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Original article

Synthesis and biological screening of 5-(alkyl(1H-indol-3-yl))-2-(substituted)-1,3,4-oxadiazoles as antiproliferative and anti-inflammatory agents

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ABSTRACT

A series of 5-(alkyl(1H-indol-3-yl))-2-(substituted)-1,3,4-oxadiazoles were efficiently synthesized by oxidative cyclisation of N′-benzylidene-(1H-indol-3-yl)alkane hydrazides using di(acetoxy)iodobenzene. N′-Benzylidene-(1H-indol-3-yl)alkane hydrazides themselves were derived from simple indole-3-carboxylic acids. The 5-(alkyl(1H-indol-3-yl))-2-(substituted)-1,3,4-oxadiazoles were evaluated for their anti-inflammatory and anti-proliferative activities. Based on the results obtained structure and activity relationship (SAR) was established and a correlation between the activities was observed. Compound **6i** and **6t** showed best activity against proliferation of human cancer cell lines and as well as inflammation of rat paw edema.

1. Introduction

Inflammation is a major cause in chronic illnesses, including diabetes, cardiovascular disease, arthritis, psoriasis, and cancer [1]. Cancer, one of the prime life threat causes in the present society. Chronic inflammation increases the threat of various cancers, indicating that eliminating inflammation may represent a valid strategy for cancer prevention and therapy [2]. Investigation of new therapeutic agents for the treatment of cancer has become a major area of research owing to its resistance to conventional single drug chemotherapeutic agents [3]. Since mono-therapy is generally insufficient for treating cancer, the combined use of anti-inflammatory agents and conventional cancer therapy is gaining attention as a new therapeutic approach. Moreover, non-steroidal anti-inflammatory drugs (NSAIDs) are in general recognized as prototypical chemo-preventive agents against many forms of cancers [4].

Indole, the most active pharmacodynamic nucleus in nature [5], has been a major constituent of number of bio-molecules such as a plant growth regulator hormone indole-3-acetic acid, an amino acid tryptophan, the hormones serotonin and melatonin. It is also incorporated in various natural products such as alkaloids [6]. In particular, 3-substituted indole derivatives have been found to play an important role in many biologically active compounds especially with anti-inflammatory, anti-tumour, hypoglycemic, analgesic and antipyretic activities [7]. Clinically effective agents like anti-inflammatory drug indomethacin, the psychotropic drug LSD and the anti-tumour agent vinblastine are few of the representative compounds possessing 3-substituted indoles nucleus [8]. Recent reports on naturally occurring 5-(3′-indolyl)oxazoles indicate that they are endowed with diverse biological activities [9,10], where Labradorin 1 (**a**) and Labradorin 2 (**b**) (Fig. 1) were found to exhibit anticancer activity against NCI-H 460 (lung-NSC) with GI_{50} values 9.8 and 9.6 μg/mL, respectively [11]. Among the azoles, 1,3,4-oxadiazoles have received a great deal of attention owing to their simple structure and wide variety of pharmacological activities such as anti-inflammatory and anti-edema [12], anticancer [13], analgesic, antibacterial [14], antifungal, anticonvulsant [15] etc.

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Non-steroidal anti-inflammatory drugs like indomethacin (**c**) and tenidap (**d**) have led to exploration of indole ring, especially by modifying it at C-3 position. These modifications mainly include the introduction of alkyl and heterocyclic groups to increasing its pharmacological efficacy [16,17]. Encouraged by the above observations and considering the interesting anti-inflammatory and cytotoxic profiles of 3-substituted indoles, a project has been conceived to synthesize 1,3,4-oxadiazole skeleton appended to the C-3 position of the indole ring with varying alkyl linkers between the two active heterocyclic units. The program also aims to introduce diversity in substitutions at C-2 position of oxadiazole ring with (un)substituted phenyl group to study the synergic effect on the pharmacological profile. These newly synthesized compounds would be evaluated for anti-inflammatory and antiproliferative activities, with a view to study the structure–activity relationship.

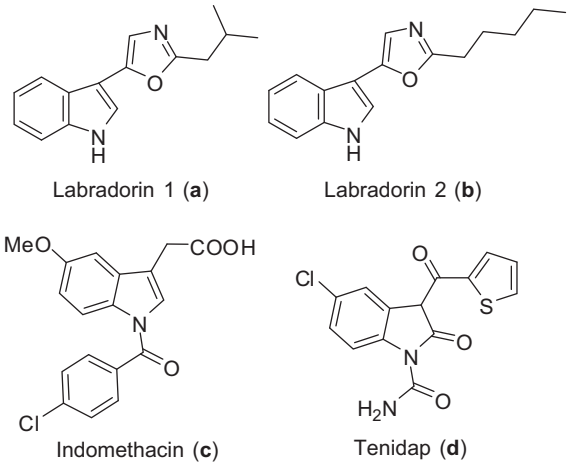

Fig. 1. Some of the biologically active 3-substituted indole representatives.

2. Results and discussion

2.1. Chemistry

Synthesis of the 5-(alkyl(1H-indol-3-yl))-2-(substituted)-1,3,4-oxadiazoles, has been accomplished by the simple chemical reactions outlined in Scheme 1 below. Indole-3-carboxylic acids namely, indole-3-propionic and indole-3-butyric acids are selected as starting materials thus fixing the two different alkyl chain linkers for present study. Diversity at C-2 position of oxadiazole ring was envisaged by introducing various aromatic and hetero aromatic aldehydes at an appropriate stage in the synthesis.

Esterification of the indole-3-carboxylic acids **1**(**a**–**b**) was achieved with ethanol and catalytic amount of con. H_2SO_4, to afford the corresponding esters **2**(**a**–**b**). The reaction of esters with 99% hydrazine monohydrate in ethanol medium produced the corresponding hydrazides **3**(**a**–**b**). Refluxing the hydrazides with various aldehydes **4**(**a**–**r**) in ethanol for 5 h resulted in the formation of hydrazone intermediates **5**(**a**–**v**). Target compounds **6**(**a**–**v**) (Table 1) were accessed by oxidative cyclisation of hydrazones using a benign and non-toxic hypervalent iodine reagent, di(acetoxy) iodobenzene (DIB) [18–20]. All the synthesized compounds were characterized by ^{1}H NMR and MS data.

3. Pharmacology

3.1. In vivo anti-inflammatory activity

The *in vivo* anti-inflammatory screening of the synthesized compounds was carried out by employing the carrageenan-induced paw edema bioassay in rats [21]. From the results obtained (Table 2), it has been noticed that all the tested oxadiazole derivatives exhibit considerable inhibition of inflammation i.e., paw-edema. In addition, it has been noticed that, several synthetic compounds (**6f**, **6h**, **6i**, **6k**, **6q**, **6t**) reveal remarkable inhibition of paw-edema (61.0–70.9% inhibition of edema) comparable to that of Ibuprofen which is used as a reference standard (72.8% inhibition of edema). Compound **6i** exhibit better inhibition of inflammation (78.3% inhibition of edema) compared to that of the reference standard (Ibuprofen) at the end of 3rd hour (Table 3).

Another feature observed during the study is that, the analogues derived from indole-3-propionic acid ($n = 2$), show better anti-inflammatory properties than the analogues derived from indole-3-butyric acid ($n = 3$). Among the substitutions at C-2 position of the oxadiazole ring, **6i** having 4-chlorophenyl and **6t** having *m*-phenoxyphenyl substitution shows inhibition of inflammation comparable to that of the standard employed (Fig. 2).

3.2. In vitro antiproliferative activity

The *in vitro* antiproliferative activity of the synthesized compounds was evaluated against four human cancer cell lines, i.e., human epithelial lung carcinoma (A549), human epithelial cervical cancer (HeLa), human liver carcinoma (HepG2), and human prostate cancer (Du145) cells and the IC_{50} values determined are summarized in Table 4. From the results obtained (Table 4), it is clear that, all the 1,3,4-oxadiazoles reduce the cancer cell viability significantly with IC_{50} values ranging from 20μM to 100 μM. MTT assay reveals that compounds **6a**, **6e**, **6i**, **6m**, **6p**, **6r**, **6t** and **6u** are potent against all the cell lines at IC_{50} values less than 50 μM. Interestingly, majority of these active compounds are derived from indole-3-propionic acid i.e., analogues having two carbon linker between the indole and oxadiazole ($n = 2$).

The SAR study reveals that, alkyl linkers at C-5 position and substitutions on phenyl ring at C-2 of the oxadiazole ring are playing an important role. Comparing the inhibitory concentrations of the pairs (with similar substitution and variation in *n*) **6e** and **6f**, **6i** and **6j**, **6u** and **6v** clearly indicate that, a shorter alkyl side chain ($n = 2$) show relatively enhanced activity than longer alkyl side chain ($n = 3$). Compound **6i** having 4-chlorophenyl substitution at C-2 position of the oxadiazole ring exhibited the effective cytotoxicity against all the four cell lines, with the best inhibition concentrations 23.4 μM and 21.2 μM against HeLa and HepG2, respectively. Compound **6q** with 4-methoxyphenyl substitution at C-2 of oxadiazole ring showed IC_{50} values 23.2 μM and 22.2 μM against A549 and HeLa, respectively, while **6t** with C-2 *meta*-phenoxyphenyl group also inhibited the proliferation of A549 (23.3 μM) and HeLa (25.4 μM) cell lines. Comparison of these two compounds indicates that there is no selectivity between 4-methoxyphenyl and 3-phenoxyphenyl groups with respect to bulkiness of the group or substituent position. Compound **6a** with a phenyl group at C-2 showed good activity against all the cell lines. While **6b** having 1-naphthyl ring at C-2 exhibited moderate activity against HepG2 (101.9 μM) and relatively good activity against all other. Introduction of 2,6-dichlorophenyl group at the C-2 position of 1,3,4-oxadiazole ring in **6n** results in reduction of activity against different cancer cell lines except against HeLa (47.5 μM). On the other hand, 3,4-dichlorophenyl group at the C-2 position in **6o** proved to be better in inhibition of cell proliferation against all cell lines when compared 2,6-dichlorophenyl group. 2,4,6-Trimethoxyphenyl group in **6s** displayed moderate activity against HepG2 (366.16 μM) and good with other cell lines (between 26.3 and 31.5 μM). Comparing the anti-inflammatory and