European Journal of Medicinal Chemistry 89 (2015) 701–720

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of novel substituted purine derivatives and identification of the cell death mechanism

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ARTICLE INFO

Article history:
Received 24 November 2013
Received in revised form
19 September 2014
Accepted 29 October 2014
Available online 30 October 2014

Keywords:
Adenine
Purine derivatives
Cytotoxic activity
Senescence
Hepatocellular carcinoma

ABSTRACT

Novel 9-(substituted amino/piperazinoethyl)adenines (**4**–**12**), 6-(substituted piperazino/amino)purines (**15**–**27**), 9-(*p*-toluenesulfonyl/cyclopentyl/ethoxycarbonylmethyl)-6-(substituted amino/piperazino)purines (**28**–**34**, **36, 37**, **38**–**41**) were synthesized and evaluated initially for their cytotoxic activities on liver Huh7, breast T47D and colon HCT116 carcinoma cells. N^6-(4-Trifluoromethylphenyl)piperazine derivative (**17**) and its 9-(p-toluene-sulfonyl)/9-cyclopentyl analogues (**28**, **36**) had promising cytotoxic activities. Compounds **17**, **28** and **36** were further analysed for their cytotoxicity in a panel of a liver cancer cell lines. The compound **36** had better cytotoxic activities ($IC_{50} \leq 1$ μM) than the nucleobase 5-FU and nucleosides fludarabine, cladribine, and pentostatine on Huh7 cells. Cytotoxicity induced by **36** was later identified as senescence associated cell death by SA-β-Gal assay.

1. Introduction

Nucleobase analogues, which are structurally, metabolically and pharmacodynamically similar, are known to have different biological activities [1]. These diverse effects have been reported to be associated with anti-cancer, anti-viral, anti-fungal and anti-bacterial activities due inhibition of the enzymes involved in cell proliferation [2–24]. The nucleobase analogues induce apoptosis during growth and division, which is a common inhibitory mechanism observed in the presence of these molecules [25]. A well-known pioneer fluorinated nucleobase analogue, 5-fluorouracil, is highly preferred in clinics for the treatment of various cancers [26]. Later, other pyrimidine analogues such as arabinofuranosyl cytidine (Ara-C) and gemcitabine have been identified as antimetabolite chemotherapeutic agents in cancer [1]. Purine derivatives, 6-mercaptopurine and 6-thioguanine have been used as an inhibitor of nucleic acid metabolism in paediatric acute lymphoblastic leukaemia [27]. Furthermore purine nucleoside analogues such as fludarabine, cladribine, and pentostatine, emerged as a group of antimetabolites against haematological malignancies in clinics [28].

Nucleoside analogues interfere with the integrity of DNA by impairing dNTP pools and ultimately DNA synthesis through ribonucleotide reductase (RR) inhibition [29]. Due to the altered DNA integrity, which is detected as damaged by cellular machinery, the treatment with nucleoside analogues induces apoptosis [1]. There are also nucleoside analogues such as toyocamycin and decitabine, which have been reported to induce senescence, associated cell death [30,31]. Recently senescence-associated cell death, which is a cellular event in tumour development and progression as well as treatment, was reported as premature senescence in cancer cells [32]. Therefore, senescence induced cell death through pro-senescence therapy is currently the target of small molecule inhibitors [33,34].

Primary liver cancer, hepatocellular carcinoma (HCC), is the sixth most common and the third lethal cancer [35]. Sorafenib, a kinase inhibitor, is the only FDA approved drug for HCC treatment and extends the mean survival of the patients only for 3 months [36]. Thus, it is essential to discover new chemotherapeutic agents for the treatment of this cancer. Here, we synthesized a series of 9-substituted adenines (**4**–**12**), 6-substituted purines (**15**–**27**) and

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http://dx.doi.org/10.1016/j.ejmech.2014.10.080
0223-5234/

6,9-disubstituted purine analogues (**28**–**34**, **36**–**41**) and evaluated their cytotoxic activities against liver (Huh7), colon (HCT116), and breast (T47D) carcinoma cell lines; and the most active purine analogues (**17**, **28**, and **36**) were further tested on a panel of liver cancer cells. Moreover, we further characterized the most bioactive compound **36** an agent inducing senescence associated cell death with a remarkable cytotoxicity ($IC_{50} \leq 1$ μM).

2. Result and discussion

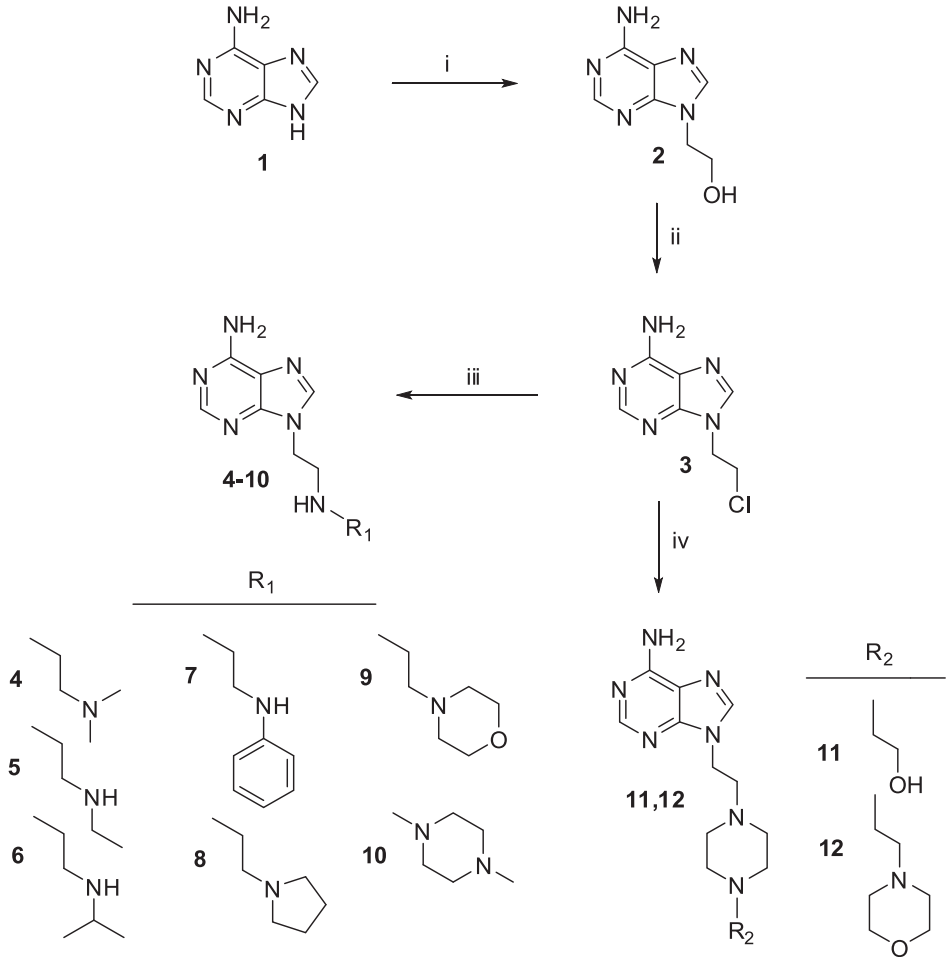
2.1. Chemistry

The synthesis of the 9-(substituted amino/piperazinoethyl) adenine derivatives **4**–**12** was carried out starting from commercially available adenine (**1**) (Scheme 1). The base catalysed nucleophilic addition of **1** to ethylene carbonate afforded 9-(2-hydroxyethyl)-9*H*-adenine (**2**) [37]. The nucleophilic addition reaction occurred only at the N-9 atom. The hydroxyethyl compound (**2**) was chlorinated with $SOCl_2$ to give intermediate 9-(2-chloroethyl)-9*H*-adenine (**3**) [37]. Compounds **4**–**12** were synthesized by nucleophilic substitution of chlorine of (**3**) with the appropriate amine and piperazines.

6-Chloro-9-*p*-toluensulfonyl-9*H*-purine (**14**) was prepared from 6-chloropurine and *p*-toluensulfonyl chloride under Schotten-Baumann conditions [38]. The amination of **14** with 1-(2-hydroxyethyl)piperazine did not afford the desired product and compound **15** was formed (Scheme 2). This reaction sequence was not applicable for the synthesis of 6,9-disubstituted purine derivatives **28**–**34.** Thus, 9-(*p*-toluene-sulfonyl)-6-substituted amino/piperazinopurines (**28**–**34**) were first synthesized as shown in Scheme 3. Purines substituted at C-6 (**15**–**27**) were synthesized by nucleophilic substitution of the chlorine of 6-chloropurine (**13**) with the appropriate amine and piperazines in the presence of base. Compounds **15**–**27** were N-sulfonylated with complete regioselectivity applying the same set of reaction conditions as reported for the sulfonylation of adenine [39]. The sulfonylation reaction occurred only at the N-9 atom, without the concurrent N-7 sulfonylation, as proved by the X-ray crystallographic analysis of the structure of compound **28** (Figs. 1 and 2).

9-Cyclopentyl-substituted purines **36**, **37** [24] were synthesized via N-9 alkylation of **13** with cyclopentyl bromide, and by amination of 6-chloro-9-cyclopentylpurine **35** with 4-(4-trifluoromethylphenyl)piperazine or 4-methylpiperidine (Scheme 4). The alkylation reaction occurred only at the N-9 atom. X-ray analysis [24] also confirmed the structure of compound **35**.

Compounds **15**, **17**, **22**, **24** could be alkylated with ethyl chloroacetate in DMF by first generating the anion with NaH (Scheme 5). This procedure yielded only one isolable compound which was identified as the expected 9-acetat substituted purines (**38**–**41**). ^{1}H NMR Nuclear Overhauser Effect Spectroscopy (NOESY) also supported the structure of N-9 regioisomer **41**. The NOE interaction showed coupling between purine N–CH_2 and H-8 protons, but no such interactions between any of the piperazine and purine N–CH_2 protons eliminate N-7 acetylation. On the other hand, the



Scheme 1. Reagents: i) Ethylene carbonate, NaOH, DMF; ii) $SOCl_2$; iii) the appropriate amine, EtOH; iv) the appropriate piperazine, EtOH.