Bioorganic & Medicinal Chemistry Letters 19 (2009) 393–396

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Combining 3D-QSAR, docking, molecular dynamics and MM/PBSA methods to predict binding modes for nonsteroidal selective modulator to glucocorticoid receptor

Yong Xu [a], Tao Zhang [b,*], Minbo Chen [b]

[a] Laboratory of Structural Sciences, Van Andel Research Institute, 333 Bostwick Avenue, Grand Rapids, MI 49503, USA
[b] Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Computational Chemistry Group, 354 Fenglin Rd., Shanghai 200032, PR China

ARTICLE INFO

Article history:
Received 21 June 2008
Revised 11 October 2008
Accepted 19 November 2008
Available online 24 November 2008

Keywords:
Glucocorticoid receptor
AL-438
Molecular modeling

ABSTRACT

AL-438 is a selective and dissociated GR (glucocorticoid receptor) agonist. In this letter, the binding mode of AL-438 to GR is predicted by using multiple computational methods including 3D-QSAR, molecular docking and molecular dynamics simulation. This provides a guideline for rational design of novel and dissociated nonsteroidal GR ligand.

Glucocorticoid receptor (GR, NR31C1[1,2]) is a promising pharmaceutical target to treat inflammatory and other related diseases. Several steroidal GR agonists such as dexamethasone (DEX), prednisolone (PRED) and fluticasone propionate (FP) have been widely and successfully used in the treatment of acute and chronic inflammatory diseases for decades of years. However, the long-term usage of these drugs are also accompanied by serious and unpleasant side-effects including diabetes mellitus, peptic ulcer, skin atrophy, etc. As a consequence of these side-effects, there has recently been considerable interest in a hypothesis of selective glucocorticoid agonism where the beneficial anti-inflammatory effects are postulated to derive from transrepression (TR) pathways and may be separated from the side-effects derived from transactivation (TA) pathways. Compounds which display selectivity for TR over TA are often referred to as dissociated agonists. Recently, researchers in both academics and industries are engaged in the search for novel GR ligands that preferentially induce the TR activities of the GR, while having reduced TA activities. For recent progress of dissociated GR agonist see review papers.[3–5]

Based on dihydroquinoline scaffold that is initially used in development of PR agonists,[6] Abbott Laboratories and Ligand Pharmaceuticals cooperatively develop a series of four-ring ligands (AL-series)[7–10] with dissociated properties more or less (Fig. 1). One of them, AL-438 that has been tested in many experiments presents a hopeful dissociated profile and a potential of practical usage.[11] A reasonable GR/ligand model would be very helpful for designing and optimizing lead compounds. However, the limited GR/ligand complex[12–16] structural information strongly hamper our understanding of how known GR ligands interact with GR. At GSK, researchers have recently described nonsteroidal GR modulators designed by using an agreement docking method.[17–20] In this

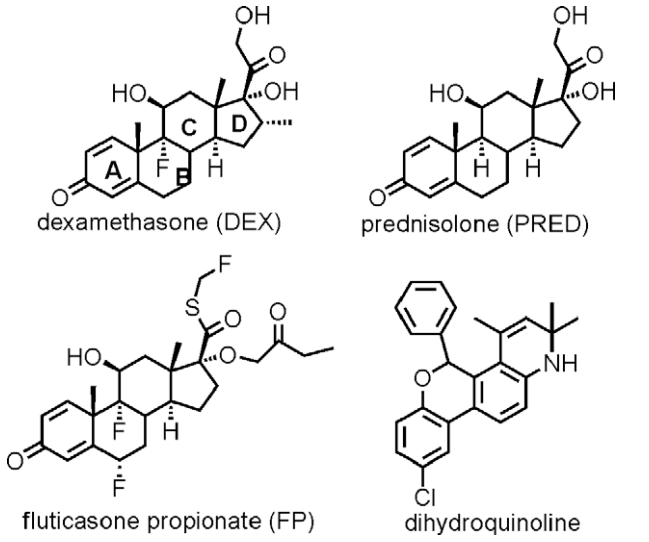

Figure 1. The chemical structures of dexamethasone, prednisolone, fluticasone propionate and dihydroquinoline.

* Corresponding author.
E-mail address: zhangcybely@hotmail.com (T. Zhang).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.11.069

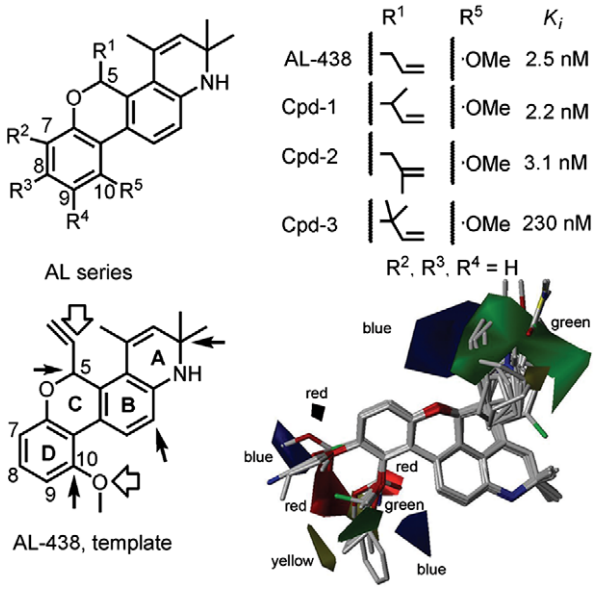

Figure 2. The chemical structures of AL-series molecules (top-left). The representative compounds of AL-series (top-right). AL-438 was used as template for rigid superimposition in CoMFA studies (bottom-left). Four atoms pointed by solid arrows are necessary, while two atoms pointed by hollow arrows are optional. The graphic view of the CoMFA results (bottom-right). The contours of the steric map are shown in yellow and green, and those of the electrostatic map are shown in red and blue. Greater values are correlated with more bulk near green; less bulk near yellow; more positive charge near blue, and more negative charge near red. The relative contribution fraction data are indicated.

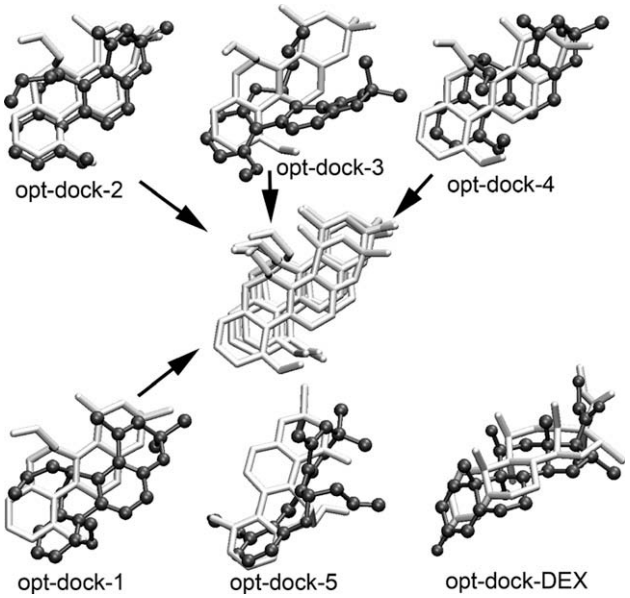

Figure 3. Comparison of the conformations change of each docking pose before (black) and after (white) molecular dynamics simulations.

study, we have used a protocol combining three-dimensional structure–activity relationship (3D-QSAR), molecular docking, molecular dynamics (MD) and MM/PBSA methods to investigate the detailed interactions of GR with AL-438 analogues.

To more fully explore the specific contributions of electrostatic, steric and hydrophobic effects for these dihydroquinoline analogues binding to GR, 3D-QSAR analyses was performed on these agonists using comparative molecular field analysis (CoMFA)[21,22] based on the rigid alignment as shown in Figure 2. The study contains 79 molecules which C-5, C-9 and C-10 substitution on the dihydroquinoline scaffold. The CoMFA provide statistically valid model with good correlation and predictive power. The cross-validated q^2 value reaches 0.633 for CoMFA (for details see Supporting information). It should be pointed out that this 3D-QSAR study is not to make a 'model' to predict activities of new AL analogs, but to get a whole impression about the structure–activity relationship underling the structural information and biological data of the tens of molecules in the references. The most valuable information disclosed by CoMFA study is related to the part of GR ligand-binding-site that interacts with R^1 group attached 5-site (Fig. 2). The big green region indicates that this part have some space available or there are some flexible residues to accommodate bulky groups, while the blue region indicates that there might be some negative charge residues nearby.

Secondly, molecular docking studies for all AL-series molecules studied presently were performed by using GOLD program.[23] The crystal structure of GR/DEX complex (PDB code is 1M2Z[12]) is taken as the receptor. Here, the combination of position, orientation of a ligand relative to the receptor, along with its conformation is referred to as a ligand pose in a receptor. In this docking study, the flexibility of the receptor is not considered. The objects are to explore several initial poses of ligands relative to the receptor, to give a whole impression about GR/AL-series binding modes, and to prepare starting points for the following molecular dynamics simulations. For all AL-series molecules, the docking studies show that poses of AL-series could be clustered into several groups (for details see the Supporting information.). Just for the specific AL-438, careful investigations also show that it can adopt five different poses (dock-1, dock-2, dock-3, dock-4 and dock-5) in the ligand-binding-site (Fig. 3). In comparison, the binding mode of GR/DEX is exactly reproduced by GOLD docking program.

Thirdly, six molecular dynamics simulations including five GR/AL-438 systems and one GR/DEX system are performed by using the Amber program[24] to get more reasonable GR/AL-438 binding mode, where the flexibility of receptor is considered. In each system, the whole structure of the receptor including key H12 is kept stable, while the ligand takes conformational changes more or less (Fig. 4). The DEX always keeps in its original position, while AL-438 in five GR/AL-438 systems take minor or major conformational changes to optimize the protein–ligand interactions. In opt-dock-5 systems, the variation of AL-438 indicates that it reaches a stable position after a sharp leap at about 1.9 ns when the 5-vinly group slips into a new pocket. For other four systems (opt-dock-1, opt-dock-2, opt-dock-3 and opt-dock-4), although the simulations start from different poses, the very similar final poses of AL-438 are obtained. Figure 4 show that these conformational changes happened at early stage of the simulations. After visual inspection, we can summarize these five poses into two binding modes. MD_mode_I denotes MD simulation binding mode from opt-dock-1, opt-dock-2, opt-dock-3 and opt-dock-4, while MD_mode_II denotes that from opt-dock-5.

In comparison, DEX and AL-438 have very different binding modes with GR because AL-438 does not have a classic steroidal 4 ring. As shown in Figures 5 and 6, in both binding modes predicted by docking and MD simulation, the D ring of AL-438 takes the place of A ring of DEX. Figure 5, DEX and GR can form perfect hydrogen bond interactions, where all polar atoms (N and O) and polar hydrogen atoms on DEX could form hydrogen bonds with polar residues with ARG611, GLN570, ASN564, THR739 and GLN642 of GR. For AL-438, in MD_mode_I, only one moderately stable hydrogen bond forms between N atom of AL-438 and GLN642 of GR. This is less conserved hydrogen bond in GR–glucocorticoids interaction. In MD_mode_II, two more conserved hydrogen bonds are formed. The O atom on D ring forms hydrogen bond with GLN570, while N atom of A ring forms hydrogen bond with residue ASN564 of GR. There are two important electrostatics regions distributed in our

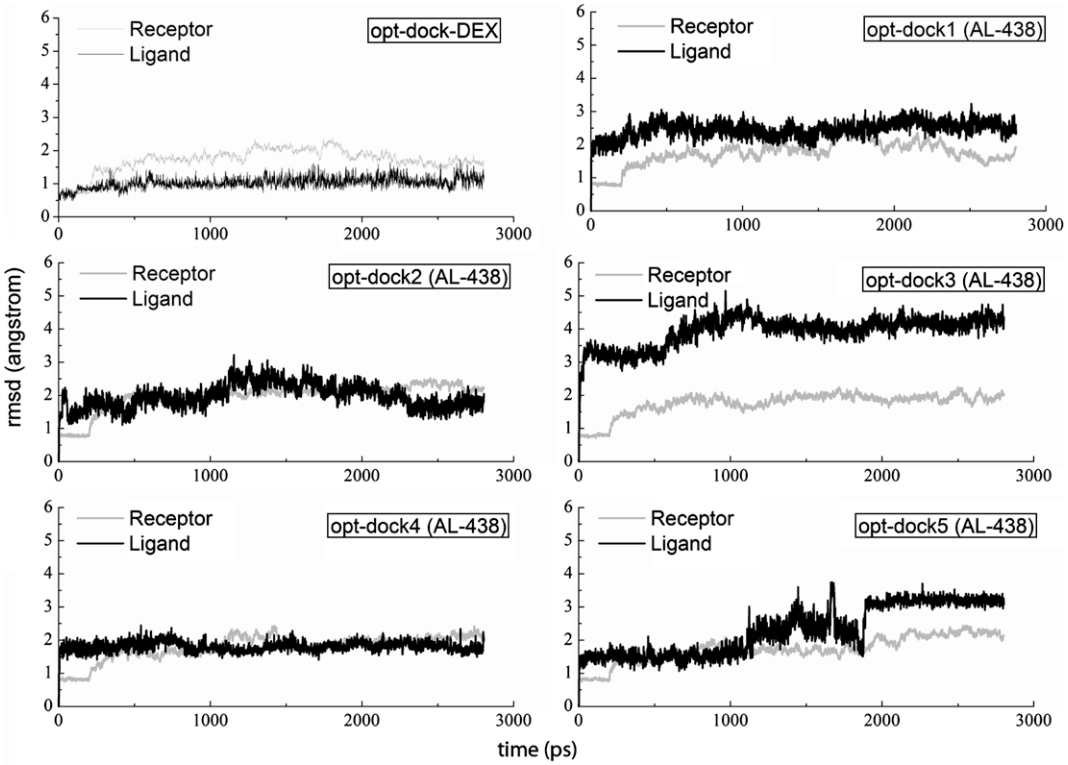

Figure 4. Variations of the rmsd of the backbone of the receptor (grey), and the ligand (black) in MD simulations. The backbone atoms (C, N, Cα) of the initial structure (X-ray) are taken as the reference in least-square-fitting.

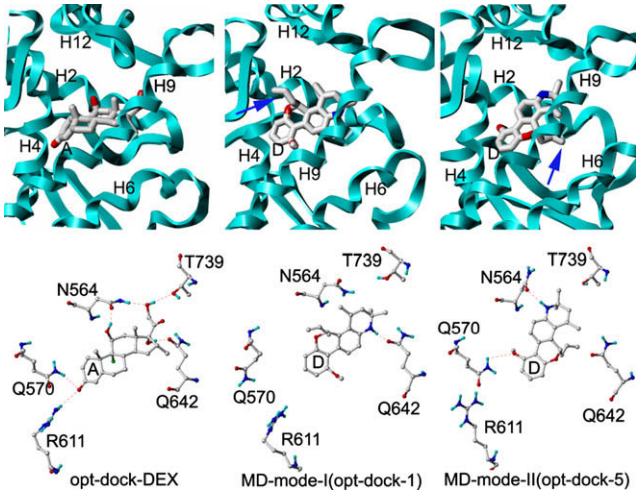

Figure 5. Representative binding mode of DEX and AL-438 in the GR ligand-binding-site. The upper part shows the relative orientation, the lower part show some key residues. H-bonds are shown with red line.

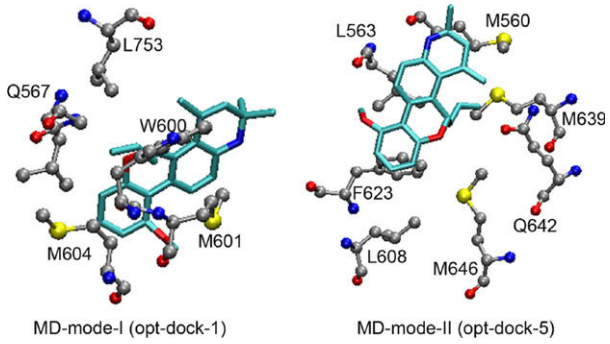

Figure 6. Comparison of the residues close to R^1 group in MD-mode-I (left) and in MD-mode-II (right).

CoMFA contour map (Fig. 2). The blue one close to the substituted group on 5-site (C ring) is surrounded by MET646, MET639, MET560, LEU563, PHE623, LEU608 and GLN642 (Fig. 6). The thiol group on MET and GLN642 might construct a negatively electrostatic environment. A smaller red region near ring D corresponds to residue ARG611 that may form a positively electrostatic environment. Considering the conserved property of the hydrogen bonds in GR–glucocorticoids interactions, we conclude that MD_mode_II is more close to the real binding mode of AL-438 with GR.

In order to confirm this, the binding free energies of these two binding modes are computed by using MM/PBSA method and Normal-mode analysis based on the molecular dynamics trajectories. Table 1 summarizes the results of our MM/PBSA calculation, including the energy terms given by the MM/PBSA method for DEX and AL-438. Two things are suggested by the binding free

Table 1
The binding free energy computed by MM/PBSA methods (unit: kcal/mol)

System code	$\Delta G_{MM/PBSA}$	$T\Delta S$	ΔG_{total}
Dock-DEX	−37.21	−26.35	−10.86
GR/AL-438 (opt-dock-1)	−34.58	−22.69	−11.89
GR/AL-438 (opt-dock-2)	−30.43	−18.53	−11.90
GR/AL-438 (opt-dock-3)	−35.50	−15.76	−19.74
GR/AL-438 (opt-dock-4)	−29.47	−23.86	−5.61
MD-mode-I[a]	−32.50	−20.21	−12.29
MD-mode-II[b]	−35.71	−20.82	−14.89

[a] Averaged from opt-dock-1 to opt-dock-4.
[b] Opt-dock-5.

energies (ΔG_{total}) presented in Table 1. (1) As GR ligands, DEX looks weaker than AL-438 for ΔG_{total} of GR/DEX (−10.86 kcal/mol) is smaller than those in GR/AL-438 systems. (2) The binding free energy of MD_mode_I is around 2.6 kcal/mol lower than that of MD_mode_II for AL-438. Between AL-438 and DEX, the difference of ΔG_{total} is originated from the changes of entropy, which are calculated by Normal-mode. As described above, DEX forms much more hydrogen bonds with GR than AL-438. In the view point of computational chemistry, the hydrogen bonds would restrict the configuration space of the complex, which should be characterized by configurational entropy calculation. Unfortunately, the contribution from configurational entropy is completely ignored by Normal-mode. Therefore, we cannot expect that the entropy calculation at this level is able to give correct numbers for the two quite different molecules. Differently, the data for AL-438 looks more reasonable. The difference of ΔG_{total} is mainly originated from $\Delta G_{MM/PBSA}$ calculation. As expected, their changes of entropy in binding process (calculated) are similar (−20.21 and −20.82 kcal/mol) and looks reasonable as the ligands are same. The lower binding free energy in MD_mode_II confirms that it is the favorable binding mode.

As mentioned above, CoMFA study indicates that the region of the receptor, with which the R^1 group contact directly, should have some space available or some flexible residues to accommodate bulky groups. Obviously, MD_mode_II matched with this structural information very well than MD_mode_I (Figs. 2, 5 and 6). In MD_mode_I, the 5-vinyl (R^1) could directly contact with the side chain of LEU753 on H12, and insert a pocket mainly constituted by the backbone atoms from TRP600, MET601, MET604 on H4, GLY567 on H2, and side chain atoms from LEU753 on H12. It is difficult for this pocket to contain bulky groups since both H2 and H4 are belonged to the most conserved structural unit of nuclear receptor. Oppositely, in MD_mode_II, 5-vinyl group insert into a small pocket formed by the terminal atoms from MET646, MET639, MET560, LEU563, PHE623, LEU608 and GLU642. It is reasonable to believe this pocket has potential to accommodate bulky group as most of these resides are flexible and extended currently.

Experimentally, a little structural modification on the 5-alpha site would strikingly affect the activity of AL-438 analogs. The K_i value of AL-438 whose R^1 group is 2-propenyl is about 2.5 nM (Fig. 2).[9] The activities are kept when just a methyl is added on the R^1 (3.1 nM for 2-methylallyl and 2.2 nM for but-3-en-2-yl), while if two methyl groups added on the 5-alpha site, the activity decreased (230 nM for 2-methylbut-3-en-2-yl). The sharp changes strongly indicated that there are some special structural factors on the receptor to strictly control the region around R^1. Given MD_mode_II could represent the real binding mode, the big, rigid and deeply-hidden PHE623 may very likely play this role. The crystallographic study of GR/FP and GR/FF has proved the existence of the small pocket that accommodates the R^1 group in MD_mode_II.[15,16] Furthermore, in a recent modeling work, using docking and simulated annealing refinement, an AL-438 derivative developed by Ligand Pharmaceuticals was predicted to have similar protein–ligand binding mode as that of MD_mode_II.[25]

In summary, molecular docking method was used to produce several possible binding poses for AL-438 and GR. The molecular dynamics, MM/PBSA method and 3D-QSAR were combinational used to confirm the reasonable binding mode of AL-438/GR complex. This model provides a structural framework for understanding the SARs of these compounds. The elucidation of protein–ligand interaction in this study will be helpful for rational design of novel glucocorticoid receptor modulator.

Supplementary data

A document that gives more details about CoMFA model and 2D structures of all the compounds used in this study. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.11.069.

References and notes

1. Nuclear Receptors Nomenclature Committee. *Cell* **1999**, 97, 161.
2. Lu, N. Z.; Wardell, S. E.; Burnstein, K. L.; Defranco, D.; Fuller, P. J.; Giguere, V.; Hochberg, R. B.; McKay, L.; Renoir, J. M.; Weigel, N. L.; Wilson, E. M.; McDonnell, D. P.; Cidlowski, J. A. *Pharmacol. Rev.* **2006**, *58*, 782.
3. Schacke, H.; Rehwinkel, H.; Asadullah, K. *Curr. Opin. Investig. Drugs* **2005**, *6*, 503.
4. Schacke, H.; Rehwinkel, H.; Asadullah, K.; Cato, A. C. *Exp. Dermatol.* **2006**, *15*, 565.
5. Schacke, H.; Berger, M.; Rehwinkel, H.; Asadullah, K. *Mol. Cell. Endocrinol.* **2007**, *275*, 109.
6. Edwards, J. P.; West, S. J.; Marschke, K. B.; Mais, D. E.; Gottardis, M. M.; Jones, T. K. *J. Med. Chem.* **1998**, *41*, 303.
7. Coghlan, M. J.; Kym, P. R.; Elmore, S. W.; Wang, A. X.; Luly, J. R.; Wilcox, D.; Stashko, M.; Lin, C. W.; Miner, J.; Tyree, C.; Nakane, M.; Jacobson, P.; Lane, B. C. *J. Med. Chem.* **2001**, *44*, 2879.
8. Elmore, S. W.; Coghlan, M. J.; Anderson, D. D.; Pratt, J. K.; Green, B. E.; Wang, A. X.; Stashko, M. A.; Lin, C. W.; Tyree, C. M.; Miner, J. N.; Jacobson, P. B.; Wilcox, D. M.; Lane, B. C. *J. Med. Chem.* **2001**, *44*, 4481.
9. Elmore, S. W.; Pratt, J. K.; Coghlan, M. J.; Mao, Y.; Green, B. E.; Anderson, D. D.; Stashko, M. A.; Lin, C. W.; Falls, D.; Nakane, M.; Miller, L.; Tyree, C. M.; Miner, J. N.; Lane, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1721.
10. Kym, P. R.; Kort, M. E.; Coghlan, M. J.; Moore, J. L.; Tang, R.; Ratajczyk, J. D.; Larson, D. P.; Elmore, S. W.; Pratt, J. K.; Stashko, M. A.; Falls, H. D.; Lin, C. W.; Nakane, M.; Miller, L.; Tyree, C. M.; Miner, J. N.; Jacobson, P. B.; Wilcox, D. M.; Nguyen, P.; Lane, B. C. *J. Med. Chem.* **2003**, *46*, 1016.
11. Coghlan, M. J.; Jacobson, P. B.; Lane, B.; Nakane, M.; Lin, C. W.; Elmore, S. W.; Kym, P. R.; Luly, J. R.; Carter, G. W.; Turner, R.; Tyree, C. M.; Hu, J.; Elgort, M.; Rosen, J.; Miner, J. N. *Mol. Endocrinol.* **2003**, *17*, 860.
12. Bledsoe, R. K.; Montana, V. G.; Stanley, T. B.; Delves, C. J.; Apolito, C. J.; McKee, D. D.; Consler, T. G.; Parks, D. J.; Stewart, E. L.; Willson, T. M.; Lambert, M. H.; Moore, J. T.; Pearce, K. H.; Xu, H. E. *Cell* **2002**, *110*, 93.
13. 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.
14. Suino-Powell, K.; Xu, Y.; Zhang, C. H.; Tao, Y. G.; Tolbert, W. D.; Simons, S. S. J.; Xu, H. E. *Mol. Cell. Biol.* **2008**, *28*, 1915.
15. Bledsoe, R. K.; Lambert, M. H.; Montana, V. G.; Stewart, E. L.; Xu, H. E. Eur. Pat. Appl. 1375517, 2004.
16. Biggadike, K.; Bledsoe, R. K.; Hassell, A. M.; Kirk, B. E.; McLay, I. M.; Shewchuk, L. M.; Stewart, E. L. *J. Med. Chem.* **2008**, *51*, 3349.
17. Biggadike, K.; Boudjelal, M.; Clackers, M.; Coe, D. M.; Demaine, D. A.; Hardy, G. W.; Humphreys, D.; Inglis, G. G.; Johnston, M. J.; Jones, H. T.; House, D.; Loiseau, R.; Needham, D.; Skone, P. A.; Uings, I.; Veitch, G.; Weingarten, G. G.; McLay, I. M.; Macdonald, S. J. *J. Med. Chem.* **2007**, *50*, 6519.
18. Clackers, M.; Coe, D. M.; Demaine, D. A.; Hardy, G. W.; Humphreys, D.; Inglis, G. G.; Johnston, M. J.; Jones, H. T.; House, D.; Loiseau, R.; Minick, D. J.; Skone, P. A.; Uings, I.; McLay, I. M.; Macdonald, S. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4737.
19. Barker, M.; Clackers, M.; Copley, R.; Demaine, D. A.; Humphreys, D.; Inglis, G. G.; Johnston, M. J.; Jones, H. T.; Haase, M. V.; House, D.; Loiseau, R.; Nisbet, L.; Pacquet, F.; Skone, P. A.; Shanahan, S. E.; Tape, D.; Vinader, V. M.; Washington, M.; Uings, I.; Upton, R.; McLay, I. M.; Macdonald, S. J. *J. Med. Chem.* **2006**, *49*, 4216.
20. Barker, M.; Clackers, M.; Demaine, D. A.; Humphreys, D.; Johnston, M. J.; Jones, H. T.; Pacquet, F.; Pritchard, J. M.; Salter, M.; Shanahan, S. E.; Skone, P. A.; Vinader, V. M.; Uings, I.; McLay, I. M.; Macdonald, S. J. *J. Med. Chem.* **2005**, *48*, 4507.
21. Cramer, R. D.; Patterson, D. E.; Bunce, J. D. *J. Am. Chem. Soc.* **1998**, *110*, 5959.
22. Cho, S. J.; Tropsha, A. *J. Med. Chem.* **1995**, *38*, 1060.
23. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727.
24. Case, D. A.; Darden, T. A.; Cheatham, T. E., III; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Wang, B.; Perlman, D. A.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, J. W.; Ross, W. S.; Kollman, P. A. *AMBER 8*, University of California, San Francisco, **2004**.
25. Hudson, A. R.; Roach, S. L.; Higuchi, R. I.; Phillips, D. P.; Bissonnette, R. P.; Lamph, W. W.; Yen, J.; Li, Y.; Adams, M. E.; Valdez, L. J.; Vassar, A.; Cuervo, C.; Kallel, E. A.; Gharbaoui, C. J.; Mais, D. E.; Miner, J. N.; Marschke, K. B.; Rungta, D.; Negro-Vilar, A.; Zhi, L. *J. Med. Chem.* **2007**, *50*, 4699.