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Research paper

(*E*)-1,3-diphenyl-1*H*-pyrazole derivatives containing O-benzyl oxime moiety as potential immunosuppressive agents: Design, synthesis, molecular docking and biological evaluation

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ABSTRACT

A series of novel (*E*)-1,3-diphenyl-1*H*-pyrazole derivatives containing O-benzyl oxime moiety were firstly synthesized and their immunosuppressive activities were evaluated. Among all the compounds, **4n** exhibited the most potent inhibitory activity ($IC_{50} = 1.18\ \mu M$ for lymph node cells and $IC_{50} = 0.28\ \mu M$ for PI3Kγ), which was comparable to that of positive control. Moreover, selected compounds were tested for their inhibitory activities against IL-6 released in ConA-simulated mouse lymph node cells, **4n** exhibited the most potent inhibitory ability. Furthermore, in order to study the preliminary mechanism of the compounds with potent inhibitory activity, the RT-PCR experiment was performed to assay the effect of selected compounds on mRNA expression of IL-6. Among them, compound **4n** strongly inhibited the expression of IL-6 mRNA.

1. Introduction

Immunosuppressant has been one of the most prescribed drugs in the world, widely used in the treatment of autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type I diabetes mellitus, psoriasis, and inflammatory bowel disease [1]. Although immunosuppressive drugs have been used for the clinical treatment of autoimmune diseases, their side effects including liver toxicity, nephro toxicity, infection, cardiovascular toxicity and others which limit their clinical applications [2–4]. Thus, there is an urgent need for the design and development of novel and less toxic anti-inflammatory agents.

Phosphoinositide 3-kinases (PI3Ks) phosphorylate the 3-hydroxyl of the head group of phosphatidylinositol (PtdIns), and of phosphorylated derivatives of PtdIns termed phosphoinositides [5]. PI3Ks are key components of the PI3K/AKT pathway which plays essential roles in various cellular activities including cell proliferation, cell survival, membrane trafficking, glucose transport, neurite outgrowth, membrane ruffling, superoxide production [6,7]. The dysregulation of the PI3K pathway is associated with numerous cancers as well as inflammatory and autoimmune diseases [8,9]. PI3Ks are grouped into three classes (I, II, and III) according to their structure preference and substrate specificity, class I PI3Ks are separated into two subfamilies (IA and IB) depending on the receptors to which they couple [9–11]. Among these isoforms, PI3Kγ (the only isoform of class IB) plays a pivotal role in inflammation, and it is involved in allergy, development of chronic inflammation, autoimmune diseases [12,13]. Therefore, there is important significance to discover new agents that can influence PI3Kγ.

The oxime ether moiety have received significant attention for this structural scaffold makes up the core structure of numerous biologically active compounds in chemical, food and pharmaceutical research. Some oxime-ether derivatives of hydroxylated benzaldehydes and acetophenones were found to possess

Abbreviations: PI3K, Phosphoinositide 3-kinase; IL-6, interleukin-6; AKT, protein kinase B; NaOAc, CH_3COONa; CsA, cyclosporin A; ConA, Concanavalin A; SAR, structure–activity relationships; CADD, computer assistant drug design; SI, selective index, CC_{50}/IC_{50}; mp, melting point; DMF, N,N-Dimethylformamide.

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pronounced nonsteroidal anti-inflammatory activities and favorable toxicities [14–18]. On the other hand, it was found that a number of other 1,3-substituted 1*H*-pyrazole derivatives (Fig. 1. A, B and C) showed potent anti-inflammatory activity [19–21]. In continuation of our research work on the development of new anti-inflammatory agents [22–24], we report the design and SAR of a series of novel (*E*)-1,3-diphenyl-1*H*-pyrazole oxime ether derivatives (Fig. 2) in this study. Introducing the oxime ether moiety into the 1,3-substituted 1*H*-pyrazole skeleton leads to a positive result in preliminary *in silico* screening. The binding model generated by molecular modeling process revealed that the designed (*E*)-1,3-diphenyl-1*H*-pyrazole oxime ether derivative was tightly embedded in the binding sites of PI3Kγ via hydrogen bonds and π–cation interactions (Fig. 3).

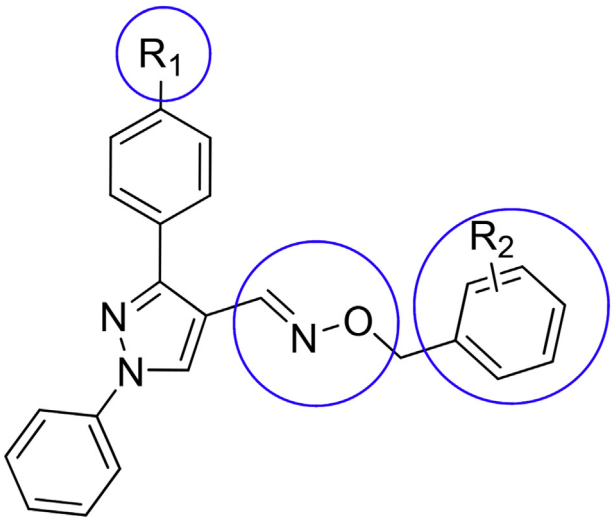
2. Results and discussion

2.1. Chemistry

Twenty (*E*)-1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde O-benzyl oxime derivatives (**4a**–**4t**) were firstly synthesized and the general pathway outlined in Scheme 1. The requisite intermediates **3** and **4** were prepared by the reaction of the Vilsmeier–Haack reagent ($DMF/POCl_3$) and phenyl hydrazones **1** and **2,** respectively [25]. Then target compounds **4a**–**4t** were synthesized by direct addition–elimination reactions of 1.0 equivalent of corresponding intermediates **3**/**4** and 1.2 equivalents of O-benzylhydroxylamine hydrochloride in the presence of NaOAc as catalytic agent. Then compounds **4a**–**4t** were obtained by subsequent purification with recrystallisation in methanol. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures. Compound **4m** was successfully crystallized and its structure was determined by single-crystal X-ray diffraction analysis. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods using SHELXL-97 [26]. The crystal data, data collection, and refinement parameter for the compound **4m** are listed in Table 1, the crystal structure is shown in Fig. 4.

2.2. Cytotoxicity and inhibitory activity

The inhibitory activity of tested compound is sometimes a result of their toxic effect and consequently might cause an erroneous conclusion, so we performed cytotoxicity test before inhibitory activity assay of target compounds. (*E*)-1,3-diphenyl-1*H*-pyrazole oxime ether derivatives **4a**–**4t** were screened for their cyotoxicities on lymph node cells with cyclosporin A (CsA) as the positive control, the cytotoxicity of each compound was expressed as the CC_{50} values which were summarized in Table 2. The results depicted that most of the compounds were low toxic.



4a-4t

Fig. 2. Title compounds.

T cells play a pivotal role in immune response. Excessive T-cell proliferation and activation has been implicated in the pathogenesis of a variety of inflammatory diseases. Next, all the synthetic (*E*)-1,3-diphenyl-1*H*-pyrazole oxime ether derivatives **4a**–**4t** were tested for evaluated for their *in vitro* inhibitory activity on murine lymphocyte proliferation induced by Concanavalin A (ConA). The immunosuppressive activity of each compound was expressed as the concentration of the compound that inhibited ConA stimulated T cells proliferation to 50% (IC_{50}) of the control value, and the results were summarized in Table 2. In general, it was observed that a number of synthesized pyrazole oxime ether analogues displayed potent immunosuppressive activities in the low micromolar range. Inspection of the chemical structure of the compounds **4a**–**4t** suggested that it could be divided into two subunits: A and B rings. A comparison of the *para* substituents on the A-ring demonstrated that an electron-withdrawing group have improved immunosuppressive activity and the potency order is F > Cl > OMe > H > Me. SAR also suggested that compounds bearing the same substituents on phenyl ring A exhibited distinct inhibitory activity due to the difference of the substituents on the ring B. Among these compounds, compounds with *ortho* electron-withdrawing substitution (**4m**–**4t**) showed stronger anti-inflammatory activities in the following order: F > Cl > H. Furthermore, introduction of substituent to *para*-position of ring B results in less active analog (**4d**, **4h**, **4p** and **4t**). Among all the compounds, **4n** and **4o** exhibited the most potent inhibitory activities (2.16 and 1.74 μM, respectively),

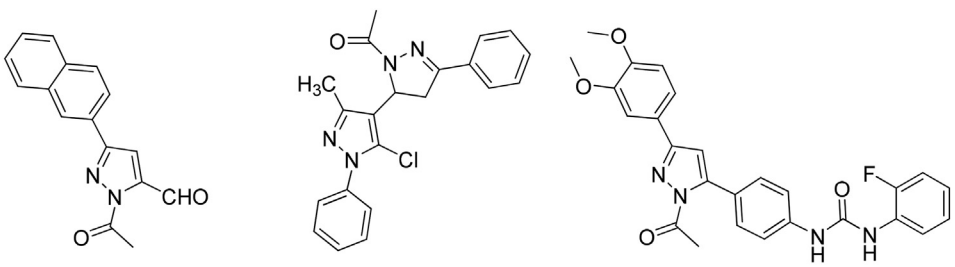


Fig. 1. The structure of compounds A–C.