Bioorganic & Medicinal Chemistry Letters 18 (2008) 3687–3690

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Insight from molecular modeling into different conformation and SAR of natural steroids and unnatural 7-oxa-steroids

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ARTICLE INFO

Article history:
Received 21 March 2008
Accepted 19 May 2008
Available online 22 May 2008

Keywords:
Progesterone receptor antagonist
Mifepristone
Oxa-steroid
Molecular modeling

ABSTRACT

Replacement of the 7-CH_2 group of natural steroid with an oxygen atom led to identification of unnatural 7-oxa-steroids as potent and selective progesterone receptor antagonists. The unnatural 7-oxa-steroids exhibited a different structure–activity relationship (SAR) from natural steroids. Molecular modeling demonstrated that the switch of carbon to oxygen in the B-ring results in a subtle conformational change of the tetracyclic skeleton and induces a remarkable spatial shift at the terminal end of the side chain of the D-ring. This shift causes the phenyl ring on the D-ring to form a perfect parallel-displaced form of π–π interaction with the phenyl ring of Phe794. The unnatural 7-oxa-steroids were orally active in a rat complement C3 assay and showed comparable pharmacokinetic and metabolic profiles to those of the natural steroid, mifepristone.

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Progesterone plays an important role in the maintenance and development of female reproductive functions, and its biological activity is mediated by the progesterone receptor. Progesterone antagonists or antiprogestins are agents that inhibit the action of progesterone. Blocking the function of the progesterone receptor by using an antiprogestin should allow the modulation of diverse reproductive processes. Therefore, progesterone receptor antagonists have great potential as therapeutic drugs for numerous gynecological, obstetrical, and oncological indications.[1]

Mifepristone (RU-486) is a derivative of the progestin norethindrone, and has a high affinity for the progesterone and glucocorticoid receptors. It is the first and so far the only commercial synthetic drug that is an effective antiprogestin at the receptor level. It has been proven to be useful for cervical priming, emergency contraception, and therapeutic abortion, and has been studied for a variety of indications including breast cancer, meningioma, endometriosis, leiomyoma, and refractory ovarian cancer.[2] It has recently been shown that treatment with mifepristone can potentially prevent *brca1*-mediated breast cancer.[3] However, current progesterone antagonists, including mifepristone, are compromised as clinically useful agents by overt glucocorticoid receptor antagonism.[4] Therefore, new compounds with antiprogestational activity devoid of antiglucocorticoid activity are highly desirable for both clinical applications and basic endocrine research.[5]

Our approach to discover novel progesterone receptor antagonists was to modify the natural steroid skeleton by replacing one of the CH_2 groups with an oxygen atom to form an unnatural oxa-steroid.[6] It was hoped that the resulting oxa-steroid would exert a fine-tuning modulation of its biological activities toward the steroid receptors, and potentially leads to differentiation between the progesterone and glucocorticoid receptors. The mifepristone-like 7-oxa-steroid, compound **1** (Fig. 1), was designed, synthesized, and found to be a potent antiprogestin.[7] Although compound **1** was a slightly less potent antiprogestin than mifepristone, it showed promising improved selectivity between the progesterone and glucocorticoid receptors. Molecular modeling demonstrated that compound **1** had a similar binding mode to that of mifepristone in the progesterone receptor.[7] Moreover, it also suggested that there was an open space around the C17-ethynylmethyl group that could tolerate further modification with larger functional groups at this site.[8] This information, as well as the belief that the C11-aniline group is likely to be responsible for the antiprogestin action of mifepristone,[9] prompted us to focus on modification at the C17-ethynyl position, while keeping the rest of the molecule the same as that of mifepristone.[7,8]

Interestingly, among the 7-oxa-steroids synthesized and evaluated, it was found that compound **2** with a phenyl group and compound **3** with a *para*-fluorophenyl group were more potent and selective antiprogestins than mifepristone and compound **1**. It is known that antiprogestin RU-746 with a phenyl group is less potent than RU-486 with a methyl group in the natural steroid series.[10] Therefore, it is notable to find that a different SAR exists in the unnatural 7-oxa-steroid series.

To rationalize the different SAR observed for the natural steroid series and the unnatural 7-oxa-steroid series, ab initio quantum mechanism (QM) calculations were performed to investigate the influence of this subtle structural change from CH_2 to O at the 7-

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doi:10.1016/j.bmcl.2008.05.070

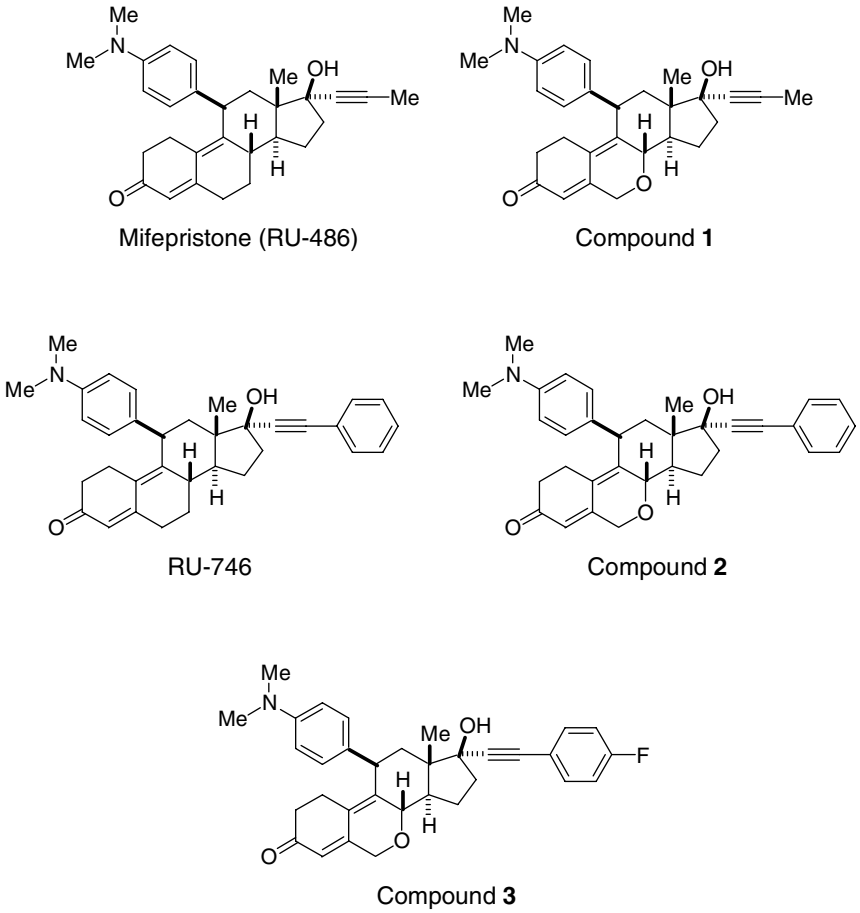

Figure 1. Natural steroids and unnatural 7-oxa-steroids.

position of the B-ring between the two scaffolds. The overlap of the QM optimized structures of RU-486 and compound **1** is presented in Figure 2a. The two structures were aligned by minimizing the root mean square deviation (RMSD) of the heavy atoms in the A-ring, since they are believed to bind in the most rigid part of the ligand-binding pocket of the progesterone receptor,[11] and therefore should have maximum overlap.

As shown in Figure 2a, the single heavy atom change in the B-ring, from CH_2 (RU-486) to O (compound **1**), induced a spatial shift of ca. 1.371 Å at the terminal end of the ethynylmethyl group. In general, it seems that the natural steroid skeleton is more buckled, while the unnatural 7-oxa-steroid skeleton is more extended. However, this difference in shape led to a major change of binding modes at the D-ring binding region. The possible binding modes of RU-746 and compound **2** suggested by molecular modeling are shown in Figure 2b and c. Molecular modeling indicated that the phenyl ring of compound **2** forms a perfect parallel-displaced form of π–π interaction[12] with the phenyl side chain of Phe794. The horizontal and vertical distances between the centers of these two phenyl rings are 1.4 Å and 3.4 Å, respectively. According to the reported high-level QM studies of benzene dimers,[13] there may be ca. 2.5 kcal/mol favorable interaction energy between the two phenyl rings in this parallel-displaced configuration. This may well explain the enhanced potency of compounds **2** and **3** in binding to the progesterone receptor. On the other hand, the phenyl ring of RU-746 may be directed to a position that may not form a favorable π–π interaction with Phe794. Instead, an unfavorable collision with the binding pocket may be present, leading to the decreased potency of RU-746 compared to RU-486. The subtle conformational change and π–π interaction may also be responsible for the boosted selectivity of compounds **2** and **3**, probably due to altered unfavorable binding modes in the glucocorticoid receptor. This is a unique example of a subtle structural change at one end of a molecule being magnified at the other end of the molecule, so as to change the SAR.

To investigate the in vivo activity of these novel 7-oxa-steroids, compounds **1** and **2** were tested orally in ovariectomized Sprague–Dawley rats in a rat complement C3 assay.[14] In this assay, ethinyl estradiol (EE) is used to stimulate C3 expression. Progestins inhibit EE-induced expression. In turn, antiprogestins counteract progestin-dependent inhibition. When compounds **1** and **2** were administered via the oral route along with EE and progesterone, they had similar dose–responsive antiprogestin activities (Fig. 3) and comparable efficacy to mifepristone (Table 1). In a standard rat pharmacokinetic study, compound **3** at 10 mg/kg had a similar profile to that of mifepristone at 30 mg/kg (Table 2). This result shows that the 7-oxa-steroids, particularly the more potent ones with the relatively large phenyl groups at the C17-ethynyl position, have good oral exposure from a pharmacokinetic perspective. Following incubation with rat liver microsomes, compound **3** was rapidly metabolized by oxidative demethylation of the C11-*N,N*-dimethylaniline group, affording two metabolites: compound **3a** with the C11-*N*-methylaniline group and compound **3b** with the C11-aniline group (Fig. 4). This is consistent with the metabolism of mifepristone.[15] It is worth noting that the O7–ether linkage in the 7-oxa-steroids would apparently prevent hydroxylation at this site, as in the case of mifepristone.[15]

Molecular modeling. The molecular geometries of RU-486 and compound **1** were optimized using the program Jaguar.[16] The LMP2 (Local Moller–Plesset second-order perturbation theory)

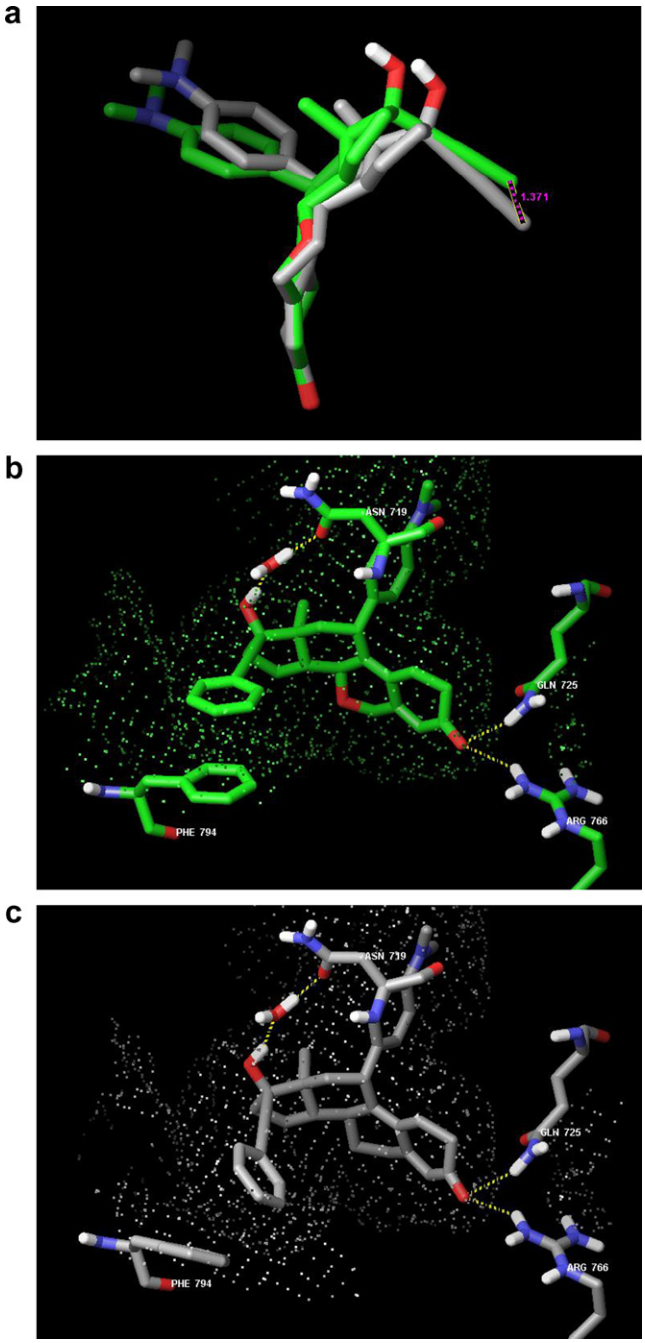

Figure 2. Illustration of the possible impact of the change from CH_2 to O at the B-ring on the binding mode of the D-ring binding region, as suggested by molecular modeling. (a) LMP2/6-311G**++ geometries of RU-486 and compound **1**, overlapped by minimizing the RMSD of their A-ring heavy atoms, (b) the binding mode of compound **2** in the progesterone receptor, (c) the binding mode of RU-746 in the progesterone receptor. The binding pocket is represented as a dotted molecular surface. The ligands and the key residues that may form hydrogen bonds with them are represented as stick models. (Color scheme: oxygen in red, nitrogen in blue, hydrogen in white, carbon of compounds **1** and **2** in green, and carbon of RU-486 and RU-746 in gray.)

method was used with the 6-311G**++ basis set. All the calculations were done in the gas-phase, considering the fact that the progesterone receptor ligands studied herein are mostly buried in the ligand-binding pocket. The human progesterone receptor model in its antagonist conformation was built based on the X-ray crystal structures of the human progesterone receptor–norethindrone complex (PDB ID:1SQN).[11] Using the X-ray crystal structure of the human estrogen receptor–raloxifene complex

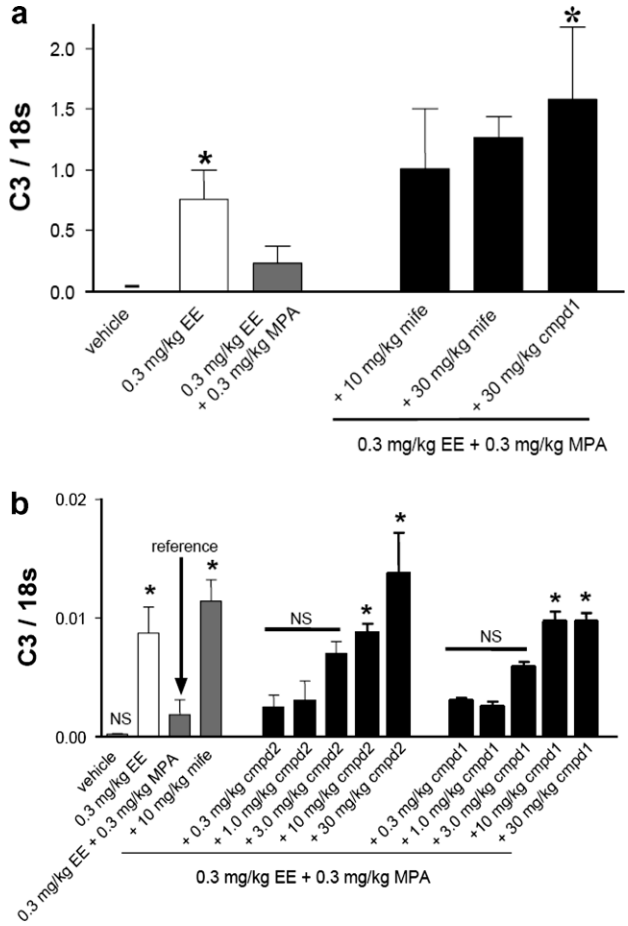

Figure 3. In vivo activities of mifepristone, compounds **1** and **2** in a rat complement C3 assay (n = 3 rats, 6 uterine horns/group).

Table 1
ID_{50} and efficacy of mifepristone, compounds **1** and **2** in a rat complement C3 assay following oral administration

	Mifepristone	Compound **1**	Compound **2**
ID_{50} (mg/kg)	2.1	2.4	2.5
Efficacy at 10 mg/kg (%)	130	110	100

(PDB ID:1ERR)[17] as a reference, it was found that rotation of a single Cα–C bond (Pro906) can easily translocate helix-12 of the progesterone receptor from agonist conformation to the antagonist conformation. The binding conformation of RU-486 in the progesterone receptor was then determined by aligning the progesterone receptor model to the X-ray crystal structure of the human glucocorticoid receptor–mifepristone complex (PDB ID:1NHZ)[18] and copying the mifepristone binding pose in the glucocorticoid receptor X-ray structure to the progesterone receptor model. Later on, the binding pose of compound **1** was determined by aligning it to RU-486 to minimize the RMSD of the A-ring heavy atoms. The final complex structures were optimized by energy minimization using the program MacroModel,[19] holding the core scaffolds rigid as determined by QM calculations. The OPLS2005 force field was used.[20] The effect of an aqueous solution was treated using a GB/SA model.[21] The binding modes of RU-746 and compound **2** were generated by mutating the terminal methyl groups of RU-486 and compound **1** into phenyl groups, followed by full energy minimization.

Biology. In vivo rat complement C3 assay. Ovariectomized 2-month-old Sprague–Dawley rats were purchased from Harlan

Table 2
Pharmacokinetic profiles of mifepristone and compound **3**

Compound	Model	Route	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	MRT (h)	AUC last (ng/h/mL)	AUC inf (ng/h/mL)
Mifepristone	Rat	Po	30	53.8	12.5	5.56	8.0	446	3571
Compound **3**	Rat	Po	10	48.7	3.0	6.88	9.4	460	505

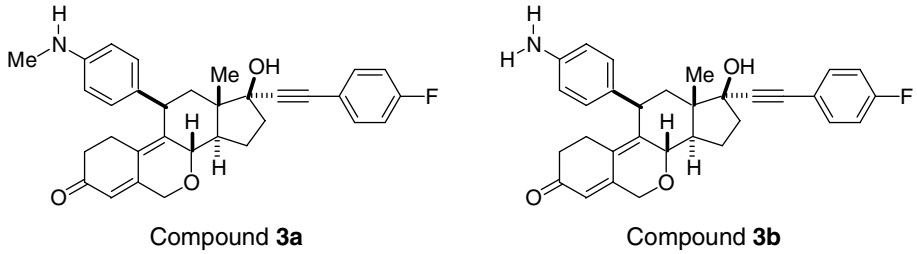

Figure 4. Metabolites of compound **3** following incubation with rat liver microsomes.

(Indianapolis, IN). Five to seven days after surgery, the rats were dosed once with the test compound or control. All of the test compound-treated animals also received 0.3 mg/kg EE and 0.3 mg/kg medroxyprogesterone acetate (MPA) by the oral route. The test compounds were administered orally. After 24 h, the rats were euthanized by carbon dioxide asphyxiation. The uteri were removed, trimmed of fat, and frozen on dry ice prior to storage at −80 °C. The uteri were homogenized in 1–2 mL each of TRIzol (Invitrogen Life Technologies, Carlsbad, CA), and the homogenates were processed for RNA preparation according to the manufacturer's directions. Quantitative PCR was performed using rat complement C3 primers, from Applied Biosystems (Foster City, CA) and an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The level of 28S ribosomal RNA in each sample was determined for normalization, and a dilution series of one of the estrogen-treated samples were used to generate a standard curve.

In conclusion, a subtle structural change from CH_2 to O led to improved potency and selectivity of the resulting 7-oxa-steroids as novel progesterone receptor antagonists. Molecular modeling provided a unique insight into the different SAR of the natural steroids and unnatural 7-oxa-steroids, which is caused by conformational change and π–π interaction. The 7-oxa-steroids had comparable oral efficacy, pharmacokinetics, and metabolism profiles to mifepristone.

Acknowledgment

We thank JNJ PRD ADME group for the pharmacokinetic and metabolic studies of the compounds herein.

References and notes

1. (a) Hess-Stumpp, H.; Hoffmann, J.; Fuhrmann, U. *Drugs Future* **2002**, *27*, 1113; (b) Spitz, I. M. *Endocrinologist* **2005**, *15*, 391.
2. (a) Brogden, R. N.; Goa, K. L.; Faulds, D. *Drugs* **1993**, *45*, 384; (b) Spitz, I. M.; Bardin, C. W. *N. Engl. J. Med.* **1993**, *329*, 404; (c) Tang, O. S.; Ho, P. C. *Gynecol. Endocrinol.* **2006**, *22*, 655.
3. (a) Poole, A. J.; Li, Y.; Kim, Y.; Lin, S.-C. J.; Lee, W.-H.; Lee, E. Y.-H. P. *Science* **2006**, *314*, 1467; (b) Marx, J. *Science* **2006**, *314*, 1370.
4. Lazar, G.; Lazar, G., Jr.; Husztik, E.; Duda, E.; Agarwal, M. K. *Ann. N. Y. Acad. Sci.* **1995**, *761*, 276.
5. Michna, H.; Parczyk, K.; Schneider, M. R.; Nishino, Y. *Ann. N. Y. Acad. Sci.* **1995**, *761*, 224.
6. Ibrahim-Ouali, M. *Steroids* **2007**, *72*, 475.
7. Kang, F.-A.; Allan, G.; Guan, J.; Jain, N.; Linton, O.; Tannenbaum, P.; Xu, J.; Zhu, P.; Gunnet, J.; Chen, X.; Demarest, K.; Lundeen, S.; Sui, Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 907.
8. Kang, F.-A.; Guan, J.; Jain, N.; Allan, G.; Linton, O.; Tannenbaum, P.; Xu, J.; Zhu, P.; Gunnet, J.; Chen, X.; Demarest, K.; Lundeen, S.; Sui, Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2531.
9. Baulieu, E. E. *Science* **1989**, *245*, 1351.
10. Philibert, D.; Costerousse, G.; Gaillard-Moguilewsky, M.; Nedelec, L.; Nique, F.; Tournemine, C.; Teutsch, G. Antihormones in Health and Disease. In *Frontiers of Hormone Research*; Agarwal, M. K., Ed.; Karger: Basel, 1991; Vol. 19, pp 1–17.
11. Madauss, K. P.; Deng, J.-S.; Austin, R. J. H.; Lambert, M. H.; McLay, I.; Pritchard, J.; Short, S. A.; Stewart, E. L.; Uings, I. J.; Williams, S. P. *J. Med. Chem.* **2004**, *47*, 3381.
12. Sinnokrot, M. O.; Sherrill, C. D. *J. Phys. Chem. A* **2006**, *110*, 10656.
13. Sinnokrot, M. O.; Sherrill, C. D. *J. Phys. Chem. A* **2004**, *108*, 10200.
14. Lundeen, S. G.; Zhang, Z.; Zhu, Y.; Carver, J. M.; Winneker, R. C. *J. Steroid Biochem. Mol. Biol.* **2001**, *78*, 137.
15. Wiechert, R.; Neef, G. *J. Steroid Biochem.* **1987**, *27*, 851.
16. Jaguar, Schrodinger, 1500 S.W. First Avenue, Suite 1180, Portland, OR 97201, USA.
17. Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J. A.; Carlquist, M. *Nature* **1997**, *389*, 753.
18. Kauppi, B.; Jakob, C.; Farnegardh, M.; Yang, J.; Ahola, H.; Alarcon, M.; Calles, K.; Engstrom, O.; Harlan, J.; Muchmore, S.; Ramqvist, A.-K.; Thorell, S.; Ohman, L.; Greer, J.; Gustafsson, J.-A.; Carlstedt-Duke, J.; Carlquist, M. *J. Biol. Chem.* **2003**, *278*, 22748.
19. MacroModel, Schrodinger, 1500 S.W. First Avenue, Suite 1180, Portland, OR 97201, USA.
20. Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.
21. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.