Synthesis of Novel Quinolinecarboxamide Derivatives with Estrogenic Activity

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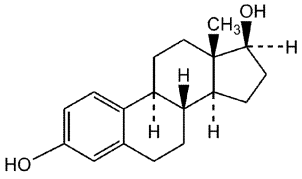
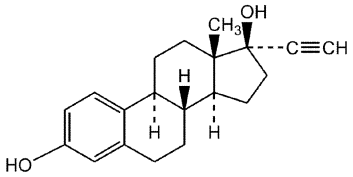
Even though estrogens have a well-established role in the growth of hormone-dependant tumors by the estrogen receptor (ER)-dependant mitogenic effect in cells containing ER,[1] a member of the nuclear receptor superfamily,[2] they exert numbers of favorable activities in women. From a therapeutical point of view, estrogen and its derivatives are well known not only as oral contraceptives, but also as a major component in hormone replacement therapy (HRT) required for bone loss prevention and for the control of cardiovascular diseases in postmenopausal women.[3]



17 -Estradiol (E2)



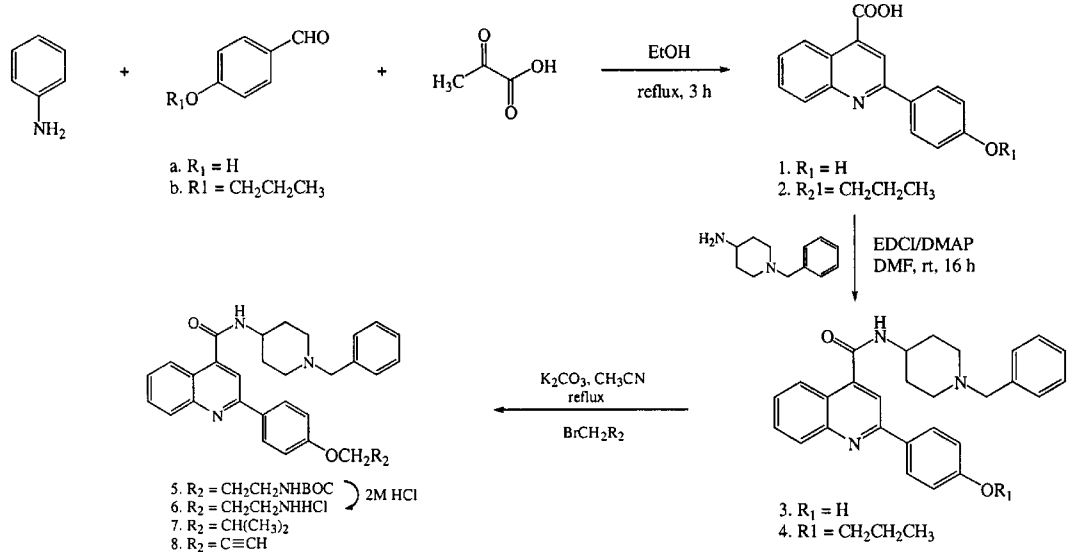
Ethynylestradiol

The most widely used estrogens are 17β-estradiol[4] and ethynylestradiol,[5] a synthetic steroidal compound. These estrogen agonists have been used for postmenopausal women required for HRT. However, it has been known that long-term usage of estrogen causes high incidence of breast cancer,[6] which leads to development of antiestrogen such as tamoxifen, the first selective ER modulator (SERM).[7] Recently, raloxifene as a second SERM was developed to alleviate menopausal symptoms without risk of breast cancer.[8] Afterwards, the more effective SERMs are being developed and tested that act as antiestrogens on breast and endometrium while having estrogenic effects on bones, the lipid profile and the central nervous system.[9-11]

In this point of view to develop antiestrogens, we used virtual screening system based on the structure of ER. From the initial screening, we identified compound **4** that has non-steroidal quinoline structure. More compounds were synthesized from the leader compound **4** and tested for estrogenic activity using ERα and ERβ.

Results and Discussion

Synthesis of quinolinecarboxamide derivatives. Application of such multi-component condensation approach based Doebner reation[12] for the synthesis of a clinically useful



Scheme 1

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pharmacophore, 2-(4-hydroxyphenyl)quinoline-4-carboxylic acid **1** is shown in scheme 1.

The quinolinecarboxylic acid **1** was obtained using the condensation of aniline and pyruvic acid with 4-hydroxy benzaldehyde in EtOH under reflux,[13] and coupled with 4-amino-1-benzylpiperidine using EDCI/DMAP as reagent,[14] to give quinolinecarboxamide **3** in 76% yield. Following to the same method, propoxy quinolinecarboxamide **4** was obtained from propoxy quinolinecarboxylic acid **2** in 70% yield.

The synthesis of target compound **6-8** was prepared by *O*-alkylation of the bromo-substitued function groups with quinolinecarboxamide **3** in the presence of anhydrous potassium carbonate.

N-Boc-aminopropoxy quinolinecarboxamide **5** was carried out the *O*-alkylation of quinolinecarboxamide **3** with *N*-Boc-3-bromopropoxyamine using K_2CO_3 in refluxing acetonitrile,[15] followed by the treatment with 2 M HCl etherate[16] generating aminopropoxy quinolinecarboxamide salt **6** in 93 % overall yield. It should be noted that the solubility of quinolinecarboxamide **3** was transformated from organic solubility to the enhanced aqueous solubility for the pharmacological assay.

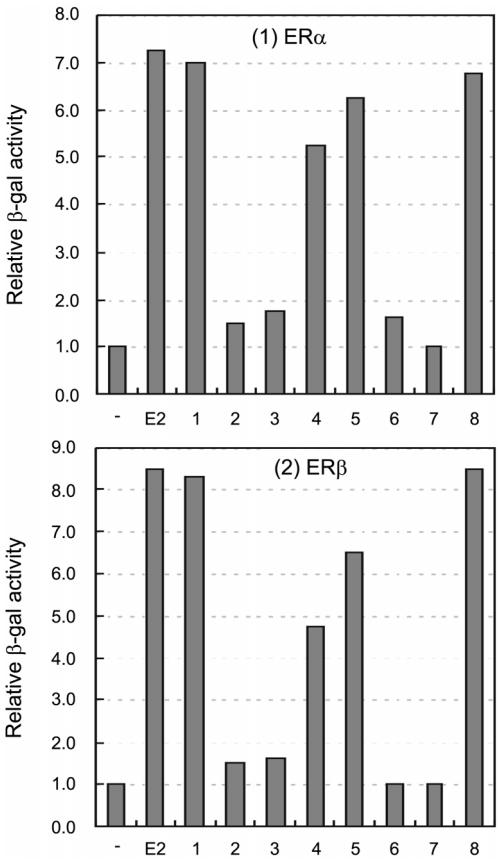

Figure 1. Estrogenic effect of synthetic compounds. NIH3T3 cells were cotransfected with 3XERE-tk-β-gal reporter gene and ERα (A) or ERβ (B) expression vector in pSG5, and treated with 1 μM E2 or synthetic compounds using Lipofectamine PLUS reagent. Estrogenic effect was determined by transcriptional activities coupled with ER, shown by β-gal activity, and expressed as a relative activity compared with that of the DMSO control (average of 3 independent assays ± SD).

Aiming to improve the binding activity of **4**, further work was in progress involving structural modifications of quinolinecarboxamide. We expected that the activity of compound **7**, **8** would be also affected by the size changes in the quinolinecarboxamide basic side chain.

In order to introduce isobutyl or propylnyl group in 4'-OH position, these same conditions applied to **5** gave 77% and 36%, respectively, of the corresponding quinolinecarboxamide **7**, **8** to compare their size effect.

Estrogen agonist test. Compound **4** was identified from virtual screening for estrogen antagonist. More derivatives were synthesized using compound **4** as a leader. To determine whether our synthesized compounds act as estrogen agonist or antagonist, we performed transcription assays[17] in which NIH3T3 cells were transiently transfected with 3XERE-tk-β-gal reporter plasmid, and ERα or ERβ expression vector. As a positive control, 17β-estradiol (E_2) was used in DMSO. As expected, 1 μM E_2 activated the transcriptional activities of both ERα and ERβ. Compounds **1**, **4**, **5**, and **8** were active, whereas **2**, **3**, **6**, and **7** were

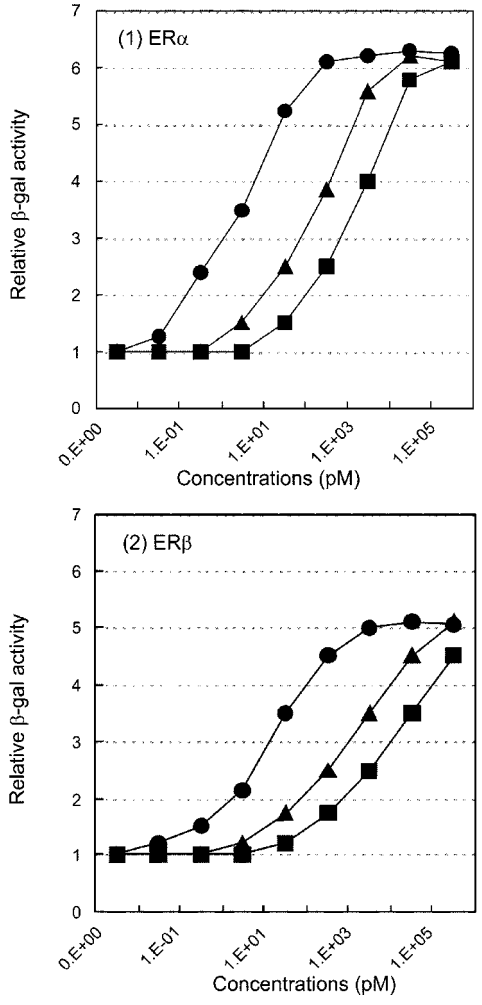

Figure 2. EC_{50} of synthetic compound **1** and **8**. Transfections were conducted as described by increasing concentrations of 17β-estradiol (E2) (●), compound **1** (■), or compound **8** (▲) from 0.01 to 100,000 *p*M. EC_{50} value, the concentration activating ER by 50%, was determined for ERα (A) and ERβ (B).

inactive for both ERα and ERβ (Figure 1).

Although compound **4** was virtually screened as an antagonist, it acted as an agonist in our assay system. Of active compounds, **1** and **8** were selected for further dose-dependent assays to determine EC_{50} value, the concentration activating ER by 50% (Figure 2). When compared to EC_{50} of E2 (0.6 *p*M for ER*a* and 2.5 *p*M for ERβ), those of compound **1** were 500 and 1,000 *p*M for ERα and ERβ, respectively. EC_{50} values of compound **8** were 40 and 100 *p*M for ERα and ERβ, respectively. Therefore, compound **1** is 833.3 and 12.5 times less active than E2 and compound **8** for ERα, respectively. For ERβ, compound **1** is 400 and 10 times less active than E2 and compound **8**, respectively. These data indicate that compound **1** and **8** are about two times more active to ERα than ERβ likewise E2. Furthermore, compound **8** is about 10 times more active than compound **1** to both ERs. As mentioned, compound **4**, a leader for compound **1** and **8**, was designed as an antiestrogen. However, no antagonist effect was found in our assays (data not shown), suggesting that more works should be followed to design antiestrogen.

Throughout our works, we found that non-steroidal quinoline structure could be estrogen agonist. These results were unexpected as virtual screening was conducted to identify estrogen antagonists. Although the reason for the unexpected results is not clarified yet, our screened compound **4** may not possess rigid, bulky, and extended structure in vivo that is required for preventing coactivator from associating with ER for the enhanced transcriptional activity. Therefore, our data may be important and better be considered for virtual screening of real antagonists. Our data also indicate that instead of antagonist, compound **8** can be used to design more estrogenic compounds as a second leader.

Experimental Section

All commercially available reagents and solvents were used without further purification. All reactions were conducted under an Ar atmosphere, except for those reactions utilizing water as a solvent. They were monitored by TLC (Merck *Kieselgel* 60, F254). All the products prepared were purified by flash column chromatography on silica gel 60 (Merck, 230-400 mesh). Melting points were determined with a Büchi 510 hot stage apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL *JNM* EX-400 using $CDCl_3$ as the solvent. All chemical shifts (δ) are quoted in ppm downfield from TMS and coupling constants (*J*) are given in Hz. Mass spectra were measured on a Agilent 1100 LC/MSD (API-ES) mass spectrometer.

2-(4-Hydroxyphenyl)-4-quinolinecarboxylic acid (1). A solution of aniline (9.30 g, 0.10 mol) in EtOH (30mL) was added to a solution of pyruvic acid (13.19 g, 0.15 mol) and 4-hydroxybenzaldehyde (12.20 g, 0.10 mol) in EtOH (80 mL), and the mixture was heated under reflux for 3 h and allowed to cool overnight. The resulting solid was collected by filtration, washed sequentially with cold EtOH and benzene, and dried to give **1** (13.51 g, 51%) as a light yellow powder. mp = > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.76 (br s, 1H), 8.71 (d, 1H, *J*=8.30), 8.37 (s, 1H), 8.13 (d, 2H, *J* = 8.79), 8.09 (d, 1H, *J* = 8.30), 7.76 (dd, 1H, *J* = 7.81, 7.32), 7.65 (dd, 1H, *J* = 8.30, 6.84), 6.95 (d, 2H, *J* = 8.79).^{13}C NMR (100 MHz, DMSO-d_6): δ 167.56, 159.24, 155.65, 148.46, 136.61, 129.42, 129.31, 128.83, 128.42, 126.55, 125.29, 123.08, 118.72, 115.58, 115.53. MS: *m/z* (%) = 265 (27, M^+), 204 (100).

2-(4-Propoxyphenyl)-4-quinolinecarboxylic acid (2). Yield: 3.90 g (65%) as a light yellow powder. mp = 194-198 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.80 (d, 1H, *J* = 8.30), 8.41 (s, 1H), 8.18 (d, 2H, *J*=8.79), 8.14 (d, 1H, *J* = 8.30), 7.74 (t, 1H, *J* = 7.32), 7.57 (t, 1H, *J* = 7.32), 7.04 (d, 2H, *J* = 8.79), 4.01(dd, 2H, *J* = 6.84, 0.49), 1.85 (m, 2H), 1.07 (t, 3H, *J* = 7.32).^{13}C NMR (100 MHz, DMSO-d_6): δ 167.89, 160.17, 155.63, 148.62, 136.29, 130.50, 129.34, 129.19, 128.24, 126.57, 125.30, 123.36, 119.25, 114.32, 69.07, 22.03, 10.08. MS: *m/z* (%) = 307 (18, M^+), 262 (68), 204 (100), 128 (35).

***N*4-(1-Benzyl-4-piperidyl)-2-(4-hydroxyphenyl)-4-quinolinecarboxamide (3)**. To a solution of EDCI (0.43 g, 2.26 mmol) in dry DMF (5 mL) was added quinolinecarboxylic acid (0.20 g, 0.75 mmol) in dry DMF (5 mL). The solution was stirred at room temperature for 30 min. To this mixture were added 4-amino-1-benzylpiperidine (0.77 mL, 3.77 mmol) in dry DMF (2 mL) and DMAP (cat.), stirred at room temperature for 16 h. The reaction was quenched with H_2O (100 mL), extracted with EtOAc (20 mL × 2). The extracts were washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (10% MeOH : EtOAc) to give **3** (0.25 g, 76%) as a white powder. mp = 222-226 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, 1H, *J* = 8.79), 8.13 (d, 1H, *J* = 8.79), 8.08 (d, 2H, *J* = 8.79), 7.83 (s, 1H), 7.74 (t, 1H, *J* = 7.32), 7.54 (t, 1H, *J* = 7.32), 7.27 (m, 5H), 6.98 (d, 2H, *J* = 8.79), 5.93 (d, 1H, *J* = 7.64, NH), 4.13 (m, 1H), 3.55 (s, 1H), 2.91 (m, 2H), 2.25 (m, 2H), 2.15 (m, 2H), 1.65 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.08, 164.83, 157.34, 150.57, 144.70, 140.03, 134.24, 130.31, 129.78, 129.13, 128.30, 128.15, 126.94, 126.76, 125.53, 123.41, 115.86, 113.99, 113.87, 62.96, 52.26, 47.53, 32.20, 22.52. MS: *m/z* (%) = 437 (28, M^+), 420 (31), 218 (52), 204 (100), 174 (44), 128 (40).

***N*4-(1-Benzyl-4-piperidyl)-2-(4-propoxyphenyl)-4-quinolinecarboxamide (4)**. Yield: 0.26 g (70%) as a white powder. mp = 162-164 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, 1H, *J* = 8.30), 7.99 (d, 2H, *J* = 8.79), 7.93 (d, 1H, *J* = 8.30), 7.65 (t, 1H, *J* = 7.32), 7,61 (s, 1H), 7.39 (t, 1H, *J* = 7.32), 7.33 (m, 4H), 7.28 (m, 1H), 6.97 (d, 2H, *J* = 8.79), 6.44 (d, 1H, *J* = 7.81, NH), 4.07 (m, 1H), 3.97 (t, 2H, *J* = 6.84), 3.52 (s, 2H), 2.89 (m, 2H), 2.18 (m, 2H), 2.10 (m, 2H), 1.85 (m, 2H), 1.66 (m, 2H), 1.07 (t, 3H, *J* = 7.32). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.04, 160.63, 156.14, 148.37, 142.76, 138.33, 130.84, 129.91, 129.57, 129.02, 128.75, 128.22, 127.04, 126.69, 124.73, 122.81, 115.78, 114.69, 69.57, 62.96, 52.26, 47.53, 32.20, 22.52, 10.50. MS: *m/z* (%) = 479 (39, M^+), 436 (21), 420 (43), 218 (63), 204 (100), 174 (40), 128 (38).

***N*4-(1-Benzyl-4-piperidyl)-2-[4-(3-aminopropoxy)phenyl]-4-quinolinecarboxamide hydrochloride (6)**. To a solution

of 1 M NaOH (20.10 mL, 20.10 mmol) in *tert*-butyl alcohol (6 mL) was added 3-bromopropylamine hydrobromide (2.00 g, 9.14 mmol) and di-*tert*-butylcarbonate (2.19 g, 10.05 mmol), the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was washed with 0.1*N* HCl and 5% $NaHCO_3$, Brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give *N*-Boc-3-bromopropylamine (1.40 g, 64%) as a oil.

A solution of *N*-Boc-3-bromopropylamine (0.14 g, 0.32 mmol) in CH_3CN (10 mL) was added to a mixture of **3** (0.14 g, 0.32 mmol) and K_2CO_3 (0.14 g, 0.96 mmol) in CH_3CN (20 mL), and the mixture was heated under reflux for 12 h. The inorganic material was filtered off and the solvent was evaporated *in vacuo*. The crude was extracted with EtOAc (20 mL × 2), washed with H_2O (100 mL) and Brine. The extract was dried over Na_2SO_4 and concentrated *in vacuo* to give **5** (0.17 g, 89%) as a white powder.

A solution of **5** (0.14 g, 0.24 mmol) in dry THF (10 mL) was cooled to −20 °C and stirred for 30 min. Etherate HCl (1*M*) was added dropwise to the reaction mixture under pH=1. The precipitate was collected by suction filtration, washed with ether (5 mL × 2) and dried in desiccator to give **6** (0.12 g, 96%) as a light yellow solid. mp = 174-179 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (d, 1H, *J* = 8.30), 8.41 (s, 1H), 8.36 (d, 1H, *J* = 8.30), 8.26 (d, 2H, *J* = 8.79), 8.15 (t, 1H, *J* = 7.81), 7.93 (t, 1H, *J* = 7.81), 7.60 (m, 2H), 7.51 (m, 3H), 7.32 (d, 2H, *J* = 8.79), 4.34 (m ,1H), 4.30 (m, 2H), 3.61 (m, 2H), 3.30 (s, 2H), 3.28 (m, 2H), 3.21 (m, 2H), 2.40 (m, 2H), 2.24 (m, 2H), 2.07 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6): δ 166.39, 164.65, 156.45, 151.42, 140.64, 136.05, 132.97, 132.76, 132.54, 132.41, 131.30, 131.23, 131.03, 130.50, 127.67, 125.29, 124.60, 122.15, 121.33, 120.70, 117.02, 66.93, 61.59, 52.70, 46.81, 38.39, 29.82, 28.24, 24.22. MS: *m/z* (%) = 531 (69, M^+), 420 (54), 218 (41), 204 (100), 174 (36), 128 (21).

***N*4-(1-Benzyl-4-piperidyl)-2-(4-isobutoxyphenyl)-4-quinolinecarboxamide (7)**. A solution of 1-bromo-2-methylpropane (0.07 g, 0.50 mmol) in CH_3CN (10 mL) was added to a mixture of **3** (0.20 g, 0.46 mmol) and K_2CO_3 (0.19 g, 1.37 mmol) in CH_3CN (20 mL), and the mixture was heated under reflux for 12 h. The inorganic material was filtered off and the solvent was evaporated *in vacuo*. The crude was extracted with EtOAc (20 mL × 2), washed with H_2O (100 mL) and Brine. The extract was dried over Na_2SO_4 and concentrated *in vacuo* to give **7** (0.17 g, 77%) as a white powder.

mp = 112-114 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, 1H, *J* = 8.30), 7.99 (d, 2H, *J* = 8.79), 7.93 (d, 1H, *J* = 8.30), 7.65 (t, 1H, *J* = 7.32), 7.62 (s, 1H), 7.39 (t, 1H, *J* = 7.32), 7.33 (m, 4H), 7.28 (m, 1H), 6.97 (d, 2H, *J* = 8.79), 6.48 (d, 1H, *J* = 8.30, NH), 4.09 (m, 1H), 3.77 (d, 2H, *J* = 6.35), 3.52 (s, 3H), 2.89 (m, 2H), 2.23 (m, 3H), 2.10 (m, 2H), 1.68 (m, 2H), 1.06 (d, 6H, *J* = 6.35). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.05, 160.76, 156.13, 148.35, 142.75, 138.31, 130.79, 129.89, 129.55, 129.02, 128.72, 128.20, 127.03, 126.66, 124.73, 122.80, 115.78, 114.70, 62.94, 60.34, 52.25, 47.52, 32.16, 28.24, 19.24, 14.13. MS: *m/z* (%) = 493 (47, M^+), 420 (50), 218 (38), 204 (100), 174 (40), 128 (29).

***N*4-(1-Benzyl-4-piperidyl)-2-[4-(2-propylnyloxy)phenyl]-4-quinolinecarboxamide (8)**. Yield: 0.80 g (36%) as a white powder. mp = 104-106 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 8.06 (d, 1H, *J* = 8.79), 8.02 (d, 2H, *J* = 8.30), 7.86 (s, 1H), 7.68 (t, 1H, *J* = 7.32), 7.50 (t, 1H, *J* = 7.32), 7.18 (m, 4H), 7.11 (m, 1H), 7.03 (d, 2H, *J* = 8.79), 4.73 (s, 2H), 3.98 (m, 1H), 3.21 (s, 2H), 2.90 (s, 1H), 2.84 (m, 2H), 2.03 (m, 2H), 1.65 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ 169.53, 160.77, 158.07, 149.63, 143.05, 133.25, 131.47, 130.46, 130.29, 130.22, 130.10, 129.28, 128.15, 128.07, 127.62, 126.02, 124.58, 117.87, 116.33, 79.55, 77.08, 60.51, 56.73, 46.98, 44.23, 40.41, 29.47, 20.85. MS: *m/z* (%) = 475 (16, M^+), 420 (63), 218 (44), 204 (100), 174 (48), 128 (36).

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References

1. Dickinson, R. B.; McManaway, M. E.; Lippman, M. E. *Science* **1986**, *232*, 1540.
2. Tsai, M. J.; OMalley, B. W. *Annul. Rev. Biochem.* **1994**, *63*, 451.
3. Sun, J.; Meyers, M. J.; Fink, B. E.; Rajendran, R.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Endocrinology* **1999**, *140*, 800.
4. Wiese, T. E.; Polin, L. A.; Palomino, E.; Brooks, S. C. *J. Med. Chem.* **1997**, *40*, 3659.
5. Li, J. J.; Hou, X.; Bentel, J.; Yazlovitskaya, E. M.; Li, S. A. *Carcinogenesis* **1998**, *19*, 471.
6. Lippman, M. E.; Bolan, G. *Nature* **1975**, *256*, 592.
7. Jordan, V. C. *Eur. J. Cancer* **1976**, *12*, 419.
8. Jordan, V. C.; Morrow, M. *Br. Med. J.* **1999**, *319*, 331.
9. Munster, P. N.; Buzdar, A.; Dhingra, K.; Enas, N.; Ni, L.; Major, M.; Melemed, A.; Seidman, A.; Booser, D.; Theriault, R.; Norton, L.; Hudis, C. *J. Clin. Oncol.* **2001**, *19*, 2002.
10. Labrie, F.; Labrie, C.; Belanger, A.; Simard, J.; Gauthier, S.; Luu-The, V.; Merand, Y.; Giguere, V.; Candas, B.; Luo, S.; Martel, C.; Singh, S. M.; Fournier, M.; Coquet, A.; Richard, V.; Charbonneau, R.; Charpenet, G.; Tremblay, A.; Tremblay, G.; Cusan, L.; Veilleux, R. *J. Steroid Biochem. Mol. Biol.* **1999**, *69*, 51.
11. Ke, H. Z.; Qi, H.; Chidsey-Frink, K. L.; Crawford, D. T.; Thompson, D. D. *J. Bone Miner. Res.* **2001**, *16*, 765.
12. Doebner, O. *Ber.* **1883**, *16*, 2357.
13. Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 396.
14. (a) Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962. (b) Desai, M. C.; Stephens Stramiello, L. M. *Tetrahedron Lett.* **1993**, *34*, 7685. (c) Akaji, K.; Kuriyama, N.; Kiso, Y. *Tetrahedron Lett.* **1994**, *35*, 3315. (d) Norman, B. H.; Hemscheidt, T.; Schultz, R. M.; Andis, S. L. *J. Org. Chem.* **1998**, *63*, 5288.
15. Saari, W. S.; Schwering, J. E.; Lyle, P. A.; Smith, S. J.; Engelhardt, E. L. *J. Med. Chem.* **1990**, *33*, 97.
16. (a) Fletcher, S. R.; Burkamp, F.; Blurton, P.; Cheng, S. K. F.; Clarkson, R.; O'Connor, D.; Spinks, D.; Tudge, M.; van Niel, M. B.; Patel, S.; Chapman, K.; Marwood, R.; Shepheard, S.; Bentley, G.; Cook, G. P.; Bristoww, L. J.; Castro, J. L.; Hutson, P. H.; MacLeod, A. M. *J. Med. Chem.* **2002**, *45*, 492. (b) Salvatore, R. N.; Yoon, C. H.; Jung, K. W. *Tetrahedron* **2001**, *57*, 7785.
17. Um, S. J.; Kim, E. J.; Hwang, E. S.; Kim, S. J.; Namkoong, S. E.; Park, J. S. *Int. J. Cancer* **2000**, *85*, 416.