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Synthesis and biological evaluation of novel pyridine derivatives as potential anticancer agents and phosphodiesterase-3 inhibitors

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ABSTRACT

Phosphodiesterases (PDEs) have been studied in a variety of tumours; data have suggested that the levels of PDE activities are elevated and, therefore, the ratios of cGMP to cAMP are affected. In addition, PDE inhibitors are potential targets for tumour cell growth inhibition and induction of apoptosis. Nonselective PDE inhibitors, such as theophylline or aminophylline, are known regulators of growth in a variety of carcinoma cell lines, suggesting a potential role for PDE inhibitors as anticancer drugs.

In the current study, we reported the synthesis of novel derivatives of 6-aryl-4-imidazolyl-2-imino-1,2-dihydropyridine-3-carbonitriles (Ia,b,c) and their 2-oxo isosteres (IIa,b,c,d). All the compounds were evaluated for their PDE3A inhibitory effects, as well as their cytotoxic effects on MCF-7 and HeLa cell lines. Moreover, structure–activity relationships were studied.

4-(1-benzyl-2-ethylthio-5-imidazolyl)-6-(4-bromophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (Ib) exhibited the strongest PDE3A inhibitory effects with an IC_{50} of 3.76 ± 1.03 nM. Compound Ib also showed the strongest cytotoxic effects on both the HeLa and MCF-7 cells with an IC_{50} of 34.3 ± 2.6 μM and 50.18 ± 1.11 μM, respectively. There was a direct correlation between PDE3 inhibition and anticancer activity for the synthesised compounds.

The data reported here support our view that PDEs represent promising cellular targets for antitumor treatment.

1. Introduction

Eleven cyclic nucleotide phosphodiesterase gene families (PDE1-11) have been identified in various tissues or cells. PDEs are characterised based on their primary amino acid sequences, their affinities for cAMP and cGMP, their sensitivities to specific inhibitors, their biochemical and physical properties and their biological regulatory pathways [1–11]. PDE isoforms can influence disease pathogenesis and be novel therapeutic targets [12]. Impaired cAMP and/or cGMP generation upon overexpression of PDE isoforms have been described in various cancer pathologies [13].

Selective inhibition of PDE isoforms, which raises the levels of intracellular cAMP and/or cGMP, may regulate the tumour microenvironment and induce apoptosis and cell cycle arrest in a broad spectrum of tumour cells. Therefore, development and clinical application of inhibitors specific for individual PDE isoenzymes may selectively restore normal intracellular signalling and provide antitumour therapy with reduced adverse effects [14,15]. PDE3 isoforms are found in a variety of tissues, including myocardium, platelets and adipose tissue [1–3]. Two PDE3 genes, PDE3A and PDE3B have been discovered in humans and these genes are located on human chromosomes 11 and 12, respectively [16]. Studies on specific PDE3 inhibitors suggest that PDE3s are important in the regulation of cAMP-modulated processes, including myocardial contractility, platelet aggregation and antilipolytic action [1–3].

Recent studies have shown that PDE3, PDE4 and PDE5 are overexpressed in cancer cells. In addition, inhibition of PDE3 along with other PDEs may lead to inhibition of tumour cell growth and angiogenesis [17–21].

In this study, we reported the synthesis of novel derivatives of 6-aryl-4-imidazolyl-2-imino-1,2-dihydropyridine-3-carbonitriles (I) and their 2-oxo isosteres (II). The PDE3A inhibitory effects, as well as the cytotoxic effects, of synthesised compounds on

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MCF-7 and HeLa cell lines were evaluated. Moreover, structure–activity relationships were studied.

2. Results and discussion

For many cancers, there has been a shift from management with traditional, nonspecific cytotoxic chemotherapies to treatment with molecule-specific targeted therapies that are used either alone or in combination with traditional chemotherapies [22,23] here, we designed and synthesised some new PDE3A inhibitors. The cytotoxicity and PDE3A inhibitory effect of these compounds were evaluated both in the lab and in silico.

2.1. Chemistry

The general synthesis of 6-aryl-4-imidazolyl-2-imino-1,2-dihydropyridine-3-carbonitriles (Ia–c) and 6-aryl-4-imidazolyl-2-oxo-1,2-dihydropyridine-3-carbonitriles (IIa–d) is illustrated in Fig. 1. Briefly, 4-bromoacetophenone or 1-(1,3-benzodioxol-5-yl) ethanone were reacted with the appropriate aldehyde, namely 2-akyl thio-1-benzyl-5-formyl imidazolyl, in the presence of malononitrile or ethyl cyanoacetate and ammonium acetate. The infrared (IR) spectra of all derivatives showed bands at a stretching frequency (m) around 3400 cm^{-1} corresponding to the NH, which showed relatively lower values of the carbonyl with a stretching frequency (m) around 1632 cm^{-1}.

2.2. Biology

The cytotoxic effects of synthesised compounds on HeLa and MCF-7 cell lines were determined using MTT assay. PDE3A activity was analysed using an IMAP TR-FRET phosphodiesterase assay kit and cAMP as the substrate. As shown in Fig. 2, there was a direct correlation between PDE3 inhibition and cytotoxic activities of the synthesised compounds ($r^2 = 0.89$). 4-(1-benzyl-ethylthio-5-imidazolyl)-6-(4-bromophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (Ib) exhibited the strongest PDE3A inhibition ($IC_{50} = 3.76 \pm 1.03$ nM). Compound Ib was also the most cytotoxic compound against both the HeLa and MCF-7 tumour cells, as the IC_{50} values were measured to be 34.3 ± 2.6 μM and 50.18 ± 1.11 μM, respectively. The electronic and steric effects, as well as the H-bond capabilities of the synthesised compounds, were all crucial in PDE3 inhibition and cytotoxicity. Our data are summarised in Table 1.

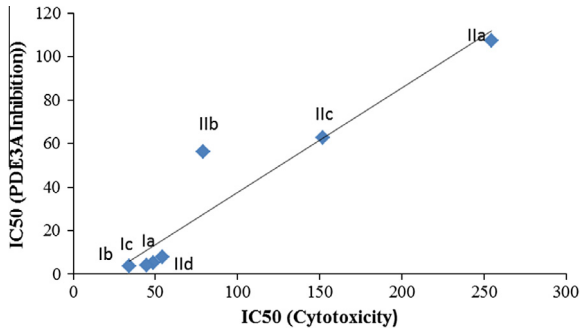

Fig. 2. Correlation between PDE3 inhibition and cytotoxic activities of synthesized compounds on Hela cell line.

The data summarised here may support our view that PDEs represent promising cellular targets for antitumour treatment (Table 1).

For investigating the structure–activity relationship, different substitutes are compared according to the potency of the compounds. As demonstrated, all the compounds in the imino group have shown higher IC_{50}s than the chemicals with the oxo substitute. In addition, a comparison of IIc with IIa and IIb with IId in the oxo group and Ic with Ia in imino group demonstrated that a benzodioxol substitute can make chemicals more potent than a bromobenzene group. On the other hand, compound Ib is more potent than compound Ia, and compounds IIb and IId are more potent than compounds IIa and IIc, respectively. This proves that the SEt group is better than the SMe group in PDEs inhibition. In conclusion, chemicals with imino, benzodioxol and SEt groups are more potent than compounds with oxo, bromobenzene and SMe groups.

2.3. Docking

The accuracy of the docking procedure was examined by calculating the correlation between theoretical ki and the experimental IC_{50} of the compounds. A high correlation between ki and IC_{50} values has been considered to be evidence of the validity of the docking procedure (Figs. 3–5). On the other hand, the IC_{50} values of compounds were in micromolar range whereas theoretical ki values were in nanomolar range. Really several approximations have been made in theoretical Ki calculation and in real in vitro experiment several factors like low cell permeability; removal by ABC exporters and metabolism by cells may be involved.

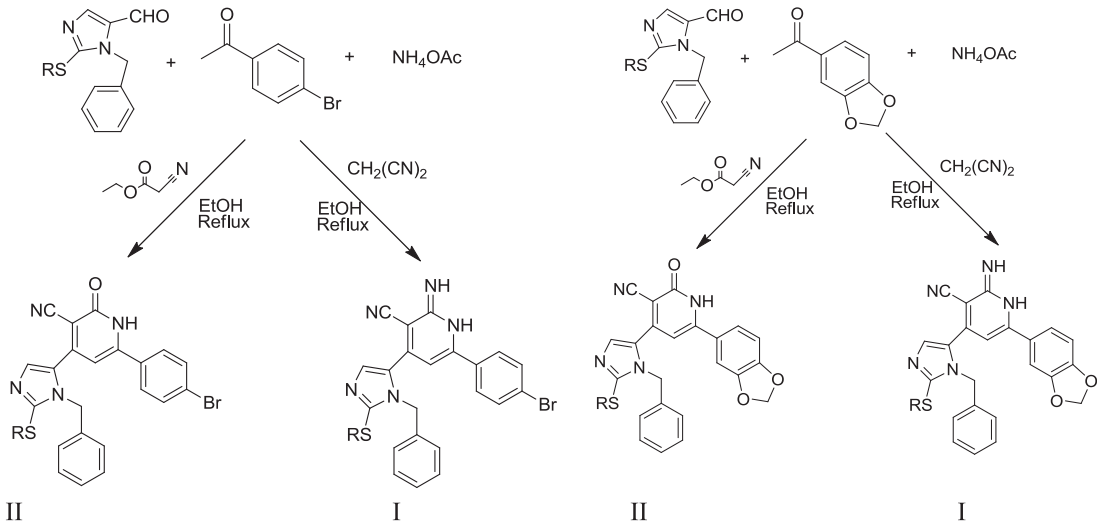

Fig. 1. Synthesis of 4-imidazolyl-6-aryl-2-imino-1,2-dihydropyridine-3-carbonitriles (Ia,b,c) and their 2-oxo isosteres (IIa,b,c,d).