Bioorganic & Medicinal Chemistry Letters 23 (2013) 4990–4995

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and cytotoxic activity evaluation of 2,3-thiazolidin-4-one derivatives on human breast cancer cell lines

Marina Sala [a,†], Adele Chimento [b,†], Carmela Saturnino [a], Isabel M. Gomez-Monterrey [c], Simona Musella [d], Alessia Bertamino [a], Ciro Milite [a], Maria Stefania Sinicropi [b], Anna Caruso [b], Rosa Sirianni [b], Paolo Tortorella [e], Ettore Novellino [c], Pietro Campiglia [a,*], Vincenzo Pezzi [b,*]

[a] Department of Pharmaceutical Science, Division of Biomedicine, University of Salerno, Fisciano, SA 84084, Italy
[b] Department of Pharmacy, Health and Nutrition Sciences, University of Calabria, Arcavacata di Rende, Cosenza 87036, Italy
[c] Department of Pharmaceutical and Toxicological Chemistry, University of Naples "Federico II", Naples 8013, Italy
[d] Department Pharmaco-Biological, University of Messina, 98168 Messina, Italy
[e] Department Pharmaceutical Chemistry, University of Bari "Aldo Moro", 70125 Bari, Italy

ARTICLE INFO

Article history:
Received 24 April 2013
Revised 11 June 2013
Accepted 16 June 2013
Available online 26 June 2013

Keywords:
Resveratrol analogs anticancer drugs
2,3-Thiazolidin-4-one derivatives
Polyphenol
Breast cancer cells
MCF-7
SKBR3

ABSTRACT

It is well known that resveratrol (RSV) displayed cancer-preventing and anticancer properties but its clinical application is limited because of a low bioavailability and a rapid clearance from the circulation. Aim of this work was to synthesize pharmacologically active resveratrol analogs with an enhanced structural rigidity and bioavailability. In particular, we have synthesized a library of 2,3-thiazolidin-4-one derivatives in which a thiazolidinone nucleus connects two aromatic rings. Some of these compounds showed strong inhibitory effects on breast cancer cell growth. Our results indicate that some of thiazolidin-based resveratrol derivatives may become a new potent alternative tool for the treatment of human breast cancer.

Epidemiological and current laboratory studies suggest that consumption of certain types of fruits and vegetables, containing phytochemicals, is associated with reduced cancer risk.[1] Furthermore, it is postulated that dietary phytochemicals can function as chemopreventive and/or adjuvant chemotherapeutic agents. One such phytochemical is resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) (RSV), (Fig. 1) a naturally occurring phytoalexin, readily available in the diet and a lot of health-promoting effects have been ascribed to it.

Resveratrol, first identified as a bioactive compound in 1992, is found in several plants, particularly in the skin of red grapes.[2]

This compound has elicited much attention in recent years, as a potential anticancer agent, since its inhibitory effect on carcinogenic processes (initiation, promotion, and progression) was first reported in 1997.[3] Thereafter extensive studies have verified the cancer-preventing and anticancer properties of resveratrol in various murine models of human cancer, including skin cancer (both chemically and ultraviolet B-induced), gastric and colorectal cancer, lung cancer, breast cancer, ovarian and prostate cancer, hepatoma, neuroblastoma, fibrosarcoma, pancreatic cancer, and leukemia.[4] Several studies, using both in vitro and in vivo model systems, have illustrated resveratrol's capacity to modulate a multitude of signaling pathways associated with cellular growth and division, apoptosis, angiogenesis, invasion, and metastasis.[5]

In particular, it exhibits an action in both hormone-sensitive and hormone-resistant breast cancer cells and shows cytostatic activity and determines cell growth arrest; these properties seem to be related to regulation of xenobiotic carcinogen metabolism and antiinflammatory, antiproliferative, and pro-apoptotic effects.[6] The phytoestrogenic character of RSV was confirmed by its capacity to bind and activate α- and β-estrogen receptors (ERs) regulating transcription of estrogen-responsive target genes. However,

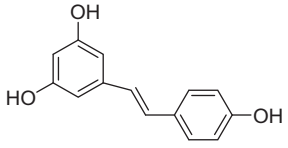


Figure 1. Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) (RSV).

* Corresponding authors. Fax: +39 089 969353 (P.C.); fax: +39 0984 493271 (V.P.).
E-mail addresses: pcampigl@unisa.it (P. Campiglia), v.pezzi@unical.it (V. Pezzi).
† These authors equally contributed to this work.

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.06.051

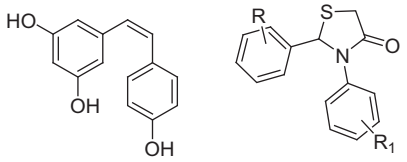
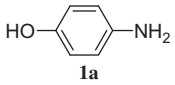
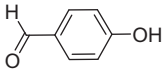
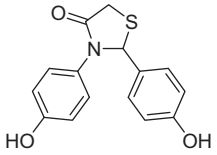
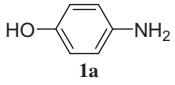
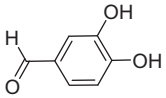
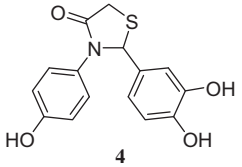
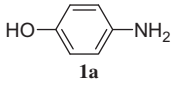
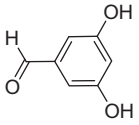
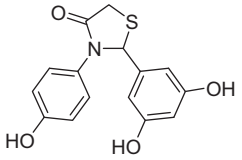
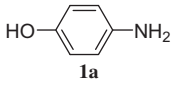
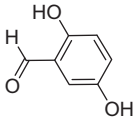
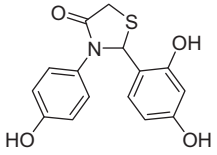
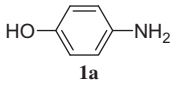
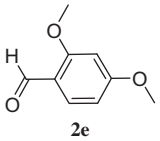
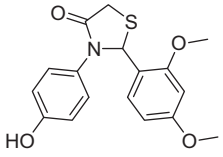
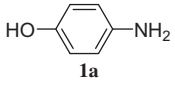
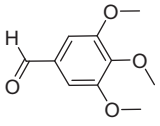
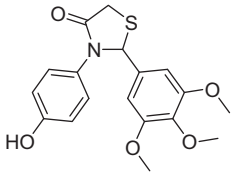
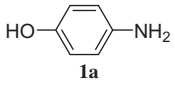
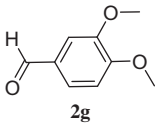
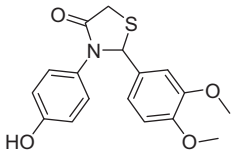


Figure 2. Structure of *cis*-resveratrol (**I**) and a *cis*-conformation mimetic of resveratrol containing an thiazolidin-4-one moiety.

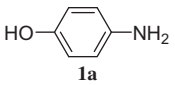
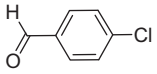
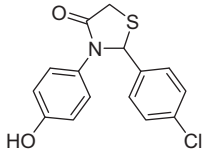
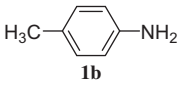
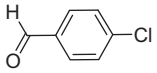
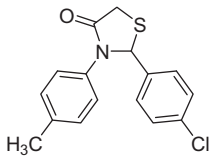
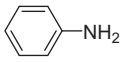
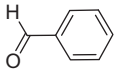
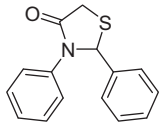
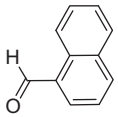
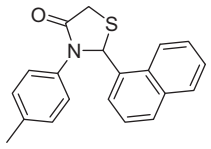
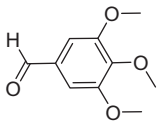
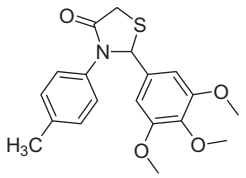
although a number of studies have been conducted, the effects of RSV on ERs remain controversial. For example, with MCF-7 cells in culture, Gehm et al.[7] showed that RSV (3–10 μM) is a superagonist when combined with estradiol (E2), while Lu and Serrero[8] reported ER antagonism of RSV (5 μM) in the presence of E2 and partial agonism in its absence.[8] Bowers et al.[9] observed partial to full agonism in CHO-K1 cells transfected with ERα or ERβ and reporter genes based on various estrogen receptor element (EREs). The authors showed that RSV (100 μM) acts as a mixed agonist/antagonist in cells transiently transfected with ER and mediates higher transcriptional activity when bound to ERβ than to ERα. Moreover, RSV showed antagonist activity with ERα, but not with ERβ.[9] Based on these reports, it appears that the ability of RSV to act as an ER agonist varies between different cell types and dosage. Resveratrol acts as an estrogen-agonist or antagonist that depends

Table 1
Library of synthesized 2,3-thiazolidin-4-one (**3**–**14**)

Entry	Arylamine	Aryl-aldehyde	2,3-Thiazolidin-4-one derivative	Yield (%)
1	 **1a**	 **2a**	 **3**	93
2	 **1a**	 **2b**	 **4**	47
3	 **1a**	 **2c**	 **5**	63
4	 **1a**	 **2d**	 **6**	80
5	 **1a**	 **2e**	 **7**	85
6	 **1a**	 **2f**	 **8**	90
7	 **1a**	 **2g**	 **9**	90

(*continued on next page*)

Table 1 (*continued*)

Entry	Arylamine	Aryl-aldehyde	2,3-Thiazolidin-4-one derivative	Yield (%)
8	 **1a**	 **2h**	 **10**	90
9	 **1b**	 **2h**	 **11**	56
10	 **1c**	 **2i**	 **12**	80
11	 **1b**	 **2j**	 **13**	48
12	 **1b**	 **2f**	 **14**	85

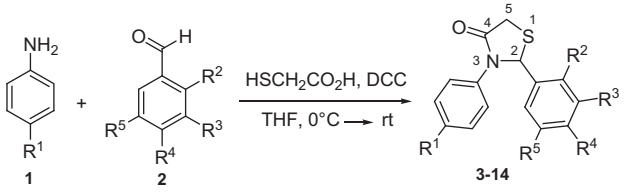
upon the relative abundance of ER-α and -β and the *cis*-regulatory sequences they target.[10] Furthermore, resveratrol was also shown to inhibit the proliferation of the estrogen-receptor negative human breast carcinoma cell line, MDA-MB-468, by inhibiting the levels of autocrine growth stimulators.[11]

Although the anticancer effects of resveratrol have been demonstrated, its clinical application is limited because of a low bioavailability and a rapid clearance from the circulation.[12] In fact, in humans, three metabolic pathways have been identified, that is, sulfate and glucuronic acid conjugation of the phenolic groups and hydrogenation of the aliphatic double bond, the latter likely produced by the intestinal microflora.[13] Extremely rapid sulfate conjugation by the intestine/liver appears to be the rate-limiting step in resveratrol's bioavailability. Virtually no unconjugated resveratrol was detected in urine or serum samples, which could have implications regarding the significance of in vitro studies that used only unconjugated resveratrol.

The structural alteration of the stilbene moiety of resveratrol has been found to be a promising strategy for generation of several synthetic analogs with improved pharmacokinetic parameters. Moreover, the structure-activity studies have shown that methoxy groups added to the trihydroxystilbene scaffold of resveratrol, significantly potentiated its cytotoxic activity.[14] In view of the significant biological and anticancer potential of RSV, we considered its structure as lead compound for the design and synthesis of small molecules with structural rigidity, enhance bioavailability and antitumoral activity. Recently, Mayhoub et al.[15] described a series of 2,3-thiadiazol analogs of resveratrol using a strategy by the replacement of the alkene linker between the two aromatic rings with a heterocyclic system. This approach keeps the geometry of these rings relatively unchanged and close to that of the *cis* stilbene template. Now, we prepared a library of 2,3-diaryl-4-thiazolidinone derivatives in which a thiazolidin-4-one nucleus connects two aromatic rings (Fig. 2).

This design results in an increase in the structural rigidity of the new analogs, fixing the *cis*-conformation of resveratrol. Modifications on both aryl moieties have been also considered in order to define which functional groups are critical for cell proliferation control. These variations could lead to new restricted conformation analogs of resveratrol with higher cytotoxic activity that this and, hence, higher ability to inhibit the growth of cancer cells in vitro.

The synthesis of designed compounds[16] (**3**–**14**, Table 1) was carried out by condensation of anilines (**1**) and aldehydes (**2**) with thioglycolic acid in THF using *N*,*N*-dicyclohexylcarbodiimide (DCC) as a dehydrating agent (Scheme 1). This protocol, described by Srivastava et al.[17] has the advantage of mild reaction conditions,



Scheme 1. Synthesis of 2,3-thiazolidin-4-one derivates (**3**–**14**).

a very short reaction time (1 h) and product formation in high yields (47–93%).

We evaluated the effects on cell proliferation of these new derivatives against estrogen receptor positive (ER+) MCF-7 (Fig. 3) and estrogen receptor negative (ER−) SKBR3 (Fig. 4) human breast cancer cell line at different concentrations (0.1, 1, 10 μM) using the MTT assay.[18]

Therefore, we treated for 72 h MCF-7 and SKBR3 cells with each compounds and also with RSV in order to compare the anticancer effects of the chemicals used to this well-known anticancer agent.

In MCF7 cells, **5**, **7**–**8** (Fig. 3a), **9**–**12** (Fig. 3b) determined a clear dose-response inhibitory effect on cell growth. Low doses of**4** (Fig. 3a) and **13** (Fig. 3b) elicited an inhibition while higher doses do not favor it. **14** (Fig. 3b) decreased cell viability only at 1 and 10 μM concentrations. As showed in Figure 3b, the compounds **9** and **10** are more active.

In fact, the most interesting results were obtained for **9** and **10** characterized by the presence of –OMe groups in R_3 and R_4 and of an chlorine atom on the aromatic ring bonded at C-2 position, of the thiazolidinone moiety, respectively, as evidenced by half-maximal inhibitory concentration (IC_{50}) values (Table 2).

In particular, the thiazolidin-4-one derivative **9** showed the best pattern of dose-dependent inhibition among substance tested with an IC_{50} of 2.58 μM, value much more relevant respect to RSV (IC_{50} = 28.07 μM).

In SKBR3 cells, **3**–**8** (Fig. 4a) and **9**–**11** (Fig. 4b) did not change cell proliferative behavior while only 10 μM of **5**, **8**–**11** decreased

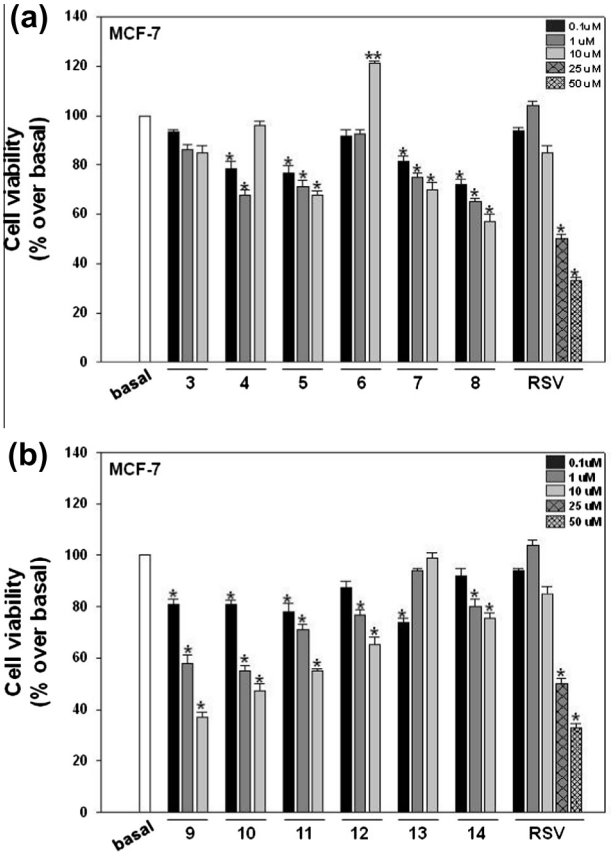


Figure 3. Effects of different doses of 2,3-thiazolidin-4-one derivatives on MCF-7 cell proliferation. Cells treated for 72 h with the indicated concentrations of compounds **3**–**8** (a), **9**–**14** (b) and RSV (a and b). Cell viability was evaluated by MTT assay. Statistically significant differences are indicated. Columns, mean of three independent experiments each performed with triplicate samples expressed as percent of basal; bars, SD. (*,**$P < 0.01$ compared with basal).

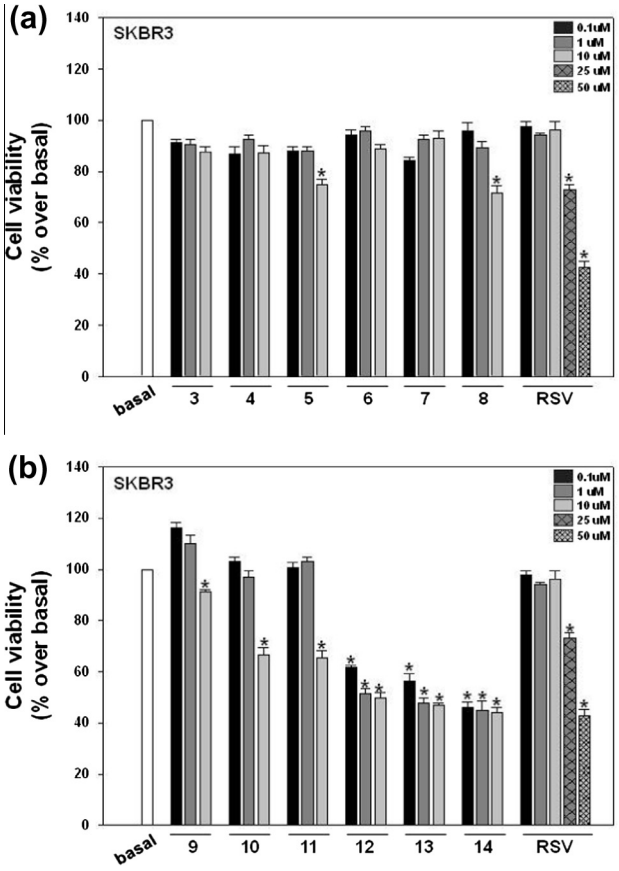


Figure 4. Effects of different doses of 2,3-thiazolidin-4-one derivatives on SKBR3 cell proliferation. Cells treated for 72 h with the indicated concentrations of compounds **3**–**8** (a), **9**–**14** (a) and RSV (a and b). Cell viability was evaluated by MTT assay. Statistically significant differences are indicated. Columns, mean of three independent experiments each performed with triplicate samples expressed as percent of basal; bars, SD. (*$P < 0.01$ compared with basal).

significantly cell viability. Among all tested compounds in SKBR3 cells particularly **12**–**14** (Fig. 4b) determined a significant inhibition starting from the lowest dose. As showed in Figure 4 the presence of a phenyl unsubstituted (**12**), a naphthyl (**13**) or a trimethoxy-phenyl groups (**14**) at C2 position of the thiazolidinonic core were important for the inhibitory activity as indicated by IC_{50} values (Table 3).

In particular, the derivative **14** showed the best pattern of dose-dependent inhibition among tested substances with an IC_{50} of

Table 2
IC_{50} of resveratrol and its derivatives **9**–**10** for MCF-7 cells on cell viability

Compounds	IC_{50} (μM)	95% Confidence interval
9	2.58	1.85–3.6
10	5	2.14–11.73
RSV	28.07	23.85–33.04

Table 3
IC_{50} of resveratrol and its derivatives **12**–**14** for SKBR-3 cells on cell viability

Compounds	IC_{50} (μM)	95% Confidence interval
12	0.81	0.19–3.53
13	0.25	0.06–1.1
14	0.23	0.06–0.94
RSV	41.42	34.59–49.61

0.23 μM, a value much more relevant respect to IC_{50} value of RSV (41.42 μM).

It is evident that the dose at which RSV shows antiproliferative effects on MCF7 and SKBR3 cells is relatively higher (>10 μM) (Tables 2 and 3)[23] compared to that of the tested compounds.

In addition, a control experiment using 3T3 mouse embryonic fibroblast cells has been performed; no effects on cell viability was obtained using all thiazolidin-based resveratrol derivatives of 0.1 μM from to 10 μM after 72 h of treatment (data not shown), suggesting that these compounds have specific inhibitory effect on breast cancer cells.

Because of the widespread chemopreventive and chemotherapeutic applications of resveratrol, a strong demand exists to search for other pharmacologically active resveratrol analogs with enhanced potency and selectivity. The findings arising from the studies described in this work could open a possible approach to the design and development of new restricted resveratrol analogs, such as 2,3-thiazolidin-4-one derivatives,[24] as anticancer agents for the treatment of human breast cancer. In this effort, we have identified **9**–**10** compounds as potent anticancer agents against MCF-7 breast cancer cells and **12**–**14** compounds with cytotoxic activity on SKBR3 cells. It was demonstrated that changes in the structure of the RSV derivatives may be responsible for the different ERα-mediates biological responses observed in estrogen-sensitive cancer cells.[25] Moreover, the different inhibitory effects of the **9**, **10**, **12**, **13** and **14** compounds in the two breast tumor cell lines may suggest that the biological action of these molecules could be also influenced by the different estrogenic receptor pattern. In particular, in ER positive MCF-7 cells **9**–**10** compounds could interfere with ERα-dependent pathway, while in ER negative and GPER positive SKBR3 cells **12**, **13** and **14** compounds could antagonize with GPER-dependent pathway that is involved in E2 dependent SKBR3 cell growth.[26,27] This last aspect is currently under investigation in our laboratory.

Our data outline a promising perspective: these thiazolidin-based resveratrol derivatives may become an alternative tool to modulate complex signal transduction pathways and interfere with their activation in cancer. Modifications of the two structural aromatic domains on the thiazolidin-4-one core aimed to identify more potent and selective antitumor agents are currently underway. Further experiments are needed to clarify the molecular mechanism involved in the growth responses to the tested compounds and to evaluate in vivo bioavailability and chemotherapeutic potential.

Acknowledgments

This work was supported by grants from the Italian Ministry of Education (MIUR) (PRIN n° 20098SJX4F) and by Associazione Italiana per la Ricerca sul Cancro (AIRC) project n. IG10344.

References and notes

1. Sporn, M. B.; Suh, N. *Nat. Rev. Cancer* **2002**, *2*, 537.
2. Baur, J. A.; Sinclair, D. A. *Nat. Rev. Drug Disc.* **2006**, *5*, 493.
3. Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W.; Fong, H. H.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. *Science* **1997**, *275*, 218.
4. (a) Joe, A. K.; Liu, H.; Suzui, M.; Vural, M. E.; Xiao, D.; Weinstein, I. B. *Clin. Cancer Res.* **2002**, *8*, 893; Athar, M.; Back, J. H.; Tang, X.; Kim, K. H.; Kopelovich, L.; Bickers, D. R.; Kim, A. L. *Toxicol. Appl. Pharmacol.* **2007**, *224*, 274.
5. (a) Kundu, J. K.; Surh, Y. J. *Cancer Lett.* **2008**, *269*, 243; (b) Athar, M.; Back, J. H.; Kopelovich, L.; Bickers, D. R.; Kim, A. L. *Arch. Biochem. Biophys.* **2009**, *486*, 95.
6. Le Corre, L.; Chalabi, N.; Delort, L.; Bignon, Y. J.; Bernard-Gallon, D. J. *Mol. Nutr. Food Res.* **2005**, *49*, 462.
7. Gehm, B. D.; McAndrews, J. M.; Chien, P. Y.; Jameson, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14138.
8. Lu, R.; Serrero, G. *J. Cell. Physiol.* **1999**, *179*, 297.
9. Bowers, J. L.; Tyulmenkov, V. V.; Jernigan, S. C.; Klinge, C. M. *Endocrinology* **2000**, *141*, 3657.
10. Gougelet, A.; Mueller, S. O.; Korach, K. S.; Renoir, J. M. *J. Steroid Biochem. Mol. Biol.* **2007**, *104*, 110.
11. Serrero, G.; Lu, R. *Antioxid. Redox Signal.* **2001**, *3*, 969.
12. Walle, T.; Hsieh, F.; DeLegge, M. H.; Oatis, J. E., Jr.; Walle, U. K. *Drug Metab. Dispos.* **2004**, *32*, 1377.
13. (a) De Santi, C.; Pietrabissa, A.; Mosca, F.; Pacifici, G. M. *Xenobiotica* **2000**, *30*, 1047; (b) De Santi, C.; Pietrabissa, A.; Spisni, R.; Mosca, F.; Pacifici, G. M. *Xenobiotica* **2000**, *30*, 609; (c) Yu, C. W.; Shin, Y. G.; Chow, A.; Li, Y. M.; Kosmeder, J. W.; Lee, Y. S.; Hirschelman, W. H.; Pezzuto, J. M.; Mehta, R. G.; Van Breemen, R. B. *Pharm. Res.* **1907**, *2002*, 19.
14. (a) Lee, S. K.; Nam, K. A.; Hoe, Y. H.; Min, H. Y.; Kim, E. Y.; Ko, H.; Song, S.; Lee, T.; Kim, S. *Arch. Pharm. Res.* **2003**, *26*, 253; (b) Roberti, M.; Pizzirani, D.; Simoni, D.; Rondanin, R.; Baruchello, R.; Bonora, C.; Buscemi, F.; Grimaudo, S.; Tolomeo, M. *J. Med. Chem.* **2003**, *46*, 3546.
15. Mayhoub, A. S.; Marler, L.; Kondratyuk, T. P.; Park, E. J.; Pezzuto, J. M.; Cushman, M. *Bioorg. Med. Chem.* **2012**, *20*, 510.
16. Experimental section: Reagents, starting material and solvents were purchased from Sigma–Aldrich (Milano, Italy) and used as received. Analytical TLC was performed on plates coated with a 0.25 mm layer of silica gel 60 F254 Merck and preparative TLC on 20 × 20 cm glass plates coated with a 2 mm layer of silica gel PF254 Merck. Silica gel 60 (300–400 mesh, Merck) was used for flash chromatography. Melting points were measured with a Köfler apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded with a Bruker 300 spectrometer operating at 300 and 100 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si and J values are reported in Hz. Mass spectra were obtained using a ESI mass spectrometer: Finnigan LCQ Advantage max (Thermo Finnigan;San Jose, CA, USA).
General Procedure for the synthesis of 2,3-diaryl-thiazolidin-4-ones. Arylamine (1 mmol) and arylaldehyde (2 equiv) appropriately substituted were stirred in THF at 0 °C for 5 min, then thioglycolic acid (3 equiv) was added. After 5 min *N*,*N*-dicyclohexylcarbodiimide (DCC) (1.3 equiv) was added to the reaction mixture at 0 °C and it was stirred for additional 50 min at room temperature. DCC was removed by filtration and the filtrate was concentrated to dryness under reduced pressure and the residue was taken up in ethyl acetate. The organic layer was successively washed with 10% aq. citric acid, water, 10% aq. sodium hydrogen carbonate and finally with brine. Finally, the organic layer was dried over sodium sulphate anhydrous and the solvent was removed under reduced pressure to get a crude product that was purified by column chromatography on silica gel using *n*-hexane/ethyl acetate 4:1 as eluent.
2,3-Bis-(4-hydroxy-phenyl)-thiazolidin-4-one (**3**). White solid. Yield 93%. Mp 230–231 °C. ^1H NMR (300 MHz, CD_3OD): δ 3.87 (d, J = 12.3 Hz, 1H), 3.95 (d, J = 12.3 Hz, 1H), 6.08 (s, 1H), 6.70-6.73 (m, 4H), 6.93 (d, J = 6.6 Hz, 2H), 7.20 (d, J = 6.4 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 33.5, 72.6, 115.8, 116.3, 123.0, 129.8, 132.0, 134.3, 154.1, 156.9, 171.2. ESI m/z calcd for $C_{15}H_{13}NO_3S$: 287.06; found: 286.10.
2-(3,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl)thiazolidin-4-one (**4**). White solid. Yield 47%. Mp 214–215 °C. ^1H NMR (300 MHz, CD_3OD): δ 3.77 (d, J = 15.7 Hz, 1H), 3.87 (d, J = 15.7 Hz, 1H), 6.00 (s, 1H), 6.19–6.21 (m, 2H), 6,90 (s, 1H) 6.93 (d, J = 6.6 Hz, 2H), 7.20 (d, J = 6.4 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 33.5, 72.9, 115.6, 115.8, 116.1, 123.1, 133.2, 134.3, 146.0, 154.1, 171.2. ESI m/z calcd for $C_{15}H_{13}NO_4S$: 303.06; found 304.31.
2-(3,5-Dihydroxyphenyl)-3-(4-hydroxyphenyl)thiazolidin-4-one (**5**). White solid. Yield 63%. Mp. 214–215 °C. ^1H NMR (300 MHz, CD_3OD): δ 3.80 (d, J = 15.7 Hz, 1H), 3.94 (d, J = 15.7 Hz, 1H), 5.96 (s, 1H) 6.00 (s, 1H), 6.26–6.29 (m, 2H), 6.73–6.76 (m, 2H), 6.97–6.99 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 33.5, 73.2, 102.0, 107.1, 116.1, 123.2, 134.3, 142.0, 155.1, 158.4, 171.2. ESI m/z calcd for $C_{15}H_{13}NO_4S$: 303.06; found 304.12.
2-(2,4-Dihydroxy-phenyl)-3-(4-hydroxy-phenyl)-thiazolidin-4-one (**6**). White solid. Yield 80%. Mp 214–215 °C. ^1H NMR (300 MHz, CD_3OD): δ 3.84 (d, J = 15.6 Hz, 1H), 3.97 (d, J = 15.7, 1H), 6.00 (s, 1H), 6.39–6.67 (m, 3H), 6,82–6,93 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD): δ 33.5, 72.9, 102.0, 107.1, 116.1, 123.2, 134.3, 145.6, 154.1, 171.2. ESI m/z calcd for $C_{15}H_{13}NO_4S$: 303.06; found 304.09.
3-(4-Hydroxyphenyl)2-(2,4-dimethoxyphenyl)thiazolidin-4-one (**7**). White solid. Yield 85%, Mp. 234–235 °C. ^1H NMR (300 MHz, DMSO): δ 3.76 (s, 3H), 3.82 (s, 3H), 3.93 (d, J = 14.47 Hz, 1H) 3.99 (d, J = 15.39 Hz, 1H), 6.45 (s, 1H), 6.63–6.65 (m, 1H) 6.66–6.69 (m, 2H), 6.98–6.95 (m, 2H), 7.15–7.12 (m, 2H). ^{13}C NMR (75 MHz, DMSO): δ 33.5, 55.8, 56.1, 66.7, 101.0, 107.1, 108.8, 116.1, 123.2, 134.3, 154.1, 160.2, 171.2. ESI m/z calcd for $C_{17}H_{17}NO_4S$: 331.09; found 332.12.
3-(4-Hydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)thiazolidin-4-one (**8**). White solid. Yield 90%. Mp. 240–241 °C. ^1H NMR (300 MHz, DMSO): δ 3.78 (s, 9H), 3.96 (d, J = 15.39, 1H), 4.01 (d, J = 15.47, 1H), 5.89 (s, 1H), 6.49–6.56 (m, 4H), 6.82 (s, 2H). ^{13}C NMR (75 MHz, DMSO): δ 33.5, 56.1, 60.8, 73.2, 106.1, 116.1, 123.2, 133.1, 134.3, 137.6, 152.8, 154.1, 171.2. ESI m/z calcd for $C_{18}H_{19}NO_5S$: 361.10; found 362.10.
3-(4-Hydroxyphenyl)-2-(3,4-dimethoxyphenyl)thiazolidin-4-one (**9**). White solid. Yield 90%. Mp. 240–241 °C. ^1H NMR (300 MHz, DMSO): δ 3.79 (s, 6H), 3.93 (d, J = 15,39, 1H), 3.95 (d, J = 15.47, 1H), 6.16 (s, 1H), 6.70–6.78 (m, 2H), 6.82–6.97 (m, 5H). ^{13}C NMR (75 MHz, DMSO): δ 33.5, 56.1, 72.9, 112.3, 113.8, 116.1, 122.3, 123.2, 132.5, 134.3, 148.2, 198.7, 154.1, 171.2. ESI m/z calcd for $C_{17}H_{17}NO_4S$: 331.09; found 332.30.
2-(4-Chlorophenyl)-3-(4-hydroxyphenyl)thiazolidin-4-one (**10**). White solid. Yield 90%. Mp. 244–245 °C. ^1H NMR (300 MHz, DMSO): δ 3.88 (d, J = 15.39, 1H), 3.99 (d, J = 15.47, 1H), 6.20 (s, 1H), 6.69–6.70 (m, 2H), 6.95–6.97 (m, 2H), 7.29–7.39 (m, 4H). ^{13}C NMR (75 MHz, DMSO): δ 33.5, 72.6, 116.1, 123.2, 128.7, 130.1, 132.7, 134.3, 137.0, 154.1, 171.2. ESI m/z calcd for $C_{15}H_{12}ClNO_2S$: 305.03; found 306.10.

2-(4-Chlorophenyl)-3-p-tolylthiazolidin-4-one (**11**). White solid. Yield 56%. Mp. 215–217 °C. ^{1}H NMR (300 MHz, DMSO): δ 2.28 (s, 3H), 3.86 (d, J = 15.7 Hz, 1H), 4.01 (d, J = 15.7 Hz, 1H), 6.05 (s, 1H), 7.01–7.03 (d, J = 8.8 Hz, 2H), 7.09–7.12 (d, J = 8.6 Hz, 2H), 7.26–7.28 (m, 4H). ^{13}C NMR (75 MHz, DMSO): δ 21.4, 33.8, 65.3, 126.0, 128.9, 129.4, 130.1, 134.9, 135.0, 137.7, 138.5, 171.2. ESI m/z calcd for $C_{16}H_{14}ClNOS$: 303.05; found 304.16.

2,3-Diphenylthiazolidin-4-one (**12**). White solid. Yield 80%. Mp. 250–251 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.86 (d, J = 15.7 Hz, 1H), 4.00 (d, J = 15.7 Hz, 1H), 6.11 (s, 1H), 7.27–7.15 (m, 6H), 7.32–7.29 (m, 4H). ^{13}C NMR (75 MHz, DMSO): δ 33.5, 72.6, 126.9, 127.1, 127.5, 128.0, 128.6, 128.9, 139.2, 141.7, 171.2. ESI m/z calcd for $C_{15}H_{13}NOS$: 255.07; found: 256.20.

2-(Naphthalen-1-yl)-3-*p*-tolylthiazolidin-4-one (**13**). White solid. Yield 48%. Mp. 240–241 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.23 (s, 3H), 3.90 (d J = 15.7 Hz, 1H), 4.05 (d, J = 15.7 Hz, 1H), 6.23 (s, 1H), 7.05–7.11 (m, 3H), 7.48 (s, 1H) 7.65–7.77 (m, 3H), 7.80 (s, 1H), 7.86–7.80 (m, 3H). ^{13}C NMR (75 MHz, DMSO): δ 21.3, 33.5, 56.1, 60.8, 73.2, 106.1, 116.1, 129.2, 133.4, 136.8, 137.6, 152.8, 171.2. ESI m/z calcd for $C_{20}H_{17}NOS$: 319.10; found 320.20.

2-(3,4,5-Trimethoxyphenyl)-3-*p*-tolylthiazolidin-4-one (**14**). White solid. Yield 85%. Mp. 244–245 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H), 3.80 (s, 9H), 3.89 (d, J = 15.7 Hz, 1H), 4.01 (d, J = 15.7 Hz, 1H), 6.01 (s, 1H), 6.50 (s, 2H), 7.07–7.10 (m, 4H). ^{13}C NMR (75 MHz, DMSO): δ 21.3, 33.5, 56.1, 60.8, 73.2, 106.1, 116.1, 129.2, 133.4, 136.8, 137.6, 152.8, 171.2. ESI m/z calcd for $C_{19}H_{21}NO_4S$: 359.12; found 360.13.

17. Srivastava, T.; Haq, W.; Katti, S. B. *Tetrahedron* **2002**, *58*, 7619.
18. Cell culture and treatments. MCF-7 breast cancer cells (a ER positive breast cancer cells, provided by Dr. E. Surmacz, Sbarro Institute for Cancer Research and Molecular Medicine, Philadelphia, USA) were maintained as previously described.[19] SKBR3 breast cancer cells (a ER negative breast cancer cells, obtained from American Type Culture Collection (ATCC), Manassas, VA, USA) were maintained in RPMI1640 without phenol red supplemented with 10% fetal bovine serum (FBS), 1% glutamine and 1% penicillin/streptomycin (Sigma–Aldrich, Milano, Italy) (complete medium). Cells were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 and were screened periodically for *Mycoplasma* contamination. All compounds were dissolved in dimethylsulfoxide (DMSO) (Sigma, St. Louis, Missouri, USA) at a concentration of 10 mM and diluted in DMEM/F12 (for MCF-7 cells) or in RPMI (for SKBR3 cells) medium supplemented with 1% DCC-FBS (dextran-coated charcoal-treated newborn calf serum) to obtain the working concentration.

    Assessment of cell viability. MCF-7 and SKBR3 cells were seeded on twenty-four well plates (0.2×10^5 cells/well) and grown for 48 h in complete medium. Before being treated, cells were starved in DMEM/F12 (for MCF-7 cells) or in RPMI (for SKBR3 cells) serum free medium for 24 h to the purpose of cell cycle synchronization. The effect of the different doses of compounds on cell viability was measured using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) assay as previously described.[20–22] Seventy two hours after treatments, fresh MTT (Sigma), re-suspended in PBS, was added to each well (final concentration (0.33 mg/ml). After 3 h incubation, cells were lysed with 1 ml of DMSO. Each experiment was performed in triplicate and the optical density was measured at 570 nm in a spectrophotometer.

    Statistical analyses. All experiments were conducted at least three times and the results were from representative experiments. Data were expressed as mean values ± standard deviation (SD), and the statistical significance between control (basal) and treated samples was analyzed using the GraphPad Prism 5 software program. The unpaired Student's t-test was used to compare two groups. $P < 0.05$ was considered statistically significant.
19. Sirianni, R.; Capparelli, C.; Chimento, A.; Panza, S.; Catalano, S.; Lanzino, M.; Pezzi, V.; Andò, S. *Mol. Cell. Endocrinol.* **2012**, *363*, 100.
20. Sirianni, R.; Zolea, F.; Chimento, A.; Ruggiero, C.; Cerquetti, L.; Fallo, F.; Pilon, C.; Arnaldi, G.; Carpinelli, G.; Stigliano, A.; Pezzi, V. *J. Clin. Endocrinol. Metab.* **2012**, *97*, E2238.
21. Caruso, A.; Chimento, A.; El-Kashef, H.; Lancelot, J. C.; Panno, A.; Pezzi, V.; Saturnino, C.; Sinicropi, M. S.; Sirianni, R.; Rault, S. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 60.
22. Sinicropi, M. S.; Caruso, A.; Conforti, F.; Marrelli, M.; El Kashef, H.; Lancelot, J. C.; Rault, S.; Statti, G. A.; Menichini, F. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 1148.
23. (a) Pozo-Guisado, E.; Lorenzo-Benayas, M. J.; Fernández-Salguero, P. M. *Int. J. Cancer* **2004**, *109*, 167; (b) Pozo-Guisado, E.; Alvarez-Barrientos, A.; Mulero-Navarro, S.; Santiago-Josefat, B.; Fernandez-Salguero, P. M. *Biochem. Pharmacol.* **2002**, *64*, 1375.
24. Verma, A.; Saraf, S. K. *Eur. J. Med. Chem.* **2008**, *43*, 897.
25. Lappano, R.; Rosano, C.; Madeo, A.; Albanito, L.; Plastina, P.; Gabriele, B.; Forti, L.; Stivala, L. A.; Iacopetta, D.; Dolce, V.; Andò, S.; Pezzi, V.; Maggiolini, M. *Mol. Nutr. Food Res.* **2009**, *53*, 845.
26. Vivacqua, A.; Romeo, E.; De Marco, P.; De Francesco, E. M.; Abonante, S.; Maggiolini, M. *Breast Cancer Res. Treat.* **2012**, *133*(3), 1025.
27. Lappano, R.; De Marco, P.; De Francesco, E. M.; Chimento, A.; Pezzi, V.; Maggiolini, M. *J. Steroid Biochem. Mol. Biol.* **2013 (Mar 28)**. pii: S0960-0760(13)00054-X. 10.1016/j.jsbmb.2013.03.005 [Epub ahead of print].