Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2153–2157

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Rational Design of 4,5-Disubstituted-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-ones as a Novel Class of Inhibitors of Epidermal Growth Factor Receptor (EGF-R) and Her2(p185^{erbB}) Tyrosine Kinases

Li Sun,* Jean Cui, Congxin Liang, Yong Zhou, Asaad Nematalla, Xueyan Wang, Hui Chen, Cho Tang and James Wei*

SUGEN, Inc., 230 East Grand Avenue, South San Francisco, CA 94080, USA

Received 12 March 2002; accepted 30 April 2002

Abstract—A novel class of 4,5-disubstituted-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-ones has been discovered as potent and selective inhibitors of the EGF-R tyrosine kinase family. These compounds selectively inhibit EGF-R kinase activity at low nanomolar concentration and tyrosine autophosphorylation in cells expressing EGF-R or Her2 (p185^{erbB}). Structure–activity relationships (SARs) for this class of compounds are presented.

In the past decade, a numerous diverse small molecule scaffolds have been discovered as selective ATP competitive inhibitors against the tyrosine kinases.[1–6] The extensive SAR studies and co-crystallization of various inhibitors within the catalytic domain of the kinases (i.e., FGF-R1, Bcr-Abl, Src) provided rich information on inhibitor–kinase interaction at the molecular level which allowed further rational design of highly selective tyrosine kinase inhibitors.[1,2] Currently, at least 10 ATP competitive tyrosine kinase inhibitors are on the market or under clinical evaluations at different stages.[7]

Since overexpression of the EGF-R tyrosine kinase family and their ligands have been implicated in many disease indications such as tumors and psoriasis, specific inhibitors of this tyrosine kinase family may demonstrate therapeutic utility in the treatment of these diseases. In our search for novel inhibitors of EGF-R/Her2 (p185^{erbB}), we have designed 4,5-disubstituted-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-ones (5,7-diazaindolinones) based on the SAR information from both indolinone and quinazoline scaffolds. The 4-substituted pyrimidine ring of this hybrid compound originated from quinazoline while the five-membered lactam ring from indolin-2-one core. The binding mode for these hybrid compounds is proposed in this report. The synthesis of the target molecules in Table 1 consists of three steps: (1) preparation of the substituted 5,7-diazaindolinone core (**4a**, **4b**, and **5** in Scheme 1) (2) preparation of substituted pyrrol-2-carboxaldehyde (**8**, **9**, **13**, **19**, **21**, and **25** in Scheme 2) and (3) condensation of the substituted 5,7-diazaindolinone cores with the substituted pyrrol-2-carboxaldehydes to afford the target molecules (Tables 1 and 2).

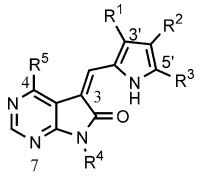
The 5,7-diazaindolinone core **4a** or **4b** was prepared via an intermediate, **1** (Scheme 1), which was synthesized according to a literature report.[8] Chlorination of **1** with phosphorus oxychloride yielded **2a** which was methylated to afford **2b**. Amination of **2a** or **2b** with substituted aniline catalyzed by silver triflate afforded **3a** and **3b**, which were then oxidized with pyridinium bromide perbromide (PBPB) followed by reduction with zinc dust to give, **4a** or **4b**. Direct oxidation of **2a** with PBPB followed by reduction with zinc dust afforded another core **5**.

3,5-Dimethyl-1*H*-pyrrole-2-carbaldehyde, for preparation of **26d** (Table 1), is commercially available and (5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)-propionic acid, for synthesizing **26b** (Table 1), was synthesized according our previous report.[4] Other substituted pyrrol-2-carboxaldehydes were prepared using various methods depicted

*Corresponding authors. Fax: +1-650-837-3348; e-mail: connie-sun@sugen.com

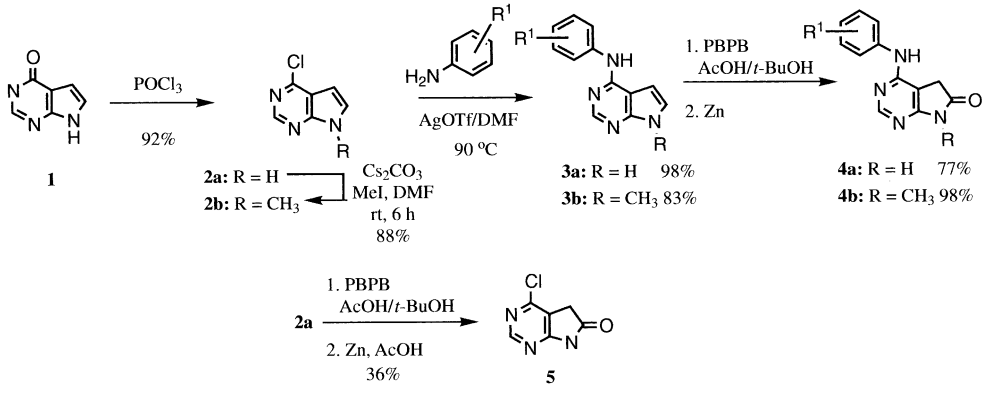
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00364-5

Table 1. Inhibition of biochemical kinases



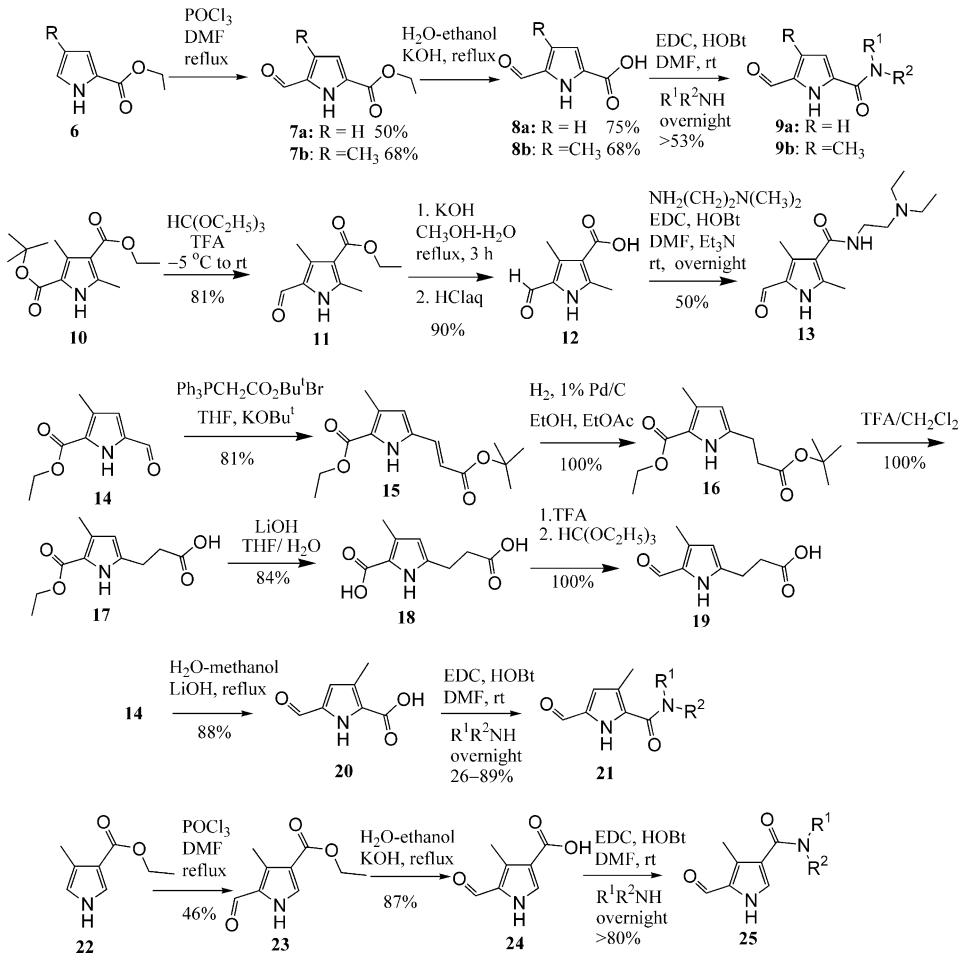
ID	R^1	R^2	R^3	R^4	R^5	Biochemical kinase inhibition (tyrosine phosphorylation) IC_{50}, μM[a]		
						EGF-R	PDGF-R	VEGF-R2 (Flk-1/KDR)
26a	No substitution at the C-3 position of the core				3-Cl-4-F-phenylamino	>20	>20	>20
26b	CH_3	CH_2CH_2COOH	CH_3	H	3-Cl-4-F-phenylamino	0.18	>20	>20
26c	CH_3	$CONH(CH_2)_2N(C_2H_5)_2$	CH_3	H	3-Cl-4-F-phenylamino	0.20	>20	3.47
26d	CH_3	H	CH_3	H	3-Cl-4-F-phenylamino	0.041	>100	16.5
26e	CH_3	H	CH_2CH_2COOH	H	3-Cl-4-F-phenylamino	0.0047	>20	>20
26f	CH_3	H	COOH	H	3-Cl-4-F-phenylamino	0.029	>20	8.92
26g	H	H	$CONHCH_2CH_2N(CH_2CH_2)_2O$	H	3-Cl-4-F-phenylamino	0.022	>100	>20
26h	CH_3	H	$CONHCH_2CH_2N(CH_2CH_2)_2O$	H	3-Cl-4-F-phenylamino	0.0065	>100	>20
26i	CH_3	H	CH_3	CH_3	3-Cl-4-F-phenylamino	>20	>20	5.82
26j	CH_3	H	$CONHCH_2CH_2N(CH_2CH_2)_2O$	CH_3	3-Cl-4-F-phenylamino	5.31	>20	10.1
26k	CH_3	H	$CON(CH_2CH_2)_2O$	H	3-Cl-4-F-phenylamino	0.17	>20	>20
26l	CH_3	H	$CON(CH_2CH_2)_2NCH_3$	H	3-Cl-4-F-phenylamino	0.0028	>20	>20
26m	H	CH_3	$CON(CH_2CH_2)_2NCH_3$	H	3-Cl-4-F-phenylamino	0.21	>20	>20
26n	CH_3	$CON(CH_2CH_2)_2NCH_3$	H	H	3-Cl-4-F-phenylamino	0.41	>20	>20
26o	CH_3	H	$CON(CH_2CH_2)_2NC_2H_5$	H	3-Cl-4-F-phenylamino	0.013	>20	>20
26p	CH_3	H	$CONH(CH_2)_2N(C_2H_5)_2$	H	3-Cl-4-F-phenylamino	0.0012	>20	0.3
26q	CH_3	H	$CON[CH_2CH(CH_3)]_2N$	H	3-Cl-4-F-phenylamino	0.0059	>20	1.63
26r	CH_3	H	$CONHCH_2CH(OH)CH_2N(C_2H_5)_2$	H	3-Cl-4-F-phenylamino	0.00045	>20	0.36
26s	CH_3	H	$CONHCH_2CH_2N(CH_2CH_2)_2O$	H	3-Ethynyl-phenylamino	0.018	>20	>20
26t	CH_3	H	$CONH(CH_2)_2N(C_2H_5)_2$	H	4-Cl-2-F-phenylamino	0.45	5.62	0.04
28a	CH_3	H	$CON(CH_2CH_2)_2O$	H	Indan-5-amino	1.41	>20	3.46
28b	CH_3	H	$CON(CH_2CH_2)_2O$	H	1-Benzyl-1*H*-indol-5-amino	0.0045	>20	2.05
28c	CH_3	H	$CON(CH_2CH_2)_2O$	H	1-Benzyl-1*H*-indazol-5-amino	0.0018	>10	2.1
28d	CH_3	H	$CONHCH_2CH_2N(CH_2CH_2)_2O$	H	1-Benzyl-1*H*-indol-5-amino	0.0011	>100	3.47
29	CH_3	H	$CON(CH_2CH_2)_2O$	H	Piperidine-1-yl	>20	>20	>20

[a]IC_{50} values were determined by at least two separate tests and are reported as mean values.



Scheme 1. Synthesis of substituted 5,7-diazaindolinone cores.

in Scheme 2. Aldehyde **9** was prepared starting from commercially available **6** followed by Vilsmeier, hydrolysis, and amidation with selected amines (Scheme 2). Aldehyde **13** was synthesized from commercially available **10** by hydrolytic formylation with triethyl orthoformate in the presence of trifluoroacetic acid at −5 °C to room temperature followed by base hydrolysis of the ethyl ester and an amidation reaction (Scheme 2). Aldehyde **19** was synthesized from commercially available **14** via Wittig condensation followed by hydrogenation, deprotection of the Boc group, hydrolysis of the ethyl ester, decarboxylation, and formylation. Furthermore, hydrolysis of **14** followed by amidation, as described for the preparation of **9**, afforded aldehyde **21**. Starting



Scheme 2. Synthesis of functionalized pyrrole aldehydes.

from commercially available **22**, aldehyde **25** was synthesized following the same procedure for the preparation of **9**.

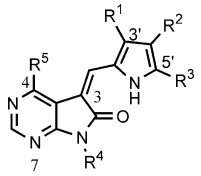
The final compounds (Table 1) were synthesized by condensation of substituted pyrrol-2-carboxaldehyde (**8**, **9**, **13**, **19**, **21**, and **25**) and the substituted 5,7-diazaindolinone (**4a**, **4b**, and **5**) using either one of the methods depicted in Scheme 3.

Compounds were evaluated for their inhibitory activity towards tyrosine phosphorylation for the EGF-R, VEGF-R2 (Flk-1/KDR), and PDGF-Rβ kinases according to previously reported methods.[6] The results for these compounds are summarized in Tables 1 and 2. Initial SAR studies indicated that substitution at the C-3 position of the core is essential for inhibitory activity against the EGF-R kinase. The core molecule without substitution at the C-3 position (**26a** in Table 1) is inactive against any kinases in the study ($IC_{50} > 20$ μM) whereas condensation of the core with substituted pyrrole-2-carboxaldehyde at the C-3 position afforded compound **26d** (Table 1), which was observed to be active against the EGF-R kinase ($IC_{50} = 0.041$ μM). In addition, the proton at the N-1 position was found to be essential for the EGF-R kinase inhibitory activity, as demonstrated by comparison of **26d** to **26i** or **26h** to **26j**.

The substitution pattern on the pyrrole ring (at the C-3 position of the core) has a dramatic impact on the inhibitory potency against the EGF-R kinase. Compounds with amide or acid functionality at the C-5′ position of the pyrrole ring have been found to be more potent than the compounds with the same substituent at the C-4′ position of the pyrrole ring (**26b** vs **26e**, **26c** vs **26p**, **26n** vs **26l**). This observation is opposite to the SAR results on indolin-2-ones as VEGF-R inhibitors (data not shown), which implied 5,7-diazaindolinones might have different binding mode from indolin-2-ones. Further modification of the side chain at the C-5′ position with different alkylaminoalkylamides, in general, retained the potency against the EGF-R kinase (e.g., **26h**, **26l**, **26p**, **26r**, and **26q**) but could change the kinase selectivity profile. Compounds **26p** and **26r** have also displayed inhibitory activity against the Flk-1 kinase in the submicromolar concentration range. Compound **26k** (with morpholine amide), which does not have a terminal basic amine moiety at the C-5′ position, has shown decreased potency against the EGF-R kinase when compared to **26l** (with a basic *N*-methyl piperazine amide). All of these results indicated that a terminal basic amino functionality of the 5,7-diazaindoliones might be important for inhibitory potency against the EGF-R kinase.

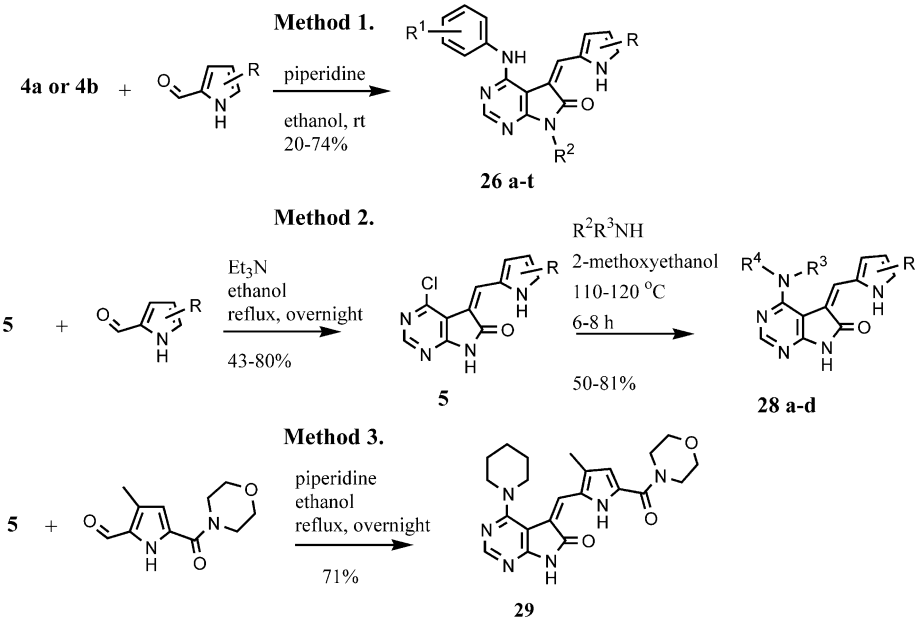
Modification at the C-4 position of the core by replacing 3-chloro-4-fluoroaniline with other aryl or aliphatic

Table 2. Inhibition of cellular kinases



ID	R^1	R^2	R^3	R^4	R^5	Cellular assays IC_{50}, μM[a]			
						Tyrosine phosphorylation		3T3 Proliferation	
						EGF-R	Her-2	EGF	Her2
26d	CH_3	H	CH_3	H	3-Cl-4-F-phenylamino	0.3	0.3	7.82	2.50
26f	CH_3	H	COOH	H	3-Cl-4-F-phenylamino	>5	>5	>50	49.3
26h	CH_3	H	$CONHCH_2CH_2N(CH_2CH_2)_2O$	H	3-Cl-4-F-phenylamino	0.04	0.1	2.05	1.24
26k	CH_3	H	$CON(CH_2CH_2)_2O$	H	3-Cl-4-F-phenylamino	0.04	0.4	0.44	1.21
26l	CH_3	H	$CON(CH_2CH_2)_2NCH_3$	H	3-Cl-4-F-phenylamino	0.1	0.2	0.75	0.37
26o	CH_3	H	$CON(CH_2CH_2)_2NC_2H_5$	H	3-Cl-4-F-phenylamino	0.2	0.1	7.50	0.35
26p	CH_3	H	$CONHCH_2CH_2N(C_2H_5)_2$	H	3-Cl-4-F-phenylamino	0.2	0.2	1.29	0.37
26q	CH_3	H	$CON[CH_2CH(CH_3)]_2N$	H	3-Cl-4-F-phenylamino	0.2	0.5	2.58	0.51
26s	CH_3	H	$CONHCH_2CH_2N(CH_2CH_2)_2O$	H	3-Ethynyl-phenylamino	0.04	0.04	0.62	0.50
26t	CH_3	H	$CONHCH_2CH_2N(C_2H_5)_2$	H	4-Cl-2-F-phenylamino	0.5	NT[b]	NT	NT
28a	CH_3	H	$CON(CH_2CH_2)_2O$	H	Indan-5-amino	5	>5	4.32	2.78
28b	CH_3	H	$CON(CH_2CH_2)_2O$	H	1-Benzyl-1*H*-indol-5-amino	0.1	0.1	NT	NT
28c	CH_3	H	$CON(CH_2CH_2)_2O$	H	1-Benzyl-1*H*-indazol-5-amino	0.1	0.1	NT	NT
28d	CH_3	H	$CONHCH_2CH_2N(CH_2CH_2)_2O$	H	1-Benzyl-1*H*-indol-5-amino	0.2	0.04	1.95	1.56

[a]IC_{50} values were determined by at least two separate tests and are reported as mean values.
[b]NT = not tested.



Scheme 3. Synthesis of the target molecules: 5,7-diazaindolinones.

amines revealed that 1-benzyl-1*H*-indol-5-ylamino or 1-benzyl-1*H*-indazol-5-ylamine substitution dramatically enhanced the inhibitory activity against the EGF-R kinase (**28b** or **28c** vs **26k** and **28d** vs **26h**). Saturated piperidine substitution at the C-4 position of the core abolished the kinase inhibitory activity (**29**). These SAR results indicated that arylamino substitution at the C-4 position is essential for kinase inhibitory activity. Interestingly, kinase potency and selectivity could be dramatically altered by a subtle change in the substitution pattern on the aniline side chain at the C-4 position of the core. In this respect, **26p** is a very potent and selective inhibitor of EGF-R whereas **26t** is a selective inhibitor of the Flk-1 kinase. This SAR trend is similar to the one observed for the 4-phenylaminoquinazoline scaffold,[9] which implies the similar binding mode to quinazoline scaffold.

A few compounds have also been assessed for their ability to inhibit EGF-R or Her2 ($p185^{erbB}$) autophosphorylation in A431 or SK-OV-3 cells, respectively, as well as ligand induced BrdU incorporation in 3T3 cells (Table 2). In general, most of the compounds showed submicromolar inhibitory activity against autophosphorylation of both

EGF-R and Her2 ($p185^{erbB}$) in cells. Compound **28d** showed some selectivity against autophosphorylation of Her2 ($p185^{erbB}$) over the EGF-R kinase. It should be noted, however, there is no direct correlation between biochemical and cellular results (Table 2). For example, **26k** was 60-fold less potent than **26l** in the biochemical assay, but has been found to be equally potent, if not more potent, against EGF-R stimulated BrdU incorporation in 3T3 cells and 25-fold more potent against EGF-R autophosphorylation in cells. This might be the result of factors such as chemical stability, solubility, cell membrane permeability, and in vitro assay conditions. Of particular interest, most of the compounds have been found to show submicromolar inhibitory activity against Her-2 ($p185^{erbB}$) autophosphorylation in cells.

In conclusion, we have discovered 5,7-diazaindolinones as a novel class of potent and selective inhibitors of EGF-R and Her2 ($p185^{erbB}$) tyrosine kinases. These compounds exhibited low nanomolar IC_{50} values against the EGF-R kinase without inhibiting PDGF-R and other related kinases. Of particular interest, these compounds have been found to show inhibitory activity toward not only the EGF-R but also Her2 ($p185^{erbB}$) kinases in cells. Moreover, **26q** has been found to exhibit efficacy in tumor-bearing mice.[10] This later finding suggests that these substituted 5,7-diazaindoliones may be developed for the treatment of human cancers that require both EGF-R and Her2 ($p185^{erbB}$) kinase for growth and survival.

Acknowledgements

The authors would like to thank Dr. Gerald McMahon and Dr. Ken Lipson for their valuable input and discussion.

References and Notes

1. Sun, L.; McMahon, G. *Drug Discov. Today* **2000**, *5*, 344.
2. Traxler, P.; Furet, P. *Pharmacol. Ther.* **1999**, *82*, 195.
3. Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. *Science* **1994**, *265*, 1093.
4. Sun, L.; Tran, N.; Tang, T.; App, H.; Hirth, P.; McMahon, G.; Tang, C. *J. Med. Chem.* **1998**, *41*, 2588.
5. Sun, L.; Tran, N.; Liang, C.; Tang, F.; Rice, A.; Schreck, R.; Waltz, K.; Shawver, L. K.; McMahon, G.; Tang, C. *J. Med. Chem.* **1999**, *42*, 5120.
6. Sun, L.; Tran, N.; Liang, C.; Hubbard, S.; Tang, F.; Lipson, K.; Schreck, R.; Zhou, Y.; McMahon, G.; Tang, C. *J. Med. Chem.* **2000**, *43*, 2655.
7. Morin, M. J. *Oncogene* **2000**, *19*, 6574.
8. Davoll, J. *J. Chem. Soc.* **1960**, 131.
9. Hennequin, L. F.; Thomas, A. P.; Johnstone, C.; Stokes, E. S. E.; Ple, P. A.; Lohmann, J.-J. M.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Curwen, J. O.; Kendrew, J.; Brempt, C. L. D. *J. Med. Chem.* **1999**, *42*, 5369.
10. Christensen, J. G.; Schreck, R. E.; Chan, E.; Wang, X.; Yang, C.; Liu, L.; Cui, J.; Sun, L.; Wei, J.; Cherrington, J. M.; Mendel, D. B. *Clin. Cancer Res.* **2001**, *7*, 4230.