

Synthesis of Novel Class of Flavone-Pyrimidinone Analogues

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Abstract

The new concept to synthesize a molecular hybrid led me to synthesize novel molecules with wide spectrum biological activities. 2-(2-chlorophenyl)-5, 7-dihydroxy-4-oxo-4H-chromene-8-carbaldehyde is easily synthesized in the laboratory by Duff reaction. Flavone-Dihydropyrimidinone novel derivatives are prepared by one pot Biginelli reaction. Reaction was carried out thermally as well as under ultrasound condition. I prepared novel molecules with very interesting skeleton. Both (chrysin and monastrol/Dihydropyrimidinone) molecules have anticancer activities. So this discovery will give new direction to current anticancer drug synthesis.

KEYWORDS: Flavone, Duff reaction, Biginelli reaction.

Introduction

Natural products have wide spectrum biological activities. Drugs derived from alkaloids, terpenoids, flavonoids, carbohydrates, proteins and amino acids have large number of biological activities. It contains two oxygen atoms, carbonyl and ether in the ring B (**Figure 1**). Flavonoids are classified as flavone, flavanones, flavane, isoflavone etc. (**Figure1, structures 1-3**)³⁹. Some basic examples are given below (**Figure1, structures 4-9**),

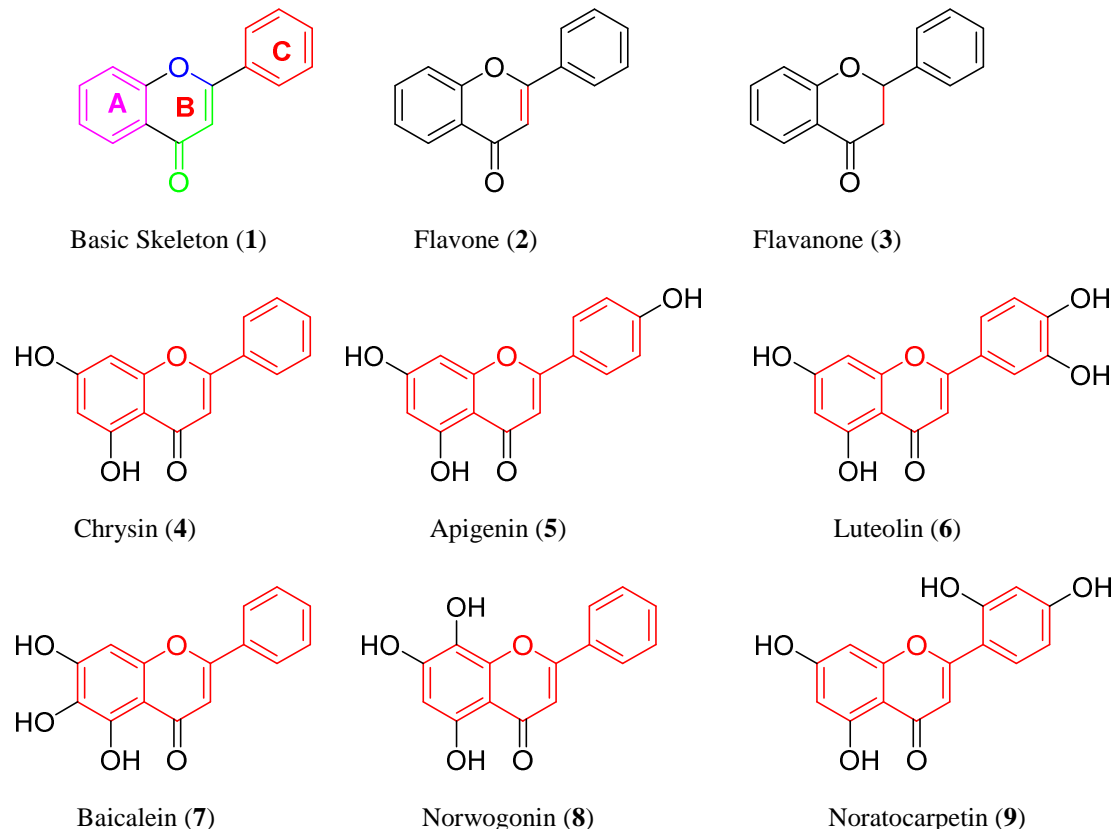


Figure 1. Examples of flavone molecules.

Flavone is a natural product having large number of activities like, anti-cancer⁴², anti-fungal⁵², anti-malarial⁵¹, anti-bacterial, anti-HIV, anti-tuberculosis, anti-oxidant⁵⁰, etc.¹⁻⁸ Some examples of flavonoids are Chrysin, Naringenin, Luteolin, Apigenine, Tangeretin, Myricetin, Quercetin, Rhamnazin etc. as shown in figure 1. Among which, I selected a Chrysin (3) (Figure 1.) as basic skeleton having hydroxyl group at 5th and 7th positions. From ancient times, this is well known anticancer molecule. It has added advantages over doxorubicin, cisplatin which have side effects like cardiotoxicity, nephrotoxicity, hepatotoxicity, kidney damage, tissue damage² etc. Chrysin is less toxic compared to current anticancer drugs. It is need of the time to synthesize anticancer drug molecules without any side effects. Some synthetic drugs like flavone-8-acetic acid and Flavopiridol are anticancer agents (Figure 2. structures 1&2)^{7,8}.

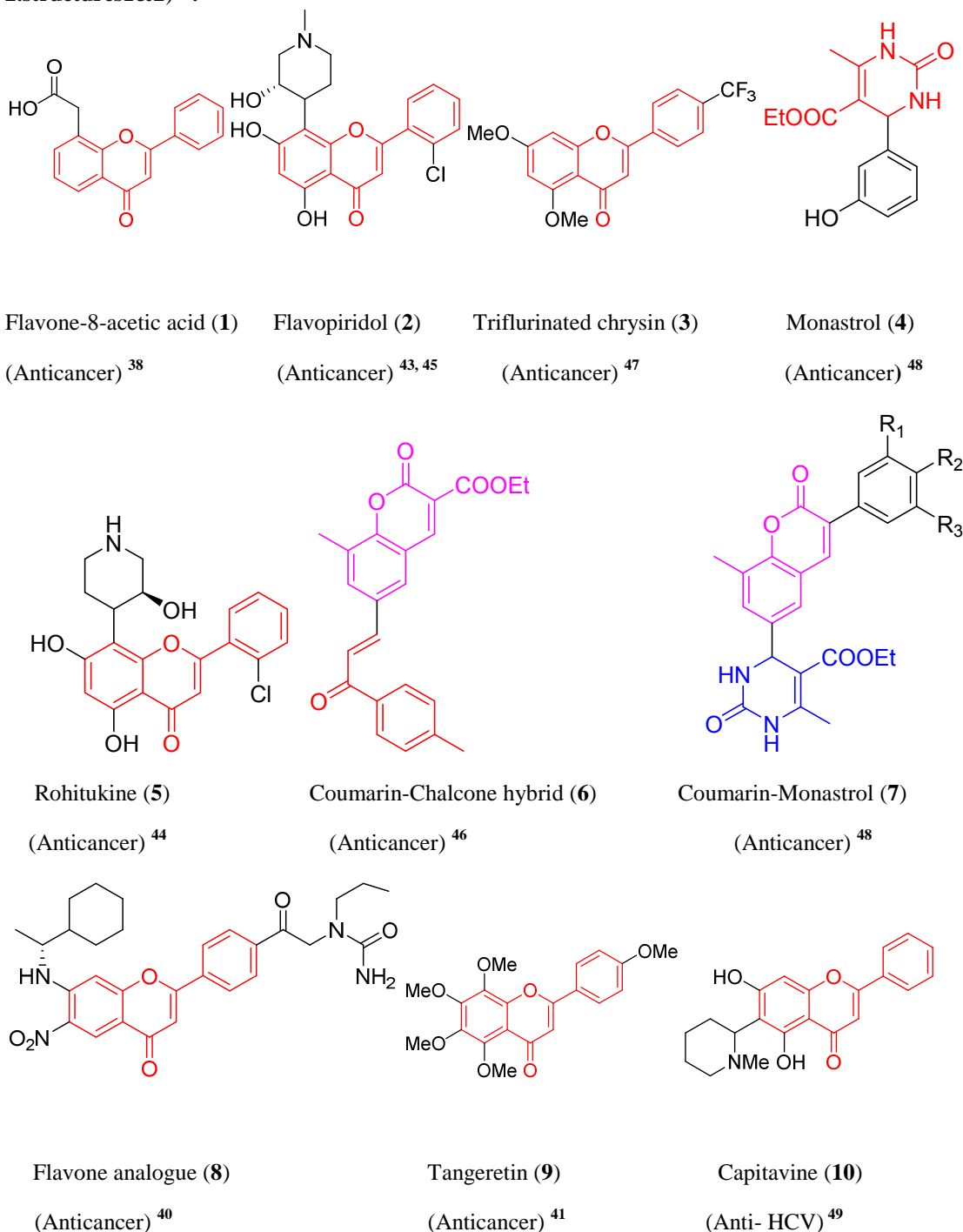


Figure2. Examples of anticancer hybrid molecules.

Breast cancer is the second leading cause of death in female today. It also causes malignancy in woman. Estrogen causes a breast cancer. Hence, efforts are made to block estrogen formation. The commonly used therapy to antagonizing estrogens receptor (ER) function is the anti-estrogen tamoxifen (TAM) which binds to ER and blocks downstream signalling. But with TAM we get only 37% result of patients. The cause is drug resistance which usually develops in the patients in a couple of years. This resistance occurs by various mechanisms, such as induction of estrogens independent pathway for breast cancer cell growth, over expression of human epidermal growth factor receptor2 (HER2), the functional crosstalk between ER and HER2 etc. In recent period, combination chemotherapy is a common clinical strategy adopted to treat cancer or to overcome drug resistance (e.g. combination of anticancer drug with cytotoxic drug). Hence, novel potent anti-breast cancer drug that has selective tissue effects with new mechanism are current need in drug discovery. Currently, very few drugs of cancer are available and they also have resistance in patient. So by accepting this challenge, we synthesized hybrid molecules as a leading solution for the cancer. Hybrid molecules play dual role and reduce side effect¹. Chrysin molecule is naturally occurring drug with anticancer activity. Comparatively, it has fewer side effects than the present drugs. If these side effects occur in chrysin, they will be overcome by connecting it with dihydropyrimidinone like skeleton.

Monastrol is well known drug having activities like anti-inflammatory, anticancer, antifungal, prosthetic hyperplasia, antitumor etc. It is known as novel cell permeable molecule that causes mitotic arrest by blocking bipolar mitotic spindle in mammalian cells. It is first mitotic kinesin Eg5 also called as kinesin 5 or kinesin spindle protein (KSP) inhibitor causing a specific and reversible cell cycle block¹¹⁻¹³. Now days, it is considered as new lead in the synthesis of anticancer drugs as it inhibits mitotic kinesin Eg5 motor protein¹². Dihydropyrimidinone ring also inhibits inflammation related neurotoxicity which is caused by most of anticancer drugs. Hence, we prepared flavone-Dihydropyrimidinone hybrid molecules which will give more positive results and fewer side effects in the field of anticancer^{1,14}.

Experimental

Synthetic route for the synthesis of chrysin derivatives and flavone-dihydropyrimidinone (monastrol) derivatives are illustrated in (**Scheme 1, 2, 3 & 4**).

For the synthesis of Flavone various methods are known. But the most popular methods are Baker – Venkatraman (**Scheme 1**) and Claisen-Schmidt (**Scheme 2**)¹²⁻²². Chrysin is synthesized by using both methods (scheme1&2). In Claisen Schmidt reaction (**Scheme 1**) 2, 4, 6-trihydroxy-acetophenone (1mole), benzaldehyde (1.2 mole) & KOH (2 mole) is dissolved in ethanol as a good solvent for this reaction. Reaction mixture is refluxed under stirring till completion of TLC. After reaction is complete it poured on to crush ice. Stirred and dilute HCl is added to neutralize the reaction mixture. Filtered and recrystallized by ethanol. The compound formed is chalcone. Chalcone converted into flavone by oxidative coupling method. Chalcone reacts with 10 mole % iodine in DMSO as a solvent. Reaction is carried at 115⁰C temperature, (**Scheme 2**).

Reaction is completed within 30-45 minutes. Mixture of Benzene & ethyl acetate is used as solvent system for TLC (15). The flavone formed in above reaction is known as chrysin (**Scheme 2**). Chrysin hydrogen is replaced by formyl group by well known Duff reaction²³⁻²⁸. The Duff reaction²³⁻²⁸ on chrysin is carried out by using hexamethylenetetramine (HMTA) & acetic acid by suitable molar ratio as mentioned in literature (scheme 3). Reaction was carried out at suitable temperature. Reaction is monitored by TLC. It takes 8 hours to complete the reaction.¹² Reaction mixture is hydrolyzed by using dilute HCl (10%) and further heated for 30 minute. 5,7dihydroxy-8-formyl,flavone(chrysin) precipitate out.Filter and purified with suitable solvent. This compound also called 8-formyl chrysin. Finally, the novel compounds were synthesized by Biginelli reaction. Biginelli reaction is multi-component reaction. Here we used one pot strategy to synthesis novel compounds (**Scheme 4**).

In this reaction 8-formyl chrysin, 1, 3-diketones and urea/thiourea were dissolved in ethanol. To this reaction mixture catalytic amount of pTSA was added. Reaction mass refluxed for 9-10 hours to yield flavone-dihdropyrimidinone novel hybrid molecules (1) (**Scheme 4**).

*Representative procedure for the (4a)*²³⁻²⁸

Chrysin (1 equiv.) and hexamethylenetetramine (1 equiv.) were dissolved in acetic acid and the solution was heated 90⁰c for 6 hrs.Then check the TLC.After completion of TLC.Reaction mixture

cooled to RT. Then add 10% solution of HCl for the hydrolysis purpose. Heat and stir for 30 minute. Solid is formed. Filter and purify with AcOH. Product further purified over column (60-120 mesh).

Representative procedure for compound (5a).³¹⁻³²

8- Formylchrysin (1 mmole), urea/thiourea (1.5mmole), /Ethyl/methyl acetoacetate/acetyl acetone (1mmole) were charged in round bottom flask. Then add small amount ethanol to dissolve the components. Stir for 5 minute and add catalytic amount of pTSA. Reflux the reaction mass for appropriate time till the completion of TLC. Solid appears filter and dry. Purify with methanol. Compound was identified by IR & NMR Spectral data.

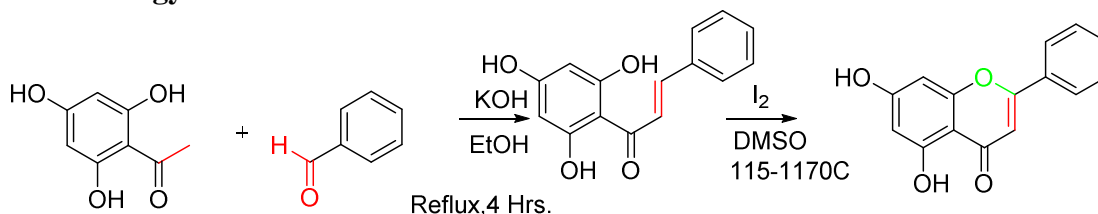
Results & Discussion

All the new synthesized compounds (shown in table 2, 5a-f) were characterized using IR and NMR spectral data (see the supporting information). Spectral data of all synthesized hybrid compounds were in good agreement with the proposed structures. IR spectrum reveals the presence of N-H bond at (3400-3580 cm^{-1}), C=O and C=S (1690-1715 cm^{-1}) in the novel synthesized hybrid compounds. This was further conformed by HNMR spectrum which displayed singlet around δ (4.2 - 5 ppm) for chiral CH in Dihydropyrimidinone (monastrol) ring respectively.

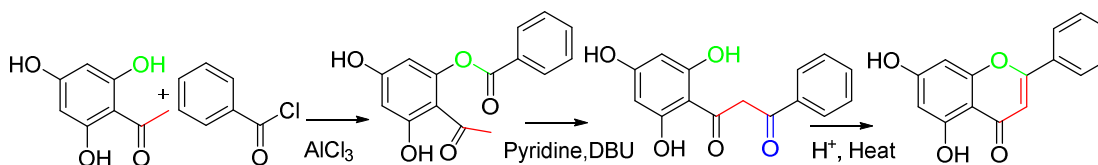
Conclusion

In summary, we have developed novel drug molecules with eco friendly process (Ultrasound condition) (table 1). We tried this reaction under ultra sound process (**Scheme 4**). Surprisingly, we got very good results. We first time used ultra sound for the Duff formylation and for the synthesis of novel flavone-Dihydropyrimidinone (monastrol) drug analogues. We set up Duff formylation by AcOH as well as TFA (trifluoroacetic acid) under Ultra sound (**Scheme 4**). We optimize the process for thermal and ultrasound. (**Table1**).

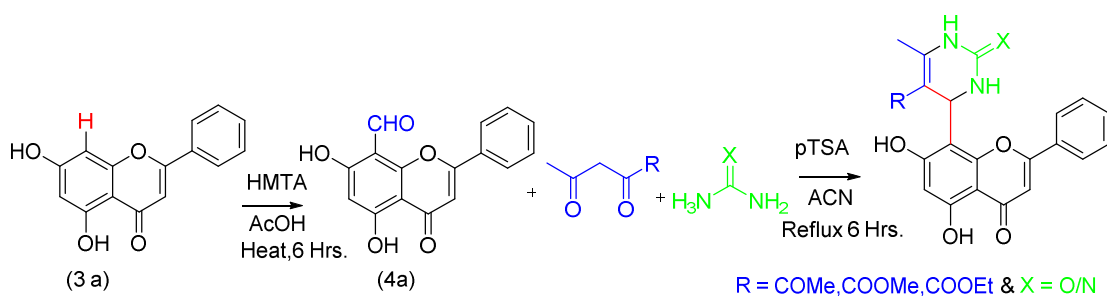
Synthetic Methodology



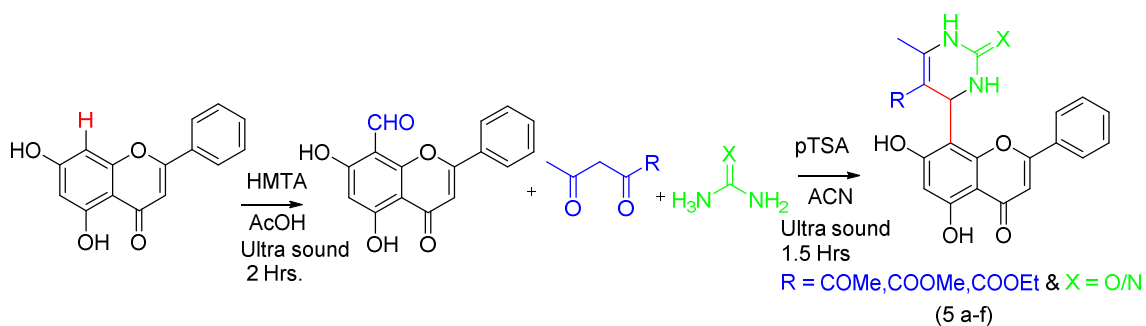
Scheme1. Claisen-Schmidt Synthesis & Oxidative Coupling^{1, 20}.



Scheme2. Baker-Venkatraman Synthesis of Chrysin^{9, 10}



Scheme3. Duff formylation and Biginelli reaction ²³⁻²⁸ (Thermal Condition)



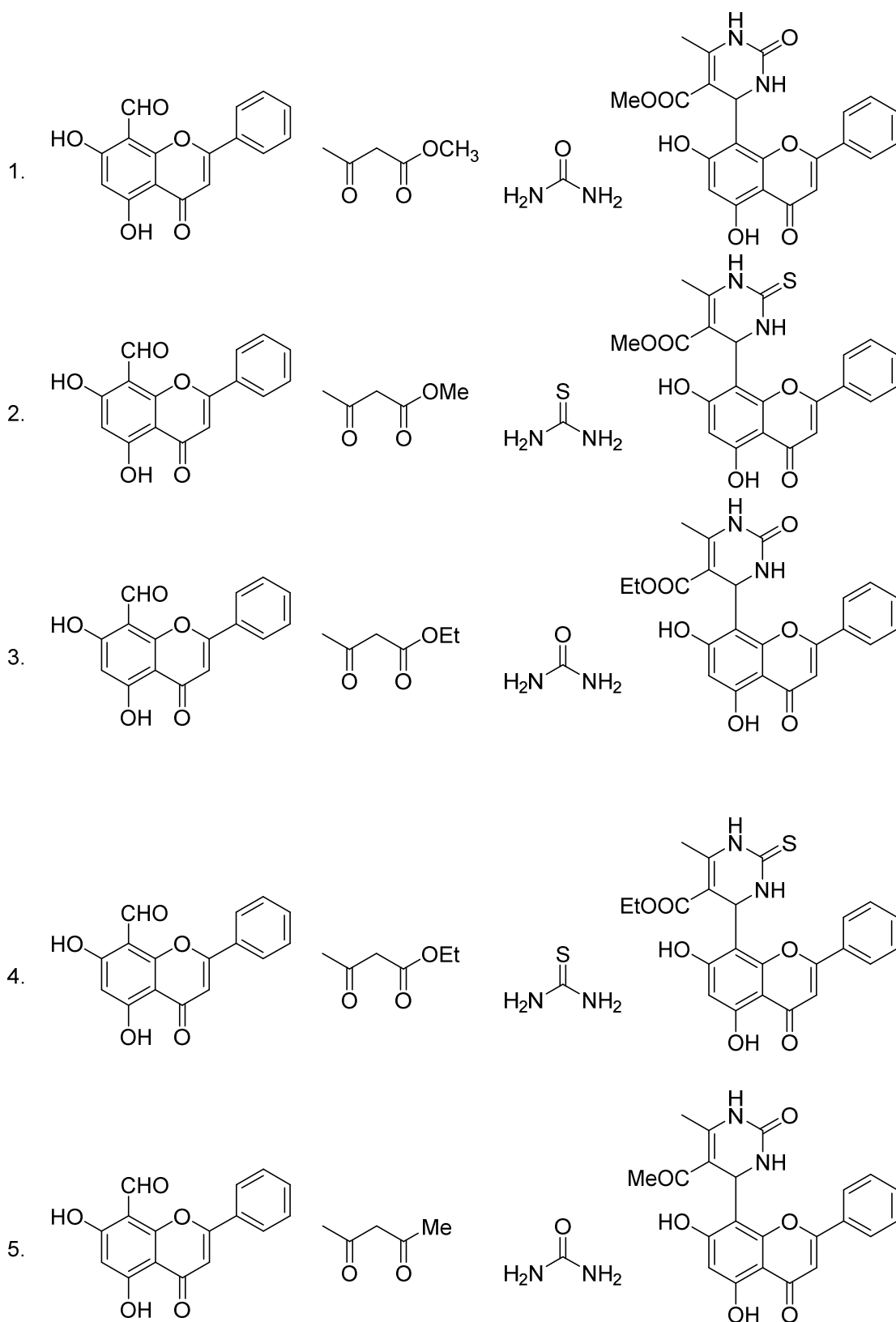
Scheme4. Duff Formylation and Biginelli Reaction ²³⁻²⁸ (Ultra Sound condition)

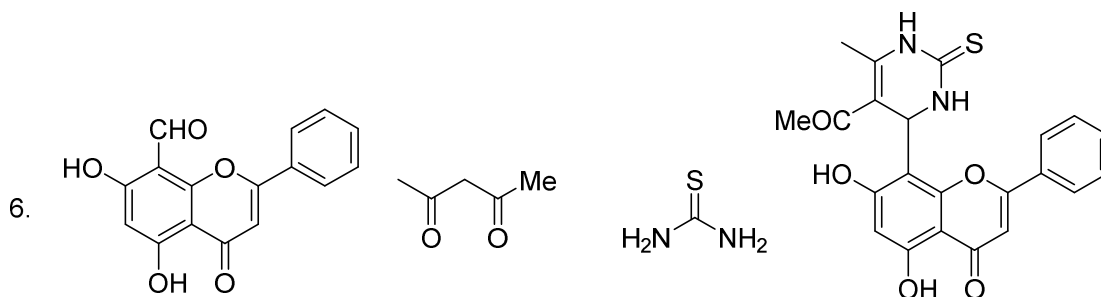
Table1. Reaction condition for Biginelli reaction (5a-f) produced via (Scheme 4.)

Entry	Thermal (Hrs.)	Ultrasound (Hrs.)	Yield (%)
(5a)	8	1.5	50
(5b)	9	1.5	60
(5c)	8	1.5	60
(5d)	8	2.0	60
(5e)	7	1.5	50
(5f)	10	2.5	60

Table2. Chrysin-monastrol hybrid analogues (5a-f) produced via Biginelli reaction condition.

Entry	Reactant 1	Reactant 2	Reactant 3	Product





Supplementary data

Supplementary data of all the compounds associated with this article is available as supplementary data

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