Synthesis of biologically active compounds | results chapter

One pot synthesis using 3-chloro benzaldehyde, malononitrile and 3-(dimethylamino)phenol has yielded molecule 1 with moderately good yield after purification using preparative TLC. Preliminary biological evaluations such as in vitro anti-oxidant and anti-inflammatory studies were carried out. The IC50 value for the molecule (25. 2µg/mL) was found to be good when compared with ascorbic acid (IC50 = 24. 8µg/mL). The dimethyl amino group and presence of chlorine seems to be favorable for in vitro biological studies. The activities demonstrated by the compound suggest that it could be useful for anti-cancer studies and give way towards the synthesis other substituted chromenes.

1. Introduction

Synthesizing biologically active compounds is one of the main focuses in organic and medicinal chemistry. Among all known compounds, chromenes and its derivatives possess a unique importance. Chromenes shows wide range of biological and pharmacological properties1 such as diuretic, anticoagulant, anticancer, and antianaphylactic activity. 2 Many substituted benzochromenes were proposed for the treatment of immune system diseases and diabetic complications resulted from an increase in permeability of blood vessels and a change in blood pressure. 3 The current interest in 2-amino-4H-chromenes arises from their application in the treatment of human inflammatory TNFα-mediated diseases, such as rheumatoid and psoriatic arthritis and in cancer therapy. 4-5

Based on the previous report on in silico target profiling, a series of molecules were identified targeting estrogen receptor (ER), TNFR and tubulin

which contains chromene unit. 6 This paper illustrates the synthesis and preliminary biological studies of 1.

2. Results and discussion

2. 1 Chemistry

The target molecule 1 was prepared using 3-(dimethylamino)phenol, 3-chlorobenzaldehyde and malonitrile as and as per the scheme 1. The reaction proceeds via Knoevenagel condensation wherein a nucleophilic addition of an active hydrogen compound takes place onto the carbonyl group which when followed by the dehydration reaction and subsequent elimination of water molecule would afford us the condensate which is often an α, β-conjugated enone. In this, the anionic compound of malononitrile added to the carbonyl carbon of the benzaldehyde leads to the formation of benzylidene malononitrile.

It is a conventional method of three component one-pot synthesis. The reactants 2, 3 and 4 were dissolved in ethanol with few drops of piperidine at room temperature and heated to 75-80ºC for two hours. The progress of reaction was monitored through TLC, after the completion of the reaction the solid separated was filtered, purified by column chromatography and recrystallized using ethanol. 7

As the resultant product shows more than three spots, it was then purified using a preparative TLC using ethyl acetate and hexane (7: 3) as solvents. The major two spots, from preparative TLC were collected, after mass analysis it was found that one among is the expected product 1 (Fig. 2).

Further, the structure of the expected product was confirmed by its IR, 1H and 13C NMR spectral analysis.

2. 2 In vitro Antioxidant Studies

The main feature of an antioxidant is the ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems. These free radicals may oxidize nucleic acids, proteins or lipids which could initiate degenerative disease. Antioxidant compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. In this study, in vitro antioxidant activity of 4-phenyl-4H-chromene derivative was assessed by nitric oxide and hydrogen peroxide free radical scavenging activity. Ascorbic acid and butylated hydroxytoluene (BHT) were used as standards.

2. 2. 1 Nitric oxide free radical scavenging method8

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Excess concentration of nitric oxide is implicated in the cytotoxic effects observed in various disorders like cancer, Alzheimer’s and arthritis. The nitric oxide radical can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OHË™ and NOË™.

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated

using Griess reagent. 9 Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of NOË™ may lead to tissue damage. The results of this experiment for the target molecule 1 and references were shown in table1. The experiment was done in triplicate, mean and standard deviation was calculated. From the result, IC50 value was calculated using Graph Pad Prism software (Table 1).

The graphical representation of table 1 was given in fig. 3. The result from this experiment reveals that the inhibitory activity of 1 is in good comparison with ascorbic acid and butylated hydroxytoluene (BHT).

The IC50 value of 1 is found to be 24. 8µg/mL while for ascorbic acid is 20. 6µg/mL which is in good comparison. Further, 1 shows more inhibitory activity than standard BHT (IC50 = 30. 4µg/mL)

2. 2. 2 Hydrogen peroxide free radical scavenging method10

The hydrogen peroxide plays an important role in many biological systems and may oxidize many compounds by converting into the singlet oxygen and hydroxy radicals. Although H2O2 itself is not very reactive, but it can sometimes be toxic to cell since it may give rise to hydroxyl radical in the cells.

Scavenging of H2O2 by synthesized compounds may be attributed to their radical scavenging activity, which can donate electrons to H2O2, thus neutralizing it to water. Scavenging of hydrogen peroxide by 1 may be attributed to its radical scavenging activity, which can donate electrons to H2O2, thus neutralizing it to water. The experiment was done in triplicate,

mean and standard deviation was taken (Table 2). The analysis revealed that 1 exhibits significant level of antioxidant activity when compared to standard substances.

The graphical representation of table 1 was given in fig. 4. The results demonstrates that the inhibitory activity of 1 is in line with ascorbic acid and better than butylated hydroxytoluene.

In short, we described a convenient route towards the synthesis of 4-aryl-4H-chromene in moderately good yield. From the biological analyses, it was observed the compound possess high anti-oxidant and anti-inflammatory activity. This behaviour may be due to the presence of dimethyl amino group and electronegative chlorine. This preliminary analysis indicates that substituted 2-amino-4H-chromenes has a potency to be an anti-cancer molecule. Synthesis of various substituted 4-Aryl-4H-chromenes are under way in our laboratory.