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Synthesis and antiproliferative activity of 6-phenylaminopurines

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ABSTRACT

A series of novel 6-phenylaminopurines have been efficiently synthesized in 3 steps exploring different groups at positions 2, 8 and 9 of the purine ring and at the exocyclic nitrogen atom at position 6. Among the newly described purines, five compounds showed antiproliferative activity with IC_{50} values below 10 μM, the tetrahydroquinoline derivative at position 6 of phenylaminopurine being the most active of the series in the six cell lines tested. Moreover, the compounds induced G_2/M phase arrest in human cervical carcinoma HeLa cells as reported for tubulin depolymerizing agents.

1. Introduction

Microtubules are cytoskeletal filaments consisting of α,β-tubulin heterodimers and are involved in a wide range of fundamental cellular processes, such as formation and maintenance of cell shape, regulation of motility, cell signaling, secretion, intracellular transportation, and cell division and mitosis [1,2]. Due to the different functions of microtubules during cell cycle progression, tubulin has become an attractive target for the discovery of anticancer drugs. For instance, taxoids and vinca alkaloids, first-line therapies for a large variety of tumors, exert their antitumor action by altering microtubule dynamics, either by promoting the course of microtubule assembly or disassembly. Indeed taxoids and vinca alkaloids target two distinct binding sites at the α,β-tubulin heterodimers: while the taxol site is located at the β subunit, the vinca site is situated at the interface of two heterodimers [3,4]. Despite of the clinical efficacy of these drugs, they have serious deficiencies, including narrow therapeutic indexes and emergence of drug resistance, mainly mediated by P-glycoprotein (P-gp) or βIII-tubulin overexpression [5,6]. Another serious disadvantage is their poor water solubility, which forces them to be administered with a surfactant that may cause hypersensibility reactions and long-time administration [7].

A third binding site in tubulin, where colchicine specifically binds, has gained prominence in the last few years. The colchicine binding site is located at the interface of the α and β-subunits [8] and compounds binding at this site act as microtubule depolymerization agents [9,10]. Although colchicine itself cannot be used in the treatment of cancer owing to its high toxicity [11], a number of compounds binding at this site such as CA-4P, ZD6126, AVE-8062, ABT-751 and MPC-6827 are in clinical trials for antitumor indications [9]. It should be mentioned that until now no P-glycoprotein (Pgp) or βIII-tubulin overexpression mediated resistance phenomena have been described with the best studied colchicine site binders [9,12]. Moreover, compounds binding at the colchicine site in tubulin have an additional value as anticancer agents since many of them have shown vascular disrupting properties targeting the tumor endothelium [13,14].

Among the colchicine-binding site compounds, we have centered our attention on MPC-6827 (**1**, Fig. 1), a quinazoline derivative identified through an anticancer screening apoptosis platform. Studies performed with MPC-6827 have shown that the compound binds at the colchicine binding site in tubulin [15]. This compound, also named Verubulin or Azixa, and whose structure significantly differs from colchicine or combretastatins, is currently undergoing clinical evaluation for the treatment of malignancies such as glioblastoma multiforme [16,17]. Other quinazoline

Abbreviations: HeLa, human cervical carcinoma cells; MEP, molecular electrostatic potential; MW, microwave irradiation; P-gp, P-glycoprotein.

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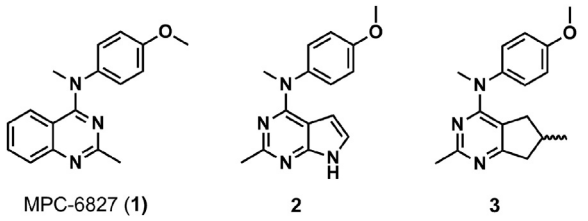


Fig. 1. Chemical structure of MPC-6827 (**1**) and related compounds **2** and **3**.

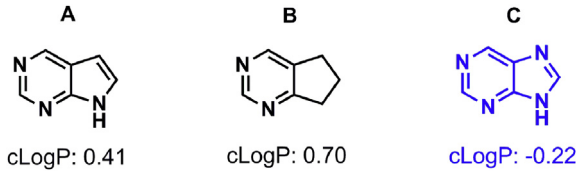


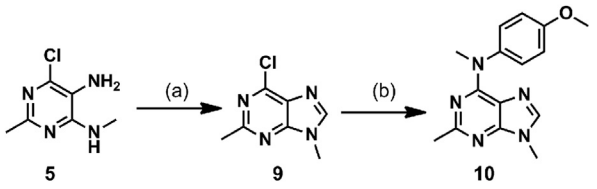
Fig. 2. Theoretical cLogP values [25] of the pyrrole[2,3-*d*]pyrimidine (A), cyclopenta[*d*]pyrimidine (B) and purine (C) scaffolds.

derivatives continue to be explored as antimitotic and vascular disrupting agents [18–21].

Interestingly, Gangjee et al. have reported on some pyrimidine derivatives fused to a 5 membered ring (a pyrazole such as in compound **2**, or a cyclopentane as in compound **3** (Fig. 1)), that have also shown microtubule depolymerization properties by binding at the colchicine site and are characterized by cancer cell growth inhibitory activity. The substitution pattern in these compounds very much resembles the one in MPC-6827 [22].

A comparison of the central scaffold present in compounds **1**, **2** and **3** led us to propose, as a simplistic example of "scaffold hoping" [23], that replacement of such scaffolds by a purine ring [24] might allow a similar distribution of the outsider substituents while solubility could be improved due to the inclusion of an imidazole at the central core. A calculation of the logP values [25] of the skeleton present in compounds **2** and **3**, and a comparison with that of a purine ring (Fig. 2), supported a positive contribution of the latest to an increase in solubility.

Therefore we have addressed the synthesis and antiproliferative evaluation of a series of 6-phenylaminopurine derivatives towards tumor and endothelial cells. In addition, cell-cycle analysis has been performed to establish the mechanism of action of this family of purines.



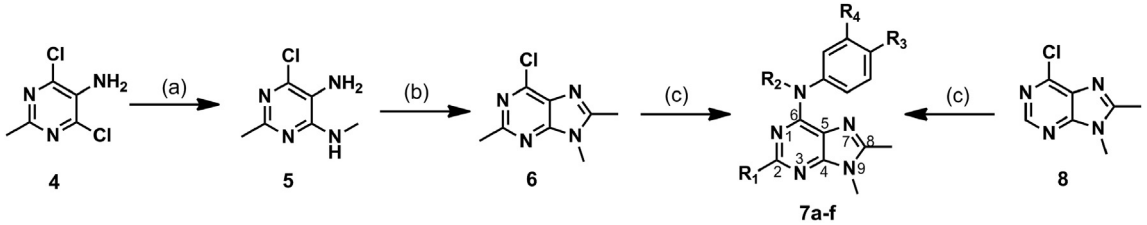
Scheme 2. *Reagents and conditions*: (a) $CH(OMe)_3$, $EtSO_3H$, Ac_2O, MW, 120 °C, 15 min; (b) 4-methoxy-*N*-methylaniline, HCl, ^{i}PrOH, MW, 80 °C, 10 min.

2. Results and discussion

2.1. Chemistry

Starting from 2-amino-4,6-dichloro-2-methylpyrimidine (**4**) a first series of 6-phenylaminopurines were obtained in just 3 steps (Scheme 1). Thus, reaction of **4** with aqueous methylamine in 1,4-dioxane at 80 °C overnight afforded the pyrimidine **5** in an excellent yield, considerably better than previously described [26]. Then, reaction of **5** with triethyl orthoacetate in the presence of ethanesulphonic acid and acetic anhydride under MW irradiation for 15 min [27–29] afforded the 8-methylpurine **6**. Finally, reaction of **6** with substituted anilines in isopropanol in the presence of HCl at 80 °C afforded the 6-phenylamino derivatives **7a**–**e**, in moderate to good yields (65–90%). Under the same conditions, reaction of the 6-chloropurine **8** [30] with 4-methoxy-*N*-methylaniline afforded compound **7f**. Similarly, when compound **5** (Scheme 2) reacted with trimethyl orthoformate, the purine **9** was obtained, that was further treated with 4-methoxy-*N*-methylaniline to yield the 8-unsubstituted compound **10**. In addition, the unsubstituted compound at *N*-9 was obtained as depicted in Scheme 3. Reaction of the 4,6-dichloropyrimidine **4** with *p*-methoxybenzylamine afforded the benzyl derivative **11** in 64% yield. Then, reaction of **11** with triethyl orthoacetate led to the 8-methyl purine **12** in modest yield (31%). Finally, reaction with 4-methoxy-*N*-methylaniline, followed by catalytic hydrogenation to remove the *p*-methoxybenzyl group, afforded the purine **14** in 64% yield.

According to some recent papers on pyrazolopyrimidines related to compounds **2** and **3** (Fig. 2), the introduction of an ethyl group at the NH at position 6 of the purine ring, or even the replacement of the *N*-methylaniline by a tetrahydroquinoline, might lead to an improvement in the antiproliferative activity [31]. Therefore reaction of **7b** with ethyl iodide in the presence of $CsCO_3$ [32] afforded the *N*-ethyl derivative **15** (Scheme 4). On the other



7a R_1= R_2=CH_3; R_3=OCH_3; R_4=H
7b R_1=CH_3;R_2=H; R_3=OCH_3; R_4=H
7c R_1= R_2=CH_3; R_3= H; R_4=OCH_3
7d R_1= R_2=R_3= CH_3; R_4=H
7e R_1= R_2=CH_3; R_3= OCF_3; R_4=H
7f R_1= H; R_2=CH_3; R_3= OCH_3; R_4=H

Scheme 1. *Reagents and conditions*: (a) CH_3NH_2, 1,4-dioxane, 80 °C, overnight; (b) $CH_3C(OEt)_3$, $EtSO_3H$, Ac_2O, MW, 120 °C, 15 min; (c) the corresponding aniline, HCl, ^{i}PrOH, MW, 80 °C, 10 min.