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ARTICLE

An efficient synthesis of 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-ylidene) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones and investigation of their interactions with β-lactoglobiulin

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An efficient method for synthesis of 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-ylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones has been developed by treatment of 2-hydroxychalcones and 1,3-dimethylbarbituric acid in refluxing toluene in presence of amberlyst-15 as catalyst in air. The process involves the occurrence of a domino sequence of Michael addition, cyclization and aerial oxidation. The compounds synthesized showed an interesting property of free rotation around the bond linking the two heterocyclic moieties and three of them were found to show binding property with the milk protein β–lactoglobulin (β-lg). DFT and docking (with β-lg) studies of one of the compounds have been done.

Introduction

Chromenes are well-known for their important biological activities like anticancer, antitumor, antidiabetic, antioxidant, anti-inflammatory, antianxiety, antitubercular, antitrypanosomal, antiviral, antidyslipidemic, antihelmenthic, anticonvulsant, anti-depressant, antiproliferative, anti-Alzheimer's, anti-Perkinson's anti-Huntington's, anti-HIV, anticoagulant, antianaphylatic, antibacterial/antimicrobial, monoamineoxidase (MAO) inhibitor, fungicidal, diuretic, hypothermal, vasodilatory, TNF-α inhibitor, estrogenic, herbicidal and analgesic activitities[1]. Besides, some of them have drawn attention as photochromic materials[2]. Though both the 2*H*- and 4*H*-chromene moieties are often found to be present in the structural motif of a variety of natural products[1a-c,3], development of synthetic routes to differently substituted chromenes is still an important area of research for organic chemists[1a-c,4].

In the late 19th and early 20th centuries barbiturates (C-5 substituted barbituric acids) became very well-known for their sedative and hypnotic properties[5,6], though barbituric acid itself is biologically inactive[5,6]. Throughout the 20th century, 50 out of more than 2500 barbiturates synthesized were found to be used often for clinical purpose and many of them are used till today[7]. The pharmacological properties of barbiturates hugely depend on the substituent(s) present at their C-5[7]. There are a number of strategies known in literature to get C-5 substituted barbiturates[8]. One of the methylene hydrogens of barbituric acid is quite acidic (pKa = 4.03)[9]. The acidic property of these methylene hydrogens is commonly utilized to construct C-5 substituted barbiturates through reactions like alkylation, Knoevenagel condensation, Michael addition etc.[8]

Construction of molecules by joining two or more biologically active moieties has been growing as an important strategy for getting access to newer molecules of pharmaceutical interest[10]. Recently, we have developed methods for synthesis of such molecules where an indole, cyclopentan-1,3-dione or indane-1,3-dione moiety is attached to 2-chromenes[11] at the 4-position of the latter. In case of synthesis of 2-aryl-4-(indol-3-yl)-4*H*-chromenes (**1**), an acid-catalysed condensation of a 2-hydroxychalcone (**3**) and an indole by use of amberlyst-15 as catalyst was the method[11a]. However, in the synthesis of 2-(2-phenyl-4*H*-chromen-4-ylidene)-2*H*-indene-1,3-diones (**2**), analogous condensation of the two constituent units was followed by an interesting aerial oxidation[11b]. Considering the versatile biological activities of 5-substituted barbiturates[5,6] and 2-chromenes[1-4], we got interested to synthesise compounds incorporating these two moieties. So, we undertook

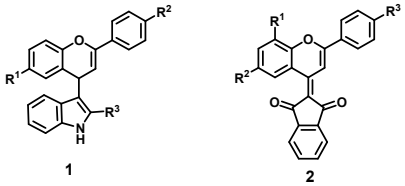
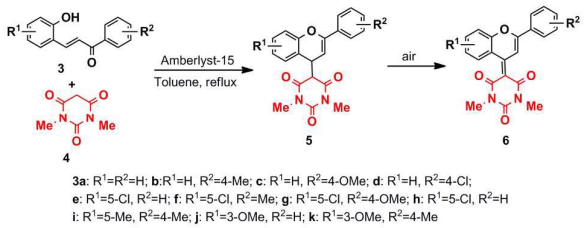


Fig. 1 Some 4*H*-chromenes attached to a biologically important moiety at the 4-position.



3a: R^1=R^2=H; **b**:R^1=H, R^2=4-Me; **c**: R^1=H, R^2=4-OMe; **d**: R^1=H, R^2=4-Cl; **e**: R^1=5-Cl, R^2=H; **f**: R^1=5-Cl, R^2=Me; **g**: R^1=5-Cl, R^2=4-OMe; **h**: R^1=5-Cl, R^2=H **i**: R^1=5-Me, R^2=4-Me; **j**: R^1=3-OMe, R^2=H; **k**: R^1=3-OMe, R^2=4-Me

Scheme 1 Novel synthesis of 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-ylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones.

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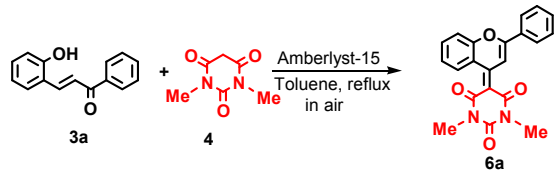
the present work, wherein a 2-hydroxychalcone (**3**) was made to undergo reaction with barbituric acid or its 1,3-dimethyl derivative in presence of amberlyst-15 as catalyst under aerial condition. Though the reaction with barbituric acid did not give any impressive result, that with its 1,3-dimethyl derivative yielded an interesting class of compounds, *viz.*, 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-ylidene)-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**6**) in good yield through the involvement an aerial oxidation in the last step (Scheme 1). Three compounds of the series **6** have been found to possess an interesting binding ability with the milk protein β–lactoglobulin (β-lg). DFT and docking studies of one compound (**6a**) with β-lg have been done to get an insight about its structural features and its binding site with the said protein. All these results are presented herein.

Results and discussion

Barbituric acid, a highly polar compound, is insoluble in most of the common organic solvents and also in water. The main problem in carrying out the reaction between a 2-hydroxychalcone (**3**) and barbituric acid was to get a suitable solvent where both the components are soluble. Ethyl alcohol was found to be a solvent where both of them are somewhat soluble. Thus, we carried out reaction of each of the two 2-hydroxychalcones, (*E*)-3-(2-hydroxyphenyl)-1-phenylprop-2-en-1-one (**3a**) and (*E*)-1-(4-chlorophenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one (**3d**), with barbituric acid in ethanol using amberlyst-15 as catalyst, and here we got highly polar non-crystalline materials (sparingly soluble even in DMSO) which were difficult to purify and characterize. 1,3-Dimethylbarbituric acid (**4**), which is comparatively less polar than barbituric acid, was then chosen for the reaction and this study started by exploring the reaction of **3a** and **4** in toluene using amberlyst-15 , fused $ZnCl_2$ and anhy. $FeCl_3$ as catalysts under aerial condition (Table 1). Among these catalysts amberlyst-15 showed superior activity, and hence scope of the reaction using this catalyst in solvents of different polarities was investigated. Reaction in a highly polar solvent like water did not lead to the product **6a**, but when solvents like ethanol, isopropanol, THF, acetonitrile, dioxane, DMF and *o*-xylene were used, the yield of **6a** gradually improved (entries 9-16, Table 1). As toluene was found to be the best solvent for the reaction, optimization of catalyst load was then done (Table 1). Thus, when **3a** was allowed to react with **4** in the presence of amberlyst-15 (40 mg) in toluene under reflux for 8 h in open air, 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-ylidene)pyrimi-dine-2,4,6-(1*H*,3*H*,5*H*)-trione (**6a**) was formed in 75% yield, without formation of any 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-yl)pyrimi-dine-2,4,6-(1*H*,3*H*,5*H*)-trione (**5a**, **5** with R^1=R^2=H) (entry 18 in Table 1). The reaction was found to take place also in the absence of any catalyst, but significantly poor yield of product was obtained even in solvents like toluene or xylene under refluxing condition (entries 21 & 22, Table 1). After getting the optimised reaction conditions, the developed methodology was tested on a series of 2-hydroxychalcones (**3**) and 1,3-dimethylbarbituric acid (**4**) and the results are summarized in Table 2. 2-Hydroxychalcones (**3**) used contained both electron donating and electron withdrawing groups in their phenyl rings. It was observed that the yield of compound **6** was significantly high in cases of 2-hydroxychalcones containing a OMe group at different positions of the phenyl rings.

It is very interesting to note that for each of the compounds of the series **6** only one ^{1}H NMR signal and only one ^{13}C NMR signal were observed for its two N-Me groups. Again, the

Table 1. Optimization of reaction condition for synthesis of **6a**[a]



Entry	Solvent	Catalyst	Amt. of Cat.[a]	Time (h)	Yield (%)[b]
1	Toluene	Amberlyst-15	10	8	58
2	Toluene	Amberlyst-15	15	8	65
3	Toluene	Amberlyst-15	20	8	70
4	Toluene	Fused $ZnCl_2$	5	8	35
5	Toluene	Fused $ZnCl_2$	7	8	39
6	Toluene	Fused $ZnCl_2$	9	8	41
7	Toluene	Anhy. $FeCl_3$	5	8	38
8	Toluene	Anhy. $FeCl_3$	7	8	33
9	Water	Amberlyst 15	40	8	-
10	EtOH	Amberlyst 15	40	8	25
11	*i*-PrOH	Amberlyst 15	40	8	30
12	THF	Amberlyst 15	40	8	51
13	MeCN	Amberlyst 15	40	8	57
14	Dioxane	Amberlyst 15	40	8	68
15	DMF	Amberlyst 15	40	8	59
16	*o*-Xylene	Amberlyst 15	40	8	70
17	Toluene	Amberlyst-15	30	8	72
18	**Toluene**	**Amberlyst-15**	**40**	**8**	**75**
19	Toluene	Amberlyst-15	50	9	75
20	Toluene	Amberlyst-15	60	10	74
21	Toluene	-	-	10	42
22	*o*-Xylene	-	-	10	35

[a]Reaction conditions: 2-hydroxychalcone (**3a**) (1.0 mmol) , 1,3-dimethylbarbituric acid (**4**) (1.0 mmol), solvent, catalyst [amberlyst-15 (in mg $mmol^{-1}$ of **3** or **4**), fused $ZnCl_2$ or anhy. $FeCl_3$ (in mol % with respect of **3** or **4**), reflux under atmospheric oxygen. [b]Yield of isolated pure product.

carbonyl carbons at the 4 and 6 positions of the barbiturate moiety of each of **6a-i** gave only one ^{13}C NMR signal[12]. These are very much indicative of the occurrence of free rotation around the double bond linking the two heterocyclic moieties in the compounds at room temperature. Thus, in the structure of the interesting compounds being reported the canonical form **6′** contributes to a great extent. This view is supported by the calculated C4-C5″ bond length for the compound **6a** (considering the optimized structure obtained from DFT study), which is 1.421 Å, quite longer than a normal C=C bond (*e.g.*, 1.35 Å for ethene[13]). It may be commented here that a gain of aromaticity by the oxacyclic ring of **6′** may be the reason for its remarkable contribution. Thus, the compounds of the series **6** behave as interesting examples of push-pull alkenes which in general show low barrier of rotation around the double bond[14].

A thorough literature survey revealed that some fifty years back reaction between flavones and 1,3-dimethylbarbituric acid (**4**) by heating them in acetic anhydride was reported as a valuable colour reaction for the former group of compounds[15].

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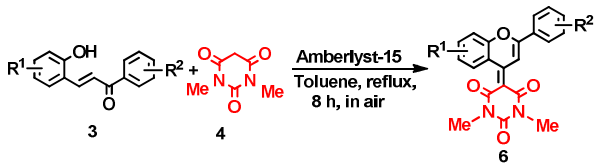
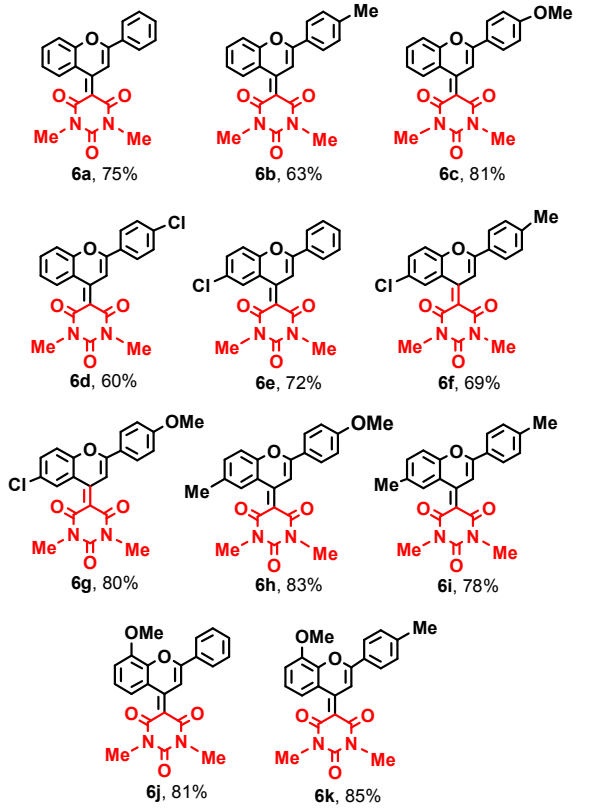
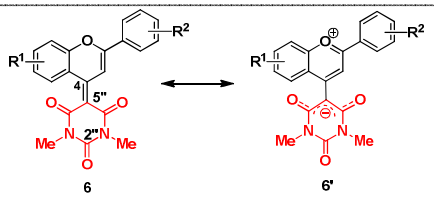
Table 2: Reaction of 2-hydroxychalcones (**3**) and 1,3-dimethylbarbituric acid (**4**)[a].



3 4 6

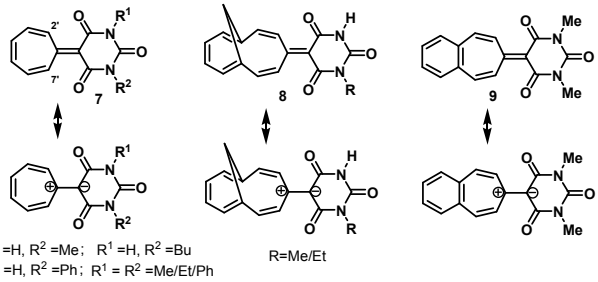


[a]Reaction conditions: 2-hydroxychalcone (**3a**) (1.0 mmol) and 1,3-dimethylbarbituric acid (**4**) (1.0 mmol), amberlyst-15 (40 mg), toluene, reflux under atmospheric oxygen, 8 h.



6 6'

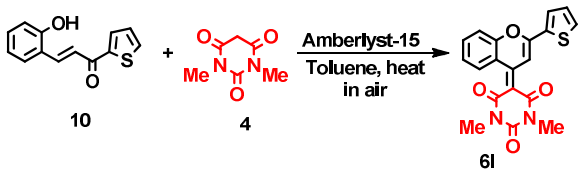
The products of this reaction were assigned the general structure **6** on the basis of their analytical and UV spectral data only[15]. Subsequently, condensations of tropolone, 4,9-methano[11]annulenone and benzo[*d*]tropolone with **4** and related compounds under similar reaction conditions were also studied[16]. The products were assigned the structures **7**-**9**, all having significant contribution of their zwitterionic canonical forms where barbituric acid part bears negative charge, just like that of **6'**. In the unsymmetrical compounds of series **7**, H-2′ and H-7′ appeared as two broad signals at room temperature, as two sharp 1H doublets at –60 °C and as a 2H doublet at 55 °C. These observations indicated slow rotation around the exocyclic double bond of such compounds at room



temperature. Our view about the occurrence of free rotation around the C4–C5′′ bond of **6** at room temperature as mentioned above thus gets support from these reported results. It may be mentioned here that x-ray crystallographic studies on any compound of the series **6** could not be done due to non-availability of quality crystals.

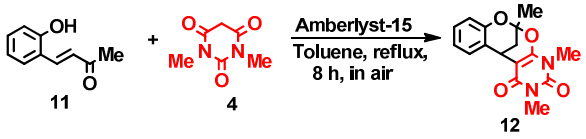
Reactions were carried out also with two 2-hydroxychalcone analogues, *viz*., (E)-3-(2-hydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**10**) and (E)-4-(2-hydroxyphenyl)but-3-en-2-one (**11**). The optimized condition shown in Table 1 was found to be unsuitable for **10** and a dark brown gummy material resulted from which no pure product could be isolated. However, the expected product **6l** could be obtained in lower yield by carrying out the reaction at lower temperature [40 mg catalyst, 85°C, 14 h, 21 %] or by use of lesser proportion of catalyst [5 mg, reflux, 14 h, 25 %] or by avoiding the catalyst [reflux, 14 h, 17%] using toluene as solvent (Scheme 2). In the reaction of **11,** no product analogous to **6** could be obtained, instead, a 2,8-dioxabicyclo[3.3.1]nonane derivative **12** resulted in moderate yield (40 mg catalyst, reflux, 8 h, 42 %) (Scheme 3).

The mechanistic aspect for formation of **6** may be rationalized in the following way. In an aprotic solvent toluene, the enol content of **4** may be increased in the presence of amberlyst-15, a polymer-based sulfonic acid. Compound **3** may also be activated by amberlyst-15 and it can take part in a Michael reaction with the enol form of **4**[11]. The Michael adduct **13** undergoes cyclisation followed by water elimination to afford 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-yl)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione (**5**). The compound **5** is then oxidised by air to give the final product **6**. Here, this irreversible aerial oxidation



10 4 6l

Scheme 2 Outcome of the reaction of **10** and **4**



11 4 12

Scheme 3 Formation of a 2,8-dioxabicyclo[3.3.1]nonane derivative **12** in the reaction of **11** and **4**

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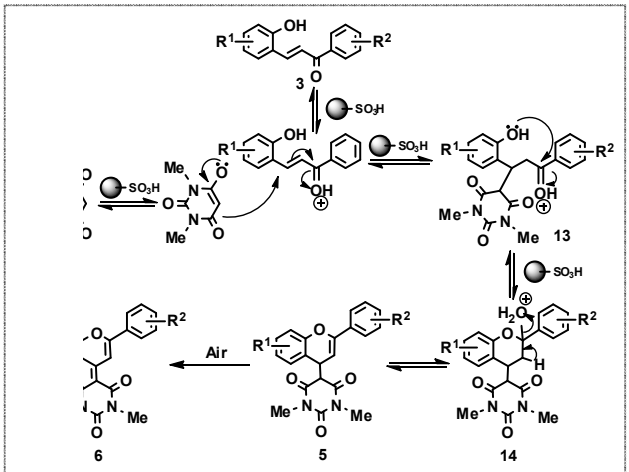

Scheme 4 Plausible mechanistic pathway for formation of 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-ylidene)pyrimidine-2,4,6 (1*H*,3*H*,5*H*)-triones (**6**).

(all other steps are reversible) and poor solubility of the product **6** in the solvent toluene may be responsible for the success of the reaction. To get a better understanding about the role of aerial oxygen in the oxidation step, we repeated the experiment under the optimised conditions employing **3a** and **4** in an argon atmosphere and found the formation of only a trace amount of **6a**. However, when the supernatant liquid of the reaction mixture was separated from the catalyst and kept at room temperature for 12 h with an exposure to air, the product **6a** was obtained as precipitate (yield: 41%). Again, when the same experiment was conducted in an oxygen atmosphere, it was observed that the reaction was complete within 6 h affording **6a** in a higher yield (83 %). These observations definitely indicated that the presence of aerial oxygen was indispensable for formation of **6a** and a plausible mechanism for the overall reaction may be delineated (Scheme 4). The very good yield of the methoxy-containing compounds (**6c**, **6g-h**, **6j-k**) may be attributed to greater reactivity of the intermediate **5** towards aerial oxidation in these cases. In the reaction involving **11,** an intermediate analogous to **14** fails to undergo elimination reaction leading to a 2-chromene, rather it undergoes a cyclisation reaction generating the bicyclic compound **12**. The suggested mechanism shows that the overall process restores the catalyst, which is used in the next cycle of reactions. Thus, it has been observed that the catalyst can be recovered after completion of the reaction and then it can be used further without any significant reduction of activity (Table 3).

Table 3 Recycling of the catalyst for the synthesis of **6a**

Entry	Condition of the catalyst[a]	Time (h)	Yield[b] (%)
1	Fresh	8	75
2	Run 1	8	73
3	Run 2	9	73
4	Run 3	10	70
5	Run 4	10	68

[a] Amberlyst-15, [b] Isolated yield

Interaction with β–lactoglobulin (β-lg).

β–Lactoglobulin (β-lg), one of the main lipocalin proteins of whey, is of great interest in the food and dairy industry due to its nutritional and functional properties[17]. Its properties can variously be favorable or unfavorable in dairy products and their processing[18]. High abundance, easy purification, high water solubility, owning of natural nutrient binding sites and unresponsiveness toward peptic digestion make β-lg an attractive model protein as an encapsulator and carrier of various agents, bioavailability enhancer and target protein for ligand-binding as well as other studies[17]. Its binding property with small organic molecules has been studied in a widespread way in naturally occurring, isolated and chemically modified forms[19]. With a view to understanding the protein-ligand binding of the compounds synthesized by us, β–lg was used as a model protein. In this study, the presence or absence of interaction was investigated using UV-vis absorbance and fluorescence in 5% ethanolic aqueous phosphate buffer (pH = 7.4). It was evident from fluorescence study that among the compounds **6a-k,** only **6a**, **6b** and **6i** showed significant interaction, **6a** giving the best result. Interaction with the chlorine containing compounds **6d-f** was very insignificant. The remaining compounds were only slightly soluble in ethanol, which debarred the study of their interaction.

As evident from the topmost line (black in colour) of Fig. 2d, the UV-vis spectrum of pure **6i** has three λ_{max} values at 255.8, 383.7 and 484.9 nm in 5% ethanol-aqueous phosphate buffer (pH = 7.4). A gradual decrease and saturation (after certain level) of intensity of the peaks at 383.7 and 484.9 with increasing concentration of protein in the same buffer was observed. We performed the same experiment by addition of water instead of the protein solution to investigate the dilution effect on the UV-vis spectrum, and a very insignificant decrease in intensity of the peaks at 383.7 and 484.9 was found. Indole moieties of tryptophan in β-lg are mainly responsible for its fluorescence property. This protein gets excited at 295 nm to give fluorescence at λ_{max} 340 nm in the said buffer system. The gradual decrease to a certain extent and then

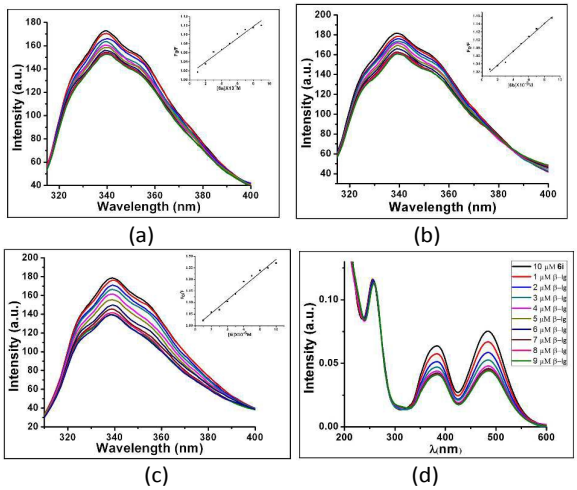

Fig. 2 Fluorescence titration of 10μM of *β*-lg with (a) 1-9 μM of **6a**, (b) 1-9 μM of **6b**, (c) 1-10 μM of **6i** and (d) UV-Vis spectrum of **6i** and its change with addition of protein solution. Insets: Plot of F_0/F *vs* [compound **6a** or **6b** or **6i**] as per the Stern-Volmer equation.

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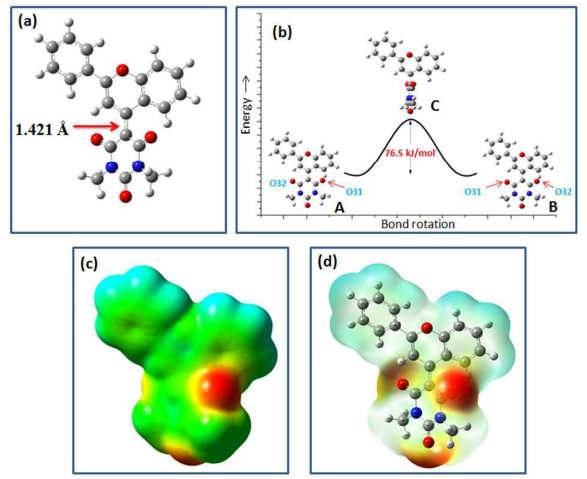

Fig. 3 (a) Optimized geometry of **6a**. (b) Potential energy curve for the rotation about the C=C bond in **6a**. (c) & (d) Molecular electrostatic potential map of **6a**.

saturation of fluorescence intensity of β-lg at the λ_{max} with increasing concentration of **6a**, **6b** and **6i** were found. The above results of UV-vis and fluorescence studies (Fig. 2) definitely suggest that the compounds **6a**, **6b** and **6i** originally present in a hydrophilic environment in aqueous ethanolic solution move toward more hydrophobic environment inside the protein. Depending upon the characteristics of ligands, the binding constant for ligand-binding with β-lg varies widely (from 1.5×10^2 to 5×10^7 M^{-1})[20]. The binding constant for the interaction can be calculated from the ratio of intercept and slope of the plot of (F_0/F) vs [ligand] (where F_0 is the fluorescence intensity at λ_{max} without addition of ligand and F is the fluorescence intensity at λ_{max} with different concentrations of ligand) by using Stern-Volmer equation.[21] From this plot, shown in the insets of Figs. 2a, b and c for the compounds **6a**, **6b** and **6i**, the binding constants were calculated as 7.8×10^5, 5.9×10^5 and 3.4×10^5 M^{-1}, respectively. These binding constant values indicated a lofty level of affinity of ligand towards the protein.

DFT study: Ground-state geometry optimization and molecular electrostatic potential (MEP) calculation.

A theoretical investigation utilizing density functional theory (DFT) was performed to get a better understanding of the structural features and chemical properties of **6a,** a representative member of the series **6**. The structure of the compound, shown in Fig. 3a, was optimized by DFT at the B3LYP/6-31G level of theory using Gaussian 09W**.** The pyrimidine ring of the molecule is tilted away from the plane of chromene moiety by an angle of 36.8°. The bond linking the two heterocyclic moieties is somewhat longer (1.421 Å) than a normal C=C bond (1.35 Å). Calculations for interconversion of the conformers **A** and **B** shows that in between them the most unstable point is **C** which is at 76.50 kJ mol^{-1} higher level than either of **A** and **B** (Fig. 3b). It is therefore reasonable that the potential energy of the conformer having the two heterocyclic moieties in coplanar arrangement will be less than this value and free rotation around the C4-C5″ bond will be possible at room temperature[22]. In reality, this is evident from the ^{1}H and ^{13}C NMR spectral features of **6a** and other compounds of the series.

The molecule **6a** having four oxygen and two nitrogen atoms may show hydrogen bonding interaction with proteins through these heteroatoms, and the rest part is hydrophobic in nature and may be responsible for electrostatic interactions like π-π, π-H-C and other interactions. The parts of the molecule which would interact with β-lg can be predicted by exploiting MEP calculation (a tool for predicting the chemical activity of a molecule when it interacts with another molecule potentially)[23]. In this electrostatic potential surface of constant electron density the red colour represents the electronegative region, yellow the less electronegative region and green the neutral region (Figs. 3c and 3d). From the MEP map, it was found that the chromene oxygen (O15) and the two nitrogen atoms of pyrimidine which are buried in the hydrophobic environment, show no red colour, and may not be able to form any hydrogen bond with protein. Among the remaining oxygen atoms of the pyrimidine moiety, oxygen-31 forms the most electronegative region and it may interact with a protein through hydrogen bonding (Figs. 3c and d). The compound **6a** is a neutral molecule having large hydrophobic part (green regions) which can interact with a protein mainly through electrostatic interactions.

Modeling of 6a Binding Site in* β-lg*: Docking Study.

The structure of the protein β-lg has been determined several times. Its monomeric form is globular in nature with 18400 Da molecular mass and having 162 amino acid residues. The A and B genetic variants of β-lg differ just by amino acids at 64 and 118 positions. Eight long antiparallel *β*-strands build a *β*-barrel, called calyx which is a pocket having ability to accommodate small

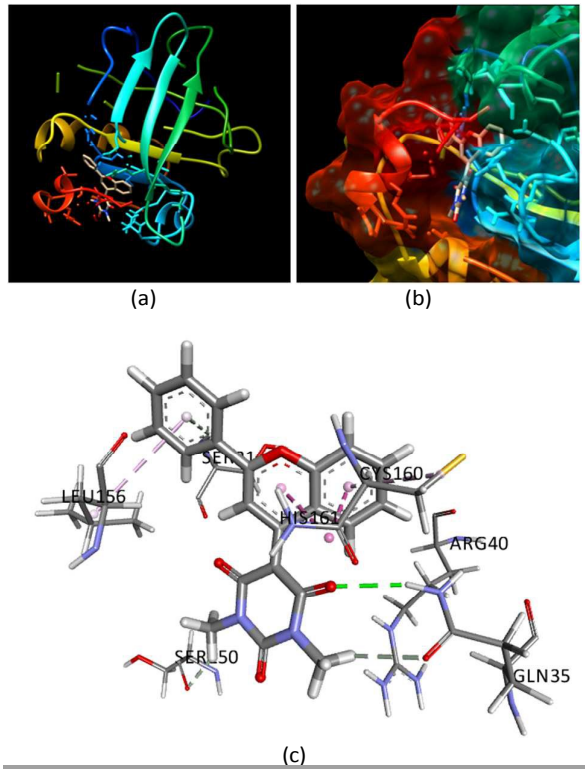

Fig. 4 (a) Docking pose showing the interaction site of the compound **6a** inside the protein (b) A close-up to the binding pocket of the protein (c) Mode of interaction of **6a** with some constituent amino acids at the binding site of β-lg.

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hydrophobic molecules inside it, along with this, there is one α-helix at the outer surface of the barrel in both the varients[17]. This tertiary framework has three potential binding pockets for ligands: the calyx, a channel between α-helix and the barrel and the region near Trp19-Arg124. The last two pockets are at the outer surface of the protein[24] and polar aromatic compounds prefer the outer surface binding sites[25]. Generally, hydrophobic and amphiphilic compounds interact with β-lg via electrostatic and hydrogen bonding interactions[21].

The molecular docking study has drawn extraordinarily large attention of theoretical and experimental researchers. This study was carried out to get better understanding about the protein environment where the molecule **6a** interacts. The docked pose shows that the molecule prefers the outer surface channel of β-lg between α-helix and the barrel to bind in the fashion shown in Fig. 4a. It is involved in hydrogen bonding with amide hydrogen of the side chain of GLN35 residue and electrostatic interaction with SER21, GLN35, ARG40, SER150, LEU156, CYS160 and HIS161 residues as apparent from the docked pose represented in Fig. 4b. The docking study shows that oxygen-31 of the molecule is involved in hydrogen bonding, which was predicted from MEP calculation also (Fig. 3c and 3d). The neutral nature, lack of effective polar atoms and presence of large hydrophobic part in the molecule of **6a** may be responsible for its efficiency of interaction through the hydrophobic part. Thus, the electrostatic interactions with the molecule become very important for showing affinity toward the protein. This is reflected in the high binding constant values obtained from fluorescence experiment. Using the docking tools, the binding constant of this ligand-protein interaction was calculated to be 3.05×10^5 M^{-1}.

Experimental

General

Standard literature procedures were used to dry the solvents used in the experiments. An oven-dried round-bottomed flask was used to perform the reactions and they were conducted under the atmospheric oxygen. Thin-layer chromatography plates (Silica gel G) were visualized by exposure to ultraviolet light and/ or iodine vapor. IR spectra were recorded on a Perkin Elmer FT-IR Spectrophotometer (Spectrum BX II) as KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker 300 MHz or a Bruker 500 MHz NMR spectrometer. Chemical shifts (δ) were reported in parts per million (ppm) taking $CHCl_3$ peak at δ 7.26. Coupling constants (*J*) are quoted in hertz (Hz). ESMS were recorded on a Jeol MS Station 700 mass spectrometer by electron spray ionization (ESI) and HRMS on a Waters Xevo G2QT mass spectrometer. Elemental analyses were done using two Perkin-Elmer 2400 Series II C, H, N analyzers.

General Procedure for the Synthesis of 1,3-Dimethyl-5-(2-phenyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-triones (**6**). To a solution of 2-hydroxychalcone (**1**) (1 mmol) and 1,3-dimethyl-barbituric acid (1 mmol) in anhydrous toluene (10 mL) was added amberlyst-15 (40 mg) at room temperature. The resulting mixture was refluxed with stirring under atmospheric oxygen for 8 h. After completion of reaction, sufficient amount of dichloromethane was added to dissolve the product and then amberlyst-15 was filtered off. The filtrate was concentrated by removal of solvent and the resulting crude product was purified by crystallization from DCM hexane solvent system.

1,3-Dimethyl-5-(2-phenyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (**6a**): Red crystals (yield: 75 %), m.p. 171-172 °C, IR (KBr pellet): 1700, 1630, 1544, 1467, 1440, 1401, 1358, 1129, 1060, 861, 765 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.43 (6H, s, 2 × >NCH_3), 7.43 (1H, t, *J*=8.2 Hz), 7.56-7.61(3H, m, Ar-H), 7.67 (1H, d, *J*=8.4 Hz), 7.78 (1H, t, *J*=8.4 Hz), 8.05-8.11(3H, m, Ar-H), 9.13 (1H, s, H-3 of 2-chromene moiety). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.4, 99.6, 111.9, 118.0, 121.4, 124.8, 127.2, 129.4, 131.0, 131.8, 132.7, 135.1, 152.0, 154.8, 161.6, 162.6, 162.8, ESMS: *m/z* calculated for $C_{21}H_{17}N_2O_4$ $[M + H]^+$: 361.1188, found 361.1180, Anal. Calcd for $C_{21}H_{16}N_2O_4$: C, 69.99; H, 4.48; N, 7.77. Found: C, 70.13; H, 4.67; N, 7.52.

1,3-Dimethyl-5-(2-p-tolyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (**6b**): Red crystals (yield: 63 %), m.p. 175-176 °C, IR (KBr pellet): 1699, 1630, 1544, 1509, 1463, 1440, 1356, 1279, 1192, 1128, 1057, 763, 721, 615; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47 (3H, s, CH_3), 3.43 (6H, s, 2 × >NCH_3), 7.37 (2H, d, *J* = 8.1Hz, Ar-H), 7.42 (1H, t, *J* = 7.5Hz, Ar-H), 7.66 (1H, d, *J* = 8.7Hz, Ar-H), 7.78 (1H, br. t, J=7.2 Hz Ar-H), 8.00 (2H, d, *J* =8.2 Hz, Ar-H), 8.05 (1H, br. d, *J* = 8.4 Hz, Ar-H), 9.07 (1H, s, H-3 of 2-chromene moiety), ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.7, 28.3, 98.9, 111.8, 118.0, 121.5, 124.8, 127.3, 128.1, 130.2, 131.8, 135.0, 144.0, 152.1, 154.7, 162.2, 162.9; Anal. Calcd for $C_{22}H_{18}N_2O_4$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.72; H, 5.02; N, 7.61.

5-(2-(4-methoxyphenyl)-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**6c**) Red crystals (yield: 81 %), m.p. 199-200 °C ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.42 (6H, s, 2 × >NCH_3), 3.92 (3H, s, OCH_3), 7.05 (2H,d, *J*=8.5 Hz, H-3′, 5′), 7.42 (1H, t, *J*=8.0 Hz, H-6), 7.65 (1H, d, *J*=8.5 Hz, H-8), 7.76 (1H, t, *J*=7.5 Hz, H-7), 8.05 (1H, d, *J*=8.5 Hz, H-5), 8.08 (2H,d, *J*=8.5 Hz, H-2′, 6′), 9.00 (1H, s, H-3 of 2-chromene moiety). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 55.7, 98.2, 111.5, 115.0, 117.9, 121.6, 123.1, 124.8, 129.5, 131.8, 134.8, 152.2, 154.6, 162.3, 162.9, 163.8; ESMS: *m/z* calculated for $C_{22}H_{19}N_2O_5$ $[M + H]^+$: 391.1294, found 391.1214. Anal. Calcd for $C_{22}H_{18}N_2O_5$: C, 67.69; H, 4.65; N, 7.18. Found: C, 67.49; H, 4.79; N, 7.03.

5-(2-(4-Chlorophenyl)-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**6d**): Red crystals (yield:60%), m.p. 217-218 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.42 (6H, s, 2 × >NCH_3), 7.42 (1H, t, *J*=6.9 Hz), 7.53 (2H, d, *J*=8.7 Hz, H-3′& H-5′), 7.64 (1H, d, *J*=8.1Hz, Ar-H), 7.78 (1H, t, *J*=7.5 Hz), 8.01-8.05 (3H, m, Ar-H), 9.12 (1H, s, H-3 of 2-chromene moiety). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.5, 100.4, 111.6, 119.5, 122.3, 127.2, 129.4, 130.4, 130.7, 130.9, 132.9, 134.9, 151.8, 153.1, 160.8, 161.5, 162.7, Anal. Calcd for $C_{21}H_{15}ClN_2O_4$: C, 63.89; H, 3.83; N, 7.10. Found: C, 64.03; H, 3.76; N, 6.81.

5-(6-Chloro-2-phenyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**6e**): Red crystals (yield: 72%), m.p. 215-206 °C, IR (KBr pellet): 2920, 2850, 1701, 1649, 1627,1515, 1467, 1366, 1262, 1192, 1120, 1022, 836, 753, 702, 641 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.42 (6H, s, 2 × >NCH_3), 7.54-7.61(4H, m, Ar-H),7.69 (1H, dd, *J*=8.0 & 2.4 Hz, H-7), 8.01(1H, d, *J*=2.4 Hz, H-5), 8.06 (2H, dd, *J*=8.2 & 2 Hz, H-2′& H-6′), 9.16 (1H, s, H-3 of 2-chromene moiety). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.5, 99.6, 111.8, 118.0, 119.4, 125.0, 128.3, 129.5,129.6, 131.9, 135.4, 139.1, 153.0, 154.8, 160.0, 161.3, 163.1, Anal. Calcd for $C_{21}H_{15}ClN_2O_4$: C, 63.89; H, 3.83; N, 7.10. Found: C, 63.61; H, 3.67; N, 7.03.

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5-(6-Chloro-2-p-tolyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**6f**): Red crystals (yield: 69%), m.p. 203-204°C;^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (3H, s, CH_3), 3.42 (6H, s, 2 × >NCH_3), 7.36 (2H, d, *J*=9 Hz, H-3′, 5′), 7.58 (1H, d, *J*=9 Hz, H-8), 7.68 (1H, dd, *J*=9.0 &2.3 Hz, H-7), 7.96 (2H, d, *J*=8.2 Hz, H-2′, 6′), 8.01 (1H, d, *J*=2.3 Hz, H-5), 9.10 (1H, s, H-3 of 2-chromene moiety). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.7, 28.4, 99.7, 111.4, 119.4, 122.4, 127.3, 127.8, 130.2, 130.4, 130.9, 134.8, 144.2, 151.9, 153.0, 161.0, 162.0, 162.7. ESMS: *m/z* calculated for $C_{22}H_{18}ClN_2O_4$ $[M + H]^+$: 409.09, found 409.39

5-(6-Chloro-2-(4-methoxyphenyl)-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**6g**): Red crystals (yield: 80%), m.p. 209-210 °C,^{1}H NMR (300 MHz, $CDCl_3$): δ 3.42 (6H, s, 2 × >NCH_3), 3.92 (3H, s, OCH_3), 7.03 (2H, d, *J*=9.0 Hz, H-3′, 5′), 7.58 (1H, d, *J*=9 Hz, H-8), 7.67 (1H, dd, *J*=9 & 2.4 Hz, H-7), 8.00 (1H, br.s, H-5), 8.05 (2H, d, *J*=9 Hz, H-2′, 6′), 9.03 (1H, s, H-3 of 2-chromene moiety). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.4, 55.7, 99.6, 111.2, 115.0, 119.3, 122.4, 122.8, 129.4, 130.4, 130.9, 134.7, 152.0, 152.9, 161.0, 162.2, 163.9. Anal. Calcd for $C_{22}H_{17}ClN_2O_5$: C, 62.20; H, 4.03; N, 6.59. Found: C, 62.07; H, 4.18; N, 6.68.

5-(2-(4-Methoxyphenyl)-6-methyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**6h**): Red crystals (yield: 83%), m.p. 217-218 °C, IR (KBr pellet):1699, 1628, 1551, 1515, 1430, 1466, 1363, 1262, 1224, 1120, 1025, 904, 838, 862, 804, 776, 754, 719, 643 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47 (3H, s, CH_3), 3.42 (6H, s, 2 × >NCH_3), 3.92 (3H, s, OCH_3), 7.04 (2H,d, *J*=9.0 Hz, H-3′, 5′), 7.56 (2H, br.s, H-7 & H-8), 7.82 (1H, br.s, H-5), 8.07(2H,d, *J*=8.9 Hz, H-2′, 6′), 8.93 (1H, s, H-3 of 2-chromene moiety). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.4, 28.3, 55.7, 97.7, 111.9, 114.9, 117.6, 121.5, 123.1, 129.4, 131.0, 134.9, 136.5, 152.3, 153.1, 162.2, 162.8, 136.0, 163.7 Anal. Calcd for $C_{23}H_{20}N_2O_5$: C, 68.31; H, 4.98; N, 6.93. Found: C, 68.14; H, 4.88; N, 7.11.

1,3-Dimethyl-5-(6-methyl-2-p-tolyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (**6i**): Red crystals (yield: 78%), m.p. 181-182 °C, IR: 1701, 1630, 1546, 1546, 1511, 1358, 1275, 1192, 1131, 1054, 816, 778, 764, 719, 643; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (3H, s, CH_3), 2.48 (3H, s, CH_3), 3.42 (6H, s, 2 × >NCH_3), 7.36 (2H, d, *J*=7.8 Hz, H-3′, 5′), 7.58 (2H, s, H-7,8), 7.82 (1H, s, H-5), 7.98 (2H, d, *J*=7.8 Hz, H-2′, 6′), 9.00 (1H, s, H-3 of 2-chromene moiety), ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.4, 21.7, 28.3, 98.3, 112.1, 117.7, 121.5. 127.3, 128.1, 130.1, 131.0, 134.9, 136.7, 143.9, 152.2, 153.2, 162.1, 162.8, 163.1 ESMS: *m/z* calculated for $C_{23}H_{20}N_2O_4$ $[M + H]^+$: 389.1501, found 389.1541.

5-(8-Methoxy-2-phenyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**6j**): Red crystals (yield: 81%), m.p. 219-220 °C, IR: 2923, 2852, 1692, 1550, 1501, 1402, 1450682, 701, 723, 755, 775, 802, 863, 1068, 1140, 122874, 1353;^{1}H NMR (300 MHz, $CDCl_3$): δ 3.42 (6H, s, 2 × >NCH_3), 4.06 (3H, s, OCH_3), 7.22 (1H, d, *J*=7.9 Hz, H-7), 7.32 (1H, t, *J*=8.4 Hz, H-6), 7.53-7.59 (4H, m, Ar-H), 8.14(2H, dd, *J*= 7.8 & 2.0 Hz, H-2′, 6′), 9.14 (1H, s, H-3 of 2-chromene moiety). ESMS: *m/z* calculated for $C_{22}H_{18}N_2O_5$ $[M + H]^+$: 391.12, found 391.20.

5-(8-Methoxy-2-p-tolyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**6k**): Red crystals (yield: 85%), m.p. 231-232 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (3H, s, CH_3), 3.41 (6H, s, 2 × >NCH_3), 4.05 (3H, s, OCH_3), 7.20-7.37 (4H, m, Ar-H), 7.54 (1H, d, *J*=8.0 Hz, H-5), 8.04 (2H, d, *J*= 8.1Hz, H-2′, 6′), 9.08 (1H, s, H-3 of 2-chromene moiety), ESMS: *m/z* calculated for $C_{23}H_{20}N_2O_5$ $[M + H]^+$: 405.13, found 405.11

1,3-Dimethyl-5-(2-(thiophen-2-yl)-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (**6l**): Red crystals (yield: 25%), m.p. 243-244 °C,^{1}H NMR (300 MHz, $CDCl_3$): δ 3.42 (6H, s, 2 × >NCH_3), 7.26-7.28 (1H, m,), 7.41 (1H, t, *J* =7.5 Hz, Ar-H), 7.61 (1H, d, *J* = 8.1 Hz), 7.72-7.76 (2H, m, Ar-H), 7.96 (1H, d, *J* = 3.6 Hz, H-5 of thiophene moiety), 8.01 (1H, d, *J* = 8.3 Hz, Ar-H), 8.93 (1H, s, H-3 of 2-chromene moiety) ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.5, 99.1, 111.3, 118.0, 121.6, 124.9, 129.4, 131.0, 132.0, 133.1, 135.1, 152.5, 154.3, 158.2, 162.1, 162.9. HRMS: *m/z* calculated for $C_{19}H_{15}N_2O_4S$ $[M + H]^+$: 366.0674, found 367.0747

2,4,6-Trimethyl-4,12-dihydro-1H-6,12-methanobenzo[7,8][1,3]dioxocino[4,5-d]pyrimidine-1,3(2H)-dione (**12**): Colourless crystals (yield: 42%), m.p. 98-99 °C,^{1}H NMR (300 MHz, $CDCl_3$): δ 1.91 (3H, s, CH_3), 2.14 and 2.23 (each 1H, br. d, *J* =13.5 Hz, CH_2), 3.28 (3H, s, >NCH_3), 3.34 (3H, s, >NCH_3), 4.21 (1H, br. s, aliph. CH), 6.85 (1H, d, *J* = 7.4 Hz, Ar-H), 6.91 (1H, t, *J* = 7.4 Hz, Ar-H), 7.12 (1H, t, *J* = 7.4 Hz, Ar-H), 7.40 (1H, d, *J* = 7.5 Hz, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.5, 26.7, 28.0, 28.7, 31.2, 91.8, 102.3, 116.0, 122.0, 126.3, 127.8, 128.0, 150.7, 155.1, 161.6. HRMS: *m/z* calculated for $C_{16}H_{17}N_2O_4$ $[M + H]^+$: 301.1110, found 301.1295.

Recovery of the catalyst.

The catalyst separated from the reaction mixture was washed thoroughly with ethyl acetate and acetone successively until the washings became colorless. It was then dried in a hot air oven at 100 °C for 8 h. The dried catalyst was used further.

Sample Preparation for UV–Vis and Fluorescence Study.

The solutions of β–lg (Sigma Aldrich, St. Louis, MO), used without further purification, were prepared in 10 mM phosphate buffer of 3% ethanol-water mixture at pH 7.4. This pH level was chosen to replicate the physiological conditions in which β-lg exist its stable conformation. β-lg was dissolved directly into this buffer, and the final concentrations were found 108μM for β-lg. The compound **6a**, **6b** and **6i** was dissolved in absolute ethanol and the concentration was 1 mM.

UV–Vis Absorption Spectroscopy.

A solution of compound **6i** was placed in a four-clear-sided quartz cell (2 mL) of 1 cm path length using a Shimadzu UV1700 spectrophptometer. Spectra were recorded with a matched pair of silica cuvettes against a solvent blank reference from 200 to 650 nm.

Fluorescence Spectroscopy.

The compounds **6a-k** were successively added from 1 to 10 μM to the 10 μM concentration of β-lg for fluorescence studies at room temperature using Shimadzu spectrofluorimeter (model RF 5301) with the bandwidths of excitation and emission slits at 3 and 5 nm. The excitation wavelength of β-lg (10 μM, with phosphate buffer, pH 7.4, with 5% ethanol) was 295nm and emission scans were recorded in the range 310-400 nm.

DFT Calculations.

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For the DFT calculations in this paper GAUSSIAN 09 program package was used. All the calculations was done using Becke three parameters hybrid exchange and the Lee–Yang–Parr correlation functional (B3LYP). To optimize the geometry of **6a**, 6-31G basis set was used. The same method was used also for calculation of MEP for the compound **6a**.

Docking study.

The molecular docking of **6a** into the three dimensional structures of β-lg (downloaded from RCSB website, PDB ID: 2BLG)[26] was carried out using AutoDock 4.2.0 software package. The lowest energy conformation of **6a** obtained from DFT optimisation was used for docking study. It was done using an efficient and durable algorithm, Lamarckian Genetic Algorithm (LGA). The conformation with the lowest binding energy was used to analyze ligand placement.

Conclusions

In conclusion, we report a very simple and new methodology for synthesis of 1,3-dimethyl-5-(2-phenyl-4*H*-chromen-4-ylidene)-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones, an interesting class of compounds having novel structural features. The flavylium cation part of the compounds is responsible for their intense red colour and the zwitterionic structure for their high polarity. They have potential to find important applications for their biological activities and material properties.

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Notes and references

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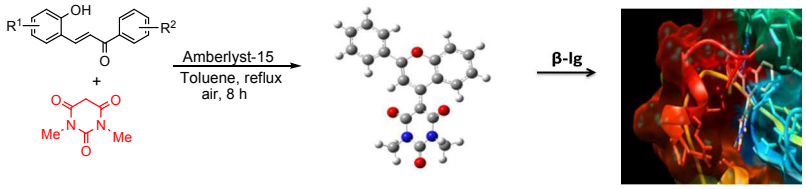

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