Arch. Pharm. Res. Vol.20, No.3, pp. 283-287, 1997

Note

Planar Structural Requirement at 4-Position of 1-Arylsulfonyl-4-phenyl-4,5-dihydro-2-imidazolones for Their Cytotoxicity

Sang-Hun Jung and Suhk-Jun Kwak

College of Pharmacy, Chung-Nam National University, Taejon 305-764, Korea

(Received March 28, 1997)

In order to identify the structural requirement at 4-position of 1-arylsulfonyl-4-phenyl-4,5-dihydro-2-limidazolones **3** for their cytotoxicity, the corresponding 1-arylsulfonyl-4-cyclohexyl-4,5-dihydro-2-imidazolones **4** were synthesized and their *in vitro* cytotoxicity against human solid tumor cell lines were measured. Unlike compounds **3a-c**, cyclohexyl analogues **4a-c** do not show the cytotoxicity. This dramatic loss of activity of these analogues on the volume change with the bulkier cyclohexyl group indicates that the planar structure at 4-position of 1-arylsulfonyl-4-pheny-4,5-dihydro-2-limidazolones **3** is required for their activity as an important pharmacophoric moiety.

Key word : Cytotoxic activicity, 4-Phenyl-1-arylsulfonylimidazolone, Phenyl pharmacophore

INTRODUCTION

Diarylsulfonylureas LY186641 (**1**, sulofenur) (Grindey, 1988; Munshi, *et al.*, 1991; Howbert, *et al.*, 1989) and LY295501 (**2**) (Shultz, *et al.*, 1993) have been extensively investigated for the treatment of nonhematogeneous cancers in the past decade. Although the exploration of the clinical utility of these compounds has been seriously delayed by the occurance of the unexpected methemoglobinemia and anemia in clinical trials (Munshi, *et al.*, 1991, Talbot, *et al.*, 1993, Taylor, *et al.*, 1989, Kamthan, *et al.*, 1992), these analogues are considered to be very important lead for the development of novel anticancer agent due to their unique mechanism of action, which is completely different from those of antineoplastic agents being clinically used (Grindey, *et al.*, 1987, Grindey, 1990, Houghton, *et al.*, 1990, Houghton, *et al*, 1990, Howbert, *et al.*, 1991). Such advantageous characteristics of these diarylsulfonylureas led us to investigate new structural entity containing sulfonylurea motif (Jung, *et al.*, 1996, Jung, *et al.*, 1996). As the results, three 1-arylsulfonyl-4-phenyl-4,5-dihydro-2-imidazolones (**3**) have shown very potent activity against the various human cancer cell lines shown in Table I and murine leukemias *in vitro*. Introduction of electron releasing or electron withdrawing groups at 4-phenyl group of these analogues reduced the activity in these series (Jung, *et al.*, 1996). This trend might be originated from the increment of bulkness at this site by the substituents. Therefore the necessity of planar structure at 4 position of these analogues was examined through comparison of the cytotoxicity of compounds **3** and analogues **4** against human solid tumor cell lines.

Correspondence to: Sang-Hun Jung, Ph. D. College of Pharmacy, Chung-Nam National University, Taejon 305-764, Korea

MATERIALS AND METHODS

Melting points (m.p.) were determined on Electrothermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures prior to use (Perrin, 1982). Thin-layer chromatography was performed on E Merck silica gel GF-254 precoated plates and the identification was done with UV light and colorization with spray 10% phosphomolybdic acid followed by heating. Flash column chromatography was performed with E. Merck silica gel (200~430 mesh). IR spectra were recorded with Jasco IR-Report-100 IR spectrometer in cm^{-1} and corrected against peak at 1601 cm^{-1} of polystyrene. NMR spectra were measured in against the peak of tetramethylsilane by Varian Gemini 200 NMR spectrometers. Mass spectra (Ms) were obtained by GC-Mass PP-1000 mass spectrometer.

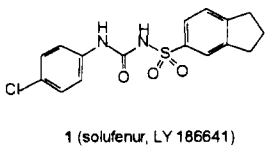
Synthesis

Snthesis of bromocyanamide 6a and 6b: The solution of vinylcyclohexane (**5**) 3.00 g (27.3 mmol) in dichloromethane was added to the mixture of N-bromosuccinimide 6.30 g (35.4 mmol) and cyanamide 2.30 g (54.8 mmol) in dichloromethane over one hour

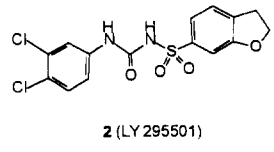
Table I. Cytotoxicity of 1-arylsulfonylimidazolidinones **3** and **4**

Entry No[a].	Compound		IC_{50} (μg/mL)[c]						
			3a[d]	**4a**	**3b**[d]	**4b**	**3c**[d]	**4c**	LY186641(**1**)
	cell[b]		R_1: Cl		R_1: Me		R_1: indane[e]		
1	ovary	SK-OV-3	1.99	>100	0.91	>100	0.03	>100	7.49
2	brain	XF-498	2.55	>100	1.95	85.90	0.37	>100	3.46
3	colon	HCT-15	0.65	>100	0.15	29.80	0.04	>100	2.95
4	lung	A549	3.19	>100	3.94	>100	0.31	>100	3.97
5	melanoma	SK-MEL-2	0.48	>100	0.80	>100	0.0007	>100	4.53

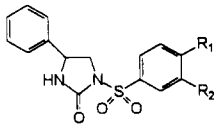
[a]SRB assay method was used for the measurement of cytotoxicity. [b]Cell lines used for the test are human cancer cell lines. [c]IC_{50} values are the mean value of three times measurement. [d]Compounds **3a**, **b**, and **c** were prepared according to the method previously reported (Jung, *et al.*, 1996). [e]Indane is represented for 5-indanyl connected to sulfonyl group as a substituted phenyl.



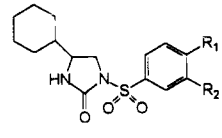
1 (solufenur, LY 186641)



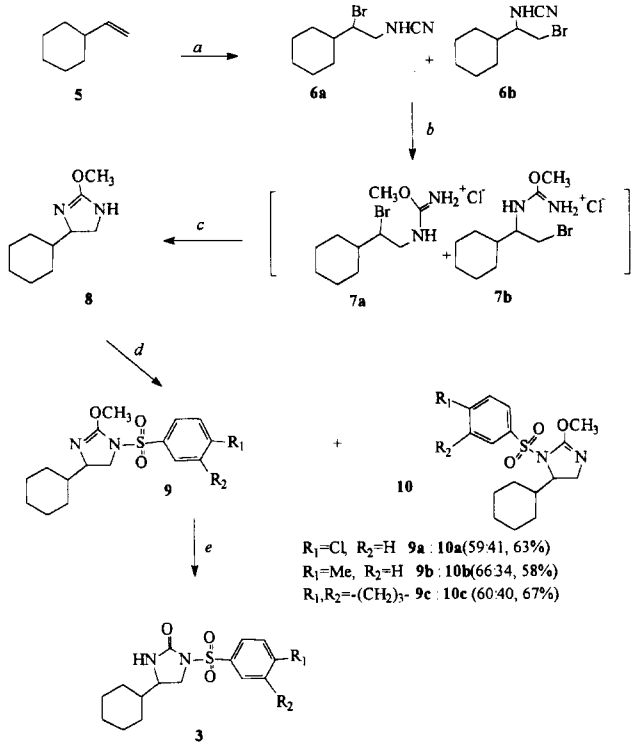
2 (LY 295501)



3a: R_1=Cl, R_2=H
3b: R_1=CH_3, R_2=H
3c: R_1, R_2= -$(CH_2)_3$-



4a: R_1=Cl, R_2=H
4b: R_1=CH_3, R_2=H
4c: R_1, R_2= -$(CH_2)_3$-



a. N-bromosuccinimide, NH_2CN, rt 80% (6a:6b=3:4); b. HCl, MeOH, rt; c. Na_2CO_3 70% for step b and c; d arylsulfonyl chloride, $NaHCO_3$, rt; e. HCl, rt.

Scheme 1. Synthesis of 1-arylsulfonyl-4-cyclohexyl-4,5-dihydro-2-imidazolones **4**.

at room temperature under nitrogen atmosphere. The resulting mixture was stirred at room temperature overnight and washed with 5% aqueous sodium thiosulfate and brine. After dehydration with anhydrous sodium sulfate, the solvent was evaporated under vacuum. The crude products were purified with flash column chromatography. *2-Bromo-2-cyclohexylcyanamide (**6a**)-Rf 0.33 (hexane-ethyl acetate=3:1)*; yield 35%; yellow oil; IR (KBr) 3160, 2920, 2250 cm^{-1}; NMR($CDCl_3$) δ 1.03 1.83 (m, 11H), 3.46 (m, 2H), 4.06 (m, 1H) 4.11 (exchangeable proton with D_2O); MS m/z (rel %) 234 (4.5), 233 (33.8), 231 (34.4), 177 (4.6), 175 (5.9), 151 (100): *2-Bromo-1-cyclo hexylethylcyanamide* (**6b**)-Rf 0.42 (hexane-ethyl acetate=3:1); yield 44%; yellow oil; IR (KBr) 3120, 2920, 2250 cm^{-1}; NMR ($CDCl_3$) δ 0.92 1.99 (m, 11H), 3.06 (broad s, 1H), 3.54 (dd, J=6.2, 11.0 Hz, 1H), 3.65 (dd, J=3.6, 11.0 Hz, 1H), 4.02 (exchangeable proton); MS m/z (rel %) 234 (4.6), 233 (34.9) 231 (37.9), 191 (6.2), 189 (8.3), 137 (100), 69 (100).

Synthesis of 4-cyclohexyl-2-methoxy-4,5-dihydroimidazole 8: The solution of bromocyanamide **6a** and **6b** (3.90 g, 14.0 mmol) mixture in methanol was added to the methanolic hydrochloride (9.80 g, 9.7%w/w, 1.5 equivalent) at room temperature. The reaction mixture was stirred for 8 hours and then methanol was evaporated. The residue was dispersed in dichloromethane (30 ml) and extracted with water (30 ml×6). The aqueous layers were combined and then sodium carbonate (2.20 g, 2.5 equivalent) was added. The mixture was stirred for 4 hours and extracted with dichlomethane three times. The organic layers combined were dehydrated with anhydrous sodium sulfate and evaporated under vacuum to give the crude yellowish oil (yield 70%). The product **8** was directly used for the next step without further purification due to the difficulty in chromatographic separation and decomposition in bulb to bulb vacuum distillation.

Synthesis of N-arylsulfonylimidazoline 9 and 10: The appropriate arylsulfonyl chloride (1 equivalent) was added to the mixture of compounds **8** and sodium bicarbonate (1.5 equivalent) in acetone-water (1:1). The resulting mixture was stirred for two hours at room temperature and then extracted with dichloromethane three times. The organic layer was dehydrated with anhydrous sodium sulfate and evaporated under vacuum. The residue was then separated by flash column chromatography to give compounds **9** and **10** in approximate ratio of 3:2.

1-(p-Chlorobenzenesulfonyl)-4-cyclohexyl-2-methoxy-4,5-dihydroimidazole (**9a**)-Rf 0.31 (hexane:acetone=5:1); yield 37%; white solid; m.p. 113.3~117.8°C; δ IR (KBr) 3400, 2920, 2850, 1665 cm^{-1}; NMR ($CDCl_3$) δ 0.85 1.77 (m, 11H), 3.59 (m, 2H), 3.81 (m, 1H), 3.87 (s, 3H) 7.52 (d, J=8.6 Hz, 2H), 7.85 (d, J=8.6 Hz, 2H): *3-(p-Chloro benzenesulfonyl)-4-cyclohexyl-2-methoxy-4,5-dihydroimidazole* (**10a**)-Rf 0.24 (hexane:acetone=5:1); yield 26%; white solid; m.p. 56.2~59.5°C; yield 26%; IR (KBr) 3430, 1620, 1380 cm^{-1}; NMR (200 MHz, $CDCl_3$) δ 1.11 1.80 (m, 11H), 3.55 (ddd, J=5.5, 8.7, 14.4 Hz, 1H), 3.70 (ddd, J=4.1, 6.1, 14.4 Hz, 1H), 3.80 (s, 3H), 3.90 (m, 1H), 7.43 (d, J=8.7 Hz, 2H), 7.87 (d, J=8.7 Hz, 2H).

1-(p-Methylbenzenesulfonyl)-4-cyclohexyl-2-methoxy-4,5-dihydroimidazole (**9b**)-Rf 0.26 (hexane:acetone=4:1); yield 38%; white solid; m.p. 101.4~104.2°C; yield 38%; IR (KBr) 3320, 2920, 2850, 1660 cm^{-1}; NMR ($CDCl_3$) δ 0.81 1.76 (m, 11H), 2.44 (s, 3H), 3.56 (m, 2H), 3.86 (m, 4H), 7.33 (d, J=7.9 Hz, 2H), 7.78 (d, J=8.3 Hz, 2H): *3-(p-Methylbenzenesulfonyl)-4-cyclohexyl-2-methoxy-4,5-dihydroimidazole* (**10b**)-Rf 0.20 (hexane:acetone=4:1); yield 20%; white solid; m.p. 87.6~89.5°C; yield 20%; IR (KBr) 3320, 2920, 2850, 1618, cm^{-1}; NMR ($CDCl_3$) δ 1.15 1.81 (m, 11H), 2.40 (s, 3H), 3.53 (ddd, J=5.6, 8.4, 14.4 Hz, 1H), 3.67 (dd, J=4.4, 6.2, 14.4Hz, 1H), 3.80 (s, 3H), 3.91 (m, 1H), 7.27 (d, J=8.0 Hz, 2H), 7.83 (d, J=8.4 Hz, 2H).

1-(5-indanesulfonyl)-4-cyclohexyl-2-methoxy-4,5-dihydroimidazole (**9c**)-Rf 0.35 (hexane:acetone=4:1); yield 33%; brown oil; IR (KBr): 3340, 2910, 2840, 1660 cm^{-1}; NMR($CDCl_3$) δ 0.78 1.73 (m, 11H), 2.27 (m, 2H), 2.96 (t, J=7.4 Hz, 4H), 3.55 (m, 2H), 3.79 (m, 1H), 3.85 (s, 3H), 7.33 (d, J=8.0 Hz, 1H), 7.66 (d, J=8.1 Hz, 1H), 7.71 (s, 1H): *3-(5-indanesulfonyl)-4-cyclohexyl-2-methoxy-4,5-dihydroimidazole* (**10c**)-Rf 0.30 (hexane:acetone=4:1); yield 33%; white solid; m.p. 86.3 89.5oC; yield 37%; IR (KBr) 3310, 2920, 2900, 1615, cm^{-1}; NMR ($CDCl_3$) δ 1.08 1.80 (m, 11H), 2.11 (m, 2H), 2.94 (t, J=7.4 Hz, 4H), 3.56 (m, 1H), 3.69 (m, 1H), 3.81 (s, 3H), 3.91 (m, 1H), 7.29(d, J=7.7 Hz, 1H), 7.72 (d, J=7.8 Hz, 1H), 7.78 (s, 1H).

General procedure of the synthesis of imidazolidinones 4: Compounds **9** were dispersed in ether and then hydrochloride (1.5 equivalent) in ether (more than 5% w/w concentration) was added. The resulting mixture was stirred for 5 hours at room temperature. During the reaction, the reaction mixture became clear solution and then reprecipitated. The white solid was collected, washed with ether, and dried in vacuum oven below 60°C. These reactions can be done in methanolic hydrochloride instead of ethereal hydrochloride.

1-(p-Chlorobenzesulfonyl)-4-cyclohexyl-4,5-dihydro-2-imidazolone (**4a**)-Rf 0.25 (hexane:ethyl acetate=1:1); yield 96%; white solid; m.p. 228.4~230.1°C; IR (KBr) 3420, 2920, 2850, 1715 cm^{-1}; NMR ($CDCl_3$) δ 0.83 1.78 (m, 11H), 3.39 (m, 1H), 3.58 (dd, J=6.5, 9.2 Hz, 1H), 4.00 (dd, J=8.7, 9.2 Hz, 1H), 5.57 (broad s, exchangeable with D_2O), 7.49 (d, J=8.7 Hz, 2H), 7.97 (d, J=8.7 Hz, 2H).

1-(p-Toluenesulfonyl)-4-cyclohexyl-4,5-dihydro-2-imidazolone (**4b**)-Rf 0.28 (hexane: ethyl acetate=2:1); yield 98%; white solid; m.p. 212.5~213.6°C; IR (KBr) 3220, 3110, 2925, 2850, 1710 cm^{-1}; NMR ($CDCl_3$) δ 0.83 1.76 (m, 11H), 2.42 (s, 3H), 3.36 (m, 1H), 3.56 (dd, J=6.6, 9.3 Hz, 1H), 3.94 (dd, J=8.8, 9.3 Hz, 1H), 5.54 (broad s, exchangeable with D_2O), 7.31 (d, J=8.3 Hz, 2H), 7.89 (d, J=8.3Hz, 2H).

1-(5-indanesulfonyl)-4-cyclohexyl-4,5-dihydro-2-imidazolone (**4c**)-Rf 0.34 (hexane:ethyl acetate=1:1); yield 95%; white solid; m.p. 240°C decomposed; IR (KBr) 3205, 2920, 2840, 1718 cm^{-1}; NMR ($CDCl_3$) δ 0.85 1.76 (m, 11H), 2.12 (m, 2H), 2.96 (t, J=7.5 Hz, 4H), 3.36 (m, 1H), 3.58 (dd, J=6.5, 9.2 Hz, 1H), 3.95 (dd, J=8.6, 9.2 Hz, 1H), 5.39 (broad s, exchangeable with D_2O), 7.33 (d, J=7.8 Hz, 1H), 7.79 (d, J=7.9 Hz, 1H), 7.84 (s, 1H).

Biological assay

Cytotoxicity of compounds **1**, **3**, and **4**, was measured against human ovarian (SK-OV-3), brain (XF 498), colon (HCT-15), lung carcinoma (A549), and human melanoma (SK-MEL-2) cell lines *in vitro* using sulforhodamine B(SRB) assay (Everitt *et al.*, 1987, Skehan *et al.*, 1990). The cytotoxicity of these compounds is shown as IC_{50} value (μg/mL) in Table 1.

RESULTS AND DISCUSSION

In order to identify the necessity of planar structure at 4-position of 4-phenyl-1-arylsulfonylimidazolones **3**, compounds **4** were prepared as shown in scheme 1 using the procedure previously reported (Jung, *et al.*, 1996) from vinylcyclohexane (5) and measured their

cytotoxicity against five human cancer cell lines as shown in Table 1. Compared to the structures of 4-phenyl-1-arylsulfonylimidazolones **3**, those of compounds **4** are differentiated with containing cyclohexyl group at 4 position of imidazolone ring in place of phenyl group. This substitution resulted in the complete loss of biological activity of these analogues as shown in Table 1. Such replacement could be considered mainly to alter the volume and π-π stacking ability with the variation from the planar structure to the bulkier one at 4 position of these imidazolones rather than the change of other properties such as lipophilicity. Rf values of **4a** (Rf 0.38) and **4c** (Rf 0.28) are nearly the same as those of the corresponding **3a** (Rf 0. 40) and **3c** (Rf 0.28) on silica plate with an eluant, hexane-ethyl acetate (1:1). These Rf values certainly imply that the replacement of phenyl with cyclohexyl group would not much alter the lipophilicity of π compounds **3**, although the constants for phenyl (1.96) and cyclohexyl (2.51) groups are not equivalent (Hansch, 1995). Therefore the loss of cytotoxicity along with the saturation of phenyl group of **3** should mainly originated from the loss of planarity or π-π stacking ability of aromatic ring with a putative receptor. This type of activity change was also notified in the diarylsulfonylurea **1** analogues (Howbert, *et al.*, 1990). Thus the planar structure at 4 position of imidazolione ring of 4-phenyl-1-arylsulfonylimidazolones **3** should play an essential role for binding of these compounds to the putative receptor and be an important pharmacophoric moiety for their activity.

ACKNOWLEDGEMENT

This work was supported in part by Korea Science and Engineering Foundation through Research Center for New Drug Development at Seoul National University of Korea and Institute of Drug Research and Development of the College of Pharmacy of the Chungnam National University. We thank Dr. C. O. Lee at the Korea Research Institute of Chemical Technology for measuring the cytotoxicity.

REFERENCES CITED

Everitt, E. and Wohlfart, C., Spectrophotometric quantitation of anchorage-dependent cell numbers extraction of naphthol blue-black-stained cellular protein. *Anal. Biochem.*, 162(1), 122-9 (1987).

Grindey, G. B., Boder, G. B., Grossman, C. S., Howbert, J. J., Poore, G. A., Shaw, W. H., Todd, G. C., and Worzalla, J. F., Further development of diarglsulfonylureas as novel anticancer drugs. *Proc. Am. Assoc. Cancer Res.*, 28, 309 (1987).

Grindey, G. B., Identification of diarysulfonylureas as novel anticancer drugs. *Proc. Am. Assoc. Cancer Res.*, 29, 535(1988).

Grindey, G. B., Current status of cancer drug development: Failure or limited success. *Cancer Cells*, 2, 163-171 (1990).

Hainsworth, J. D., Handle, K. R., Satterlee, W. G., Kuttesch, J., Johnson, D. H., Grindey, G. B., Jackson, L. E., and Greco, F. A., Phase I clinical study of N-[(4-chlorophenylamino)]carbonyl-2,3-dihydro-1H-indene-5-sulfonamide (LY186641). *Cancer Res.*, 49, 5217-5220 (1989).

Hansch, C., Leo, A., *Exploring QSAR*, ACS Professional Reference Book, American Chemical Society, Washington, DC, U. S. A., 1995, pp.522-528.

Houghton, P. J., Bailey, F. C., Germain, G. S., Grindey, G. B., Witt, B. C., and Houghton, J. A., N-(5-indanylsulfonyl)-N'-(4-chlorophenyl)-urea, A novel agent equally cytotoxic to nonproliferlating human colon adenosarcoma cells. *Cancer Res.*, 50, 318-322 (1990).

Houghton, P. J., Bailey, F. C., Houghton, J. A., Murti, K. G., Howbert, J. J., and Grindey, G. B., Evidence for mitochondrial localization of N-(4-Methylphenylsulfonyl)-N'-(4-chlorophenyl) urea in human colon adenosarcoma cells. *Cancer Res.* 50, 664-668 (1990).

Howbert, J. J., Grossman, C. S., Crowell, T. A., Rieder, B. J., Harper, R. W., Kramer, K. E., Tao, E. V., Aikins, J., Poore, G. A., Rinzel, S. M., Grindey, G. B., Shaw, W. N., Todd, G. C., Novel Agents Effective against Solid Tumors: The Diarylsulfonylureas. Synthesis, Activities, and Analysis of Quantitative Structure-Activity Relationships. *J. Med. Chem.*, 33, 2393-2407 (1990).

Howbert, J. J., Sulofenur. *Drugs Future.*, 16, 517-520 (1991).

Jung, S. H., Song, J. S., Lee, H. S., Choi, S. U., and Lee, C. O., Synthesis and evaluation of cytotoxic activity of novel arylsulfonylimidazolidinones. *Bioorg. Med. Chem. Lett.* 6, 2553-2558 (1996).

Jung, S. H., Song, J. S., Lee, H. S., Choi, S. U., and Lee, C. O., Synthesis and evaluation of cytotoxic activity of novel arylsulfonylimidazolidinones containing sulfonylurea pharmacophore. *Arch. Pharm. Res.*, 19, 570-580 (1996).

Kamthan, A., Scarffe, J. H., Walling, J., Hatty, S., Peters, B., Coleman, R., and Smyth, J. F., A phase II study of solufenur (LY186641) in gastric cancer. *Anti-cancer Drugs*, 3, 331-335 (1992).

Munshi, N. C., Seitz, D. E., Fosella, F., Lippman, S. N., and Einhorn, L. H., Phase II study of sulofenur (LY186641). A novel antineoplastic agent in advanced non-small lung cancer. *Proc. Am. Assoc. Cancer Res.* 32, 189 (1991).

Perrin, D. D., Armarego, W. L. F., and Perrin, D. R., *Purification of laboratory chemicals, 2nd edition.* Pergamon Press, Oxford, England, 1982.

Schultz, R. M., Merriman, R. L., Toth, J. E., Zimmermann, J. E., Hertel, L. W., Andis, S. L., Dudley, D. E., Rutherford, P. G., Tanzer, L. R., and Grindey, G. B., Evaluation of new anticancer agents against the MIA Paca-2 and PANC-1 human pancreatic carcinoma xenografts. *Oncol. Res.*, 5, 223 (1993).

Skehan, P., Storeng, R., Scudiero, D. A., Monks, A., McMahon, J., Vista, D. T., Warren, J. T., Kenny, S., and Boyd, M. R., New Colorimetric cytotoxicity assay for anticancer drug screening, *J. Natl. Cancer. Inst.*, 82, 1107-1112 (1990).

Talbot, D. C., Smith, I. E., Nicolson, M. C., Powles, T. J., Button, D., and Walling, J., Phase II trial of the novel sulphonylurea sulofenur in advanced breast cancer. *J. Cancer Chemother. Pharmacol.*, 31, 419-422 (1993).

Taylor, C. W., Alberts, D. S., Ketcham, M. A., Satterlee, W. G., Holdsworth, M. T., Plazia, P. M., Peng, Y. M., Mccloskey, T. M., Roe, D. J., Hamilton, M., and Salmaon, S. E., Clinical pharmacology of a novel diarylsulfonylurea anticancer agent. *J. Clin. Oncol.*, 7, 1733-1740 (1989).