography CH_2Cl_2:CH_3OH 9:1 and 1% NEt_3); ^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.45–7.43 ppm (m, 2H), 7.25–7.23 (m, 2H), 3.87 (s, 2H), 3.03–2.94 (m, 3H), 2.46 (s, 3H), 2.38 (s, 3H), 2.20–2.11 (m, 2H), 1.90–1.82 (m, 3H), 1.48–1.41 (m, 1H); ^{13}C NMR (100 MHz, CD_2Cl_2): δ = 144 ppm, 132.8, 130.5, 128.5, 127.5, 62.3, 55.8, 55.3, 46.1, 42.1, 33.4, 30.6, 25.5; IR (NaCl): $\tilde{\nu}$ = 3396 cm^{-1}, 2933, 2852, 2779, 2359, 1684, 1517, 1465, 1375, 1284, 1242, 1198, 1157, 1130, 1090, 1060, 1024, 985, 912, 822, 798; MS (CI, CH_5^+): *m/z* (%) = 219 (100) $[M+H]^+$, 188 (30); MS (EI, 70 eV): *m/z* (%) = 218 (12) $[M]^+$, 58 (100); HR-MS (EI, 70 eV): *m/z* = 218.1778 $[M]^+$: calcd for $C_{14}H_{22}N_2$: 218.1783.

5.7. N-(4-Chlorobenzyl)-N-methyl-4-(1-methylpiperidine-3-yl)-benzylamine (**9g**)

Prepared following General Procedure B using **8** (200 mg, 0.628 mmol) and 4-chlorobenzaldehyde (111 μL, 0.942 mmol) to yield 220 mg (70%) of **9g** as yellow oil; ^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.32–7.28 ppm (m, 6H), 7.20–7.18 (m, 2H), 3.47 (s, 2H), 3.46 (s, 2H), 3.07–2.97 (m, 3H), 2.39 (s, 3H), 2.18–2.12 (m, 5H) 1.95–1.79 (m, 3H), 1.46 (qd, *J* = 3.9 and 12.3 Hz, 1H); ^{13}C NMR (100 MHz, CD_2Cl_2): δ = 142.7 ppm, 138.8, 138.1, 132.7, 130.6, 129.4, 128.6, 127.4, 62.5, 61.8, 61.3, 55.7, 45.9, 42.3, 41.9, 30.9, 25.2; IR (NaCl): $\tilde{\nu}$ = 3416 cm^{-1}, 2933, 2781, 1597, 1513, 1498, 1463, 1364, 1284, 1241, 1130, 1089, 1060, 1014, 985, 804, 670; MS (CI, CH_5^+): *m/z* (%) = 343 (100) $[M+H]^+$, 231 (6), 188 (18), 168 (16), 125 (12); MS (EI, 70 eV): *m/z* (%) = 342 (18) $[M]^+$, 58 (100); HR-MS (EI, 70 eV): *m/z* = 342.1863 $[M]^+$: calcd for $C_{21}H_{27}ClN_2$: 342.1863.

5.8. tert-Butyl N-methyl-N-[4-(pyridin-4-yl)-benzyl]-carbamate (**25**)

Prepared following General Procedure A using **24** (200 mg, 1.01 mmol) to yield 264 mg (88%) of **25** as yellow oil; (flash column chromatography ethyl acetate/hexanes 1:1 and 2% NEt_3); ^{1}H NMR (CD_2Cl_2, 400 MHz): δ = 8.62 ppm (dd, *J* = 1.6 and 4.5 Hz, 2H), 7.68–7.62 (m, 2H), 7.52 (dd, *J* = 1.6 and 4.5 Hz, 2H), 7.38–7.32 (m, 2H), 4.47 (s, 2H), 2.83 (s, 3H), 1.53–1.39 (m, 9H); ^{13}C NMR (CD_2Cl_2, 125 MHz): δ = 156.4 ppm, 150.7 (2C), 148.1, 140.0, 137.3, 128.5 (2C), 127.5 (2C), 121.8 (2C), 79.9, 52.6 (rotamer 1), 52.0 (rotamer 2), 34.3, 28.5 (3C); IR (NaCl): $\tilde{\nu}$ = 3028 cm^{-1}, 2974, 2928, 2358, 1690, 1597, 1487, 1391, 1365, 1245, 1171, 1145, 879, 801; MS (CI, CH_5^+): *m/z* (%) = 299 (100) $[M+H]^+$; MS (EI, 70 eV): *m/z* (%) = 242 (48); HR-MS (EI, 70 eV): *m/z* = 298.1652 $[M]^+$, calcd for $C_{18}H_{22}N_2O_2$: 298.1681.

5.8.1. tert-Butyl N-methyl-N-[4-(piperidin-4-yl)-benzyl]-carbamate (**26**)

Pyridine **25** (1.96 g, 6.57 mmol) was dissolved in methanol (5 mL) and acetic acid (50 mL). Palladium on carbon (10%, 50 mg) was added and the mixture was stirred in a pressure vessel under an atmosphere of hydrogen (40 bar) for 24 h at room temperature. The mixture was filtered over celite, brought to pH 10 with NaOH, and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. Purification was done by flash column chromatography (CH_2Cl_2/NEt_3 9:1) to yield 578 mg (29%) of **26** as a brown oil; (flash column chromatography CH_2Cl_2/NEt_3 9:1); ^{1}H NMR (CD_2Cl_2, 500 MHz): δ = 7.20–7.14 ppm (m, 4H), 4.36 (s, 2H), 3.16–3.10 (m, 2H), 2.78 (s, 3H), 2.73–2.66 (m,

2H), 2.63–2.55 (m, 1H), 1.80–1.74 (m, 3H), 1.63–1.52 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (CD_2Cl_2, 100 MHz): δ = 156.5 ppm, 146.4, 136.4, 127.9 (2C), 127.3 (2C), 79.6, 52.5 (rotamer 1), 51.9 (rotamer 2), 47.5 (2C), 43.2, 35.0 (2C), 34.0, 28.6 (3C); IR (NaCl): $\tilde{\nu}$ = 2974 cm^{-1}, 2930, 2851, 2807, 2735, 1694, 1480, 1449, 1391, 1365, 1246, 1173, 1143, 880; MS (CI, CH_5^+): *m/z* (%) = 305 (22) $[M+H]^+$; MS (EI, 70 eV): *m/z* (%) = 304 (2) $[M]^+$; HR-MS (EI, 70 eV): *m/z* = 304.2148 $[M]^+$, calcd for $C_{18}H_{28}N_2O_2$: 304.2151.

*5.8.2. tert-Butyl N-methyl-N-[4-(1-methylpiperidin-4-yl)-benzyl]-carbamate (**27**)*

Prepared following General Procedure D using **26** (185 mg, 0.608 mmol) to yield 136 mg (70%) of **27** as colourless oil (flash column chromatography ethyl acetate/hexanes 1:1 and 2% NEt_3); ^{1}H NMR (CD_2Cl_2, 500 MHz): δ = 7.21–7.18 ppm (m, 2H), 7.16–7.13 (m, 2H), 4.36 (s, 2H), 2.94–2.89 (m, 2H), 2.77 (s, 3H), 2.49–2.41 (m, 1H), 2.25 (s, 3H), 2.04–1.97 (m, 2H), 1.81–1.68 (m, 4H), 1.45 (s, 9H); ^{13}C NMR (CD_2Cl_2, 100 MHz): δ = 156.3 ppm, 146.0, 136.4, 127.8 (2C), 127.3 (2C), 79.6, 56.7 (2C), 52.5 (rotamer 1), 51.9 (rotamer 2), 46.7, 42.1, 34.0 (3C), 28.6 (3C); IR (NaCl): $\tilde{\nu}$ = 2973 cm^{-1}, 2934, 2846, 2780, 2735, 2678, 2361, 2342, 1697, 1448, 1420, 1391, 1365, 1278, 1246, 1174, 1142, 880; MS (CI, CH_5^+): *m/z* (%) = 319 (74) $[M+H]^+$; MS (EI, 70 eV): *m/z* (%) = 318 (18) $[M]^+$; HR-MS (EI, 70 eV): *m/z* = 318.2294 $[M]^+$, calcd for $C_{19}H_{30}N_2O_2$: 318.2307.

*5.8.3. N-Methyl-1-[4-(1-methylpiperidin-4-yl)-phenyl]-methanamine (**28**)*

Prepared following General Procedure E using **27** (118 mg, 0.371 mmol) to yield 62 mg (75%) of **28** as colourless wax; ^{1}H NMR (CD_2Cl_2, 500 MHz): δ = 7.25–7.22 ppm (m, 2H), 7.20–7.17 (m, 2H), 3.68 (s, 2H), 2.96–2.91 (m, 2H), 2.62 (s, 1H), 2.49–2.41 (m, 1H), 2.40 (s, 3H), 2.27 (s, 3H), 2.07–1.99 (m, 2H), 1.81–1.70 (m, 4H); ^{13}C NMR (CD_2Cl_2, 100 MHz): δ = 145.7 ppm, 138.2, 128.7 (2C), 127.2 (2C), 56.7 (2C), 55.8, 46.5, 42.0, 36.0, 33.8 (2C); IR (NaCl): $\tilde{\nu}$ = 3012 cm^{-1}, 2934, 2844, 2780, 2735, 2680, 2361, 2342, 1688, 1514, 1464, 1445, 1378, 1200, 1133, 994, 824, 764; MS (CI, CH_5^+): *m/z* (%) = 219 (100) $[M+H]^+$; MS (EI, 70 eV): *m/z* (%) = 218 (40) $[M]^+$; HR-MS (EI, 70 eV): *m/z* = 218.1832 $[M]^+$, calcd for $C_{14}H_{22}N_2$: 218.1783.

*5.8.4. N-(4-Chlorobenzyl)-N-methyl-1-[4-(1-methylpiperidin-4-yl)-phenyl]-methanamine (**29**)*

Prepared following General Procedure B using **28** (50 mg, 0.23 mmol) and 4-chlorobenzaldehyde (80 mg, 0.57 mmol) to yield 58 mg (74%) of **29** as colourless oil (flash column chromatography ethyl acetate/hexanes 9:1 and 2% NEt_3); ^{1}H NMR (CD_2Cl_2, 400 MHz): δ = 7.34–7.25 ppm (m, 6H), 7.20–7.16 (m, 2H), 3.46 (s, 2H), 3.45 (s, 2H), 2.94–2.87 (m, 2H), 2.48–2.39 (m, 1H), 2.24 (s, 3H), 2.12 (s, 3H), 2.03–1.94 (m, 2H), 1.80–1.67 (m, 4H); ^{13}C NMR (CD_2Cl_2, 100 MHz): δ = 145.8 ppm, 138.9, 137.4, 132.6, 130.6 (2C), 129.2 (2C), 128.6 (2C), 127.1 (2C), 61.9, 61.3, 56.8 (2C), 46.7, 42.3, 42.2, 34.1 (2C); IR (NaCl): $\tilde{\nu}$ = 3022 cm^{-1}, 2934, 2883, 2842, 2780, 2735, 2679, 1513, 1489, 1461, 1445, 1378, 1364, 1279, 1134, 1087, 1068, 1031, 1015, 994, 837, 803; MS (CI, CH_5^+): *m/z* (%) = 345 (40) $[M+H]^+$, 343 (100) $[M+H]^+$; MS (EI, 70 eV): *m/z* (%) = 344 (12) $[M]^+$, 342 (34) $[M]^+$; HR-MS (EI, 70 eV): *m/z* = 342.1865 $[M]^+$, calcd for $C_{21}H_{27}ClN_2$: 342.1863.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.12.025.

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