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## Discovery of 4-methyl-2-oxo-2H-chromen-7-yl-2-benzamidoacetate as Anti-proliferative Agents

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### ABSTRACT

Coumarin scaffolds are well known for exhibiting tremendous anti-tumor activity. The present work involved synthesis of a coumarin hybrid utilizing hippuric acid and screening of its anti-neoplastic potential. The coumarin scaffold (**3**) was fabricated utilizing the Pechman-Condensation method. In this method, the phenolic derivative (**1**) and 3-keto esters (**2**) were made to react in the presence of a Lewis acid catalyst (Conc. H<sub>2</sub>SO<sub>4</sub>). To facilitate substitution at the 7th position of the coumarin scaffold, the hydroxyl group was replaced by the chloride function utilizing POCl<sub>3</sub>. In the ultimate step, hippuric acid (**5**) was made to react in the presence of conc. HCl to form hippuric acid-coumarin hybrid (**6**). The *in vitro* anti-cancer screening was performed against breast cancer cell line MCF-7 employing Sulforhodamine B (SRB) assay. The study represented the synthesis of coumarin hybrid utilizing hippuric acid. The spectroscopic, physicochemical, and elemental analyses data were found to be quite complimentary to the proposed structures. The compound showed a very promising anti-cancer activity (IC<sub>50</sub> value of 31.88 μM) as compared to the marketed drug capecitabine (IC<sub>50</sub> value of 6.87 μM). The study will surely endorse investigators across the globe in designing more analogs with pronounced activities in coming future.

### KEYWORDS

*Coumarin; Hippuric acid; Anti-cancer; Anti-neoplastic; Anti-proliferative; Anti-tumor*



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## INTRODUCTION

Cancer is a terrible chronic disease which is the major reason of fatality across the globe. For treating various kinds of this leading health hazard, numerous anticancer agents have been approved by USFDA<sup>1</sup>. Although, at present an arsenal of drugs is available to combat the proliferation, but insufficient pharmacodynamics activity, the high occurrence of side effects, multidrug resistance, and poor pharmacokinetic aspects restricted their use<sup>2</sup>. In spite of the massive endeavor to discover some novel chemotherapeutic molecules/approach, cancer continues to be the foremost concerns universally<sup>3</sup>. Due to heterogeneity of cancer, the look for the development of novel drugs for the treatment is the foremost requirement<sup>4</sup>. As a result, there is an imperative requirement to discover the uninvestigated molecules having pronounced anti-cancer activity with attributes in modulating molecular targets, prevent cell proliferation, and apoptosis.

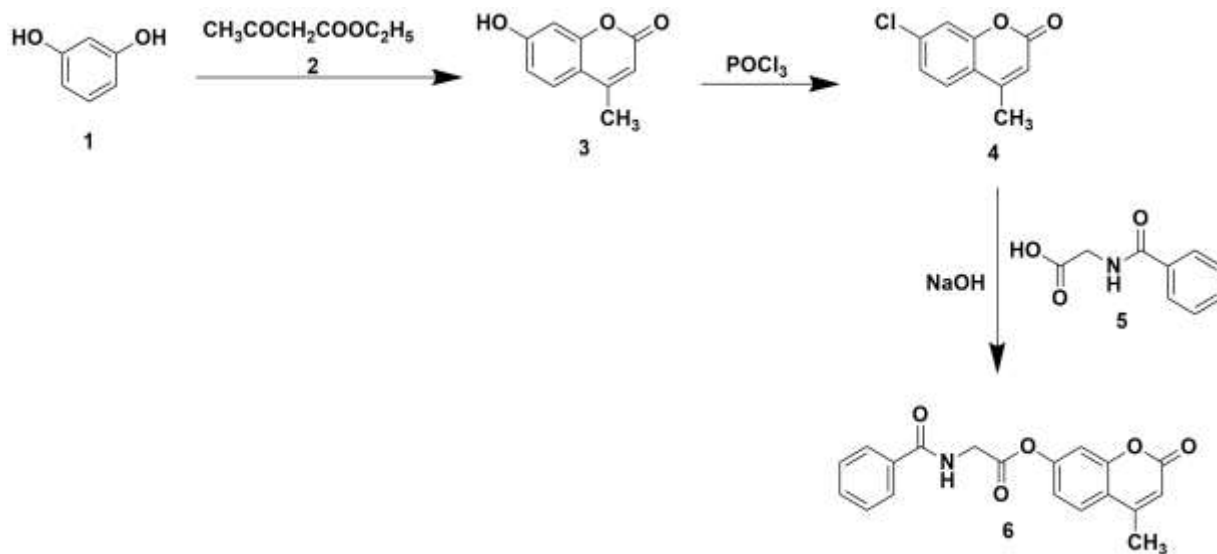
Coumarins are heterocyclic scaffolds with fused benzene and pyrone ring systems with prospective therapeutic importance<sup>5</sup>. As a result of multifarious pharmacological activities like anti-tumor, anti-microbial, anti-inflammatory, anti-viral, anti-oxidant, anti-coagulant, etc., coumarin has gained

citadel positions and is a prominent player in drug discovery<sup>6</sup>. In current clinical practice, coumarin derivatives find importance in the therapy for blood related facet and arthritis<sup>7</sup>. They are naturally produced and are often considered to be a secondary metabolite or plant growth regulators, present in leaves, seeds and roots of a number of higher plant families like rutaceae and umbrelliferae<sup>8</sup>. Many anticancer compounds of plant origin with vast potential and least side effects are coumarin derivatives, which made coumarins among the hot point research subject in the scientific community over the period of time<sup>9</sup>. Taking this coumarin based natural products as the lead material; various research groups around the world have designed several active analogs for treating numerous cancer forms<sup>10</sup>. The pattern of substitution over the basic nucleus regulates the selective anticancer activity by modulating diverse explored cellular pathways.

Coumarin scaffolds are well known for exhibiting tremendous anti-tumor activity. The present work involved synthesis of a coumarin hybrid utilizing hippuric acid and screening of its anti-neoplastic potential. Utilizing Pechman-Condensation method, the coumarin scaffold was fabricated. Hippuric acid is a carboxylic acid found in

the urine of mammal. It is an endogenous ligand in the body produced by the xenobiotic modification of toluene and benzoic acid<sup>11</sup>. With the characteristics of safety, bio-compatibility, and economic

aspect, the moiety was utilized for hybridization. The prepared derivative was screened for *in vitro* anti-cancer activity against breast cancer cell line MCF-7 employing Sulforhodamine B (SRB) assay.



**Scheme 1** Protocol for the synthesis of 4-methyl-2-oxo-2H-chromen-7-yl-2-benzamidoacetate

## MATERIALS AND METHODS

### Materials

Resorcinol and ethyl acetoacetate were procured from HiMedia India Ltd., Mumbai. Hippuric acid was purchased from Sigma-Aldrich Ltd., Germany. All other chemicals, solvents, and reagents were procured from Merck India Ltd., Mumbai.

### Instruments

The structure of the prepared coumarin derivative was determined using sophisticated analytical techniques and

analytical tools. FTIR spectra (IRAffinity-1), <sup>1</sup>H-NMR spectra (Bruker spectrosin NMR DPX-300), mass spectra (JEOL-JMS-DX 303), melting point (Perfit), elemental analyses (Perkin-Elmer 240C), and silica gel G-coated thin layer chromatography plates (Merck) were employed.

### Synthesis of target compounds

The coumarin scaffold (**3**) was fabricated utilizing the Pechman-Condensation method. In this method, the phenolic derivative (**1**) and 3-keto esters (**2**) were



made to react in the presence of a Lewis acid catalyst (Conc. H<sub>2</sub>SO<sub>4</sub>). To facilitate substitution at the 7<sup>th</sup> position of the coumarin scaffold, the hydroxyl group was replaced by the chloride function utilizing POCl<sub>3</sub>. In the ultimate step, hippuric acid (**5**) was made to react in the presence of sodium hydroxide solution to form hippuric acid-coumarin hybrid (**6**). The **Scheme 1** describes the outline of synthesis.

*Synthetic protocol for 7-hydroxy-4-methyl-2H-chromen-2-one (3)*

20 ml of concentrated sulfuric acid was taken in a flask and temperature was kept at 4-5°C by keeping it in a chiller. Powdered resorcinol (0.01 M) (**1**) was dissolved in 0.01 M of ethyl acetoacetate (liquid) (**2**) with constant stirring to form a complete solution. This solution was added to the previously prepared sulfuric acid very slowly with multiple small lots maintaining the temperature of less than 10°C. The reaction mixture was stirred using a magnetic stirrer for 1 hr and finally the contents was poured in a very thin-stream to the crushed ice with vigorous stirring, leading to the separation of the product. The compound was filtered off on a Büchner funnel under suction and washed thoroughly with cold water.

Yield 89%; FTIR (KBr)  $\nu$  (cm<sup>-1</sup>): 3404 (-OH), 3034 (C-H, aromatic), 1713 (C=O), 1635 (C=C, aromatic), 1262 (C-O); <sup>1</sup>H NMR ( $\delta$ , ppm, CDCl<sub>3</sub>): 6.9-7.8 (aromatic, 4H), 5.49 (hydroxyl group, 1H), 2.40 (methyl group, 3H); MS: M<sup>+</sup> 176. Anal.Calcd.for C<sub>10</sub>H<sub>8</sub>O<sub>3</sub>: C, 68.18; H, 4.58; N, 0.00. Found: C, 67.92; H, 4.36; N, 0.00.

*Synthetic protocol for 7-chloro-4-methyl-2H-chromen-2-one (4)*

7-hydroxy-4-methyl-2H-chromen-2-one (**3**) (0.01 M) and 75 mL of POCl<sub>3</sub> were refluxed for an hr. The reaction content was further cooled and was poured slowly into the crushed ice with vigorous stirring. The solid product was filtered off and washed consecutively with ice cold water. The reaction byproduct was separated by azeotropic distillation with n-hexane, filtered, the solvent was evaporated and duly crystallized.

Yield 72%; FTIR (KBr)  $\nu$  (cm<sup>-1</sup>): 3075 (C-H, aromatic), 1693 (C=O), 1628 (C=C, aromatic), 747 (C-Cl); <sup>1</sup>H NMR ( $\delta$ , ppm, CDCl<sub>3</sub>): 6.8-7.7 (aromatic, 4H), 2.47 (methyl group, 3H); MS: M<sup>+</sup> 194. Anal.Calcd.for C<sub>10</sub>H<sub>7</sub>ClO<sub>2</sub>: C, 61.72; H, 3.63; N, 0.00. Found: C, 61.47; H, 3.55; N, 0.00.

*Synthetic protocol for 4-methyl-2-oxo-2H-chromen-7-yl 2-benzamidoacetate (6)*

An equal amount (0.01 M) of 7-chloro-4-methyl-2H-chromen-2-one (**4**) and hippuric acid (**5**) were refluxed in the presence of concentrated HCl with continuous stirring for 12 hrs. The progress of the reaction was monitored by TLC plates. The content was cooled successively and the precipitate was collected suitably. The obtained product was washed with cold water thoroughly, dried, and recrystallized with aqueous ethanolic solution.

Yield 51%; FTIR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3181 (-NH, stretch), 3119 (C-H, aromatic), 1716 (C=O), 1673 (C=C, aromatic), 1557 (-NH, bending), 1280 (C-O), 1244 (C-N);  $^1\text{H}$  NMR ( $\delta$ , ppm,  $\text{CDCl}_3$ ): 8.46 (amide, 1H), 7.3-8.2 (aromatic, 9H), 4.61 (aliphatic CH, 2H), 2.55 (methyl group, 3H). MS:  $\text{M}^+$  337. Anal. Calcd. for  $\text{C}_{19}\text{H}_{15}\text{NO}_5$ : C, 67.65; H, 4.48; N, 4.15. Found: C, 67.28; H, 4.17; N, 4.01.

#### Anti-cancer activity

The *in vitro* anti-cancer screening was performed against breast cancer cell line MCF-7 employing Sulforhodamine B (SRB) assay to determine the cell sensitivity towards the experimental compound. The standard protocol was followed according to Mahapatra *et al.*<sup>12</sup> which involved culturing the cells in RPMI1640 media containing 10% fetal bovine serum maintained in a

controlled environment ( $37^\circ\text{C}$ , 5%  $\text{CO}_2$ ) utilizing capecitabine as the standard drug. The cells were initially harvested, counted, and plated using a 96-wells plate. The cells were treated with the experimental compound and 10% trichloroacetic acid solution was applied to attach at a reduced temperature of  $4^\circ\text{C}$ . Subsequently, the cells were further treated with deionized water and stained with 0.4% SRB solution for 15 min. Afterwards, the wells were washed thoroughly with the glacial acetic acid solution so as to remove unbound stain and dried at room temperature. The bound protein stain was solubilized using tris-base (tris(hydroxymethyl)aminomethane) solution. The optical density was measured at 540 nm using a microplate reader and  $\text{IC}_{50}$  value was estimated.

## RESULTS AND DISCUSSION

### Chemistry

The characterization of the structure revealed some prominent features which further confirmed the formation of compounds. In the structure of 7-hydroxy-4-methyl-2H-chromen-2-one (**3**), a carbonyl group was prominently observed at  $1713\text{ cm}^{-1}$ , representing the absolute transformation of C-OH into coumarin functionality. The presence of single -OH



group was detected at  $3404\text{ cm}^{-1}$  and the carbonyl group in the spectra completely supported the formation of intermediate. The appearance of C-O bonding represented the successful conversion of resorcinol to coumarin. Additionally, the appearance of 3 protons for the  $\text{CH}_3$  group at 2.46 ppm also represented the accomplishment of intermediate structure. In the structure of 7-chloro-4-methyl-2H-chromen-2-one (**4**), the disappearance of -OH group in the structure and marked appearance of -Cl group at  $746\text{ cm}^{-1}$  confirmed the replacement of the hydroxyl group with halogen function. The terminal product, 4-methyl-2-oxo-2H-chromen-7-yl-2-benzamidoacetate (**6**) was best characterized by the appearance of an amide peak in NMR spectra (8.46 ppm) and IR spectra ( $3181\text{ cm}^{-1}$ ), followed by identification of a C-N bonding at  $1244\text{ cm}^{-1}$ . The aromatic protons were recognized in the range 7.3-8.2 ppm. The mass spectra established the manifestation of several fragmented products in the m/z range of 90-120 along with the base peak, which match with the molecular weight of the compound. The % elemental analyses of the intermediately formed products and destination compound were observed to be having closer compliance with the theoretical values. Thus, the characterization

studies wholly supported the formation of the novel molecule.

### Biological activity

The SRB anti-cancer assay of the novel synthesized compound demonstrated potent anti-proliferative activity against MCF-7 cell line with  $\text{IC}_{50}$  value of  $31.88\text{ }\mu\text{M}$  which was comparable with the standard drug capecitabine having  $\text{IC}_{50}$  value of  $6.87\text{ }\mu\text{M}$ .

### CONCLUSION

The study represented the synthesis of coumarin hybrid utilizing hippuric acid. The spectroscopic, physicochemical, and elemental analyses data were found to be quite complimentary to the proposed structures. The compound showed a very promising anti-cancer activity as compared to the marketed drug. The study will surely endorse investigators across the globe in designing more analogs with pronounced activities in coming future.



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