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Synthesis of 5-arylidene-2-amino-4-azolones and evaluation of their anticancer activity

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ABSTRACT

Series of novel 5-arylidene-2-arylaminothiazol-4(5*H*)-ones and 2-aryl(benzyl)amino-1*H*-imidazol-4(5*H*)-ones were synthesized from appropriate 2-alkylthioazol-4-ones using nucleophilic substitution in position 2 by various anilines and benzylamines and Knoevenagel reaction. X-ray structural studies of **22** revealed the structure to be intermediate between amino and imino tautomeric forms. All the target compounds were evaluated for the anticancer activity in vitro in standard National Cancer Institute 60 cancer cell lines assay. Majority of compounds showed significant antitumor cytotoxicity effect at micromolar and submicromolar level (Mean Log GI_{50} ranges −5.77 to −4.35). Some of the most potent compounds, namely **10** and **13**, possessed selectively high effect on all leukemia cell lines at submicromolar level (Mean Log GI_{50} [leukemia lines], respectively, −6.41 and −6.29), which are probably associated with immunosuppressive activity. Individual cancer cell lines sensitivity to synthesized compounds and SAR studies are discussed. COMPARE analysis allowed to disclose probable modes of anticancer action for synthesized compounds, in particular showed number of high correlations with activity patterns of alkylating agents (PCC ~ 0.606–0.731).

1. Introduction

The increasing diversity of small molecule libraries is an important source for the discovery of new drug candidates. In terms of this trend, azolidinone heterocycles are of great importance in modern medicinal chemistry. Particularly thiazolidinone[1,2] and imidazolidinone[3–7] derivatives (as members of azolidinones class) have been widely investigated for a range of pharmacological activities such as anti-inflammatory, antimicrobial (incl. antimycobacterial), antiviral, anticonvulsant, antiarrhythmic, neuroprotective, antihypertensive, antidiabetic, and antiproliferative. During last five years special attention of medicinal chemists was attracted by investigations of azolidinones as potential lead-compounds for novel anticancer agents. Random screening of commercially available compounds as well as target-oriented drug design allowed identifying anticancer hits among azolidinone-related compounds and their potential biotargets (Fig. 1).

Recently, it was communicated that PPARγ agonist troglitazone (2,4-thiazolidinedione derivative) mediated the suppression of cyclin D1 in MCF-7 breast cancer cells by facilitating proteasome-facilitated proteolysis. The PPARγ-independent mechanism provided a molecular basis for troglitazone, which was further used as scaffold for development of compound STG28 (**I**)—cyclin D1-ablative agent with micromolecular potency and inhibitory activity on MCF-7 cell proliferation.[8] Several 5-benzylidene-thiazolidine-2,4-diones and -2-thiones (rhodanines) **III** inhibit cell growth with low micromolar GI_{50} mediated by inhibition of translation initiation, which involves partial depletion of intracellular Ca^{2+} stores and eIF2α phosphorylation.[9] Interestingly, benzylidenerhodanine derivatives **IV** related to previous compounds showed strong inhibition (IC_{50} = 0.9 μM) of PRL-3 (protein tyrosine phosphatase), one of putative prognostic markers and therapeutic targets for metastatic tumors.[10] A series of rhodanine based hits **V** were found as potent and selective inhibitors of the 'atypical' dual-specificity phosphatases (DSP) family member—JNK-stimulating phosphatase-1 (JSP-1). Compounds of this class may be useful for the treatment of inflammatory and proliferative disorders.[11]

The Pim protein kinases are frequently overexpressed in prostate cancer and certain forms of leukemia and lymphoma. 5-Arylidene-2,4-thiazolidinediones **II** was identified by screening to be a Pim-1 inhibitor and was found to attenuate the autophosphorylation of

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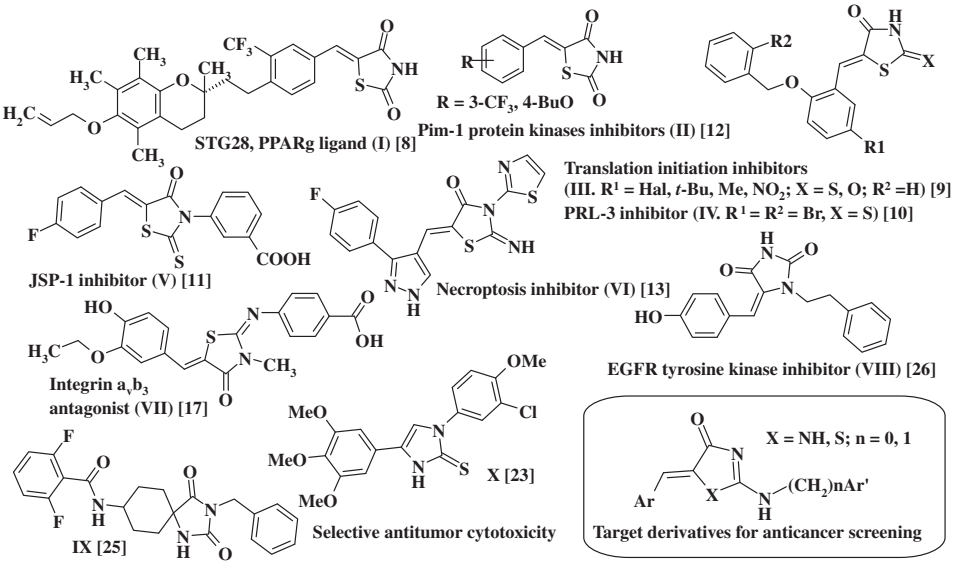


Figure 1. Examples of structure lead compounds among azolidinones with anticancer properties.

target Pim-1 in intact cells. These studies may provide promising leads for novel anticancer agents.[12]

Necrostatin-7 (Nec-7, **VI**) was found to be a novel 'small-molecule' inhibitor of necroptosis, regulated caspase-independent cell death mechanism. The further progress in this field led to development of a number of Nec-7 derivatives, inhibiting TNFα-induced necroptosis in FADD-deficient variant of human Jurkat T cells.[13]

Among thiazolidinone derivatives 2-arylaminothiazol-4-ones were one of the most promising group in anticancer drug discovery process. One of them (MMPT) was identified as early hit, effectively inhibiting the growth of several human lung cancer cell lines (H460 and H460/TaxR) but not normal fibroblasts in a dose-dependent manner.[14,15] Moreover, 2-arylaminothiazol-4-ones with selectivity to NSCL cancer cell line H-460 at submicromolar concentrations and lower toxicity to normal human fibroblasts were discovered.[16] Among 3-substituted 2-arylimino-4-thiazolidinones integrin $\alpha_v\beta_3$ antagonists (**VII**) were shown to be perspective novel anticancer agents.[17] 2-Phenylimino-3-alkyl-4-thiazolidinone derivatives demonstrated inhibition of the HT29 cell line (colon cancer), characterized by a high COX-2 expression,[18] as well as CDK1/cyclin B inhibition.[19,20] These effects were achieved by block of cell cycle progression at the G2/M phase border in reversible manner and induction of apoptosis.[21] Antagonizing stimulatory effect of free fatty acids at cell proliferation (inhibitory effect on tumor survival) in human breast cancer cell line (MDA-MB-231) was reported as well for the benzylidene-2-arylaminothiazol-4-ones.[22]

Antitumor activity of imidazolone and hydantoin analogs is intensively studied. In this group, a variety of compounds have been identified as both cytostatic and cytotoxic agents (**VIII**–**IX**) active in various models of cancer.[23–27]

Our search was aimed at optimization of anticancer activity profile of 5-arylidene-2-aminoazolones and SAR analysis within these series in accordance with our systematic study of anticancer activity of azolidinone derivatives and related/fused heterocyclic systems.[28,29]

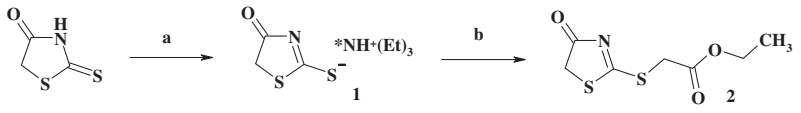
2. Results and discussion

2.1. Chemistry

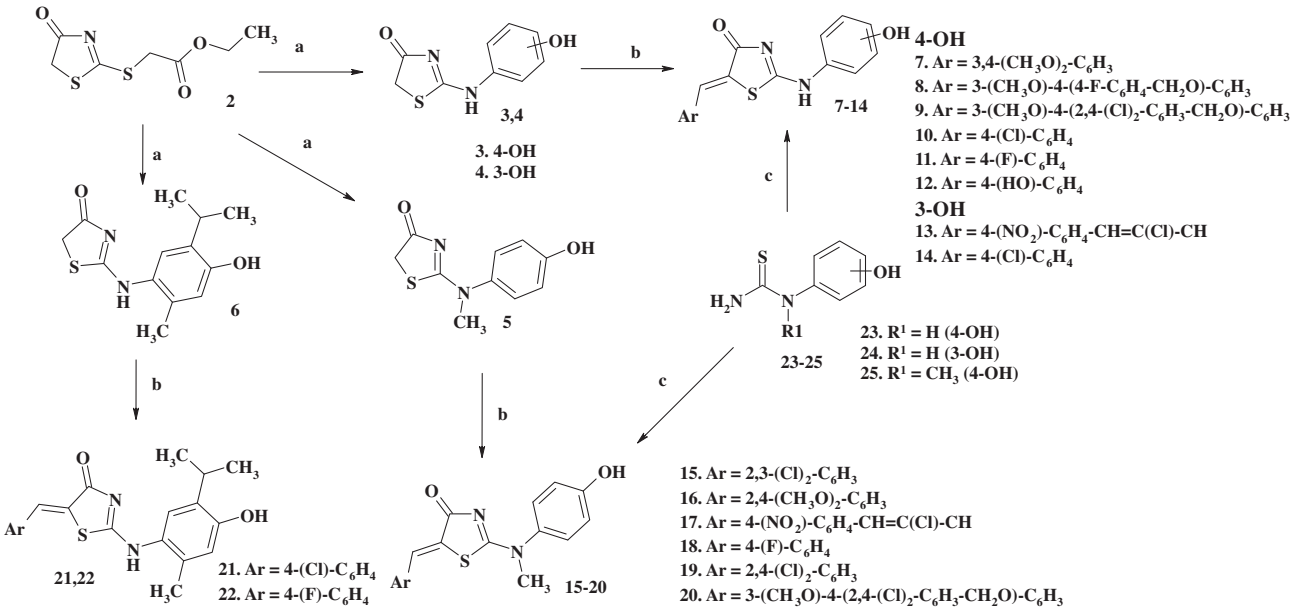
The synthetic approach to 2-substituted azolones design was based on exploration of methylthio and ethoxycarbonylmethylthio moieties as leaving groups. The starting 2-carbethoxymethylthio-2-thiazol-4(5*H*)-one (**2**) was obtained by the reaction of 2-thioxo-4-thiazolidinone triethylammonium salt with ethyl chloroacetate in acetone (Scheme 1).[30]

Reaction of **2** with a range of aminophenols provided target 2-arylaminothiazol-4(5*H*)-ones (**3–6**) with high yields (Scheme 2). Synthesized compounds **3–6** are methylene active heterocycles. Previously it was shown that the presence and nature of moiety in position 5 of thiazolidinones play the key role in realization of pharmacological effects.[1,2,29–31] The abovementioned served as a background for the synthesis of new 5-arylidene derivatives **7–22**, using standard Knoevenagel reaction procedure (medium: acetic acid, catalyst: fused sodium acetate).[1,32] 5-Arylidene derivatives **7–22** were prepared also alternatively by one-pot methodology involving reaction of arylthioureas **23–25** with chloroacetic acid and appropriate aromatic aldehydes in the presence of fused sodium acetate in refluxing acetic acid (Scheme 2).

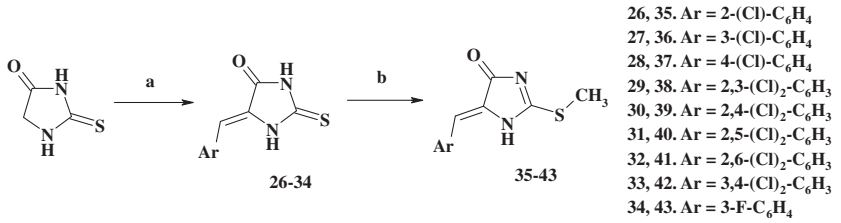
To explore the effect of substitution of sulfur by nitrogen in position 1 of core heterocycle on anticancer activity, we designed library of 5-arylidene-2-aryl(benzyl)amino-1*H*-imidazol-4(5*H*)-ones **44–59**. The starting 5-arylidene-2-thiohydantoins (**26–34**) prepared in the Knoevenagel condensation of the appropriate benzaldehydes with 2-thiohydantoin,[33] were then treated with methyl iodide



Scheme 1. Synthesis of 2-carbethoxymethylthio-2-thiazol-4(5*H*)-one. Reagents and conditions: (a) triethylamine (1.0 equiv), *i*-PrOH, 80 °C, 2 h, 88%; (b) $ClCH_2COOEt$ (1.0 equiv), acetone, reflux 2 h, 62%.



Scheme 2. Synthesis of 2-arylaminothiazol-4(5*H*)-ones and their 5-arylidene derivatives. Reagents and conditions: (a) aromatic amine (1.0 equiv), *i*-PrOH, reflux, 2 h, 70–95%; (b) ArCHO (1.1 equiv), AcONa anhyd (1.0 equiv), AcOH, reflux 3 h, 55–95%; (c) ArCHO (1.1 equiv), $ClCH_2COOH$ (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux 3 h, 71–78%.



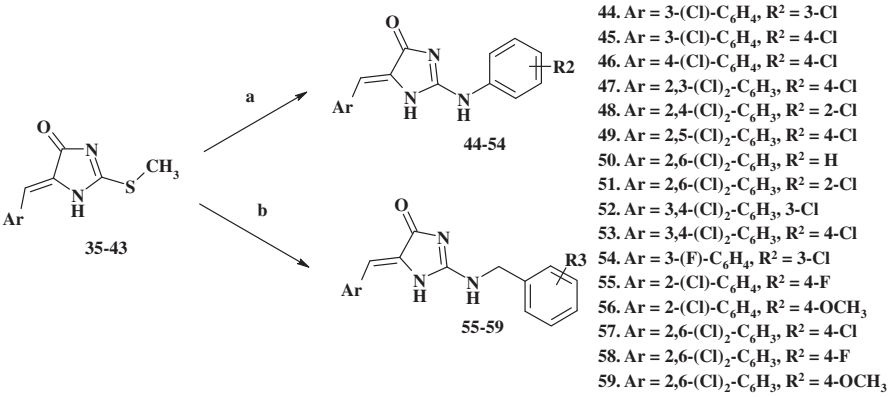
Scheme 3. Synthesis of 5-arylidene-2-methylthio-1*H*-imidazol-4(5*H*)-ones. Reagents and conditions: (a) Ar–CHO (1.1 equiv), AcONa (4.0 equiv), AcOH, reflux 1–3 h; (b) EtONa (1.0 equiv), CH_3I (1.0 equiv), EtOH, rt, 0.5 h.

(Scheme 3). Obtained intermediate 5-arylidene-2-methylthio-products (**33–43**) underwent reaction with series of anilines and benzylamines in acetic acid or toluene to produce **44–59** (Scheme 4).[34–37]

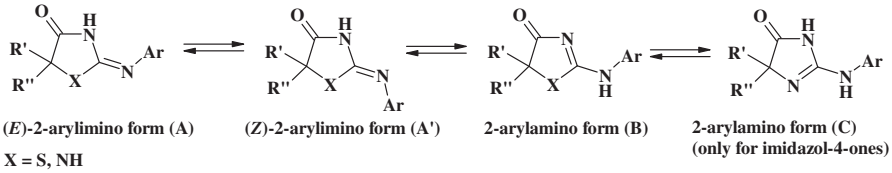
Synthesized azolones derivatives were characterized by ^1H, ^{13}C NMR, IR spectra and microanalyses and some of them by UV and mass spectroscopy and presented in experimental part.

2.2. Structural investigations

The group of 2-aminoazolones presents an interesting target for studies on both molecular structures and spectroscopic properties. In the case of 2-amino-4-thiazolone derivatives, compounds carrying hydrogen atom on an exocyclic nitrogen atom exist in the form of two tautomers differing by the position of C=N double bond. In the group of 2-amino-4-imidazolones, including guanidine Y-shaped fragment built-in five-membered heterocyclic ring, studied derivatives can appear in at least three tautomeric forms with two hydrogens assigned to two of three nitrogen atoms in the structure. Additionally, tautomers with exocyclic C=N double bond exist as mixture of *Z*- and *E*-stereoisomers (Scheme 5). Introduction of an arylidene fragment into position 5 of heterocyclic ring generates more structural questions concerning both stereochemistry and electron distribution.



Scheme 4. Synthesis of 5-arylidene-2-aryl(benzyl)amino-1*H*-imidazol-4(5*H*)-ones. Reagents and conditions: (a) Ar–NH_2 (1.1 equiv), AcOH, reflux 7–9 h, 54–87%; (b) Ar–CH_2NH_2 (1.1 equiv), toluene, reflux 7–9 h, 70–90%.



Scheme 5. Prototropic tautomerism of 2-arylamino-4-azolones.

The prototropic tautomerism and stereoisomerism among this class of compounds was previously studied, both in solutions and in the solid state.[30,38–40] Spectroscopic studies revealed characteristic multiplication of signals in IR, ^{1}H and ^{13}C NMR spectra, observed for some compounds and connected with co-existence of two different tautomeric forms. This phenomenon was also observed in the case of described here 2-amino-4-thiazolones **3**, **4**, **6–14**, **21** and **22**.

Structural features of synthesized 5-arylidene-2-hydroxyphenylaminothiazol-4(5*H*)-ones were confirmed by X-ray crystallographic analysis of exemplified compound **22**. As follows from the X-ray analysis the compound obtained has the structure of 5-(4-fluorobenzylidene)-2-(2-methyl-4-hydroxy-5-isopropylphenylamino)thiazol-4(5*H*)-one (**22**) and crystallizes as dimethylformamide solvate (Fig. 2). In the asymmetric part of the unit cell there are two symmetry-independent host (solute) molecules and one-half of the guest (solvent) molecule as the latter is at the special position in the twofold axis.

Molecules of the compound **22** can exchange an H atom between a ring and side-chain N atoms (side-chain tautomerism). In the crystal lattice the molecules take a structure intermediate between two tautomeric forms **A** and **B** (Scheme 5). This conclusion was based because of the comparable values of the interatomic distances C2–N3 and C2–N6 [molecule *A*: 1.3260(15) and 1.3210(16) Å; molecule *B*: 1.3308(15) and 1.3177(16) Å], indicating that these two bonds have in molecules partially double character. Only the localization of H atoms at the exocyclic N6 atoms make the structures of the molecules in the crystal closer to that of the tautomeric form **A** of compound **22**. Additional confirmation of the presence of H atoms at N6 and their absence at N3 atoms comes from the hydrogen bonds N6A^{i}–H6A^{i}···N3*B* and N6*B*–H6*B*···N3A^{i}, in which N6 atoms act as proton donors, while N3 atoms as proton acceptors (Table 1).

Partly double character of the C2–N6 bonds, hindering the rotation, explains the values of the torsional angles N3–C2–N6–C7 and S1–C2–N6–C7 [molecule *A*: 177.20(11)° and −3.40(17)°, molecule *B*: 165.94(12)° and −13.18(17)°] and small deviation of the system of S1, C2, N3, N6 and C7 atoms from planarity {rms deviation 0.0144 Å (molecule *A*) and 0.0691 Å (molecule *B*)}.

Two symmetry-independent molecules *A* and *B* of the compound studied differ significantly in conformation. The weighted rms deviation for the superposition of the non-H atoms in both molecules is 1.7122 Å.[41] The differences concern the angular arrangement of the system of 2-thiazolin-4-one, at a smaller

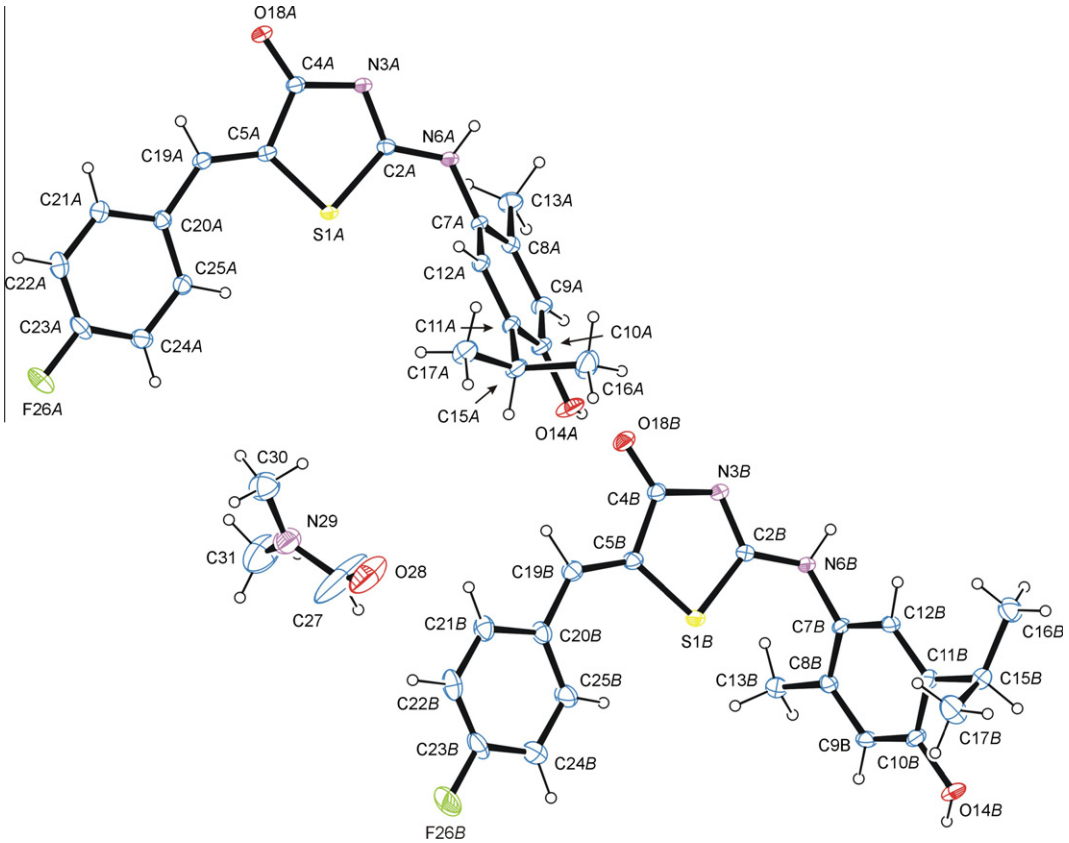


Figure 2. The structure of **22**, showing the atomic labeling scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms, treated as isotropic, are on an arbitrary scale.

Table 1
Hydrogen- and halogen–halogen bond data (Å, °)

D–H···*A*	*D*–H	H···*A*	*D*···*A*	*D*–H···*A*
N6*A*^{i}–H6*A*^{i}···N3*B*	0.84(2)	2.04(2)	2.878(2)	175.6(17)
N6*B*–H6*B*···N3*A*^{i}	0.89(2)	1.96(2)	2.844(2)	170.3(17)
O14*A*–H14*A*···O18*B*	0.84(2)	1.88(2)	2.6995(14)	165(2)
O14*B*–H14*B*···O18*A*^{ii}	0.86(2)	1.88(2)	2.7120(16)	165(2)
C13*A*^{i}–H13*C*^{i}···O18*B*	1.01(3)	2.44(3)	3.393(2)	157.8(16)
C21*B*^{iii}–H21*B*^{iii}···O14*A*	0.923(19)	2.557(18)	3.2112(18)	128.2(15)
C16*A*^{iii}–H16*B*^{iii}···O28	1.05(2)	2.50(2)	3.411(3)	144(2)
C25*B*–H25*B*···O28^{iv}	0.95(2)	2.53(2)	3.259(3)	134.3(18)
F26*A*···F26*B*^{v}			2.7405(18)	

Symmetry codes: (i) 0.5 − *x*, 0.5 − *y*, 1 − *z*; (ii) *x*, 1 + *y*, *z*; (iii) 1 − *x*, *y*, 1.5 − *z*; (iv) 1 − *x*, 1 − *y*, 1 − *z*; (v) 1.5 − *x*, −0.5 + *y*, 1.5 − *z*.

degree towards the 4-fluorophenyl substituent [molecule *A*: 15.43(5)°, molecule *B*: 14.25(5)°], while at a greater angle towards 2-methyl-4-hydroxy-5-isopropyl-phenylamine [molecule *A*: 63.11(4)°, molecule *B*: 110.24(4)°].

The main factors that determine the crystal packing and take part in the formation of the supramolecular structure of **22** are the system of hydrogen bonds, both strong N–H···N and O–H···O and weak C–H···O, and dihalogen F···F interactions. The symmetry-independent molecules *A* and *B* are connected by two strong N6*A*^{i}–H6*A*^{i}···N3*B*, N6*B*–H6*B*···N3*A*^{i} and one weak C13*A*^{i}–H13*C*^{i}···O18*B* hydrogen bonds into non-centrosymmetric dimers (Table 1). Using graph-set notation[42,43] for the description of the hydrogen-bond network, there are three first-level dimeric hydrogen bonds, *DDD*, and more interesting higher-order rings $R_2^2(8)$ and $R_2^2(9)$ (Fig. 3). The dimers are linked into continuous ribbons by O14*A*–H14*A*···O18*B* and O14*B*–H14*B*···O18*A*^{ii} hydrogen bonds. At the second level, there are two centrosymmetric $R_4^4(16)$ and $R_4^4(22)$ rings, closed by two *A*–*B* pairs of molecules in each case. The adjacent ribbons are further connected by F26*A*···F26*B*^{v} contacts to form two-dimensional layers that expand in parallel to the (−1 0 2) plane. These layers are connected by means of weak C21*B*^{iii}–H21*B*^{iii}···O14*A* interactions into three-dimensional structure (Table 1, Fig 3).

The solvent (dimethylformamide) molecule is disordered with a 50% site occupancy factor. Although the C=O group in the latter is a potential acceptor, there are no strong hydrogen bonds between solute and solvent molecules (Table 1, Fig. 3).

2.3. Evaluation of anticancer activity in vitro

The synthesized compounds (**3**, **10–12**, **15–18**, **21**, **22**) were submitted and evaluated against three human tumor cell lines panel, consisting of NCI-H460 (non-small cell lung cancer), MCF7 (breast cancer), and SF-268 (CNS cancer) cell lines. Primary anticancer assays were performed according to the US NCI protocol, as described elsewhere.[44–48] The substances which reduced the growth of the cell lines to 32% or less (negative numbers indicate cell kill) were passed on for evaluation in the full panel of 60 human tumor cell lines. The results of the primary screening (Table 2) and the full panel screening (Table 3) are summarized below.

Compounds **10–12**, **15–18**, **21**, **22** were selected for in vitro testing against a full panel of about 60 tumor cell lines at 10-fold dilutions of five concentrations (100 μM, 10 μM, 1 μM, 0.1 μM and

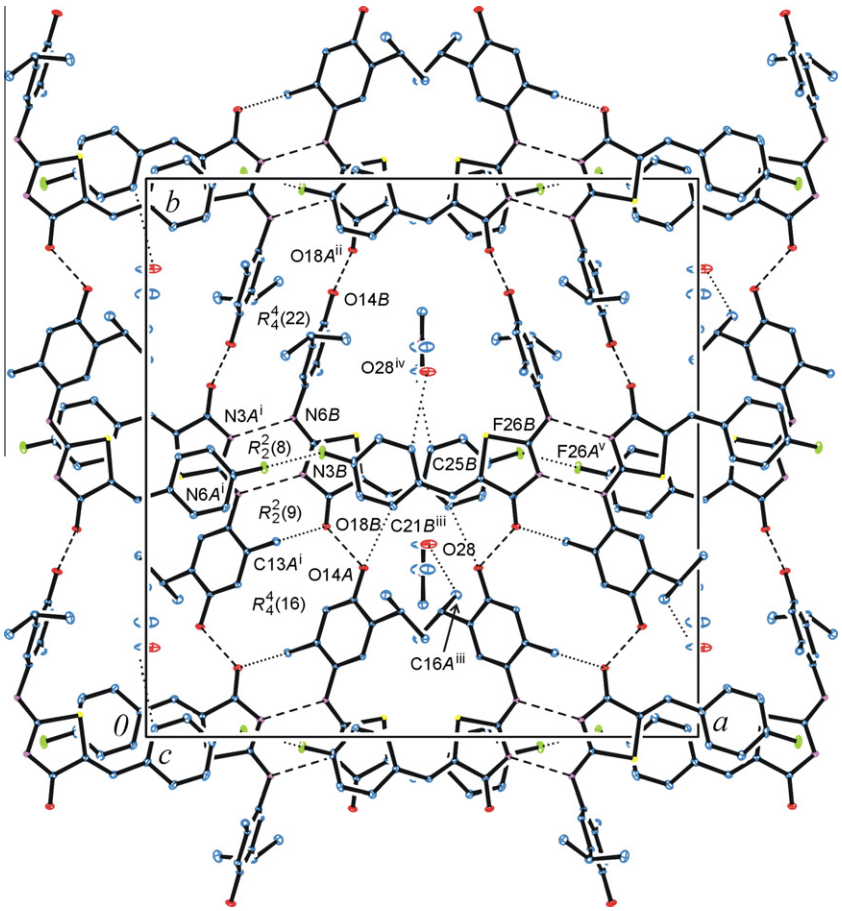


Figure 3. The view of the hydrogen-bonding in the unit cell. Molecules linked by N–H···N, O–H···O and C–H···O hydrogen bonds are depicted by dashed and dotted lines, respectively. The symmetry codes are explained in Table 1. The ring graph symbols are shown.

Table 2
Preliminary screening of anticancer activity of the target compounds

Compd	NSC (NCI code)	Three cell lines assay[a]			Selected for 60 cell lines assay
		A	B	C	
3	725368	108	116	112	N
10	725366	25	50	66	Y
11	728395	11	43	38	Y
12	733556	28	28	89	Y
15	728392	4	34	8	Y
16	733390	12	18	34	Y
17	733585	0	0	4	Y
18	733579	0	40	77	Y
21	728398	0	0	0	Y
22	728393	0	9	23	Y

[a] The results for each compound are reported as the percent growth of treated cells when compared to untreated control cells accordingly for the following human tumor cell lines: (A) NCI-H460 (lung cancer); (B) SF-268 (CNS cancer) and (C) MCF7 (breast cancer).

0.01 μM), compound **3** appeared to be inactive in the prescreening stage. Moreover, compounds **7–9**, **13**, **14**, **19**, **20**, **44–59** were tested at the latter 60 cell lines assay without primary prescreening.[44–48] The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. Based on the cytotoxicity assays, three antitumor activity dose–response parameters were calculated for each experimental agent against each cell line: GI_{50}—molar concentration of the compound that inhibits 50% net cell growth; TGI—molar concentration of the compound leading to total inhibition; and LC_{50}—molar concentration of the compound leading to 50% net cell death. Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value was expressed as greater or less than the maximum or minimum concentration tested. Furthermore a mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an averaged activity parameter over all cell lines for each compound. For the calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested.

The most potent inhibition of human tumor cells was found for compounds **8**, **10**, **11**, **13**, **15**, **20**, **44**, **46**, **52** and **54**, which showed the average activity (GI_{50} level) at less than 10 μM concentration (MG_MID GI_{50} <−5.0). All the tested compounds showed a broad spectrum of growth inhibition activity against human tumor cells, as well as some distinctive patterns of selectivity. In general, the majority of the compounds, especially 2-amino-4-thiazolinone derivatives selectively inhibit the growth of leukemia cell lines. Of note, we found three compounds: **8** ($Mean_{Leukemia}$ Log GI_{50} = −6.12), **10** ($Mean_{Leukemia}$ Log GI_{50} = −6.41) and **13** ($Mean_{Leukemia}$ Log GI_{50} = −6.29) with submicromolar potency level against leukemia and compound **11** with the same potency against breast cancer ($Mean_{Breast}$ Log GI_{50} = −6.12) and colon cancer ($Mean_{Colon}$ Log GI_{50} = −6.21). Certain selectivity tendencies are shown in Figure 4, where mean Log GI_{50} values for separate cancer type's subpanels, which are lower than Mean Graph Midpoints are depicted for the most active compounds. Compounds **7**, **13**, **46** act selectively against prostate cancer cell lines panel also, compounds **10** and **11** possess higher than average growth inhibition activity at breast and CNS cancer cell lines, while potency profile of the compound **13** is directed against colon and renal cancers, as well as melanoma cell lines.

Furthermore individual cell lines have a variable sensitivity towards the tested compounds. The most potent and selective

Table 3
In vitro anticancer activity of the target compounds

Compd	NSC (NCI code)	Log GI_{50} Range	MG_MID	Log TGI Range	MG_MID	Log LC_{50} Range	MG_MID
7	735677	−6.40 to −4.15	−5.39	−5.69 to −4.28	−4.27	−4.34	−4.01
8	**735703**	**−6.70** to **−4.33**	**−5.29**	**−5.94** to **−4.28**	**−4.55**	**−4.65** to **−4.10**	**−4.10**
9	735704	−5.65 to −4.03	−4.66	−5.20 to −4.06	−4.25	−4.38 to −4.06	−4.04
10[a]	**725366**	**−7.30** to **−4.13**	**−5.54**	**−6.66** to **−4.06**	**−4.52**	**−5.24** to **−4.14**	**−4.07**
11	**728395**	**−7.50** to **−4.26**	**−5.77**	**−6.94** to **−4.33**	**−4.66**	**−6.15** to **−4.01**	**−4.12**
12	733556	−5.71 to −4.19	−4.8	−5.28 to −4.24	−4.13	−4.55 to −4.19	−4.01
13	**728398**	**−6.74** to **−4.72**	**−5.56**	**−6.36** to **−4.13**	**−4.98**	**−5.98** to **−4.12**	**−4.55**
14	728393	−6.31 to −4.15	−4.94	−5.56 to −4.07	−4.47	−5.02 to −4.01	−4.15
15	**740762**	**−6.36** to **−4.37**	**−5.1**	**−5.58** to **−4.36**	**−4.6**	**−4.48** to **−4.01**	**−4.14**
16	740761	−5.72 to −4.16	−4.96	−4.94 to −4.04	−4.04	−4.00	−4.00
17[b]	728392	**−8.00** to −4.05	−4.77	−8.00 to −4.07	−4.44	−4.61 to −4.03	−4.09
18	733390	−5.69 to −4.20	−4.63	−4.66 to −4.01	−4.09	-4.06	−4.00
19	733585	−5.70 to −4.49	−4.83	−4.96 to −4.11	−4.4	−4.45 to −4.01	−4.11
20	**733579**	**−5.62** to **−4.50**	**−5.03**	**−5.09** to **−4.01**	**−4.32**	**−4.30 to −4.04**	**−4.01**
21	735598	−5.64 to −4.22	−4.76	−4.89 to −4.13	−4.29	−4.32 to −4.11	−4.06
22	735605	**−7.46** to −4.45	−4.93	−5.35 to −4.04	−4.4	−4.58 to −4.02	−4.09
44	**740738**	**−5.68** to **−4.88**	**−5.29**	**−5.31** to **−4.00**	**−4.73**	**−4.72** to **−4.00**	**−4.24**
45	740739	−5.52 to −4.66	−4.97	−4.94 to −4.13	−4.55	−4.40 to −4.00	−4.17
46	**740743**	**−6.42** to **−4.24**	**−5.05**	**−5.36** to **−4.00**	**−4.25**	**−4.41** to **−4.00**	**−4.02**
47	740745	−5.68 to −4.38	−4.98	−4.90 to −4.00	−4.39	−4.33 to −4.00	−4.05
48	716495	**−6.91** to −4.00	−4.35	−5.74 to −4.00	−4.05	−4.00	−4.00
49	740737	−5.84 to −4.62	−4.98	−5.13 to −4.18	−4.57	−4.43 to −4.00	−4.19
50	716498	−5.48 to −4.00	−4.64	−4.74 to −4.00	−4.15	−4.27 to −4.00	−4.02
51	716494	−5.26 to −4.22	−4.57	−4.33 to −4.00	−4.07	−4.00	−4.00
52	**716496**	**−5.72** to **−4.85**	**−5.34**	**−5.37** to **−4.45**	**−4.81**	**−5.07** to **−4.00**	**−4.33**
53	740744	−4.99 to −4.59	−4.76	−4.56 to −4.00	−4.42	−4.28 to −4.00	−4.12
54	**740751**	**−5.98** to **−4.58**	**−5.18**	**−6.09** to **−4.00**	**−4.68**	**−4.45** to **−4.00**	**−4.17**
55	713592	−5.27 to −4.49	−4.77	−4.62 to −4.00	−4.32	−4.29 to −4.00	−4.07
56	740742	**−7.04** to −4.44	−4.74	−4.56 to −4.00	−4.29	−4.25 to −4.00	−4.04
57	740749	−5.97 to −4.37	−4.62	−4.53 to −4.00	−4.15	−4.21 to −4.00	−4.0
58	740753	−5.39 to −4.41	−4.65	−4.62 to −4.00	−4.24	−4.22 to −4.00	−4.02
59	740750	−5.33 to −4.13	−4.54	−4.49 to −4.00	−4.08	−4.16 to −4.00	−4.00

[a] The most active substances are marked in bold.
[b] 728392 showed GI_{50} less than 0.01 μM for cell line SK-MEL-5 (melanoma).

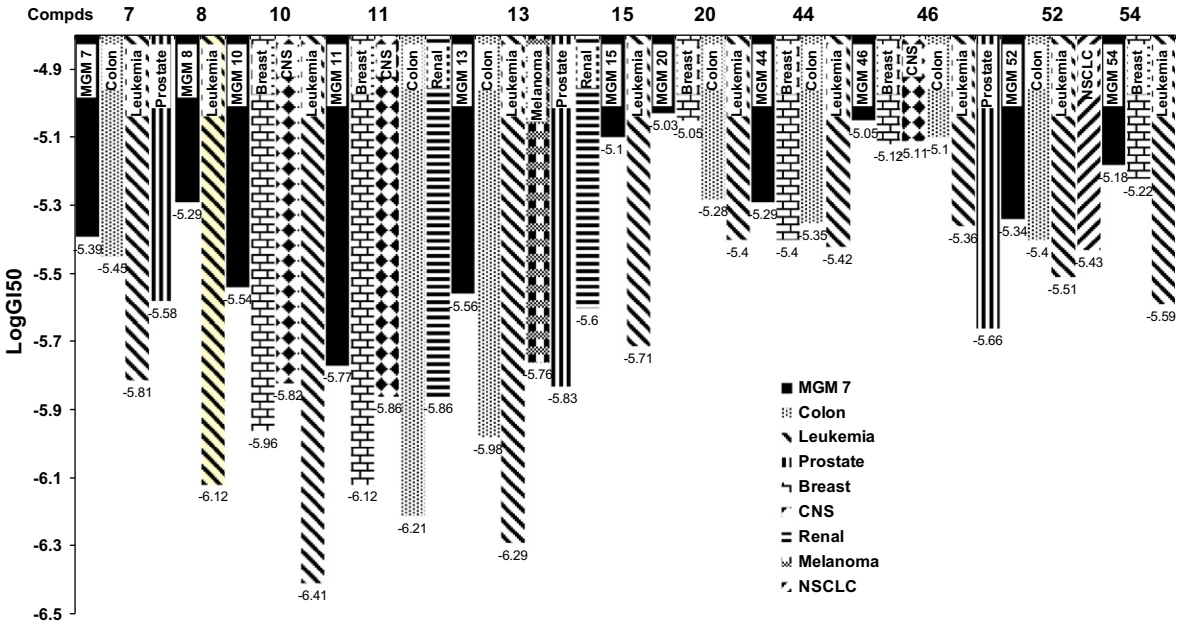


Figure 4. Selectivity of antitumor activity for some tested compounds (Log GI_{50} values). (a) Mean graph midpoints ('MGM') are colored in black. (b) NSCLC: non-small cell lung cancer.

cytotoxic activities against separate tumor cell lines are shown in Table 4.

In the cancer panel of 2-arylamino-4-azolones influence on leukemia cells is the most prominent. The considerable impact on such activity could be due to immunosuppressant component of 2-*R*-amino(imino)-substituted-4-azolones, which was reported recently.[49]

Empirical SAR study revealed that the presence of OH-substituent at *para* position of arylamino fragment in 2-amino-4-thiazolinones is more favorable than in *meta* position. Effect of elimination of HBD by introduction of methyl into secondary amino group gave ambiguous influence on the activity (compounds **15–20**), depending on the ylidene substituent in position 5 of thiazoline heterocycle. The loss of anticancer activity caused by additional substitution of arylamino-moiety by small alkyl groups (compounds **21** and **22**), which most probably make a steric hindrance with eventual biotarget. Variation of substituents of the arylidene moiety is more tolerated, however, small lipophiles in positions 3 and 4 (F, Cl, OMe) were more favored. The most active compounds **10** and **11** inhibited 50% tumor cells in submicromolar concentrations (Table 4) at 25% and 37% of total studied cancer cell lines, respectively.

Importantly, compounds **13** and **17**, which contain 2-chloro-3-(4-nitrophenyl)propenylidene substituent in combination with 4-hydroxyphenylamino moiety exhibit one of the most potent activities with specific selectivity against certain cancer cell lines. At the same time in the series of 2-amino-4-imidazolinone derivatives with Cl-substituent in position 3 of arylamino moiety (compounds **44**, **52**, **54**) have the most preferable level of activity comparing to the average one. Elongation of NH linker by methylene group (compounds **55–59**) did not give positive influence on the level of antitumor cytotoxicity.

2.4. COMPARE analysis and molecular mechanism assumptions

Cellular cytotoxicity assays allowed us to discover selective antitumor activity of certain 2-arylamino-4-azolones. Thus the question of their potential molecular target became actual.

In our previous studies,[30] anti-inflammatory activity was studied for some of compounds within series of 2-arylaminothiazolidones (**10**, **11**, **13**, **14**), mentioned in this paper. It was determined that considerable level of anti-inflammatory activity at in vivo mouse model possessed only compound **13**, so no correlation could be found between anti-inflammatory and antitumor activities of these series and no assumption of molecular mechanism could be made based on these data so far.

There exist a number of target identification approaches from traditional HTS and in vitro target profiling to different in silico methodologies of pharmacological target profiling, including chemical proteomics, chemogenomics, or their combinations. Hence advanced target identification studies are very time- and resources-consuming as well as not always allow to reliably define proper target, the latter were beyond the scope of this article, and for the first assumption of molecular mechanism we used accessible on-line tool—NCI COMPARE analysis.

NCI's COMPARE algorithm[50–53] allows to assume biochemical mechanisms of action of novel compounds on the basis of their in vitro activity profiles when comparing with those of standard agents. It determines Pearson correlation coefficient (PCC) for the degree of similarity of mean graph fingerprints obtained from in vitro anticancer screen with patterns of activity of standard agents. We performed COMPARE computations for synthesized compounds against the NCI 'Standard Agents' database at the GI_{50} level, correlations where considered as significant when PCC >0.6 (Table 5).

COMPARE analysis hypothesis precludes, that the compound may have the same mechanism of action as the agent with known action mechanism, if the data pattern of a compound correlates well with the data pattern of compounds belonging to the standard agent database.

The majority of significant correlations of tested compounds, in particular for 2-amino-1*H*-imidazol-4(5*H*)-one derivatives, have been found with numerous alkylating agents with different modes of action. This may suggest that studied compounds influence DNA transcription and alkylation could be the most probable action of the majority of compounds. Interestingly, mean graph fingerprints of compounds **15**, **45**, **47**, **49** showed significant similarity with several topoisomerase II inhibitors, as well as CTP-synthase inhibitor (compound **47**) in COMPARE test. These molecular targets should be considered as the first priority and be explored for the

Table 4
Cytotoxicity of the studied compounds against individual tumor cell lines

Disease	Cell line	Compd	Log GI_{50}	Log TGI
Breast cancer	MCF7	**10**	−7.30	−6.66
	MCF7	**11**	−7.45	−6.94
	MDA-MB-435	**7**	−6.39	−5.69
	MDA-MB-435	**10**	−6.40	−5.78
	MDA-MB-435	**11**	−7.50	−6.84
	NCI/ADR-RES	**11**	−6.20	−5.50
	T-47D	**11**	−6.18	>−4.00
CNS cancer	SF-268	**11**	−6.31	−5.36
	SF-295	**11**	−6.42	−5.72
	SNB-75	**11**	−6.39	>−4.00
	U251	**10**	−6.31	—
Colon cancer	COLO-205	**11**	−6.20	>−4.00
	HCC-2998	**11**	−6.25	−5.67
	HCT-116	**11**	−6.66	−5.36
	HCT-15	**10**	−6.37	>−4.00
	KM12	**11**	−6.88	−6.13
	SW-620	**11**	−6.28	>−4.00
	SW-620	**13**	−6.08	−5.58
Leukemia	CCRF-CEM	**8**	−6.70	−5.94
	CCRF-CEM	**10**	−6.07	−4.99
	CCRF-CEM	**22**	−7.46	—
	CCRF-CEM	**57**	−5.97	−4.53
	HL-60(TB)	**8**	−6.22	−4.99
	HL-60(TB)	**10**	−6.36	−4.99
	HL-60(TB)	**48**	−6.91	−5.74
	K-562	**7**	−6.40	−5.17
	K-562	**10**	−6.59	—
	K-562	**13**	−6.34	>−4.00
	MOLT-4	**10**	−6.22	−4.77
	MOLT-4	**54**	−5.98	−5.41
	RPMI-8226	**10**	−6.39	>−4.00
	RPMI-8226	**22**	−6.23	>−4.00
	SR	**10**	−6.84	−6.28
	SR	**11**	−6.27	>−4.00
	SR	**15**	−6.36	−5.58
Melanoma	M-14	**11**	−6.21	−4.94
	SK-MEL-5	**17**	<−8.00	<−8.00
Non-small cell lung cancer	HOP-62	**11**	−6.37	−4.88
	HOP-92	**22**	−7.12	−5.35
	NCI-H23	**10**	−6.25	−5.49
	NCI-H23	**11**	−6.43	−5.68
	NCI-H460	**10**	−6.01	−4.06
	NCI-H460	**11**	−6.58	−6.02
Ovarian cancer	OVCAR-3	**10**	−6.79	−6.31
	OVCAR-3	**11**	−6.49	−6.25
	OVCAR-3	**13**	−6.43	−6.02
	OVCAR-3	**14**	−6.31	−5.56
	OVCAR-4	**11**	−6.08	>−4.00
Prostate cancer	PC-3	**46**	−6.42	−5.36
	PC-3	**56**	−7.04	−4.56
Renal Cancer	786-0	**11**	−6.51	−5.00
	CAKI-1	**10**	−6.30	−5.19
	CAKI-1	**11**	−6.32	−5.61
	RXF-393	**10**	−6.93	−5.40
	UO-31	**8**	−6.05	−4.54
	UO-31	**11**	−6.02	>−4.00

hit-to-lead optimization using further in silico and in vitro studies. Comparison of other substituted 2-aminothiazol-4(5*H*)-ones, not mentioned in the Table 5, did not yield any significant activity correlations with any standard agents, which may indicate their unique mode of anticancer action.

3. Conclusions

In the present paper, 32 novel 5-arylidene-2-arylaminothiazol-4(5*H*)-ones and 2-aryl(benzyl)amino-1*H*-imidazol-4(5*H*)-ones were described. Majority of compounds showed significant antitumor cytotoxicity effect at micromolar and submicromolar level on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines. The most potent compounds **10** and **13** possessed selectively high effect on all leukemia cell lines at submicromolar level, which probably associated with immunosuppressive activity.

SAR study allowed to identify optimal H-bond donating OH-substituent at *para* position of arylamino fragment in 2-amino-4-thiazolinones, as well as small lipophilic substituents of the arylidene moiety at positions 3 and 4 are more favored. COMPARE analysis allowed to disclose probable modes of anticancer action for synthesized compounds, in particular showed number of high correlations with patterns of alkylating agents.

4. Experimental

4.1. Materials and methods

All starting materials were purchased from Merck and used without purification. The chemical structures of the obtained compounds were confirmed by elemental and spectral analyses (IR, UV, ^{1}H and ^{13}C NMR). IR spectra were recorded with FT/IR-410 Spectrophotometer (Jasco Corp., Japan) and OMNIC-510 spectrometer using potassium bromide pellets (frequencies are expressed in cm^{-1}). UV spectra were recorded on a UVIDEC-510 spectrophotometer (1% w/v solution in ethanol), wave length are expressed in nm. ^{1}H NMR spectra were determined with Varian Mercury 300 MHz and Varian Gemini 75 Hz spectrometers, in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard (chemical shifts values are reported in ppm units, coupling constants (J) are in Hz). Abbreviations are as follows: s—singlet; d—doublet; dd—double doublet; t—triplet; q—quartet, m—multiplet; br—broad. Elemental analyses (C, H, N) performed at the Perkin–Elmer 2400 CHN and Carlo-Erba 1106 CHN analyzer and were within ± 0.4% from the theoretical values. LC–MS were obtained on Agilent 1100 instrument.

The purity of the compounds was checked by thin-layer chromatography performed with Merck Silica Gel GF254 aluminum sheets. Spots were detected by their absorption under UV light. The melting points (uncorrected) were determined on Mel-Temp melting point apparatus (Laboratory Devices Inc., USA).

(*Z*)-Configuration of the exocyclic C=C bond of the final compounds was confirmed by spectroscopic and X-ray studies performed for chosen structures and described before.[34,39] Coexistence of tautomeric forms for compounds **3**, **4**, **6–11**, **19–22** is confirmed by characteristic doubling of signals observed in IR, ^{1}H and ^{13}C NMR spectra.

4.2. Chemistry

The starting compounds: 2-thioxo-4-thiazolidinone,[54] arylthioureas (**23–25**)[55] and 2-carbethoxymethylthio-4-thiazol-4(5*H*)-one (**2**)[30] were obtained according to described procedures. 2-Arylaminothiazol-4(5*H*)-ones (**3–6**) and their 5-arylidene derivatives (**7–22**) were synthesized in analogy to the previously reported methods.[30]

4.3. General procedure for the preparation of 2-arylaminothiazol-4(5*H*)-ones (3–6)

A mixture of 21.9 g (0.1 mol) of **1** and 0.1 mol of the appropriate aminophenol in 150 mL of isopropanol was heated under reflux for 2 h. After cooling, the obtained precipitated solid was filtered off, washed with isopropanol, water, and then recrystallized from acetic acid.

Substances **3–6** were isolated as white or gray powders.

Table 5
COMPARE analysis results for tested compounds[a]

Compd No.	PCC	Target	Target vector NSC		Seed StDev	Target StDev	Target mechanism of action[b]
15	0.64	*S*-Trityl-L-cysteine	S83265	52	0.337	0.337	Reversible binding inhibitor of the human kinesin Eg5, antimitotic agent
	0.636	*N,N*-Dibenzyldaunomycin	S268242	55	0.334	0.556	Topoisomerase II inhibitor
	0.606	Fluorodopan	S73754	53	0.336	0.248	Alkylating agent, alkylates DNA at the N7 position of guanine
22	0.642	Piperazine alkylator	S344007	55	0.494	0.613	Alkylating agent, alkylates DNA at the N7 position of guanine
45	0.676	Dichloroallyl lawsone	S126771	57	0.21	0.397	DNA/RNA antimetabolite
	0.644	Mitindomide	S284356	54	0.211	0.316	Topoisomerase II inhibitor
	0.643	Methyl-CCNU	S95441	47	0.217	0.494	Chloroethylating alkylator, alkyl transferase-dependent cross-linkers
	0.619	Menogaril	S269148	48	0.217	0.491	DNA intercalator and topoisomerase II inhibitor
	0.608	Deoxyspergualin	S356894	51	0.213	0.07	Immunosuppressive agent, inhibition of growth of activated naive CD4 T cells
	0.607	*N,N*-Dibenzyldaunomycin	S268242	56	0.209	0.556	Topoisomerase II inhibitor, prodrug of daunomycin, active on acute leukemias
	0.604	Fluorodopan	S73754	54	0.212	0.209	Alkylating agent, alkylates DNA at the N7 position of guanine
47	0.676	Fluorodopan	S73754	55	0.255	0.207	Alkylating agent, alkylates DNA at the N7 position of guanine
	0.656	Anguidine	S141537	55	0.251	0.286	Inhibitor of protein synthesis
	0.636	D-Tetrandrine	S77037	55	0.251	0.605	Inductor of apoptosis, reversal activity for MDR tumors
	0.629	*N,N*-dibenzyldaunomycin	S268242	57	0.248	0.555	Topoisomerase II inhibitor, prodrug of daunomycin, active on acute leukemias
	0.617	Methyl-CCNU	S95441	48	0.261	0.49	Chloroethylating alkylator, alkyl transferase-dependent cross-linkers
	0.611	Dichloroallyl lawsone	S126771	58	0.253	0.395	DNA/RNA antimetabolite
	0.602	Cyclopentenylcytosine	S375575	51	0.256	0.872	CTP synthetase inhibitor (conversion UTP to CTP)
48	0.731	Methyl-CCNU	S95441	52	0.488	0.384	Chloroethylating alkylator, alkyl transferase-dependent cross-linkers
	0.677	Indicine *N*-oxide	S132319	56	0.474	0.146	A natural pyrrolizidine alkaloid with antineoplastic properties, alkylates and crosslinks DNA
	0.613	Fluorodopan	S73754	56	0.474	0.242	Alkylating agent, alkylates DNA at the N7 position of guanine
49	0.636	CCNU	S79037	47	0.248	0.55	Chloroalkylating agent (lomustine)
	0.633	Mitindomide	S284356	55	0.237	0.314	Topoisomerase II inhibitor
	0.621	Methyl-CCNU	S95441	48	0.248	0.49	Chloroethylating alkylator, alkyl transferase-dependent cross-linkers
	0.62	Anguidine	S141537	55	0.237	0.286	4,15-Diacetoxyscirpen-3-ol, ANG 66, Mycotoxin, diacetoxyscirpenol, apoptosis inducer
	0.612	Deoxyspergualin	S356894	52	0.242	0.07	Immunosuppressant, inhibits antigen processing in monocytes, interacts with heat shock protein 70 family Hsc70, and NFκβ
54	0.635	Dichloroallyl lawsone	S126771	56	0.276	0.384	DNA/RNA antimetabolite
57	0.617	D-Tetrandrine	S77037	54	0.216	0.607	Inductor of apoptosis, reversal of MDR
58	0.723	CCNU	S79037	46	0.157	0.557	Chloroalkylating agent
	0.682	Didemnin B	S325319	54	0.15	0.345	Caspase activator, apoptosis inductor, protein synthesis inhibitor
	0.659	BCNU	S409962	57	0.147	0.24	Chloroalkylating agent (carmustine)
	0.647	Chlorozotocin	S178248	55	0.146	0.573	Chloroalkylating agent
	0.605	*S*-Trityl-L-cysteine	S83265	53	0.143	0.334	Reversible binding inhibitor of the human kinesin Eg5, antimitotic agent
59	0.722	CCNU	S79037	46	0.172	0.557	Chloroalkylating agent
	0.619	Didemnin B	S325319	54	0.176	0.345	Caspase activator, apoptosis inductor, protein synthesis inhibitor

[a] Only correlations with PCC $\geq$ 0.6 were selected, as significant.
[b] Putative mechanisms of action were identified with the use of literature sources.

4.3.1. 2-(4-Hydroxyphenylamino)thiazol-4(5*H*)-one (3)

Yield 95%, mp 274–276 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.79, 3.88 (2*s, 2H, CH_2), 6.79 (d, 2H, J = 8.4 Hz, arom.), 6.90, 7.46 (2*d, 2H, J = 8.4 Hz, arom.), 9.20, 9.24 (2*s, 1H, OH), 10.78, 11.33 (2*s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 36.94, 39.08, 115.97, 116.44, 122.67, 124.91, 131.29, 134.74, 155.20, 156.06, 177.89, 180.06, 188.66. IR (KBr): ν (cm^{-1}) 3676 (OH), 3568 (OH), 3402 (NH), 3084, 3054, 3026, 3005 (CH), 2817 (CH_2), 2774 (CH_2), 1884, 1828 (arom.), 1664 (C=O), 1616 (C=N), 1464, 1432 (CH_2), 1320 (CN), 1244 (CH_2–S), 1196 (C–O) cm^{-1}. Anal. ($C_9H_8N_2O_2S$) C, H, N.

4.3.2. 2-(3-Hydroxyphenylamino)thiazol-4(5*H*)-one (4)

Yield 80%, mp 218–220 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.76 (s, 2H, CH_2), 6.45–7.22 (m, 4H, arom.), 9.50 (br s, 1H, OH), 11.00, 12.25 (2*s, 1H, NH). UV spectra, λ (lg ε): 267 (4.25), 277 (4.24), 375 (3.32). Anal. ($C_9H_8N_2O_2S$) C, H, N.

4.3.3. 2-[(4-Hydroxyphenyl)methylamino]thiazol-4(5*H*)-one (5)

Yield 73%, mp 238–240 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.47 (s, 3H, CH_3), 3.91 (s, 2H, CH_2), 6.85 (d, 2H, J = 8.4 Hz, arom.), 7.27 (d, 2H, J = 8.4 Hz, arom.), 9.94 (s, 1H, OH). IR (KBr): ν (cm^{-1}) 3560 (OH), 3107, 3079 (arom.), 2991, 2860 (CH_3), 2847, 2843 (CH_2), 1716 (C=O), 1621 (C=N), 1448 (CH_3), 1320 (CN), 1204 (C–$O_{phen.}$), 1096 (C–N) cm^{-1}. Anal. ($C_9H_8N_2O_2S$) C, H, N.

4.3.4. 2-(2-Methyl-4-hydroxy-5-isopropylphenylamino)thiazol-4(5*H*)-one (6)

Yield 70%, mp >200 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 1.20 (d, 6H, $(CH_3)_2$), 2.08, 2.12 (2*s, 3H, CH_3), 3.20 (m, 1H, CH), 3.80, 3.88 (2*s, 2H, CH_2), 6.64, 6.75 (2*s, 2H, arom.), 9.20, 9.30 (2*s, 1H, OH), 10.70, 11.75 (2*s, 1H, NH). Anal. ($C_{13}H_{16}N_2O_2S$) C, H, N.

4.4. General procedure for the preparation of 5-arylidene-2-arylaminothiazol-4(5*H*)-ones (7–22)

Method ***A:*** A mixture of 0.1 mol of the appropriate 2-arylaminothiazol-4(5*H*)-one, 8.2 g (0.1 mol) of fused sodium acetate, 0.11 mol of the respective aromatic aldehyde in 150 mL of acetic acid was heated under reflux for 3 h. The precipitated solid 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.

Method B: A mixture of 0.1 mol of the appropriate arylthiourea, 0.11 mol of the respective aromatic aldehyde, 9.4 g (0.1 mol) of chloroacetic acid and 8.2 g (0.1 mol) of fused sodium acetate 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.

Substances **7–22** were isolated as white or yellowish powders.

4.4.1. 5-(3,4-Dimethoxybenzylidene)-2-(4-hydroxyphenylamino)thiazol-4(5*H*)-one (7)

Yield: 73% (method **B**), mp 288–289 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.75, 3.77, 3.85, 3.87 (4*s, 6H, 2*OCH_3), 6.77 (d, 2H, J = 8.0 Hz, arom.), 6.88, 7.57 (2*d, 2H, J = 8.0 Hz, arom.), 7.00–7.21 (m, 3H, arom.), 7.54, 7.60 (2*s, 1H, =CH), 9.43 (br s, 1H, OH), 11.22 (br s, 1H, NH). UV spectra, λ (lg ε): 244 (3.98), 245 (3.95), 371 (4.53). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 56.17, 56.25, 56.33, 56.41, 112.76, 113.30, 114.70, 115.10, 116.14, 116.57, 122.77, 123.87, 129.99, 149.46, 149.58, 150.88, 155.50, 158.40, 170.18, 181.19. Anal. ($C_{18}H_{16}N_2O_4S$) C, H, N.

4.4.2. 5-[4-(4-Fluorobenzyloxy)-3-methoxybenzylidene]-2-(4-hydroxyphenylamino)thiazol-4(5*H*)-one (8)

Yields: 95% (method **A**), 71% (method **B**), mp >270 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.85, 3.90 (2*s, 3H, OCH_3), 5.00, 5.10 (2*s, 2H, CH_2), 6.70–6.90, 6.95–7.15, 7.40–7.53 (3*m, 11H, arom.), 7.56, 7.59 (2*s, 1H, =CH), 8.95 (br s, 1H, OH), 10.95, 11.65 (2*s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 56.16, 56.27, 69.81, 113.49, 114.28, 115.05, 115.88, 116.12, 116.58, 122.65, 122.78, 123.75, 124.32, 124.83, 125.70, 127.38, 127.71, 130.86, 130.94, 133.56, 149.74, 149.86, 155.51, 155.99, 163.75, 170.17, 181.17. LC–MS: *m/z 451,2* (98.85%, M^++1). Anal. ($C_{24}H_{19}FN_2O_4S$) C, H, N.

4.4.3. 5-[4-(2,4-Dichlorobenzyloxy)-3-methoxybenzylidene]-2-(4-hydroxyphenylamino)thiazol-4(5*H*)-one (9)

Yields: 95% (method **A**), 71% (method **B**), mp >270 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.85, 3.90s (2*s, 3H, OCH_3), 5.10, 5.15 (2*s, 2H, CH_2), 6.75d (d, 2H, J = 8.2 Hz, arom.), 6.82, 7.57 (2*d, 2H, J = 8.2 Hz, arom.), 6.95–7.20, 7.25–7.35, 7.40–7.60 (3*m, 6H, arom.), 7.56, 7.59 (2*s, 1H, =CH), 8.95 (br s, 1H, OH), 10.95, 11.70 (2*s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 56.25, 56.37, 67.60, 113.73, 114.39, 115.17, 116.15, 116.58, 122.79, 124.34, 128.31, 129.63, 129.90, 130.65, 132.08, 133.91, 134.35, 149.41, 149.88, 155.53, 156.00, 170.12, 181.20. LC–MS: *m/z 502.*0 (96.79%, M^++1). Anal. ($C_{24}H_{18}N_2Cl_2O_4S$) C, H, N.

4.4.4. 5-(4-Chlorobenzylidene)-2-(4-hydroxyphenylamino) thiazol-4(5*H*)-one (10)

Yields: 83% (method **A**), 78% (method **B**), mp >320 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 6.80, 6.81 (2*d, 2H, J = 8.2 Hz, arom.), 6.96, 7.57 (2*d, 4H, J = 8.2 Hz, arom.), 7.53, 7.62 (2*d, 4H, J = 8.0 Hz, arom.), 7.61, 7.67 (2*s, 1H, =CH), 9.54, 9.59 (2*s, 1H, OH), 11.53, 12.12 (2*s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 116.17, 116.65, 123.00, 124.39, 126.31, 128.57, 128.97, 129.21, 130.86, 129.95, 129.99, 131.80, 131.94, 133.17, 133.69, 134.83, 134.87, 155.69, 156.17, 170.12, 180.84. UV spectra, λ (lg ε): 241 (3.82), 248 (3.58), 272 (3.58), 346 (4.40). EI-MS: *m/z* 331 (35%, M^++2), 330 (80%, M). Anal. ($C_{16}H_{11}ClN_2O_2S$) C, H, N.

4.4.5. 5-(4-Fluorobenzylidene)-2-(4-hydroxyphenylamino) thiazol-4(5*H*)-one (11)

Yield: 85% (method **A**), mp 268–269 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 6.77 (d, 2H, J = 7.2 Hz, arom.), 6.90, 7.58 (2*d, 2H, J = 7.2 Hz, arom.), 7.25, 7.32 (2*t, 2H, J = 8.2 Hz, arom.), 7.52–7.60 (m, 2H, arom.), 7.64, 7.67 (2*s, 1H, =CH), 9.36, 9.40 (2*s, 1H, OH), 11.30, 12.05 (2*s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 116.15, 116.63, 117.03, 122.97, 124.40, 128.17, 128.84, 129.26, 130.90, 131.37, 132.62, 155.64, 156.14, 161.97, 164.37, 170.06, 180.92. UV spectra, λ (lg ε): 239 (3.74), 339 (4.21). Anal. ($C_{16}H_{11}Cl_2N_2O_2S$) C, H, N.

4.4.6. 5-(4-Hydroxybenzylidene)-2-(4-hydroxyphenylamino) thiazol-4(5*H*)-one (12)

Yields: 95% (method **A**), 71% (method **B**), mp >220 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 6.78 (d, 2H, J = 8.0 Hz, arom.), 6.90, 7.58 (2*d, 2H, J = 8.0 Hz, arom.), 6.84 (d, 2H, J = 8.0 Hz, arom.), 7.34, 7.43 (2*d, 2H, J = 8.0 Hz, arom.), 7.49, 7.56 (2*s, 1H, =CH), 9.36, 9.40 (2*s, 1H, OH), 11.17, 11.85 (2*s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 116.12, 116.60, 116.89, 122.83, 124.21, 125.11, 125.50, 130.40, 130.90, 131.00, 132.35, 132.50, 155.45, 155.92, 159.89, 162.99, 170.36, 180.84. Anal. ($C_{16}H_{11}N_2O_3S$) C, H, N.

4.4.7. 5-[2-Chloro-3-(4-nitrophenyl)-2-propenylidene]-2-(3-hydroxyphenylamino)thiazol-4(5*H*)-one (13)

Yield: 55% (method **A**), mp 310 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 6.40–6.52, 6.55–6.65 (2*m, 2H, arom.), 7.05–7.23, 7.95–8.05, 7.39 (3*m, 2H, arom.), 7.90, 8.02 (2*d, 2H, J = 8.6 Hz, arom.), 8.22–8.31 (m, 2H, arom.), 7.53, 7.62 (2*s, 1H, =CH), 7.74, 7.80 (2*s, 1H, =CH), 9.53s, 9.62s (1H, OH), 11.41s, 12.45s (1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 108.32, 111.89, 112.88, 113.13, 124.47, 127.74, 129.77, 130.58, 131.25, 135.56, 147.64, 158.60, 158.86, 170.30, 180.80. UV spectra, λ (lg ε): 258 (3.89), 272 (3.67), 378 (4.37), 386 (4.39). IR (KBr): ν (cm^{-1}) 3408 (N–H), 3212 (N–H), 1672 (C=O), 1632 (C=N), 1604 (C=N), 1576 (C=C), 1484 (OH), 1344 (CN), 1268 (NO_2), 1152 (C–O) cm^{-1}. Anal. ($C_{18}H_{12}ClN_3O_4S$) C, H, N.

4.4.8. 5-(4-Chlorobenzylidene)-2-(3-hydroxyphenylamino) thiazol-4(5*H*)-one (14)

Yield: 64% (method **A**), mp 272–276 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 6.34 (m, 1H, arom.), 6.45–6.55 (m, 1H, arom.), 6.70–6.80 (m, 1H, arom.), 6.95–7.10 (m, 2H, arom.), 7.15–7.30 (m, 3H, arom.), 7.45, 7.50 (2*s, 1H, =CH), 9.15, 9.22 (2*s, 1H, OH), 11.00, 11.90 (2*s, 1H, NH). UV spectra, λ (lg ε): 240 (4.02), 272 (3.99), 278 (3.97), 374 (3.10). Anal. ($C_{16}H_{11}ClN_2O_2S$) C, H, N.

4.4.9. 5-(2,3-Dichlorobenzylidene)-2-[(4-hydroxyphenyl) methylamino]thiazol-4(5*H*)-one (15)

Yields: 95% (method **A**), 71% (method **B**), mp >240 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.60 (s, 3H, CH_3), 6.90 (d, 2H, J = 8.7 Hz, arom.), 7.20 (d, 2H, J = 8.2 Hz, arom.), 7.35 (d, 2H, J = 8.7 Hz, arom.), 7.50 (t, 1H, J = 8.2 Hz, arom.), 7.90 (s, 1H, CH=), 9.80 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 42.87, 116.99, 125.61, 127.59, 127.87, 129.20, 129.61, 131.90, 132.39, 133.48, 135.32, 135.59, 159.15, 176.89, 179.35. Anal. ($C_{17}H_{12}Cl_2N_2O_2S$) C, H, N.

4.4.10. 5-(2,4-Dimethoxybenzylidene)-2-[(4-hydroxyphenyl) methylamino]thiazol-4(5*H*)-one (16)

Yields: 95% (method **A**), 71% (method **B**), mp >250 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.55 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 7.80 (s, 1H, arom.), 6.88 (d, 2H, J = 8.8 Hz, arom.), 6.64 (dd, 1H, J = 8.0 Hz, J = 2.0 Hz, arom.), 7.17 (d, 1H, J = 8.0 Hz, arom.), 7.35 (d, 2H, J = 8.8 Hz, arom.), 7.80 (s, 1H, CH=), 10.01 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 42.40, 56.18, 54.54, 99.21, 106.97, 115.77, 116.94, 124.93, 127.40, 129.73, 133.76, 158.92, 160.10, 162.99, 177.04, 180.58. Anal. ($C_{19}H_{18}N_2O_4S$) C, H, N.

4.4.11. 5-[2-Chloro-3-(4-nitrophenyl)-2-propenylidene]-2-[(4-hydroxyphenyl)methylamino]thiazol-4(5*H*)-one (17)

Yields: 95% (method **A**), 71% (method **B**), mp >300 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.57 (s, 3H, CH_3), 6.90 (d, 2H, J = 8.7 Hz, arom.), 7.37 (d, 2H, J = 8.7 Hz, arom.), 7.54 (s, 1H, CH=), 7.74 (s, 1H, CH=), 7.94 (d, 2H, J = 9.0 Hz, arom.), 8.27 (d, 2H, J = 9.0 Hz, arom.), 10.04 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 42.81, 117.00, 124.43, 129.22 129.34, 130.90, 131.20, 133.38, 133.96, 135.28, 140.94, 147.58, 158.97, 177.74, 179.88. Anal. ($C_{19}H_{14}ClN_3O_4S$) C, H, N.

4.4.12. 5-(4-Fluorobenzylidene)-2-[(4-hydroxyphenyl)methylamino]thiazol-4(5*H*)-one (18)

Yields: 95% (method **A**), 71% (method **B**), mp >270 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.55 (s, 3H, CH_3), 6.88 (d, 2H, J = 8.6 Hz, arom.), 7.30 (d, 2H, J = 8.6 Hz, arom.), 7.23 (t, 2H, J = 8.2 Hz, arom.), 7.57 (s, 1H, =CH), 7.30 (m, 2H, arom.), 9.92 (s, 1H, OH). Anal. ($C_{17}H_{13}FN_2O_2S$) C, H, N.

4.4.13. 5-(2,4-Dichlorobenzylidene)-2-[(4-hydroxyphenyl)methylamino]thiazol-4(5*H*)-one (19)

Yields: 95% (method **A**), 71% (method **B**), mp >260 °C. Anal. ($C_{17}H_{12}Cl_2N_2O_2S$) C, H, N. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.59 (s, 3H, CH_3), 6.88 (d, 2H, J = 8.8 Hz, arom.), 7.37 (d, 2H, J = 8.8 Hz, arom.), 7.39 (d, 1H, J = 8.4 Hz, arom.), 7.52 (dd, 1H, J = 8.4 Hz, J = 1.6 Hz, arom.), 7.72 (s, 1H, =CH), 7.75 (d, 1H, J = 1.6 Hz, arom.), 10.06 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 42.87, 117.00, 124.43, 129.06, 129.21, 130.37, 131.62, 133.45, 134.67, 135.34, 135.62, 159.05, 176.82, 179.56. Anal. ($C_{17}H_{12}Cl_2N_2O_2S$) C, H, N.

4.4.14. 5-[4-(2,4-Dichlorobenzyloxy)-3-methoxybenzylidene]-2-[(4-hydroxyphenyl)methylamino]thiazol-4(5*H*)-one (20)

Yields: 95% (method **A**), 71% (method **B**), mp 227–230 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 3.58 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 5.14 (s, 2H, CH_2), 6.89 (d, 2H, J = 8.8 Hz, arom.), 6.97 (d, 1H, J = 8.8 Hz, arom.), 7.16 (dd, 1H, J = 8.8 Hz, J = 1.5 Hz arom.), 7.20 (d, 1H, J = 1.5 Hz, arom.), 7.37 (d, 2H, J = 8.8 Hz, arom.), 7.48 (dd, 1H, J = 8.2 Hz, J = 2 Hz, arom.), 7.58 (s, 1H, =CH), 7.58 (d, 1H, J = 8.2 Hz, arom.), 7.69 (d, 1H, J = 2 Hz, arom), 9.98 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 42.49, 56.33, 67.53, 114.45, 115.24, 116.92, 122.09, 128.06, 128.27, 128.47, 129.23, 129.60, 130.76, 131.99, 133.71, 133.84, 134.27, 149.32, 149.74, 158.89, 177.01, 180.38. Anal. ($C_{25}H_{20}Cl_2N_2O_4S$) C, H, N.

4.4.15. 5-(4-Chlorobenzylidene)-2-(2-methyl-4-hydroxy-5-isopropylphenylamino)thiazol-4(5*H*)-one (21)

Yield: 77% (method **A**), mp 294–295 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 1.22 (d, 6H, $(CH_3)_2$), 2.12, 2.16 (2*s, 3H, CH_3), 3.20 (m, 1H, CH), 6.65, 6.70 (2*s, 2H, arom.), 7.35d (d, 2H, J = 8.2 Hz, arom.), 7.40d (d, 2H, J = 8.2 Hz, arom.), 7.51, 7.55 (2*s, 1H, CH=), 8.75 (br s, 1H, OH), 10.30, 11.60 (2*br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 17.82, 23.14, 26.80, 117.56, 128.55, 129.93, 131.84, 133.29, 134.80, 153.65, 153.73, 161.82, 164.30, 170.26, 180.98. LC–MS: m/z 387.2 (99.8%, M^++1). Anal. ($C_{20}H_{19}ClN_2O_2S$) C, H, N.

4.4.16. 5-(4-Fluorobenzylidene)-2-(2-methyl-4-hydroxy-5-isopropylphenylamino)thiazol-4(5*H*)-one (22)

Yield: 59% (method **A**), mp 278–280 °C. ^{1}H NMR (DMSO-d_6 + CCl_4): δ (ppm) 1.18 (d, 6H, $(CH_3)_2$), 2.09, 2.12 (2*s, 3H, CH_3), 3.20 (m, 1H, CH), 6.65, 6.75 (2*s, 2H, arom.), 7.22, 7.31 (2*t, 2H, J = 8.7 Hz, arom.), 7.52 (m, 2H, arom.), 7.58, 7.60 (2*s, 1H, CH=), 9.19, 9.30 (2*s, 1H, OH), 10.76, 11.83 (2*s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 17.84, 18.10, 19.23, 23.14, 26.80, 56.71, 116.87, 117.09, 117.56, 122.09, 126.30, 128.80, 131.01, 132.50, 132.97, 133.33, 153.58, 161.81, 164.28, 170.06, 180.92. Anal. ($C_{20}H_{19}FN_2O_2S$) C, H, N.

The synthesis of 5-arylidene-2-arylaminoimidazol-4-ones **48**, **50**, **51**, **55–59** was previously reported,[34,35] while the remaining derivatives of imidazol-4-one were obtained in analogy to the described procedure.

4.5. General procedure for the preparation of 5-arylidene-2-aryl(methyl)amino-1*H*-imidazol-4(5*H*)-ones (44–59)

To a stirred solution of Na (0.01 mol, 0.23 g) in 60 mL of EtOH 5-arylidene-2-thiohydantoin **26–34** (0.04 mol) and CH_3I (0.04 mol, 0.62 mL) were added. After stirring at room temperature for 30 min the solid of **33–43**, respectively, was filtered off, washed with water and dried. Methylthio derivatives were shown to be analytically pure.

A mixture consisting of 5 mmol appropriate methylthio derivative (**33–43**), 5.5 mmol of amine in 30 mL of acetic acid (**44–54**) or toluene (**55–59**) was refluxed for 7–9 h and then allowed to cool, formed precipitate was filtered off. Substances **44–59** were recrystallized from AcOH or EtOH and isolated as yellowish or yellow powders.

4.5.1. (*Z*)-5-(3-Chlorobenzylidene)-2-(3-chlorophenylamino)-1*H*-imidazol-4(5*H*)-one (44)

Yield: 73%, mp 275–277 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 6.56 (s, 1H, =CH), 7.16 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.3 Hz, arom.), 7.35–7.46 (m, 3H, arom.), 7.56 (d, 1H, J = 8.0 Hz, arom.), 7.87 (d, 1H, J = 7.4 Hz, arom.), 8.26 (s, 1H, arom.), 8.44 (s, 1H, arom.), 10.24 (br s, 1H, 7-NH), 10.98 (br s, 1H, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 113.51, 118.44, 119.67, 123.15, 128.04, 129.47, 129.68, 130.57, 130.81, 133.79, 133.82, 137.90, 140.61, 141.80, 155.97, 170.47. IR (KBr): ν (cm^{-1}) 3413, 1697, 1670, 1639, 1479. Anal. ($C_{16}H_{11}Cl_2N_3O$) C, H, N.

4.5.2. (*Z*)-5-(3-Chlorobenzylidene)-2-(4-chlorophenylamino)-1*H*-imidazol-4(5*H*)-one (45)

Yield: 73%, mp 288–289 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 6.53 (s, 1H, =CH), 7.36 (d, 1H, J = 8.0 Hz, arom.), 7.42–7.48 (m, 3H, arom.), 7.85 (d, 2H, J = 8.2 Hz, arom.), 7.93 (d, 1H, J = 7.4 Hz, arom.), 8.37 (s, 1H, arom.), 10.17 (br s, 1H, 7-NH), 10.95 (br s, 1H, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 113.05, 121.78, 127.33, 127.85, 129.11, 129.24, 129.82, 130.66, 133.66, 133.81, 137.97, 141.98, 155.99, 176.62. IR (KBr): ν (cm^{-1}) 3342, 1697, 1664, 1643, 1508. Anal. ($C_{16}H_{11}Cl_2N_3O$) C, H, N.

4.5.3. (*Z*)-5-(4-Chlorobenzylidene)-2-(4-chlorophenylamino)-1*H*-imidazol-4(5*H*)-one (46)

Yield: 54%, mp 338–339 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 6.54 (s, 1H, =CH), 7.41–7.47 (m, 2H, arom.), 7.50 (d, 2H, J = 8.3 Hz, arom.), 7.84 (d, 2H, J = 8.5 Hz, arom.), 8.13 (d, 2H, J = 8.5 Hz, arom.), 10.09 (br s, 1H, 7-NH), 10.89 (br s, 1H, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 113.60, 121.65, 127.16, 129.01, 129.27, 132.19, 132.70, 134.70, 138.18, 141.26, 155.78, 170.71. IR (KBr): ν (cm^{-1}) 3324, 1697, 1652, 1601, 1506. Anal. ($C_{16}H_{11}Cl_2N_3O$) C, H, N.

4.5.4. (*Z*)-5-(2,3-Dichlorobenzylidene)-2-(4-chlorophenylamino)-1*H*-imidazol-4(5*H*)-one (47)

Yield: 87%, mp 296–298 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 6.78 (s, 1H, =CH), 7.43 (d, 2H, J = 8.7 Hz, arom.), 7.46–7.48 (m, 1H, arom.), 7.54 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.5 Hz, arom.), 7.77 (d, 2H, J = 8.7 Hz, arom.), 8.75 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.5 Hz, arom.), 11.07 (br s, 2H, 7-NH, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 107.92, 121.99, 127.56, 128.50, 129.28, 129.61, 129.93, 131.22, 132.49, 135.62, 137.88, 143.38, 156.88, 170.61. IR (KBr): ν (cm^{-1}) 3291, 1712, 1654, 1641, 1493. Anal. ($C_{16}H_{10}Cl_3N_3O$) C, H, N.

4.5.5. (*Z*)-5-(2,5-Dichlorobenzylidene)-2-(4-chlorophenylamino)-1*H*-imidazol-4(5*H*)-one (49)

Yield: 81%, mp 303–304 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 6.66 (s, 1H, =CH), 7.32 (dd, 1H, J_1 = 8.7 Hz, J_2 = 2.6 Hz, arom.), 7.39 (d, 2H, J = 8.7 Hz, arom.), 7.50 (d, 1H, J = 8.7 Hz, arom.), 7.80 (d, 2H, J = 8.7 Hz, arom.), 8.73 (d, 1H, J = 2.6 Hz, arom.), 11.13 (br s, 2H, 7-NH, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 106.40, 122.09, 127.71, 128.77, 129.03, 130.87, 131.30, 131.96, 132.28, 134.92, 137.81, 143.57, 157.01, 170.50. IR (KBr): ν (cm^{-1}) 3423, 3291, 1714, 1658, 1639, 1491. Anal. ($C_{16}H_{10}Cl_3N_3O$) C, H, N.

4.5.6. (*Z*)-5-(3,4-Dichlorobenzylidene)-2-(3-chlorophenylamino)-1*H*-imidazol-4(5*H*)-one (52)

Yield: 82%, mp 301–302 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 6.51 (s, 1H, =CH), 7.13 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.1 Hz, arom.), 7.37 (t, 1H, J = 8.2 Hz, arom.), 7.53 (d, 1H, J = 8.2 Hz, arom.), 7.60 (d, 1H, J = 8.5 Hz, arom.), 7.86 (d, 1H, J = 8.5 Hz, arom.), 8.16 (br s, 1H, arom.), 8.58 (br s, 1H, arom.), 10.25 (br s, 1H, 7-NH), 10.99 (br s, 1H, 3-NH).). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 112.25, 118.45, 119.70, 123.18, 130.34, 130.73, 130.91, 131.33, 131.50, 131.71, 133.82, 136.53, 140.48, 142.18, 156.08, 170.35. IR (KBr): ν (cm^{-1}) 3401, 1697, 1672, 1641, 1483. Anal. ($C_{16}H_{10}Cl_3N_3O$) C, H, N.

4.5.7. (*Z*)-5-(3,4-Dichlorobenzylidene)-2-(4-chlorophenylamino)-1*H*-imidazol-4(5*H*)-one (53)

Yield: 77%, mp 300–301 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 6.52 (s, 1H, =CH), 7.57 (dd, 2H, J_1 = 6.7 Hz, J_2 = 2.2 Hz, arom.), 7.67 (d, 1H, J = 8.2 Hz, arom.), 7.84 (d, 2H, J = 8.5 Hz, arom.), 8.00 (br s, 1H, arom.), 8.52 (s, 1H, arom.), 10.22 (br s, 1H, 7-NH), 11.00 (br s, 1H, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 111.80, 121.71, 127.32, 127.37, 129.03, 130.15, 130.81, 131.58, 131.66, 136.60, 137.99, 142.32, 156.03, 170.45. IR (KBr): ν (cm^{-1}) 3423, 3291, 1718, 1658, 1621, 1492. Anal. ($C_{16}H_{10}Cl_3N_3O$) C, H, N.

4.5.8. (*Z*)-5-(3-Fluorobenzylidene)-2-(4-chlorophenylamino)-1*H*-imidazol-4(5*H*)-one (54)

Yield: 72%, mp 298–300 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 6.55 (s, 1H, =CH), 7.14 (dt, 1H, J_1 = 8.4 Hz, J_2 = 2.5 Hz, arom.), 7.43–7.50 (m, 3H, arom.), 7.82 (d, 2H, J = 8.5 Hz, arom.), 7.86 (s, 1H, arom.), 8.02 (d, 1H, J = 8.4 Hz, arom.), 10.98 (br s, 2H, 7-NH, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 113.49, 115.19, 116.52, 121.82, 126.90, 127.35, 129.20, 130.72, 130.83, 138.11, 141.92, 156.07, 164.19, 170.68. IR (KBr): ν (cm^{-1}) 3308, 1717, 1654, 1614, 1492. Anal. ($C_{16}H_{11}ClFN_3O$) C, H, N.

4.6. Crystal structure determination of 5-(4-fluorobenzylidene)-2-(2-methyl-4-hydroxy-5-isopropylphenilamino)thiazol-4(5*H*)-one (22)

Crystal data: 2($C_{20}H_{18}FN_2O_2$),1/2(C_3H_7NO), M_r = 1554.82, monoclinic, space group *C*2/*c*, a = 26.8044(17), b = 22.5054(17), c = 15.0934(8) Å, β = 123.301(4)°, V = 7609.9(9) Å^3, T = 100(2) K, Z = 8.

Data collection: A yellow planar crystal of 0.53 × 0.44 × 0.15 mm was used to record 35149 (Mo Kα radiation, θ_{max} = 28.6°) intensities on a KM4CCD κ-axis diffractometer. The crystal was positioned at 62 mm from the CCD camera. 600 Frames were measured at 1° intervals with a counting time of 20 s. The data were corrected for Lorentz and polarization effects. Multi-scan absorption correction has been applied too. The minimum and maximum transmissions were 0.9013 and 0.9706. Data reduction and analysis were carried out with the OXFORD DIFFRACTION programs.[56] The 8973 total unique reflections (R(int) = 0.051) were used for further calculations. The positions of the H atoms in the solute molecules were obtained from difference Fourier map and were refined freely. The solvent H atoms were positioned geometrically, and were refined with a riding model (C–H = 0.95 and 0.98 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,[57] and refinement was done against F^2 for all data using SHELXL-97.[57] The final refinement converged with R = 0.0348 (for 7017 data with F^2 >4σ(F^2)), wR = 0.1013 (on F^2 for all data), and S = 1.062 (on F^2 for all data). The largest difference peak an hole were 0.320 and −0.340 e Å^{-3}. The molecular illustration was drawn using ORTEP-3 for Windows.[58] Software used to prepare material for publication was WINGX.[59]

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 736034).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.073.

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