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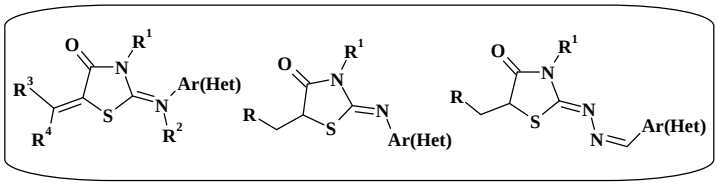

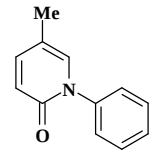

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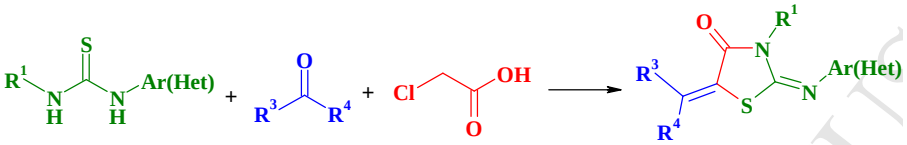

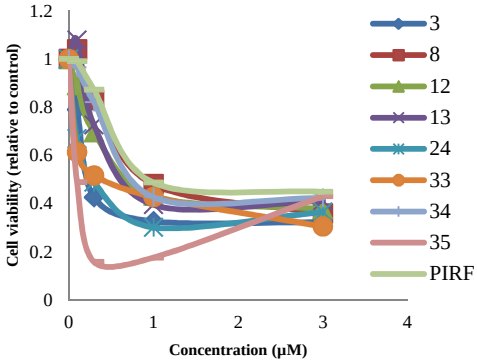

Antifibrotic and anticancer action of 5-ene amino/iminothiazolidinones

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Keywords

Amino/amino-thiazolidinones, synthesis, one-pot method, antifibrotic activity, superoxide radicals, antiproliferative effects

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Abstract

Here we describe the synthesis and the antifibrotic and anticancer activity determination of amino(imino)thiazolidinone derivatives. An efficient one-pot three-component reaction which involved [2+3]-cyclocondensation and Knoevenagel condensation was used for the synthesis of 5-ene-2-amino(imino)-4-thiazolidinones. Following amino-imino tautomerism, the compound structures were confirmed by X-ray analysis. Comparison of SRB assays on fibroblasts and cancer cells revealed that compounds which significantly reduced the viability of fibroblasts did not possess an anticancer effect. A series of thiazolidinone derivatives as interesting candidates for further testing has been identified. Among the tested compounds 2-{3-furan-2-ylmethyl-2-[(2-methyl-3-phenylallylidene)hydrazono]-thiazolidin-4-one-5-yl}-N-(3-trifluoromethylphenyl)-acetamide (**5**), N-(2-methoxyphenyl)-2-[5-(4-oxothiazolidin-2-ylideneamino)[1,3,4]thiadiazol-2-ylsulfanyl]-acetamide (**12**), 3-[3-allyl-4-oxo-2-(thiazol-2-ylimino)thiazolidin-5-ylidene]-1,3-dihydroindol-2-one (**33**), and 5(*Z*)-(thiophen-2-ylmethylene)-4-(4-chlorophenylamino)thiazol-2(5*H*)-one (**34**) possessed high antifibrotic activity levels, had a similar effect as Pirfenidone, and did not scavenge superoxide radicals. Their antifibrotic potential was confirmed using the xCelligence system.

1. Introduction

Structure based approach is an efficient tools for new hit- and lead-compounds design. Utilizing so-called privileged structures can be considered beneficial in such an approach, and it also greatly increases the chances of success [1]. 4-Thiazolidinones are prime examples of such compounds. Achievements in the medical chemistry of the latter significantly increased and are illustrated by the rapid growth of the number of scientific papers and patents [2-5]. Most of the studies on these compounds are devoted to the 2,4-thiazolidinedione, rhodanine and 2-alkyl(aryl)-substituted 4-thiazolidinone derivatives as leads for antimicrobial, antidiabetic, anti-inflammatory and anticancer agents [5-7]. The variety of thiazolidinones allows to diversify the modification directions of the mentioned heterocycles and makes them attractive scaffolds in the privileged substructure-based diversity oriented synthesis [8]. Among the wide range of 4-thiazolidinones two subtypes are of special interest, namely the 5-ene- and 2-amino(imino)-4-thiazolidinone derivatives [9].

Pharmacological attractiveness of the 5-ene derivatives is reasoned and confirmed by the thesis about the crucial role of the presence/nature of C5 substituent of a basic core for the biological activity realization [10-12]. Conjugation of the 5-ene fragment to the C4 carbonyl group renders the compounds electrophilic and potentially reactive by enabling Michael addition of nucleophilic protein residues to the exocyclic double bond. This enables for example, reactions with glutathione or other free thiols. Simultaneously this property may cause 5-ene-4-thiazolidinones to be frequent hitters or pan assay interference compounds that are useless in drug discovery process because of their insufficient selectivity [2,13,14]. However, this view has been disproved in many studies and a large number of lead-compounds belongs to the mentioned 5-ene-4-thiazolidinones. The positive perspective also is linked to a poly-pharmacological approach, where affinity toward various targets is regarded advantageously [14,15]. In this context, it is worth mentioning that Michael acceptors are among the most effective activators of Nrf2, which opens new perspectives in the treatment of inflammation and cancer [16].

The other promising subtype of 4-thiazolidinone derivatives comprises the 2-amino(imino)-4-thiazolidinones. These compounds possess antimicrobial [17], anti-inflammatory [18], and anticancer [19] activities. Members of this group are the 2-arylaminothiazol-4-ones which are effective growth inhibitors of HT29 adenocarcinoma cells [20] and the human lung cancer cell lines H460 and H460/TaxR [21,22]. Furthermore, 4-thiazolidinone derivatives are efficient integrin $\alpha_v\beta_3$ antagonists [23] and inhibitors of CDK1/cyclin B [24].

Currently, the search for compounds possessing combined types of activities is of special interest, especially for compounds with simultaneous antiproliferative or cytostatic. Combination of anticancer and antioxidant, anti-inflammatory [2,3,5], antiprotozoal [25] activities have been discussed. In this field

the search for new 4-thiazolidinone based compounds with anticancer and antifibrotic activities could be a promising direction.

Fibroblasts are mesenchymal cells that reside in the pulmonary interstitium and produce collagen and other extracellular matrix proteins. They play a pivotal role in the normal wound healing or tissue repair process and excessive stimuli for fibroblasts lead to the tissue remodeling or fibrous tissue that is observed in bronchial asthma, chronic obstructive pulmonary disease [26] and idiopathic pulmonary fibrosis. Moreover fibroblasts are associated with cancer cells and cancer progression, and their structural and functional contributions to this process are discussed [27,28] and are associated with an array of cytokines produced by fibroblasts. Targeting the so-called cancer-associated fibroblasts (promoters of tumour growth and progression) is a novel and promising therapeutic strategy against cancer. In this context, ideal therapeutic molecule will have reduced cancer-promoting properties in combination with optimized antifibrotic action. Currently, therapeutic options in lung fibrosis are extremely limited [29]: Pirfenidone, the only registered antifibrotic drug, has proven antifibrotic efficacy in idiopathic pulmonary fibrosis patients [30,31] but is also associated with side effects viz. gastrointestinal disturbances and photo toxicity. Key findings in the creation of new antifibrotic agents are mainly related with inhibition of Wnt- and TGFβ-mediated β-catenin signaling [30], inhibition of the TGFβ/CTGF axis [33,34] and are considered as attractive direction for the development of new therapeutics against not only various fibro-proliferative diseases but also cancer. Another argument for search for anticancer and antifibrotic activities combination is utilization of proven anticancer agents in the new antifibrotic agents design. For instance, nintedanib (a potent and specific tyrosine kinase inhibitor) has clinical efficacy in the treatment of pulmonary fibrosis [35]. Thiazolidinone based compounds with antiproliferative effect were much less toxic to fibroblasts [22,36-38] but possessed significant antitumor and antifibrotic activities [39]. In addition to the antiproliferative and antifibrotic activity, superoxide scavenging potency appears to be a beneficial feature to include during the design of new anticancer and antifibrotic agents. Superoxide anion radicals have been shown to play an important role in fibrotic disorders by activating fibroblasts to produce collagen and the profibrotic cytokine transforming growth factor β (TGFβ) and with simultaneous activation of α-smooth muscle actin [40-42] as well as in cancer growth.

The work described herein is an extension of our ongoing efforts towards a search for new therapeutic agents bearing the thiazolidinone frame. The aim of study was to evaluate the potential antifibrotic and anticancer effects of 4-thiazolidinones and to determine their effects on lung fibroblast viability and proliferation as well as their superoxide scavenging potency.

2. Results and discussion

2.1. Chemistry

Synthetic approach to the target compounds was based on the results of our previous studies in the field of 2-amino(imino)-4-thiazolidinones and their isomeric 4-aminothiazol-2(5*H*)-ones [4,12,19,43]. The synthetic protocols for compounds **1-11** involved [2+3]-cyclocondensation of S,N-dinucleophiles (thioureas, thiosemicarbazones) with a row of dielectrophilic synthons $[C_2]^{2+}$ (Schemes 1, 2). The reactions were performed in glacial acetic acid medium in the presence of anhydrous sodium acetate. Presented non-conjugated structures (**1-7**, **9-11**) contain a stereo-center at the C-5 position of the heterocyclic system and occur in racemic form.

Scheme 1

Compounds **12-16** were obtained by the interaction of the N-heteryl-chloroacetamides with ammonium thiocyanate in the acetone medium. Following the hypothesis about crucial role of the presence/nature of C5 position moiety, 5-ene derivatives (**23-27**, **30**, **33**) were obtained with high yields via Knoevenagel reaction based on the CH-acidic site of thiazolidinone core (Scheme 2). For the most of the compounds (**17-22**, **28**, **29**, **31**, **32** – bearing simple arylidene, aryl-alkylidene, isatin or pyrazolone fragments) the efficient one-pot method based on the three-component reaction of S,N-dinucleophile, electrophilic reagent and oxocompound was used. The latter include the abovementioned [2+3]-cyclocondensation and Knoevenagel condensation phases. The reaction was performed in the medium of glacial acetic acid in the presence of sodium acetate (Scheme 2). Mentioned protocol is the useful approach for the target compounds synthesis based on the various types of thiourea or thiosemicarbazide derivatives as well as the various oxo-compounds including heterocycles, e.g. isatin [19,44,45]. Moreover, the cyclic analogues of thiourea also have been successfully utilized in such approach in the synthesis of compounds bearing 5-ene-4-thiazolidinone fragment [46].

Scheme 2

The regioselectivity of the cyclization is influenced by the reaction conditions, choice of the haloacetic acid derivative and also by the nature of the two substituents R^1, R^2 and Ar(Het) of the thioureas (Scheme 2). Therefore, the products of the reaction may be the mixture of isomers which differ by the substituents at the positions 2 and 3 [47,48]. Based on the described protocols only one presented form of compounds was isolated in this study. Synthesized 2-substituted-4-thiazolidinones, bearing hydrogen atom on an exocyclic nitrogen atom exist in the form of two tautomers differing by the position of C=N double bond (2-amino- and 2-amino tauthomers); tautomers with exocyclic C=N double bond exist as mixture of *Z*- and *E*-stereoisomers [12,19,49]. Following the aforesaid the large number of works has been devoted to the investigation of the structure of mentioned compounds. The latter are represented both in amino and imino forms; predominance of one or another form depends usually on the nature of substituent in starting thiourea. Presented spectral data are not sufficient

argument to assert only one form, moreover ^{1}H NMR spectral analysis of the data allows affirming the presence of two mentioned forms. Main argument for amino form presentation is X-ray data for such type of compounds [19].

The analytical and spectral data (^{1}H NMR, ^{13}C NMR, LCMS) confirmed the structure and purity of compounds. ^{1}H NMR spectra of target compounds showed characteristic patterns of protons. In the most cases there is a doubling of signals corresponding to appropriate tautomeric forms. The location of CH= group (C5 ylidene fragment compounds **17-27**) signal (singlet) at ~7.50-8.50 ppm confirms the formation of compounds with *cis*-position of ylidene residue (*Z*-configuration), similar to other 5-ene-4-azolidinones [10,12,19,50].

For compounds which include oxo(thio)diazole/thiazole/pyrazole residue (**12-16**, **23-27**, **29**, **30**, **33**) it was shown the existence of imino form in the solid state based on the **13** X-ray data (Fig. 1). This was the main argument for structures representation (Scheme 2).

Figure 1

The X-ray analysis of **13** geometry showed that the hydrogen atom is attached to N3 which is in agreement with its localization in tautomer **13-1** (Fig. 1 and 2), containing a secondary amide in the 4-thiazolidinone system and an exocyclic imine nitrogen. Relevance of this finding is supported by the values of interatomic distances N3–C4 [1.371(2)] and C2–N3 [1.373(2) Å] as well as C2–N6 [1.298(2) Å] that are close to the mean values for the single bonds *(O=)*C–NH[1.369(4)Å] and NH–C*(=N)* [1.374(3) Å] as well as double bond C=N [1.280(2) Å], respectively, acquired from the structures containing 2-imino-1,3-thiazolidine-4-one moiety that are accessible from the Crystal Structure Database (refcodes: EHITZO, HEGMEN, HEGMIR, HEGMOX, ROMXUN, ULACEQ, VAMPUV, CSD Cambridge, Version 5.35 , R < 0.07) [51].

Figure 2

A shortening of the distance C4=O20 [1.212(2) Å] and lengthening of the distance C4–N3 [1.371(2) Å], bond in comparison with the typical *(HN–)*C=O, 1.235(2) Å, and *(O=)*C–NH, 1.331(2) Å, bond lengths [52] for the secondary amide group within γ-lactam ring are noteworthy. The difference is about 8σ for the former and 14σ for the latter bond. This proves that the conjugation of the lone electron pair on N3 with the C4=O19 carbonyl group is significantly weakened in favor of the conjugation with the C2=N6 double bond and further with the 1,3,4-oxadiazole ring. The molecule of **13** as a whole is exactly flat, as it lies on the mirror plane. Concurrently, the S1–C2 and N6–C7 bonds are in *Z* configuration and conjugated double bond system C2=N6–N7=C8 is in *s-cis* conformation. Both torsion angles S1–C2–N6–C7 and C2–N6–C7–N11 are 0.0º.

The next phase was the synthesis 5-ylidene-4-aminothiazol-2(5*H*)-ones (**34**, **35**), which are positional isomers of target compounds. The synthetic protocol included the phase of 4-aminothiazol-

2(5*H*)-ones formations followed by Knoevenagel condensation according to the known procedure [12,50].

Scheme 3

In contrast to the 2-amino(imino)-4-thiazolidinones, compounds **34**, **35** exist only in amino form and possibility of amino-imino tautomerism had been refuted [12]. This thesis is also confirmed by the **35** X-ray data (fig. 3)**.**

Figure 3

The X-ray studies showed that compound **35** exists in the crystal in the tautomeric form **35-1** with the H atom located on the exocyclic atom N7 (Fig. 3 and 4). This observation is supported by approximately similar C4–N3 [1.3287(15) Å] and C4–N7 [1.3210(15) Å] bond lengths, a typical feature for this tautomeric form, as well as by intermolecular N7–H7···O6^{i} and O15–H15···N3^{ii} hydrogen bonds (Table 1), in which the N7 atom is a hydrogen donor and the N3 atom is a hydrogen acceptor.

Figure 4

Partly double-bond character of the C4–N7 linkage between the thiazolidinone and 2-methyl-4-hydroxy-5-isopropylphenylamine moieties slows rotation and forces the N3,C4,C5,N7,C8 atoms to adopt almost a planar conformation (r.m.s.: 0.0443 Å) with atoms N3 (thiazolidinone system) and C8 (2-methyl-4-hydroxy-5-isopropylphenyl residue) synperiplanar to each other [torsion angle: N3–C4–N7–C8: -9.43(17)°]. The planarity of the N3,C4,C5,N7,C8 framework results in an unfavorable spatial arrangement of the N7–H7 and C19–H19 groups of atoms which form a short H7···H19 contact (2.08 Å). The phenyl ring of the *p*-chlorobenzylidene group and thiazolidinone system are approximately coplanar [dihedral angle: 4.54(6)°]. Furthermore, the *p*-chlorophenyl group is in a *Z* configuration [torsion angle S1–C5–C19–C20: -1.7(2)°]. The second phenyl ring of the 2-methyl-4-hydroxy-5-isopropylphenylamine substituent forms with the flat thiazolidinone system a dihedral angle of 69.39(5)°. Such mutual orientation is stabilized by an intermolecular hydrogen bond O15–H15···N3^{ii} (Table 1, Supplementary Fig. S1).

Table 1

In the crystal of **35-1** the solute molecules are connected by hydrogen bonds O15–H15···N3^{ii} [(ii) 1.5-*x*,0.5-*y*,1-*z*] (Table 1) into centrosymmetric dimers which are further linked through three-centre hydrogen bonds N7–H7···O6^{i}···H19–C19 [(i) 1.5-*x*,0.5+*y*,0.5-*z*] (Table 1, Fig. 3) into two-dimensional layers growing parallel to the *bc* plane. The layer thickness is about half of the *a* parameter length. The methanol molecules, located in the symmetry centre of the crystal lattice, are connected by three-centre hydrogen bonds O6···H27–O27···H21–C21^{iii} [(iii) 1.5-x, -0.5+y,0.5-z] with the solute molecules from the neighbouring layers.

The synthesis of some compounds (**16-18, 20-22, 34**) was described in our previous publications (see experimental part).

2.2. Pharmacology

2.2.1. Fibroblasts viability determined by SRB assay

The need for novel drugs for treatment of idiopathic pulmonary fibrosis [29] prompted the present search for new compounds that display antifibrotic properties in cultured lung fibroblasts as well as superoxide scavenging activity. A series of thiazolidinones and Pirfenidone as a referent drug were evaluated for their effect on fibroblast viability using the SRB assay. In the SRB assay the test compounds showed a wide range of activities. Based on their activity, the tested compounds were divided into 3 categories: *i*) good inhibitors (compounds that inhibit proliferation by at least 50%) for which the level of inhibition was comparable with Pirfenidone; *ii*) moderate inhibitors (compounds that inhibit proliferation by at least 10% but less than 50%) and *iii*) stimulators (compounds that do not reduce or stimulate proliferation). The results for these groups are shown in Figure 5. Interestingly, in the cellular assays Pirfenidone displayed potent antifibrotic activity as evidenced by its inhibition of fibroblast viability as well as its moderate effect on fibroblast proliferation (see below) which are in line with clinical findings.

Figure 5

2.2.2. Anticancer activity screening

A series of compounds was screened within *Developmental Therapeutic Program* (NCI, NIH, USA; https://dtp.cancer.gov/) according to the procedure described elsewhere [53-55] and involved several phases. First, tested compounds were evaluated at one-dose primary anticancer assay towards: a three cell line panel (the human tumor cell lines: *MCF7* – breast cancer (BC), *NCI-H460* – lung cancer (NSCLC) and *SF-268* CNS cancer (CNSC)) at concentration 10^{-4}M or sixty cell line panel (the human tumor cell lines were derived from nine different cancer types: leukemia (L), melanoma (M), NSCLC, colon (CC), CNSC, ovarian (OC), renal (RC), prostate (PC) and BC) at concentration 10^{-5}M. In the screening assay, each cell line was inoculated and preincubated for 24-48 h. Then tested compounds were added at the mentioned concentration the culture was incubated for further 48 h. SRB was used as endpoint in a cytotoxicity assay. Results of screening assay were presented as growth percent (GP) of the treated cells when compared to the untreated control cells (Tables 2 & 3). Based on 3 cell lines assay only compounds **17**, **20** [19] and **24** were selected for further phases.

Tables 2, 3

In a 60 cancer cell lines assay tested compounds including Pirfenidone did not exhibit significant activity. Only compound **31** possesses selective action on UO-31 (RC) cell line. This finding is

consistent with previously data regarding high sensitivity of UO-31 cells to active thiazolidinone [10,12,50].

At the next phase compounds (**4**, **17** [19], **18, 20** [19], **30** and **34** [12]) were tested towards a sixty tumor cell lines panel at 5 different concentrations (100μM, 10μM, 1.0 μM, 0.1μM and 0.01μM) [53-55]. The same screening protocol was used and GPs were evaluated for each cell lines at each compounds concentrations. The results were presented as three dose-dependent parameters – GI_{50}, TGI and LC_{50} (see experimental part) (Table 4).

Table 4

Among the tested compounds **17** [19], **18** and **20** [19] possessed the prominent activity levels and inhibited cancer cell lines at concentration less than 1.0 μM. Comparison of SRB assays on fibroblasts and cancer cell lines revealed that compounds which significant reduced the viability of fibroblasts (Fig. 5a) (**13**, **24**, **33**, **34** and Pirfenidone) did not possess a significant anticancer effect. Conversely, compounds with anticancer activity (**18**, **20**) belong to the group 3 ("stimulators" Fig. 5c) which did not suppress fibroblast proliferation. Only compound **17**, which possesses significant anticancer activity, displayed moderate antifibrotic action.

2.2.3. Superoxide scavenging

Superoxide scavenging was determined for all compounds by measuring their ability to reduce superoxide induced reduction of nitroblue tetrazolium (NBT) as described previously [56]. In Figure 6, the results are shown for the compounds that inhibited NBT reduction. IC_{50} and Emax values for inhibition of NBT reduction were calculated as described above. Results from the compounds that did not inhibit NBT reduction were omitted from this graph for clarity.

Figure 6

Pirfenidone, although being a good inhibitor in the SRB assay, did not scavenge superoxide radicals. A similar picture emerged for compounds **12** and **34**. Compounds that combined good activity on fibroblast viability as well as superoxide scavenging activity were **13**, **24** and **33**. While compound **3** was a good inhibitor of fibroblast viability and it did not scavenge any superoxide radicals. This is remarkable because this compound contains a phenol moiety attached to the thiazolidinone core. Given the previously recognized importance of superoxide radicals in the pathophysiology of idiopathic pulmonary fibrosis [40,41] lack of this feature may make this compound probably less interesting for further testing. Mode of action of such compounds most probably is not related with directed superoxide inhibition/scavenging. Among the 4-thiazolidinones with anticancer activity only **20** and **24** show superoxide radicals scavenging. This finding is not consistent with a known approach: search for new radical scavenging agents as active anticancer agent, including thiazolidonones [57]. While the anticancer effect of "classical" anticancer drugs (cisplatin and doxorubicin) is accompanied by an

increase in the intracellular level of ROS that may contribute to their therapeutic effect [58]. Increasing ROS production represents also an alternative among the chemotherapeutic strategies against cancer cells [59]. This effect is also associated with the mode of action of related thiazolidinones. It was reported that ROS levels increased under the action of 5-ene-4-thiazolidinones in HT29 and CEM cells [60,61]. Moreover, generation of ROS could activate the NF-2E related factor 2/Kelch like ECH-associated protein 1 (Nrf2/Keap1) pathway, involved to protect cells against oxidative stress [16,62].

2.2.4. Proliferation determined by xCelligence analysis

Using the xCelligence system, the effects of set of thiazolidinones and pirfenidone on human lung fibroblast proliferation were monitored (Fig. 7).

Figure 7.

Four of the tested compounds (**5**, **17**, **21**, and Pirfenidone) concentration-dependently reduced proliferation over the 150 h time course (P150). Compounds **12**, **13**, **24**, **33** and **34** (belong to group 1 – Fig. 5a – "good inhibitors") also reduced P150 but with these compounds no concentration dependency was observed. Compounds **21** and **34** also concentration dependently reduced the maximum rate of proliferation (Pdot). Compounds that combined good activity on cell viability as well as superoxide scavenging activity, namely **13**, **24** and **33** also inhibited cell proliferation although this effect did not show a good correlation with the concentration tested. Testing these compounds in the xCelligence system confirmed the antifibrotic potential of **5**, **12**, **13**, **24**, **33**, and **34** which had a similar effect as Pirfenidone.

3. Conclusion

A series of 2-amino(imino)-4-thiazolidinone derivatives and 4-R-aminothiazol-2(5*H*)-ones were synthesized. An efficient one-pot method based on three-component reaction of S,N-dinucleophile, electrophilic reagent and oxocompound was used as synthetic protocol for 5-ene-2-amino(imino)thiazolidin-4-ones. Following the amino-imino tautomerism the compound structures were confirmed by X-ray analysis. Compounds were screened for their antifibrotic and anticancer activities. Comparison of SRB assays on lung fibroblasts and cancer cells revealed that compounds which significantly reduced the viability of fibroblasts did not possess antiproliferative or antineoplastic effects. Among the tested compounds 2-{3-furan-2-ylmethyl-2-[(2-methyl-3-phenylallylidene)hydrazono]-thiazolidin-4-one-5-yl}-N-(3-trifluoromethylphenyl)-acetamide (**5**), N-(2-methoxyphenyl)-2-[5-(4-oxothiazolidin-2-ylideneamino)[1,3,4]thiadiazol-2-ylsulfanyl]-acetamide (**12**), 3-[3-allyl-4-oxo-2-(thiazol-2-ylimino)thiazolidin-5-ylidene]-1,3-dihydroindol-2-one (**33**), and 5(*Z*)-(thiophen-2-ylmethylene)-4-(4-chlorophenylamino)thiazol-2(5H)-one (**34**) possessed high activity levels, had effects similar to Pirfenidone and did not scavenge superoxide radicals. xCelligence system

testing confirmed their antifibrotic potential. Thus a range of thiazolidinone derivatives as interesting candidates for further testing and for design of new antifibrotic agents has been identified.

4. Experimental

4.1. Chemistry

4.1.1. Materials and methods

The starting 4-thioxo-2-thiazolidinone [63], 2-(thiazol-2-ylimino)-thiazolidin-4-one (**15**) [64] were obtained by the methods described previously. Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer and were within ±0.4% of the theoretical values. The ^{1}H-NMR spectra were recorded on Varian Gemini 400 MHz and ^{13}C NMR spectra on Varian Mercury-400 100 MHz in DMSO-d_6 using tetramethylsilane as an internal standard. Chemical shifts are reported in ppm units with use of δ scale. Mass spectra were obtained using electrospray ionization (ESI) techniques on an Agilent 1100 Series LCMS.

4.1.2. General procedure for the preparation of 5-unsubstituted and 5-alkyl-2-R-amino(imino)thiazol-4(5H)-one derivatives (**1-16**).

Method 1 (compounds **1-11**). A mixture of appropriate substituted thiourea or thiosemicarbazone (10 mmol) and appropriate carboxylic acid derivative (β-aroylacrylic acid, N-arylmaleinimide, γ-bromobutyrolactone, chloroacetic, 2-bromopropionic and 2-bromobutyric acids) (10 mmol) and sodium acetate (10 mmol) in 50 mL of acetic acid was refluxed for 3 h. Crystalline precipitate was filtered off, washed with acetic acid, water, ethanol and diethyl ether and then recrystallized from a mixture DMF : acetic acid (1:2) or DMF : ethanol (1:2) or glacial acetic acid.

Method 2 (compounds **12-16**). A mixture of N-heteryl-2-chloroacetamide (10 mmol) and ammonium thiocyanate (20 mmol) was refluxed for 3 h in acetone (20 mL). After the reaction was completed, the mixture was cooled. The precipitate was collected by filtration, washed with water and recrystallized with glacial acetic acid.

4.1.2.1. 3-(4-Ethoxyphenyl)-2-(4-ethoxyphenylimino)-5-[2-(4-methoxyphenyl)-2-oxoethyl]thiazolidin-4-one (**1**). Yield 69%, mp 147-149 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.29 (t, 3H, J = 7.0 Hz, CH_3), 1.35 (t, 3H, J = 6.8 Hz, CH_3), 3.80 (dd, 1H, J = 18.0, 9.2 Hz, $CHCH_2$), 3.85 (s, 1H, CH_3), 3.96 (m, 3H, CH_2, $CHCH_2$), 4.07 (q, 2H, J = 7.0 Hz, CH_2), 4.68 (dd, 1H, J = 9.3, 3.0 Hz, $\underline{CH}CH_2$), 6.77 (d, 2H, J = 8.8 Hz, arom.), 6.84 (d, 2H, J = 8.8 Hz, arom.), 7.00-7.07 (m, 4H, arom.), 7.38 (d, 2H, J = 8.8 Hz, arom.), 7.95 (d, 2H, J = 8.2 Hz, arom.). ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.3, 165.8, 155.3, 144.3,

141.2, 140.5, 130.6, 129.6, 128.7, 128.0, 121.9, 114.9, 114.7, 114.1, 63.4, 63.2, 55.7, 44.7, 42.8, 14.8, 14.7. LCMS (ESI) *m/z* 505 (99.0%, $(M+H)^+$). Calcd. for $C_{28}H_{28}N_2O_5S$: C, 66.65; H, 5.59; N 5.55; Found: C, 66.80; H, 5.70; N, 5.70%.

4.1.2.2. 5-[2-(4-Fluorophenyl)-2-oxoethyl]-2-(4-hydroxyphenylamino)-4,5-dihydrothiazol-4-one (**2**). Yield 76%, mp >230 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 3.54 (m, 1H, $CHCH_2$), 3.95 (m, 1H, $CHCH_2$), 4.39 (m, 1H, $CHCH_2$), 6.67 (m, 2H, arom.), 6.89 (d, 1H, *J* = 8.0 Hz, arom.), 7.30 (m, 2H, arom.), 7.48 (d, 1H, *J* = 8.0 Hz, arom.), 8.02-8.12 (m, 2H, arom.), 9.31, 9.33 (s, 1H, OH), 10.87, 11.40 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.5, 189.4, 176.8, 166.4, 164.4, 154.7, 144.3, 132.6, 131.3 (d, *J* =9.8 Hz), 130.6, 122.2, 115.9 (d, *J* = 22 Hz), 115.8, 115.4, 49.2, 42.5. LCMS (ESI) *m/z* 345 (100.0%, $(M+H)^+$). Calcd. for $C_{17}H_{13}FN_2O_3S$: C, 59.29; H, 3.81; N, 8.13; Found: C, 59.10; H, 3.90, N, 8.00%.

4.1.2.3. N-(4-Bromophenyl)-2-[2-(3-hydroxyphenylamino)-4-oxo-4,5-dihydrothiazol-5-yl]acetamide (**3**). Yield 61%, mp 210-211 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 2.65-2.75 (m, 1H, $CHCH_2$), 3.30-3.40 (m, 1H, $CHCH_2$), 4.30-4.38 (m, 1H, $CHCH_2$), 6.96-7.02 (m, 2H, arom.), 7.06 (t, 1H, *J* = 8.1 Hz, arom.), 7.22 (brs, 2H, arom.), 7.42 (brs, 1H, arom.), 7.45-7.50 (m, 2H, arom.), 10.80, 11.50 (brs, 1H, NH), 9.06, 9.18 (s, 1H, OH), 9.90, 9.92 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.7, 173.5, 168.1, 158.1, 154.0, 149.3, 138.1, 131.7, 130.0, 121.1, 116.7, 111.7, 107.8, 44.3, 43.4. LCMS (ESI) *m/z* 420 (95.9%, $(M+H)^+$). Calcd. for $C_{17}H_{14}BrN_3O_3S$: C, 48.58; H, 3.36;N, 10.00; Found: C, 48.45; H, 3.40; N, 10.20%.

4.1.2.4. N-(4-Bromophenyl)-2-[2-(4-sulfamoylphenylamino)-4-oxo-4,5-dihydrothiazol-5-yl]acetamide (**4**).Yield 80%, mp 222-224 ^oC. ^1HNMR (400 MHz, DMSO-d_6): δ 3.54 (m, 1H, $CHCH_2$), 3.90 (m, 1H, $CHCH_2$), 4.36 (m, 1H, $CHCH_2$), 6.68 (m, 2H, arom.), 6.95 (d, 1H, *J* = 8.1 Hz, arom.), 7.28 (m, 2H, arom.), 7.52 (d, 1H, *J* = 8.0 Hz, arom.), 7.62 (s, 2H, NH_2), 8.07-8.14 (m, 2H, arom.), 10.92, 11.38 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.7, 165.7, 158.3, 144.3, 139.7, 131.7, 127.2, 121.5, 121.0, 119.2, 115.0, 42.1, 43.7. LCMS (ESI) *m/z* 483/485 (100.0%, $(M+H)^+$). Calcd. for $C_{17}H_{15}BrN_4O_4S_2$: C, 42.24; H, 3.13; N, 11.59; Found: C, 42.30; H, 3.30; N, 11.70%.

4.1.2.5. 2-{3-Furan-2-ylmethyl-2-[(2-methyl-3-phenylallylidene)hydrazono]-thiazolidin-4-one-5-yl}-N-(3-trifluoromethylphenyl)-acetamide (**5**). Yield 68%, mp 176-177 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 2.11 (s, 3H, CH_3), 3.03 (dd, 1H, *J* = 9.0, 16.8 Hz, $CHCH_2$), 3.27-3.33 (m, 1H, $CHCH_2$), 4.56 (dd, 1H, *J* = 3.6, 9.2 Hz, $CHCH_2$), 4.93 (s, 2H, CH_2), 6.43 (m, 2H, arom.), 7.04 (brs, 1H, arom.), 7.31 (m, 1H, arom.), 7.41-7.48 (m, 5H, arom.), 7.56 (t, 1H, *J* = 7.9 Hz, arom.), 7.60 (s, 1H, arom.), 7.72 (d, 1H, *J* = 8.6 Hz, arom.), 8.07 (s, 1H, arom.), 8.25 (s, 1H, arom.), 10.52 (s, 1H, NH). ^{13}C NMR (100 MHz,

DMSO-d_6): δ 173.6, 168.64, 163.0, 162.5, 148.9, 142.6, 139.5, 139.3, 137.5 (q, *J* = 121 Hz), 136.3, 134.3, 130.2, 129.6, 128.6, 128.1, 125.2, 122.7, 121.6, 110.7, 108.7, 42.5, 38.9, 34.8, 13.0. LCMS (ESI) *m/z* 541 (100.0%, $(M+H)^+$). Calcd. for $C_{27}H_{23}F_3N_4O_3S$: C, 59.99; H, 4.29; N, 10.36; Found: C, 60.10; H, 4.10; N, 10.50%.

4.1.2.6. 3-Furan-2-ylmethyl-2-(pyridin-3-ylmethylenehydrazono)-thiazolidin-4-one (**6**). Yield 80%, mp 156-158 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 3.96 (s, 2H, CH_2), 4.92 (s, 2H, CH_2), 6.37 (d, 2H, *J* = 12.1 Hz, arom.), 7.41-7.44 (m, 1H, arom.), 7.47 (s, 1H, arom.), 8.14 (d, 1H, *J* = 7.9 Hz, arom.), 8.51 (s, 1H, arom.), 8.58 (m, 1H, arom.), 8.87 (s, 1H, CHN). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.6, 171.9, 164.8, 155.6, 151.5, 148.8, 142.7, 134.2, 129.9, 124.2, 110.8, 109.0, 50.3, 32.2. LCMS (ESI) *m/z* 301 (98.6%, $(M+H)^+$). Calcd. for $C_{14}H_{12}N_4O_2S$: C, 55.99; H, 4.03; N, 18.65; Found: C, 55.80; H, 4.10; N, 18.80%.

4.1.2.7. 5-Ethyl-3-furan-2-ylmethyl-2-(pyridin-3-ylmethylenehydrazono)-thiazolidin-4-one (**7**). Yield 79%, mp 175-177 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 1.00 (t, 3H, *J* = 7.0 Hz, CH_3), 1.90 (dd, 1H, *J* = 13.6, 6.3 Hz, $CHCH_2$), 2.01 (dd, 1H, *J*= 13.4, 7.3 Hz, $CHCH_2$), 4.27 (m, 1H, $CHCH_2$), 4.93 (s, 2H, CH_2), 6.35 (s, 2H, arom.), 7.44 (m, 2H, arom.), 8.15 (d, 1H, *J* = 7.6 Hz, arom.), 8.51 (s, 1H, arom.), 8.59 (s, 1H, arom.), 8.88 (s, 1H, arom.). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.3, 172.3, 163.4, 155.0, 152.4, 148.0, 145.2, 133.0, 129.8, 124.6, 113.7, 109.2, 48.6, 32.2, 25.4, 13.0. LCMS (ESI) *m/z* 329 (97.0%, $(M+H)^+$). Calcd. for $C_{16}H_{16}N_4O_2S$: C, 58.52; H, 4.91; N, 17.06; Found: C, 58.70; H, 5.00; N, 16.90%.

4.1.2.8. 2-(N'-Pyridin-3-ylmethylenehydrazono)thiazolidin-4-one (**8**). Yield 68%, mp >250 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 3.81 (s, 3H, CH_2), 7.40 (s, 1H, arom.), 8.10 (s, 1H, arom.), 8.41 (s, 1H, arom.), 8.57 (s, 1H, arom.), 8.86 (s, 1H, arom.), 11.88 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.5, 153.9, 153.8, 151.3, 149.2, 134.1, 130.1, 124.1, 33.1.LCMS (ESI) *m/z* 221 (100.0%, $(M+H)^+$). Calcd. for $C_9H_8N_4OS$: C, 49.08; H, 3.66; N, 25.44; Found: C, 47.90; H, 3.80; N, 25.20%.

4.1.2.9. 5-Methyl-2-(N'-pyridin-3-ylmethylenhydrazono)thiazolidin-4-one (**9**). Yield 59%, mp 241-243 ^oC.^1H NMR (400 MHz, DMSO-d_6): δ 1.56 (d, 3H, *J* = 7.1 Hz, CH_3), 4.10 (q, 1H, *J* = 7.1 Hz, CH), 7.42 (m, 1H, arom.), 8.11 (d, 1H, *J* = 7.3 Hz, arom.), 8.41 (s, 1H, arom.), 8.57 (m, 1H, arom.), 8.84 (s, 1H, arom.), 11.93 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.7, 157.3, 153.8, 151.3, 149.2, 134.1, 129.6, 124.1, 33.3, 18.8. LCMS (ESI) *m/z* 235 (98.4%, $(M+H)^+$). Calcd. for $C_{10}H_{10}N_4OS$: C, 51.27; H, 4.30; N, 23.91; Found: C, 57.40; H, 4.40; N, 24.00%.

4.1.2.10. 5-Ethyl-2-(N'-pyridin-3-ylmethylenehydrazono)thiazolidin-4-one (**10**). Yield 61%, mp 192-194 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.04 (t, 3H, J = 7.0 Hz, CH_3), 1.87 (m, 1H, $CHCH_2$), 2.04 (m, 1H, $CHCH_2$), 4.10 (m, 1H, $CHCH_2$), 7.43 (m, 1H, arom.), 8.14 (d, 1H, J = 7.9 Hz, arom.), 8.47 (s, 1H, arom.), 8.57 (m, 1H, arom.), 8.86 (s, 1H, arom.), 11.93 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.3 154.03, 151.1, 149.1, 144.3, 134.3, 130.2, 124.2, 49.4, 25.5, 10.4. LCMS (ESI+) *m/z* 249 (98.0%, $(M+H)^+$). Calcd. for $C_{11}H_{12}N_4OS$: C, 53.21; H, 4.87; N, 22.56; Found: C, 53.30; H, 5.00; N, 22.60%.

4.1.2.11. 5-(2-Hydroxyethyl)-2-(N'-pyridin-3-ylmethylenehydrazono)thiazolidin-4-one (**11**). Yield 68%, mp 249-251 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.85 (m, 1H, CH_2), 2.29 (m, 1H, aliph.), 3.57 (m, 1H, aliph.), 3.65 (m, 1H, aliph.), 4.14 (m, 1H, aliph.), 4.62 (brs, 1H, OH), 7.42 (t, 1H, J = 6.6 Hz, arom.), 8.13 (d, 1H, J = 7.9 Hz, arom.), 8.41 (s, 1H, CH), 8.84 (s, 1H, arom.), 11.90 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.3, 165.1, 153.9, 151.3, 149.3, 134.1, 130.2, 124.1, 58.5, 45.5, 35.8. LCMS (ESI) *m/z* 265 (100.0%, $(M+H)^+$). Calcd. for $C_{11}H_{12}N_4O_2S$: C, 49.99; H, 4.58; N, 21.20; Found: C, 50.10; H, 4.50; N, 21.10%.

4.1.2.12. N-(2-Methoxyphenyl)-2-[5-(4-oxothiazolidin-2-ylideneamino)-[1,3,4]thiadiazol-2-ylsulfanyl]-acetamide (**12**). Yield 73%, mp 180-181 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H, CH_3), 4.11 (s, 2H, CH2), 4.30 (s, 2H, CH_2), 6.89-6.93 (m, 1H, arom.), 7.04-7.09 (m, 2H, arom.), 7.99 (d, 1H, J = 8.1 Hz, arom.), 9.64 (s, 1H, NH), 12.35 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.7, 165.8, 163.2, 149.3, 146.4, 138.6, 127.1, 124.7, 121.3, 120.4, 111.3, 55.8, 37.6, 35.8. LCMS (ESI) *m/z* 396 (95.4%, $(M+H)^+$). Calcd. for $C_{14}H_{13}N_5O_3S_3$: C, 42.52; H, 3.31; N, 17.71; Found: C, 42.60; H, 3.20; N, 17.90%.

4.1.2.13. 2-[5-(4-Methoxyphenyl)-[1,3,4]oxadiazol-2-ylimino]thiazolidin-4-one (**13**). Yield 72%, mp 261-263 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 7.11 (d, 2H, J = 8.2 Hz, arom.), 7.88 (d, 2H, J = 7.9 Hz, arom.), 12.2 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.8, 174.4, 169.8, 164.1, 161.8, 127.9, 116.2, 114.9, 55.6, 36.1. LCMS (ESI) *m/z* 291 (100.0%, $(M+H)^+$). Calcd. for $C_{12}H_{10}N_4O_3S$: C, 49.65; H, 3.47; N, 19.30; Found: C, 49.80; H, 3.60; N, 19.10%.

4.1.2.14. N-(4-Chlorophenyl)-2-[5-(4-oxothiazolidin-2-ylideneamino)[1,3,4]thiadiazol-2-ylsulfanyl]-acetamide (**14**). Yield 69%, mp 239-241 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.04 (s, 2H. CH_2), 4.23 (s, 2H, CH_2), 7.13 (d, 2H, J = 8.2 Hz, arom.), 7.47 (d, 2H, J = 8.2 Hz, arom.), 10.28 (s, 1H, NH), 12.25

(s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.8, 186.7, 165.6, 162.4, 150.7, 137.8, 128.9, 127.3, 120.8, 35.8, 30.9. LCMS (ESI) m/z 400/402 (100.0%, $(M+H)^+$). Calcd. for $C_{13}H_{10}ClN_5O_2S_3$: C, 39.05; H, 2.52; N, 17.51;Found: C, 38.90; H, 2.40; N, 17.60%.

4.1.2.15. 2-*(6-Methylbenzothiazol-2-ylimino)thiazolidin-4-one* (**16**). Spectral and analytical data are described [43].

4.1.3. General procedure for the preparation of 5-ene-2-R-amino(imino)thiazol-4(5H)-ones (**17-33**)

Method 1 (compounds **23-27**, **30**, **33**). A mixture of appropriate 5-unsubstituted thiazolidinone (compound **12-16**) (3 mmol), appropriate oxocompond (4 mmol) and anhydrous sodium acetate (3 mmol) was refluxed for 3 h in glacial acetic acid (30 mL). Powder obtained after cooling was filtered off, washed with methanol and recrystallized with DMF : ethanol (1:2).

Method 2 (compounds **17-22**, **28**, **29**, **31**, **32**). A mixture of the appropriate thiourea derivative (10 mmol), chloroacetic acid (10 mmol) and appropriate oxocompound (10 mmol), and sodium acetate (10 mmol) in 100 mL of acetic acid was heated under reflux for 3 h. Crystalline precipitate was filtered off, washed with acetic acid, water, ethanol and diethyl ether and then recrystallized from a mixture DMF : acetic acid (1:2) or acetic acid.

4.1.3.1. (Z)-5-(4-Chlorobenzylidene)-2-(4-hydroxyphenylamino)thiazol-4(5H)-one (**17**). Spectral and analytical data are described [19].

4.1.3.2. (Z)-5-Benzylidene-2-(4-hydroxyphenylamino)thiazol-4(5H)-one (**18**). Spectral and analytical data are described [65].

4.1.3.3. (Z)-5-(3-Phenylallylidene)-2-(4-hydroxyphenylamino)thiazol-4(5H)-one (**19**). Yield 78%, mp 276-278 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.78, 6.88, 7.58 (d, 4H, J = 7.6 Hz, 4-OH-$\underline{C_6H_4}$), 6.80-6.65, 7.05-7.16, 7.25-7.43, 7.47-7.63 (m, 8H, C_6H_5CH=CH-CH=), 9.48, 9.53 (s, 1H, OH), 11.34, 11.91 (s, 1H, NH).^{13}C NMR (100 MHz, DMSO-d_6): δ 179.9, 169.1, 162.4, 154.9, 141.6, 136.0, 129.3, 129.0, 127.7, 125.5, 124.1, 122.3, 116.0, 115.5. LCMS (ESI) *m/z* 323 (100.0%, $(M+H)^+$). Calcd. for $C_{18}H_{14}N_2O_2S$: C, 67.06; H, 4.38; N, 8.69; Found: C, 67.20; H, 4.30; N, 8.80%.

4.1.3.4. (Z)-5-[2-Chloro-3-(4-nitrophenyl)-allylidene]-2-[(4-hydroxyphenyl)-methylamino]thiazol-4(5H)-one (***20***).Spectral and analytical data are described [19].

4.1.3.5. (Z)-5-(2-Methyl-3-phenylallylidene)-2-[(4-hydroxyphenyl)methylamino]-thiazol-4(5H)-one (**21**). Spectral and analytical data are described [65].

4.1.3.6. (Z)-5-(3-Hydroxybenzylidene)-2-(4-hydroxyphenylamino)thiazol-4(5H)-one (**22**). Spectral and analytical data are described [65].

4.1.3.7. (Z)-5-(3-Ethoxy-4-hydroxybenzylidene)-2-(6-methylbenzothiazol-2-ylamino)thiazol-4(5H)-one (**23**).Yield 71%, mp 202-204 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.42 (t, 3H, J = 6.9 Hz, CH_3), 2.42 (s, 3H, CH_3), 4.15 (q, 2H, J = 6.8 Hz, CH_2), 6.96 (d, 1H, J = 8.2 Hz, arom.), 7.17 (d, 1H, J = 8.2 Hz, arom.), 7.30 (m, 2H, arom.), 7.67-7.74 (m, 3H, arom.), 9.93 (s, 1H, OH), 12.73 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.6, 167.5, 167.3, 158.6, 149.8, 148.9, 147.2, 134.1, 133.6, 127.9, 124.8, 124.6, 121.8, 121.2, 120.2, 116.4, 115.5, 64.0, 21.1, 14.8. LCMS (ESI) *m/z* 412 (98.3%, $(M+H)^+$). Calcd. for $C_{20}H_{17}N_3O_3S_2$: C, 58.38; H, 4.16; N, 10.21; Found: C, 58.50; H, 4.10; N, 10.40%.

4.1.3.8. 5-(4-Chlorobenzylidene)-2-(thiazol-2-ylimino)-thiazolidin-4-one (**24**). Yield 68%, mp 256-258 ^{o}C, lit 257.5-258.5 ^{o}C [66]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.40 (d. 1H, J = 3.9 Hz, thiazol.), 7.55-7.60 (m, 2H, arom.), 7.63 (brs, 1H, arom.), 7.64-7.66 (m, 2H, arom.), 7.68 (s, 1H, arom.), 12.63 (brs, 1H, NH). Calcd. for $C_{13}H_8ClN_3OS_2$: C, 48.52; H, 2.51; N, 13.06; Found: C, 48.40; H, 2.60; N, 12.90%.

4.1.3.9. 2-{5-[5(Z)-(4-Chlorobenzylidene)-4-oxothiazolidin-2-ylideneamino][1,3,4]thiadiazol-2-ylsulfanyl}-N-(2-methoxyphenyl)acetamide (**25**). Yield 63%, mp 245-246 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 3H, CH_3), 4.33 (brs, 2H, CH_2), 6.93 (d, 1H, J = 8.0 Hz, arom.), 7.05-7.11 (m, 2H, arom.), 7.66 (brs, 4H. arom.), 7.79 (s, 1H, arom.), 8.00 (d, 1H, J = 7.5 Hz, arom.), 9.65 (s, 1H, arom.), 13.02 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.8, 182.8, 163.2, 131.9, 129.6, 129.6, 124.7, 121.2, 120.4, 112.6, 112.6, 111.3, 55.8, 38.5. LCMS (ESI) *m/z* 518/520 (96.4%, $(M+H)^+$). Calcd. for $C_{21}H_{16}ClN_5O_3S_3$: C, 48.96; H, 3.11; N, 13.52; Found: C, 49.10; H, 3.00; N, 13.40%.

4.1.3.10. Z-5-(4-Dimethylaminobenzylidene)-2-[5-(4-methoxyphenyl)[1,3,4]oxadiazol-2-ylimino]-thiazolidin-4-one (**26**). Yield 74%, mp 291-292 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05 (brs, 6H, 2*CH_3), 3.85 (s, 3H, CH_3), 6.88 (brs, 2H, arom.), 7.12 (brs, 2H, arom.), 7.50 (brs, 2H, arom.), 7.66 (brs. 1H, arom.), 7.90 (brs, 2H, arom.). LCMS (ESI) *m/z* 412 (97.4%, $(M+H)^+$). Calcd. for $C_{21}H_{19}N_5O_3S$: C, 59.84; H, 4.54; N, 16.62; Found: C, 60.00; H, 4.50; N,16.50%.

4.1.3.11. N-(4-Chlorophenyl)-2-{5-[5(Z)-(4-methoxybenzylidene)-4-oxothiazolidin-2-ylideneamino][1,3,4]thiadiazol-2-ylsulfanyl}-acetamide (**27**). Yield 59%, mp 238-239 ^{o}C. ^{1}H NMR

(400 MHz, DMSO-d_6): δ 3.84 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 7.15 (d, 2H, *J* = 8.3 Hz, arom.), 7.39 (d, 2H, *J* = 8.3 Hz, arom.), 7.60-7.64 (m, 4H, arom.), 7.73 (s, 1H, =CH-Ar), 10.54 (s, 1H, NH), 12.35 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.0, 170.2, 167.2, 165.6, 165.5, 161.2, 160.5, 158.7, 137.8, 133.2, 132.4, 128.9, 127.3, 120.8, 115.2, 55.6, 37.8. LCMS (ESI) *m/z* 516/518 (95.1%, $(M-H)^+$). Calcd. for $C_{21}H_{16}ClN_5O_3S_3$: C, 48.69; H, 3.11; N, 13.52; Found: C, 48.60; H, 3.20; N, 13.70%.

4.1.3.12. 3-[2-(4-Hydroxyphenylamino)-4-oxo-4H-thiazol-5-ylidene]-1,3-dihydroindol-2-one (**28**). Yield 70%, mp >260 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.79, 6.82, 7.62 (d, 4H, *J* = 8.3 Hz, arom), 6.89, 6.93 (d, 1H, *J* = 7.5 Hz, arom.), 6.95-7.07, 7.28-7.35 (m, 2H, arom.), 8.89, 8.97 (d, 1H, *J* = 7.9 Hz, arom.), 9.45 (s, 1H, OH), 11.01, 11.04 (s, 1H, NH), 11.40, 12.10 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.7, 180.4, 172.7, 169.0, 155.2, 143.0, 136.9, 131.6, 130.2, 128.0, 124.9, 122.6, 120.6, 115.6, 110.2. LCMS (ESI) *m/z* 338 (95.6%, $(M+H)^+$). Calcd. for $C_{17}H_{11}N_3O_3S$: C, 60.53; H, 3.29; N, 12.46; Found: C, 60.70; H, 3.40; N,12.60%.

4.1.3.13. 3-[2-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)-4-oxothiazolidin-5-ylidene]-1,3-dihydroindol-2-one (**29**). Yield 68%, mp >260 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.41 (s, 3H, CH_3), 3.20 (s, 3H, CH_3), 6.88 (d, 2H, *J* = 8.4 Hz, arom.), 6.90-7.00 (m, 4H, arom.), 7.24-7.32 (m, 2H, arom.), 9.00 (d, 1H, *J* = 7.0 Hz, arom.), 10.90 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.3, 180.1, 169.0, 168.4, 164.4, 153.8, 144.2, 134.3, 133.1, 129.4, 129.1, 128.1, 127.8, 127.4, 124.9, 122.2, 119.9, 110.7, 35.2, 10.5. LCMS (ESI) *m/z* 432 (96.2%, $(M+H)^+$). Calcd. for $C_{22}H_{17}N_5O_3S$: C, 61.24; H, 3.97; N, 16.23; Found: C, 61.40; H, 3.80, N, 16.50%.

4.1.3.14. 5*-Bromo-3-[4-oxo-2-(thiazol-2-ylimino)thiazolidin-5-ylidene]-1,3-dihydroindol-2-one* (**30**). Yield 71%, mp >250 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.88 (d, 1H, *J* = 8.1 Hz, arom.), 7.48-7.53 (m, 2H, arom.), 7.73 (brs, 1H, arom.), 9.00, 9.17 (s, 1H, arom.), 11.12, 11.21 (s, 1H, NH), 12.95 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3, 162.3, 142.6, 140.8, 136.5, 134.2, 130.2, 125.1, 122.1, 118.2, 113.5, 112.1, 35.9, 30.9. LCMS (ESI) *m/z* 407/409 (98.0%, $(M+H)^+$). Calcd. for $C_{14}H_7BrN_4O_2S_2$: C, 41.29; H, 1.73; N, 13.76; Found: C, 41.10; H, 1.80. N, 13.90%.

4.1.3.15. 3-{2-[(4-Hydroxyphenyl)-methylamino]-4-oxo-4H-thiazol-5-ylidene}-1,3-dihydroindol-2-one (**31**). Yield 70%, mp >300 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.65 (s, 3H, CH_3), 6.85 (d, 2H, *J* = 8.8 Hz, arom.), 7.00 (d, 2H, *J* = 8.8 Hz, arom.), 6.80-7.00 (m, 2H, arom.), 7.24-7.26 (m, 1H, arom.), 9.00 (d, 1H, J = 7.0 Hz, arom.), 9.65(s, 1H, OH), 10.80 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.6, 179.5, 179.0, 168.9, 158.5, 143.1, 138.9, 132.6, 131.6, 128.6, 128.1, 125.3, 121.9, 120.5, 116.4, 110.1,

42.1. LCMS (ESI) *m/z* 352 (100.0%, $(M+H)^+$). Calcd. for $C_{18}H_{13}N_3O_3S$: C, 61.53; H, 3.73; N, 11.96; Found: C, 61.60; H, 3.60; N, 12.10%.

4.1.3.16. 3-{2-[(4-Hydroxyphenyl)-methylamino]-4-oxo-4H-thiazol-5-ylidene}-5-methyl-1,3-dihydroindol-2-one (**32**). Yield 63%, mp >300 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 3.60 (s, 3H, CH_3), 6.91 (d, 2H, *J* = 8.2 Hz, arom.), 7.31 (d, 2H, *J* = 8.2 Hz, arom.), 6.75 (d, 1H, *J* = 8.8 Hz, arom.), 7.05 (d, 1H, *J* = 8.8 Hz, arom.), 8.80 (s, 1H, arom.), 9.80 (s, 1H, OH), 10.65 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.7, 168.9, 158.4, 140.9, 138.6, 132.6, 132.1, 130.5, 128.7, 128.5, 125.4, 122.0, 120.5, 116.3, 109.8, 42.1, 21.1. LCMS (ESI) *m/z* 366 (99.0%, $(M+H)^+$). Calcd. for $C_{19}H_{15}N_3O_3S$: C, 62.45; H, 4.14; N, 11.50; Found: C, 62.60; H, 4.20; N, 11.30%.

4.1.3.17. 3-[3-Allyl-4-oxo-2-(thiazol-2-ylimino)thiazolidin-5-ylidene]-1,3-dihydroindol-2-one (**33**). Yield 71%, mp >230 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 4.98 (d, 2H, *J* = 4.8 Hz, CH_2), 5.23 (q, 2H, *J* = 10.3 Hz, CH), 5.81 (m, 1H, CH), 6.88 (d, 1H, *J* = 8.1 Hz, arom.), 7.48-7.53 (m, 2H, arom.), 7.62 (m, 1H, arom.), 7.73 (m, 1H, arom.), 8.92 (s, 1H, arom.), 11.12 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.7, 168.4, 165.3, 156.8, 143.8, 140.9, 132.5, 132.2, 131.4, 128.2, 127.2, 122.0, 120.3, 118.6, 117.4, 110.4, 44.6. LCMS (ESI) *m/z* 369 (100.0%, $(M+H)^+$). Calcd. for $C_{17}H_{12}N_4O_2S_2$: C, 55.42; H, 3.28; N, 15.21; Found: C, 55.50; H, 3.40; N, 15.30%.

*4.1.4. General procedure for synthesis of 5-aryliden-4-amino-1,3-thiazol-2(5H)-ones (**34, 35**)*

A mixture of 4-thioxo-2-thiazolidinone (10 mmol) and appropriate amine (10 mmol) was refluxed for 1 h in 25 mL of ethanol. After cooling to the room temperature formed precipitate (4-R-amino-thiazol-2(5*H*)-one) was filtered off, washed with methanol and recrystallized with appropriate solvent.

A mixture of appropriate 4-R-aminothiazol-2(5*H*)-one (3 mmol), aldehyde (3.3 mmol), and anhydrous sodium acetate (3 mmol) was refluxed for 3 h in glacial acetic acid (10 mL). Obtained precipitate was filtered off, washed with acetic acid, water and methanol and recrystallized with DMF : ethanol or DMF : acetic acid (1:2) mixtures.

4.1.4.1. (Z)-5-(Thiophen-2-ylmethylene)-4-(4-chlorophenylamino)thiazol-2(5H)-one (**34**). Spectral and analytical data described [12].

4.1.4.2. (Z)-5-(4-Chlorobenzylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino)thiazol-2(5H)-one (**35**). Yield 71%, mp 242-245^oC. ^1H NMR (400 MHz, DMSO-d_6): δ 1.20 (d, 6H, *J* = 7.0 Hz, 2*CH_3), 2.08 (s, 3H, CH_3), 3.18 (m, 1H, CH), 6.71 (s, 1H, arom.), 7.01 (s, 1H, arom.), 7.59 (d, 2H, *J* = 8.7 Hz, arom.), 7.62 (d, 2H, *J* = 8.7 Hz, arom.), 8.02 (s, 1H, CH=), 9.45 (s, 1H, OH), 10.67 (s, 1H, NH). ^{13}C

NMR (100 MHz, DMSO-d_6): δ 176.9, 172.9, 153.8, 134.5, 133.2, 132.4, 132.2, 131.3, 129.8, 129.5, 127.9, 126.7, 124.3, 116.6, 26.3, 22.6, 17.6. LCMS (ESI) *m/z* 387/389 (99.8%, $(M+H)^+$). Calcd. for $C_{20}H_{19}ClN_2O_2S$: C, 62.09; H, 4.95; N, 7.24; Found: C, 62.20; H, 4.80; N, 7.30%.

4.1.5. Crystal structure determination

4.1.5.1. Crystal structure determination of 2-[5-(4-methoxyphenyl)-[1,3,4]oxadiazol-2-ylimino]thiazolidin-4-one (**13**).

Crystal data: $C_{12}H_{10}N_4O_3S$, M_r = 290.30, monoclinic, space group $P2_1/m$, a = 7.23203(19), b = 6.35516(16), c = 13.4288(3) Å, β = 104.275(3)º, V = 598.14(3) Å^3, Z = 2, D_{calc} = 1.612 g/cm^3, μ = 0.285 mm^{-1}, T = 130(2) K.

Data collection: A yellow lath crystal (DMF) of 0.23×0.13×0.03 mm was used to record 5141 (Mo$K\alpha$-radiation, θ_{max}= 29.10°) intensities on an Xcalibur Atlas diffractometer (Agilent (2011). CrysAlis PRO. Oxford Diffraction Ltd, Yarnton, England). Intensity data collection employed the ω–scans mode with Enhance (Mo) X-ray Source. The data were corrected for Lorentz and polarization effects. Multi-scan absorption correction has been applied too. The minimum and maximum transmissions were 0.9844 and 1.0000. Data reduction and analysis were carried out with the Oxford Diffraction programs (Agilent (2011). CrysAlis PRO. Oxford Diffraction Ltd, Yarnton, England). The 1567 total unique reflections (R(int) = 0.019) were used for further calculations. H atom bonded to N atom was located from difference map and refined freely. All H atoms of CH groups were placed in calculated positions. As the molecule lies on the mirror plane in the crystal, symmetry-independent methylene and methyl H atoms were refined with the atomic displacement parameters fixed at 1.2U_{eq}(C) and 1.5U_{eq}(C), respectively. The remaining H atoms were refined as riding on their parent C atoms, with C–H = 0.93 Å and U_{iso}(H) = 1.2U_{eq}(C).

Structure solution and refinement. The structure was solved by the direct methods using the program SHELXS-97 [67], and refinement was done against F^2 for all data using SHELXL-97 [67]. The final refinement converged with R = 0.0302 (for 1352 data with $F^2 > 4\sigma(F^2)$), wR = 0.0852 (on F^2 for all data), and S = 1.039 (on F^2 for all data). The largest difference peak and hole were 0.367 and -0.232 eÅ^{-3}. The molecular illustrations were drawn using ORTEP-3 for Windows [68]. Software used to prepare material for publication was WINGX [68], OLEX [69] and PLATON [70].

The supplementary crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC), 12 Union ROAD, Cambridge CB2 1EZ (UK), Tel.: (+44) 1223/336-408, Fax: (+44) 1223/336-033, E-mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk (deposition No. **CCDC 992833**).

4.1.5.2. Crystal structure determination of (Z)-5-(4-chlorobenzylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino)-5H-thiazol-2-one methanol semisolvate (**35**).

Crystal data: $C_{20}H_{19}ClN_2O_2S$, ½(CH_4O), M_r = 402.90, monoclinic, space group *C*2/*c*, *a* = 20.6265(18), *b* = 13.6576(6), *c* = 17.8624(16) Å, β = 126.789(14)°, *V* = 4029.8(5) Å^3, Z = 8, D_{calc} = 1.328 g/cm^3, μ = 0.314 mm^{-1}, *T* = 130(2) K.

Data collection: A yellow block crystal (0.55 x 0.43 x 0.30 mm) was used to record 9665 (Mo*Kα* radiation, θ_{max} = 29.09º) intensities on an Agilent Xcalibur Atlas diffractometer (Agilent (2011). CrysAlis PRO. Oxford Diffraction Ltd, Yarnton, England)). The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlisPro (Agilent (2011). CrysAlis PRO. Oxford Diffraction Ltd, Yarnton, England). The intensities were collected in the ω scan mode with Enhance (Mo) X-ray Source. The data were corrected for Lorentz, polarization effects. Multi-scan absorption corrections have been applied too. The minimum and maximum transmissions were 0.9611 and 1.0000. The 4678 unique reflections (*R*(int) = 0.016) were used for further calculations. The position of the H atom bonded to N and O atoms in solute molecule were obtained from difference Fourier map and was refined freely. The remaining H atoms were positioned geometrically and were refined within the riding model approximation: C_{methyl}–H = 0.96 Å, $C_{methine}$–H = 0.98 Å, C(sp^2)–H = 0.93 Å and U_{iso}(H) = 1.2U_{eq}(C) or 1.5U_{eq}(C) for methyl H atoms. The methyl groups were refined as rigid groups, which were allowed to rotate.

Structure solution and refinement: The structure was solved by the direct methods using the program SHELXS-97 [67], and refinement was done against F^2 for all data using SHELXL-97 [67]. The final refinement converged with *R* = 0.0303 (for 3939 data with $F^2 > 4\sigma(F^2)$), *wR* = 0.0840 (on F^2 for all data), and *S* = 1.092 (on F^2 for all data). The largest difference peak and hole were 0.339 and -0.224 eÅ^{-3}. The molecular illustration was drawn using ORTEP-3 for Windows [68]. Software used to prepare material for publication was WINGX [68], OLEX2 [69] and PLATON [70].

The supplementary crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC), 12 Union ROAD, Cambridge CB2 1EZ (UK), Tel.: (+44) 1223/336-408, Fax: (+44) 1223/336-033, E-mail: deposit@ccdc.cam.ac.uk, World Wide Web: http://www.ccdc.cam.ac.uk (deposition No. **CCDC 992832**).

4.2. Pharmacology

4.2.1. Cell culture

Human fetal lung fibroblast (HFL-1) cells were obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured in tissue culture flasks (Falcon; Becton–Dickinson

Labware, Lincoln Park, NJ) with DMEM supplemented with 10% FCS, 100 mg/mL penicillin and 250 µg/mL streptomycin. The cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 and passaged every 4–5 days at a 1:4 ratio. HFL-1 cells were used between the 14th and 18th passages.

4.2.2. Sulphorhodamine B assay

SRB assay was performed according to Jacobs et al. [71] in 96-well plates in triplicate after 48 h treatment. In brief, cells were seeded at a density of 8000 cells/well, allowed to attach overnight, and treated with the various concentrations of compound. Afterwards, 100µL of 20% trichloroacetic acid was added to each well and refrigerated at 4 ºC for 3 h, then the supernatant was discarded and the plate was washed for 5 times with water and air dried. 100 µL of SRB solution 0.4% (w/v) in 1% acetic acid was added to each well and incubated for 30 min at room temperature. Unbound SRB was removed and the plates were air dried. Bound SRB was solubilized with 150 µL of 10 mM Tris-HCl and then the plates were shaken for 5 min. The optical density at 570 nm wavelength was measured and the ratio of cell proliferation to control group was calculated. IC_{50} and Emax values for inhibition of cell viability were calculated by 3 parameter curve fitting using Prism 5 for Windows (GraphPad Software Inc.).

4.2.3. Superoxide scavenging

The ability of the compounds to scavenge superoxide radicals was determined in a system consisting of xanthine (1 mM), xanthine oxidase (0.11 U/mL) and nitro blue tetrazolium (50 µM). Test compounds (dissolved in DMSO) were added at concentrations up to 10 µM. The DMSO concentration was kept constant at 0.1%. The NBT reduction was monitored spectrophotometrically for 3 min at 560 nm, the rate (dA560/dt) was determined from the linear part of the absorption vs. time plot. IC_{50} and Emax values for inhibition of NBT reduction were calculated by 3 parameter curve fitting as described above.

4.2.4. xCelligence

The effect of the test compounds on cell proliferation was determined by real time monitoring using the xCelligence system (Roche GmbH, Darmstadt) as described by Ke et al. [72]. In short, HLF-1 were inoculated in the 96 well plate at a density of 20,000 cells per well. Cell attachment was maximal after approximately 30 hours. Test compounds or the vehicle (DMSO) were then added to the cells and proliferation was monitored for another 150 h. The difference in signal between t = 30 h and t = 180 h was calculated and represents the 150 hour proliferation (P150). The maximum rate of proliferation Pdot was calculated by determining the maximum slope of the curve.

4.2.5. Anticancer activity screening

A primary anticancer assay was performed for three and an approximately 60 human tumor cell line panels derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD [53-55]. Tested compounds were added to the culture at a single concentration (10^{-4} and 10^{-5}M), and the cultures were incubated for 48 h. End point determinations were made with a protein binding SRB. The results for each tested compound were reported as the growth percentage of the treated cells when compared to that of the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active selected compounds were tested in vitro against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8}M. A 48 h continuous drug exposure protocol was followed, and an SRB protein assay was used to estimate cell viability or growth. By use of the seven absorbance measurements [time zero, (T_z), control growth in the absence of drug (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$[(T_i - T_z) / (C - T_z)] \times 100$ for concentrations for which $T_i \geq T_z$;

$[(T_i - T_z) / T_z] \times 100$ for concentrations for which $T_i < T_z$.

Three dose response parameters (GI_{50}, TGI, LC_{50}) were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(T_i - T_z) / (C - T_z)] \times 100 = 50$, which was the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(T_i - T_z) / T_z] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less for the maximum or minimum concentration tested. The lowest values are obtained with the most sensitive cell lies. The compounds having $GI_{50} \leq 100$ μM were considered active (https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm) [53-55].

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Scheme 1. Synthesis of 2-amino(imino)-4-thiazolidinones. Reagents and conditions: (*i*) – substituted thiourea or thiosemicarbazone (1.0 equiv), appropriate carboxylic acid derivative (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h.

Scheme 2. Synthesis of 5-ylidene-2-amino(imino)-4-thiazolidinones. Reagents and conditions: *(i*) – substituted thiourea (1.0 equiv), chloroacetic acid (1.0 equiv), appropriate oxocompound (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h; *(ii)* – N-heteryl-2-chloroacetamide (1.0 equiv), ammonium thiocyanate (2.0 equiv), acetone, reflux, 3 h; *(iii)* – 5-unsubstituted-4-thiazolidinone (**12-16**) (1.0 equiv), appropriate oxocompound (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h.

Scheme 3. Synthesis of 5-ylidene-4-R-aminothiazol-2(5*H*)-ones. Reagents and conditions: *(i*) – 4-thioxo-2-thiazolidinone (isorhodanine) (1.0 equiv), appropriate amine (1.0 equiv), EtOH, reflux, 1 h; *(ii)* – 4-arylaminothiazol-2(5*H*)-one (1.0 equiv), appropriate oxocompound (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h.

Figure 1. ORTEP view of **13** showing displacement ellipsoids at the 30% probability level. H atoms are shown as spheres of arbitrary radii.

Figure 2. Possible tautomeric structures of **13**.

Figure 3. ORTEP view of **35** showing displacement ellipsoids at the 30% probability level. H atoms are shown as spheres of arbitrary radii.

Figure 4. Possible tautomeric forms of **35**.

Figure 5. Effects on cell viability by the tested compounds determined with the SRB assay.

a. Compounds that reduced cell proliferation by at least 50%. **b.** Compounds that reduced cell proliferation less than 50%. **c.** Compounds that did not reduce cell viability or increased viability at concentrations > 1 μM. Viability was calculated relative to the control**.** Data are presented as means of 4 independent experiments.

Figure 6. Superoxide scavenging by tested compounds.

Scavenging was measured as inhibition of the reduction of NBT by xanthine/xanthine oxidase generated superoxide levels. NBT reduction rate is plotted on the y-axis as dA560/dt (min^{-1}). Data are presented as means of 4 independent experiments.

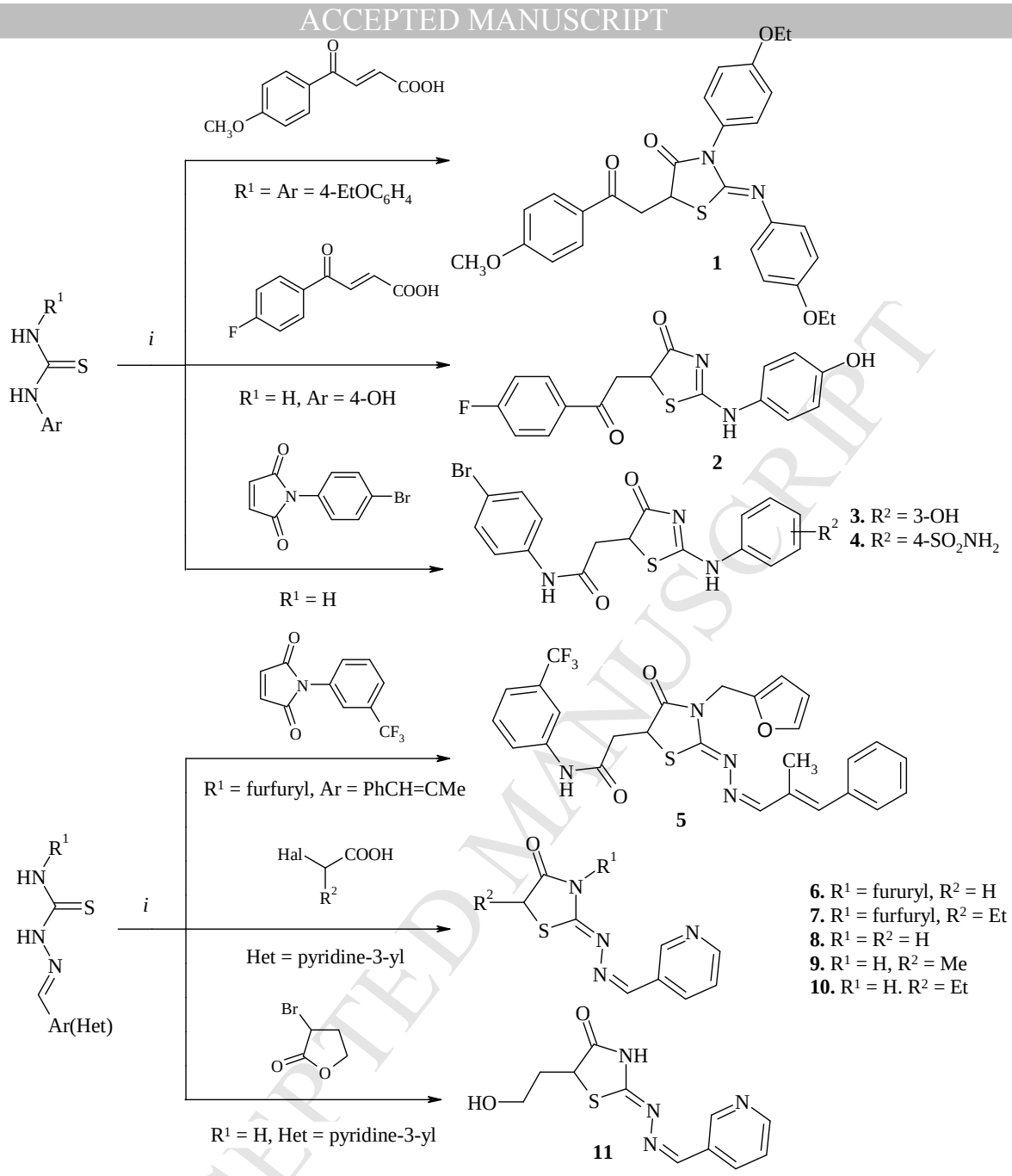
Figure 7. Effects of test compounds on cell proliferation measured by the xCelligence system. Upper panel: Effect of compounds on the 150 h HLF proliferation relative to control. Lower panel: Effect of compounds on the maximum rate of proliferation.

Table 1. Hydrogen-bond geometry (Å, º) for **35** structure.

Table 2. Cytotoxic activity towards 3 cancer cell lines (10^{-4}M).

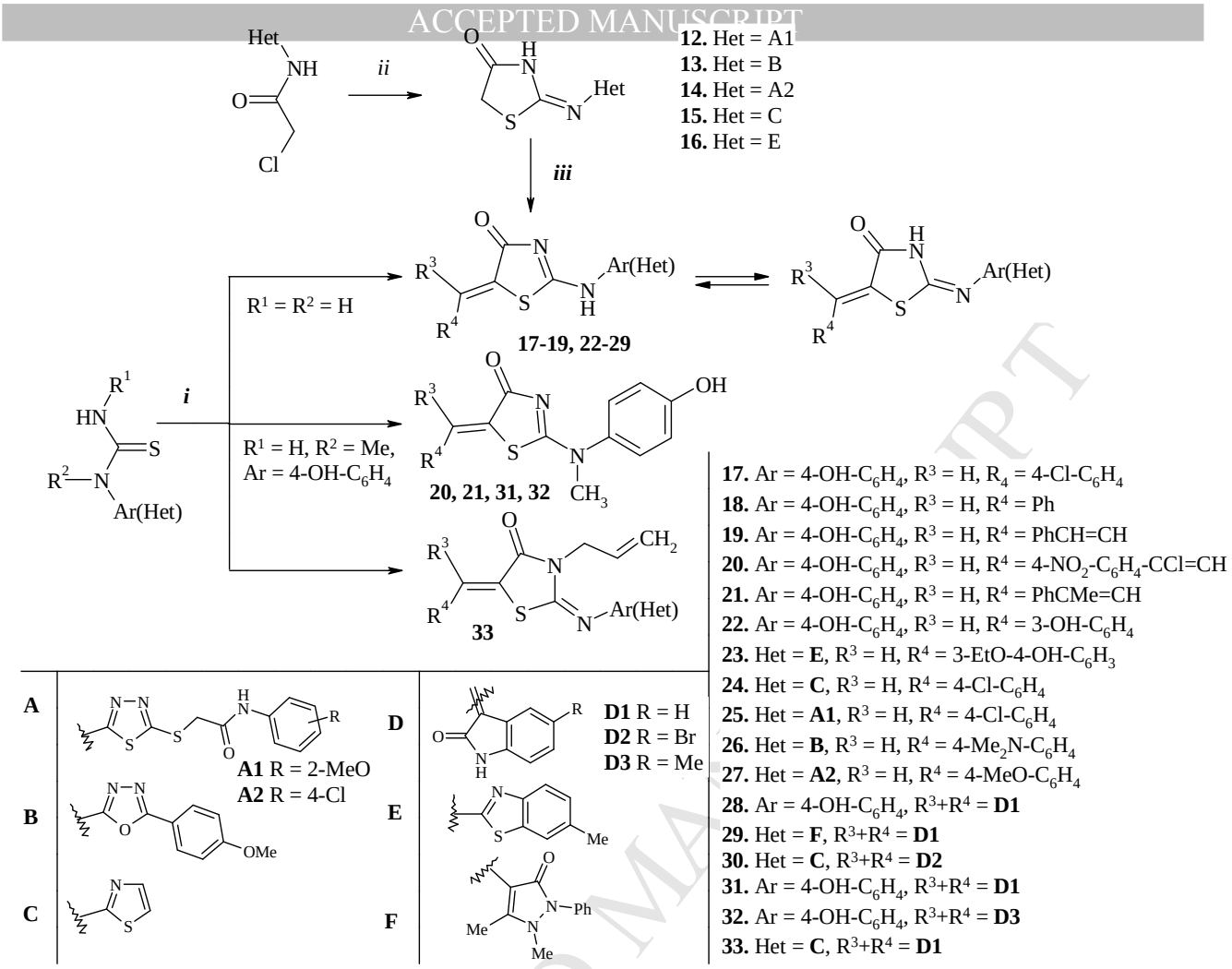
Table 3. Cytotoxic activity towards 60 cancer cell lines (10^{-5}M).

Table 4. Anticancer activity against 60 tumor cell lines (10-fold dilutions/ five concentrations).



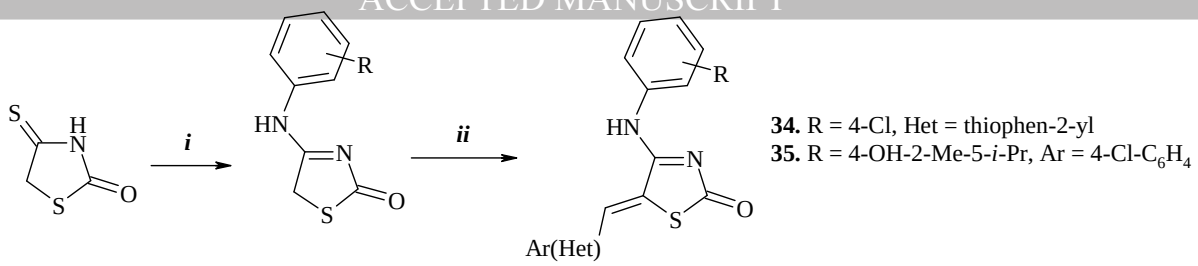
Scheme 1. Synthesis of 2-amino(imino)-4-thiazolidinones.

Reagents and conditions: (*i*) – substituted thiourea or thiosemicarbazone (1.0 equiv), appropriate carboxylic acid derivative (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h.



Scheme 2. Synthesis of 5-ylidene-2-amino(imino)-4-thiazolidinones.

Reagents and conditions: *(i*) – substituted thiourea (1.0 equiv), chloroacetic acid (1.0 equiv), appropriate oxocompound (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h; *(ii)* – N-heteryl-2-chloroacetamide (1.0 equiv), ammonium thiocyanate (2.0 equiv), acetone, reflux, 3 h; *(iii)* – 5-unsubstituted-4-thiazolidinone (**12-16**) (1.0 equiv), appropriate oxocompound (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h.



Scheme 3. Synthesis of 5-ylidene-4-R-aminothiazol-2(5*H*)-one. Reagents and conditions: *(i*) – 4-thioxo-2-thiazolidinone (isorhodanine) (1.0 equiv), appropriate amine (1.0 equiv), EtOH, reflux, 1 h; *(ii)* – 4-arylaminothiazol-2(5*H*)-one (1.0 equiv), appropriate oxocompound (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h.

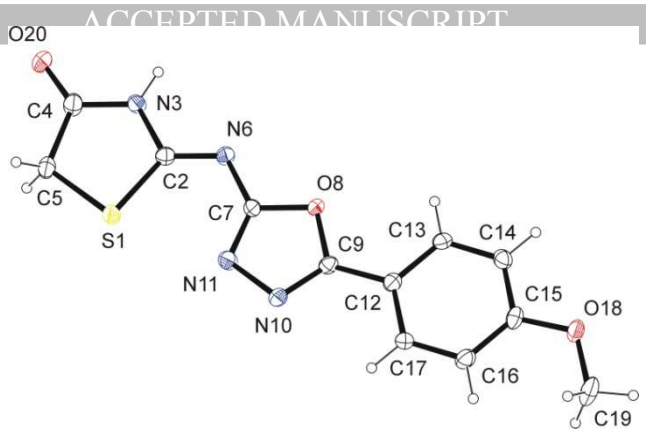


Figure 1. ORTEP view of **13** showing displacement ellipsoids at the 30% probability level. H atoms are shown as spheres of arbitrary radii.

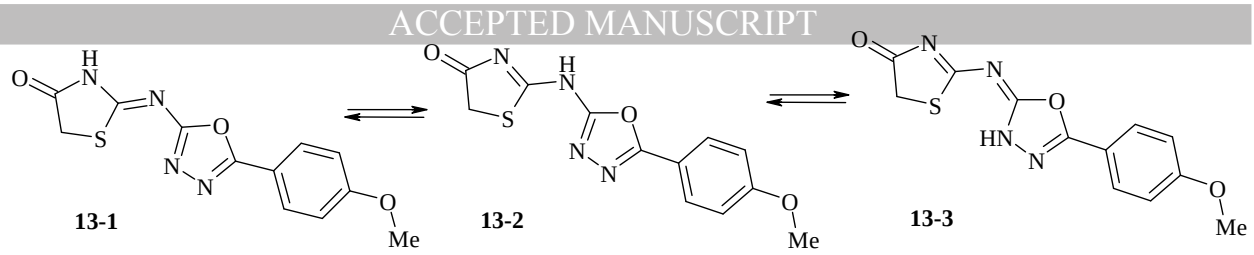


Figure 2. Possible tautomeric structures of **13**.

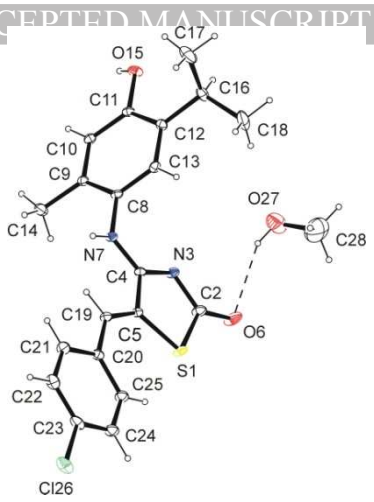
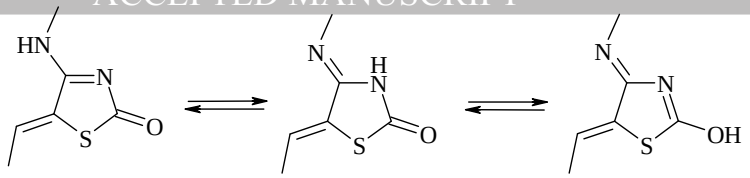
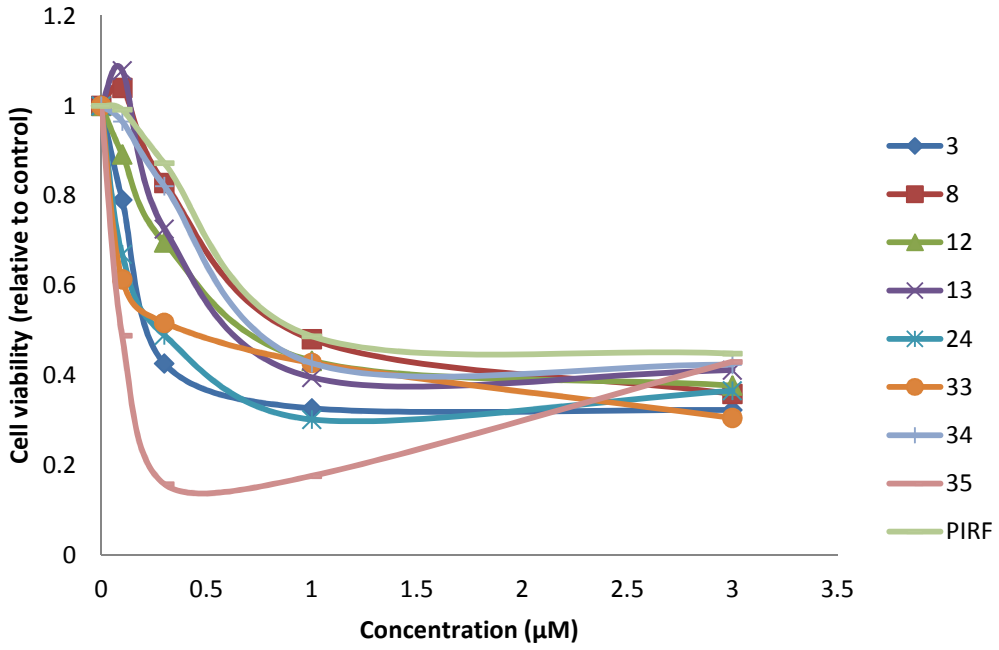


Figure 3. ORTEP view of **35** showing displacement ellipsoids at the 30% probability level. H atoms are shown as spheres of arbitrary radii.



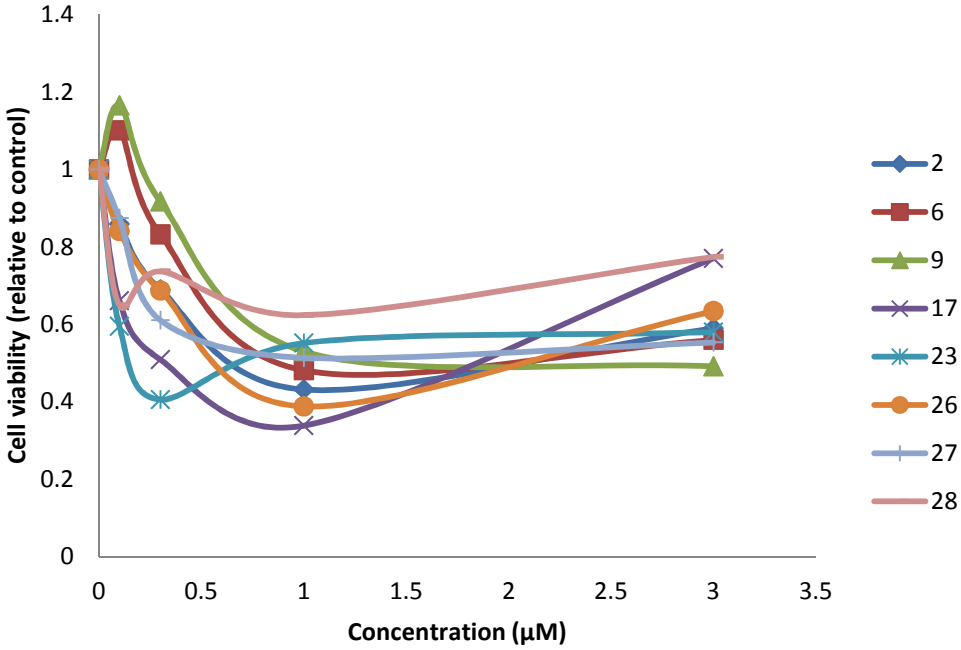
35-1 **35-2** **35-3**

Figure 4. Possible tautomeric forms of **35**.



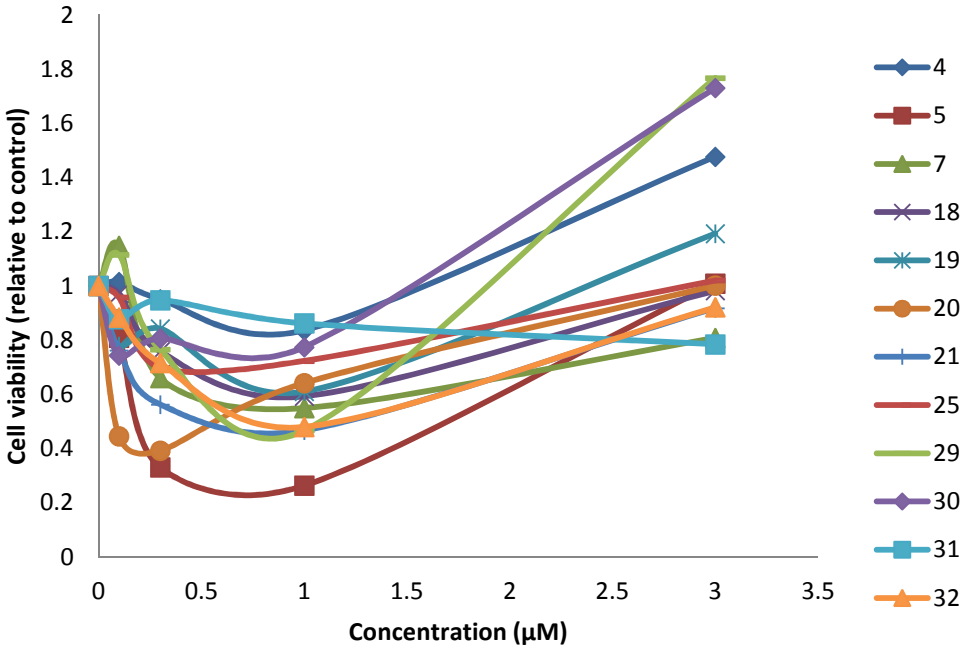
Compound	IC_{50} (μM)	Emax (%)
3	0.16	77.6
8	0.81	92.1
12	0.40	75.0
13	0.47	84.6
24	0.11	70.6
33	0.09	65.9
34	0.57	79.0
35	0.03	75.5
PIRF	0.71	78.5

A



Compound	IC_{50} (μM)	Emax (%)
2	0.19	54.2
6	0.50	69.4
9	0.83	84.6
17	0.02	46.1
23	0.01	48.1
26	0.15	52.4
27	0.16	53.8
28	nd	nd

B

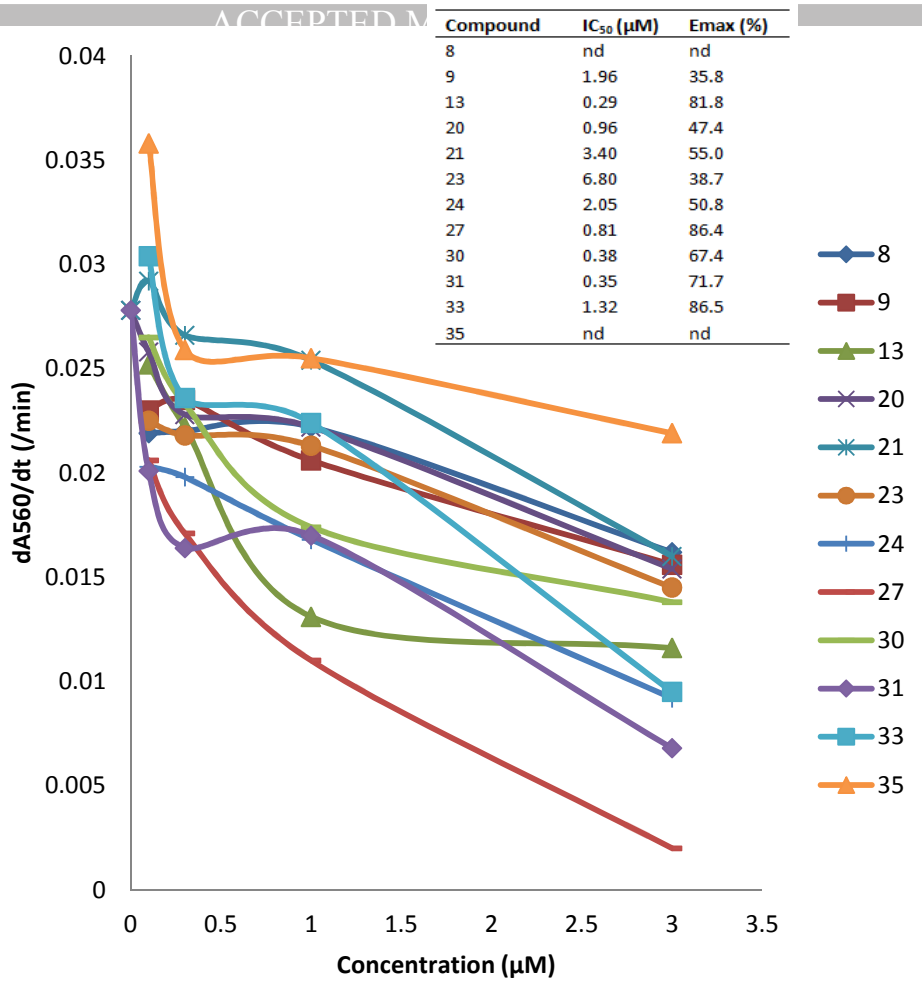


C

Compound	IC_{50} (µM)	Emax (%)
4	nd	nd
5	0.03	44.3
7	0.21	44.7
18	0.09	23.8
19	nd	nd
20	nd	nd
21	0.01	34.0
25	0.04	17.4
29	nd	nd
30	nd	nd
31	1.78	27.3
32	0.07	31.3

Figure 5. Effects on cell viability by the tested compounds determined with the SRB assay. **a.** Compounds that reduced cell proliferation by at least 50%. **b.** Compounds that reduced cell proliferation less than 50%. **c.** Compounds that did not reduce cell viability or increased viability at concentrations > 1 µM.

Viability was calculated relative to the control. Data are presented as means of 4 independent experiments.



Compound	IC_{50} (µM)	Emax (%)
8	nd	nd
9	1.96	35.8
13	0.29	81.8
20	0.96	47.4
21	3.40	55.0
23	6.80	38.7
24	2.05	50.8
27	0.81	86.4
30	0.38	67.4
31	0.35	71.7
33	1.32	86.5
35	nd	nd

Figure 6. Superoxide scavenging by tested compounds.

Scavenging was measured as inhibition of the reduction of NBT by xanthine/xanthine oxidase generated superoxide levels. NBT reduction rate is plotted on the y-axis as dA560/dt (min^{-1}). Data are presented as means of 4 independent experiments.

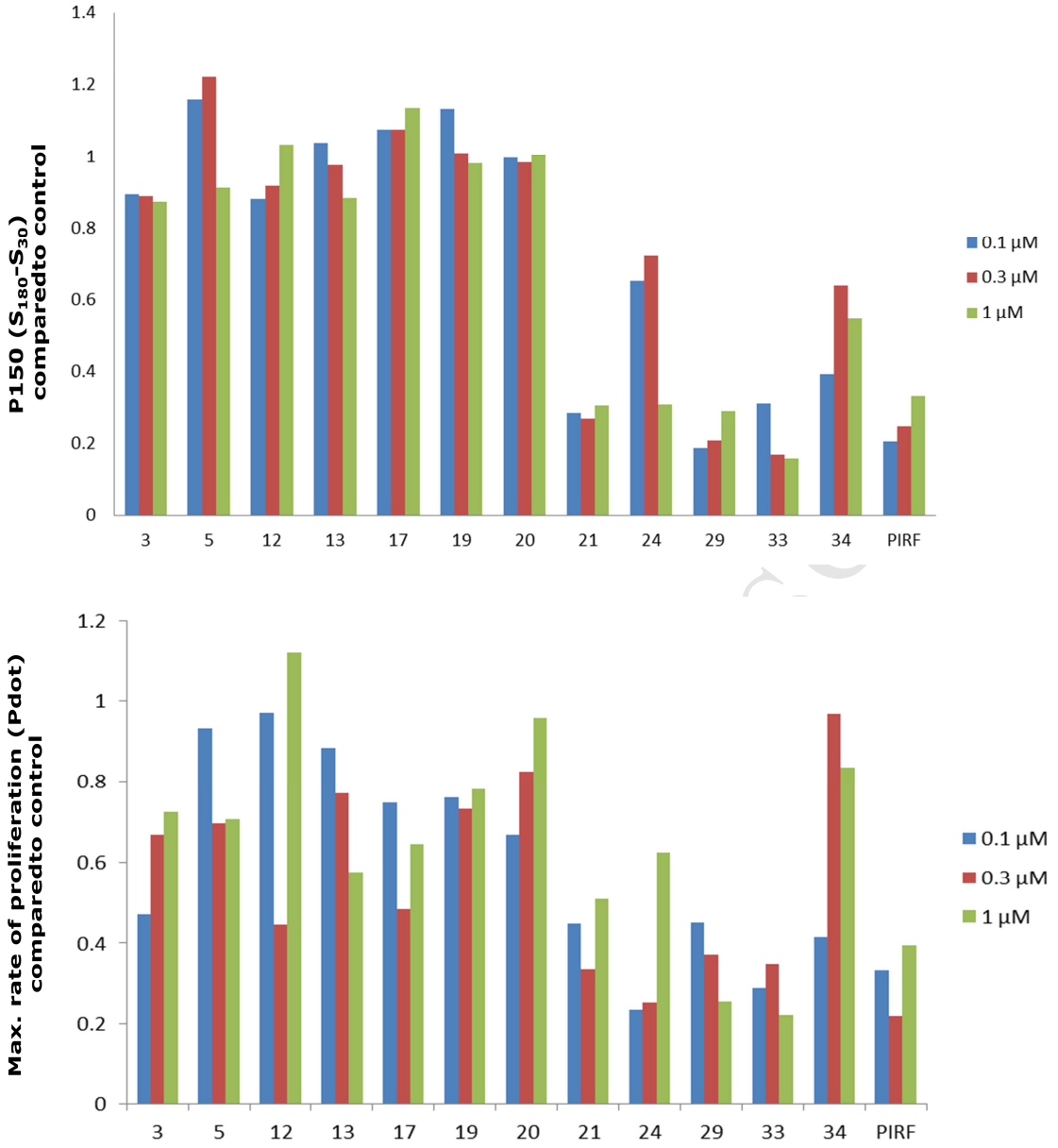


Figure 7. Effects of test compounds on cell proliferation measured by the xCelligence system. **Upper panel**: Effect of compounds on the 150 h HLF proliferation relative to control. **Lower panel**: Effect of compounds on the maximum rate of proliferation.

Table 1. Hydrogen-bond geometry (Å, º) for **35** structure.

D–H···A	D–H	H···A	D···A	D–H···A
C25–H25···S1	0.93	2.54	3.2428(14)	133
N7–H7···O6^{i}	0.818(18)	1.996(18)	2.7879(15)	162.9(18)
O15–H15···N3^{ii}	0.81(3)	2.06(3)	2.849(2)	165.1(18)
O27–H27···O6	0.82	2.31	3.066(3)	153
C19–H19···O6^{i}	0.93	2.42	3.3183(16)	164
C21–H21···O27^{i}	0.93	2.59	3.458(3)	154

Symmetry codes: (i) 1.5–*x*,0.5+*y*,0.5–*z;* (ii) 1.5–*x*,0.5–*y*,1–*z*

Table 2. Cytotoxic activity towards 3 cancer cell lines (10^{-4}M).

compound/ NSC	GP, %			compound/ NSC	GP, %		
	NCI-H460	*MCF7*	*SF-268*		*NCI-H460*	*MCF7*	*SF-268*
2 731891	62	66	53	**24** 733559	**23**	**71**	**33**
4 733380	**88**	**24**	**45**	**28** 725360	96	108	118
17 725366 [17]	**25**	**50**	**66**	**32** 725378	95	89	74
19 728388	140	124	103	**33** 731885	49	76	82
20 728392 [17]	**4**	**34**	**8**				

Hereinafter: NSC – DTP code (https://dtp.cancer.gov/)

Table 3. Cytotoxic activity towards 60 cancer cell lines (10^{-5}M).

Comp./ NSC	Mean growth, %	Range of growth, %	The most sensitive cell line / cancer type	Growth of the most sensitive cell lines, %
13 760863	101.94	71.89 to 120.52	*SNB-75*/CNSC	71.89
26 760867	92.84	47.76 to 129.37	*SNB-75*/CNSC *SF-295*/CNSC *MDA-MB-435/*M	60.43 69.84 47.76
31 745054	85.57	21.38 to 119.11	*MOLT-4/*L *RPMI-8226/*L *NCI-H522/*NSCLC *UO-31/*RC	60.18 55.22 65.24 21.38
pirfenidone 748456*	96.32	76.0 to 126.4	*RPMI-8226/*L *NCI-H522/*NSCLC *UO-31/*RC	76.0 79.8 76.9

* data available at https://dtp.cancer.gov/

Table 4. Anticancer activity against 60 tumor cell lines (10-fold dilutions / five concentrations).

Comp. NSC	MG_MID / range			The most sensetive cell lines / cancer type	GI_{50}, μM	TGI, μM	LC_{50}, μM
	pGI_{50}	pTGI	pLC_{50}				
4 733380	4.23/ 0.81	4.05/ 0.45	4.01/ 0.18	*HOP-62*/NSCLC	15.6	31.7	64.3
				HOP-92/NSCLC	24.3	58.3	>100
				HCT-116/CC	21.8	43.5	86.7
				U-251/CNSC	20.7	44.2	94.3
				MDA-MB-231/BC	18.5	38.2	78.6
17 725366 [17]	5.54/ 3.30	4.52/ 2.66	4.07/ 1.24	*K-562*/L	0.26	-	>100
				SR/L	0.14	0.52	>100
				SF-539/CNSC	0.24	1.32	36.0
				OVCAR-3/OC	0.16	0.49	61.8
				RFX 393/RC	0.12	3.95	>100
				MCF7/BC	0.05	0.22	-
18 749066	4.89/ 3.07	4.05/ 2.04	4.00/ 0.00	*RPMI-8226*/L	0.60	>100	>100
				SR/L	0.09	-	>100
				HCT-116/CC	0.32	>100	>100
				KM12/CC	0.87	>100	>100
				SW-620/CC	0.47	>100	>100
				U251/CNSC	0.68	>100	>100
				MDA-MB-435/M	0.21	0.92	>100
				OVCAR-3/OC	0.71	>100	>100
				MCF-7/BC	0.25	>100	>100
20 728392 [17]	6.16/ 2.08	5.49/ 2.55	4.77/ 2.25	*HOP-62*/NSCLC	0.23	0.60	6.70
				NCI-H23/NSCLC	0.21	0.41	0.81
				NCI-H460/NSCLC	0.20	0.42	0.89
				NCI-H522/NSCLC	0.25	-	>100
				SW-620/CC	0.27	-	>100
				SF-268/CNSC	0.22	0.46	0.96
				M14/M	0.27	0.93	5.40
				786-0/RC	0.22	0.51	2.61
				CAKI-1/RC	0.25	0.83	4.68
				NCI/ADR-Res/BC	0.20	0.46	1.39
				MDA-MB-435/BC	0.14	0.28	0.58
24 733559	4.05/ 0.97	4.00/ 0.00	4.00/ 0.00	*HCT-116*/CC	10.8	>100	>100
				MALME-3M/M	22.5	>100	>100
				HS 578T/BC	87.5	>100	>100
30 735606	4.02/ 0.74	4.00/ 0.07	4.00/ 0.00	-	-	-	-
34 743117 [10]	4.09/ 2.50	4.00/ 0.00	4.00/ 0.00	*U251*/CNSC	41.7	>100	>100
				OVCAR-3/OC	42.5	>100	>100
				MCF-7/BC	0.32	>100	>100
				T-47D/BC	1.46	>100	>100

* - data of double assay; MG_MID (mean graph midpoint) – average level of all tested cell lines

Highlights for the manuscript **"Antifibrotic and anticancer action of 5-ene amino/iminothiazolidinones"**

by **D. Kaminskyy**, **G.J.M. den Hartog, M. Wojtyra**, **M. Lelyukh, A. Gzella**, **A. Bast, R. Lesyk**

- One-pot three-component reaction for 5-ene-2-amino(imino)-4-thiazolidinones
- Compounds **5,12,33,34** with antifibrotic activity have a similar effect as Pirfenidone
- Compounds reduced the viability of fibroblasts did not possess an anticancer effect
- Thiazolidinones as candidates for further testing have been identified