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Research paper

Novel vitexin-inspired scaffold against leukemia

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in children. Up to a quarter of ALL patients relapse and face poor prognosis. To identify new compound leads, we conducted a phenotypic screen using terrestrial natural product (NP) fractions against immortalized ALL cellular models.

We identified vitexin, a flavonoid, as a promising hit with biological activity ($EC_{50} = 30\ \mu M$) in pre-B cell ALL models with no toxicity against normal human tissue (BJ cells) at the tested concentrations. To develop more potent compounds against ALL and elucidate its potential mode of action, a vitexin-inspired compound library was synthesized. Thus, we developed an improved and scalable protocol for the direct synthesis of 4-quinolone core heterocycles containing an *N*-sulfonamide using a one-pot condensation reaction protocol. The newly generated compounds represent a novel molecular scaffold against ALL as exemplified by compounds **13** and **15**, which demonstrated EC_{50} values in the low micromolar range (0.3–10 μM) with little to no toxicity in normal cellular models. Computational studies support the hypothesis that these compounds are potential CDK inhibitors. The compounds induced apoptosis, caused cell arrest at G0/G1 and G2/M, and induced ROS in cancer cells.

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in children [1]. Up to a quarter of ALL patients relapse and face poor prognosis [2]. Relapsed ALL cases are associated with initial poor response and chemotherapy resistance [1–4]. New potent compounds are required to effectively treat relapsed ALL. Our drug discovery program objective is to identify new chemical scaffolds to advance the bench-to-bedside therapeutic agent development process in high-risk and drug-resistant leukemia.

Based on a phenotypic screen using stable B cellular ALL models (representatives of this high-risk patient cohort), we expected to identify specific molecular scaffolds to expand our knowledge on current treatment modalities against ALL. We identified the natural product vitexin (**1**, Fig. 1) as a hit compound. Vitexin has been isolated from several sources including *Acer palmatum* [5]. Vitexin was reported as a potent hypotensive inhibitor of ganglion activity, with anti-inflammatory, *anti*-histamic, anti-cancer and anti-bradykinin among a broad range of other biological activities including antioxidant properties [5–7]. Some glycosylated flavonoids have a direct bond between the sugar and the anomeric carbon (O-C bond), while others such as vitexin feature the sugar bond at C6 or C8 (C-C bond). Studies of natural products have led to the development of clinical candidates such as flavopiridol (*Alvocidib*, **4**, with FDA orphan drug designation to treat AML, Fig. 1), flavonoid-like and FDA approved ATP-mimetic compound **5** (Fig. 1) [8]. Mechanistic studies of flavonoid compounds suggest a two-prone mode of action, CDK inhibition (particularly CDK9) and various cellular effects from metabolic changes to antioxidant activity [8]. Recently, the treatment of chronic lymphocytic leukemia by flavopiridol in clinical trials was successfully reported [9]. Compounds **4** and **5** were used as control compounds in this study.

The antioxidant activity of flavonoids depends on the molecular structure, the degree of hydroxylation/glycosylation, and the positions of hydroxyl groups as they provide resonance effects on the aromatic ring for radical atom engagement. Some of the most widely studied isoflavones are genistein (**2**, Fig. 1) and baicalein (**3**, Fig. 1), which reacts with topoisomerase II via radical mechanism, disrupting the maintenance of DNA stability, and wogonin induces autophagy [10]. However, their biological effects are observed in

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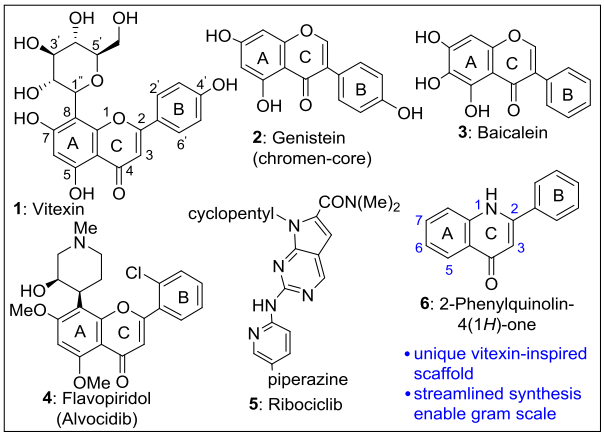

Fig. 1. Vitexin and related molecular scaffolds.

the high micromolar range as solubility/cellular permeability are some of the obstacles for the chromen core compounds [6]. While natural products (NPs) show potent bioactivities, their modest bioavailability properties can be affected by the presence and number of hydrophilic moieties (i.e. sugars, which can modulate water solubility and cell permeability). Thus, the introduction of heteroatoms and polar functional groups could lead to modified NPs with improved bioavailability properties.

The lack of scaffold diversity among standard care agents against leukemia and relapsed patients strongly supports the investigation of new chemical matter. We developed a vitexin-inspired library based on the hypothesis that a quinolone core (**6**, Fig. 1) instead of the chromen core (**2**, Fig. 1) would display improved biological activity against ALL since such compounds would a) decrease overall electronegativity due to the nitrogen, b) enable hydrogen bonding via donor rather than acceptor, and c) provide a handle to introduce functional groups to survey the chemical space. Thus, the resulting compounds would be more potent than vitexin, potentially enabling the development of a new class of chemical agents against high-risk and relapsed ALL.

Our studies describe an efficient synthesis, structure-activity relationship (SAR) studies of quinolin-4(1H)-one derivatives inspired by vitexin against ALL cellular models and the potential biological target(s).

2. Results and discussion

The study began by developing suitable synthetic conditions to generate the core synthons required for biological evaluation. On the basis of their chemical structures, the quinolone derivatives were divided into 3 group series (Scheme 1). Series I consisted of derivatives with 2-(4-methoxyphenyl) quinolone and 6, 8-dimethoxy on the A ring to explore the biological effect of the *N*-1 substituents and potentially improve biological potency. Series II derivatives focused on the role of C2 substituents via amide formation and evaluation of the electronic effects of these substituents. Series III included the evaluation of C6 to explore alternative functional groups to the methoxy groups and reduce potential metabolic liabilities, while maintaining biological activity. Thus, the combined group series would enable the generation of the corresponding *N*-sulfonamides, 2-amino-benzenethiazoles connected at C2 and urea moiety at C6 for a thorough biological evaluation.

Synthetic methodologies to access 4-quinolone derivatives are frequently based on intramolecular cyclization reactions mediated by acid or base (i.e. Conrad-Limpach or Gould-Jacobs reactions). Once we identified 3, 5-dimethoxy aniline and 4-nitroaniline as the synthetic building blocks for our 4-quinolone libraries, reaction conditions were evaluated to access the desired core compounds [11a]. Nucleophilic addition of 3, 5-dimethoxyaniline with aryl acid chlorides in the presence of triethyl amine provided intermediate A, which was treated with acyl chloride in the presence of tin chloride, followed by condensation reaction mediated by potassium *t*-butoxide under refluxing conditions (Scheme 1). The reaction sequence involves the amide bond formation via Schotten-Baumann reaction or aza-Michael addition followed by acylation/condensation reaction to provide the functionalized cores for series I-III in good yield. The intramolecular cyclization/condensation reaction of the aniline with an electrophile (intermediate B, Scheme 1), was mediated by modified thermal cyclization reaction conditions (diphenyl ether at 250 °C, in one-pot reaction sequence). Although, polyphosphoric acid can mediate such cyclization reactions, its corrosive nature and its high viscosity render work-up and the purification process challenging [11].

For the synthesis of the core quinolones for series II and III, substrate 3,5-dimethoxyaniline and 4-nitroaniline respectively were treated with dimethyl but-2-ynedioate in the presence of diphenyl ether to afford intermediate B, which was heated to form the desired product via intramolecular cyclization reaction in one-pot reaction sequence [11]. Consequently, the challenges posed by the purification of the products were circumvented by cooling the reaction vessel to RT, and rapidly adding hexane. The net effect of this binary solvent mixture enabled the precipitation of the desired product as a dark red powder, which was filtered and dried. Thus, our one-pot protocol afforded the quinolone product in nearly quantitative yield and avoided purification steps. To complete the synthesis of series III core, the corresponding nitro compound was reduced with Pd/C under a hydrogen atmosphere to yield the expected aniline product in excellent yield.

A preliminary survey of SAR was designed to assess potential liabilities related to the following concerns a) similar quinolone compounds display abroad range of biological properties, namely antimicrobial, antiviral, antimalarial activity, and mediated cyto- and geno-toxicity. Thus, our compounds were required to be evaluated in normal tissue (BJ cellular model) to avoid globally cytotoxicity, and b) comprehensive information regarding steric and electronic factors at the C2-C3 centers to provide guidance in generating a more appropriate quinolone system (Fig. 2). Compounds **7**–**16** were synthesized to evaluate the role of an aromatic versus the methyl ester at C-2, and the relevance of the introduction of the sulfonamide at *N*-1, followed by the evaluation of halogen groups at C3 center. Compounds **16**–**21** were synthesized to evaluate whether protecting groups influence biological activity.

To expand our evaluation of the molecular diversity that could be introduced at *N*-1, we proceeded to focus on sulfonamide synthesis (Fig. 3). Although numerous efforts have been made to modify quinolones [11], there are currently few reports concerning electrophilic substitutions at C-3 and direct *N*-modifications, which are governed by the C2-C3 substituents of the quinolone. Direct *N*-sulfonylation upon 2-aryl quinolone compound **7** was challenging using conventional conditions such as sulfonyl chloride in the presence of triethyl amine, as it provided *O*-sulfonylated compound **7b** as the sole product. After extensive experimentation, we were pleased to identify that copper mediated *N*-sulfonylation was highly efficient for this quinolone substrate **7** with great functional group tolerance, enabling the synthesis of quinolones with electron withdrawing or donating groups on the aryl sulfonamide moiety. Our findings extend the scope of utility for *N*-sulfonylation of quinolones without C-3 electron withdrawing directing groups, and also enable the direct *N*-sulfonylation on substituted anilines in