Journal of Medicinal Chemistry

Article

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Conicasterol E, a Small Heterodimer Partner Sparing Farnesoid X Receptor Modulator Endowed with a Pregnane X Receptor Agonistic Activity, from the Marine Sponge *Theonella swinhoei*

Valentina Sepe,[†] Raffaella Ummarino,[†] Maria Valeria D'Auria,[†] Maria Giovanna Chini,[‡] Giuseppe Bifulco,[‡] Barbara Renga,[§] Claudio D'Amore,[§] Cécile Debitus,[∥] Stefano Fiorucci,[§,⊥] and Angela Zampella*[,†,⊥]

[†]Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", Via D. Montesano 49, 80131 Napoli, Italy

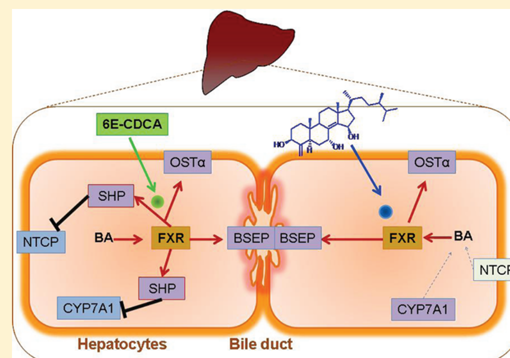
[‡]Dipartimento di Scienze Farmaceutiche e Biomediche, Università di Salerno, Via Ponte don Melillo, 84084 Fisciano (SA), Italy

[§]Dipartimento di Medicina Clinica e Sperimentale, Nuova Facoltà di Medicina e Chirurgia, Università di Perugia, Via Gerardo Dottori 1, S. Andrea delle Fratte, 06132 Perugia, Italy

[∥]Institut de Recherche pour le Dèveloppement (IRD), Polynesian Research Center on Island Biodiversity, BP529, 98713 Papeete, Tahiti, French Polynesia

Ⓢ *Supporting Information*

ABSTRACT: We report the isolation and pharmacological characterization of conicasterol E isolated from the marine sponge *Theonella swinhoei*. Pharmacological characterization of this steroid in comparison to CDCA, a natural FXR ligand, and 6-ECDCA, a synthetic FXR agonist generated by an improved synthetic strategy, and rifaximin, a potent PXR agonist, demonstrated that conicasterol E is an FXR modulator endowed with PXR agonistic activity. Conicasterol E induces the expression of genes involved in bile acids detoxification without effect on the expression of small heterodimer partner (SHP), thus sparing the expression of genes involved in bile acids biosynthesis. The relative positioning in the ligand binding domain of FXR, explored through docking calculations, demonstrated a different spatial arrangement for conicasterol E and pointed to the presence of simultaneous and efficient interactions with the receptor. In summary, conicasterol E represents a FXR modulator and PXR agonist that might hold utility in treatment of liver disorders.



■ INTRODUCTION

Among nuclear receptors, farnesoid X receptor (FXR) has emerged as a valuable pharmacological target[1−5] because of its role in regulating bile acids (BAs), lipid, and glucose homeostasis. Activation of FXR, highly expressed in the liver, intestine, kidney, and adrenals, leads to complex responses, the most relevant of which is the inhibition of bile acids synthesis through the indirect repression of the expression of cytochrome 7A1 (CYP7A1), the rate limiting enzyme of this pathway. After its deorphanization[6−8] a number of nonsteroidal[9−14] and steroidal compounds,[15−17] have been shown to interact with the ligand binding domain (LBD) of the receptor and to promote FXR mediated gene transcription. Among these, 6-ECDCA has emerged as a potent, orally bioavailable FXR agonist,[18] and ongoing clinical trials have shown its utility in the treatment of type 2 diabetes.[19]

In this scenario the discovery of FXR modulators represents an important answer to the urgent demand of new drugs for the treatment of relevant human diseases including dyslipidemia, cholestasis, nonalcoholic steatohepatitis (NASH), and type 2 diabetes. Nevertheless the use of potent FXR ligands holds some potential risk. Indeed, it has been shown that FXR activation in mammalian cells and tissues inhibits biosynthesis of endogenous bile acids by indirect transrepression of cholesterol 7α-hydroxylase (CYP7A1), a gene encoding for the first and rate limiting enzyme involved in their biosynthesis. This effect is indirect and mediated by activation of SHP, small heterodimer partner, an atypical nuclear receptor that lacks the DNA binding domain and that binds to liver X receptor (LXR), causing its displacement from a positive regulatory element in the CYP7A1 promoter.[1,3] Despite the effect of SHP, which has been shown to be dispensable in some settings, it is well recognized that SHP activation amplifies the effects of FXR on bile acids uptake and biosynthesis, strongly suggesting that identification of SHP-sparing FXR modulators might have the potential to promote bile acid detoxification without interfering with the biosynthesis (Figure 1).

Besides the significant contribution derived from high-throughput screenings of chemical libraries and chemical strategy based on extensive modifications of the BAs body and side chain, only few natural FXR modulators have been

Received: July 27, 2011
Published: November 29, 2011

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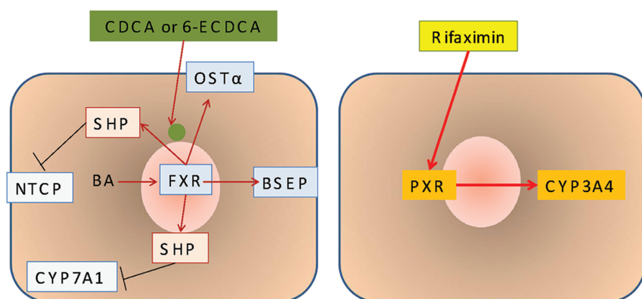


Figure 1. Schematic representation of the activity of FXR and PXR on target genes. For abbreviations, see list in the paper.

described. Guggulsterone, the active component of the resin extract of the tree *Commiphora mukul*,[20−23] and xanthohumol,[24] the principal prenylated chalcone from beer hops, are two well characterized examples of FXR modulators isolated from the vegetal realm. Recently the marine environment has also emerged as a source of human nuclear receptor ligands, and several molecules, including scalarane sesterterpenes,[25] isoprenoids,[26] and polyhydroxylated sulfated steroids,[27] have been shown to act as FXR antagonists, whereas to the best of our knowledge, no examples of marine derived FXR agonists are known.

As part of our systematic study on the chemical diversity and bioactivity of secondary metabolites from marine organisms collected at Solomon Islands,[28] we have found a single specimen of the sponge *Theonella swinhoei* as an extraordinary source of NRs steroidal ligands (Figure 2). Analysis of the polar extracts afforded solomonsterols A and B,[29] two potent PXR agonists and new leads in the treatment of immune-driven inflammatory bowel diseases,[30] whereas analysis of the apolar extracts allowed the isolation of a small library of 4-methylene steroids.[31,32] By means of a deep in vitro pharmacological analysis and docking calculations, we have demonstrated that the steroidal molecules shown in Figure 2 are potent PXR agonists and FXR antagonists able to antagonize the effect of CDCA on human FXR. Importantly, even if the junction between A/B rings is trans and the OH group at position 3 is in the β position with respect to the natural ligand CDCA, our docking studies demonstrated that all these compounds could be accommodated in the ligand binding domain of FXR, establishing hydrophobic and hydrogen bond contacts with the catalytic triad.[33] Furthermore, within this series, we have demonstrated that a methyl group at position 24 (conicasterols B−D in Figure 2) allows stronger interactions with a shallow groove on the FXR molecular surface with respect to the ethyl group in theonellasterol-like compounds (theonellasterols B−H in Figure 2).

In this paper we report the isolation and the molecular characterization of conicasterol E (**1**), a 7α,15β-dihydroxyconicasterol analogue (Figure 3), as the first example of an SHP-sparing FXR modulator endowed with PXR agonistic activity from the same specimens of *Theonella swinhoei*. Furthermore, in order to prove its efficiency in transactivation assays in comparison to a well validated FXR agonist, we have generated a novel synthetic strategy to obtain 6-ECDCA (**2**), a widely used derivative of endogenus CDCA (**3**) (Figure 3).

■ CHEMISTRY. ISOLATION AND STRUCTURAL CHARACTERIZATION OF CONICASTEROL E (1)

The initial processing of the *Theonella swinhoei* (R3159) was conducted according to procedures described previously.[34] The *n*-hexane extract from a solvent partitioning Kupchan procedure was chromatographed by silica gel, and the fraction that eluted with CH_2Cl_2/MeOH (96:4) was further purified by reverse phase HPLC to give 2.1 mg of conicasterol E (**1**) as a colorless amorphous solid ($[\alpha]_D^{25}$ +59.6).

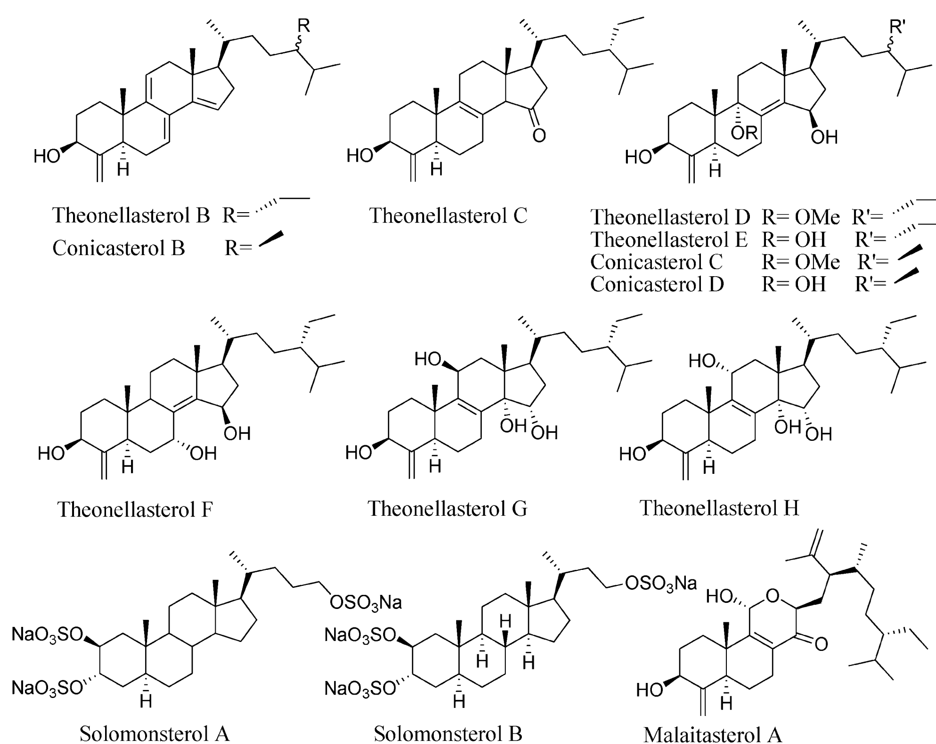


Figure 2. Nuclear receptors ligands previously isolated from the marine sponge *Theonella swinhoei*.

dx.doi.org/10.1021/jm201004p | J. Med. Chem. 2012, 55, 84−93

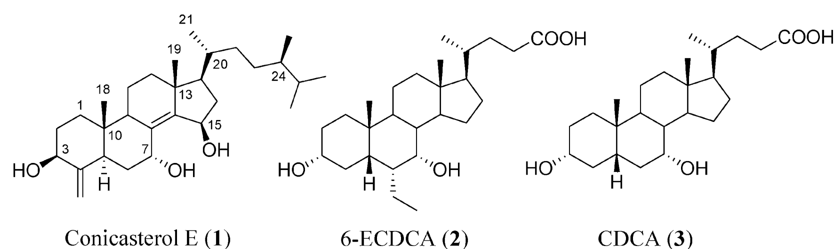


Figure 3. Conicasterol E (**1**), the first example of SHP-sparing marine FXR modulator, 6*α*-ethyl-chenodeoxycholic acid (**2**), a synthetic FXR agonist and chenodeoxycholic acid (**3**), the endogenus FXR ligand.

The molecular formula of $C_{29}H_{48}O_3$, established by HR ESIMS ($[M + Li]^+$ at m/z 451.3769 (calculated 451.3763)) and NMR data (Table 1), were compatible with a steroidal tetracyclic nucleus, two double bonds, and three hydroxyl groups in the molecule. COSY correlations delineated the spin system H-1 through H-7 and the spin system H-15/H-17 with OH substitutions at C-7 (δ_H 4.63) and at C-15 (δ_H 4.57), whereas the presence of a Δ8(14) double bond was inferred from careful analysis of HMBC data reported in the Table 1 and in Figure 4. The small vicinal coupling constant of H-7 (br t, J = 2.9 Hz) allowed us to establish an equatorial disposition for this proton, thereby placing the hydroxyl group in an axial *α*-orientation, while the ROE effect H-15/H-17*α* (Figure 3) was indicative of a *β*-orientation of the hydroxy group at C-15.

Table 1. NMR Data (700 MHz, C_6D_6) for 1[a]

position	δ_H	δ_C	key HMBC
1	1.11 m, 1.52 ovl	36.7	
2	1.30 ovl, 1.82 m	33.4	
3	3.77 m	73.4	
4		153.3	
5	2.41 m	43.2	
6	1.65 ovl, 1.91 ovl	31.7	
7	4.63 br t (2.9)	66.6	
8		136.7	
9	2.40 m	45.5	
10		40.0	
11	1.54 ovl	20.3	
12	1.31 ovl, 1.92 ovl	38.1	
13		43.8	
14		151.5	
15	4.57 br d (4.8)	70.1	C13, C14, C17
16	1.50 ovl, 1.66 ovl	39.5	C13, C14, C15, C17
17	1.64 ovl	53.4	C13, C14, C15
18	0.79 s	19.8	C12, C13, C14, C17
19	0.59 s	12.7	C1, C5, C9, C10
20	1.43 m	34.6	
21	1.00 d (6.3)	19.1	
22	1.20 m, 1.48 m	33.7	
23	1.16 m, 1.47 m	30.6	
24	1.33 m	39.3	
25	1.59 m	32.8	
26	0.88 d (6.7)	18.6	C24, C25
27	0.94 d (6.7)	20.4	C24, C25
28	0.87 d (6.8)	15.7	C23, C24, C25
29	4.72 br s, 5.32 br s	103.3	C3, C4, C5

[a]Coupling constants are in parentheses and given in hertz. ^{1}H and ^{13}C assignments are aided by COSY, TOCSY, ROESY, HSQC, and HMBC experiments. Ovl: signals overlapped.

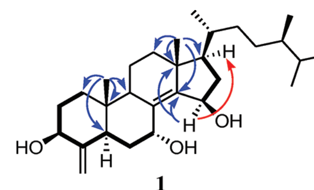


Figure 4. COSY connectivities (bold bonds) and HMBC (blue arrows) and ROESY correlations (red arrows) for conicasterol E (**1**).

The configuration at C-24 was determined by comparison of ^{13}C NMR data (Table 1) with literature data for epimeric steroidal side chains.[35]

Retrospective analysis of NMR data of theonellasterol F (Figure 2), previously isolated from the same sponge,[31] indicated a strong resemblance with conicasterol E (see Supporting Information). The difference between **1** and theonellasterol F lies in the side chain with a methyl group (δ_H 0.88, 3H, d, J = 6.7 Hz) replacing the C-24 ethyl group present in all theonellasterol-like compounds.

NEW SYNTHETIC STRATEGY OF 6-ECDCA (2)

So far, two synthetic procedures of 6-ECDCA (**2**) have been reported. The first process was based on the alkylation of the 3-tetrahydropyranyloxy derivative of 7-ketolithocholic acid with lithium diisopropylamide and ethyl bromide followed by standard reduction and hydrolysis steps.[16,18]

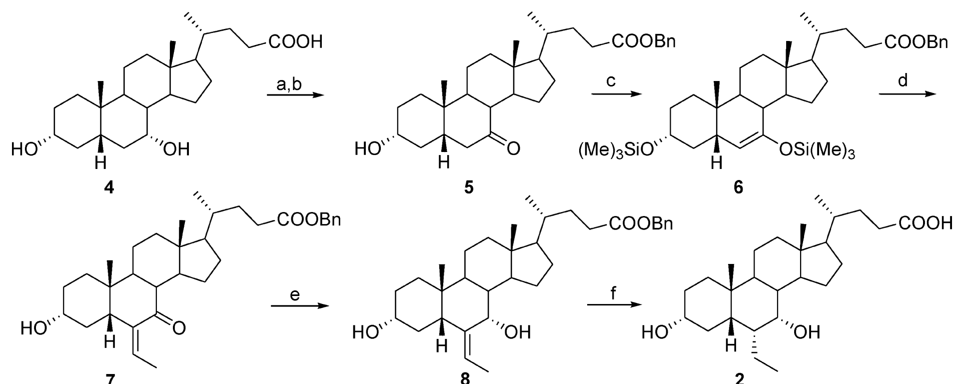
Our preliminary screening of this synthesis revealed low-yielding steps, especially in the alkylation of 7-ketolithocholic acid, and the need for chromatographic purification of every step.

Recently, an alternative procedure[36] via aldol-type addition of a silyl enol ether derivative of 7-ketolithocholic acid methyl ester with acetaldehyde followed by hydrogenation with PtO_2, alkaline hydrolysis (10% NaOH in refluxing methanol), and selective reduction of the C7-ketone with sodium borohydride has been reported (58% overall yield from methyl 7-keto-lithocholate).

Despite our extensive effort, we faced several problems in reproducing the reported high yield in the hydrolysis and reduction steps. In fact the hydrolysis of methyl ester in alkaline condition proceeded with low yields and extensive epimerization at C-6 position due to the presence of the carbonyl group at C-7.

Alternatively we tested the possibility of inverting the last two steps of the reported protocol and of performing first the C-7 reduction followed by methyl ester hydrolysis. Despite the reported regio- and stereoselectivity of $NaBH_4$ reduction, in our hands the reduction of methyl 3*α*-hydroxy-6*α*-ethyl-7-keto-5*β*-cholan-24-oate proceeded with the formation of a large amount of the over-reduced product with the primary alcoholic function

Scheme 1[a]



[a]Reagents and conditions: (a) NaClO/$Bu_4N^+Br^-$, NaBr, 0 °C; (b) BnBr, Cs_2CO_3, CH_3CN, reflux, 60% over two steps; (c) DIPA, *n*-BuLi, TMSCl, Et_3N, THF, −78 °C; (d) MeCHO, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −60 °C, 70% over two steps; (e) $NaBH_4/CeCl_3$, THF/MeOH (4:1), 95%; (f) H_2, Pd/C, THF/MeOH (1:1), 80%.

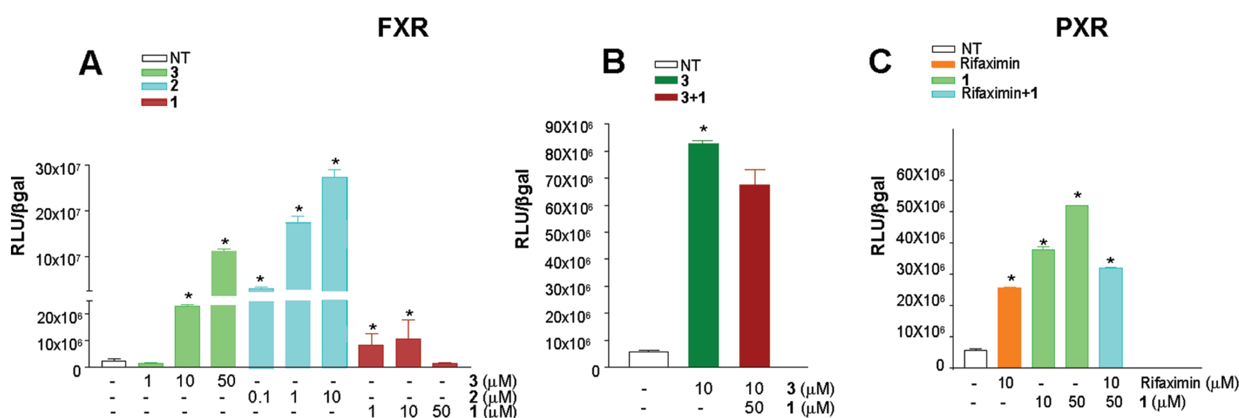


Figure 5. (A) Relative potency of FXR activation by CDCA (**3**), 10 μM, conicasterol E (**1**), 10 μM, and 6-ECDCA (**2**), 1 μM, as measured by transactivation assay in HepG2 cells. (B) Conicasterol E (**1**), 50 μM, does not revert the effect of CDCA (**3**), 10 μM, on FXR transactivation in HepG2 cells. (C) Relative potency of PXR activation by rifaximin, 10 μM, and conicasterol E (**1**) alone, 10 μM, or in combination, 50 μM. Data are the mean ± SE of four experiments: (*) $P < 0.05$ versus untreated cells (NT).

at C-24 and with scarce stereoselectivity in the introduction of hydroxyl group at C-7 with the required α-configuration.

To overcome the inconvenience of the coexistence of C-7 ketone and C-24 methyl ester, we initially attempted to perform the generation of silyl enol ether on the 7-ketolithocholic acid without the protection of the carboxyl function at C-24, but despite our several efforts,[37] no transformation occurred.

At this point we decided to protect the carboxyl function at C-24 as benzyl ester. The choice of benzyl as protecting group addressed two essential demands: reduction of synthetic steps in order to achieve better chemical yield and, importantly, improvement of regio- and stereoselectivity of the entire process. The synthesis of **2**, starting from commercially available chenodeoxycholic acid (**4**), is outlined in Scheme 1. Oxidation with sodium hypochlorite solution/NaBr and tetrabutylammonium bromide in a mixture of methanol/acetic acid/water/ethyl acetate as solvent, followed by benzylation of C-24 carboxylic acid, afforded benzyl ester of 7-ketolithocholic acid (**5**) in 60% chemical yield (two steps) and without traces of 3-keto regioisomer and dioxidated product. Generation of silyl enol ether (**6**) and subsequent aldol addition with acetaldehyde in the presence of $BF_3{\cdot}OEt_2$ gave the desired benzyl 3α-hydroxy-6-ethylidene-7-keto-5β-cholan-24-oate (**7**) in 70% yield over two steps.

High selective reduction of the C7-ketone with $NaBH_4/CeCl_3$ in a mixture of THF/MeOH at room temperature gave diol **8** (95% chemical yield, >98% de). Hydrogenation (H_2 on Pd/C Degussa type) of exocyclic double bond and concomitant removal of the benzyl-protecting group afforded 6-ECDCA (**2**) in six steps with a 32% overall yield.

The identity of **2** was secured by comparison of its NMR and MS spectra with those previously reported.[16,18] In a reporter assay we demonstrated that the so obtained 6-ECDCA (**2**) is a potent FXR agonist (Figure 5).

■ BIOLOGICAL ACTIVITY

Conicasterol E (**1**) was tested in vitro using an hepatocarcinoma cell line (HepG2 cells) transfected with FXR, RXR, β-galactosidase expression vectors (pSG5FXR, pSG5RXR, and pCMV-βgal) and with p(hsp27)TKLUC reporter vector that contains the promoter of the FXR target gene heat shock protein 27 (hsp27) cloned upstream the luciferase gene.

As shown in Figure 5, conicasterol E (**1**) activates FXR in transactivation assay. However, in contrast to CDCA (**3**), the results of these experiments demonstrate that conicasterol E (**1**) activates FXR with a bell shaped concentration−response curve, the agonistic activity being partially reduced at 50 μM. At 10 μM, **1** was as effective as CDCA (**3**) in transactivation assay

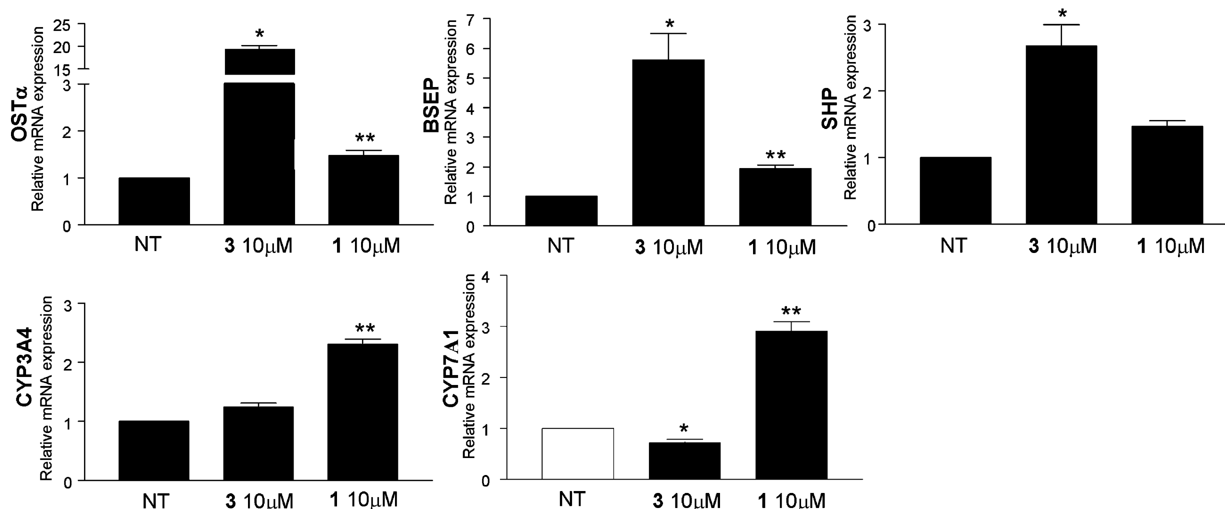


Figure 6. RT-PCR analysis of effects of CDCA (**3**), 10 μM, and conicasterol E (**1**), 10 μM, on expression of FXR and PXR-regulated genes in HepG2 cells. Conicasterol E (**1**) does not induce SHP, whereas it induces the expression of CYP7A1. Data are the mean ± SE of four experiments: (∗) $P < 0.05$ versus untreated cells (NT); (∗∗) $P < 0.05$ versus CDCA alone.

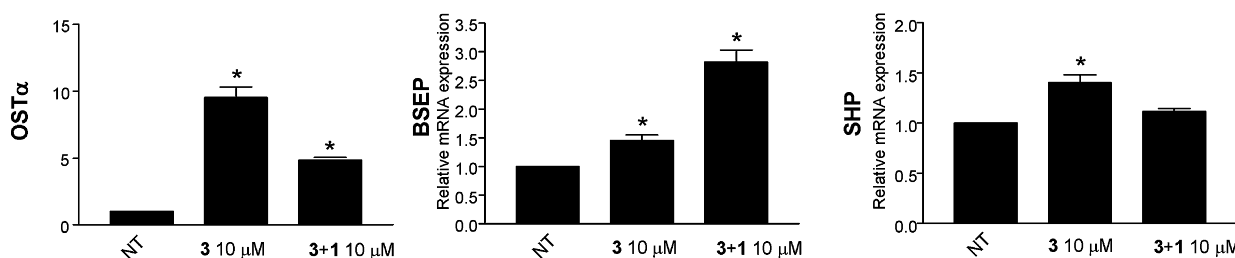


Figure 7. RT-PCR analysis of effects of CDCA (3), 10 μM, alone or in combination with conicasterol E (**1**), 10 μM, on expression of FXR-regulated genes in HepG2 cells. Conicasterol E (**1**) does not induce SHP even when cells were co-incubated with **3**, while the association of the two agents partially attenuated the expression of OSTα but increased the expression of BSEP. Data are the mean ± SE of four experiments: (∗) $P < 0.05$ versus untreated (NT).

but significantly less potent than the synthetic FXR ligand 6-ECDCA (**2**) and GW4064 (data not shown). We then tested whether conicasterol E (**1**) exerted any antagonistic activity against FXR. Because the above-mentioned results revealed a bell-shaped curve in the concentration−response effect of compound **1** in transactivating FXR, we tested the effects of this agent at 10 μM (data not shown) and 50 μM and found that compound **1** was devoid of any antagonistic activity when coadministered with CDCA (**3**) (Figure 5B) and 6-ECDCA (data not shown) to HepG2 cells. In addition to an FXR agonistic activity, conicasterol E (**1**) effectively induced PXR expression, being as effective as rifaximin in inducing PXR transactivation (Figure 5C). Thus, conicasterol E (**1**) is a dual FXR and PXR agonist.

To further characterize the biological activity of the conicasterol E (**1**), we have examined the effect of this agent on the expression of canonical FXR and PXR target genes in hepatocytes, and as shown in Figure 6, we found that exposure to conicasterol E slightly increased the expression of OSTα and BSEP mRNAs (two FXR regulated genes) and the expression of CYP3A4 mRNA (a PXR-regulated gene), while no effect was observed on SHP mRNA expression.

In addition, in contrast to CDCA, **1** failed to repress CYP7A1. Thus, while the expression of this gene was reduced by 30% by CDCA, exposure to conicasterol E (**1**) increased CYP7A1 mRNA by 2- to 3-fold. These data are further evidence that in HepG2 cells repression of CYP7A1 by FXR is indirect and requires induction of SHP.

Further on, when administered in combination with a concentration of CDCA of 10 μM, conicasterol E exerted an additive effect with CDCA on the expression of OSTα and BSEP while no further changes were observed in the expression of SHP (Figure 7). Taken together, these data highlight that conicasterol E (**1**) is a FXR modulator whose potency on selective target genes is very close to that of the endogenous mammalian ligand CDCA (**3**) and lower than that of the synthetic agonist 6-ECDCA (**2**). Interestingly, conicasterol E (**1**) failed to stimulate SHP even when coadministered in combination with CDCA (**3**).

Finally, analysis of CYP3A4 expression, shown in Figure 8, demonstrated that conicasterol E (**1**) has no antagonistic effects on expression of CYP3A4 mRNA induced by rifaximin, a potent PXR agonist.

■ DOCKING STUDIES

As reported in previous studies,[31] 4-methylene sterols isolated from *Theonella swinhoei* are able to modulate in different ways the FXR activity depending on the steroid skeleton substitutions. On this basis and in order to describe at atomic level the interactions of **1** with FXR macromolecule, we performed a molecular docking calculations using Autodock 4.2 software.[38] As shown in Figure 9A, the FXR binding site, located between helixes 2, 3, 5−7, and 10/11, is occupied by **1**,

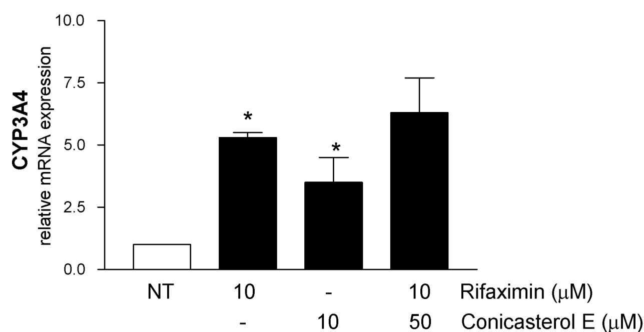


Figure 8. Activation of CYP3A4 by the PXR agonist rifaximin, 10 μM, is not modulated by conicasterol E (**1**), 50 μM. Data are the mean ± SE of four experiments: (*) $P < 0.05$ versus untreated (NT).

and as previously reported,[31] the β-OH groups at positions 3 and 15 and the trans junction between A/B rings cause a different positioning with respect to the cocrystallized molecule 6-ECDCA (**2**). In particular (Figure 9B) conicasterol E (**1**), compared to the synthetic agonist 6-ECDCA (**2**), is able to interact with two amino acids of the catalytic triad formed by Tyr358 in helix 7, His444 in helix 10/11, Trp466 helix 12, responsible for the activation of FXR.[33] Specifically, the 3-OH group at β position forms a hydrogen bond with Tyr358 in helix 7, while the trans junction between the A/B ring allows a hydrophobic interaction with His444 (helix 10/11). In our previous study,[31] we have described the influence of the side chain on the FXR binding. In fact, the methyl at position 24 of conicasterol E (**1**) (yellow, Figure 9B) relating to 6-ECDCA (**2**) (red, Figure 9B) is able to simultaneously interact with the Met262 (coil 2), His291 (helix 3), and Met287 (helix 3) present on the shallow groove of the FXR molecular surface protruding toward the solvent.

Moreover, the OH at position 15β in **1** forms an additional hydrogen bond with the CO of Leu284 (helix 3), and the steroid skeleton is in close contact with Leu345, Ala288, Met447, Phe326, and Trp451 relating to the 6-ECDCA (**2**). On the other hand, the OH at 7α position does not seem to exert further polar interactions with the FXR binding site.

In summary, conicasterol E (**1**) presents a different spatial arrangement relating to the cocrystallized molecule 6-ECDCA; however, our docking calculations point out that its simultaneous and efficient hydrophobic and hydrophilic additional interactions with the receptor binding site might be responsible for its agonist activity on FXR. Further on, the exclusive amino acid interactions exerted by conicasterol E (**1**) might support the notion that the compound is an FXR modulator endowed with the ability to activate OSTα and BSEP without effect on SHP expression.

CONCLUSION

In this paper, we have identified a micromolar potent FXR modulator that induces both BSEP and OSTα. These studies pave the way to further elaborating on the critical interactions on the FXR-LBD aimed at the identification of a site specific ligand that could be used to specifically induce selective genes. Because activation of BA transporters along with induction of detoxification pathways (CYP3A4) might hold potential in the treatment of nonobstructive cholestasis, a clinical condition associated with intake of many drugs including, among others, estrogens, these results support the notion that the development of selective FXR modulators represents a feasible strategy for treating these disorders. In addition the lack of activity on SHP is of interest and further supports a functional role for differential amino acid interactions in comparison with 6-ECDCA shown by our docking studies. Indeed, it is recognized that SHP induction mediates transrepression of CYP7A1 by FXR. In the present study we have shown that conicasterol E (**1**) enhances CYP7A1 expression. This effect is likely PXR dependent[39] because liver expression of this gene increases significantly in PXR$^{-/-}$ mice in comparison to wild type mice, further highlighting the dual nature of FXR and PXR ligands of this agent. Because inhibition of CYP7A1 impairs bile acid synthesis, our data strongly support the notion that conicasterol E (**1**) is acting mainly on the BAs excretion from hepatocytes without impacting bile acid synthesis. Inhibition of BAs synthesis is a well recognized drawback of FXR agonism, raising concern on clinical use of its ligands.

The SHP sparing activity shown by conicasterol E (**1**) is of particular interest. The lack of effects of this sterol on SHP likely explains the lack of inhibition of CYP7A1 we have observed in hepatocytes. SHP is an orphan nuclear receptor that lacks the DNA-binding domain, whose activation by FXR is responsible for some unwanted effects seen with potent FXR agonists including inhibition of bile acids synthesis which leads to bile acid pool shrinking. Because a reduction of bile acid pool holds potential for side effects including cholestasis and

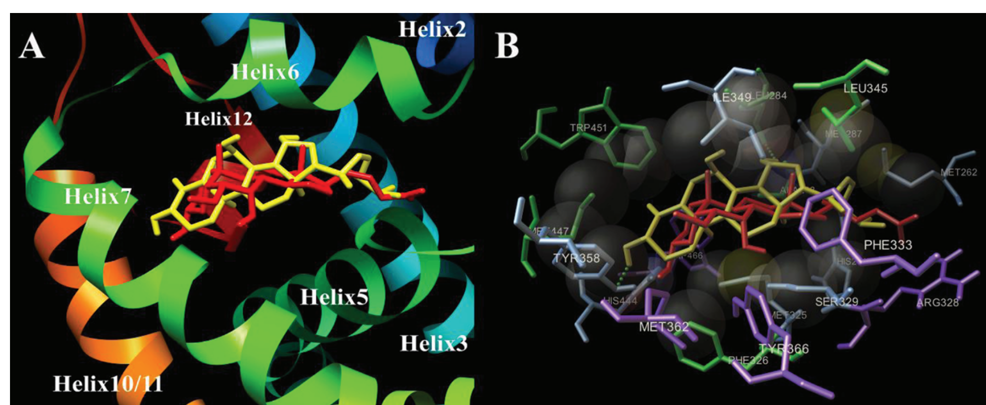


Figure 9. (A) Superimposition of **1** (yellow) with 6-ECDCA (**2**) (red) in the binding pocket of FXR (PDB code 1OSV). (B) Amino acids interacting with 6-ECDCA (red) are depicted in purple. Amino acids interacting with **1** (yellow) are depicted in green, and amino acids interacting with both molecules are depicted in light blue.

impaired glucose and lipid homeostasis, present findings provide SAR insights for development of SHP-sparing FXR agonists.

In addition, we have shown that conicasterol E (**1**) is a potent PXR agonist. PXR is an essential regulator of hepatic detoxification of endo- and xenobiotics,[40] and the ability of conicasterol E (**1**) to transactivate PXR was confirmed by the ability of this agent to induce the expression of CYP7A1 and CYP3A4 mRNAs. Because CYP3A4 is a canonical PXR responsive gene, these data strongly support the notion that **1** is a PXR agonist. Activation of PXR along with FXR modulation holds some potent benefit. Thus, induction of endobiotic clearance, including excretion of bilirubin, a typical PXR-dependent effect, might help to prevent accumulation of toxic intermediates, a condition that occurs in several liver diseases.

We have previously reported the isolation and characterization of theonellasterol derivatives endowed with FXR antagonism and PXR agonism.[31] The further decodification of this family of steroidal metabolites has now allowed the identification of a SHP-sparing FXR modulator (i.e., an FXR agonist that modulates the expression of FXR's target genes in a different manner in comparison to CDCA) endowed with a potent PXR activity, indicating that detailed characterization of these unusual steroids from *Theonella swinhoei* could be an important tool in deciphering the biology of nuclear receptors in mammals.

In summary, we have provided evidence that conicasterol E (**1**), a marine steroid isolated from the sponge *Theonella swinhoei*, is a dual FXR and PXR agonist that triggers bile acid detoxification without impairing uptake or biosynthesis. This compound selectively activates BSEP and OSTα without inducing SHP. In addition we have reported a novel synthetic pathway to generate 6-ECDCA (**2**), a potent FXR agonist. Present data might be exploited for further designing of multilevel regulators of nuclear receptors.

EXPERIMENTAL PROCEDURES

General Procedures. Specific rotations were measured on a Perkin-Elmer 243 B polarimeter. High-resolution ESI-MS spectra were performed with a QTOF Micromass spectrometer. ESI-MS experiments were performed on an Applied Biosystem API 2000 triple−quadrupole mass spectrometer. NMR spectra on conicasterol E were obtained on Varian Inova 700 NMR spectrometer (^{1}H at 700 MHz, ^{13}C at 175 MHz) equipped with Sun hardware, with δ (ppm), J in Hz, and spectra referenced to C_6HD_5 as internal standard (δ_H 7.16, δ_C 128.4). Through-space ^{1}H connectivities were evidenced using a ROESY experiment with mixing times of 200 ms. NMR spectra on all synthetic intermediates were obtained on Varian Inova 400 and Varian Inova 500 NMR spectrometers (^{1}H at 400 and 500 MHz, ^{13}C at 100 and 125 MHz, respectively) and recorded in $CDCl_3$ (δ_H =7.26 and δ_C =77.0 ppm) and CD_3OD (δ_H = 3.30 and δ_C = 49.0 ppm).

HPLC was performed using a Waters model 510 pump equipped with Waters Rheodine injector and a differential refractometer, model 401.

Reaction progress was monitored via thin-layer chromatography (TLC) on Alugram silica gel G/UV254 plates. Silica gel MN Kieselgel 60 (70−230 mesh) from Macherey-Nagel Company was used for column chromatography. All chemicals were obtained from Sigma-Aldrich, Inc. Solvents and reagents were used as supplied from commercial sources with the following exceptions. Tetrahydrofuran, toluene, dichloromethane, ether, and triethylamine were distilled from calcium hydride immediately prior to use. All reactions were carried out under argon atmosphere using flame-dried glassware.

The purities of compounds were determined to be greater than 95% by HPLC.

Sponge Material and Separation of Conicasterol E (1). *Theonella swinhoei* (order Lithistida, family Theonellidae) was collected at a depth of 22 m, on an isolated reef off the western coast of Malaita Island, Solomon Islands, in July 2004, and reference specimens are on file (R3159) at the ORSTOM, Centre of Noumea. The samples were frozen immediately after collection and lyophilized to yield 207 g of dry mass. Taxonomic identification was performed by Dr. John Hooper at Queensland Museum, Brisbane, Australia.

The lyophilized material (207 g) was extracted with methanol (3 × 1.5 L) at room temperature, and the crude methanolic extract was subjected to a modified Kupchan's partitioning procedure as follows. The methanol extract was dissolved in a mixture of MeOH/H_2O containing 10% H_2O and partitioned against n-hexane (4.5 g). The water content (% v/v) of the MeOH extract was adjusted to 30% and partitioned against $CHCl_3$ (6.0 g). The aqueous phase was concentrated to remove MeOH and then extracted with n-BuOH (10.3 g).

The hexane extract was chromatographed in two runs by silica gel MPLC using a solvent gradient system from CH_2Cl_2 to $CHCl_2$/MeOH, 1:1.

Fractions eluted with CH_2Cl_2/MeOH, 96:4 (18.5 mg), were further purified by HPLC on a Nucleodur 100-5 C18 (5 μm, 10 mm i.d. × 250 mm) with MeOH/H_2O (92:8) as eluent (flow rate of 5 mL/min) to give 2.1 mg of conicasterol E (**1**) (t_R = 14.5 min).

Conicasterol E (1). White amorphous solid; $[\alpha]_D^{25}$ +59.6 (c 0.06, MeOH); ^{1}H and ^{13}C NMR data in C_6D_6 given in Table 1. ESI-MS: m/z 451.4 $[M + Li]^+$. HRMS (ESI): calcd for $C_{29}H_{48}LiO_3$, 451.3763; found, 451.3769 $[M + Li]^+$.

Total Synthesis of 6-ECDCA (2). Benzyl 3α-Hydroxy-7-keto-5β-cholan-24-oate (5). An oven-dried 250 mL flask was charged with chenodeoxycholic acid **4** (2.00 g, 5.1 mmol), sodium bromide (30 mg, 0.25 mmol), tetrabutylammonium bromide (5.4 g, 16.8 mmol), and 43 mL of a solution of MeOH/CH_3COOH/H_2O/AcOEt, 3:1:0.25:6.5 v/v. The mixture was stirred at room temperature until a homogeneous solution formed and then cooled at 0 °C. Sodium hypochlorite solution (10%, 5 mL, 5.6 mmol) was added, until the test for hypochlorite (peroxide test paper) was positive, and the yellow suspension was stirred. The mixture was stirred at room temperature for 6 h. Aqueous sodium bisulfite (3.3%) was added to afford a white suspension (negative test for peroxide). Water (50 mL) was added and the mixture stirred at 15 °C for 5 min. Aqueous solution was extracted with AcOEt (3 × 50 mL). The combined organic layer was washed with aqueous sodium bisulfite (50 mL) and water (50 mL) and then dried over anhydrous $MgSO_4$ and evaporated in vacuo to give 2.0 g of 3α-hydroxy-7-keto-5β-cholan-24-oic acid, which was subjected to the next step without any purification. To a solution of this latter intermediate (2.0 g, 5.1 mmol) in CH_3CN dry (30 mL), Cs_2CO_3 (2.5 g, 7.6 mmol) was added. The solution was heated to 150 °C, and BnBr (3.0 mL, 25.5 mmol) was added under reflux. The solution was stirred at this temperature for 24 h and then afterward cooled to room temperature and, after removal CH_3CN in the rotavapor, poured into saturated $NaHCO_3$ solution (50 mL) and extracted with AcOEt (3 × 30 mL). The combined organic layer was washed with water (30 mL) and then dried over anhydrous $MgSO_4$ and evaporated in vacuo. Purification on silica gel column, eluting with hexane/AcOEt (7:3) and 0.5% Et_3N, afforded pure **5** (1.5 g, 60% over two steps). $[\alpha]_D^{25}$ −10.9 (c 0.99, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (5H, m), 5.09 (2H, dd, J = 12.9, 15.6 Hz), 3.57 (1H, m), 2.83 (1H, m), 2.37 (1H, m, ovl), 2.26 (1H, m), 2.15 (1H, m), 1.37 (3H, s), 0.89 (3H, d, J = 6.0 Hz), 0.61 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 212.0 (s), 174.0 (s), 136.2 (s), 128.5 (2C) (d), 128.2 (2C) (d), 128.1 (d), 70.7 (d), 66.0 (t), 54.7 (d), 49.4 (d), 48.8 (d), 46.0 (d), 45.3 (t), 42.7 (s), 42.5 (t), 38.9 (d), 37.3 (s), 35.1 (d), 34.1 (t), 31.2 (t), 30.9 (t), 29.8 (2C) (t), 28.2 (t), 24.7 (t), 23.0 (t), 21.6 (q), 18.3 (q), 11.9 (q). HRMS-ESI m/z 480.3240 ($[M + H]^+$, $C_{31}H_{44}O_4$ requires 480.3235.

Benzyl 3α,7-Trimethylsilyloxy-5β-cholan-6-en-24-oate (6). To a solution of diisopropylamine (5.5 mL, 39 mmol) in dry THF (50 mL) was added dropwise a solution of n-butyllithium (15 mL, 2.5 M in

hexane, 37.2 mmol) at −78 °C under nitrogen atmosphere. After 30 min, trimethylchlorosilane (3.9 mL, 31 mmol) was added, and the resulting mixture was reacted for an additional 20 min. A solution of benzyl 3α-hydroxy-7-keto-5β-cholan-24-oate (**5**) (1.5 g, 3.1 mmol) in dry THF (20 mL) was added dropwise in 10 min. The mixture was stirred at −78 °C for an additional 45 min, and then triethylamine (7.8 mL, 56 mmol) was added. After 1 h, the reaction mixture was allowed to warm to −20 °C, treated with aqueous saturated solution of $NaHCO_3$ (10 mL), and brought up to room temperature in 2 h. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed several times with a saturated solution of $NaHCO_3$, water, and brine. After the mixture was dried over anhydrous Na_2SO_4, the residue was evaporated under vacuum to give 1.8 g of **6** as a yellow residue, which was subjected to next step without any purification. $[\alpha]^{25}_D$ +4.3 (*c* 0.58, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.35 (5H, m), 5.10 (1H, dd, *J* = 15.8, 12.4 Hz), 4.72 (1H, d, *J* = 6.1 Hz), 3.50 (1H, m), 2.40 (1H, m), 2.28 (1H, m), 0.90 (3H, d, *J* = 6.3 Hz), 0.81 (3H, s), 0.64 (3H, s), 0.18−0.001 (18H, ovl). ^{13}C NMR (100 MHz $CDCl_3$): δ 174.0 (s), 151.7 (s), 136.2 (s), 128.5 (2C) (d), 128.2 (2C) (d), 128.1 (d), 108.8 (d), 71.5 (d), 66.1 (t), 54.8 (d), 54.1 (d), 44.3 (t), 42.6 (s), 41.0 (d), 40.9 (t), 40.3 (d), 40.1 (s), 35.2 (d), 34.6 (t), 32.9 (d), 31.4 (t), 31.0 (t), 30.7 (t), 28.6 (t), 27.0 (t), 22.5 (t), 20.9 (q), 18.4 (q), 12.4 (q), 1.4 (3C) (q), 0.4 (2C) (q), 0.2 (q). HRMS-ESI *m/z* 624.4030 ($[M + H]^+$, $C_{37}H_{60}O_4Si_2$ requires 624.4047.

Benzyl 3α-Hydroxy-6-ethyliden-7-keto-5β-cholan-24-oate (7). To a cooled (−60 °C) and stirred solution of acetaldehyde (328 μL, 5.86 mmol) and benzyl 3α,7-trimethylsilyloxy-5β-cholan-6-en-24-oate (**6**) (1.8 g, 2.93 mmol) in dry CH_2Cl_2 (30 mL) was added dropwise $BF_3{\cdot}OEt_2$ (3.7 mL, 29.3 mmol). The reaction mixture was stirred for 2 h at −60 °C and allowed to warm to room temperature. The mixture was quenched with saturated aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. Purification on silica gel column, eluting with hexane/AcOEt (9:1) and 0.5% Et_3N, afforded pure **7** (1.09 g, 70% over two steps). $[\alpha]^{25}_D$ −42.5 (*c* 0.12, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.34 (5H, m), 6.16 (1H, q, *J* = 6.7 Hz), 5.10 (2H, dd, *J* = 13.0, 17.8 Hz), 3.64 (1H, m), 2.56 (1H, m), 2.38 (1H, m), 2.27 (1H, m), 1.67 (3H, d, *J* = 6.7 Hz), 0.99 (3H, s), 0.91 (3H, d, *J* = 6.0 Hz), 0.60 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.2 (s), 174.0 (s), 143.3 (s), 140.9 (s), 129.8 (d), 128.5 (2C) (d), 128.2 (2C) (d), 128.1 (d), 70.5 (d), 66.1 (t), 54.5 (d), 50.6 (d), 48.6 (d), 45.5 (d), 43.5 (t), 39.1 (s), 38.9 (t), 37.5 (s), 35.1 (d), 34.4 (t), 31.2 (t), 30.9 (t), 29.6 (2C) (d, t), 28.4 (t), 25.9 (t), 22.8 (t), 21.3 (q), 18.4 (q), 12.6 (q), 12.0 (q). HRMS-ESI *m/z* 506.3396 ($[M + H]^+$, $C_{33}H_{46}O_4$ requires 506.3378.

Benzyl 3α,7α-Dihydroxy-6-ethyliden-5β-cholan-24-oate (8). Compound **7** (1.00 g, 1.97 mmol) was dissolved in a solution of dry tetrahydrofuran/dry methanol (50 mL, 4/1 v/v) and treated with $CeCl_3$ (1.46 g, 5.93 mmol) and $NaBH_4$ (667 mg, 2.36 mmol). After 3 h, water and MeOH were added. Then after evaporation of the solvents, the residue was diluted with water and extracted with ether (3 × 50 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel, using dichloromethane/methanol (7:3 v/v) and 0.5% Et_3N as eluent, to afford 950 mg of **8** (95% yield). $[\alpha]^{25}_D$ + 18.0 (*c* 0.06, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.34 (5H, m), 5.64 (1H, q, *J* = 6.2 Hz), 5.10 (2H, dd, *J* = 12.5, 17.3 Hz), 3.98 (1H, m), 3.64 (1H, m), 2.47 (1H, m), 2.39 (1H, m), 2.27 (1H, m), 1.60 (3H, d, *J* = 6.6 Hz), 0.91 (3H, d, *J* = 6.2 Hz), 0.77 (3H, s), 0.61 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.0 (s), 141.6 (s), 136.1 (s), 128.5 (2C) (d), 128.3 (2C) (d, d), 128.2 (d), 114.4 (d), 73.3 (d), 71.2 (d), 66.1 (t), 56.0 (d), 55.0 (d), 45.5 (d), 44.0 (t), 40.0 (s), 39.5 (d), 36.3 (s), 35.4 (d), 35.2 (d), 34.7 (t), 31.3 (t), 31.0 (t), 30.2 (t), 29.6 (t), 28.6 (t), 27.1 (t), 22.8 (t), 21.2 (q), 18.4 (q), 12.4 (q), 12.2 (q). HRMS-ESI *m/z* 508.3553 ($[M + H]^+$, $C_{33}H_{48}O_4$ requires 508.3565.

6-ECDCA (2). An oven-dried 50 mL flask was charged with 10% palladium on carbon (50 mg) and compound **8** (950 mg, 1.87 mmol), and the flask was evacuated and flushed with argon. Absolute methanol (10 mL) and dry THF (10 mL) were added, and the flask was flushed with hydrogen. The mixture was stirred at room temperature under H_2 for 4 h. The mixture was filtered through Celite, and the recovered filtrate was evaporated under vacuum to give pure 6α-ethylchenodeoxycholic acid **2** (630 mg, 80%). $[\alpha]^{25}_D$ +5.11 (*c* 1.8, CH_3OH). ^1H NMR (400 MHz, CD_3OD): δ 3.60 (1H, br s), 3.32 (1H, m), 2.33 (1H, m), 2.20 (1H, m), 0.97 (3H, d, *J* = 6.2 Hz), 0.91 (3H, s), 0.90 (3H, t, *J* = 7.0 Hz), 0.69 (3H, s). ^{13}C NMR (100 MHz, CD_3OD): δ 178.4 (s), 73.3 (d), 71.2 (d), 57.4 (d), 51.7 (d), 46.9 (d), 43.8 (t), 43.2 (s), 41.6 (d), 41.1 (d), 36.8 (2C) (d, s), 36.6 (d), 34.5 (t), 34.4 (t), 32.4 (t), 32.2 (t), 31.2 (t), 29.3 (t), 24.6 (t), 23.7 (t), 23.5 (t), 21.9 (q), 18.8 (q), 12.2 (q), 12.0 (q). HRMS-ESI *m/z* 420.3240 ($[M + H]^+$, $C_{26}H_{44}O_4$ requires 420.3237.

Plasmids, Cell Culture, Transfection, and Luciferase Assays. All transfections were made using Fugene HD transfection reagent (Roche). For FXR mediated transactivation, HepG2 cells, plated in a six-well plate at 5 × 10^5 cells/well, were transfected with 100 ng of pSG5-FXR, 100 ng of pSG5-RXR, 200 ng of pCMV-β-galactosidase and 500 ng of the reporter vector p(hsp27)-TK-LUC containing the FXR response element IR1 cloned from the promoter of heat shock protein 27 (hsp27). At 48 h after transfection, cells were stimulated 18 h with 10 μM CDCA or with **1** alone (10 μM) or in combination (50 μM) with CDCA.

For PXR mediated transactivation, HepG2 cells, plated in a six-well plate at 5 × 10^5 cells/well, were transfected with 100 ng of pSG5-PXR, 100 ng of pSG5-RXR, 200 ng of pCMV-β-galactosidase, and 500 ng of the reporter vector containing the PXR target gene promoter (CYP3A4 gene promoter) cloned upstream of the luciferase gene (pCYP3A4promoter-TKLuc). At 48 h after transfection, cells were stimulated 18 h with 10 μM rifaximin or with **1** alone (10 μM) or in combination (50 μM) with rifaximin.

Cells were lysed in 100 μL of diluted reporter lysis buffer (Promega), and 0.2 μL of cellular lysates was assayed for luciferase activity using the luciferase assay system (Promega). Luminescence was measured using an automated luminometer. Luciferase activities were normalized for transfection efficiencies by dividing the relative light units by β-galactosidase activity expressed from cells cotransfected with pCMV-βgal.

Quantitative Real-Time PCR. Template (50 ng) was added to the PCR mixture (final volume of 25 μL) containing the following reagents: 0.2 μM each primer and 12.5 μL of 2× SYBR Green qPCR master mix (Invitrogen, Milan, Italy). All reactions were performed in triplicate, and the thermal cycling conditions were 2 min at 95 °C, followed by 40 cycles of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s in iCycler iQ instrument (Biorad, Hercules, CA). The mean value of the replicates for each sample was calculated and expressed as cycle threshold (C_T, cycle number at which each PCR reaction reaches a predetermined fluorescence threshold, set within the linear range of all reactions). The amount of gene expression was then calculated as the difference (ΔC_T) between the C_T of the sample for the target gene and the mean C_T of that sample for the endogenous control (GAPDH). Relative expression was calculated as the difference ($\Delta\Delta C_T$) between the ΔC_T of the test sample and of the control sample (not treated) for each target gene. The relative quantization value was expressed and shown as $2^{-\Delta\Delta C_T}$. All PCR primers were designed with PRIMER3-OUTPUT software using published sequence data from the NCBI database. The primer sequences were as follows: hGAPDH, gaaggtgaaggtcggagt and catgggtggaatcatattggaa; hBSEP, gggccattgtacgagatcctaa and tgcaccgtcttttcactttctg; hCYP7A1, caccttgaggacggttccta and cgatccaaagggcatgtagt; hOSTα, tgttgggccctttccaatac and ggctcccatgttctgctcac; hSHP, gctgtctggagtccttctgg and ccaatgatagggcgaaagaagag; hCYP3A4, caagaccccttgtggaaaa and cgaggcgactttctttcatc.

Computational Details. We performed molecular docking calculations by Autodock 4.2 software[38] on quad-core Intel Xeon 3.4 GHz, using a grid box size of 94 × 96 × 68, with spacing of 0.375 Å between the grid points and centered at 20.689 (*x*), 39.478 (*y*), 10.921 (*z*), covering the active site of the FXR.[33] To achieve a representative conformational space during the docking studies and for taking into account the variable number of active torsions, 10 calculations

consisting of 256 runs were performed, obtaining 2560 structures for the ligand. The Lamarckian genetic algorithm (LGA) was employed for docking calculations, choosing an initial population of 600 randomly placed individuals. The maximum number of energy evaluations and of generations was set up to 5×10^6 and to 6×10^6, respectively. Results differing by less than 3.5 Å in positional root-mean-square deviation (rmsd) were clustered together and represented by the result with the most favorable free energy of binding. Illustrations of the 3D models were generated using the Chimera[41] and the Python software.[42]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of **1**, theonellasterol F, and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (0039) 081-678525. Fax: (0039) 081-678552. E-mail: angela.zampella@unina.it.

Author Contributions

⊥These authors contributed equally to this work.

■ ACKNOWLEDGMENTS

This work is part of the CRISP (Coral Reef Initiative in the South Pacific) project and is supported by a grant from the Agence Française de Développement. We thank the Solomon Islands Government for allowing us to collect there and their Fisheries Departments for their help and assistance. We thank the IRD diving team for the collection of the sponges and Dr. John Hooper for the identification of the sponges. NMR spectra were provided by the CSIAS, Centro Interdipartimentale di Analisi Strumentale, Faculty of Pharmacy, University of Naples, Italy.

■ ABBREVIATIONS USED

BAs, bile acids; BSEP, bile salt export pump; CDCA, chenodeoxycholic acid; CYP7A1, cytochrome 7A1; CYP3A4, cytochrome 3A4; 6-ECDCA, 6-ethylchenodeoxycholic acid; FXR, farnesoid X receptor; HepG2, human hepatocarcinoma cell line; HR ESIMS, high-resolution electrospray ionization mass spectrometry; LBD, ligand binding domain; LXR, liver X receptor; NASH, nonalcoholic steatohepatitis; NTCP, Na^+ taurocholate co-transporting polipeptide; NR, nuclear receptor; OSTα, organic solute transporter α; PDB, Protein Data Bank; PXR, pregnane X receptor; RT PCR, real time polymerase chain reaction; SHP, small heterodimer partner

■ REFERENCES

(1) Fiorucci, S.; Mencarelli, A.; Distrutti, E.; Palladino, G.; Cipriani, S. Targeting farnesoid-X-receptor: from medicinal chemistry to disease treatment. *Curr. Med. Chem.* **2010**, *17*, 139−159.

(2) Fiorucci, S.; Cipriani, S.; Baldelli, F.; Mencarelli, A. Bile acid-activated receptors in the treatment of dyslipidemia and related disorders. *Prog. Lipid Res.* **2010**, *49*, 171−185.

(3) Fiorucci, S.; Mencarelli, A.; Palladino, G.; Cipriani, S. Bile-acid-activated receptors: targeting TGR5 and farnesoid-X-receptor in lipid and glucose disorders. *Trends Pharmacol. Sci.* **2009**, *30*, 570−580.

(4) Fiorucci., S.; Baldelli, F. Farnesoid X receptor agonists in biliary tract disease. *Curr. Opin. Gastroenterol.* **2009**, *25*, 252−259.

(5) Fiorucci, S.; Rizzo, G.; Donini, A.; Distrutti, E.; Santucci, L. Targeting farnesoid X receptor for liver and metabolic disorders. *Trends Mol. Med.* **2007**, *13*, 298−309.

(6) Wang, H.; Chen, J.; Hollister, K.; Sowers, L. C.; Forman, B. M. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol. Cell* **1999**, *3*, 543−553.

(7) Makishima, M.; Okamoto, A. Y.; Repa, J. J.; Tu, H.; Learned, R. M.; Luk, A.; Hull, M. V.; Lustig, K. D.; Mangelsdorf, D. J.; Shan, B. Identification of a nuclear receptor for bile acids. *Science* **1999**, *284*, 1362−1365.

(8) Parks, D. J.; Blanchard, S. G.; Bledsoe, R. K.; Chandra, G.; Consler, T. G.; Kliewer, S. A.; Stimmel, J. B.; Willson, T. M.; Zavacki, A. M.; Moore, D. D.; Lehmann, J. M. Bile acids: natural ligands for an orphan nuclear receptor. *Science* **1999**, *284*, 1365−1368.

(9) Maloney, P. R.; Parks, D.; Haffner, C. D.; Fivush, A. M.; Chandra, G.; Plunket, K. D.; Creech, K. L.; Moore, L. B.; Wilson, J. G.; Lewis, M. C.; Jones, S. A.; Willson, T. M. Identification of a chemical tool for the orphan nuclear receptor FXR. *J. Med. Chem.* **2000**, *43*, 2971−2974.

(10) Nicolaou, K. C.; Evans, R. M.; Roecker, A. J.; Hughes, R.; Downes, M.; Pfefferkorn, J. A. Discovery and optimization of non-steroidal FXR agonists from natural product-like libraries. *Org. Biol. Chem.* **2003**, *1*, 908−920.

(11) Deng, G.; Li, W.; Shen, J.; Jiang, H.; Chen, K.; Liu, H. Pyrazolidine-3,5-dione derivatives as potent non-steroidal agonists of farnesoid X receptor: virtual screening, synthesis, and biological evaluation. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5497−5502.

(12) Flatt, B.; Martin, R.; Wang, T. L.; Mahaney, P.; Murphy, B.; Gu, X. H.; Foster, P.; Li, J.; Pircher, P.; Petrowski, M.; Schulman, I.; Westin, S.; Wrobel, J.; Yan, G.; Bischoff, E.; Daige, C.; Mohan, R. Discovery of XL335 (WAY-362450), a highly potent, selective, and orally active agonist of the farnesoid X receptor (FXR). *J. Med. Chem.* **2009**, *19*, 904−907.

(13) Feng, S.; Yang, M.; Zhang, Z.; Wang, Z.; Hong, D.; Richter, H.; Benson, G. M.; Bleicher, K.; Grether, U.; Martin, R. E.; Plancher, J. M.; Kuhn, B.; Rudolph, M. G.; Chen, L. Identification of an *N*-oxide pyridine GW4064 analog as a potent FXR agonist. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2595−2598.

(14) Abel, U.; Schlüter, T.; Schulz, A.; Hambruch, E.; Steeneck, C.; Hornberger, M.; Hoffmann, T.; Perović-Ottstadt, S.; Kinzel, O.; Burnet, M.; Deuschle, U.; Kremoser, C. Synthesis and pharmacological validation of a novel series of non-steroidal FXR agonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4911−4917.

(15) Fujino, T.; Une, M.; Imanaka, T.; Inoue, K.; Nishimaki-Mogami, T. Structure−activity relationship of bile acids and bile acid analogs in regard to FXR activation. *J. Lipid Res.* **2004**, *45*, 132−138.

(16) Pellicciari, R.; Costantino, G.; Camaioni, E.; Sadeghpour, B. M.; Entrena, A.; Willson, T. M.; Fiorucci, S.; Clerici, C.; Gioiello, A. Bile acid derivatives as ligands of the farnesoid X receptor. Synthesis, evaluation, and structure−activity relationship of a series of body and side chain modified analogues of chenodeoxycholic acid. *J. Med. Chem.* **2004**, *47*, 4559−4569.

(17) Pellicciari, R.; Gioiello, A.; Costantino, G.; Sadeghpour, B. M.; Rizzo, G.; Meyer, U.; Parks, D. J.; Entrena-Gaudix, A.; Fiorucci, S. Back door modulation of the farnesoid X receptor: design, synthesis, and biological evaluation of a series of side chain modified chenodeoxycholic acid derivatives. *J. Med. Chem.* **2006**, *49*, 4208−4215.

(18) Pellicciari, R.; Fiorucci, S.; Camaioni, E.; Clerici, C.; Costantino, G.; Maloney, P. R.; Morelli, A.; Parks, D. J.; Willson, T. M. 6α-Ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J. Med. Chem.* **2002**, *45*, 3569−3572.

(19) Fiorucci, S; Cipriani, S; Mencarelli, A; Baldelli, F; Bifulco, G; Zampella, A. Farnesoid X receptor agonist for the treatment of liver and metabolic disorders: focus on 6-ethyl-CDCA. *Mini-Rev. Med. Chem.* **2011**, *11*, 753−762.

(20) Singh, R. B.; Niaz, M. A.; Ghosh, S. Hypolipidemic and antioxidant effects of *Commiphora mukul* as an adjunct to dietary therapy in patients with hypercholesterolemia. *Cardiovasc. Drugs Ther.* **1994**, *4*, 659−664.

(21) Sinal, C. J.; Gonzalez, F. J. Guggulsterone: an old approach to a new problem. *Trends Endocrinol. Metab.* **2002**, *13*, 275−276.

(22) Urizar, N. L.; Liverman, A. B.; Dodds, D. T.; Silva, F. V.; Ordentlich, P.; Yan, Y.; Gonzalez, F. J.; Heyman, R. A.; Mangelsdorf, D. J.; Moore, D. D. A natural product that lowers cholesterol as an antagonist ligand for FXR. *Science* **2002**, *296*, 1703−1706.
(23) Wu, J.; Xia, C.; Meier, J.; Li, S.; Hu, X.; Lala, D. S. The hypolipidemic natural product guggulsterone acts as an antagonist of the bile acid receptor. *Mol. Endocrinol.* **2002**, *16*, 1590−1597.
(24) Nozawa, H. Xanthohumol, the chalcone from beer hops (*Humulus lupulus* L.), is the ligand for farnesoid X receptor and ameliorates lipid and glucose metabolism in KK-Ay mice. *Biochem. Biophys. Res. Commun.* **2005**, *336*, 754−761.
(25) Nam, S. J.; Ko, H.; Shin, M.; Ham, J.; Chin, J.; Kim, Y.; Kim, H.; Shin, K.; Choi, H.; Kang, H. Farnesoid X-activated receptor antagonists from a marine sponge *Spongia* sp. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5398−5402.
(26) Choi, H.; Hwang, H.; Chin, J.; Kim, E.; Lee, J.; Nam, S. J.; Lee, B. C.; Rho, B. J.; Kang, H. Tuberatolides, potent FXR antagonists from the Korean marine tunicate *Botryllus tuberatus*. *J. Nat. Prod.* **2011**, *74*, 90−94.
(27) Sepe, V.; Bifulco, G.; Renga, B.; D'Amore, C.; Fiorucci, S.; Zampella, A. Discovery of sulfated sterols from marine invertebrates as a new class of marine natural antagonists of farnesoid-X-receptor. *J. Med. Chem.* **2011**, *54*, 1314−1320.
(28) Coral Reef Initiative in The South Pacific CRISP.
(29) Festa, C.; De Marino, S.; D'Auria, M. V.; Bifulco, G.; Renga, B.; Fiorucci, S.; Petek, S.; Zampella, A. Solomonsterols A and B from *Theonella swinhoei*. The first example of C-24 and C-23 sulfated sterols from a marine source endowed with a PXR agonistic activity. *J. Med. Chem.* **2011**, *54*, 401−405.
(30) Sepe, V.; Ummarino, R.; D'Auria, M. V.; Mencarelli, A.; D'Amore, C.; Renga, B.; Zampella, A.; Fiorucci, S. Total synthesis and pharmacological characterization of solomonsterol A, a potent marine pregnane-X-receptor agonist endowed with anti-inflammatory activity. *J. Med. Chem.* **2011**, *54*, 4590−4599.
(31) De Marino, S.; Ummarino, R.; D'Auria, M. V.; Chini, M. G.; Bifulco, G.; Renga, B.; D'Amore, C.; Fiorucci, S.; Debitus, C.; Zampella, A. Theonellasterols and conicasterols from *Theonella swinhoei*. Novel marine natural ligands for human nuclear receptors. *J. Med. Chem.* **2011**, *54*, 3065−3075.
(32) De Marino, S.; Sepe, V.; D'Auria, M. V.; Bifulco, G.; Renga, B.; Petek, S.; Fiorucci, S.; Zampella, A. Towards new ligands of nuclear receptors. Discovery of malaitasterol A, an unique bis-secosterol from marine sponge *Theonella swinhoei*. *Org. Biomol. Chem.* **2011**, *9*, 4856−4862.
(33) Mi, L. Z.; Devarakonda, S.; Harp, J. M.; Han, Q.; Pellicciari, R.; Willson, T. M.; Khorasanizadeh, S.; Rastinejad, F. Structural basis for bile acid binding and activation of the nuclear receptor FXR. *Mol. Cell* **2003**, *11*, 1093−1100.
(34) Festa, C.; De Marino, S.; Sepe, V.; Monti, M. C.; Luciano, P.; D'Auria, M. V.; Debitus, C.; Bucci, M.; Vellecco, V.; Zampella, A. Perthamides C and D, two new potent anti-inflammatory cyclopeptides from a Solomon Lithistid sponge *Theonella swinhoei*. *Tetrahedron* **2009**, *65*, 10424−10429.
(35) Wright, J. L. C.; Mclnnes, A. G.; Shimizu, S.; Smith, D. G.; Walter, J. A.; Idler, D.; Khalil, W. Identification of C-24 alkyl epimers of marine sterols by carbon-13 nuclear magnetic resonance spectroscopy. *Can. J. Chem.* **1978**, *56*, 1898−1903.
(36) Gioiello, A.; Macchiarulo, A.; Carotti, A.; Filipponi, P.; Costantino, G.; Rizzo, G.; Adorini, L.; Pellicciari, R. Extending SAR of bile acids as FXR ligands: discovery of 23-*N*-(carbocinnamyloxy)-3α,7α-dihydroxy-6α-ethyl-24-nor-5β-cholan-23-amine. *Bioorg. Med. Chem.* **2011**, *19*, 2650−2658.
(37) DIPA, BuLi, TMSCl, Et_3N, THF, −78 °C one pot procedure; generation of 3α-silyl ether with Et_3N and TMSCl followed by LDA/TMSCl formation of enolether.
(38) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30*, 2785−2791.
(39) He, J.; Nishida, S.; Xu, M.; Makishima, M.; Xie, W. PXR prevents cholesterol gallstone disease by regulating biosynthesis and transport of bile salts. *Gastroenterology* **2011**, *140*, 2095−2106.
(40) Mencarelli, A.; Migliorati, M.; Barbanti, M.; Cipriani, S.; Palladino, G.; Distrutti, E.; Renga, B.; Fiorucci, S. Pregnane-X-receptor mediates the anti-inflammatory activities of rifaximin on detoxification pathways in intestinal epithelial cells. *Biochem. Pharmacol.* **2010**, *80*, 1700−1707.
(41) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, *25*, 1605−1612.
(42) Sanner, M. F. Python: a programming language for software integration and development. *J. Mol. Graphics Modell.* **1999**, *17*, 57−61.