Bioorganic & Medicinal Chemistry Letters 18 (2008) 3974–3977

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis of novel ketoconazole derivatives as inhibitors of the human Pregnane X Receptor (PXR; NR1I2; also termed SXR, PAR)

Bhaskar C. Das [a,*], Ankanahlli V. Madhukumar [b], Jaime Anguiano [a], Sean Kim [c], Michael Sinz [c], Tatyana A. Zvyaga [c], Eoin C. Power [d], C. Robin Ganellin [e], Sridhar Mani [b,*]

[a] Albert Einstein Cancer Center, Albert Einstein College of Medicine, Departments of Developmental and Molecular Biology and Nuclear Medicine, Bronx, NY 10463, USA
[b] Albert Einstein Cancer Center, Albert Einstein College of Medicine, Broux, NY 10461, USA
[c] Bristol-Myers Squibb Co., Wallingford, CT 06492, USA
[d] Sienabiotech S.P.A., Via Fiorentina, 53100 Siena, Italy
[e] Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, UK

ARTICLE INFO

Article history:
Received 30 April 2008
Revised 2 June 2008
Accepted 4 June 2008
Available online 10 June 2008

Keywords:
Pregnane X receptor
PXR
Ketoconazole
Azole
Analogs
Nuclear receptors

ABSTRACT

PXR, pregnane X receptor, in its activated state, is a validated target for controlling certain drug–drug interactions in humans. In this context, there is a paucity of inhibitors directed toward activated PXR. Using prior observations with ketoconazole as a PXR inhibitor, the target compound **3** was synthesized from (s)-glycidol with overall 56% yield. (+)-Glycidol was reacted with 4-bromophenol and potassium carbonate in DMF to yield the ring opened compound **6**. This was then heated to reflux in benzene along with 2′, 4′-difluoroacetophenone and catalytic amount of *para*-toluene sulfonic acid to yield **8**. The resultant acetal **8** was then functionalized using Palladium chemistry to yield the target compound **3**. The activity of the compound was compared with ketoconazole and UCL2158H. However, in contrast with ketoconazole ($IC_{50} \sim 0.020$ μM; ~100% inhibition), **3** has negligible effects on inhibition of microsomal CYP450 (maximum ~20% inhibition) at concentrations >40 μM. In vitro, micromolar concentration of ketoconazole is toxic to passaged human cell lines, while **3** does not exhibit cytotoxicity up to concentrations ~100 μM (viability >85%). This is the first demonstration of a chemical analog of a PXR inhibitor that retains activity against activated PXR. Furthermore, in contrast with ketoconazole, **3** is less toxic in human cell lines and has negligible CYP450 activity.

Pregnane X receptor or PXR (NR1I2; also termed SXR, PAR), a member of the adopted orphan nuclear receptor family, is the primary xenobiotic sensor in human and mammalian tissues.[1] It responds to a wide range of structurally and chemically distinct ligands that range from small lipophilic drugs (e.g., rifampicin) to potentially toxic bile acids as well as cholesterol metabolites.[1–10] This promiscuous activation of PXR by natural substances and xenobiotics has been shown as a mechanism that accelerates the metabolism of affected drugs, which leads to unanticipated adverse drug interactions and loss of efficacy.[8] Recent data also support a major role for PXR activation in controlling (decreasing) the transport of drugs across the blood brain barrier, underscoring PXR's role in drug delivery to the brain in disease state (e.g., brain metastases).[11]

Furthermore, there is increasing evidence that PXR activation leads to increased cancer cell growth and drug resistance.[12] Together, in the context of cancer therapeutics, controlling PXR activation is expected to provide many therapeutic benefits by improving both drug metabolism and delivery. Therefore, the development of novel and non-toxic inhibitors of PXR activation is warranted.

In addition to its therapeutic utility, PXR inhibitor would be useful to study the molecular basis of receptor function. To date, only few PXR inhibitors have been described: ketoconazole (and related azoles[13]), suphoraphane,[14] and ecteinascidin-743 (ET-743).[15] Ketoconazole was first described as a PXR antagonist by Takeshita et al., and was subsequently shown to disrupt the binding of co-regulators (including both coactivators and corepressors) to the surface of PXR in an agonist-dependent fashion.[16,17] In addition, in the presence of the established PXR activator rifampicin, ketoconazole, and related azoles were shown to prevent the activation of the receptor both in cell-based assays and in a humanized PXR mouse model.[18]

Scintillation proximity assays using PXR ligand binding domain co-purified with SRC-1 peptide show that ketoconazole does indeed bind to PXR over a concentration range (10–100 μM) but is concentration-dependent.[17] Since this assay is a ligand (^{3}H-SR12813) displacement assay, the concentration dependence relates to ligand displacement (by binding to the ligand binding pocket) and not to *allosteric* binding. However, our laboratory has shown that ketoconazole binds to at least a region outside the

* Corresponding authors. Tel.: +1 17184302422; fax: +1 17184308853.
E-mail address: bdas@aecom.yu.edu (B.C. Das).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.018

ligand binding pocket. We also have shown that the revertant AF-2 region double mutant of PXR (T248E/K277Q) activates with rifampicin but is not inhibited by ketoconazole.[13,19] These data establish that small molecule modulators such as ketoconazole can antagonize PXR, and that the surface AF-2 site of the receptor appears to be a target of such compounds. Based on these data and the knowledge of the PXR:SRC-1 crystal structure, we embarked on developing novel ketoconazole analogs **3** that would retain its antagonist potential on activated PXR but also have minimal cytotoxicity as well as CYP3A inhibition potential (Scheme 1).

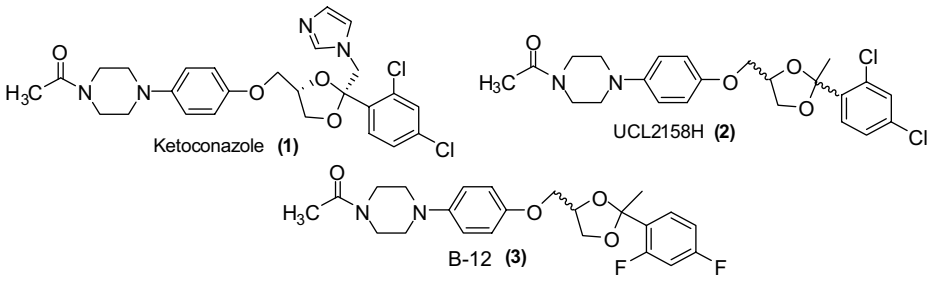
The discovery of novel ketoconazole analogs that inhibit PXR only in its ligand-activated state would serve as tools to better understand receptor function. These novel analogs would be more selective to PXR antagonism (i.e., lack cytochrome P450 inhibition effects, idiosyncratic organ toxicity, and cellular cytotoxicity in vitro). Ideally, the IC_{50} for antagonism of the lead analogs would be lower and more selective to PXR. Finally, non-toxic lead compounds could be further developed for clinical use in the setting of cancer chemotherapy. Focusing on the above objectives, we synthesized ketoconazole analogs (FL-B # 12, FL-B # 15, FL-B # 24, FL-B # 31 and FL-B # 45). From our preliminary study we found compound **3** (FL-B-12) is our lead compound shown in Scheme 2. Hence onwards, our lead compound **3** will be read as **FL-B-12** in all figures and tables.

The synthesis of compound **3** was as follows.[20–22] First, (+)-glycidol was reacted with 4-bromophenol and potassium carbonate in DMF to yield the ring opened compound **6**. This was then heated to reflux in benzene along with 2′,4′-difluoroacetophenone and catalytic toluene sulfonic acid to yield **8** as racemic mixture. The resultant acetal **8** was then functionalized using Buchwald chemistry to yield **3**, overall 56%. The general procedure for synthesis of compound **3** is briefly described in Ref. 22.

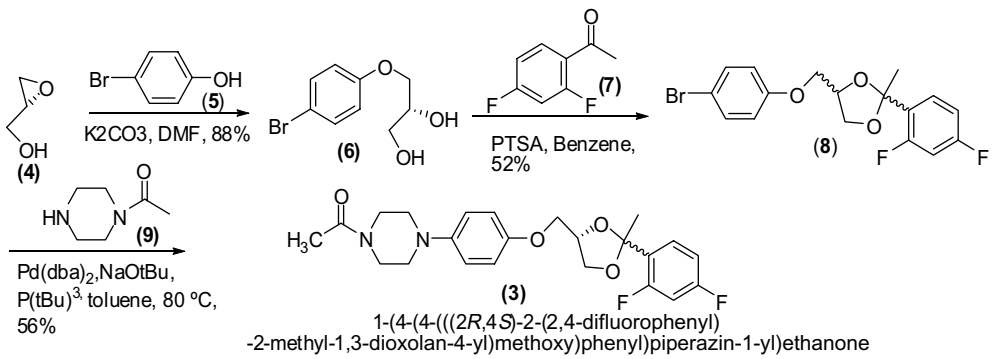
After synthesizing the compounds, we tested the biological activity of analogs and compared with parent ketoconazole and UCl2158H.

Materials: Dulbecco's modified Eagle medium (DMEM), Lipofectamine™ 2000, heat-inactivated fetal bovine serum (FBS), trypsin–EDTA (0.25%), and penicillin–streptomycin were purchased from GIBCO/Invitrogen (Carlsbad, CA). Charcoal/dextran-treated FBS was purchased from Hyclone (Logan, UT). HepG2 cells were obtained from ATCC (Manassas, VA). Steady-Glo® Luciferase Assay System was purchased from Promega (Madison, WI). Alamar-Blue reagent was purchased from Trek Diagnostics (Cleveland, OH). Rifampicin, ketoconazole, and midazolam were purchased from Sigma (St. Louis, MO). Human liver microsome and 1-hydroxymidazolam were obtained from BD Biosciences (San Jose, CA). Human PXR-pcDNA3 and luciferase reporter containing CYP3A4 promoter, CYP3A-Luc, were generated at Bristol-Myers Squibb.

Cell culture, PXR transactivation and cytotoxicity assays: HepG2 cells were cultured in DMEM containing 10% FBS. For transient transfection, a transfection mixture containing 1 μg of PXR-pcDNA3 plasmid DNA, 20 μg of Cyp3A-Luc plasmid DNA and 90 μl of Lipofectamine™ 2000 was prepared in 1 mL of serum-free DMEM. The transfection mixture was then applied to the cells in fresh medium (20 mL per flask) and was incubated at 37 °C (5% CO_2) overnight. The transfected cells were trypsinized and cryopreserved for long-term storage. On the day of experiment, vials of cryopreserved cells were thawed and then resuspended in fresh Media II (DMEM containing 5% charcoal/dextran-treated FBS, 1% penicillin/streptomycin,100 μM non-essential amino acids, 1 mM sodium pyruvate, and 2 mM L-glutamine). Approximately 8×10^3 cells were added to wells of 384-well plates containing either test compound alone or a mixture of test compound and rifampicin (10 μM) dissolved in DMSO (0.1% in final incubations). Compounds were tested at ten concentrations (50 μM–2.5 mM, 1:3 serial dilution). The plates then were incubated at 37 °C for 24 h and subsequently Alamar Blue reagent was added to each well. Plates were incubated for 2 h at 37 °C, 5% CO_2 and then 1 h at room temperature. Fluorescence was read at Ex525/Em598 to measure cytotoxicity of the test articles. Subsequently, luciferase substrate (Steady-Glo®) was added to each well. The plates were incubated for 15 min at room temperature, after which the luminescence was read on a Viewlux (Perkin-Elmer, Waltham, MA) plate reader. In addition, drug induced cytotoxicity was assessed by the MTT



Scheme 1. Structures of ketoconazole, UCL2158H, and **3**.



Scheme 2. Synthesis of **3** and derivates.

assay[17] in cancer cell lines (LS174T and SKOV3) as well as a fibroblast cell line (CRL). Cells were exposed to a concentration range of the drug(s) for 48 h. These assays were repeated three separate times each in triplicate.

Data analysis: Rifampicin (10 μM), a well known agonist of PXR, is included in each plate as an internal standard and positive control. The data were then expressed as percent activation (% Act), where the total signal is the signal from the 10 μM rifampicin and the blank signal is that from the DMSO vehicle.

$$\%\mathrm{Act} = \frac{\text{Compound signal} - \text{Blank signal}}{\text{Total signal} - \text{Blank signal}} \times 100\%$$

For PXR activation, a plot of concentration versus % Act was generated for each compound tested and from the plot, EC_{50} and the highest percent activation observed for a particular compound (E_{max}). For PXR inhibition, where the cells were incubated with a mixture of 10 M rifampicin and the test compound, a percent inhibition (%Inh) was calculated. From the concentration–%Inh plot, IC_{50} and the highest percent inhibition observed for that compound (I_{max}) were reported.

Reversible and time-dependent CYP3A4 inhibition assay in human liver microsomes: The CYP3A4 inhibition potential of ketoconazole and its two analogs, **3** and UCL3158H, were tested in human liver microsomes. Briefly, test articles were incubated in a reaction mixture containing 5 M midazolam, 0.1 mg/mL pooled human liver microsomes, and 1 mM NADPH for 5 min. The enzyme reaction was stopped by adding an equal volume of quench solution (H_2O/acetonitrile/formic-acid = 94:5:1) containing ^{13}C-1-hydroxymidazolam (0.1 M final) as the internal standard. In order to test time-dependency of CYP3A4 inhibition, an identical reaction was started without midazolam and the reaction mixture was incubated for 30 min. After the pre-incubation, 5 M midazolam was added and incubated for 5 min before the quench solution was added. The samples were centrifuged at 1500 RPM for 15 min to precipitate denatured microsomal proteins. An ultra high-throughput RapdiFireTM mass-spectrometer analysis (Biotrove, Inc., Woburn, MA) was performed to measure the quantity of 1-hydroxymidazolam present in the reaction by monitoring mass transition of 342.0 → 203.1 and 345.0 → 206.1 for 1-hydroxymidazolam and ^{13}C-1-hydroxymidazolam, respectively. A percent CYP3A4 inhibition (%Inh) was calculated by the following equation:

$$\%\mathrm{Inh} = \left(\frac{C_{\mathrm{DMSO}} - C_{\mathrm{compound}}}{C_{\mathrm{DMSO}}}\right) \times 100$$

DMSO and compounds are concentrations of 1-hydroxymidazolam in the vehicle control and compound tested, respectively. A plot of concentration–%Inh was then created and from the plot, IC_{50} and maximum % inhibition were reported.

Results: The basis for structure–function analysis of inhibitors of activated PXR comes from the initial observations that ketoconazole is a weak activator of PXR but an inhibitor of activated PXR (IC_{50} = 18.73 μM) (Table 1). Based on previous modeling studies from our laboratory, we believe that the site of inhibition may include the AF-2 surface (estimated area and volume by castP using atoms detected by GOLD ~ 88 Å^2 and 117.6 Å^3).[13,16,17,19,23,24] Ketoconazole induces toxicity at high doses in humans. This may be partly due to its inhibitory activity against heme-iron containing enzymes, which is attributed to the imidazole group.[25–27] Thus, we first designed tested analogs of ketoconazole that lacked the imidazole group to see if this modification improved on CYP inhibitory activity but still retained PXR activity. These compounds, developed at University College London, were tested in the PXR transactivation assay. Among 7 compounds tested, UCL2112H {cpd 12}[28] did not transactivate PXR or inhibit the rifampicin-mediated activation of the receptor whereas UCL2138H {cpd 17}[28] was a potent activator of PXR but was not an inhibitor. However, other five compounds behaved like ketoconazole, in that it is a weak activator of PXR and a moderate antagonist of activated PXR and UCL2158H and UCL2202D {cpd 2}[28] appeared to be the most significant inhibitors of activated PXR. UCL2158H appeared to be a better PXR antagonist than UCL2202D as UCL2203 moderately activated PXR (43%) while UCL2158H did not induce significant PXR activation (10%) (Supplementary Table 2 and 3). The new compounds are fluorinated derivatives of ketoconazole lacking the imidazole group, synthesized by our laboratory. Five new compounds were tested and all showed a varying degree of PXR antagonism. However, except for FL-B-12 (our lead compound **3** in Scheme 2), all showed moderate potent activation of PXR in addition to their activities against rifampicin-mediated PXR activation (Table 1). FL-B-12 (should read as 3) did not significantly activate PXR (10%) but inhibit PXR activation by rifampicin to a full extent (103%) (Supplementary Fig. 1 and Table 2). These results suggested that the removal of the imidazole group from ketoconazole or its analogs did not significantly alter their ability to inhibit PXR.

Table 1
Maximum activation and inhibition of PXR transactivation by ketoconazole and its derivatives

Substance (Ref.)	Maximum % activation observed	Maximum % inhibition observed
Ketoconazole	20	93
UCL2112H (28)	0	0
UCL2134D (27)	2	82
UCL2135 (23)	10	32
UCL2158H (28)	10	109
UCL2202D (28)	43	108
UCL2245 (28)	2	58
UCL2238 (27)	91	1
FL-B-12	10	103
FL-B-15	36	90
FL-B-24	50	75
FL-B-31	33	59
FL-B-45	72	48
Rifampicin	107	18

Subsequently, we examined whether the loss of imidazole group improved or eliminated CYP inhibition associated with the parent compound, ketoconazole in human liver microsome using midazolam as the CYP3A4-specific substrate. In addition, a time-dependency of a potential CYP inhibition was tested to see if the loss of the imidazole group brings about new liabilities previously not associated with ketoconazole by preincubating the compounds with microsome. Two compounds, UCL2158H and **3**, were chosen for this study along with ketoconazole as the positive control. Table 3 illustrates the IC_{50} values with and without pre-incubation with the test compounds. Ketoconazole, a prototypical reversible inhibitor of CYP3A4 (and thus midazolam 1-hydroxylase activity), completely inhibits conversion of midazolam to its metabolite (103% inhibition with IC_{50} of 0.020 μM) with no indication of time-dependency (no IC_{50} change). In the same assay performed simultaneously using the same pool of microsomes, UCL2158H was shown to be a weak inhibitor (IC_{50} of 10.8 or 19.6 μM) of CYP3A4, which demonstrated no time-dependency. FL-B-12 (compound **3**) appears to be a very weak activator of CYP3A4 (IC_{50} > 40 μM), again without a time-dependency of its CYP3A4 inhibition. Hence, elimination of the imidazole group in these analogs markedly improved CYP3A4 inhibition compared to ketoconazole without introducing new liability (time-dependent inhibition).

In a cytotoxicity screen that was performed simultaneously with PXR transactivation assay, some of the tested compounds including ketoconazole were toxic in HepG2 cells with 24 h incu-

bation especially at the highest concentration tested (50 μM) (data not shown). Thus the cytotoxic potential for the most potent PXR inhibitors (FL-B-12 and UCL-2158H) was tested in three cancer cell lines (Caco-2, LS174T and SKOV3) as well as in a transformed fibroblast cell line (CRL).[17,29] We show that these compounds (UCL2158H and FL-B-12) are less cytotoxic than ketoconazole in these epithelial cells across a concentration range up to 100 μM (Fig. 3). FL-B-12 was the least cytotoxic (~98% viability across all cell lines tested) in the effective concentration range for PXR inhibition (e.g., two times the $IC_{50} \sim 30$ μM). However, in the same concentration range, ketoconazole is cytotoxic (viability is ~65–75% across all cell lines tested). These results together showed that FL-B-12 (lead compound 3) and UCL2158H have better cytotoxic profiles than ketoconazole and also demonstrated that the PXR inhibition exhibited by these compounds was not a result of cell death induction.

We have shown that it is possible to modify the structure of ketoconazole to compounds while preserving PXR antagonist activity. Furthermore, we have demonstrated that these modifications (loss of the imidazole group) can markedly reduce CYP3A4 inhibition activity associated with ketoconazole. We conclude that it is feasible to identify PXR specific antagonists with reduced liabilities using a rationally designed structure–activity relationship.

Finally, we speculate that the mechanism of action of FL-B-12 is similar to that described for ketoconazole,[19] in that FL-B-12 may partially or wholly fit into the AF-2 site in a docking mode that may be similar or dissimilar to ketoconazole. This allosteric interaction on PXR may inhibit SRC-1 co-activator binding and receptor activation when engaged by an agonist ligand.

In summary, we have shown that it is possible to modify the structure of ketoconazole to compounds while preserving PXR antagonist activity. Furthermore, we have demonstrated that these modifications (loss of the imidazole group) can markedly reduce CYP3A4 inhibition activity associated with ketoconazole without reduction of PXR activity. The IC_{50s} obtained are identical which again suggests that the imidazole group does not contribute to binding. Also the similarities in structure between UCL 2158 and FL-B-12 (Scheme 2, compound **3**) are reflected in there IC_{50}s. Given the relatively small number of analogues we conclude that it may be feasible to identify more potent PXR specific antagonists with the design and synthesis of analogs. After obtaining this preliminary hit, we are developing a diversified library of compounds using SAR analysis on PXR.

Acknowledgment

The authors thank Dr. Matthew Redinbo, University of North Carolina, Chapel Hill, NC for insightful discussions regarding receptor structure.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.018.

References and notes

1. Chawla, A.; Repa, J. J.; Evans, R. M.; Mangelsdorf, D. J. *Science* **2001**, *294*, 1866.
2. Gronemeyer, H.; Gustafsson, J. A.; Laudet, V. *Nat. Rev. Drug Disc.* **2004**, *3*, 950.
3. Mangelsdorf, D. J.; Evans, R. M. *Cell* **1995**, *83*, 841.
4. Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schutz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P. *Cell* **1995**, *83*, 835.
5. Ingraham, H. A.; Redinbo, M. R. *Curr. Opin. Struct. Biol.* **2000**, *15*, 708.
6. McDonnell, D. P.; Connor, C. E.; Wijayaratne, A.; Chang, C. Y.; Norris, J. D. *Recent Prog. Horm. Res.* **2002**, *57*, 295 .
7. Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927.
8. Kliewer, S. A.; Moore, J. T.; Wade, L.; Staudinger, J. L.; Watson, M. A.; Jones, S. A.; McKee, D. D.; Oliver, B. B.; Willson, T. M.; Zetterstrom, R. H.; Perlmann, T.; Lehmann, J. *Mol. Cell.* **1998**, *92*, 73.
9. Fayard, E.; Auwerx, J.; Schoonjans, K. *LRH-1: Trends Cell Biol.* **2004**, *14*, 250.
10. Bertilsson, G.; Heidrich, J.; Svensson, K.; Asman, M.; Jendeberg, L.; Sydow-Backman, M.; Ohlsson, R.; Postlind, H.; Blomquist, P.; Berkenstam, A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12208.
11. Bauer, B.; Yang, X.; Hartz, A. M.; Olson, E. R.; Zhao, R.; Kalvass, J. C.; Pollack, G. M.; Miller, D. S. *Mol. Pharmacol.* **2006**, *71*, 1212.
12. Gupta, D.; Venkatesh, M. K.; Wang, H.; Kim, S.; Sinz, M.; Goldberg, G. L.; Whitney, K.; Mani, S. *Clin. Cancer Res.* **2008**, in press.
13. Wang, H.; Huang, H.; Li, H.; Teotico, D. G.; Sinz, M.; Baker, S. D.; Staudinger, J.; Kalpana, G.; Redinbo, M. R.; Mani, S. *Clin. Cancer Res.* **2007**, *13*, 2488.
14. Zhou, C.; Poulton, E. J.; Grun, F.; Bammler, T. K.; Blumberg, B.; Thummel, K. E.; Eaton, D. L. *Mol. Pharmacol.* **2007**, *71*, 220.
15. Synold, T. W.; Dussault, I.; Forman, B. M. *Nat. Med.* **2001**, *7*, 584.
16. Takeshita, A.; Taguchi, M.; Koibuchi, N.; Ozawa, Y. *J. Biol. Chem.* **2002**, *277*, 32453.
17. Huang, H.; Wang, H.; Sinz, M.; Zoeckler, M.; Staudinger, J.; Redinbo, M. R.; Teotico, D. G.; Locker, J.; Kalpana, G. V.; Mani, S. *Oncogene* **2006**, *26*, 258.
18. Xue, Y. C. E.; Zuercher, W. J.; Willson, T. M.; Collins, J. L.; Redinbo, M. R. *Bioorg. Med. Chem.* **2007**, *15*, 2156.
19. Ekins, S.; Chang, C.; Mani, S.; Krasowski, M. D.; Reschly, E. J.; Iyer, M.; Kholodovch, V.; Ni, A. ; William, J. W.; Sinz, M.; Swaan, P. W.; Patel, R.; Bachman, K. *Mol. Pharm.* **2007**, *72*, 592.
20. Power, E. C.; Ganellin, C. R.; Benton, D. C. *Biorg. Med. Chem. Lett.* **2006**, *16*, 887.
21. Camps, P.; Farres, X. *Tetrahedron: Asymmetry* **1995**, *6*, 1283.
22. Synthesis of compound **3**: A mixture of compound 8, 1-acetylpiperazine, *tert*-BuONa, P(tBu)3, and Pd(dba)2 in dry toluent was gently refluxed and stirred under a nitrogen atmosphere for 18 h. To the mixture was added 10 mL water, extracted with ethyl acetate (20 mL 3×), washed with water (10 mL), brine (15 mL). The extracts were dried over Na_2SO_4 and evaporated off. The residue was purified by column chromatography on silica gel to give 3 as thick liquid 3. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52 (m, 1H), 6.87–6.71 (m, 6H), 4.58 (m, 1H), 4.28 (m, 1H), 3.95 (m,1H), 3.76 (m,4H), 3.58 (m,2H), 3.00 (m, 4H), 2.11 (s, 3H), and 1.72 (s,3H). ^{13}C NMR $CDCl_3$: δ 169.8, 162.7,160.7, 153.7, 144.5, 127.9, 126.8, 115.3, 115.2, 110.5, 108.01, 74.7, 68.2, 67.3, 51.4, 45.8, 41.2, 26.5 and 21.2. ESI-MS: calculated for $C_{23}H_{26}F_2N_2O_4$ ($[M+H]^+$) 433:0046; found: 433.0067 Rostein, D. M.; Kertesz, D. J.; Walker, Keith A. M.; Swinney, D. C. *J. Med. Chem.* **1992**, *35*, 2818.
23. Estébanez-Perpiñá, E.; Arnold, L. A.; Nguyen, P.; Rodrigues, E. D.; Mar, E.; Bateman, R.; Pallai, P.; Shokat, K. M.; Baxter, J. D.; Guy, R. K.; Webb, P.; Fletterick, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16074.
24. Ekins, S.; Chang, C.; Mani, S.; Krasowski, M. D.; Reschly, E. J.; Iyer, M.; Kholodovych, V.; Ai, N.; Welsh, W. J.; Sinz, M.; Swaan, P. W.; Patel, R.; Bachmann, K. *Mol. Pharmacol.* **2007**, *72*, 592.
25. Eoin, C.; Power, C.; Robin, G.; David, C. H.; Benton, D. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 887.
26. Verras, A.; Alian, A.; de Montellano, P. R. *Protein Eng. Des. Sel.* **2006**, *19*, 491.
27. Heeres, J.; Backx, L. J.; Mostmans, J. H.; Van Cutsem, J. *J. Med. Chem.* **1979**, 221003.
28. Camps, P.; Farrés, X.; García, M. L.; Ginesta, J.; Pascual, J.; Mauleón, D.; Carganico, G. *Tetrahedron: Asymmetry* **1995**, *6*, 283.
29. Drug induced cytotoxicity was assessed by the MTT assay in cancer cell lines (Caco-2, LS174T and SKOV3) as well as a fibroblast cell line (CRL). Cells were exposed to a concentration range of the drug(s) for 48 h. These assays were repeated three separate times each in triplicate. For methods, see: (a) Wu, K. M.; Wang, C. G.; D'Amico, M.; Lee, R. J.; Albanese, C.; Pestell, R.; Mani, S. *Mol. Cancer Ther.* **2002**, 695; (b)Wu; Wang; D'Amico; Albanese; Pestell, R.; Mani Invest. New Drugs **2005, 23, 299,.**, *23*2005, 299.