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Original article

Purine-benzimidazole hybrids: Synthesis, single crystal determination and *in vitro* evaluation of antitumor activities

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ABSTRACT

In an effort to identify novel compounds for the treatment of cancer, a diverse array of potential bioactive hybrid, purine-benzimidazole was synthesized in good yields through nucleophilic substitution at C6 position of purine ring with versatile cyclic amines at C2 position. The structures of newly prepared compounds were confirmed by IR, ^{1}H, ^{13}C NMR, mass spectroscopy and, in case of **19**, by single crystal X-ray diffraction analysis. The newly synthesized compounds were evaluated against 60 human tumour cell lines at one dose concentration level. Compound **6** exhibited significant growth inhibition and was evaluated as 60 cell panel at five dose concentration levels. Compound **6** proved to be 1.25 fold more active than the positive control 5-FU, with GI_{50} value of 18.12 μM (MG-MID). Interaction of the compounds with Aurora-A enzyme involved in the process of propagation of cancer, has also been investigated. Compound **6** showed selectivity towards Aurora-A kinase inhibition with IC_{50} value of 0.0l μM. Molecular docking studies in the active binding site provided theoretical support for the experimental biological data acquired.

1. Introduction

Cancer is becoming a major health problem in developing and undeveloped countries [1,2]. Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the laborious task of discovering new anticancer agents remains critically important. In the course of identifying various chemical substances which may serve as leads for designing novel antitumor agents, we are particularly interested in the present work of molecular hybridizations with purine and benzimidazole derivatives which have been identified as a new class of cancer chemotherapeutic agents with significant therapeutic efficacy against solid tumors [3]. It is well known that purine derivatives are potent inhibitors of Aurora kinase [4], cyclin dependent kinase (CDK) [5], epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) [6]. Consequently, various approaches have been adopted to enhance the potency and selectivity of these inhibitors. These efforts led to discovery of many drugs such as Olomoucine, Roscovitine, R-CR8 and Dinaciclib (Fig. 1) that have also maintained selectivity towards various kinases. Olomoucine and Roscovitine are first discovered CDK inhibitors while the optically pure R-enantiomer of Roscovitine (Seliciclib) is currently being evaluated as an oncology drug candidate in patient diagnosed with non small cell lung cancer and nasopharyngeal cancer [7] or other malignancies [8]. The anti-HIV/HBV drugs *abacavir* and *penciclovir* are some of the purine drugs, also available presently in the market [9]. Similarly, benzimidazole and its derivatives are categorized as the important pharmacophores and privileged sub-structures in medicinal chemistry owing to their involvement as a key component for various biological activities [10]. Benzimidazoles are among the important heterocyclic compounds that found in natural and non-natural products such as vitamin B12, marine alkaloid kealiiquinone [11] etc. Some of their derivatives are marketed as anti-fungal agent such as Carbendazim [12], anti-helmintic agents such as Mebendazole and Thiabendazole [13] and anti-psychotic drug such as Pimozide [14].

In view of the previous rationale and in continuation of an ongoing program aiming at finding new structure leads with potential chemotherapeutic activities using molecular hybridization [15], new series of molecular hybrids using purine and benzimidazole have been synthesized and screened for *in vitro* antitumor activity as well as Aurora A kinase inhibitors. These series comprises the derived 2,6-disubstituted purine pharmacophore that are structurally related to Olomoucine and Roscovitine. In the

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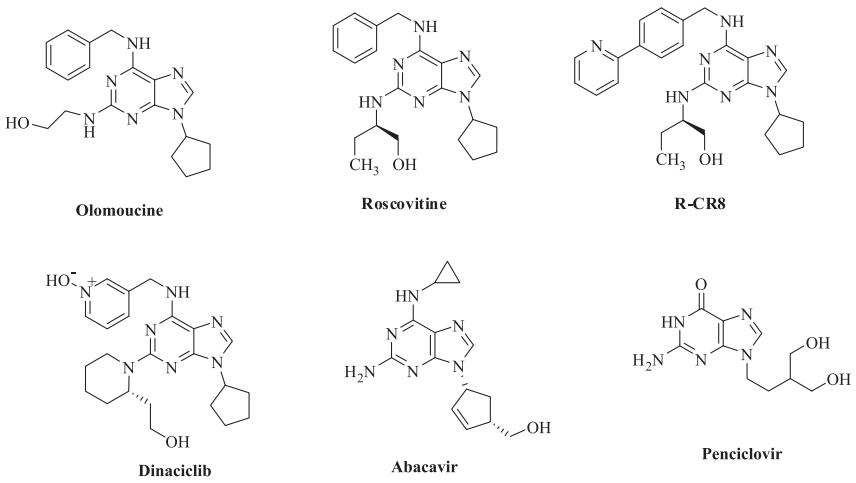


Fig. 1. Purine related drugs.

present study, the substitution pattern at the 2,6-disubstituted purine and benzimidazole pharmacophore were selected so as to confer different electronic environment that would affect the activity of target molecules. SAR, QSAR and molecular modelling studies were used to identify the structural features required for the antitumor properties of these new hybrid series.

2. Results and discussion

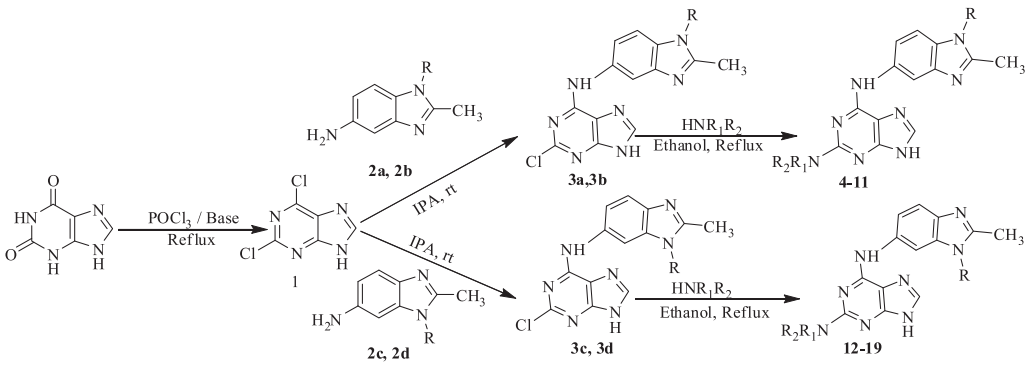
2.1. Chemistry

The synthetic strategy to prepare the purine-benzimidazole hybrids (**3a**–**d** and **4**–**19**) has been depicted in Scheme 1. The target compounds were achieved in three steps using 3,4-dihydro-1*H*-purin-2,6-(5*H*,9*H*)-dione as starting material. Refluxing of 3,4-dihydro-1*H*-purin-2,6-(5*H*,9*H*)-dione with phosphorus oxychloride in the presence of triethylamine for 5 h afforded 2,6-dichloropurine (**1**). Treatment of compound **1** with **2a** [15] in the presence of isopropyl alcohol (IPA) at room temperature for 24 h gave **3a** in 79% yield. Similarly, compound **1** was also treated with **2b**–**d** under the same reaction conditions to give compounds **3b**–**d** with 47–94% yields. Refluxing of compounds **3a**–**d** with different secondary amines in ethanol for 48 h and after purification with column chromatography gave pure compounds **4**–**19** with moderate to excellent yields (Table 1). All the synthesized compounds were well characterized by IR, ^{1}H, ^{13}C NMR, mass spectroscopy (Supporting information) and in case of compound **19**, by single crystal X-ray diffraction analysis (Fig. 2).

2.2. In vitro anticancer screening

All the synthesized compounds were submitted to National Cancer Institute (NCI) disease-oriented human cell lines for *in-vitro* evaluation as antitumor activities (Table S1). Fourteen compounds (**3a**–**d**, **4**, **6**–**11**, **16**, **18**–**19**) were evaluated against 60 cell lines at a single dose of 10 μM concentration [16–19] and their outputs were reported as a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI %). Compound **6** exhibited significant growth inhibition and was evaluated for further 60 cell panel at five dose concentration levels.

Preliminary *in vitro* antitumor screening was revealed that only compounds belonging to the series **4**–**11** showed significant inhibition for most of cancer cell lines. The percentage of inhibition for cancer cells were more than 50% in a number of the tested derivatives. On the contrary, compounds **18** and **19** showed weak activities compared with other compounds and percentage of inhibition did not reach 30% except one or two cell lines. These variations could be correlated to the difference in positions of allyl or butyl group on the benzimidazole moiety in which the distance between the core purine moiety and alkyl chains of benzimidazole



Scheme 1. Synthetic route for the preparation of target compounds **4**–**19**.