Monatshefte für Chemie - Chemical Monthly
https://doi.org/10.1007/s00706-020-02692-5

ORIGINAL PAPER

One-pot three-component reaction for facile and efficient green synthesis of chromene pyrimidine-2,4-dione derivatives and evaluation of their anti-bacterial activity

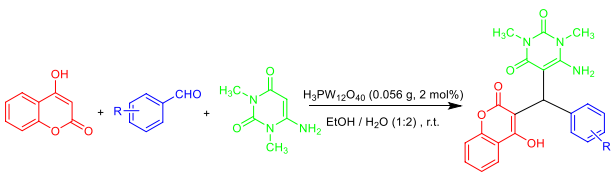
Habib Ollah Foroughi[1] · Mansoureh Kargar[1] · Zahra Erjaee[2] · Elham Zarenezhad[3]

Received: 4 August 2020 / Accepted: 14 September 2020

Abstract

A facile and highly efficient one-pot three-component synthesis of chromene pyrimidine-2,4-dione derivatives from available substrates catalyzed by heteropoly acid ($H_3PW_{12}O_{40}$) is described. In this protocol, the reaction of diverse aldehydes, 4-hydroxycoumarin, and 4-amino-1,3-dimethyluracil in the mixture of EtOH / H_2O (1:2) at room temperature in the presence of heteropoly acid ($H_3PW_{12}O_{40}$) as a highly efficient catalyst affords the corresponding chromene pyrimidine-2,4-dione in good to excellent yields. Operational simplicity, low cost, eco-friendly with the environment, easy workup and purification, short reaction time, high yield of products, green media of the reaction and mild conditions are the advantages of this method. The in vitro antibacterial activities of title compounds were obtained against *Escherichia coli* (ATTC 35218) and *Staphylococcus aureus* (ATCC 6538) as Gram-negative and Gram-positive bacteria, respectively, and their activities were compared to gentamycin and penicillin as reference drugs.

Graphic abstract



Keywords Multicomponent reaction · Heterocycles · Catalysts · Aldehydes

Introduction

Phosphotungstic acid is a heteropoly acid (HPA) with the chemical formula $H_3PW_{12}O_{40}$ is a well-known catalyst and has attracted much attention because of its redox and acidic properties [1]. HPAs have received considerable attention as an inexpensive, eco-friendly catalyst with pseudo liquid behavior, good thermal stability, which is easy to prepare and recyclable. It is widely used as an acid catalyst for different reactions in homogenous liquid media [2].

Design and synthesis of facile and efficient chemical reactions that build up maximum structural diversity and complexity with a minimum number of synthetic steps to provide chemical compounds with interesting properties is a key challenge of modern drug development [3]. Multicomponent

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00706-020-02692-5) contains supplementary material, which is available to authorized users.

✉ Habib Ollah Foroughi
foroughihabib@ymail.com

[1] Department of Science, Fasa Branch, Islamic Azad University, P. O. Box: No. 364, Fasa, Fars 7461713591, Iran

[2] Department of Food Science and Technology, Fasa Branch, Islamic Azad University, P. O. Box: No. 364, Fasa, Fars 7461713591, Iran

[3] Noncommunicable Diseases Research Center, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran

Published online: 14 October 2020

reactions (MCRs) are regarded as powerful synthetic tools for drug discovery [4]. MCRs are a synthetic methodology in which three or more different starting materials react to produce complex compounds in a one-pot setup efficiently and with good atom economy [5].

Chromene (benzopyran) is an attractive class of heterocyclic compounds because of their wide range of biological applications such as antivascular, antitumor [6], TNF-α inhibitor [7], antimicrobial [8], antifungal, anticoagulant, antioxidant, anti-viral, anti-inflammatory, anti-helminthic, herbicidal, estrogenic, antitubercular, anti-HIV, and anticonvulsant activity [9]. Pyrimidine derivatives are a major and interesting group of heterocyclic drugs, they are well known in synthetic organic chemistry as well as pharmaceutical chemistry. Additionally, pyrimidine derivatives have attracted increasing interest owing to the presence of the heterocycles in the RNA and/or DNA scaffold [5, 10]. Heterocyclic compounds containing pyrimidine structure are known as bioactive compounds, exhibiting a broad spectrum of biological activities, such as anti-proliferative, antiviral, antitumor, anti-inflammatory, antibacterial, antifungal, and anti-tubercular properties. Also, the pyrimidine ring is found in vitamins such as thiamine, riboflavin, barbitone, and folic acid [11]. Uracil is known as one of the nucleobases in the RNA, it is naturally and common occurring pyrimidine derivative can be used as a pharmaceutical in the drug discovery process. In addition, many effective antiviral and anticancer drugs contain uracil scaffold [12]. Pyrimidines are most interesting class of antibacterial compound which have made a major impact on the field of antibacterial chemotherapy especially in the past few years. Many groups of chemotherapeutic agents containing pyrimidine nucleus are in clinical use as antibacterial, as can be seen in Fig. 1. Trimethoprim is a well-known synthetic antibiotic that blocks the production of tetrahydro folic acid (THF) [13], piromidic acid is another synthetic antibacterial agent which is used to treat infections caused by gram-negative bacteria and staphylococci [14], and sulfadiazine [15] is known as an antibiotic, this drug is the treatment of choice for toxoplasmosis.

Pyrimidines are used for the synthesis of deoxyribonucleotides which are found in the DNA structure. The analogues of these nucleotide precursors have shown anticancer activity. The mechanism actions of antimetabolic pyrimidine antineoplastic drugs are well known and very similar [16]. When these compounds diffuse into cells, enzymatic reaction of the pyrimidine metabolic pathway converts them to analogues of cellular nucleotides. In conclusion, the metabolites produced by these reactions can inhibit one or more enzymes responsible for DNA synthesis. This can cause DNA destruction and induction of apoptosis [17].

One of the serious medical problems humanity faces today is microbial resistance. Additionally, the levels rate of resistance is increasing in traditional antibiotics. So, the development and discovery of novel and potent antibacterial and antifungal medicines with an effective and possibly novel mechanism of action become essential duties for antibiotic research and development programs [18–22].

Recently, a few procedures have been developed for the synthesis of chromene pyrimidine-2,4-dione derivatives via three-component condensation reaction of aldehyde, 4-hydroxycoumarin, and 4-amino-1,3-dimethyluracil using various catalysts, such as $Zr(HSO_4)_4$ [23], Fe_3O_4@TiO_2 nanocomposite [24], L-proline [25], organocatalyst [26], and ZnO nanoparticles [27]. In addition, Cai and co-worker have reported a method without any catalyst in poly(ethylene glycol) 200/H_2O [28]. However, despite their own merits some of the reported procedures suffer from certain drawbacks such as prolonged reaction time, the lower yield of products, expensive catalysts, relatively harsh reaction conditions, tedious workup and purification. Therefore, it is quite clear that developing new methodologies in terms of operational simplicity, less expensive catalyst, mild reaction conditions and applicable to a wide range of substrates could be desirable. In continuation of our research works on the development of new procedures for the synthesis of new heterocyclic compounds [29–31], herein, we wish to study the reaction of diverse aldehydes with 4-hydroxycoumarin and 4-amino-1,3-dimethyluracil in the presence of $H_3PW_{12}O_{40}$ as an efficient catalyst in green media to synthesize some chromene pyrimidine-2,4-dione derivatives (Scheme 1) and evaluated anti-bacterial activity.

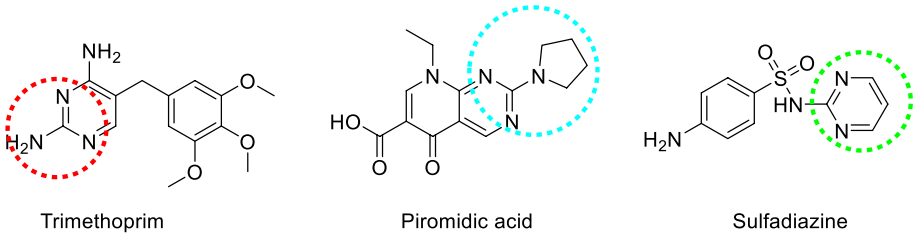
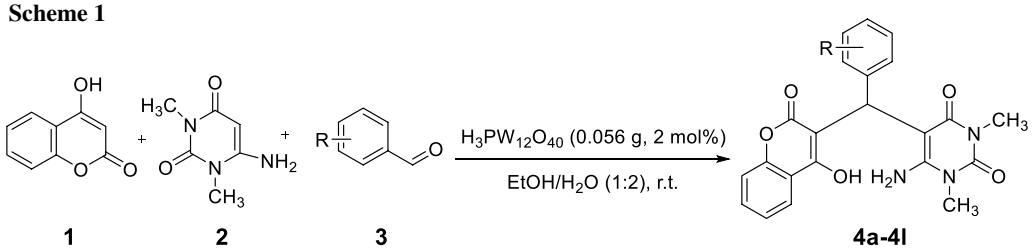


Fig. 1 Antibacterial compounds containing pyrimidine structure

Scheme 1



Results and discussion

Chemistry

In this study, facile and efficient conversion of diverse aldehydes, 4-hydroxycoumarin, and 4-amino-1,3-dimethyluracil in the presence of a catalytic amount of $H_3PMo_{12}O_{40}$ for preparing chromene pyrimidine-2,4-diones in one-pot is presented. At first, we performed the reaction between benzaldehyde, 4-hydroxycoumarin, and 4-amino-1,3-dimethyluracil as a model reaction to afford 6-amino-5-[(4-hydroxy-2-oxo-2*H*-chromen-3-yl)(phenyl)methyl]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**4a**) (Table 1). In our initial selection, we tried this model reaction without any catalyst in the mixture of EtOH / H_2O (1:2) at reflux condition but the desired product **4a** obtained in trace amount after 120 min (Table 1, entry1). When the reaction was accomplished in the presence of 2 mol% molybdatophosphoric acid ($H_3PMo_{12}O_{40}$) the yield of the product improved to 85% after 60 min under reflux (Table 1, entry 2). As can be seen in Table 1, the yield of desired product was increased to 93% when 2 mol% tungstophosphoric acid ($H_3PW_{12}O_{40}$) was added to the reaction media as a catalyst at room temperature (Table 1, entry 4). In addition, the yield of the product and reaction time was changed to some extent by increasing the amount of tungstophosphoric acid ($H_3PW_{12}O_{40}$) under the same condition (Table 1, entries 5, 7). Further attempts to decrease catalyst loading lead to lower product yields and longer reaction times (Table 1, entry 6). To evaluate the effect of the reaction temperature, the model reaction was also carried out under reflux. It increases the product yield and the reaction rate to a certain extent (Table 1, entry 3). In continuation of our study, the model reaction was performed in different solvents such as H_2O, EtOH, EtOH / H_2O (1:2), CH_2Cl_2, and CH_3CN. According to the obtained results, the mixture of EtOH / H_2O (1:2) was selected as the best solvent (Table 1, entries 4 and 8–12). However, remarkable yields were obtained using mixtures of ethanol and water

Table 1 Optimization of three-component reaction between benzaldehyde, 4-hydroxycoumarin, and 4-amino-1,3-dimethyluracil

Entry	Catalyst (mol%)	Solvent	Temp /°C	Time /min	Yield /%[a]
1	None	EtOH/H_2O (1:2)	reflux	120	Trace
2	$H_3PMo_{12}O_{40}$ (2)	EtOH/H_2O (1:2)	reflux	60	85
3	$H_3PW_{12}O_{40}$ (2)	EtOH/H_2O (1:2)	reflux	12	97
4	**$H_3PW_{12}O_{40}$ (2)**	**EtOH/H_2O (1:2)**	**r.t**	**15**	**93**
5	$H_3PW_{12}O_{40}$ (3)	EtOH/H_2O (1:2)	r.t	13	95
6	$H_3PW_{12}O_{40}$ (1)	EtOH/H_2O (1:2)	r.t	70	65
7	$H_3PW_{12}O_{40}$ (4)	EtOH/H_2O (1:2)	r.t	12	96
8	$H_3PW_{12}O_{40}$ (2)	MeCN	reflux	100	76
9	$H_3PW_{12}O_{40}$ (2)	CH_2Cl_2	reflux	120	67
10	$H_3PW_{12}O_{40}$ (2)	EtOH	reflux	25	91
11	$H_3PW_{12}O_{40}$ (2)	EtOH	r.t	30	90
12	$H_3PW_{12}O_{40}$ (2)	H_2O	reflux	60	85
13	SSA (25)	EtOH/H_2O (1:2)	reflux	120	73
14	*p*-TSA (58)	EtOH/H_2O (1:2)	reflux	150	67
15	$FeCl_3$ (30)	EtOH/H_2O (1:2)	reflux	120	42
16	$ZnCl_2$ (40)	EtOH/H_2O (1:2)	reflux	153	35

Amount of reagents in all reactions: benzaldehyde (1 mmol), 4-hydroxycoumarin (1 mmol), 4-amino-1,3-dimethyluracil (1 mmol), and 5 cm^3 solvent

The best optimized condition for the reaction are highlighted with bold

[a]Isolated yield

(Table 1, entries 10–12). During optimization of the reaction conditions, various catalysts were used to compare catalytic activity of $H_3PW_{12}O_{40}$. As shown in Table 1, silica sulfuric acid (SSA) and *p*-toluenesulfonic acid showed a significant reactivity under the same reaction conditions (Table 1, entries 13, 14). Also, $FeCl_3$ and $ZnCl_2$ as Lewis acid catalysts were tested in the model reaction and the products obtained with 42% and 35% yields, respectively, after long reaction time (Table 1, entries 15 and 16). Thus, $H_3PW_{12}O_{40}$ was chosen as the best catalyst for three-component reaction between aldehydes, 4-hydroxycoumarin and 4-amino-1,3-dimethyluracil.

To illustrate the scope of the designed protocol for the preparation of chromene-pyrimidine 2,4-dione derivatives, we extended the optimized reaction conditions for various aromatic and heteroaromatic aldehydes and the results are summarized in Table 2. As depicted in Table 2, it seems that the aromatic aldehydes with electron releasing groups reduced the yield and reaction rate to some extent (Table 2, entries 6–9). In the other hand, aromatic aldehydes with electron-withdrawing groups increased the reaction rate and the yield of products to a certain degree (Table 2, entries 2–5 and 10, 11). In addition, we used pyridine-4-carbaldehyde as a heteroaromatic aldehyde in the reaction and the desired product obtained in 90% yield after 15 min (Table 2, entry 12). Some of the synthesized compounds were known and their identity was confirmed by a comparison of their melting points and their spectral properties with literature data [19–21]. In addition, some compounds (**4c**, **4 g**, and **4i**) were new and were characterized by spectroscopic techniques. Their structures were confirmed by IR, ^1H and ^{13}C NMR spectroscopic techniques, and elemental analysis. According to the obtained results, we propose the following mechanism for three-component coupling reaction between aldehyde, 4-hydroxycoumarin, and 4-amino-1,3-dimethyluracil using heteropoly acid (HPA) as catalyst (Scheme 2). At first, heteropoly acid ($H_3PW_{12}O_{40}$) promotes the aldehyde with protonation and then 4-amino-1,3-dimethyluracil reacts with promoted aldehyde and produces the Knoevenagel intermediate **I**. Next, intermediate **I** reacts with 4-hydroxycoumarin via Michael addition and creates the desired product.

Bactericide activity

The antimicrobial activity of the synthetized compounds is shown in Table 3. DMSO is used as a negative control. The

Scheme 2

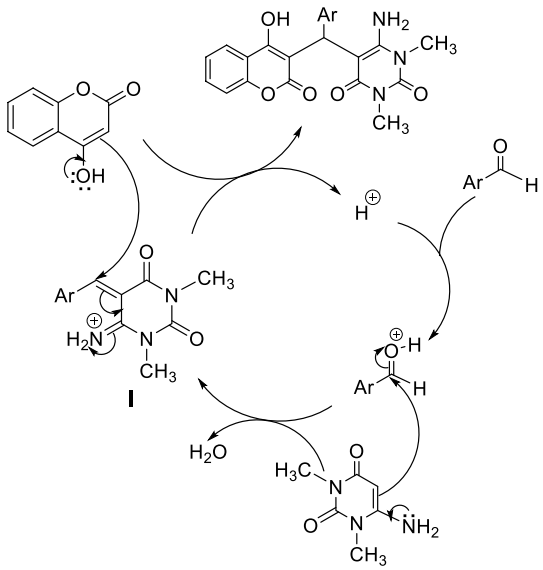


Table 2 Synthesis of chromene pyrimidine-2,4-dione derivatives via three component reaction

Entry	Aldehyde	Product	Time/min	Yield[a] /%	M.p. /°C	
					Found	Reported [Lit]
1	Benzaldehyde	**4a**	15	93	190–192	192–193 [19]
2	4-Chlorobenzaldehyde	**4b**	13	95	193–195	193–194 [19]
3	3-Chlorobenzaldehyde	**4c**	20	90	252–254	–
4	4-Bromobenzaldehyde	**4d**	18	89	235–237	233–234 [19]
5	4-Fluorobenzaldehyde	**4e**	12	96	245–247	248–250 [21]
6	4-Methylbenzaldehyde	**4f**	25	88	204–206	203–205 [19]
7	3-Methylbenzaldehyde	**4g**	17	90	170–173	–
8	4-Methoxybenzaldehyde	**4h**	25	86	199–202	205–207 [19]
9	4-*t*-Butylbenzaldehyde	**4i**	23	89	245–246	–
10	3-Nitrobenzaldehyde	**4j**	14	91	255–257	257–259 [19]
11	2- Nitrobenzaldehyde	**4k**	10	93	238–239	240–242 [20]
12	Pyridin-4-carbaldehyde	**4l**	15	90	226–228	227–229 [19]

Reaction conditions: aldehyde (1 mmol), 4-hydroxycoumarin (1 mmol), 4-amino-1,3-dimethyluracil (1 mmol), and 0.056 g $H_3PW_{12}O_{40}$ (2 mol%) in 5 cm^3 EtOH / H_2O (1:2)

[a]Isolated yield

Table 3 MIC and MBC of synthetic compounds against *Staphylococcus aureus* and *Escherichia coli*

Product	MIC /μg cm^{-3}		MBC /μg cm^{-3}	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
4a	250	500	500	500
4b	250	500	500	500
4c	250	500	500	500
4d	125	250	250	250
4e	125	250	500	500
4f	125	250	250	250
4g	125	250	500	250
4h	125	500	500	1000
4i	250	500	500	1000
4j	62.5	500	125	1000
4k	62.5	500	125	1000
4l	62.5	500	125	1000
Penicillin	125	250	500	1000
Tetracycline	1.95	125	125	500
DMSO	500	500	1000	1000

MIC and MBC of the compounds are compared to gentamycin and penicillin as reference drugs.

In general, the effect of these compounds on Gram-negative bacteria (*Escherichia coli*) was less than that of Gram-positive bacteria (*Staphylococcus aureus*) (Table 3). This maybe due to the differences in the bacterial cell wall; Gram-negative bacteria in have an outer membrane in addition to the peptidoglycan wall. Gram-positive bacteria, however, have only peptidoglycan walls [32]. The bacterial cell surface has a negative charge, which is more available in gram-positive bacteria. The negative charge on the cell surface gives the bacterium an attractive charge. Therefore, it is expected that with drawing electron groups such as halogens, nitro groups and also the pyrimidine rings be a good factor for destroying these bacteria. The inhibitory effect and bactericidal effect of some of the compounds in Tables 1 and 2 are better than the reference antibiotic used in this study. By comparing the compounds, it is clear that the halogens did not significantly affect the MIC and MBC. Some even have no inhibition as compared to the negative control. Only **4d** had the same effect as penicillin. Compounds **4i**, **4j**, **4 l** showed inhibition at concentrations of 62.5 mg/cm^3, which exhibited better antimicrobial properties than penicillin. This may be due to the strong with drawing electron groups such as NO_2, which has been able to establish surface attraction with bacteria and prevent them from growing.

Conclusions

In summary, we explained the design and synthesis of a series of chromene pyrimidine-2,4-dione derivatives **4a**–**4 l** as a new type of antibacterial agent and evaluation of their antibacterial activity. In this procedure, the reaction of diverse aldehydes, 4-hydroxycoumarin, and 4-amino-1,3-dimethyluracil in the presence of heteropoly acid in green media that lead to the synthesis of a series of chromene pyrimidine-2,4-dione derivatives has been reported. We have now realized that the reaction can be carried out much more rapidly and in good to excellent yields using $H_3PMo_{12}O_{40}$ as a catalyst. Bactericide activity of this result confirms that Compounds **4i**, **4j**, **4 l** showed inhibition at concentrations of 62.5 mg/cm^3, which exhibited better antimicrobial properties than reference drugs.

Experimental

Chemicals were purchased from Fluka, Merck, and Aldrich chemical companies. ^{1}H NMR and ^{13}C NMR were run on a Bruker Avanc DRX (250 MHz) in pure deuterated $CDCl_3$ and DMSO-d_6 solvents with tetramethylsilane (TMS) as internal standards. Chemical shifts are given in the δ scale in part per million (ppm) and J in Hz. The bonds are assigned as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), doublet of doublet (dd), doublet of triplet (dt) and complex. FT-IR spectroscopy (Shimadzu FT-IR 8300 spectrophotometer), was employed for characterization of the compounds. Microanalyses were performed on a Perkin-Elmer 240-B microanalyzer. Melting points were determined in open capillary tubes in a Büchi B 545 apparatus. The reaction monitoring was accomplished by TLC on silica gel PolyGram SILG/UV254 plates).

General procedure for the synthesis of chromene pyrimidine-2,4-dione derivatives in presence of $H_3PW_{12}O_{40}$ catalyst

To a stirred solution of 0.162 g 4-hydroxylcoumarin (1 mmol), aldehyde (1 mmol), and 0.155 g 4-amino-1,3-dimethyluracil (1 mmol) in the mixture of ethanol/H_2O (1:2, 5 cm^3) was added 0.056 g $H_3PW_{12}O_{40}$ catalyst (2 mol%) and then the reaction mixture was stirred at room temperature for the indicated time in Table 2. After completion of the reaction, as indicated by TLC (eluent: EtOAc/*n*-hexane), the reaction mixture was filtered off and washed with H_2O (2 × 5 cm^3) and hot EtOH (5 cm^3) and the pure products were obtained **4a**–**4 l** (yields 86–96%).

6-Amino-5-[(3-chlorophenyl)(4-hydroxy-2-oxo-2*H*-chromen-3-yl)methyl]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (4c, $C_{22}H_{18}ClN_3O_5$) White solid; yield 0.396 g (90%); m.p.: 252–254 °C; ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 3.13$ (s, 3H, N-CH_3), 3.36 (s, 3H, N-CH_3), 5.63 (s, 1H, CH), 7.12 (d, 1H, $J = 7$ Hz, Ar–H), 7.19–7.25 (m, 3H, Ar–H and NH_2), 7.28–7.36 (m, 3H, Ar–H and NH_2), 7.41 (d, 1H, $J = 8.25$ Hz, Ar–H), 7.62 (dt, 1H, $J_1 = 8.5$ Hz, $J_2 = 1.25$ Hz, Ar–H), 7.83 (dd, 1H, $J_1 = 8$ Hz, $J_2 = 1.25$ Hz, Ar–H), 13.97 (br, 1H, OH) ppm; ^{13}C NMR (62.5 MHz, DMSO-d_6): $\delta = 28.18$ (N-CH_3), 30.50 (N-CH_3), 35.90 (CH), 86.31, 104.32, 116.08, 116.84, 123.68, 124.25, 125.20, 125.75, 126.27, 129.78, 132.42, 132.95, 141.25, 149.98, 151.92, 155.17, 163.76 (C = O), 164.01 (C = O), 165.58 (C = O) ppm; IR (KBr): $\overline{\nu} = 3386$, 3354, 3232, 1701, 1643, 1604, 1562, 1508, 1353, 1203, 1064, 894, 775 cm^{-1}.

6-Amino-5-[(4-hydroxy-2-oxo-2*H*-chromen-3-yl)(*m*-tolyl)methyl]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (4g, $C_{23}H_{21}N_3O_5$) White solid; yield 0.377 g (90%); m.p.: 170–173 °C; ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 2.29$ (s, 3H, CH_3), 3.33 (s, 3H, N-CH_3), 3.56 (s, 3H, N-CH_3), 5.71 (s, 1H, CH), 6.41 (s, 2H, NH_2), 7.00 (t, 3H, $J = 8.75$ Hz, Ar–H), 7.17 (t, 1H, $J = 7.5$ Hz, Ar–H), 7.32 (t, 2H, $J = 8.25$ Hz, Ar–H), 7.54–7.60 (m, 1H, Ar–H), 8.00 (d, 1H, $J = 8$ Hz, Ar–H), 13.42 (s, 1H, OH) ppm; ^{13}C NMR (62.5 MHz, DMSO-d_6): $\delta = 21.67$ (CH_3), 28.71 (N-CH_3), 29.80 (N-CH_3), 36.41 (CH), 86, 104.39, 116.22, 123.33, 124.27, 124.55, 126.86, 127.13, 128.26, 132.21, 133.52, 137.33, 144.69, 150.80, 152.36, 154.41, 164.64 (C = O), 165.11 (C = O), 167.66 (C = O) ppm; IR (KBr): $\overline{\nu} = 3398$, 3332, 3228, 1701, 1616, 1559, 1506, 1205, 1047, 763 cm^{-1}.

6-Amino-5-[(4-*tert*-butylphenyl)(4-hydroxy-2-oxo-2*H*-chromen-3-yl)methyl]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (4i, $C_{26}H_{27}N_3O_5$) Cream solid; yield 0.41 g (89%); m.p.: 245–246 °C; ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 1.22$ (s, 9H, *t*-Bu), 3.14 (s, 3H, N-CH_3), 3.37 (s, 3H, N-CH_3), 5.57 (s, 1H, CH), 7.04 (d, 2H, $J = 8.25$ Hz, Ar–H), 7.23 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.31 (s, 2H, NH_2), 7.35–7.44 (m, 2H, Ar–H), 7.60–7.67 (m, 1H, Ar–H), 7.83 (dd, 1H, $J_1 = 7.87$ Hz, $J_2 = 1.5$ Hz, Ar–H), 13.96 (s, 1H, OH) ppm; ^{13}C NMR (62.5 MHz, DMSO-d_6): $\delta = 28.18$ (N-CH_3), 30.51 (N-CH_3), 31.12 (3 CH_3), 33.92 (C-3CH_3), 35.59 (CH), 86.64, 104.73, 116.09, 116.87, 123.65, 124.27, 124.82, 126, 132.37, 135.08, 147.81, 150, 151.87, 155.05, 163.67 (C = O), 164 (C = O), 165.74 (C = O) ppm; IR (KBr): $\overline{\nu} = 3398$, 3244, 2981, 1685, 1639, 1608, 1569, 1504, 1203, 1064, 760 cm^{-1}.

Acknowledgements We would like to offer our special thanks to Islamic Azd University Fasa branch for partial support of this research work.

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