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Synthesis of new 4-azolidinones with 3,5-diaryl-4,5-dihydropyrazole moiety and evaluation of their antitumor activity in vitro

Synteza nowych 4-azolidynonów z cząsteczką 3,5-diaryl-4,5-dihydropirazolu i ocena ich aktywności przeciwnowotworowej *in vitro*

INTRODUCTION

A systematic study of 4-thiazolidones derivatives with pyrazoline fragment in molecules allowed us to identify a number of high-active compounds as potential antitumor agents [7]. Generally, combination of thiazolidine template with diazole heterocycles is a perspective approach to drug-like molecules design, considering an antitumor potential of 4-thiazolidinone derivatives. The mechanisms of antitumor activity of 4-thiazolidinones can be associated with their affinity to anticancer biotargets such as JNK-stimulating phosphatase-1 (JSP-1) [4], tumor necrosis factor TNFα [3], anti-apoptotic biocomplex Bcl-X_L-BH3 [6], integrin $\alpha_v\beta_3$ receptor [5], etc. Besides, affinity of diazole derivatives was determined to the number of known biotargets. Among pyrazoles or pyrazolines inhibitors of cyclin-dependent kinase [10], heat shock proteins [2], vascular endothelium growth factors [1] and P-glycoprotein [9] were identified. The aim of our research was the synthesis of novel 4-azolidinones with 3,5-diaryl-4,5-dihydropyrazole moieties in positions 3 and 5 of core scaffold and evaluation of their antitumor activity *in vitro*.

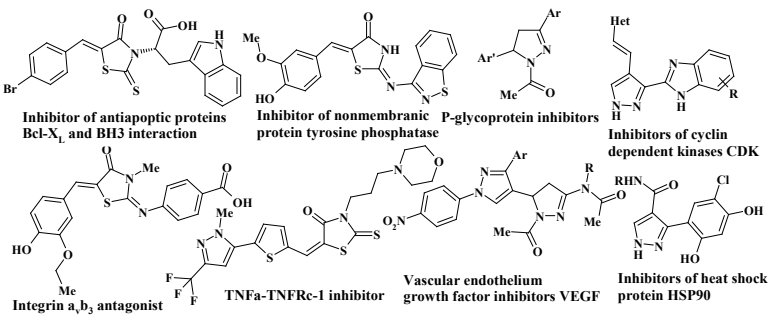


Fig. 1. 4-Thiazolidinones and diazoles as potential antitumor agents (world experience)

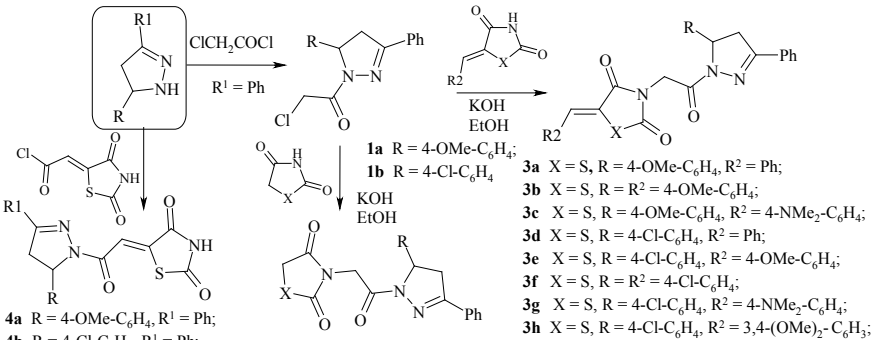
MATERIAL AND METHODS

Starting 3,5-diaryl-4,5-dihydropyrazoles [12], 5-arylidene-2,4-azolidinones potassium salts [9], (2,4-dioxothiazolidine-5-ylidene)-acetyl chloride [15] were obtained according to the methods described previously. For synthesis of new 4-thiazolidinone derivatives, the reactions of acylation and alkylation were used. The structure of the synthesized compounds was confirmed by ^{1}H NMR spectra.

RESULTS AND DISCUSSION

3,5-Diaryl-4,5-dihydro-1*H*-pyrazoles easily react with chloroacetyl chloride yielding 2-chloro-1-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-ethanones 1a-1b. Compounds 1 were tested as alkylating agents in the reactions with 2,4-azolidinones and their 5-arylidenederivatives potassium salts in refluxing ethanol or mixture DMF-ethanol. Thus, the corresponding compounds 2 and 3 were obtained. Following the reaction of 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles and (2,4-dioxothiazolidine-5-ylidene)-acetyl chloride the group of 5-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethylidene]-thiazolidine-2,4-diones 4 was synthesized (Scheme).

Scheme



Structures of the synthesized compounds were confirmed by ^{1}H NMR spectra. Protons CH_2-CH of pyrazoline fragment in the ^{1}H NMR spectra of synthesized compounds showed characteristic patterns of an AMX system. The chemical shifts of the protons H_A, H_M, and H_X were assigned to about δ~3.26-3.45, δ~3.96-4.17, and δ~5.68-6.13, respectively, with corresponding coupling constants of J_{AM} = 17.9-18.6, J_{AX} = 10.4-11.6, and J_{MX} = 2.9-4.5 Hz. The chemical shifts of the protons of the methylene group (CH_2CO) 2a-2c, 3a-3j were assigned at δ~4.45-4.86 and δ~4.56-4.94 and the protons of methylene group of azolidine cycle in compounds 2a-2c showed up as singlet at δ~3.96-4.28. The chemical shift for the methylidene group of 5-arylidenederivatives 3 is insignificantly displaced in weak magnetic

field, δ~6.90 (hydantoin derivatives 3i-3j) and δ~7.90 (2,4-thiazolidindione derivatives 3a-3h), and clearly indicated that only *Z*-isomers were obtained [13].

Primary anticancer assay of the synthesized compounds (2a, 2b, 2i, 4a, 4d, 4e) was performed at approximately sixty human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [8, 14].

The tested compounds (2a, 2b, 2i, 4a) displayed moderate antitumor activity with average values GP 51.05-97.11% (Table). Selectivity pattern analysis of cell lines of disease origin can definitely affirm selective action of compound 2a on Non-Small Lung Cancer cell line NCI-H522 (GP= -68.70%), Ovarian Cancer – IGROV1 (GP= -51.27%), Renal Cancer – CAKI-1 (GP= -50.64%), compound 2i – on CNS cancer line SF-295 (GP= -4.03%), compound 4a – on Leukemia sell line SR (GP = 39.09%). Finally, compounds 4d and 4e possessed considerable activity (mean growth for 4d – 60.11% and 4e – 51.05%) and were selected for advanced assay against a panel of approximately sixty tumor cell lines at 10-fold dilutions of five concentrations (100μM, 10μM, 1μM, 0.1μM and 0.01μM) [8, 14].

Table 1. Anticancer screening data at concentration 10^{-5}M

Comp	60 cell lines assay in 1 dose 10^{-5} M conc				Active (selected for 5-dose 60 cell lines assay)
	mean growth %	range of growth %	the most sensitive cell lines	growth % of the most sensitive cell line	
2a	84.37	-68.70 to 123.93	NCI-H522 (lung cancer) IGROV1 (ovarian cancer) CAKI-1 (renal cancer)	-68.70 -51.27 -50.64	Inactive
2b	97.11	64.96 to 119.33	SF-295 (CNS cancer)	64.96	Inactive
2i	86.17	-4.03 to 121.25	SF-295 (CNS cancer)	-4.03	Inactive
4a	96.64	39.09 to 147.38	SR (leukemia)	39.09	Inactive
4d	60.11	-27.33 to 160.47	HL-60(TB) (leukemia) SF-295 (CNS cancer)	-27.33 -13.37	Active
4e	51.05	-5.27 to 109.26	RPMI-8226 (leukemia) SF-295 (CNS cancer)	2.48 -5.27	Active

The tested compounds (4d, 4e) showed a broad spectrum of growth inhibition activity against human tumor cells, as well as some distinctive patterns of selectivity on leukemia (Fig. 2). Compound 4d was found to be a highly active growth inhibitor of the leukemia cell lines RPMI-8226 ($logGI_{50}$ = -5.67), CCRF-CEM ($logGI_{50}$ = -5.55) and SR ($logGI_{50}$ = -5.55). Compound 4e showed selectivity on leukemia cell line MOLT-4 ($logGI_{50}$ = -5.79) and non-small cell lung cancer cell line HOP-92 ($logGI_{50}$ = -5.93). Generally, pyrazoline substituted derivative 4e demonstrated the most marked

effect among all synthesized compounds and possessed significant activity with the mean $logGI_{50}$ value -5.44.

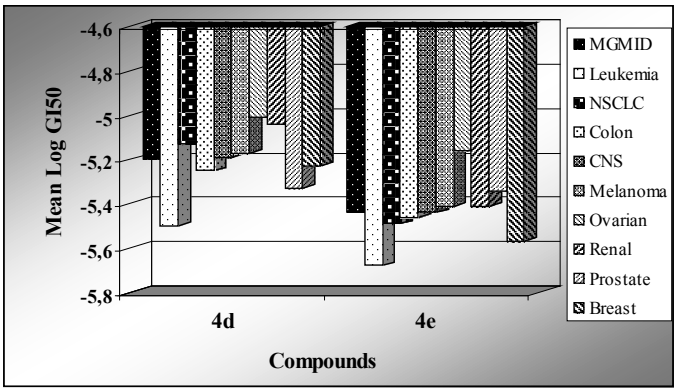


Fig. 2. Anticancer selectivity pattern of the most active compounds

CONCLUSIONS

A group of novel 4-azolidinone derivatives with pyrazoline moieties was synthesized using reactions of acylation and alkylation. Antitumor activity screening of the synthesized compounds showed their moderate activity with high selectivity to individual lung, renal, ovarian and CNS cancer cell lines. In conclusion, these preliminary results allowed to identify the most active compound 4e as a prospective antitumor agent (average $logGI_{50}$ and logTGI values -5.44 and -4.52, respectively) with selective influence on leukemia.

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SUMMARY

In the present paper, a synthetic approach for design of new pyrazoline substituted 4-azolidones were described. Six of the synthesized compounds were tested according to NCI protocol and two of them (**4d**, **4e**) displayed antitumor activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines.

Keywords: 4-azolidinones, 3,5-diaryl-4,5-dihydropyrazoles, acylation, alkylation, ^{1}H NMR spectra, antitumor activity

STRESZCZENIE

W pracy opisano proces syntezy nowego 4-azolidonu z podstawnikiem pirazolinowym. Zgodnie z protokołem NCI przebadano sześć uzyskanych substancji, przy czym dwie z nich (4d, 4e) wykazały aktywność przeciwnowotworową w stosunku do linii komórkowych białaczki, czerniaka, raka płuc, okrężnicy, OUN, jajników, nerek, prostaty i piersi.

Słowa kluczowe: 4-azolidynony, 3,5-diaryl-4,5-dihydropirazole, acylacja, alkilacja, spektra ^{1}H NMR, aktywność przeciwnowotworowa