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Short communication

Synthesis and biological evaluation of pyrazolo[4,3-*d*]pyrimidine analogues

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ABSTRACT

A series of pyrazolo[3,4-*d*]pyrimidine analogues **3**, **4**, **5a**–**f**, **6a**–**f** with various amines and ester groups at C-4 and N-1 were synthesized and evaluated for antitumour activity. They were also evaluated for xanthine oxidase inhibitory activity, with most compounds having no significant impact. Compound **5e** had the strongest activity against human hepatoma carcinoma cells 7402 and 7221, with half-maximal inhibitory concentration values of 4.55 and 6.28, respectively. Structure–activity relationship studies indicate that chlorine atoms in the structure of 4-((4-(substituted amides)phenyl)amino pyrazolo[4,3-*d*] pyrimidine analogues is crucial for antitumour activity.

1. Introduction

The term cancer encompasses a wide range of types such as lung cancer, colon cancer, and the more obscure acute leukaemia. Malignant cancers are very common and are the second largest cause of death in the West after cardiovascular disease. It is one of the major challenges of this century and is a concern for medical communities all over the world. The diversity of tumour types and their great similarity to normal cells are the main obstacles that prevent the discovery of a cure [1–6].

In the last decade or so, researchers have reported that purine derivatives of allopurinol (Fig. 1) have a certain inhibitory activity towards tumour cells [7]; they can inhibit heat shock protein 90 (Hsp90), which is involved in the degradation of somatic proteins. In addition, allopurinol is a structural isomer of hypoxanthine (a naturally occurring purine in the body) and can be used as inhibitor of the enzyme xanthine oxidase (XOD) [8–12]. Hsp90 is an important target for antitumour drugs owing to its effect on proteins, which can promote the growth and metastasis of tumours [13,14]. Purine derivative PU3 (Fig. 1), similar to geldanamycin (GA, Fig. 1), is the first purine compound discovered that can inhibit Hsp90 biological activity. Researchers have also found that PU24F–Cl has a stronger affinity to Hsp90 than PU3, and it has higher solubility than 17AAG [15,16] (Fig. 1). Although they are very structurally similar; the hydroxyl being replaced by an amino in the pyrimidine ring, and N-1 and C-2 being replaced by alkyl and 3,4,5-methoxy-1-benzyl in the pyrazole ring, respectively.

In this study, we carried out chemical modification of purine by introduction of a pyrazole ring instead of an imidazole ring to provide a specific binding mode different from that of allopurinol analogues [17–22]. We synthesized pyrazolo[3,4-*d*]pyrimidines analogues **3**, **4**, **5a**–**f**, **6a**–**f** by introducing various substituent groups at the C-4 and N-1 positions of the pyrazolopyrimidine ring (Scheme 1). The aim is to produce a potent and selective Hsp90 inhibitor. XOD inhibitory activity was evaluated, and all these new compounds were also tested for antitumour activity against the human hepatoma carcinoma cells 7402 and 7221 using the half-maximal inhibitory concentration (IC_{50}) values.

2. Results and discussion

2.1. Synthesis

The general synthetic route for preparation of two series of Hsp90 inhibitors (**5a**–**f** and **6a**–**f**) is shown in Scheme 1. In the

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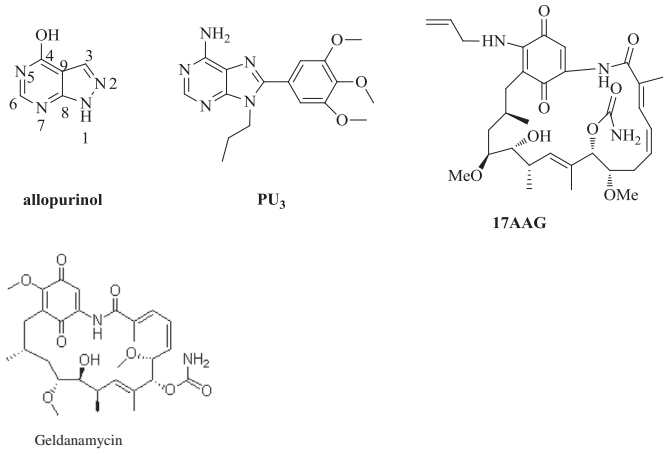


Fig. 1. Compound allopurinol, PU3, natural product Hsp90 inhibitor (17AAG) and geldanamycin.

Table 1
XOD inhibitory activity of new compounds.[a]

Compounds	IC_{50}/μg mL^{-1}
Allopurinol	1.60
3	Inactive
4	86.65
5a	Inactive
5b	>100
5c	77.74
5d	34.10
5e	40.59
5f	38.79
6a	>100
6b	89.28
6c	61.25
6d	40.32
6e	36.83
6f	33.77

[a] The IC_{50} value (μg mL^{-1}) of the reducing power assay is the effective concentration at which the absorbance is 0.5 of the reducing power.

pyrazolopyrimidine ring system, the chloro substituent, as a leaving group, was introduced at C-4, which was the most reactive site for nucleophilic attack. Heating commercially available allopurinol (**1**) with excess *N*,*N*-dimethylaniline and phosphorus oxychloride gave an intermediate product **2**, which was treated with ethyl bromoacetate to afford the corresponding ethyl 2-(4-chloro-1*H*-pyrazolo [3,4-*d*]pyrimidin-1-yl)acetate (**3**). Reacting **3** with *p*-phenylenediamine in acetonitrile at room temperature yielded compound **4**. Amination of **4** with sulfonyl chloride or chloride gave target compounds **5a**–**f**, Scheme 1.

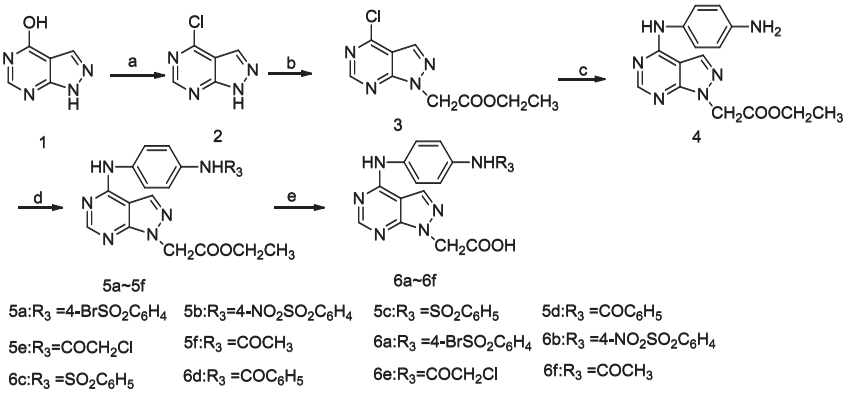
2.2. Effect on XOD inhibitory activity

Inhibition of the XOD-catalyzed conversion of xanthine to uric acid by all 1-*N*-alkyl-4-*N*-substituted amino allopurinol derivative inhibitors was evaluated and compared with the standard inhibitor allopurinol. Enzyme activity was spectrophotometrically monitored by measuring uric acid formation at 290 nm. This was done with a saturated concentration of xanthine (20 μM) as the substrate in 1 mL of 200 mM phosphate buffer at pH 7.5 and at 25 °C. All samples were tested for XOD inhibitory activity at different concentrations (5, 10, 15 and 20 μM). The test results are listed in Table 1.

On observing the data in Table 1, there seems to be no correlation between XOD inhibitory activity and the substituent size at C-4 and N-1 of the pyrazolopyrimidine skeleton, as there is no significant difference from the standard allopurinol compound. Most compounds did not show any significant XOD inhibitory activity. Compounds **5d**, **5f**, **6e** and **6f** exhibited potent XOD inhibitory activity against the target organisms, possibly because of the structural differences i.e. 4-hydroxyl versus amino. A previous literature report has shown that allopurinol derivatives such as 4-amino-substituted allopurinol have a lower XOD inhibitory activity than 4-hydroxyl-substituted allopurinol [9]. The results of these structure–activity studies suggest that the structure of purine is crucial for XOD inhibitory activity.

2.3. Effect on antitumour activity

The synthesized compounds (**3**–**6**; Scheme 1) were subjected to the human cell lines screening assay for evaluation of their in vitro antitumour activity. A single high dose (64 μM) of the test compounds were used in the full NCI two cell lines panel assay which includes human hepatoma carcinoma cells 7402 and 7221. The data were reported as a mean graph of the percent growth of treated cells and presented as IC_{50} values, Table 2. The obtained results of the tested allopurinol derivatives **3**, **4**, **5a**–**d**, **5f**, **6**–**f** showed IC_{50} values >60 μg/ml, a distinctive potential pattern of selectivity, as well as broad-spectrum antitumour activity. Compound **5e** showed potency against human hepatoma carcinoma cells 7402 and 7221 lines with IC_{50} values of 7.27 and 3.51, respectively. It also has a higher antitumour activity compared with 17AAG. This difference



Scheme 1. Reagents and conditions: (a) $POCl_3$, $(CH_3)_2NC_6H_5$, 80 °C; (b) $BrCH_2COOCH_2CH_3$, TEA, DMF, rt; (c) $H_2NC_6H_4NH_2$, CH_3CN, 80 °C; (d) NaH, THF, 0 °C, sulfonyl chloride or chloride; (e) NaOH (1 N), CH_3CH_2OH, rt.