Indole and 2,4-thiazolidinedione conjugates as potential modulators of cellular apoptosis and proliferation

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Background: Thiazolidinediones (TZDs), also called glitazones, are five-membered carbon ring molecules commonly used for the management of insulin resistance and type 2 diabetes. Recently, many prospective studies have also documented the impact of these compounds as anti-proliferative agents, though several negative side effects such as hepatotoxicity, water retention and cardiac issues have been reported. In this work, we synthesized twenty-six new TZD analogues where the thiazolidinone moiety is directly connected to an N-heterocyclic ring in order to lower their toxic effects.

Methods: By adopting a widely applicable synthetic method, twenty-six TZD derivatives were synthesized and tested for their antiproliferative activity in MTT and Wound healing assays with PC3 (prostate cancer) and MCF-7 (breast cancer) cells.

Results: Three compounds, out of twenty-six, significantly decreased cellular viability and proliferation, and these effects were even more pronounced when compared with rosiglitazone, a well-known member of the TZD class of antidiabetic agents. As revealed by Western blot analysis, part of this antiproliferative effect was supported by the anti-apoptotic protein BCL-xL.

Conclusion: Our data highlight the promising potential of these TZD derivatives as anti-proliferative agents for the treatment of prostate and breast cancer.

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ABSTRACT

Background: Thiazolidinediones (TZDs), also called glitazones, are five-membered carbon ring molecules commonly used for the management of insulin resistance and type 2 diabetes. Recently, many prospective studies have also documented the impact of these compounds as anti-proliferative agents, though several negative side effects such as hepatotoxicity, water retention and cardiac issues have been reported. In this work, we synthesized twenty-six new TZD analogues where the thiazolidinone moiety is directly connected to an N-heterocyclic ring in order to lower their toxic effects.

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Conclusion: Our data highlight the promising potential of these TZD derivatives as anti-proliferative agents for the treatment of prostate and breast cancer.

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48 **INTRODUCTION**

49 Thiazolidinediones (TZDs) are a class of medicines commonly used alongside diet and exercise
50 as therapeutic agents for the treatment of patients with type 2 diabetes mellitus, a disease in
51 which defects in both peripheral insulin resistance and pancreatic beta cell insulin secretion
52 coexist (1). Although used in clinical practice for many years, there is still much debate in the
53 medical community about when TZDs should be recommended (2). The mechanism of action of
54 TZDs is based on their activation of peroxisome proliferator-activated receptor-γ (PPARγ), a
55 member of the nuclear receptor superfamily of transcription factors (3). PPARγ receptor
56 activation by TZDs ameliorates peripheral insulin sensitivity by promoting fatty acid uptake by
57 adipocytes and adiponectin secretion, and by suppressing the inflammatory response, which
58 plays an important role in the development of insulin resistance. However, in spite of the
59 beneficial effects of these molecules on insulin sensitivity and glucose homeostasis, many
60 studies have evidenced that TZDs can lead to weight gain in several ways, including activation
61 of adipogenesis (4). Also, several studies have reported that a long-term treatment with TZDs
62 can be associated with an increased risk of cardiovascular events and cancer (5-7). For example,
63 pioglitazone, a TZD-drug in clinical use, has been associated with an increased risk for bladder
64 cancer in people with type 2 diabetes (8). However, compared with pioglitazone, other TZD
65 compounds such as rosiglitazone, were not associated with an increased risk of bladder cancer,
66 supporting the notion that some adverse effects of TZDs are specific for each compound and not
67 a class effect. On the other hand, a significant antiproliferative effect of TZDs on several types of
68 cancer has been reported (9, 10), together with the observation that some PPARγ agonists seem

to contribute to improve the chemical sensitivity of certain cancers to standard chemotherapeutic agents (11).

It is evident from the literature that the TZD moiety, which is essential for the activity, is linked to the carbocyclic rings in all marketed drugs (12). Therefore, we conceptualized that including N-heterocyclic systems like indolyl and indolinone moieties in the side chain of TZD derivatives, could result in minor side-effects of medicinal products. In this endeavor, we synthesized indolyl/indolinone TZDs by adopting an improved (13) and widely applicable synthetic method.

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95 **MATERIALS AND METHODS**

96 ***Synthesis of TZD analogues***

97 Melting points are uncorrected and were determined in open capillary tubes in sulphuric acid
98 bath for the crystalline products. TLC was run on silica gel G and visualization was done using
99 iodine or UV light. Infrared (IR) spectra were recorded using Perkin Elmer 1000 instrument in
100 KBr pellets. ^{1}H NMR spectra were recorded in DMSO-d_6 using TMS as internal standard, by
101 using 400 MHz spectrometer. Mass spectra were recorded on Agilent LC-MS instrument. As
102 reported in Scheme I, the pharmacophore TZD [1] was prepared by treating chloroacetic acid
103 and thiourea in the presence of the catalytic amount of HCl by refluxing in water. **1** was treated
104 with DMF-DMA at 100 °C for 1 h furnishing 5-dimethylaminomethylene-thiazolidine-2,4-dione
105 **[2]**. The structure of **2** was assigned on the basis of its IR, ^{1}H-NMR and LC-MS data. Treatment
106 of **2** with indole **[3]** in AcOH at 100 °C for 1 h resulted in the formation of 5-(1H-indol-3-
107 ylmethylene)-thiazolidine-2,4-dione **AC1** in 95% yield. The obtained compound **AC1** was found
108 to be identical in melting point and TLC with the corresponding derivative prepared in the
109 reported method (13). Using this strategy, various new indolylidine TZD derivatives (**AC1-20**,
110 Scheme II) have been synthesized and their structures have been assigned on the basis of spectral
111 data. However, **AC1-20** could also be synthesized without isolating **2**, using tandem approaches,
112 by adding indole **[3]** in AcOH to the reaction mixture itself. On the other hand, isatin **[4]**
113 condensed with TZD **[1]** in the presence of methanol/KOH under refluxing condition for 30 min,

leading to the formation of the TZD derivative **AC21**. The structure of **AC21** was assigned on the basis of its IR, ^{1}H-NMR and LC-MS data.

General procedure for preparation of 2

A mixture of TZD **(1)** (10 mM) and DMF-DMA (10 mM) was stirred at 100 °C for 2 h, giving rise to a colourless solid that was separated from the reaction mixture. The solid was collected by filtration, washed with hexane (10 ml) and then dried. The crude product was recrystallized from ethanol solvent to get pure **2**.

General procedure for preparation of AC1-AC20

A mixture of **2** (10 mM) and indole **(3)** (10 mM) was refluxed for 1 h in acetic acid. At the end of this period, a light yellow coloured solid separated out from the reaction mixture, which was collected by filtration. The isolated solid was washed with hot water (10 ml) and dried. The product was recrystallized from a suitable solvent to obtain **AC1**.

General procedure for preparation of AC21-26

A mixture of TZD **(1)** (10 mM), isatin **(4)** (10 mM) and methanol (10 ml), followed by 40% aqueous KOH, was refluxed for 30 min. At the end of this period, solid separated out from the reaction mixture, which was collected by filtration. The isolated solid was washed with methanol (10 ml) and dried. The product was recrystallized from a suitable solvent to obtain **AC21**.

Spectral data of compounds are detailed in the Supplemental Appendix.

137 ***Cell cultures***

138 MCF-7 human breast cancer cells and PC3 human prostate cancer cells were obtained from the
139 American Type Culture Collection (LGC Promochem, Teddington, UK). Cells were maintained
140 in DMEM, supplemented with 2 mmol/liter L-glutamine, 50 IU/ml penicillin, 50 µg/ml
141 streptomycin, and 10% fetal bovine serum (14, 15), and both viability and cell migration were
142 assessed as described elsewhere (15), in the presence of either home-made TZD compounds, or
143 commercially available rosiglitazone (rosiglitazone maleate, GSK Pharmaceuticals, Worthing,
144 UK) (15).

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146 ***MTT Assay***

147 The MTT assay is a colorimetric assay based on the transformation of tetrazolium salt by
148 mitochondrial succinic dehydrogenases in viable cells. By measuring mitochondrial function and
149 metabolic activity, the MTT test provides a reproducible quantitative indication of cellular
150 viability. In serum-free media, cultured MCF-7 and PC3 cells were tested for viability, using 12
151 mM MTT reagent (Sigma-aldrich, St. Louis, MO) for 2 h at 37 °C. Formazan crystals were
152 solved with 1 mL (12-wells plate) of an organic acid solution (40% dimethylformamide, 2%
153 glacial acetic acid, 16% sodium dodecyl sulfate, pH 4.7), in order to avoid phenol red
154 interference. Absorbance was read after 24, 48 and 72 h treatment at 540 nm

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156 ***Wound healing***

157 The wound healing assay is a simple method to study cell migration and cell interactions *in vitro*.
158 Wound healing was performed seeding 500,000 cells/well in a 12-wells plate in order to reach
159 confluence after 24 h. Afterwards, scretches were made using sterile 200-µL tips, then, wells

160 were washed three times with PBS and cells were treated with RPMI medium containing the
161 respective TZD compound (5 μM final concentration in DMSO 0.001%). RPMI plus DMSO
162 alone (0.001%) was used as vehicle control. Photos of the wound were taken at 4, 8 and 12 h.
163 Then, snapshots were analyzed with the ImageJ software (1) and percent of wound clousure was
164 calculated using the following equation:

$$\% \text{ wound closure } = \frac{\text{Area t0} - \text{Area tx}}{\text{Area t0}} \text{ x } 100$$

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166 ***Western blot analysis***

167 Western blots were performed on total extracts from PC3 and MCF-7 cells, after 24, 48, and 72 h
168 treatment. The antibodies used for these studies were: anti-BCL-xL polyclonal antibody (Cell
169 Signaling Technology, Danvers, MA, USA), and anti-β-Actin (Sigma-Aldrich Inc., St. Louis,
170 MO, USA) monoclonal antibody (16).

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RESULTS

Inhibition of cell viability in MCF-7 and PC3 cells

All the synthetic TZD-based compounds (from AC1 to AC26) were initially screened for their *in vitro* effect on cell viability, using MTT assays in MCF7 and PC3 cells, that underwent treatment with 5 μM TZD analogues over a period from 24 to 72 h of exposure (Figure 1A and 1B). As observed both in MCF-7 cells and PC3 cells, not all compounds investigated produced the same effect on cell viability, as compared with untreated control cells. In fact, whereas some of them had no influence on this parameter at all time points of the 72-h period in both cell lines, a decrease of cell viability (with slight difference among MCF-7 and PC3 cells) was observed with AC11, AC12, AC14, AC15, AC17, AC18, AC20, AC21, AC22, AC23, AC24, AC25, and AC26 (Figure 1A and 1B).

IC_{50}

Next, both cell types were incubated with increasing concentrations (1 to 33.3 μM) of each of the twenty-six compounds individually to calculate IC_{50} values (Table I). Among all the compounds evaluated, compounds AC18, AC20 and AC22 were found to be the most promising, showing a 50% reduction of cell viability in both PC3 and MCF-7 cells after 72 h exposure to 5 μM concentration of each of the three drugs. However, whereas the inhibition of cell viability in MCF-7 cells was already significant at 48 h with all three TZD analogues, no significant inhibition of cell viability by these compounds was observed before 72 h incubation in PC3 cells (Figure 2), thus suggesting some component of cell specificity amongst these synthetic drugs.

MTT assay

To further evaluate the effect of AC18, AC20 and AC22 on cellular viability, we performed MTT assays, in which the effect of each of these compounds on cell proliferation was compared to that of untreated control cells, or cells treated with either the negative AC1 TZD compound, or 5 μM rosiglitazone, a potent oral antidiabetic drug, whose inhibitory role on cell viability has been reported *in vitro*, in rat prolactin-secreting pituitary tumor cells (15). As shown in Figure 3A and 3B, treatment of both MCF-7 and PC3 cells with 5 μM of either AC18, AC20 or AC22 induced a significant decrease of cell viability as compared with control cells, which, in some instances, was even more evident than that obtained with the same dose of rosiglitazone.

To further support the antiproliferative potential of AC18, AC20 and AC22, Western blot analyses of BCL-xL were carried out in cell lysates from MCF-7 and PC3 cells. BCL-xL is an important anti-apoptotic member of the BCL-2 protein family and a potent regulator of cell death. While overexpression of BCL-xL in PC3 and MCF-7 cells has been reported to contribute to the apoptosis-resistant phenotype of these cells in response to products with antitumor activity (17, 18), the reduction of Bcl-xL levels rendered these cells more sensitive to the drugs (19, 20), thus reducing cell survival. As shown in Figue 3A and 3B, BCL-xL protein levels were reduced in both MCF-7 and PC3 cells treated with the AC22 TZD compound, and this reduction paralleled the decrease in cell viability (as monitored by the MTT assay). Additionally, as shown in Figure 3B, treatment of PC3 cells with either AC18 or AC22 showed a better inhibition of cell viability compared with cells treated with rosiglitazone, and also in this case a parallelism was observed between cell viability and BCL-xL protein expression. Therefore, it is tempting to hypothesize that the antiproliferative effect of these TZD compounds, including rosiglitazone, on MCF-7 breast cancer cells and PC3 prostate cancer cells could be mediated, at least in part, by BCL-xL.

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219 **Effects of AC18, AC20 and AC22 on cell migration**

220 The effects of AC18, AC20 and AC22 on cell migration were studied as well. To this end,
221 wound healing assays were performed *in vitro* with both MCF-7 and PC3 cells, either untreated
222 or treated with the synthetic TZD agonists, at 4 h time points (up to 12 h). As shown in Figure
223 4A and 4B, a significant inhibition of cell migration was observed in both MCF-7 and PC3 cells,
224 in the presence of compounds AC18, AC20 and AC22, as demonstrated by the decreased
225 migratory response of cells in response to wound healing. Also in this case, as for the MTT test,
226 the effect of all three compounds on cell migration was comparable or even superior than that of
227 rosiglitazone (Figure 4A and 4B), thereby indicating that these novel compounds may indeed
228 represent a new potential class of antiproliferative agents.

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243 **DISCUSSION**

244 Insulin resistance is a hallmark of obesity and type 2 diabetes (21, 22), and both these disorders
245 have an increased risk to develop some types of tumours, including breast, liver, colon, and
246 pancreatic cancers (23, 24). Many clinical trials have also documented that patients treated with
247 TZDs, while improving glycemic control and in spite of potentially drug-related adverse effects,
248 are less predisposed to these kind of cancers (3). The observation that some antidiabetic agents
249 such as TZDs (3, 9-11, 25), as well as metformin (26-28), may exert an antiproliferative effect on
250 many cell types has triggered an intense research effort in the last years. Nevertheless, our
251 knowledge and understanding of how this effect takes place remains poorly understood. This is
252 made all the more difficult by the fact that non-univocal results still exist on the antiproliferative
253 activities of these drugs. In fact, whereas a strong antiproliferative effect was seen for TZDs in
254 several studies, no significant inhibition of cell proliferation of these compounds was observed in
255 a number of other similar investigations from different research groups (5, 6, 8). Based on these
256 considerations, here, we explored the antiproliferative effects of a series of newly synthesized
257 TZD compounds, in which the carbocyclic ring was replaced by the N-heterocyclic ring in an
258 attempt to reduce their toxicity while retaining their antiproliferative abilities at the same time.
259 By adopting a simple and tunable synthetic strategy, twenty-six different TZD compounds were
260 synthesized in moderate to high yields. Among them, only three, designated AC18, AC20 and
261 AC22, showed a significant antiproliferative effect in two different human neoplastic cell lines,
262 MCF-7 breast cancer cells and PC3 prostate cancer cells, as demonstrated by cell viability
263 assays, further supporting the notion that different TZD compounds may display different effects

on cell viability. As a next step towards their characterization, these same TZD molecules were also effective in inhibiting cell migration and cell-to cell interaction, as shown by wound healing assays.

Furthermore, as the same molecule may present different efficacy and potency on different cancer cell lines, we showed that AC18 and AC22 were both more effective in MCF-7 and in PC3 cells than rosiglitazone, whereas AC20 was more powerful than rosiglitazone in MCF-7, but not in PC3 cells. This can be partially due to the poor differentiation grade of PC3 cells (29) compared with MCF-7. Also, PC3 cells are derived from a prostate cancer metastasis, while MCF-7 cell strain retains many characteristics of the mammal epithelium cells, such as the presence of estrogen receptors (30). However, since the antiproliferative activity of these compounds has been assessed *in vitro*, we can only speculate about their safety *in vivo*, and more studies are necessary to clarify this issue. In particular, further investigation in animal models would confirm both safety and efficacy of the compounds herein proposed.

CONCLUSIONS

Herein, we propose a new set of three TZD analogues as potential anticancer agents. Although more effort is needed to understand their safety, we believe that these compounds have the potential to be further developed as novel adjuvant tool for anticancer treatments.

Author Contributions

Sebastiano Messineo designed and performed the experiments, analysed the data, and prepared figures and tables.

Riyaz Syed contributed chemical compounds, analysed the data, reviewed drafts of the paper, and designed the schemes for synthesis strategy and structure of synthetized chemical compounds.

Domenica M. Corigliano performed Western blot experiments.

Rahul Patel contributed chemical compounds, and reviewed drafts of the paper.

Chittireddy V.R. Reddy contributed chemical compounds, and reviewed drafts of the paper.

Pramod K. Dubey contributed chemical compounds, and reviewed drafts of the paper.

Carmen Colica reviewed drafts of the paper and analysed the data.

Rosario Amato contributed to experiments on wound healing.

Giovambattista De Sarro contributed reagents/materials/analysis tools.

Adisherla Indrasena contributed chemical compounds, and reviewed drafts of the paper.

Antonio Brunetti conceived and designed the experiments, analysed the data and wrote the paper.

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Competing Interests

306 The authors declare that they have no competing interests.

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Schemes and Figure Legends

Scheme I: **Synthesis strategy of TZD compounds**

Scheme II: **Structures of synthetized TZD analogues**

Figure 1. **Effects of TZD compounds (AC1-AC26) on cell viability**. MTT assays were performed in MCF7 (A) and PC3 (B) cells as reported in the Materials and Methods section. Optical density (OD) was measured at 540 nm, after 24, 48 and 72 h drug treatment. Results are the mean ± SE of triplicates from three independent experiments. *p<0.05, **p<0.01 relative to untreated control cells (C=1), which received dosing vehicle alone (0.001% DMSO).

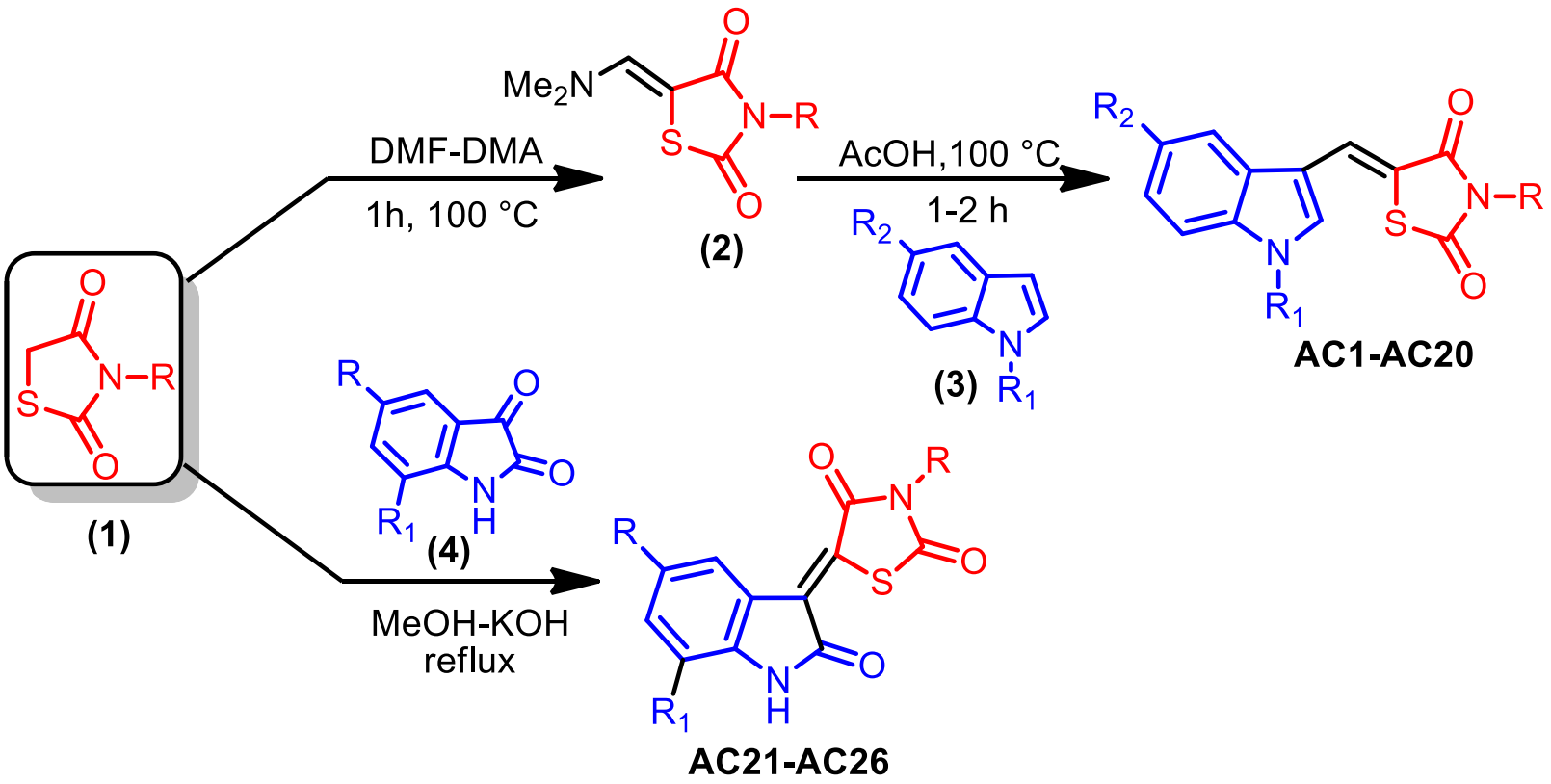
Figure 2. **Effects of AC18, AC20 and AC22 TZD analogues on cell viability**. MTT assays were performed as in Figure 1 in both MCF7 and PC3 cells, either in the absence or presence of the selected compounds. OD was measured at 540 nm, after 24, 48 and 72 h drug exposure. Results are the mean ± SE of triplicates from three independent experiments in each condition. *p<0.05, **p<0.01 relative to untreated control cells (C=1).

Figure 3. **Comparison between Rosiglitazone, AC18, AC20 and AC22 on cell viability**. MTT assays were performed in MCF7 (A) and PC3 (B) cells, either untreated or treated as indicated. OD was measured at 540 nm, after 24, 48 and 72 h drug exposure. Results are the mean ± SE of triplicates from three independent experiments. *p<0.05, **p<0.01, ***p<0.001 relative to either untreated control cells (C=1), or cells treated with the non-effective AC1 compound.

419 Representative Western blots of BCL-xL from cell lysates of untreated and treated MCF-7 and
420 PC3 cells are shown in the autoradiograms.
421
422 **Figure 4**. **Inhibition of cell migration**. Wound healing assays were carried out in MCF7 (A)
423 and PC3 (B) cells, using 200 µL pipette tips to scratch confluent cells on the base of a 12-well
424 plate. Wound healing (% wound closure) was measured and analyzed with the NIH ImageJ
425 software in both cell types, after 4, 8 and 12 h incubation *with* the compounds. Results are the
426 mean ± SE of triplicates from three independent experiments. *$p<0.05$, **$p<0.01$, ***$p<0.001$
427 relative to untreated (control) cells.
428

Figure 1(on next page)

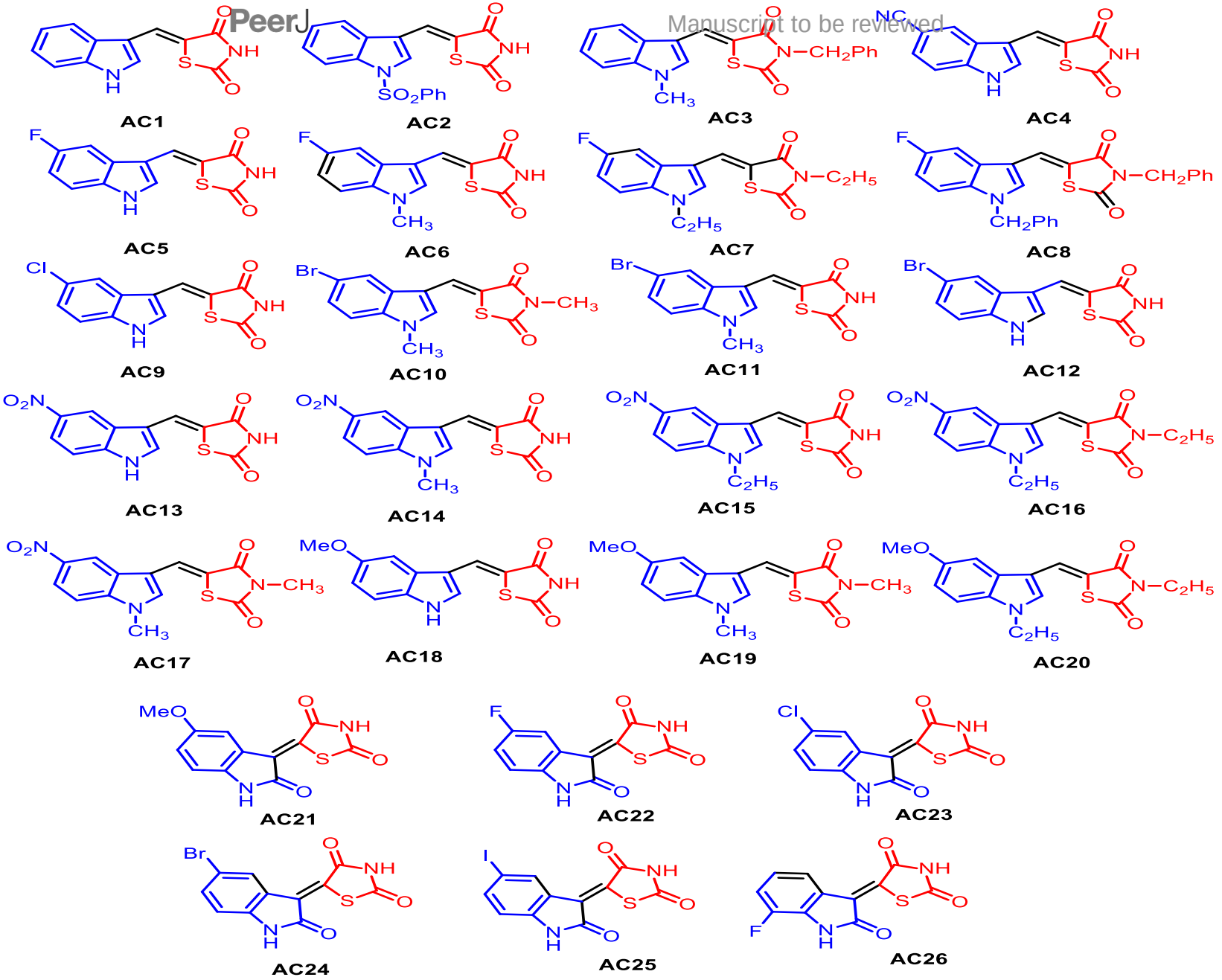
Synthesis strategy of TZD compounds


Me2N
DMF-DMA
1h, 100 °C
(2)
AcOH,100 °C
1-2 h
(3)
AC1-AC20
(1)
(4)
MeOH-KOH
reflux
AC21-AC26

Scheme I

Figure 2(on next page)

Structures of synthetized TZD analogues



Scheme II

Figure 3(on next page)

Effects of TZD compounds (AC1-AC26) on cell viability.

MTT assays were performed in MCF7 (A) and PC3 (B) cells as reported in the Materials and Methods section. Optical density (OD) was measured at 540 nm, after 24, 48 and 72 h drug treatment. Results are the mean ± SE of triplicates from three independent experiments. *$p<0.05$, **$p<0.01$ relative to untreated control cells (C=1), which received dosing vehicle alone (0.001% DMSO).

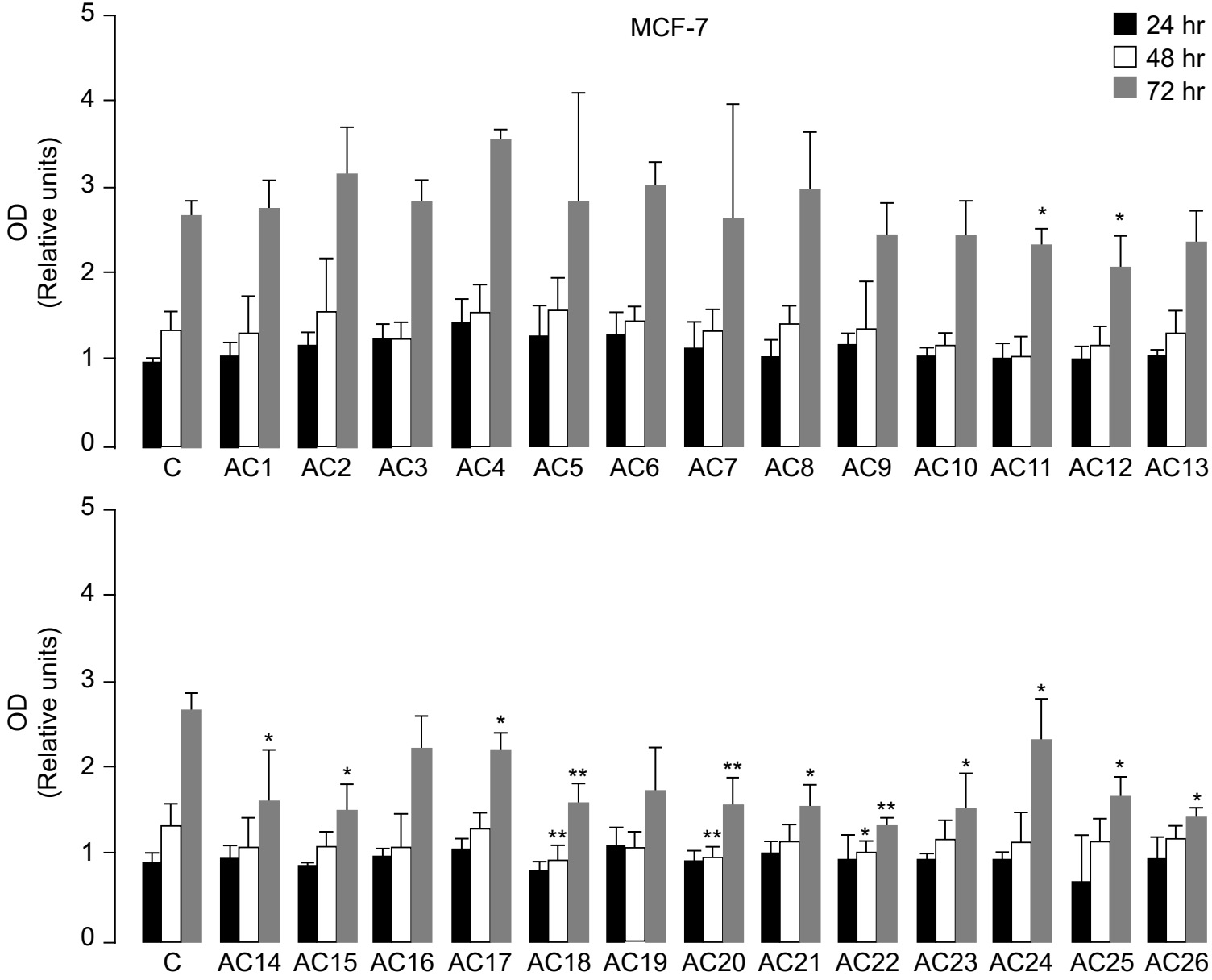

MCF-7
24 hr
48 hr
72 hr
OD
(Relative units)
0
1
2
3
4
5
C
AC1
AC2
AC3
AC4
AC5
AC6
AC7
AC8
AC9
AC10
AC11
AC12
AC13
AC14
AC15
AC16
AC17
AC18
AC19
AC20
AC21
AC22
AC23
AC24
AC25
AC26

Figure 1A

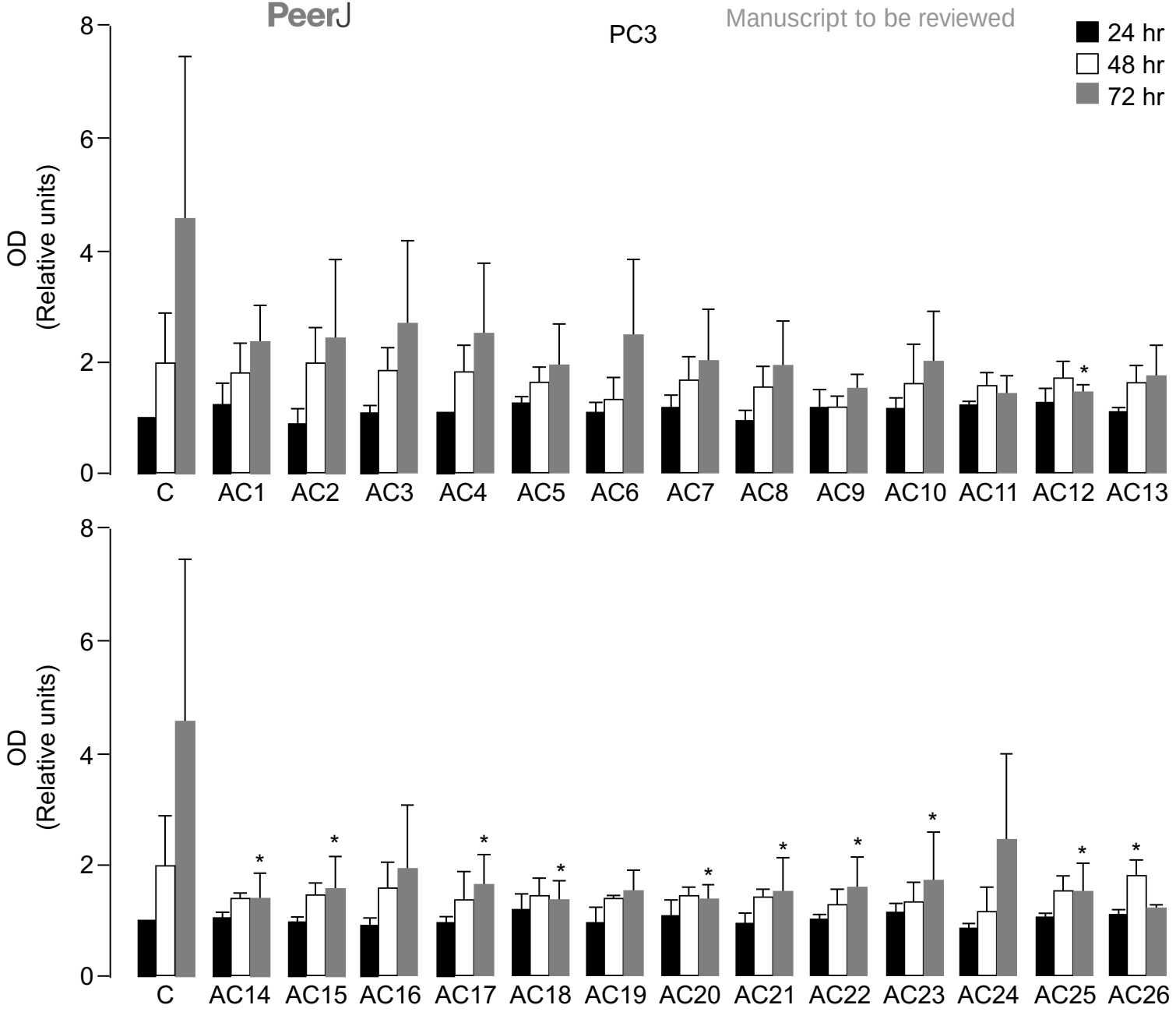

PC3
24 hr
48 hr
72 hr
OD
(Relative units)
0
2
4
6
8
C
AC1
AC2
AC3
AC4
AC5
AC6
AC7
AC8
AC9
AC10
AC11
AC12
AC13
AC14
AC15
AC16
AC17
AC18
AC19
AC20
AC21
AC22
AC23
AC24
AC25
AC26
*

Figure 1B

Figure 4(on next page)

Effects of AC18, AC20 and AC22 TZD analogues on cell viability.

MTT assays were performed as in Figure 1 in both MCF7 and PC3 cells, either in the absence or presence of the selected compounds. OD was measured at 540 nm, after 24, 48 and 72 h drug exposure. Results are the mean ± SE of triplicates from three independent experiments in each condition. *$p<0.05$, **$p<0.01$ relative to untreated control cells (C=1).

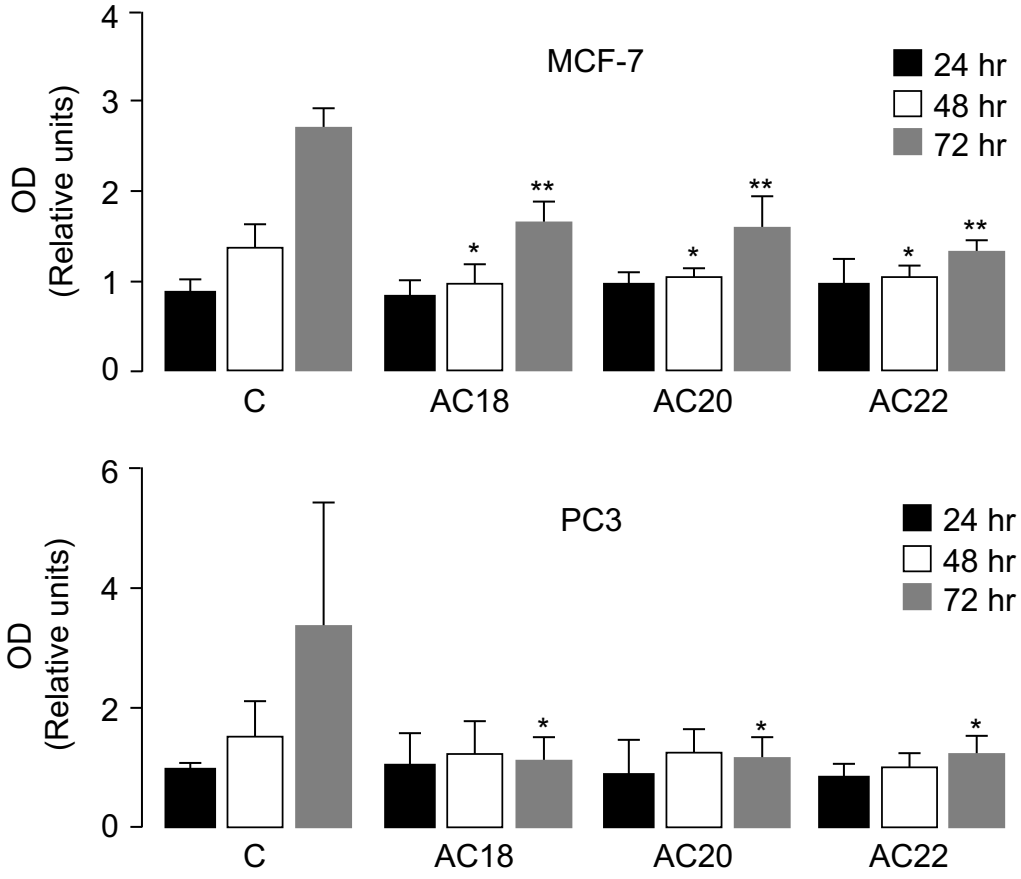

MCF-7
24 hr
48 hr
72 hr
OD
(Relative units)
0
1
2
3
4
C
AC18
AC20
AC22
*
**
PC3
24 hr
48 hr
72 hr
OD
(Relative units)
0
2
4
6
C
AC18
AC20
AC22
*

Figure 2

Figure 5(on next page)

Comparison between Rosiglitazone, AC18, AC20 and AC22 on cell viability.

MTT assays were performed in MCF7 (A) and PC3 (B) cells, either untreated or treated as indicated. OD was measured at 540 nm, after 24, 48 and 72 h drug exposure. Results are the mean ± SE of triplicates from three independent experiments. *p<0.05, **p<0.01, ***p<0.001 relative to either untreated control cells (C=1), or cells treated with the non-effective AC1 compound. Representative Western blots of BCL-xL from cell lysates of untreated and treated MCF-7 and PC3 cells are shown in the autoradiograms.

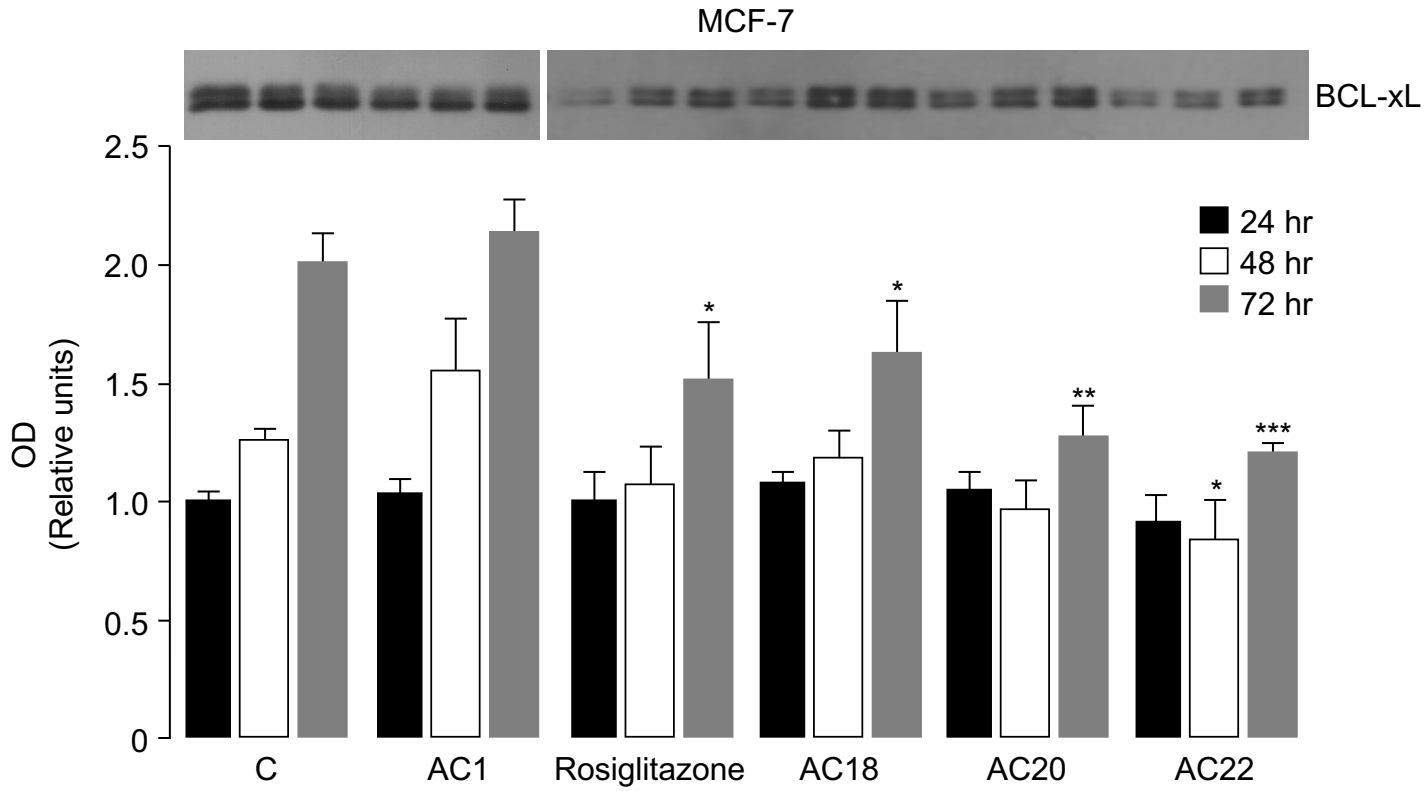

MCF-7
BCL-xL
OD
(Relative units)
2.5
2.0
1.5
1.0
0.5
0
24 hr
48 hr
72 hr
*
*
**
***
*
C
AC1
Rosiglitazone
AC18
AC20
AC22

Figure 3A

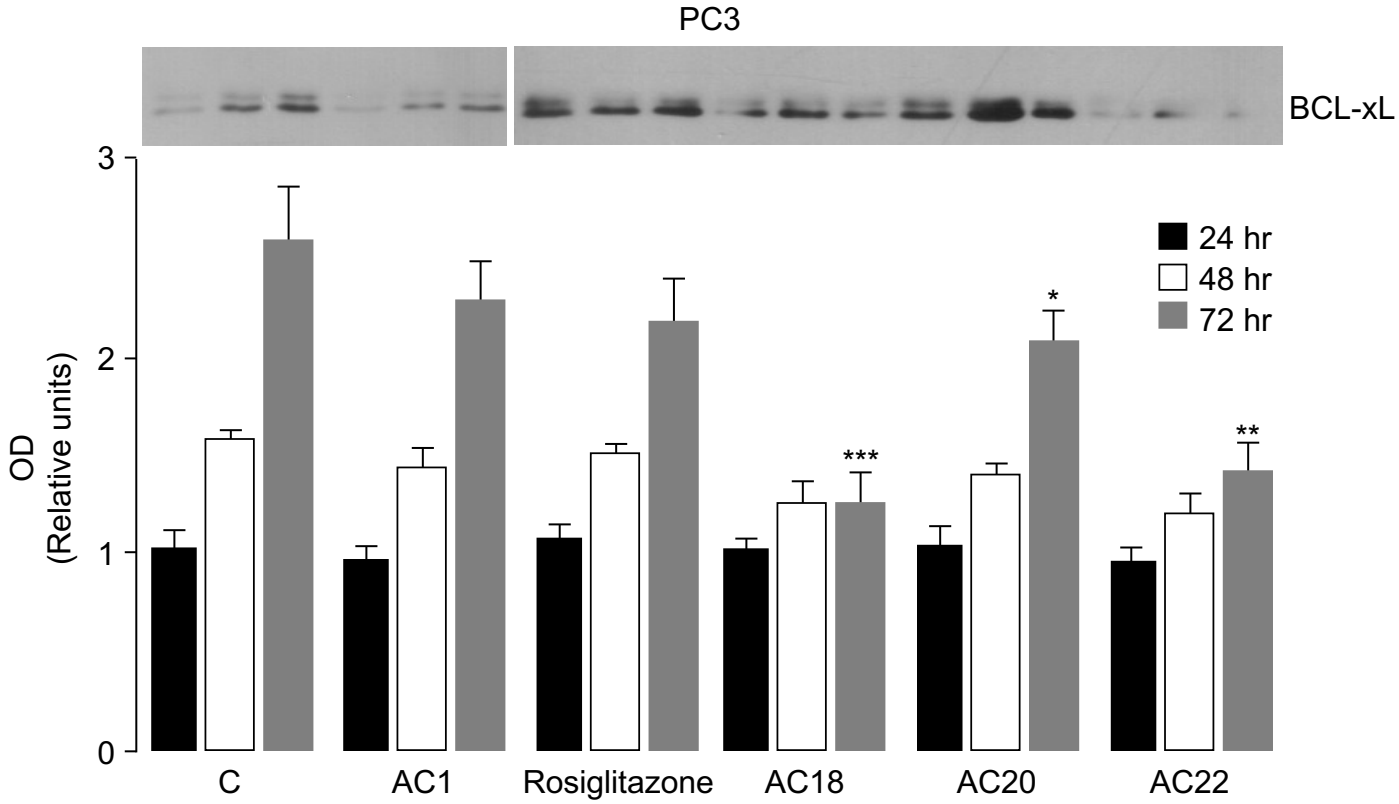

PC3
BCL-xL
OD
(Relative units)
0
1
2
3
24 hr
48 hr
72 hr
***
*
**
C
AC1
Rosiglitazone
AC18
AC20
AC22

Figure 3B

Figure 6(on next page)

Inhibition of cell migration.

Wound healing assays were carried out in MCF7 (A) and PC3 (B) cells, using 200 µL pipette tips to scratch confluent cells on the base of a 12-well plate. Wound healing (% wound closure) was measured and analyzed with the NIH ImageJ software in both cell types, after 4, 8 and 12 h incubation with the compounds. Results are the mean ± SE of triplicates from three independent experiments. *p<0.05, **p<0.01, ***p<0.001 relative to untreated (control) cells.

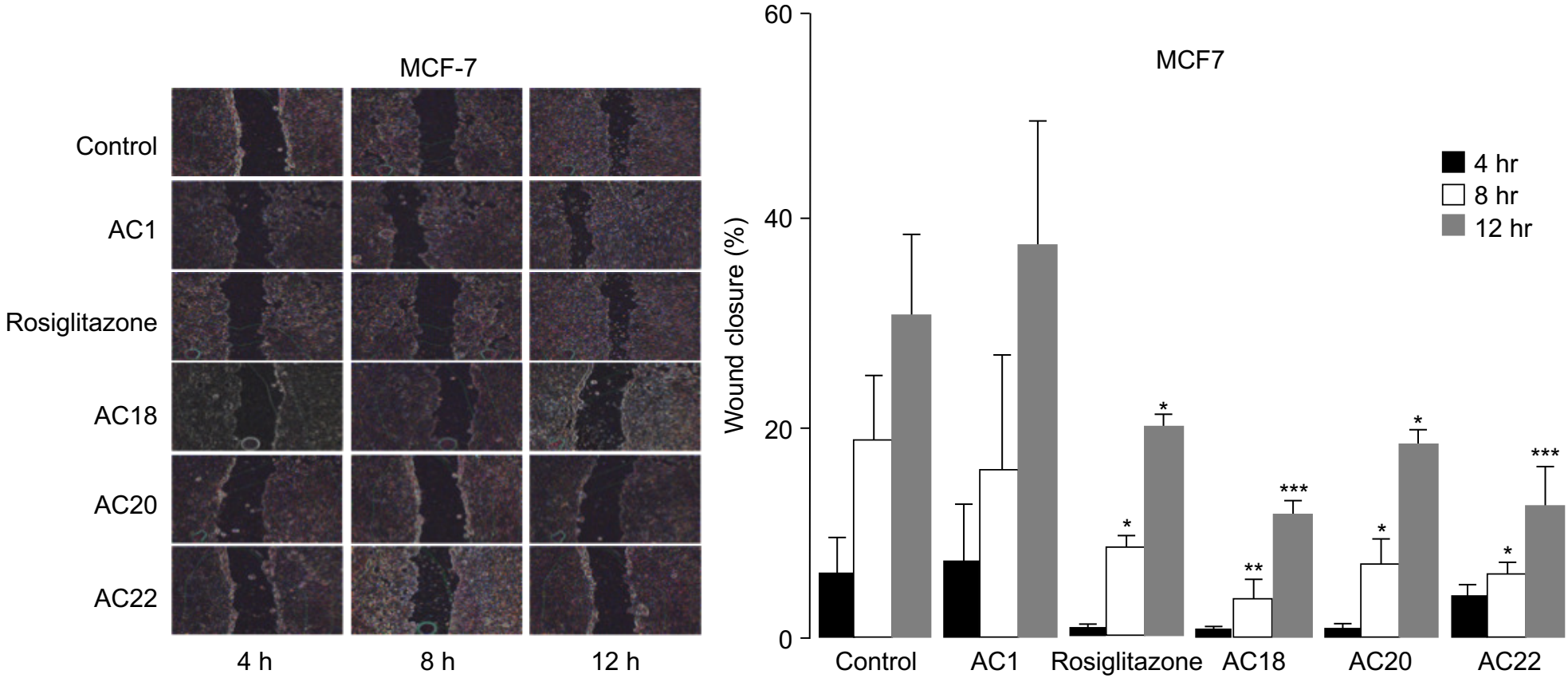

MCF-7
Control
AC1
Rosiglitazone
AC18
AC20
AC22
4 h
8 h
12 h
MCF7
Wound closure (%)
60
40
20
0
4 hr
8 hr
12 hr
Control
AC1
Rosiglitazone
AC18
AC20
AC22

Figure 4A

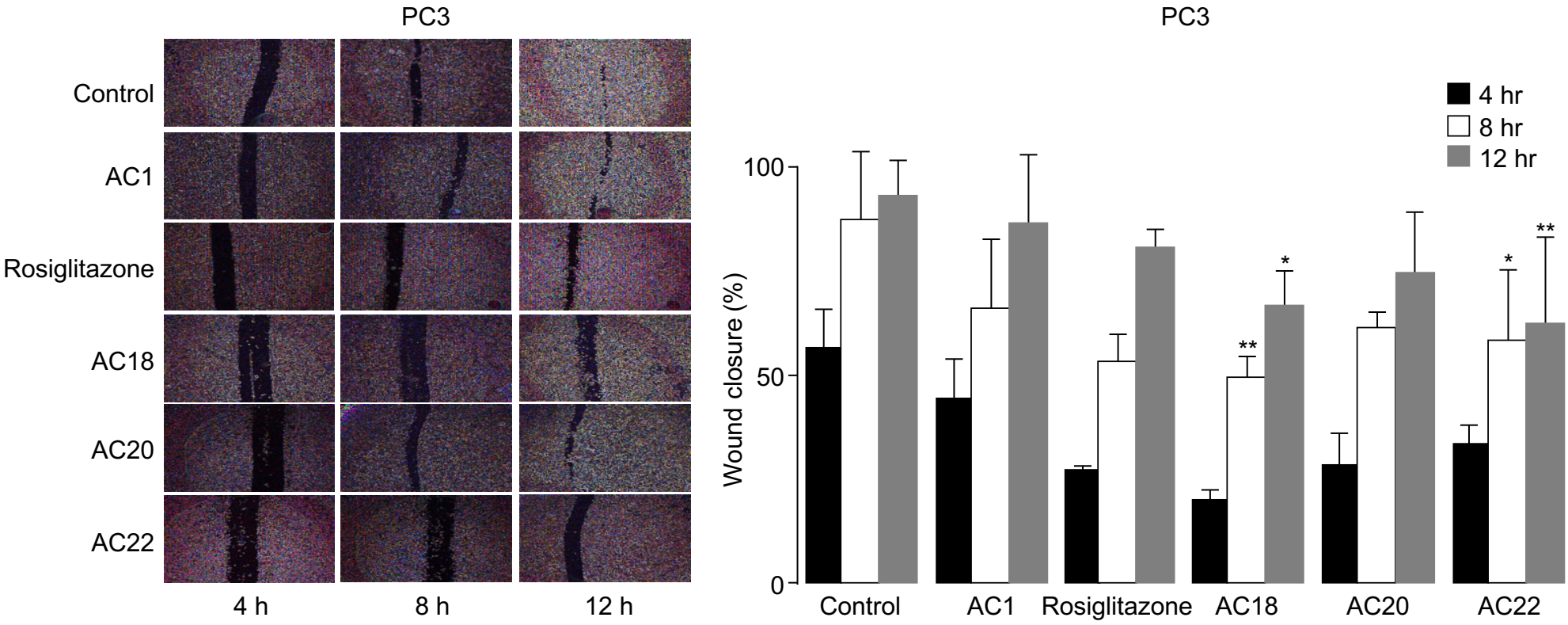

PC3
Control
AC1
Rosiglitazone
AC18
AC20
AC22
4 h
8 h
12 h
PC3
4 hr
8 hr
12 hr
Wound closure (%)
100
50
0
Control
AC1
Rosiglitazone
AC18
AC20
AC22
*
**
*
**

Figure 4B

Table 1 (on next page)

IC_{50} values of all the twenty-six compounds tested with MTT assay

Dose-response and time-course experiments were performed by exposing MCF-7 and PC3 cells to increasing doses (1.0, 3.3, 5.0, 10.0, 33.3 μM) of each TZD compound for 24, 48 and 72 h. IC_{50} values are expressed as the concentration of each compound required to produce 50% inhibition of cell viability, in relation to time of exposure to the compound. NR, not responsive.

Table I: IC_{50} values of all the twenty-six compounds tested with MTT assay

Compound	MCF-7	PC3	Compound	MCF-7	PC3
1	NR	NR	14	33.3 μM (72 h)	33.3 μM (72 h)
2	NR	NR	15	NR	NR
3	NR	NR	16	NR	NR
4	NR	NR	17	33.3 μM (72 h)	33.3 μM (72 h)
5	NR	NR	18	5 μM (48 h)	5 μM (48 h)
6	NR	NR	19	NR	NR
7	NR	NR	20	5 μM (48 h)	5 μM (48 h)
8	NR	NR	21	33.3 μM (72 h)	33.3 μM (72 h)
9	NR	NR	22	5 μM (48 h)	5 μM (48 h)
10	NR	NR	23	33.3 μM (72 h)	33.3 μM (72 h)
11	NR	NR	24	NR	NR
12	33.3 μM (72 h)	33.3 μM (72 h)	25	33.3 μM (72 h)	33.3 μM (72 h)
13	NR	NR	26	33.3 μM (72 h)	33.3 μM (72 h)

Dose-response and time-course experiments were performed by exposing MCF-7 and PC3 cells to increasing doses (1.0, 3.3, 5.0, 10.0, 33.3 μM) of each TZD compound for 24, 48 and 72 h. IC_{50} values are expressed as the concentration of each compound required to produce 50% inhibition of cell viability, in relation to time of exposure to the compound. NR, not responsive.