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Benzopyrone represents a privilege scaffold to identify novel adenosine A_1/A_{2A} receptor antagonists

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ABSTRACT

Adenosine receptor antagonists are under investigation as potential drug candidates for the treatment of certain cancers, neurological disorders, depression and potentially improve tumour immunotherapy. The benzo-γ-pyrone scaffold is well-known in medicinal chemistry with diverse pharmacological activities attributed to them, however, their therapeutic potential as adenosine receptor antagonists have not been investigated in detail. To expand on the structure–activity relationships, the present study explored the adenosine A_1 and A_{2A} receptor binding affinities of a selected series of benzo-γ-pyrone analogues. *In vitro* evaluation led to the identification of 5-hydroxy-2-(3-hydroxyphenyl)-4H-1-benzopyran-4-one with the best adenosine A_{2A} receptor affinity among the test compounds and was found to be non-selective (A_1K_i = 0.956 μM; $A_{2A}K_i$ = 1.44 μM). Hydroxy substitution on ring A and/or B play a key role in modulating the binding affinity at adenosine A_1 and A_{2A} receptors. Adenosine A_1 receptor affinity was increased to the nanomolar range with hydroxy substitution on C6 (ring A), while *meta*-hydroxy substitution on ring B governed adenosine A_{2A} receptor affinity. The double bond between C2 and C3 of ring C as well as C2 phenyl substitution was shown to be imperative for both adenosine A_1 and A_{2A} receptor affinity. Selected benzo-γ-pyrone derivatives behaved as adenosine A_1 receptor antagonists in the performed GTP shift assays. It may be concluded that benzo-γ-pyrone based derivatives are suitable leads for designing and identifying adenosine receptor antagonists as treatment of various disorders.

1. Introduction

There's a world-wide interest in identifying potential adenosine receptor (AR) drug candidates, since a variety of pharmacological applications are attributed to them. Currently four AR subtypes are known, namely A_1, A_{2A}, A_{2B} and A_3 [1]. The A_1 and/or A_{2A} AR subtypes are potential drug targets for Parkinson's disease (A_1 and A_{2A} AR) [2], Alzheimer's disease (A_1 AR) [3], depression (A_{2A} AR) [4], certain cancer therapies (A_1 and A_{2A} AR) [5] and improving tumour immunotherapy (A_{2A} AR) [5].

Initially, the A_1 and A_{2A} AR subtypes have been recognized as promising drug targets for the treatment of neurodegenerative disorders [6,7]. It is estimated that between 8.7 and 9.3 million individuals will be diagnosed with Parkinson's disease by 2030, making this disease the 2nd most common neurological condition world-wide [8]. Selective A_1 AR antagonists have shown potential to treat Parkinson's disease associated cognitive impairment [9]. In turn, selective A_{2A} AR antagonists may improve motor dysfunction of Parkinson's disease [10], exhibit neuroprotective properties [11] and lower the risk of developing dyskinesia [12]. Thus dual antagonism of the A_1 and A_{2A} ARs may find therapeutic value as Parkinson's disease therapy. The safety profile of AR antagonists has been documented in clinical trials intended to explore the validity of these drugs as Parkinson's disease treatment. For example, istradefylline (a selective A_{2A} AR antagonist) was studied in phase III clinical trials and is currently approved as Parkinson's disease therapy in Japan [13].

More recently, ARs—especially the A_{2A} AR subtype—have been implicated as a potential drug target for the development of anticancer treatment and improving tumour immunotherapy [5]. Hepatocellular carcinoma (HCC) is a common malignancy in

Abbreviations: AR, adenosine receptor; GTP, guanosine triphosphate; [^{3}H]DPCPX, [^{3}H]-dipropyl-8-cyclopentylxanthine; [^{3}H]NECA, N-[^{3}H]-ethyladenosin-5′-uronamide; CPA, N^6-cyclopentyladenosine.

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developing countries with hepatitis C and B as risk factors [14]. It's the 5th most common cancer world-wide [15] with the majority of cases occurring in Asia and Africa [14]. Approximately 600,000 deaths are attributed to HCC per year [14]. *In vivo* studies with rodents under normoxic and hypoxic conditions together with HCC (Hep3B) cells in culture have shown that adenosine via A_{2A} ARs enhance production of erythropoietin (EPO) [16,17]. This suggests that A_{2A} AR antagonists are a promising novel therapy to inhibit increased levels of EPO. It is therefore plausible that A_{2A} AR antagonists may find therapeutic relevance in the treatment of HCC and other EPO-secreting tumours (e.g. renal cell carcinoma and cerebellar hemangioblastoma) [5]. Activation of the ARs by specific ligands, agonists or antagonists, modulates tumour growth via various signalling pathways. The A_1 AR play a role in preventing the development of glioblastomas, where this antitumor effect is mediated via tumour-associated microglial cells. Activation of the A_{2A} AR results in inhibition of the immune response to tumours via suppression of T regulatory cells and by blocking A_{2A} AR, anti-tumour immunity is enhanced [18].

Benzopyrones are a class of compounds with significantly diverse biological activities and for this reason is considered a privileged scaffold in medicinal chemistry [19]. Chemically, the benzopyrones represent a class of ketone containing benzopyrone derivatives which constitute the basic framework of various natural occurring and/or synthetic compounds such as flavonoids (benzo-γ-pyrones) [20,21] and the structurally related coumarins [19] and isocoumarines (benzo-α-pyrones) [22] (Fig. 1).

Based on their chemical structure, flavonoids are primarily divided into six subgroups namely flavones, isoflavones, flavanones, flavanols and anthocyanidins [23]. Flavones and isoflavones possess a basic benzo-γ-pyrone skeleton (ring A and C is fused), with the substitution position of the phenyl side chain (ring B), at the pyrone core (ring C), dividing these flavonoids into flavones (C2 position) and isoflavones (C3 position) (Fig. 1; Table 1). In turn, flavanones are structurally related to flavones, however, they lack the double bond between the C2 and C3 position of the pyrone core (ring C) (Fig. 1; Table 1).

Flavonoids are found naturally in plants (including fruits and vegetables) and food prepared from flavonoid containing plants [21]. Consumption of flavonoids through the diet is essential, since animals and humans cannot synthesize flavonoids [24]. The beneficial effects of flavonoids, however, extend beyond dietary necessity. Various pharmacological activities have been attributed to flavonoids in the past, for instance antibacterial activity [25], anti-fungal properties [25,26], anti-inflammatory effects [26,27], antioxidant activity [20,26,28], antiviral activity [25,29], cancer chemopreventative agents [30] and hepatoprotective activity [26]. To further emphasize the therapeutic potential of flavonoids, these compounds have been under investigation for the treatment of Parkinson's disease as AR antagonists [31,32,33,34,35] and for their ability to exhibit neuroprotective properties [23]. The neuroprotective mechanism of flavonoids has been reviewed previously and may be ascribed to their ability to suppress lipid peroxidation, to inhibit inflammatory mediators, to activate endogenous antioxidant enzymes, to modulate gene expression in neuronal cells and as a mitochondrial target therapy [23]. The AR antagonistic properties of selected compounds bearing a benzo-γ-pyrone backbone were demonstrated by Ji and colleagues [35], where the flavone derivative 2-phenyl-4*H*-benzopyran-4-one (**1a**) was reported as a non-selective AR antagonist exhibiting dissociation constant (K_i) values of 3.28 μM and 3.45 μM for the A_1 and A_{2A} ARs, respectively [35]. In another study by Jacobson and co-workers [34], only the A_1 AR affinity of selected isoflavone derivatives were explored and it was found that 7-hydroxy-3-(4-hydroxyphenyl)-4*H*-benzopyran-4-one (**2c** daidzein) possess moderate A_1 AR binding [34].

Based on the above, the benzo-γ-pyrone backbone is considered a promising lead scaffold for identifying novel A_1 and/or A_{2A} AR antagonists, however, their therapeutic potential as AR antagonists have not been investigated in detail. Since, effective antagonism of the A_1 and/or A_{2A} ARs are deemed beneficial for a variety of disorders, including Parkinson's disease and anti-cancer therapy, the current study aim to further explore the structure-activity relationships for benzopyrone-based compounds and to discover high affinity AR antagonists. The present study investigated the A_1 and A_{2A} AR binding affinities of a selected series of known benzo-γ-pyrone analogues (flavone, flavanone, isoflavone and thioflavanone). In addition, the necessity of a benzo-γ-pyrone scaffold to govern AR affinity was further explored by comparing the K_i values of structurally related benzo-γ-pyrones to selected benzo-α-pyrone moieties. Table 1 summarize the experimentally determined A_1 and A_{2A} AR binding affinities (via radioligand binding assays) of the selected benzo-γ-pyrone (**1a–g**; **2a–c** and **3a–c**) and benzo-α-pyrone (**4a–c**) derivatives, respectively.

2. Results and discussion

2.1. Chemistry

All the test and reference compounds were obtained from standard commercial sources (Sigma Aldrich). These compounds were of analytical grade and used without further purification.

2.2. Radioligand binding assays

The affinities of the selected benzo-γ-pyrone (**1a–g**; **2a–c** and **3a–c**) and benzo-α-pyrone (**4a–c**) derivatives at rat A_1 and A_{2A} AR subtypes were determined with radioligand competition experiments as described previously [36,37].

The test compounds (**1a–g**, **2a–c**, **3a–c** and **4a–c**) displayed varying degrees of affinity and selectivity towards the A_1 and A_{2A} AR subtypes (Table 1). The flavone derivatives (**1a–g**) were identified as the most promising benzo-γ-pyrone based compounds, since this class exhibited the highest A_1 and A_{2A} AR affinities among the test compounds. The K_i values for the A_1 AR activity of the flavones (**1a–g**) ranged between 0.59 and 2.25 μM. Furthermore, five of the seven flavones (**1a**, **1c–f**) displayed A_{2A} AR affinity, with K_i values ranging between 1.44 and 5.14 μM. On the other hand, the benzo-α-pyrone derivatives (**4a–c**) presented one isochromenone, compound

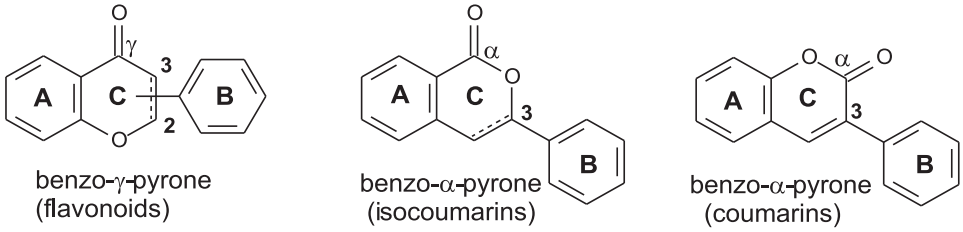

Fig. 1. The general chemical structures of benzo-γ-pyrone (flavonoids) and benzo-α-pyrone (isocoumarins and coumarins) derivatives.