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Design and environmentally benign synthesis of novel thiophene appended pyrazole analogues as anti-inflammatory and radical scavenging agents: Crystallographic, *in silico* modeling, docking and SAR characterization

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ABSTRACT

Oxidative-stress induces inflammatory diseases and infections caused by drug-resistant microbial strains are on the rise necessitating the discovery of novel small-molecules for intervention therapy. The current study presents an effective and new green protocol for the synthesis of thiophene-appended pyrazoles through 3 + 2 annulations method. Chalcones **3**(**a-g**) were prepared from 5-chloro-2-acetylthiophene and aromatic aldehydes by Claisen-Schmidt approach. The reaction of chalcones **3**(**a-g**) with phenylhydrazine hydrochlorides **4**(**a-b**) in acetic acid (30%) medium and also with freshly prepared citrus extract medium under reflux conditions produced the thiophene appended pyrazoles **5**(**a-l**) in moderate yields. Structures of synthesized new pyrazoles were confirmed by spectral studies, elemental analysis and single crystal X-ray diffraction studies. Further, preliminary assessment of the anti-inflammatory properties of the compounds showed that, amongst the series, compounds **5d, 5e** and **5l** have excellent anti-inflammatory activities. Further, compounds **5c**, **5d**, **5g**, and **5i** exhibited excellent DPPH radical scavenging abilities in comparison with the standard ascorbic acid. Furthermore, using detailed structural modeling and docking efforts, combined with preliminary SAR, we show possible structural and chemical features on both the small-molecules and the protein that might contribute to the binding and inhibition.

1. Introduction

An interest in discovery, design and synthesis of novel small-molecules with anti-inflammatory effects is propelling research in the wider research community in order to prevent the deleterious effects that free-oxide radicals can inflict upon the human body. The most important aspects in drug design are the affinity of the small-molecule for its target, the specificity of its action, drug metabolism and bioactivation. The need of the hour for the research community is to design drugs with fewer/no side effects and more advantageous attributes for the small-molecule drug than the existing ones. Occasionally, thiophenes can be deleterious and are considered a structural alert, as they form highly reactive metabolite, thiophene S-oxide. However, Duloxetine, a thiophene containing drug, is a blockbuster antidepressant without any adverse effect associated with formation of RMs [1]. This is because of the judicious conjugation of thiophene moiety with naphthalene, which facilitates the potentiality of employing this functional group for the synthesis of small-molecules with desired biological effect and the concomitant lack of any side-effects.

α, β-Unsaturated carbonyl compounds (chalcones) are the principal precursors for the biosynthesis of flavonoids and isoflavonoids. Chalcones are synthesized by various methods that

Abbreviations: TBAB, tetrabutylammonium bromide; PTC, phase transfer catalyst; SAR, structureactivity relationship.

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includes, Suzuki reaction, Witting reaction, Friedel-Crafts acylation, Aldol condensation, Photo-Fries rearrangement etc. but the most common method employed is the Claisen-Schmidt reaction of aromatic aldehyde with acetophenones [2]. Chalcones are treated as versatile building blocks in organic chemistry for the synthesis of bioactive molecules such as benzothiazepines [3], pyrazolines [4], isoxazolines [5], etc. Chalcones have gained importance due to their simple structures and diverse pharmacological applications. It has to be pointed out that they show therapeutic efficacy in the treatment of various diseases. For instance, these compounds have shown inhibitory effects on chemotaxis, phagocytosis and ROS production in human polymorphonuclear neutrophils (PMNs), which facilitated the development of potential immunomodulators [6]. They have also showed anti-inflammatory [7], tyrosinase inhibitor and anticancer [8], antimicrobial [9], antiproliferative [10], and antioxidant [11] activities.

Conventional organic synthesis involves the use of energy, petrochemical ingredients, catalysts and post-reaction separation, purification, storage, packaging, distribution etc. Green synthesis is an emerging field that endeavors to make organic synthesis efficient and effective by means of less energy consumption, higher yields and lesser wastes. The uses of greener solvents in the chemical synthesis has a major potential in benefitting both the human health and the environment [12,13]. Orange lemon (*Citrus limon*) is a species of small evergreen tree native to Asia. Lemons were the primary commercial source of citric acid before the development of fermentation based processes [14]. This manuscript demonstrates a methodology to employ the extract from *Citrus limon* for green synthesis of thiophene appended pyrazole derivatives, which were synthesized alternatively in acetic acid medium too.

Design and development of an accessible procedure for the synthesis of simple heterocycles with various functionalities is a worthwhile contribution in organic synthesis. The compounds with pyrazole moieties are the most prominent class in active pharmaceutical drugs and agrochemicals in controlling infections, diseases and pests [15]. Pyrazoles remains the choice for anti-inflammatory agents in spite of multiple attempts at exploring alternative scaffolds [16,17]. There are many protocols for the synthesis of pyrazoles we mention a few a few to mention are, the first report on a base catalyzed reaction of hydrazines with 1,3-dicarbonyl compounds to produce pyrazoles [18], 1,3-dipolar cycloaddition of hydrazones to alkenes [19]. Regioselective synthesis of phosphonylpyrazoles was achieved by the reaction of chalcones with an α-diazo-β-ketophosphonate [20], and via Vilsmeier Haack formylation reaction of hydrazones [21].

Further, it is emphasized here that pyrazoles are ubiquitous scaffolds and are regarded as promising molecules with potential applications in medicinal chemistry. Pyrazoles were known to exhibit anticancer [22], antimicrobial and antioxidant [23], anesthetic [24], and analgesic [25] activities. In view of wide range of synthetic and biological applications of pyrazoles, we herein report for the first time, the synthesis of highly substituted derivatives of pyrazoles and the results of their *in vitro* evaluation for anti-inflammatory activities. The demonstrated green synthesis paves the way for future efforts at synthesizing derivatives of pyrazoles that could find widespread applications in medicinal chemistry.

2. Materials and methods

Melting points were determined by an open capillary tube method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates pre-coated with silica gel using solvent system hexane**:** ethyl acetate (1:4). The spots were visualized under UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrometer respectively. The solvent $CDCl_3$, with TMS as an internal standard, was used to record the spectra. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on ESI/APCI-Hybrid Quadrupole, Synapt G2 HDMS ACQUITY UPLC model spectrometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer.

2.1. Extraction of lemon juice

Orange lemons were obtained from the locally grown lemon trees. Lemons were cut and squeezed to get the pulp juice (100 mL) into a beaker. The pulp juice was transferred to a jar and was diluted with water (50 mL). The mixture was well agitated to a fine solution with the help of mechanical stirrer. Then the solution was warmed for 30 min at 45–50 °C and filtered to get fine juice.

2.2. X-ray diffraction analysis

Single crystals of suitable dimensions were chosen carefully for X-ray diffraction studies. The X-ray intensity data were collected at a temperature of 293(2) K on a Bruker Proteum2 CCD diffractometer equipped with an X-ray generator operating at 45 kV and 10 mA, using CuK_α radiation of wavelength 1.54178 Å. Data were collected for 24 frames per set with different settings of φ (0° and 90°), keeping the scan width of 0.5°, exposure time of 2 s, the sample to detector distance of 45.10 mm and 2θ value at 46.6°. The complete data sets were processed using *SAINT PLUS* [26]. The structures were solved by direct methods and refined by full-matrix least squares method on F^2 using *SHELXS* and *SHELXL* programs [27]. The geometrical calculations were carried out using the program *PLATON* [28]. The molecular and packing diagrams were generated using the software *MERCURY* [29].

2.3. Pharmacological screening

2.3.1. Anti-inflammatory activity

Inflammation-mediated disorders are on the rise and hence, there is an urgent need for the design and synthesis of new anti-inflammatory drugs with higher affinity and specificity for their potential targets. Keeping this in view, the series of new synthesized pyrazole derivatives were assessed for their potential anti-inflammatory activities. In this study, we have assessed the small molecules for their ability to block the upstream enzyme $sPLA_2$. This is to ensure that multiple enzyme targets are available for combinatorial drug administration to increase potency and efficacy of blocking the pathway.

2.3.1.1. Purification of $sPLA_2$ (VRV-PL-8a) from V. Russelli venom. $sPLA_2$ (VRV-PL-8a) from *Vipera russelli* venom was purified to homogeneity by reported procedure [30] and the protein was estimated by Lowry's method [31]. Briefly, *V. russelli* venom (80 mg) was fractionated on pre-equilibrated Sephadex G-75 column (1.5 × 160 cm) using 50 mM phosphate buffer pH 7.0. The protein was resolved into major three peaks. The second peak, constitute about 30% of the total protein, which showed major $sPLA_2$ activity. This $sPLA_2$ peak fraction was lyophilized and further subjected to pre-equilibrated CM-Sephadex C-25 column (1.5 × 45 cm) chromatography. The fractions were eluted stepwise using phosphate buffers of varied ionic strength (50–200 mM) and pH (7.0–8.0). They were resolved into two fractions labeled as V and VIII respectively. The above eluted two fractions were similar to V and VIII protein profiles. The lyophilized fraction VIII was next subjected to Sephadex G-50 column (0.75 × 40 cm) chromatography and eluted using 50 mM phosphate buffer pH 7.0 and obtained