Multitarget-Directed Drug Design Strategy: A Novel Molecule Designed To Block Epidermal Growth Factor Receptor (EGFR) and To Exert Proapoptotic Effects†

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Abstract: The multifactorial mechanistic nature of cancer calls for the development of multifunctional therapeutic tools, i.e., single compounds able to interact with multiple altered pathogenetic pathways. Following this rationale, we designed compounds able to irreversibly block epidermal growth factor receptor (EGFR), and to induce apoptosis in tumor cell lines. The novel molecules were synthesized by combining the structural features of the EGFR inhibitor PD153035 (**1**) and lipoic acid, which among other therapeutic effects triggers apoptosis in human cancer cells.

Cancer, like diabetes and cardiovascular and neurodegenerative diseases, is a complex pathology. All complex diseases are multifactorial, meaning that they cannot be ascribed to mutations in a single gene, and consequently, altering the activity of a gene product might not simply alter the course of the disease. It is likely that complex diseases arise from a complicated network of interdependent biological changes occurring in a single cell over a period of time. To address this extraordinary biological complexity and to develop adequate therapeutic tools, the pharmaceutical community is directing R&D efforts toward the discovery of new drug combinations or, more likely, multifunctional drugs, namely, single compounds able to interfere with multiple altered pathways.[1,2] Following this latter approach, the so-called "multitarget-directed drug" strategy,[3] we were successful in designing multitargeted agents to hit more than one target underlying the neurodegenerative pathways in Alzheimer's disease.[4] In the present report, we have further extended this paradigm[5] to compounds able to exert different actions beneficial in the treatment of neoplastic disorders, namely, to irreversibly block the epidermal growth factor receptor (EGFR, also known as HER-1) tyrosine kinase and to induce apoptosis in tumor cell lines.

Receptor tyrosine kinases play a crucial role in signal transduction pathways that regulate cell differentiation and proliferation, and their overactivation is strongly associated with carcinogenesis.[6] In this context, the role of EGFR in a wide range of solid tumors has long been appreciated, and therefore, it was one of the first molecular targets to be identified and validated.[7,8] Structurally, EGFR is a 170 kDa glycoprotein that contains an extracellular ligand-binding domain, a transmembrane region, and an intracellular domain with kinase activity.[9] It is one of the four members of the EGFR family, which also includes HER-2 (erbB2), HER-3, and HER-4.

Inhibition of EGFR has been achieved through two main approaches: by blocking ligand binding to the extracellular domain with monoclonal antibodies and by using small-molecule inhibitors that interact at the ATP-binding site.[10] A well-studied class of these latter inhibitors is represented by 4-anilinoquinazolines, exemplified by the lead compound PD153035 (**1**) and erlotinib (**2**; Tarceva, OSI Pharmaceuticals), approved by the FDA for the treatment of advanced non-small-cell lung cancer in November 2004. Further development in this class has led to the discovery of irreversible inhibitors, such as PD168393 (**3**)[11] and canertinib (**4**),[12,13] currently in phase II clinical trials, which both carry, at the 6-position of the quinazoline core, an acrylamide moiety able to form a covalent bond with a Cys residue of the active site (see Chart 1 for structures). This irreversible binding confers a prolonged suppression of receptor activity with a resultant improved therapeutic efficacy and pan-erbB specificity, since an unpaired Cys is conserved among three members of the HER family (Cys773 of erbB-1 or the analogous Cys784 and Cys778 in erbB-2 and erB-4, respectively). Given the multiplicity of erbB receptors' expression in individual tumors, this characteristic could provide irreversible inhibitors with greater efficacy and a broader spectrum of antitumor activity.

However, the modest results of clinical trials to date suggest that targeting EGFR alone may not be sufficient to eradicate established tumors. This limited antitumor efficacy as monotherapy has led researchers to integrate anti-EGFR agents with conventional therapies and to explore combinations with other molecular targeted approaches, which interfere with the alterations in cell function that collectively dictate malignant growth.[14,15]

Oxidative stress induces a cellular redox imbalance, which has been found in various cancer cells when compared to normal cells. The role of antioxidants in the process of carcinogenesis has therefore recently gained considerable attention.[16] α-Lipoic acid (LA), a naturally occurring disulfide molecule, is a powerful antioxidant and has been reported to have beneficial effects in patients with advanced cancer by reducing reactive oxygen species levels and increasing glutathione peroxidase activity.[17,18] Moreover, LA has been shown to trigger apoptosis, another critical parameter impaired in cancer cells, and this induction is selectively exerted in transformed cell lines, whereas no apoptosis in normal cells after treatment with LA has been observed.[19,20] The differential selectivity of the proapoptotic effect, the antioxidant capacity, and the safety of this compound strongly support its use as a potential antineoplastic agent.

On the basis of these considerations, we planned to design anilinoquinazolines **5**−**7** related to **3**, in which the acrylamide group at the 6-position is replaced by a 1,2-dithiolane ring of LA (**8**) or its lower homologues **9** and **10**, allowing different spacers between the pendent disulfide ring and the anilinoquinazoline core (see Figure 1 for design strategy). In principle, the new compounds should retain the activities of parent compounds, such as the ability to covalently bind the Cys residue of EGFR via a thiol−disulfide interchange reaction operated by the dithiolane ring and to exert proapoptotic effects, leading to improved therapeutic potential due to the multiple mechanism of action. The synthesis of quinazolines **5**, **6**, and **7** was accomplished by coupling 6-aminoquinazoline **11**[21] with

† Dedicated to Professor Fulvio Gualtieri on the occasion of his 70th birthday.

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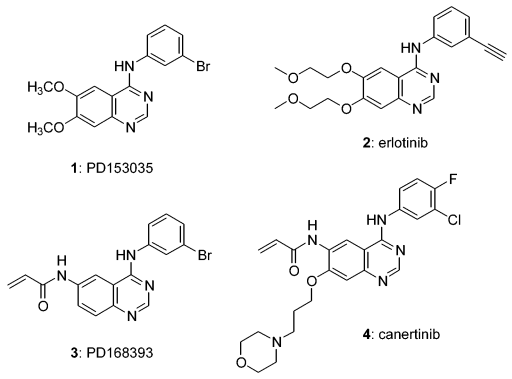
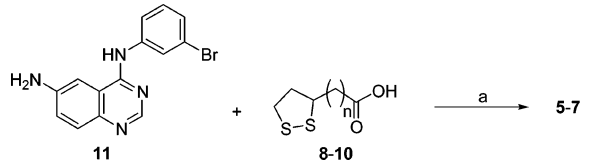
Chart 1. Chemical Structures of Four Previously Reported EGFR Inhibitors



Scheme 1[a]



[a] Reagents and conditions: (a) DMF, EDCI·HCl, 0 °C to room temp, 4 h, flash chromatography.

LA (**8**), 2-(1,2-dithiolan-3-yl)acetic acid (**9**), and 1,2-dithiolane-3-carboxylic acid (**10**), respectively, following a reported procedure (Scheme 1).[22]

The inhibition of the EGFR activity by **5**−**7** was evaluated in human epidermoid carcinoma (A431) cells in comparison with reference compounds **1** and **3**, well-known EGFR reversible and irreversible inhibitors, respectively. A431 cells are known to overexpress EGFR, which leads to continuous activation of the EGFR pathway involved in A431 cell proliferation. Initially, the inhibitory effects on A431 cell proliferation were determined after 72 h of treatment with various concentrations (0.1−30 μM) of the tested compound. The cell proliferation was measured by MTT assay, and the results were expressed as IC_{50} values. As reported in Table 1, treatment of A431 cells with **5**−**7** induced a decrease of the cell proliferation with IC_{50} ranging from 7.90 μM (**7**) to 21.56 μM (**5**), with potency highly dependent on the substitution pattern at the 6-position of the anilinoquinazoline nucleus. In parallel, EGFR tyrosine kinase (EGFR-TK) activity was evaluated by measuring the phosphorylation of synthetic peptide substrate poly(Glu,Tyr)$_{4:1}$ using EGFR isolated from A431 cells[23] after 60 min of incubation with the compounds (0.01−3 μM). As shown in Table 1, **7** was able to completely block the phosphorylation induced by EGFR-TK, with an IC_{50} in the low nanomolar range, which was comparable with that displayed by the reference compounds **1** and **3**, whereas homologues **5** and **6** were only weak inhibitors. Taken together, these results show that lengthening the chain separating the 1,2-dithiolane ring from the anilinoquinazoline nucleus is detrimental to cell proliferation and EGFR-TK activity inhibition (compare **7** with **5** and **6**). Owing to the inhibitory effect on A431 cell proliferation and EGFR-TK activity, **7** was selected for further studies.

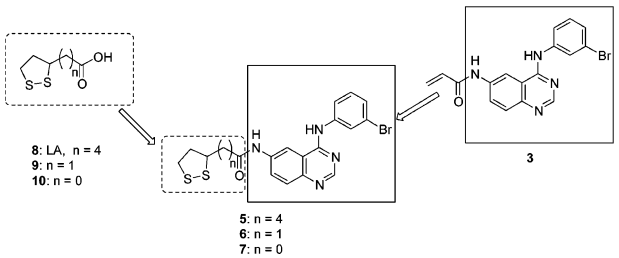


Figure 1. Design strategy for compounds **5**−**7**.

Table 1. Effects of Compounds **1**, **3**, and **5**−**7** on EGFR-TK Activity and A431 Cell Proliferation[a]

compd	IC_{50} (μM),[b] EGFR-TK	IC_{50} (μM),[c] A431
1	0.028 ± 0.004	8.31 ± 2.12
3	0.036 ± 0.005	1.96 ± 0.63
5	>3[d]	21.56 ± 3.35
6	>3[d]	13.70 ± 2.49
7	0.060 ± 0.009	7.90 ± 1.32

[a] The values are the mean ± SD of at least two independent experiments. [b] Concentration of compound resulting in 50% inhibition of EGFR-TK activity. The tyrosine kinase activity of EGFR isolated from A431 cells was determined by the nonradioactive tyrosine kinase activity assay kit (as described in the Supporting Information), after 60 min of incubation with compounds (0.01−3 μM). [c] Concentration of compound resulting in 50% inhibition of A431 cell proliferation. The cell proliferation in A431 cells was determined by the MTT assay (as described in the Supporting Information), after 72 h of incubation with compounds (0.1−30 μM). [d] IC_{50} not determined because less than 50% inhibition was observed at the highest tested concentration (3 μM).

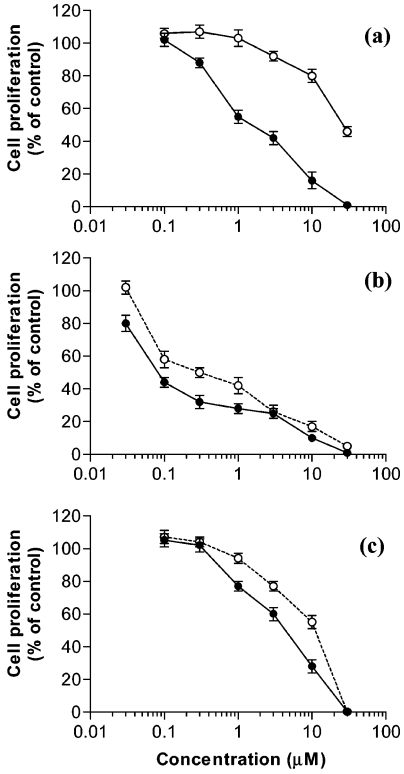


Figure 2. Reversibility of antiproliferative effects of **1** (a), **3** (b), and **7** (c) in A431 cells. Cells were incubated with various concentrations of compounds for 4 h (open circles), after which they were allowed to recover for 68 h in compound-free medium or continuously for 72 h (closed circles). Cell proliferation was determined by MTT assay (as described in the Supporting Information). Results are reported as the mean ± SD of two independent experiments.

To study the reversibility of the antiproliferative effects, A431 cells were treated with **1**, **3**, and **7** for 72 h or for 4 h followed by 68 h of recovery in compound-free medium. As reported in Figure 2, a significant reduction of activity was observed for **1** (IC_{50} = 8.31 vs 26.29 μM), indicating that it induced reversible growth inhibitory activity. In contrast, **3** and **7** showed retention of their activities. This finding supports the view that **7** might react with a sulfydryl residue within the EGFR catalytic domain, via an exchange mechanism. Subsequently, to confirm this

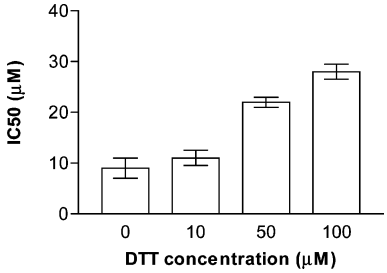


Figure 3. Effect of DTT on the inhibition of A431 cell proliferation by **7**. Cells were incubated with **7** (0.1−30 μM) for 4 h, after which they were allowed to recover for 68 h in compound-free medium with or without DTT. Cell proliferation was determined by the MTT assay (as described in the Supporting Information). At least three independent concentration response curves were done, and the concentration of compound resulting in 50% inhibition of cell proliferation (IC_{50}) was calculated: (∗) $p < 0.05$, (∗∗) $p < 0.01$ (vs untreated samples, ANOVA with Dunnett's test).

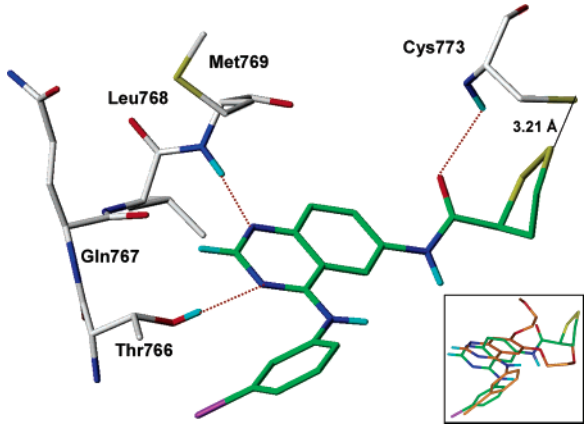


Figure 4. Low-energy docking model of the EGFR-TK/**7** complex. H-bond interactions are shown as dark-red dotted lines. The distance between the sulfur of Cys773 and a sulfur atom of the lipoyl moiety of **7** is displayed. In the inset, the relative orientations of **7**, as the outcome of docking runs, and **2** in the experimental orientation are also shown.

mechanistic rationale, A431 cells were treated with **7** for 4 h, after which they were allowed to recover for 68 h in compound-free medium with or without dithiothreitol (DTT). This reducing agent is able to cleave the intermolecular disulfide bonds at the EGFR level.[24] In addition, the use of DTT on the inhibition of EGFR-TKs by various compounds has been previously described.[25] As shown in Figure 3, increasing concentrations of DTT caused a strong decrease in the inhibition of cell proliferation induced by **7**. In particular, the inhibitory effect was concentration-dependent, with significant differences with respect to basal values ($IC_{50} = 9.23$ μM) at DTT concentrations of 50 μM ($IC_{50} = 22.41$ μM, $p < 0.05$) and 100 μM ($IC_{50} = 28.10$ μM, $p < 0.01$). Higher concentrations completely abolished the inhibition of cell proliferation by **7** (data not shown).

To further investigate the capability of **7** to covalently interact at the EGFR kinase catalytic domain, docking simulations were carried out by means of the GOLD software, using the publicly available coordinate of the EGFR-TK/**2** complex (see also the Supporting Information). One of the best-scored poses is reported in Figure 4, where some main interactions between the ligand and the receptor can be observed. In particular, the sulfur atom of Cys773 is about 3.21 Å from the closest sulfur atom of the lipoic acid moiety, demonstrating that this scaffold is suitably oriented to properly form a covalent S−S bond with Cys773. Moreover, some noncovalent interactions between **7** and the receptor were also identified: (i) the carbonyl oxygen of **7** interacts via H-bond with the amide of the Cys773 backbone; (ii) the N1 of the quinazoline moiety interacts via H-bond with NH of Met769 backbone; (iii) the hydrogen atom of C2 of the quinazoline moiety electrostatically interacts with the carbonyl oxygen of Gln767 backbone; (iv) the N3 established an H-bond with the OH group of Thr766; (v) some hydrophobic interactions mainly with Val702, Met742, and Leu764 are responsible for the stabilization of the aniline moiety of **7**. Furthermore, in Figure 4 the relative orientations of **7**, as the outcome of docking simulations, and **2**, as extracted from the crystallographic complex, are also shown. It can be seen that the two molecules adopt a very similar conformation at the EGFR-TK ATP binding pocket, showing very similar noncovalent interactions. Concerning the size and the hydrophobicity, **7** is smaller than **2**, while its log *P* value is slightly greater than that of the reference compound (estimated log *P* of 5.60 and 4.34, respectively).

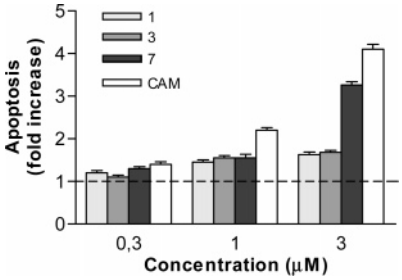


Figure 5. Apoptotic effect of **1**, **3**, and **7** in A431 cells. Cells were treated with various concentrations of compounds (0.3−3 μM) for 24 h, after which apoptosis in terms of DNA fragmentation was determined by the Cell Death Detection ELISA^{plus} (as described in the Supporting Information). As an apoptosis positive control, CAM was assayed in the same range of concentrations. The values in the treated samples are expressed as fold increases with respect to corresponding nontreated controls. Results are reported as the mean ± SD of two independent experiments.

Induction of apoptosis is a highly desirable therapeutic strategy for cancer control. In fact, many anticancer agents act through the induction of apoptosis as a mechanism to suppress cancer development, and proapoptotic effects also characterize the LA spectrum of activity. Moreover, the approach of combining EGFR inhibitors with other targeted agents to augment proapoptotic signaling has been pursued to produce a more significant clinical effect.[26] Therefore, we determined the ability of **1**, **3**, and **7** to induce DNA fragmentation, a hallmark for apoptosis, in A431 cells. In parallel, as an apoptosis-positive control, camptothecin (CAM) was used. As shown in Figure 5, treatment of A431 cells with 0.3−3 μM **7** increased DNA fragmentation in a concentration-dependent manner to a maximum of 2.8-fold. In contrast, lower levels of apoptosis were observed in cells exposed to **1** and **3**, indicating that the 1,2-dithiolane ring has conferred significant proapoptotic properties to **7**. In parallel, the necrosis, in terms of loss of membrane integrity, was determined by measuring lactate dehydrogenase (LDH) release to confirm the apoptotic events; no increase of LDH release was detected at any concentrations of compounds evaluated (data not shown). Together, these results demonstrate that the antiproliferative effects of **7** on A431 cells may arise from its multifunctional mechanism of action. Importantly, as postulated by the multitarget-directed drug design strategy,[3] we have experimental evidence that each of the pharmacophoric moieties associated with the molecular frame of **7** imparts the multifunctional ability for this agent; the anilinoquinazoline core allows molecular recognition of the receptor site, whereas the

lipoyl fragment is responsible for the proapoptotic properties and for the irreversible binding. Moreover, the use of dithiolane as Cys labeling group has inherent advantages over the highly reactive acrylamide. In fact, reaction of the inhibitor with nonspecific targets other than the thiol of the Cys residue should be reduced, and consequently, its tumor availability should be greatly enhanced.

In conclusion, we have further demonstrated the power of multifunctional drug design strategy to discover drugs to combat various diseases. To this end, following a similar approach (the "combi-targeting" concept), some nitrosourea derivatives capable of blocking EGFR and alkylating DNA were recently synthesized and selected by the U.S. National Cancer Institute for further development.[27] We believe this may represent the future direction of the drug discovery process.

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Supporting Information Available: Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Zhang, H. Y. One-compound-multiple-targets strategy to combat Alzheimer's disease. *FEBS Lett.* **2005**, *579*, 5260−5264.
(2) Keith, C. T.; Borisy, A. A.; Stockwell, B. R. Multicomponent therapeutics for networked systems. *Nat. Rev. Drug Discovery* **2005**, *4*, 71−78.
(3) Bolognesi, M. L.; Minarini, A.; Tumiatti, V.; Melchiorre, C. Lipoic acid, a lead structure for multi-target-directed drugs for neurodegeneration. *Mini-Rev. Med. Chem.*, in press.
(4) Rosini, M.; Andrisano, V.; Bartolini, M.; Bolognesi, M. L.; Hrelia, P.; Minarini, A.; Tarozzi, A.; Melchiorre, C. Rational approach to discover multipotent anti-Alzheimer drugs. *J. Med. Chem.* **2005**, *48*, 360−363.
(5) Matheson, S. L.; McNamee, J.; Jean-Claude, J. Design of a chimeric 3-methyl-1,2,3-triazene with mixed receptor tyrosine kinase and DNA damaging properties: a novel tumor targeting strategy. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 832−840.
(6) Arora, A.; Scholar, E. M. Role of tyrosine kinase inhibitors in cancer therapy. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 971−979.
(7) Ranson, M. Epidermal growth factor receptor tyrosine kinase inhibitors. *Br. J. Cancer* **2004**, *90*, 2250−2255.
(8) El-Rayes, B. F.; LoRusso, P. M. Targeting the epidermal growth factor receptor. *Br. J. Cancer* **2004**, *91*, 418−424.
(9) Wells, A. EGF receptor. *Int. J. Biochem. Cell Biol.* **1999**, *31*, 637−643.
(10) Cohen, R. B. Epidermal growth factor receptor as a therapeutic target in colorectal cancer. *Clin. Colorectal Cancer* **2003**, *2*, 246−251.
(11) Fry, D. W.; Bridges, A. J.; Denny, W. A.; Doherty, A.; Greis, K. D.; Hicks, J. L.; Hook, K. E.; Keller, P. R.; Leopold, W. R.; Loo, J. A.; McNamara, D. J.; Nelson, J. M.; Sherwood, V.; Smaill, J. B.; Trumpp-Kallmeyer, S.; Dobrusin, E. M. Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12022−12027.
(12) Smaill, J. B.; Rewcastle, G. W.; Loo, J. A.; Greis, K. D.; Chan, O. H.; Reyner, E. L.; Lipka, E.; Showalter, H. D.; Vincent, P. W.; Elliott, W. L.; Denny, W. A. Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)-quinazoline- and 4-(phenylamino)pyrido[3,2-*d*]pyrimidine-6-acrylamides bearing additional solubilizing functions. *J. Med. Chem.* **2000**, *43*, 1380−1397.
(13) Allen, L. F.; Eiseman, I. A.; Fry, D. W.; Lenehan, P. F. CI-1033, an irreversible pan-erbB receptor inhibitor and its potential application for the treatment of breast cancer. *Semin. Oncol.* **2003**, *30*, 65−78.
(14) Grandis, J. R.; Sok, J. C. Signaling through the epidermal growth factor receptor during the development of malignancy. *Pharmacol. Ther.* **2004**, *102*, 37−46.
(15) Baselga, J.; Arteaga, C. L. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J. Clin. Oncol.* **2005**, *23*, 2445−2459.
(16) Valko, M.; Rhodes, C. J.; Moncol, J.; Izakovic, M.; Mazur, M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* **2006**, *160*, 1−40.
(17) Mantovani, G.; Maccio, A.; Madeddu, C.; Mura, L.; Gramignano, G.; Lusso, M. R.; Murgia, V.; Camboni, P.; Ferreli, L.; Mocci, M.; Massa, E. The impact of different antioxidant agents alone or in combination on reactive oxygen species, antioxidant enzymes and cytokines in a series of advanced cancer patients at different sites: correlation with disease progression. *Free Radical Res.* **2003**, *37*, 213−223.
(18) Mantovani, G.; Maccio, A.; Madeddu, C.; Mura, L.; Massa, E.; Gramignano, G.; Lusso, M. R.; Murgia, V.; Camboni, P.; Ferreli, L. Reactive oxygen species, antioxidant mechanisms, and serum cytokine levels in cancer patients: impact of an antioxidant treatment. *J. Environ. Pathol., Toxicol. Oncol.* **2003**, *22*, 17−28.
(19) van de Mark, K.; Chen, J. S.; Steliou, K.; Perrine, S. P.; Faller, D. V. Alpha-lipoic acid induces p27Kip-dependent cell cycle arrest in non-transformed cell lines and apoptosis in tumor cell lines. *J. Cell. Physiol.* **2003**, *194*, 325−340.
(20) Wenzel, U.; Nickel, A.; Daniel, H. alpha-Lipoic acid induces apoptosis in human colon cancer cells by increasing mitochondrial respiration with a concomitant $O_2^{-\bullet}$-generation. *Apoptosis* **2005**, *10*, 359−368.
(21) Tsou, H. R.; Mamuya, N.; Johnson, B. D.; Reich, M. F.; Gruber, B. C.; Ye, F.; Nilakantan, R.; Shen, R.; Discafani, C.; DeBlanc, R.; Davis, R.; Koehn, F. E.; Greenberger, L. M.; Wang, Y. F.; Wissner, A. 6-Substituted-4-(3-bromophenylamino)quinazolines as putative irreversible inhibitors of the epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER-2) tyrosine kinases with enhanced antitumor activity. *J. Med. Chem.* **2001**, *44*, 2719−2734.
(22) Antonello, A.; Hrelia, P.; Leonardi, A.; Marucci, G.; Rosini, M.; Tarozzi, A.; Tumiatti, V.; Melchiorre, C. Design, synthesis, and biological evaluation of prazosin-related derivatives as multipotent compounds. *J. Med. Chem.* **2005**, *48*, 28−31.
(23) Varkondi, E.; Schafer, E.; Bokonyi, G.; Gyokeres, T.; Orfi, L.; Petak, I.; Pap, A.; Szokoloczi, O.; Keri, G.; Schwab, R. Comparison of ELISA-based tyrosine kinase assays for screening EGFR inhibitors. *J. Recept. Signal Transduction Res.* **2005**, *25*, 45−56.
(24) Kamata, H.; Shibukawa, Y.; Oka, S. I.; Hirata, H. Epidermal growth factor receptor is modulated by redox through multiple mechanisms. Effects of reductants and H_2O_2. *Eur. J. Biochem.* **2000**, *267*, 1933−1944.
(25) Showalter, H. D.; Sercel, A. D.; Leja, B. M.; Wolfangel, C. D.; Ambroso, L. A.; Elliott, W. L.; Fry, D. W.; Kraker, A. J.; Howard, C. T.; Lu, G. H.; Moore, C. W.; Nelson, J. M.; Roberts, B. J.; Vincent, P. W.; Denny, W. A.; Thompson, A. M. Tyrosine kinase inhibitors. 6. Structure−activity relationships among N- and 3-substituted 2,2′-diselenobis(1*H*-indoles) for inhibition of protein tyrosine kinases and comparative in vitro and in vivo studies against selected sulfur congeners. *J. Med. Chem.* **1997**, *40*, 413−426.
(26) Ali, S.; El-Rayes, B. F.; Sarkar, F. H.; Philip, P. A. Simultaneous targeting of the epidermal growth factor receptor and cyclooxygenase-2 pathways for pancreatic cancer therapy. *Mol. Cancer Ther.* **2005**, *4*, 1943−1951.
(27) Domarkas, J.; Dudouit, F.; Williams, C.; Qiyu, Q.; Banerjee, R.; Brahimi, F.; Jean-Claude, B. J. The combi-targeting concept: synthesis of stable nitrosoureas designed to inhibit the epidermal growth factor receptor (EGFR). *J. Med. Chem.* **2006**, *49*, 3544−3552.

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