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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF IMIDAZOLE AND TRIAZOLE DERIVATIVES OF FLAVONOIDS

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Flavones can play a potential role in estrogen dependent breast cancer due to greater reactivity of imidazole and triazole heterocycles which have been investigated in this work. We emphasized on synthesis of flavones derivatives with imidazole (**2a, 2b, 2c**) and triazole (**3a, 3b, 3c**) nucleus as a fundamental hetero-aromatic system with modifications, which have been confirmed by TLC, IR, NMR and mass spectrometry data. The synthesized compound were studied as non-steroidal aromatase inhibitors and evaluated for *in vitro* anti-breast cancer activity against MCF-7 cell line through SRB assay. The triazole derivative **3a** with nitro substitution (H-bond accepting group) was found to be more active in comparison to standard drug letrozole.

Keywords: aromatase; aromatase Inhibitors; flavones; flavonoids; imidazole; triazole; SRB assay; MCF-7 cell line.

1. INTRODUCTION

Flavonoids are a group of polyphenolic compounds that occur naturally in foods of plant origin. They show a wide variety of biological activities such as anti-cancer, anti-inflammatory, anti-microbial, anti-Alzheimer's disease, anti-malarial, antioxidant, gastro-protection and α1-adrenoceptor (α1-AR) antagonists. Due to their structural and functional similarity with endogenous estrogens, flavonoids have a potential role in ER-dependent breast cancer [1].

Aromatase enzyme is highest in or near breast tumor sites. Several studies have shown that serum level of estrogen is very low in postmenopausal women, but the concentration of estrogen in breast tissues is 5 – 6 fold higher than serum level estrogen, and concentration in the tumor is even higher as compared to normal breast tissues [2]. Therefore, there are more chances for tumor expansion in breast of postmenopausal women.

Flavonoids interact with the heme moiety of the CYP prosthetic group of the aromatase molecule. These inhibitors contain suitably positioned hetero atoms, usually in the imidazole and triazole rings, that are capable of binding to CYP enzymes so that their hetero atoms coordinate the heme iron. Non-steroidal aromatase inhibitors are reversible and the resultant estrogen blockade is dependent on continuous presence of a drug [3]. Based on the literature and structure-activity relationship [4] it was suggested to synthesize new flavonoids as possible antineoplastic agents with pharmacophore patterns required for aromatase inhibition (Fig. 1) [5].

With this objective, we report synthesis of non-steroidal aromatase inhibitors having flavone nucleus with imidazole and triazole rings as a fundamental hetero-aromatic system

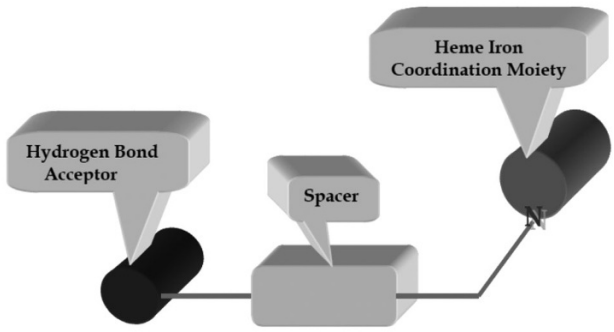

Fig. 1. Pharmacophore pattern of aromatase inhibitors with suitably positioned hetero atom, which strongly interact with heme iron, hydrophobic spacer moiety between heme coordinating group and hydrogen bond acceptor moiety, which can abstract hydrogen from Serine 478 present in active site of enzyme, and hydrophobic group is required to interact with aliphatic amino acid residue in active site [5].

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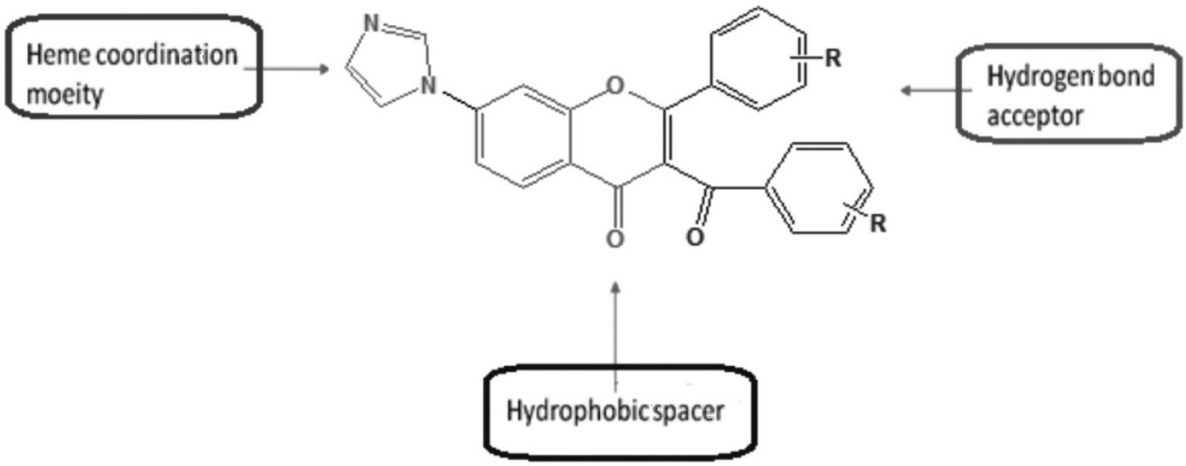

Fig. 2. Flavonoids with required pharmacophore pattern for aromatase inhibition, flavone nucleus with imidazole and triazole ring as a fundamental hetero-aromatic system with modification.

with modification and accepted pharmacophoric pattern (Fig. 2).

All synthesized compound have been evaluated for *in-vitro* antiproliferative activity against MCF-7 cell line through SRB assay and assessed for percentage cell growth inhibition on MCF-7 cell line and potency (IC_{50}) value discussed for each compound after comparing with a standard drug [4].

2. RESULTS AND DISCUSSION

2.1. Chemistry

The synthesis of substituted flavonoid scaffold was started by reacting 2,4-dihydroxy acetophenone and substituted benzoyl chloride in acetone in the presence of potassium carbonate with stirring for 30 min at room temperature to yield intermediate (2,4-bis-(substituted benzyloxy)acetophenone. Upon reflux for 24 h, three flavonoid derivatives (**1a, 1b, 1c**) were obtained by modified Baker-Venkataraman method which has been reported recently [6]. These derivatives by two pot synthesis were further reacted with imidazole in tetrahydrofuran by stirring for 24 h to yield imidazole derivatives of flavonoids (**2a, 2b, 2c**). Reacted with 1,2,4-triazole in thionyl chloride and acetonitrile by stirring for 1 h at 0 – 5°C, triazole derivatives of flavonoids (**3a, 3b, 3c**) were obtained by the scheme of synthesis shown in Fig. 3 [7].

The synthesized compounds were characterized by melting points, IR and ^{1}H NMR spectroscopy, and mass spectrometry; the completion of reaction was monitored by TLC. Analytical data for compounds **2a, 2b, 2c** and **3a, 3b, 3c** are given in Table 1.

3-Benzoyl-7-(1H-imidazol-1-yl)-2-phenyl-4H-chromen-4-one (2a). It shows characteristic peaks at 1637, 1661 cm^{-1} (C=O str. of flavones), 1584 cm^{-1} (C=N str.), 1073 cm^{-1} (C-O-C bend) in IR spectrum and M+18 peak at 411 in mass spectrum; ^{1}H NMR gives signals at 6.9 – 7.14 (m, 2H, 6,7-CH), 7.15 – 7.68 (m, 9H, Ar-H), 7.72 – 7.8 (m, 4H, Ar-H), 8.2 (S, 1H, imidazole H-2"); m.p. 212 – 214°C.

3-(4′-Nitrobenzoyl)-7-(1H-imidazol-1-yl)-2-(4′-nitrophenyl)-4H-chromen-4-one (2b). It shows characteristic peaks at 1619, 1669 cm^{-1} (C=O str. of flavones), 1580 cm^{-1} (C=N str.), 1034 cm^{-1} (C-O-C bend), 1520, 1348 cm^{-1} (NO_2) in IR spectrum and M+1 peak at 483.34 in mass spectrum; m.p., 256 – 258°C.

3-(4′-Methylbenzoyl)-7-(1H-imidazol-1-yl)-2-(4′-methylphenyl)-4H-chromen-4-one (2c).

It shows characteristic peaks at 2864, 2726 cm^{-1} (C-H str.), 1683, 1640 cm^{-1} (C=O str. of flavones), 1071 cm^{-1} (C-O-C) in IR spectrum and M+1 peak at 483.34 in mass spectrum; ^{1}H NMR give signals at 2.55 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 6.90 (s, 1H), 7.37 – 7.6 (m, 4H, Ar-H), 7.63 – 7.8 (m, 2H, Ar-H), 8.22 (m, 2H, Ar-H); m.p., 232 – 234°C.

TABLE 1. Physiochemical Data of All Synthesized Compounds

Compound No.	Mol. formula	Mol. wt.	Yield, %	R_f	Solvent system*
1a	$C_{22}H_{16}O_4$	342.09	74.00	0.89	A
1b	$C_{22}H_{12}O_7N_2$	432.06	62.90	0.94	A
1c	$C_{24}H_{18}O_4$	370.12	71.22	0.83	A
2a	$C_{25}H_{16}N_2O_3$	392.12	43.15	0.54	B
2b	$C_{25}H_{14}N_4O_7$	482.09	51.34	0.45	B
2c	$C_{27}H_{20}N_2O_3$	420.15	69.20	0.50	A
3a	$C_{24}H_{13}N_5O_7$	483.08	43.00	0.71	A
3b	$C_{26}H_{13}N_5O_7$	421.14	38.40	0.64	A

* Solvent system: (A) hexane – ethyl acetate (7:3); (B) hexane – ethyl acetate (6:4).

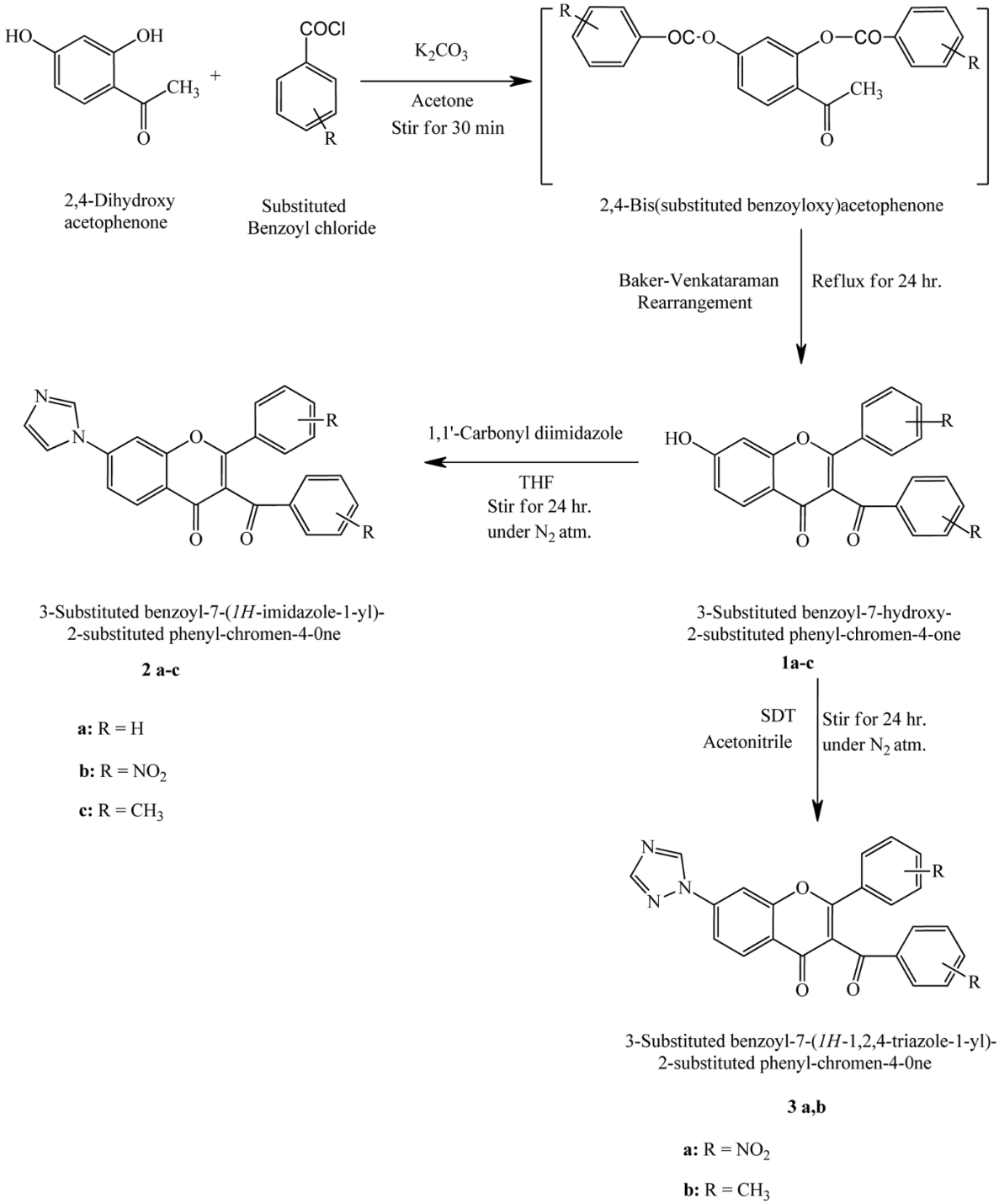

Fig. 3. Scheme of synthesis of imidazole and triazole derivatives of flavonoids. **Reagents and conditions:** (1) Potassium carbonate, acetone, stirred for 30 min; (2) Baker-Venkatraman rearrangement, refluxed for 24 h; (3) imidazole, tetrahydrofuran, stirred for 24 h under pressure of nitrogen; (4) 1,2,4-triazole, acetonitrile, stirred for 24 h [10].

3-(4′-Nitrobenzoyl)-7-(1H-1,2,4-triazol-1-yl)-2-(4′-nitrophenyl)-4H-chromen-4-one (3a). It shows characteristic peaks at 1674, 1620 cm^{-1} (C=O str.), 1524, 1346 cm^{-1} (-NO_2), 1051 (C-O-C) in IR spectrum and M+2 at 485.7 in mass spectrum; ^{1}H NMR gives signals at 6.8 – 7.0 (d, 2H, 6, 7-CH), 7.3 – 7.8 (m, 7H, Ar-H), 8.0 – 8.2 (m, 3H, Ar-H), 8.3 (s, 1H, triazole 2-H″); m.p., 228 – 23°C.

3-(4’-Methylbenzoyl)-7-(1H-1,2,4-triazol-1-yl)-2-(4′-methylphenyl)-4H-chromen-4-one (3b). It shows characteristic peaks at 1667 cm^{-1} (C=O str.), 2923, 2840 (C-H str.),

1060 (C-O-C str.) in IR spectrum and M^+ at 420.3 in mass spectrum; ^{1}H NMR shows signals at 2.34 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 6.89 (s, 1H, 8-CH), 7.0 – 7.21 (m, 4H, Ar-H), 7.28 – 7.8 (m, 7H, Ar-H), 8.25 (s, 1H, imidazole H-2''); m.p., 206 – 208°C

2.2 Biology

All synthesized compounds were evaluated for *in-vitro* antiproliferative activity against MCF-7 cell line through SRB assay. This work has been carried out in collaboration with Dr. Arti S. Juvekar, Head of Screening department at Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Khargarh and Mumbai.

MCF-7 is a breast cancer cell line (acronym of Michigan Cancer Foundation-7). For MCF-7, it is not possible for cancer researchers to obtain a mammary cell line that was capable of living longer than a few months. The cell lines were grown in RPMI (Roswell Park Memorial Institute) 1640 medium containing 10% fetal bovine serum and 2 mL L-glutamine.

SRB Assay. Sulforhodamine B (SRB) assay was carried out as per method given by Skehan, et al. [4] Experimental drugs were solubilized in 0.1% DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. Then 10-fold serial dilutions were made to provide a total of four drug concentrations plus control. Aliquots of 10 μL of these dilutions of different drugs were added to the appropriate microtiter plate with 90 wells which were previously air dried [8]. The optical density of samples in wells was determined at wavelength 540 nm by a colorimetric plate reader. Percentage growth was calculated on a plate-by-plate basis for test wells relative to control wells, expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells × 100. Using six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as [(Ti-Tz)/(C-Tz)] × 100 [%] for concentrations at which Ti ≥ Tz (Ti–Tz positive or zero) and as [(Ti-Tz)/Tz] × 100 [%] for concentrations at which Ti < Tz (Ti–Tz negative). The IC_{50} value was determined by graph pad prism software (Tables 2 and 3) [9].

TABLE 2. Percentage Inhibition of Cell Growth in MCF-7 Cell Line

	Log conc.	% Inhibition of cell growth					
		Std. letrozole	**2a**	2b	2c	3a	3b
0.05	-1.29	-27.26	-32.61	-29.06	-30.06	-28.39	-29.71
0.15	-0.82	-22.69	-28.02	-26.89	-28.32	-26.02	-27.45
0.46	-0.34	-16.88	-22.08	-23.33	-24.0	-21.36	-24.15
1.37	0.14	-10.80	-15.88	-18.18	-17.08	-13.19	-18.02
4.12	0.61	-4.89	-07.1	-11.24	-14.82	-9.08	-10.56
12.35	1.09	3.34	9.91	3.59	1.59	8.87	6.06
37.04	1.57	10.61	15.07	5.66	3.14	19.21	13.88
111.11	2.05	17.84	22.62	11.06	7.63	24.81	22.06
333.33	2.52	24.29	32.81	15.08	10.21	34.02	30.87
1000.00	3.00	32.02	46.29	21.22	17.87	49.88	41.96

3. CONCLUSION

Imidazole and triazole derivatives of flavones were synthesized, characterized by IR, ^{1}HNMR, and mass spectrometry and evaluated for antiproliferative activity. As a result, it was found that compounds **2a, 2b, 2c** and **3a, 3b** possess antiproliferative activity. Compounds with triazole ring showed good activity. The triazole derivative with nitro substitution (**3a**) showed highest activity. The imidazole ring with methyl substitution (**2a**) showed lowest activity.

4. EXPERIMENTAL SECTION

4.1. General

All the chemicals of analytical grade were purchased from LOBA chemicals, SDFCL and Spectrochem. Chemicals and solvents were purified by general laboratory techniques before use. All moisture free operations were performed in oven-dried glassware and under the nitrogen atmosphere. Synthesized compounds were characterized by melting points, IR, ^{1}H NMR, mass spectrometry, and the progression of reactions was monitored by TLC. Melting points were determined by VEEGO microprocessor based melting point apparatus having silicone oil bath and are uncorrected. IR spectra (wavenumbers in cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrophotometer using potassium bromide disks. NMR spectra were recorded on Bruker AVANCE II 400 MHz instrument in $CDCl_3$ with TMS as an internal standard for ^{1}H NMR. Chemical shift values were expressed as δ, ppm. Mass spectra were recorded on Advion Expression CMS USA at Syntel Research Solutions, Gandhinagar. Chro-

TABLE 3. Inhibition Potency (IC_{50}) of Synthesized Compounds as Compared to Standard Drug

Compounds	IC_{50} (μM)
Letrozole	10.62
2a	15.88
2b	28.48
2c	42.02
3a	11.23
3b	21.48

matographic separations were performed on columns using silica gel 100 – 200 mesh. The progress of all reactions was monitored by TLC on 2 cm × 5 cm pre-coated silica gel 60 F_{254} (Merck) plates with a thickness of 0.25 mm. The chromatograms were visualized under UV 254 nm light and/or by exposure to iodine vapors.

4.2. Synthesis

3-Benzoyl-7-hydroxy-2-phenyl-4H-chromen-4-one (1a). Anhydrous potassium carbonate (6.150 g, 0.0472 mmol) was added to a stirred solution of 2′,4′-dihydroxyacetophenone (1.0 g, 0.0065 mmol) in dry acetone (60 mL). The mixture was stirred at room temperature for 10 min and then benzoyl chloride (1.67 mL, 0.01314 mmol) was added dropwise through the pressure equalizing dropping funnel (PEDF) and the mixture was stirred at room temperature for an additional 30 min. After refluxing for 24 h, the solvent was removed under reduced pressure. The residue was cooled to room temperature and acidified in a beaker with dilute hydrochloric acid to weak acidity. The precipitate formed was filtered off, dried and then recrystallized from acetic acid [10].

3-(4'-Nitrobenzoyl)-7-hydroxy-2-(4′-nitrophenyl)-4H-chromen-4-one (1b). Anhydrous potassium carbonate (6.150 g, 0.0472 mmol) was added to a stirred solution of 2′,4′-dihydroxyacetophenone (1.0 g, 0.00657 mmol) in dry acetone (60 mL). The mixture was stirred at room temperature for 10 min and then 4-nitrobenzoyl chloride (2.438 g, 0.01314 mmol) was added dropwise through PEDF and the mixture was stirred at room temperature for an additional 30 min. After refluxing for 18 h, the solvent was recovered under reduced pressure. The residue was cooled to room temperature and acidified in a beaker with dilute hydrochloric acid to weak acidity. The precipitate so formed was filtered off, dried and then recrystallized with acetic acid.

3-(4′-Methylbenzoyl)-7-hydroxy-2-(4′-methylphenyl)-4H-chromen-4-one (1c). Anhydrous potassium carbonate (6.150 g, 0.0472 mmol) was added to a stirred solution of 2′,4′-dihydroxyacetophenone (1.0 g, 0.00657 mmol) in dry acetone (60 mL). The mixture was stirred at room temperature for 10 min and then 4-methylbenzoyl chloride (2.01 mL, 0.01314 mmol) was added dropwise through PEDF and the mixture was stirred at room temperature for an additional 30 min. After refluxing for 20 h, the solvent was evaporated under reduced pressure. The residue was cooled to room temperature and acidified in a beaker with dilute hydrochloric acid to weak acidity. The precipitate formed was filtered off, washed with acidified water dried and then recrystallized with acetic acid.

3-Benzoyl-7-(1H-imidazol-1-yl)-2-phenyl-4H-chromen-4-one (2a). To a solution of 3-benzoyl-7-hydroxy-2-phenyl-4*H*-chromen-4-one (0.5 g, 0.00146 mmol) in anhydrous THF (20 mL) was added 1,1′-carbonyldiimidazole (0.95 g, 0.00584 mol). The mixture was stirred under nitrogen atmosphere at room temperature for 24 h. Subsequently, the semi-solid mass was quenched with chloroform. Impurities were removed by filtration and the organic layer was collected and the solvent was recovered under reduced pressure. The resulting crude product was purified by passing through column chromatography using silica gel (100 – 200 mesh) and eluted with hexane – ethyl acetate (7:3)

3-(4′-Nitrobenzoyl)-7-(1H-imidazol-1-yl)-2-(4′-nitrophenyl)-4H-chromen-4-one (2b). To a solution of 3-(4′-Nitrobenzoyl)-7-(*1H*-imidazole-1-yl)-2-(4′-nitrophenyl)-4*H*-1-chromen-4-one (0.5 g, 0.00115 mmol) was stirred with, 1′-carbonyldiimidazole (0.750 g, 0.00462 mmol) in anhydrous THF (25 mL). The mixture was stirred under nitrogen atmosphere at room temperature for 20 hrs. Subsequently, the semi-solid mass was quenched with chloroform. Impurities were removed by filtration and the organic layer was collected and the solvent was recovered under reduced pressure. The resulting crude product was purified by passing through column chromatography using silica gel (100 – 200 mesh) and eluted with hexane – ethyl acetate (5:5).

3-(4′-Methylbenzoyl)-7-(1H-imidazol-1-yl)-2-(4′-methylphenyl)-4H-chromen-4-one (2c). To a solution of 3-(4′-methylbenzoyl)-7-(*1H*-imidazole-1-yl)-2-(4′-methylphenyl)-*4H*-chromen-4-one (0.4 g, 0.00108 mmol) in anhydrous THF (20 mL) was added 1,1′-carbonyldiimidazole (0.70 g, 0.00432 mol). The mixture was stirred under nitrogen atmosphere at room temperature for 30 h with the addition of 1,1′-carbonyldiimidazole. Subsequently, the semi-solid mass was quenched with chloroform. Impurities were removed by filtration and the organic layer was collected and the solvent was recovered under reduced pressure. The resulting crude product was purified by passing through column chromatography using silica gel (100 – 200 mesh) and eluted with hexane – ethyl acetate (5:5).

3-(4′-Nitrobenzoyl)-7-(1H-1,2,4-triazol-1-yl)-2-(4′-nitrophenyl)-4H-chromen-4-one (3a). To a chilled solution (0 – 5°C) of thionyl chloride (0.25 ml, 0.00173 mmol) in dry acetonitrile (3 ml) 1,2,4-triazole (0.317 g, 0.0046 mmol) in dry acetonitrile (10 mL) was added slowly maintaining the temperature at 0 – 5°C. The reaction mixture was stirred for 1 hr. at 0 – 5°C. Previously dissolved 3-(4′-nitrobenzoyl)-7-hydroxy-2-(4′-Nitrophenyl)-*4H*-chromen-4-one(0.5 g, 0.00115 mmol) in dry acetonitrile (10 mL) was added dropwise to the above reaction mixture. The reaction mixture was further stirred for 24 hr. under the nitrogen atmosphere. Subsequently, the semi-solid mass was quenched with chloroform. Impurities were removed by filtration and the organic layer was collected and the solvent was recovered under reduced pressure. The resulting crude product was purified by passing through column chromatography using silica gel (100 – 200 mesh) and eluted with hexane – ethyl acetate (8:2).

3-(4′-Methylbenzoyl)-7-(1H-1,2,4-triazol-1-yl)-2-(4′-methylphenyl)-4H-chromen-4-one (3b). To a cooled solution (0 – 5°C) of thionyl chloride (0.3 mL, 0.00202 mmol) in dry acetonitrile (3 ml) was added 1, 2,4-triazole (0.37 g, 0.0054 mmol) in dry acetonitrile (10 mL). The reaction mix-

ture was stirred for 1 hr. at 0 – 5°C. 3-(4′-Methylbenzoyl)-7-hydroxy-2-(4′-methylphenyl)-*4H*-chromen-4-one(0.5 g, 0.00135 mmol) in dry acetonitrile (10 mL) was added dropwise to the above reaction mixture. The reaction mixture was stirred for 24 hr. under the nitrogen atmosphere. Subsequently, the semi-solid mass was quenched with chloroform. The organic layer was separated by filtration and solvent was recovered under reduced pressure. The resulting crude product was purified by passing through column chromatography using silica gel (100 – 200 mesh) and eluted with hexane – ethyl acetate (8:2) [10].

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