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Synthesis and biological evaluation of novel pyrazole derivatives with anticancer activity

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ABSTRACT

We synthesized thirty-six novel pyrazole derivatives and studied their antiproliferative activity in human ovarian adenocarcinoma A2780 cells, human lung carcinoma A549 cells, and murine P388 leukemia cells.

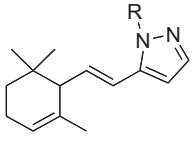
Four of these substances were selected because of their higher antiproliferative activity and further analyses showed that they were all able to induce apoptosis, although to a different extent. The expression of p53 and $p21^{waf1}$, which induce apoptosis and cell cycle arrest, was evaluated by western blot analysis in cells treated with compound **12d**.

The analysis of the cell cycle showed that all the selected compounds cause a partial G2/M block and the formation of polyploid cells. Furthermore, the four selected compounds were tested for their interaction with the microtubular cytoskeletal system by docking analysis, tubulin polymerization assay and immunofluorescence staining, demonstrating that the compound **12d**, unlike the other active derivatives, was able to significantly bind dimers of α- and β-tubulin, probably causing a molecular distortion resulting in the disassembly of microtubules.

1. Introduction

The pyrazole scaffold represents a common motif in many pharmaceutical active and remarkable compounds demonstrating a wide range of pharmacological activities; the most important activities are the anti-inflammatory [1,2], the antibacterial-antifungal [3,4], the hypoglycemic [5,6], the anti-hyperlipidemic [7], the inhibition of cyclooxigenase-2 [8], p38 MAP kinase [9] and CDK2/Cyclin A [10,11], and the antiangiogenic [12]. Heterocyclic rings and, in particular, the pyrazole ring, represent an advantageous choice for the synthesis of pharmaceutical compounds with different activities and good safety profiles [13]. Different pyrazole derivatives have also been tested for their antiproliferative activities in vitro and antitumor activity in vivo, often resulting in promising lead compounds [14–18].

Our research started some time ago with the synthesis of heterocyclic ionone-like derivatives, which have a similarity to the so-called 'short heteroretinoids'. These compounds, formed by a cyclohexenyl group linked to a heterocyclic moiety by a short ethenylic chain, have shown antimicrobial [19], anti-inflammatory and histoprotective properties [20]. The heterocyclic moiety (pyrazole, isoxazole, pyrimidine) and the substituents present in them, deeply affected the biological activities. Our researches demonstrated that these compounds have antiproliferative and pro-apoptotic activities. Our attention was focused on a class of ionone-derived 1,5-pyrazoles **1a-f** that exhibited promising antiproliferative properties in preliminary experiments on the HL-60 leukemia cell line (Chart 1) [21]. In particular **1a**, **1d-f** inhibited HL-60 cell growth and induced apoptosis in dose dependent manner, while **1b-c** displayed antiproliferative activity but were unable to induce apoptosis.



1a-f

1a: R=C(S)N(CH_3)$_2$
1b: R=4-ClC_6H_4
1c: R=4-BrC_6H_4
1d: R=4-(COOEt)C_6H_4
1e: R=3-OH$C_6H_4CH_2$
1f: R=2-pyridinyl

Chart 1. Chemical structure of previous reported compounds **1**.

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Prompted by the above-mentioned results, we further optimized this chemical series by exploring additional modifications. Firstly we introduced new substituents in the N position of the pyrazole (compd. **4**) also changing the linker moiety in one example (**4h**). Then, keeping the same pyrazole scaffold that showed the best results on HL-60 cells, we planned to synthesize two new sets of molecules: in the first set we chose to remove the cyclohexenyl ring maintaining the ethenylic chain (A), while in the second set we also removed the ethenylic chain (B). Finally we evaluated the importance of introducing a third substituent in the pyrazole ring (C) (Fig. 1).

All the synthesized compounds were preliminarily evaluated for their antiproliferative activity in A2780 (ovary, adenocarcinoma) and A549 (lung, carcinoma) human cell lines, and in murine P388 (leukemia) cells by MTT assay. **12a**, **12a1**, **12d**, **12d1**, the most active compounds in the preliminary screening, have been further evaluated for their ability to induce apoptosis in all the above-mentioned cell lines. In addition, to verify the involvement of **12d** in apoptotic triggering we analyzed the expression of p53 and $p21^{waf1}$ proteins both involved in the induction of apoptosis and cell cycle arrest. On the basis of the results of these studies, which suggest the involvement of the microtubule cytoskeletal system in the mechanism of the action of **12d**, we performed a docking analysis, using the dimer of α- and β-tubulin as the putative target for our molecules, and a tubulin polymerization assay.

2. Chemistry

Compounds **4** were synthesized by cyclisation with suitable hydrazines of the already reported [20] β-(dimethylamino)vinyl-aldehydes **2** and **3** following our method which was further modified [21]: when hydrazine derivatives were available as hydrochlorides, they were used as such (method 1), while all the other hydrazine derivatives were made to react with equimolar quantities of hydrochloridric acid 37% (method 2).

The pyrazole derivatives have been obtained in good yields (56–76%) and in a very short period of time (**1h**). Moreover, in several cases minor amounts of the 1,3-pyrazole derivative **5** have been isolated together with the predominant 1,5-pyrazoles **4** (Scheme 1). Compound **4g** was already reported as **1f** [21]. The treatment of commercially available phenylbutenone derivatives **6** with N,N-dimethylformamide dimethyl acetal (DMFDMA) afforded the dimethylaminopentadienone intermediates **7**, as reported in the literature [22]. They in turn easily reacted with the hydrazine derivatives giving the pyrazoles **8** according to method 1 or 2. In most cases the 1,3-substituted isomers **9** were also isolated (Scheme 2).

Using the same procedure on different arylmethylketones **10** and following the synthetic routes described in the literature [23–31], we first obtained the dimethylaminopropenone intermediates **11** which cyclized with the opportune hydrazines to give the pyrazoles **12**. The 1,3-substituted isomers **13** were also isolated in seven cases. When the intermediates **11c**, **d**, **f** have a hydroxy group in ortho position, the reaction with 2-hydrazinopyridine led to the chromones **14c**, **d**, **f** probably via the intramolecular cyclization of the intermediate itself (Scheme 3) [32].

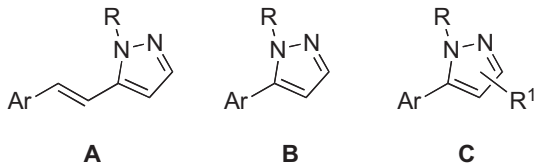
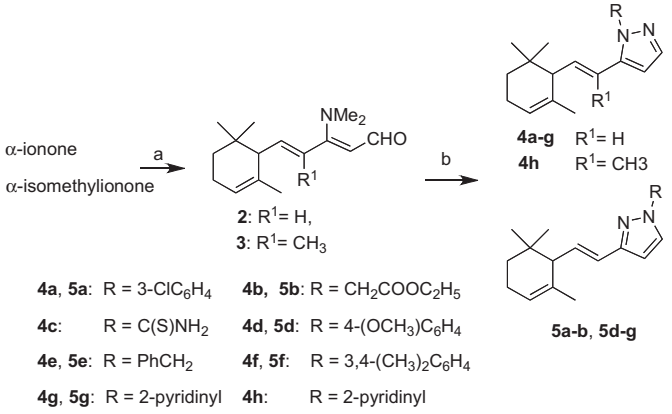


Fig. 1. General structure of new synthesized compounds.



Scheme 1. Reagents and conditions: (a) Vilsmeier reaction: $POCl_3$ (50.0 mmol), N,N-dimethylformamide (3.78 mL), ionone (25.0 mmol); (b) method 1: $RNHNH_2$*HCl; method 2: $RNHNH_2$ + HCl conc.

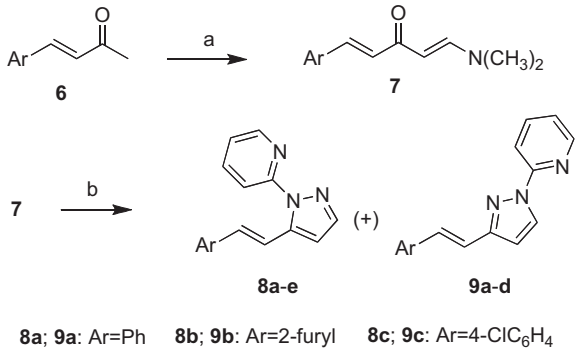
The synthetic route followed for the synthesis of the trisubstituted pyrazoles is outlined in Schemes 4 and 5. Pyrazoles **17** were easily prepared from intermediates **16a** and **16b** which, in turn, were obtained from the commercial 1-(4-methoxyphenyl)propan-1-one **15a** and from the 1-(4-methoxyphenyl)-2-phenylethanone **15b** which was synthesized by Friedel-Crafts acylation on anisole as already reported [33]. A little amount of the isomer **18** was also isolated (Scheme 4).

The intermediate 1,3-dicarbonilyc compounds **19** and **21** were obtained by Claisen condensation between 4′-methoxyacetophenone and methyl benzoate or ethyl acetate, respectively, under nitrogen atmosphere using sodium hydride as a deprotonating agent [34,35], whereas **22** was obtained using sodium amide instead of sodium hydride. Starting from those 1,3-diketones **19**, **21**, **22** we performed the cyclisation with 2-hydrazinopyridine to pyrazoles **20**, **23**, **24**, respectively, with the same procedure described above (Scheme 5).

3. Results

3.1. Inhibition of cell proliferation

The analyses of concentration-response curves obtained from each cell line treated with our 36 compounds together with the resulting calculation of mean IC_{50}s displayed a quite broad range of sensitivity (from 0.64 ± 0.31 to more than 100 μM, Table 1). The



Scheme 2. Reagents and conditions: (a) DMFDMA, xylene, reflux; (b) method 1: $RNHNH_2$*HCl; method 2: $RNHNH_2$ + HCl conc.