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Original article

Antitumor studies – Part 2: Structure–activity relationship study for flavin analogs including investigations on their in vitro antitumor assay and docking simulation into protein tyrosine kinase

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Abstract

Various analogs of flavins, 5-deazaflavins, and flavin-5-oxides were docked into the binding site of protein tyrosine kinase $pp60^{c\text{-}src}$, and some of them were assayed for their potential antitumor and PKC (protein kinase C) inhibitory activities in vitro. The results considering SAR (structure–activity relationship) revealed that the higher binding affinities obtained include compounds with the structure modifications on the flavin or 5-deazaflavin skeleton, namely, NH_2 or Ph (phenyl-) group at the C-2 position and so on. Computationally designed compounds **4a**, **6a**, **b**, **7**, **11b**, **c**, **12**, **15**, and **22c** exhibited good docking results suggesting that they are potentially active antitumor agents. These compounds have 1–3 phenyl moieties, which are thought to be responsible for the planar aromatic fitting or electrostatic attraction onto the groove of the binding pocket.

Keywords: Protein tyrosine kinase; Flavin analog; AutoDock; SAR (structure–activity relationship)

1. Introduction

A series of flavin analogs have been previously developed in our group as potential antiproliferative agents in vitro [1–4], some of which (e.g. 2-deoxo-2-phenyl-5-deazaflavins and flavin-5-oxides) have exhibited antitumor activity against different tumor cell lines (NCI-H 460, HCT 116, A 431, CCRF-HSB-2, and KB) comparable or moderately superior to cisplatin. Because we are also interested in structure-based rational design of the above-mentioned flavin analogs, the elucidation of mechanism for their action is crucial for further structure improvements in order to develop more potent antitumor compounds. In the preliminary mechanistic study, we revealed that 5-deazaflavins and 2-deoxo-2-phenyl-5-deazaflavins act as selective inhibitor of protein kinase C (PKC) and exhibited effective growth inhibition against cancer cell lines such as A 431 and HT 1080 cells [1]. Protein tyrosine kinases (PTK) are attractive targets for the design of novel therapeutic agents not only against cancer but also against many other diseases [5]. Consequently, selective inhibitors of protein tyrosine kinases have attracted significant interest for the development of potential anti-neoplastic agents [6]. Computer docking technique plays an important role in the drug design as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule in a non-covalent

Abbreviations: PKC, protein kinase C; SAR, structure–activity relationship; Ph, phenyl-; PTK, protein tyrosine kinase; RMSD, root mean square deviation.

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fashion [7]. DOCK [8] and AutoDock [9] as flexible docking programs enable us to predict favorable protein–ligand complex structures with a reasonable accuracy and speed. These docking programs, when used prior to experimental screening, can be considered as powerful computational filters to reduce labor and cost needed for the development of potent medicinal compounds. AutoDock is said to offer a reasonable result in comparison with other popular docking programs [10]. The docking technique will undoubtedly continue to play an important role in drug discovery [11]. Several drugs that were designed by intensive use of computational methods are currently under investigation for clinical trials [12].

Actually, 5-deazaflavins have attracted great interest because 5-deazaflavins were the first compounds synthesized as flavin antagonists [13]. The 10-aryl-5-deazaflavin derivatives were preliminarily reported to act as inhibitors of E 3 of HMD 2 in tumors that retain wild-type P 53 [14]. Some 5-amino-5-deazaflavin derivatives also revealed potential activity towards L 1210 and KB tumor cells [15]. However, a challenging problem remains for the design of an appropriate in vivo model to evaluate the antitumor efficacy of many non-cytotoxic PTK inhibitors [16].

In this study, we verified the binding capabilities of these compounds to the PTK using a novel in silico approach. The objective of this study is to investigate structure–activity relationships (SARs) of different compounds including 5-deazaflavins [2,3,17–19], 5-deazaalloxazines [20–22], 10-alkylated-2-deoxo-5-deazaflavins [4,19,23,24], 5-amino-5-deaza flavin derivatives [15], 10-alkylated-2-deoxoflavin-5-oxides [4], 2-methyl, 2-methylthio, 2-piperidino, and 2-(*N*-hydroxymethyl-*N*-methyl) derivatives of 2-deoxo-5-deazaflavins [23], 2-deoxo-2-phenylalloxazines [25], vertical- and horizontal-type bispyridodipyrimidines [26,27], and computationally designed 2-deoxoflavins, pyridodipyrimidines, and pyrimidopteridines. In this study, the investigated compounds include 58 synthetically known compounds, of which 37 compounds [4,15,17,19,24] revealed potential antitumor and tyrosine kinase inhibitory activities. Herein, we report the PKC inhibitory activities and the antiproliferative potency of these molecules against KB tumor cells in addition to their docking mode into the binding site of PTK pp60$^{c\text{-}src}$ (pdb code: 1skj). Moreover, a series of computations were performed to predict their binding mode and their binding affinities, and for the investigation of their structural features, which revealed the highest fitting within PTK so as to help us design potent PTK inhibitors.

2. Results and discussion

2.1. In vitro PKC inhibitory activity and antiproliferative potency of flavin analog against KB tumor cells

The biological activities of the synthetically known compounds of various 5-deazaflavins and flavin-5-oxides (Scheme 1) have not yet been well determined. Therefore, we herein prepared those compounds to study their biological activities. Generally speaking, 5-deazaflavins {pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-diones} (**1**), [2,3,17–19] 10-substituted or unsubstituted 2-deoxo-5-deazaflavins (**2**),[4,19] and 5-deazaalloxazine {pyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*)-dione} (**3**) [20–22] were synthesized by condensation of *o*-halogenobenzaldehydes and 6-*N*-alkyl- or 6-*N*-aryl-aminouracil, or by Vilsmeier–Haack reaction of 6-anilinouracils or 6-anilinopyrimidin-4(1*H*)-ones. These compounds were identified with the authentic samples by their IR and ^{1}H NMR spectra. 5-Deazaflavin derivatives (**1a–l**) and 2-deoxo-5-deazaflavins (**2a–d**, **j**) were tested for their in vitro PKC inhibitory activities and antiproliferative effect against human oral epidermoid carcinoma cell line (KB) using H-7 [1-(5-isoquinolinylsulfonyl)-2-methylpiperazine] and Ara-C as positive controls, respectively. Compounds **1d**, **2a**, **b**, **d**, and **j** showed good PKC inhibition of 10.0, 3.54, 12.9, 4.55, and 14.8 μg/mL (IC_{50}), respectively. Other compounds **1a**, **e–g**, **i–k** exhibited reasonable PKC inhibition of *ca*. 24–90 μg/mL (IC_{50}). Regarding the activity against KB tumor cells, 5-deazaflavins (**1a–l**) are inactive, while 2-deoxo-2-phenyl-5-deazaflavins (**2a–d**, **j**) exhibited the IC_{50} of 0.67, 0.79, 2.53, 1.17 and 17.9 μg/mL, respectively, as shown in Table 1.

Automated docking studies were carried out using AutoDock version 3.05 [9]. The three different search algorithms offered by AutoDock 3.05, including Monte Carlo simulated annealing (SA), Lamarckian genetic algorithm (GA), and genetic algorithm with local search (GALS). The preliminary trial runs were carried out and we found that GALS was the most efficient, reliable, and successful search algorithm. Therefore, it was selected to model the interaction/binding between PTK pp60$^{c\text{-}src}$ and flavin analogs. It showed good correlation between the IC_{50} against PKC for the potentially active compounds, i.e. **1a**, **e–g**, **i–k** and **2a**, **b**, **d**, **j**, and we obtained their estimated AutoDock binding free energies (ΔG_b) with the correlation coefficient (R^2) value of 0.593 as shown in Fig. 1. This figure was plotted based on our intention that it should include all compounds investigated so as to avoid bias or unfair comparison. It is our understanding that the positive correlation coefficient (R^2) values, especially 0.5 or above, plotting biological activity of compounds and their docking binding free energies, are regarded as reasonable [26]. In this figure, compounds **1e**, **g**, and **2d** appear to be somewhat out of the model. This discrepancy is to show the limitation of comparison between the two systems due to the fact that there are lots of unsolved areas such as inclusion of the protein movement into the computational system, incorporation of such scoring functions as atomic elasticity of the compounds, atomic vibration and rotation of the compounds and so on.

The structure–activity relationship (SAR) was studied by alternation of different structural moieties of 5-deazaflavins (**1a–s**) [2,3,17–19], 2-deoxo-5-deazaflavins (**2a–v**) [4,19,23,24], 5-deazaalloxazine (**3a–c**) [20–22], 7,10-dimethyl-2-deoxo-2-phenylflavin-5-oxide (**6c**) [4], 2-deoxo-2-phenylalloxazines (**9a**) [25], 5-alkylamino-5-deazaflavins (**10c–l**) [15], vertical- and horizontal-type bispyridodipyrimidines (**16** and **17a**, **b**) [27,28], and the computationally designed 2-deoxo-5-deazaalloxazines (**4a–r**), 3-phenylalloxazines (**5a–k**), 2-deoxo-2-phenylflavin-5-oxide (**6a**, **b**), 3,10-diphenylflavin-5-oxide (**7**), 2-deoxo-7,