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Synthesis and antitumor activity evaluation of new 2-(4-alkoxyphenylamino)thiazol-4(5H)-ones derivatives

Synteza i ocena aktywności przeciwnowotworowej nowej pochodnej 2-(4-alkoksyfenylamino) tiazol-4(5H)-onu

INTRODUCTION

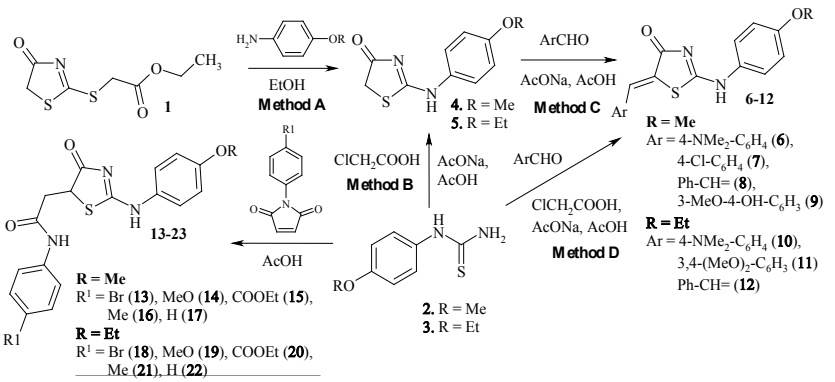
Synthetic and screening research was carried out in the field of 4-thiazolidone derivatives synthesis. Our cooperation with the U.S. National Cancer Institute (NCI) have allowed identification of significant antitumor potential of 2-(oxyphenylamino)thiazol-4(5*H*)-ones. In this group 3 lead compounds were selected, namely (5-[2-chloro-3-(4-nitrophenyl)-2-propenylidene]-2-(3-hydroxyphenylamino) thiazol-4(5*H*)-one, 5-(4-chlorophenylmethyli-dene)- and 5-(4-fluorophenylmethylidene)-2-(4-hydroxyphenylamino)thiazol-4(5*H*)-ones [5, 6]. Currently, these compounds are under in-depth study according to NCI Biological Evaluation Committee decision. The influence of the hydroxyl group replacement by the alkoxygroup in phenyl ring on antitumor activity was studied as continuation of this research.

MATERIAL AND METHODS

A synthetic approach to 2-substituted thiazolidones was based on ethoxycarbonylmethylthio moiety usage as leaving group (Scheme). Reaction of 2-carbethoxymethylthio-2-thiazol-4(5*H*)-one (1) with 4-methoxy- and 4-ethoxyanilines yielded the target 2-(4-alkoxyphenylamino)thiazol-4(5*H*)-ones (4, 5, method A). Synthesized compounds are methylene active heterocycles. On the other hand, it was previously shown that the presence and nature of moiety in position 5 of thiazolidinones played the key role in realization and character of pharmacological effects [3–7, 11]. The abovementioned became a background for the synthesis of new 5-arylidenederivatives (6-12), using standard Knoevenagel reaction procedure (method C, medium – acetic acid, catalyst – fused sodium acetate). 5-Arylidenederivatives were prepared alternatively by one-pot methodology involving reaction of arylthioureas [10] with chloroacetic acid and appropriate aromatic aldehydes in the presence of fused

sodium acetate in refluxing acetic acid (method D). It should be noted that methods A, B and C, D practically do not differ in outputs and purity of products that can alternatively be used for the synthesis of target compounds [5, 6, 8]. The condensation of appropriate arylthioureas (2, 3) with arylmaleimides gave a series of 2-[2-(4-meth(eth)oxyphenylamino)-4-oxo-4,5-dihydrothiazol-5-yl]-*N*-arylacetamides (13-22).

Scheme



The presence of amino-imino tautomerism was confimed by ^{1}H NMR spectroscopy; structures of all newly synthesized compounds were confirmed by elemental analyses, ^{1}H NMR and mass spectroscopy.

The presented row of heterocyclic compounds was studied for anticancer activity. First, the screening was carried out on 3 cancer cell lines (NCI-H460, MCF-7 and Sf-268) or on 60 cell lines covering almost the entire range of human cancers (lung, breast, ovaries, large intestine, kidney, prostate and CNS cancer lines, as well as leukemia and melanoma). Cell growth was compared with control in both cases and was determined by the percentage of growth.

Comprehensive *in vitro* screening consisted in a study of its antitumor effect in five concentrations at 10-fold dilution (100μM, 10μM, 1μM, 0.1μM and 0.01μM) for 57 lines of human cancer cells, which are the same to stage prescreening. 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. If the logarithmic value of the investigated parameters ($lgGI_{50}$, lgTGI and $lgLC_{50}$) are smaller than -4.00, compounds are considered to be active [1, 2, 9].

RESULTS AND DISCUSSION

Results of the prescreening of compounds 5, 9, 12, 19 and 20 have not shown the desired effect (Table 1), whereby some lines even stimulated cell growth. However, it is worth noting that 2-(4-metoxyphenylamino) thiazole-4(5*H*)-one (5) showed high selectivity suppression of mitotic activity line ovarian cancer IGROV1, and its 5-(3-methoxy-4-oxybenzylidene) derivative 9 – line breast cancer MBA-MB-435. As seen from the

data in Table 1, compound 15 showed high levels of cytotoxicity and was selected for in-depth *in vitro* studies. Interestingly, the substitution at position 4 ethoxygroup in aminophenol residue (compound 20) on metoxygroup (compound 15) leads to significant increase in cytotoxic effect.

Table 1. Cytotoxicity of synthesized compounds

Comp.	Mitotic activity (3 lines, 10^{-4} M), %	Average mitotic activity of 60 lines / range mitotic activity (10^{-5} M), %	The most sensitive cell line (mitotic activity,%)
5	–	100.90 / 17.89 ÷ 156.00	Ovarian Cancer: *IGROV1* (17.89%) Breast Cancer: *BT-549* (56.81%)
9	–	78.85 / 10.04 ÷ 112.63	NSC Lung Cancer: *HOP-32* (32.95%) Colon Cancer: *HCT-15* (51.82%) Leukemia: *K-562* (50.21%) Breast Cancer: *MBA-MB-435* (10.04%). MBA-MB-468 (45.93%)
12	MCF7 – 120% NCI-H460 – 112% SF-268 – 87%	–	–
15	–	59.84 / -41.66 ÷ 115.04	Renal Cancer: *UO-31* (-41.66%) Colon Cancer: *KM12* (1.50%). *HCC-2998* (-13.44%) Ovarian Cancer: *OVCAR-3* (14.31%) Breast Cancer: *MDA-MB-231/ATCC* (3.44%)
19	MCF7 – 103% NCI-H460 – 112% SF-268 – 78%	–	–
20	MCF7 – 96% NCI-H460 – 156% SF-268 – 112%	–	–

In-depth investigation of compound 15 confirmed its high antitumor potential. In general, it is worth to mention the substantial level of effective growth inhibition (Table 2) practically of all tumor cell lines (percentage of "active line" was 84.2% with an average of $lgGI_{50}$ = -5.11). With regard to individual cell lines $lgGI_{50}$ the highest value was observed for MOLT-4 (leukemia).

In evaluating the antitumor profile lead compound in various types of cancer the relative selectivity of CNS cancer line should be noted. A very interesting picture was observed for cell line U251 (CNS cancer), an effective level of inhibition, cytotoxic and cytostatic effects were approximately at the same level ($lgGI_{50}$ = -5.74, lgTGI = -5.74, $lgLC_{50}$ = 5.14). Thus, the level of antitumor profile of compound 15 may be considered as potential "lead structure", which is characterized by high effective growth inhibition of all tested cell lines (range $lgGI_{50}$ within -4.00 ÷ -5.91) and significant cytostatic (TGI range within -4.00 ÷ -5.44) activity.

Table 2. Results of in-depth *in vitro* screening compounds 15 in concentration gradient 10^{-4}-10^{-8} M

Parameter	Number of „active" lines / %	Average activity / range	The most sensitive cell line (value of the parameter effect)
$lgGI_{50}$	48 / 84.2%	-5.11 / -5.91 ÷ -4.00	*$lgGI_{50}$ < -5.50* Leukemia: *MOLT-4* (-5.91) NSC Lung Cancer: *HOP-62* (-5.54). *HOP-92* (-5.66). *NCI-H322M* (-5.52) Colon Cancer: *HCT-116* (-5.58) CNS Cancer: *SF-539* (-5.63). *SNB-75* (-5.73). *U251* (-5.74) Melanoma: *MALME-3M* (-5.85). *SK-MEL-28* (-5.57) Renal Cancer: *CAKI-1* (-5.55). *TK-10* (-5.68) Breast Cancer: *MBA-MB-231/ATCC* (-5.64)
lgTGI	26 / 45.6%	-4.36 / -5.44 ÷ -4.00	*lgTGI ≤ -5.00* NSC Lung Cancer: *HOP-62* (-5.00). *HOP-92* (-5.11) CNS Cancer: *SF-539* (-5.09). *SNB-75* (-5.16). *U251* (-5.44) Melanoma: *MALME-3M* (-5.42). *SK-MEL-28* (-5.15) Renal Cancer: *TK-10* (-5.15) Breast Cancer: *MBA-MB-231/ATCC* (-5.32)
$lgLC_{50}$	14 / 24.6%	-4.11 / -5.14 ÷ -4.00	*$lgGI_{50}$ < -4.50* CNS Cancer: *SF-539* (-4.53). *U251* (-5.14) Melanoma: *MALME-3M* (-4.79) Renal Cancer: *TK-10* (-4.58) Breast Cancer: *MBA-MB-231/ATCC* (-4.99)

CONCLUSSIONS

Results of this study prompt us to in-depth anticancer studies of 2-arylaminothiazol-4(5*H*)-one derivatives as possible "drug-like" molecules. 4-Ethoxycarbonylphenylamide of 5-carboxymethyl-2-(4-methoxyphenylamino)thiazol-4(5*H*)-one (15) was selected as a lead-compound with high antitumor activity and selective action against CNS-cancer and melanoma.

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SUMMARY

5-Substituted 2-(4-alkoxyphenylamino)thiazol-4(5*H*)-ones were synthesized. Anticancer activity of synthesized compounds toward 60 human tumor cell lines panel in National Cancer Institute was evaluated. 4-Ethoxycarbonylphenylamide of 5-carboxymethyl-2-(4-methoxyphenylamino)thiazol-4(5*H*)-one was selected as lead-compound with high antitumor activity.

Keywords: 2-arylaminothiazol-4(5*H*)-ones, [2+3]-cyclocondensation, Knoevenagel reaction, antitumor activity

STRESZCZENIE

Zsyntetyzowano 5-podstawioną pochodną 2-(4-alkoxyphenylamino)thiazol-4(5*H*)-onu. Aktywność przeciwnowotworową zsyntetyzowanego składnika w kierunku panelu 60 nowotworowych linii komórkowych zbadano w Narodowym Instytucie Raka. Jako główny składnik z najwyższą aktywnością przeciwnowotworową wskazano 4-etoksykarbonylfenylamide 5-karboksymetyl-2-(4-metoksyfenylamino) tiazol-4(5*H*)-on.

Słowa kluczowe: 2-arylaminotiazol-4(5*H*)-ony, [2+3]-cyklokondensacja, reakcja Knoevenagela, aktywność przeciwnowotworowa