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Design, synthesis and vasorelaxant evaluation of novel coumarin–pyrimidine hybrids

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ABSTRACT

The main objective of the present work depends on the hybridization of coumarin moiety as a vasorelaxant scaffold and pyrimidine ring with known potential cardiovascular activity in order to prepare some new potent antihypertensive candidates. Hence, two groups of pyrimidinyl coumarin derivatives were synthesized and evaluated for their vasorelaxing activity. These compounds were prepared via two routes; either preparation of the guanidinocoumarin **4** followed by a cocktail of cyclization reactions to yield a different array of 6-(substituted pyrimidin-2-yl)aminocoumarins **5**–**17**, or through cyclization of the precursor chalcones **22a**–**g** with guanidine hydrochloride to generate the corresponding final compounds, 8-(6-aryl-2-aminopyrimidin-4-yl)-7-methoxycoumarins **23a**–**g**. The effect of these compounds and the coumarin intermediates **3**, **4**, **21** and **22a**–**g** on nor-epinephrine induced contracture in thoracic rat aortic rings was investigated using prazocin as reference drug. Several derivatives showed promising activities either equal or even better than that of prazocin (IC_{50} 0.487 mM). The most prospective compounds; the pyrimidinylamino coumarin derivatives **8**, **17** (IC_{50} 0.411, IC_{50} 0.421 mM) and the chalcones **22b**, **22e** (IC_{50} 0.371, 0.374 mM) that displayed the highest activity can be a base for lead optimization and simple but efficient design of new compounds.

2D-QSAR analysis was applied to find a correlation between the experimental vasorelaxant activities of the newly synthesized coumarin derivatives and their different physicochemical parameters. The result of this study showed that the increase in aqueous solubility while retaining good hydrophobic character of the overall molecule is the key for maintaining high relaxation activity.

1. Introduction

Cardiovascular diseases are by far the main cause of worldwide mortality. These include coronary heart diseases, hypertension, and peripheral artery diseases in addition to others.[1] Hypertension affects approximately billions of people all around the world. This is despite the great effort and progress in developing new drugs targeting the various mechanisms of hypertension.[2] Hypertension and arrhythmia remain the major factors for cardiovascular mortality. Also, hypertension and atherosclerosis are central to the pathogenesis of coronary heart diseases (ischemia, angina and myocardial infarction), heart failure, cerebral (stroke) and peripheral vascular diseases.[3]

Therapeutic intervention is the most common approach to control hypertension and reduce hypertension-related organ damage.[4] Recent progress in developing new antihypertensive agents involves three categories of drugs: 1-diuretics and adrenergic receptor blockers, 2-calcium channel blockers, and 3-inhibitors of the rennin-angiotensin system (RAS) comprising the angiotensin converting enzyme (ACE) inhibitors and the angiotensin type-1 (AT_1) receptor antagonists.[4,5]

Relaxation of vascular smooth muscles is one of the strategies for treatment of hypertension.[6] Several agents have been developed; however they are all associated with side effects such as fatigue, mood change, sleep disturbances, etc. Therefore, there is a continuous need to explore, search and develop new vasorelaxant agents with minimal side effects.[1] Many naturally occurring and synthetic coumarin derivatives have been discovered and biologically evaluated as vasodilators. Literature survey revealed their wide spectrum and diversity of biological activities[7–15] including cardiovascular properties[16–19] of which many are selective vasodilators (Fig. 1). The remarkable relaxant effect of coumarin-resveratoral hybrid **I** on noradrenaline pre-contracted rat aortic rings was cited.[20] In addition, carbochromen (3-diethylaminoethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin) **II** is a potent specific coronary vasodilator that has been used for many years in the treatment of angina pectoris.[21,22] Several coumarin analogues structurally related to carbochromen and several furocoumarin derivatives have been synthesized and their vasorelaxant activity was measured and found to have great potency.[19] Prenyl coumarin, osthol **III**, was found to reduce high systolic blood pressure by vir-

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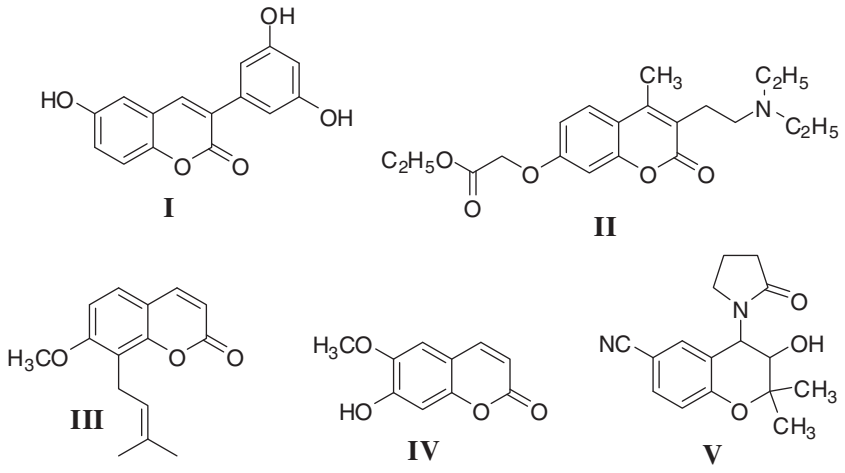


Figure 1. Examples of some coumarin-based vasorelaxants.

tue of elevation of cGMP levels in vascular smooth muscles and calcium channel blocking.[23–25] Moreover, scopoletine[26] **IV** and some coumarinolignans[27] have vasorelaxant effects by inhibiting calcium mobilization. Cromakalim, (3*R*,4*S*)-3-hydroxy-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl) chroman-6-carbonitrile **V** a potassium-channel opening vasodilator that has been used in the management of hypertension.[28,29]

Also certain α-methylene-γ-butyrolactone derivatives of coumarin were reported to exhibit potent inhibitory activities on the NE-induced phasic and tonic constrictions at very low concentrations.[30]

On the other hand, the pyrimidine ring system is a basic skeleton of therapeutic agents designed for the treatment of cardiovascular diseases, such as darusentan **VI** (Fig. 2), a selective endothelin receptor antagonist that works in a novel way by blocking the production of endothelins within the walls of the artery; thus relaxing it and causing blood pressure drop in patients suffering from resistant hypertension.[31,32] Minoxidil **VII**, is a direct vasodilator that function as potassium-channel opener causing hyperpolarization of vascular smooth muscle cells[33] (Fig. 2). Other investigational analogues viz C-linked pyrimidine derivatives **VIII**[34,35] demonstrated potent antihypertensive activities in the renal artery-ligated rat model. Also, some dihydropyrimidines and pyrimidinones were found to exhibit smooth muscle relaxation and other cardiovascular effects by virtue of their calcium channel blocking activity.[36,37] Furthermore, several studies on the vasorelaxant activity of certain heterocyclic systems containing the dihydropyrimidinone ring, such as **IX**[38] indicated that they exhibited high binding affinity and subtype selectivity for the cloned human α_{1a} receptor (Fig. 2).[39,40]

Urged by these findings, and for further exploration of the coumarin ring system as a promising nucleus for new vasodilating agents, it was of interest to hybridize this nucleus with various pyrimidine fragments either attached at position-6 of the coumarin ring through an amino spacer **5**–**17** or directly C-linked to position-8 of 7-methoxy coumarin **23a**–**g**. Furthermore, the pyrimidine moiety was substituted with various chemical groups of different lipophilic, electronic and steric properties hoping to reach safe and potent antihypertensive agents. The newly synthesized compounds were screened in vitro for their potential vasorelaxant activity. 6-Guanidinoocoumarin **4** and the chalcones **22a**–**g** are the synthetic precursors for compounds **5**–**17** and **23a**–**g**, respectively (Schemes 1–3), nevertheless, they were also screened for their vasorelaxant activity depending on the reported efficacy of many guanidine[41] and chalcone derivatives.[42–44]

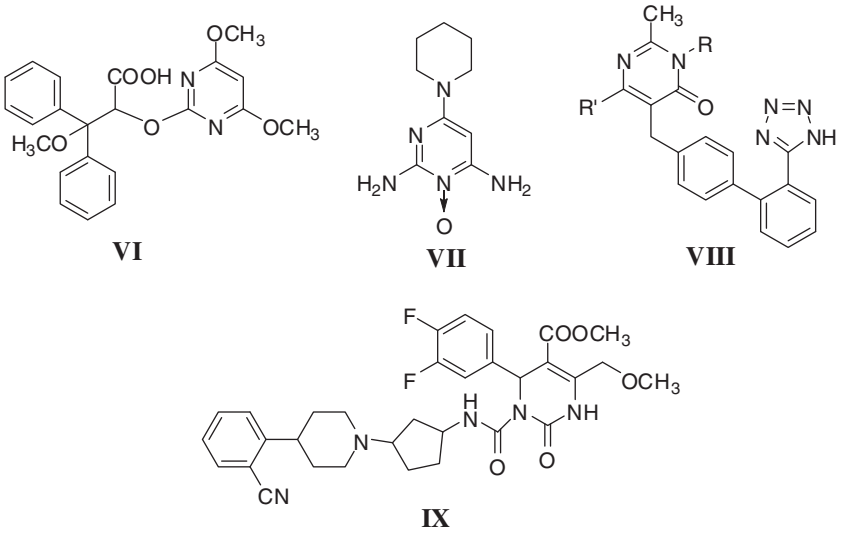
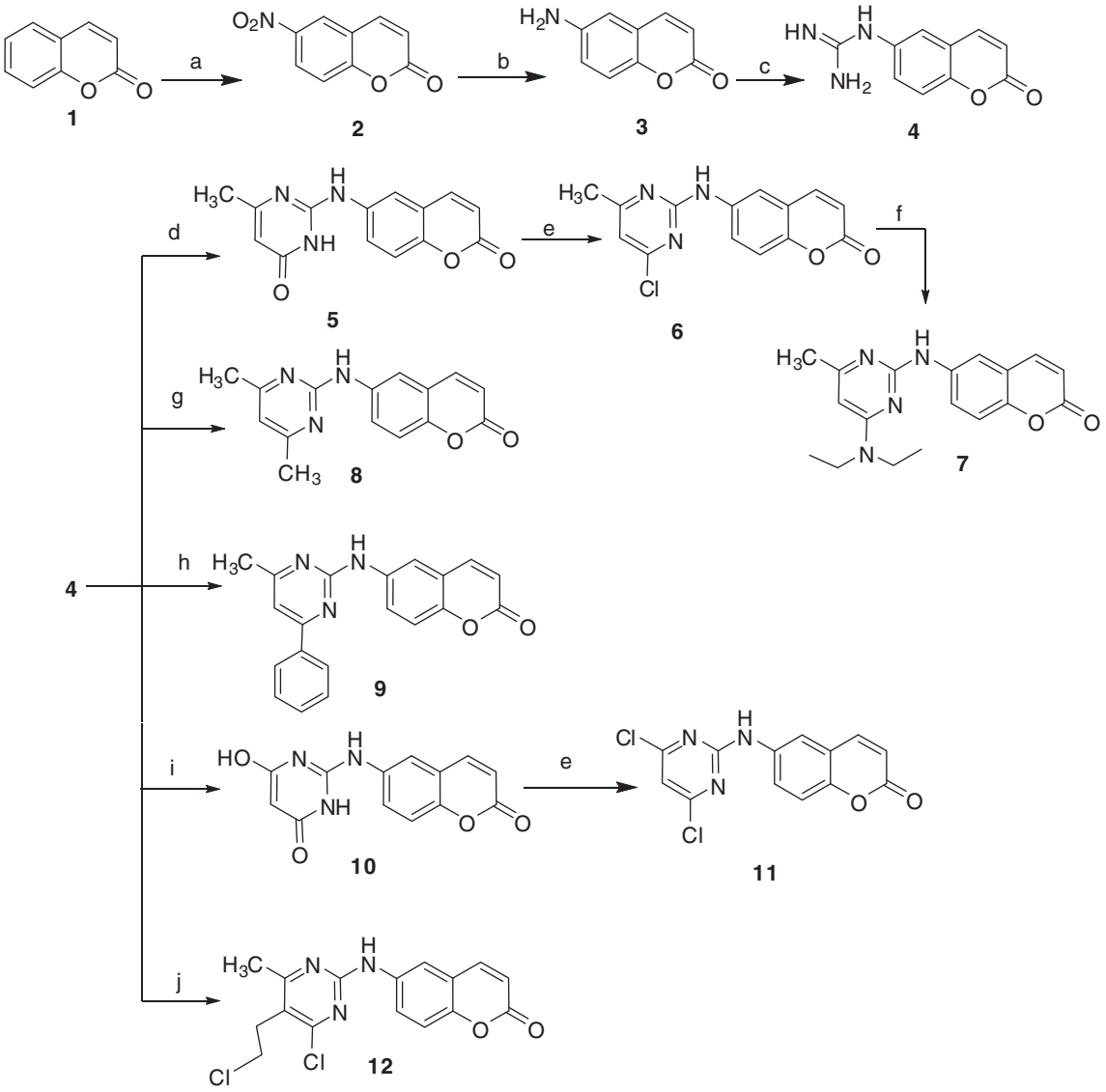


Figure 2. Examples of some pyrimidine-based vasorelaxants.



Scheme 1. Reagents and conditions: (a) HNO_3/H_2SO_4; (b) $SnCl_2/HCl$; (c) cyanamide/ethanolic HCl/reflux 24 h; (d) ethyl acetoacetate/dry DMF/reflux 24 h; (e) $POCl_3$/reflux 5 h; (f) diethyl amine/dry DMF/heat 100 °C 6 h; (g) acetyl acetone/dry DMF/reflux 24 h; (h) benzoyl acetone/heat in mineral oil at 200 °C 2 h; (i) diethyl malonate/heat in mineral oil 200 °C 2 h; (j) 2-acetylbutyrolactone, $POCl_3$/reflux 5 h.

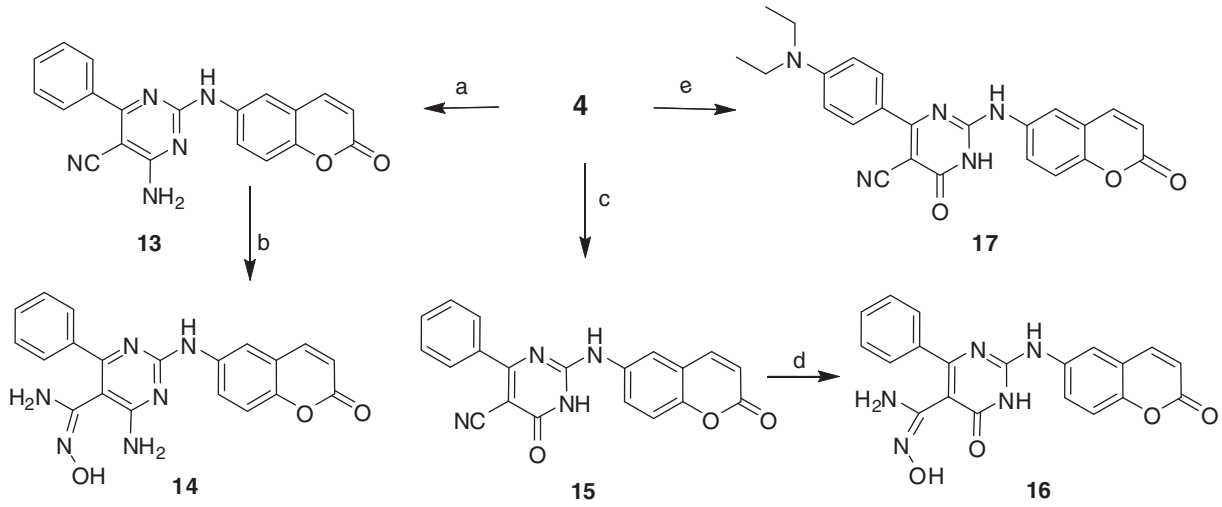
2D-QSAR analysis was performed to find a correlation between the different physicochemical parameters of the studied compounds and their experimental activity. It is noteworthy to mention that the pyrimidine fragment would be a suitable choice for reducing the overt lipophilicity of the compounds and improving the pharmacokinetic profile of the designed molecules.[45]

2. Results and discussion

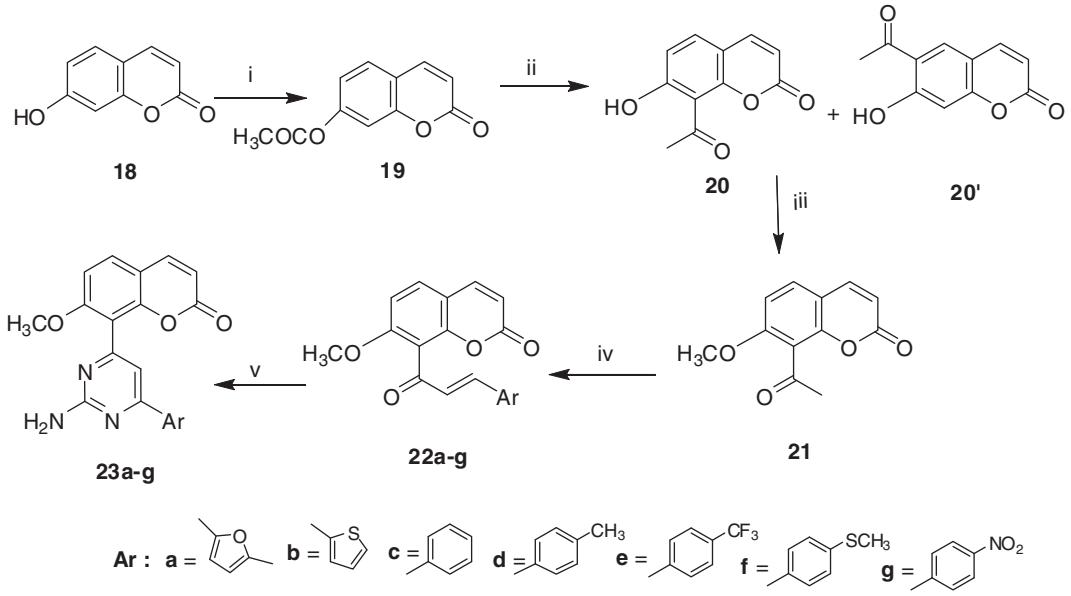
2.1. Chemistry

The first group of the target compounds **5**–**17**, was prepared as depicted in Schemes 1 and 2. The key starting 6-guanidinocoumarin **4** was prepared via the reaction of the precursor amine **3**[46–48] with cyanamide in the presence of ethanolic hydrochloric acid to give the guanidine as hydrochloride salt from which the guanidine base was liberated upon neutralization with sodium bicarbonate. The guanidine derivative **4** underwent cyclocondensation reaction with β-diketones such as ethyl acetoacetate in dry DMF to afford the pyrimidine derivative **5**. Chlorination of **5** using excess of phosphorous oxychloride furnished the chloro derivative **6**. The 4-diethylaminopyrimidine derivative **7** was obtained from the corresponding chloro compound **6** through nucleophilic substitution with diethylamine. Similarly, the reaction of **4** with acetylacetone furnished the dimethyl pyrimidine derivative **8**. Unexpectedly, the reaction of **4** with benzoyl acetone or diethyl malonate in dry DMF failed to give the substituted pyrimidines **9** and **10**, respectively. Alternatively, the reaction was attempted at higher temperature using mineral oil at 200 °C which succeeded to give the desired compounds. Reaction of **10** with phosphorous oxychloride, gave the corresponding dichloropyrimidine derivative **11**. On the other hand, the 5-(2-chloroethyl) pyrimidine analogue **12** was obtained in one-pot reaction from **4** and 2-acetylbutyrolactone in the presence of excess phosphorous oxychloride (Scheme 1).

The 5-cyano-6-(un) substituted phenylpyrimidine derivatives **13**, **15** and **17** were prepared from **4** and the appropriate arylidene derivatives[49,50] in dry DMF. Finally, the carboxamidine derivatives **14** and **16** were prepared through the nucleophilic addition of hydroxylamine to their corresponding carbonitriles **13** and **15**,



Scheme 2. Reagents and conditions: (a) benzylidene malononitrile/dry DMF/reflux 24 h; (b) hydroxylamine hydrochloride, $NaHCO_3$/dry DMF/heat 100 °C 24 h; (c) ethyl benzylidene cyanoacetate/dry DMF/reflux 24 h; (d) hydroxylamine hydrochloride, $NaHCO_3$/dry DMF/heat 100 °C 24 h; (e) ethyl 4-(*N*,*N*-diethylamino)benzylidene cyanoacetate/dry DMF/reflux 24 h.



Scheme 3. Reagents and conditions: (i) acetic anhydride/reflux 5 h; (ii) $AlCl_3$/heat 145 °C 1 h; (iii) CH_3I/dry acetone/reflux 24 h; (iv) Ar-CHO, 10% NaOH/ethanol/rt 24 h; (v) guanidine hydrochloride, few drops HCl/ethanol/reflux 8 h.

respectively; whereby the reaction was conducted in dry DMF in the presence of sodium bicarbonate (Scheme 2).

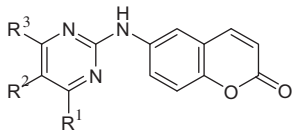
The synthesis of the second group of the target hybrids **23a**–**g** was depicted in (Scheme 3). 8-Acetyl-7-hydroxy-2*H*-chromen-2-one **20** was obtained together with its regioisomer **20′** through a Fries rearrangement of the intermediate acetate derivative **19**, which was obtained by acetylation of the commercially available 7-hydroxycoumarin **18** with acetic anhydride. The regioisomers **20** and **20′** were separated by fractional crystallization from aqueous ethanol.[51] Methylation of **20** with methyl iodide in the presence of potassium carbonate in dry acetone gave 8-acetyl-7-methoxy-2*H*-chromen-2-one **21**.[52–54] The chalcones **22a**–**g** were prepared by the reaction of **21** with the appropriate aldehydes in the presence of sodium hydroxide by the conventional Claisen–Schmidt condensation. The cyclization of the chalcones **22a**–**g** with guanidine hydrochloride under the effect of hydrochloric acid afforded the respective 2-aminopyrimidines **23a**–**g**.

All newly synthesized compounds were characterized by spectral and elemental analyses which were in full agreement with the proposed structures.

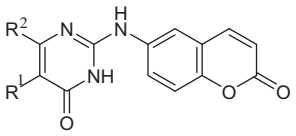
2.2. Vasorelaxant activity

The pyrimidine derivatives **5**–**17** and **23a**–**g** along with the intermediates **3**, **4**, **21** and **22a**–**g** were tested for their vasorelaxant activities against nor-adrenaline-induced spasm on thoracic rat aorta rings[55,56] and compared to the reference drug, prazocin. The results were listed in Tables 1 and 2 and illustrated in Figures 3 and 4 as IC_{50} values. The vasorelaxant procedure performed seemed to be a success ensuring the correct choice of the compounds nature and strengthening the assumption of the coumarin ring system having vasodilatation activity. This assumption was evidenced by the following screening results: Firstly, as shown in Table 1 and Figure 3, the intermediate guanidino coumarin **4**

Table 1
Vasorelaxant activity expressed in IC_{50} of synthesized compounds **3–17**



6,7,8,9,11,12,13,14

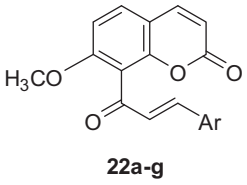


5,10,15,16,17

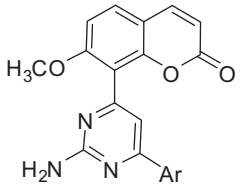
Compound	R^1	R^2	R^3	IC_{50} (mM)
3[a]	—	—	—	0.532
4[b]	—	—	—	0.418
5	H	CH_3	—	0.700
6	Cl	H	CH_3	0.468
7	$-N(C_2H_5)_2$	H	CH_3	0.495
8	CH_3	H	CH_3	0.411
9	$-C_6H_5$	H	CH_3	NA
10	H	OH	—	0.816
11	Cl	H	Cl	0.453
12	Cl	$-CH_2CH_2Cl$	CH_3	0.700
13	NH_2	CN	C_6H_5	0.595
14	NH_2	$-C(NH_2)=N-OH$	C_6H_5	0.468
15	CN	C_6H_5	—	0.547
16	$-C(NH_2)=N-OH$	C_6H_5	—	0.466
17	CN	4-$N(C_2H_5)_2C_6H_4$	—	0.421

NA = not active.
IC_{50} of prazocin = 0.487 mM.
[a] 6-Amino-2*H*-chromen-2-one.
[b] 1-(2-Oxo-2*H*-chromen-6-yl)guanidine.

Table 2
Vasorelaxant activity expressed in IC_{50} of synthesized compounds **21**, **22a–g** and **23a–g**



22a-g



23a-g

Compound	Ar	IC_{50} (mM)
21[a]	—	0.479
22a	5-CH_3–C_4H_2O	0.566
22b	C_4H_3S	0.371
22c	C_6H_5–	0.526
22d	4-CH_3–C_6H_4	NA
22e	4-CF_3–C_6H_4	0.374
22f	4-SCH_3–C_6H_4	0.705
22g	4-NO_2–C_6H_4	0.700
23a	5-CH_3–C_4H_2O	1.226
23b	C_4H_3S	0.650
23c	C_6H_5	0.800
23d	4-CH_3–C_6H_4	0.932
23e	4-CF_3–C_6H_4	1.063
23f	4-SCH_3–C_6H_4	1.142
23g	4-NO_2–C_6H_4	NA

NA = not active.
IC_{50} of prazocin = 0.487 mM.
[a] 8-Acetyl-7-methoxy-2*H*-chromen-2-one.

showed high potency value (IC_{50} 0.418 mM) even better than the amino derivative **3** (IC_{50} 0.532 mM) and prazocin (IC_{50}

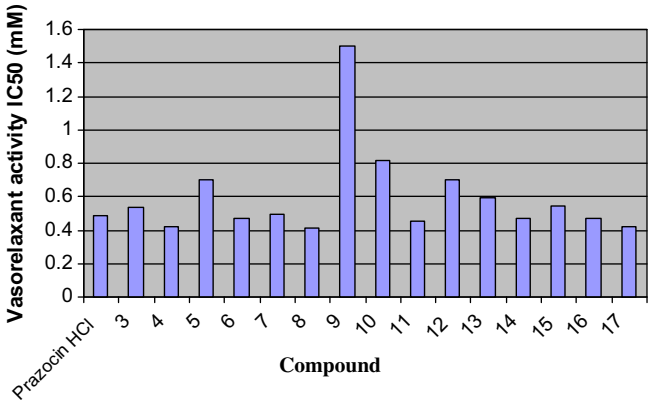


Figure 3. Vasorelaxant activity of compounds **3–17** on contracture induced by norepinephrine hydrochloride (NE·HCl) on thoracic rat aortic rings compared to prazocin HCl.

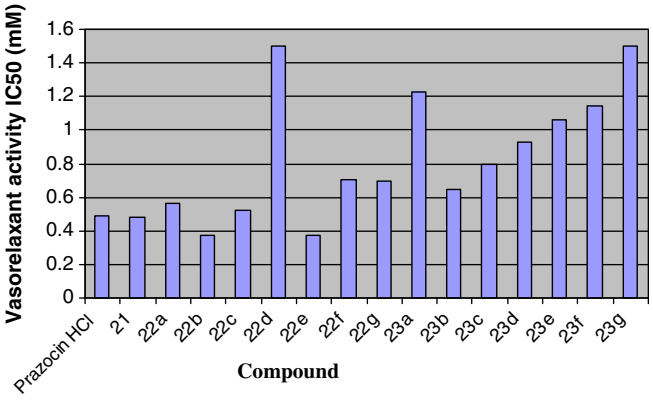


Figure 4. Vasorelaxant activity of compounds **21**, **22a–g**, and **23a–g** on contracture induced by norepinephrine hydrochloride (NE·HCl) on thoracic rat aortic rings compared to prazocin HCl.

0.487 mM), confirming the potential vasorelaxant activity of the coumarin scaffold[16–19] and guanidine function.[41] The cyclization products of the guanidine derivatives **5–17** demonstrated potent vasorelaxant activity (IC_{50} 0.411–0.816 mM), due to the presence of the coumarin core in combination with substituted pyrimidine moiety. The lead compound **8** (R^1, $R^3 = CH_3$) presented higher activity value (IC_{50} 0.411 mM) than that of **4** and the standard prazocin.

The 6-pyrimidinylaminocoumarins **5–17** could be classified into two groups: the 4,6-disubstituted pyrimidinyl derivatives **5–11** and the 4,5,6-trisubstituted ones **12–17**. Examination of the data obtained with the di-substituted derivatives **5–11** indicated that substitution at 4 and 6 positions of the pyrimidine ring with small electron donating and/or electron withdrawing groups of considerable lipophilicity resulted in high activity (Table 1, compounds **6–8** and **11**). Compound **8** (R^1, $R^3 = CH_3$) represented the most active derivative (IC_{50} 0.411 mM). Meanwhile, compound **11** (R^1, R^3 = Cl) showed almost the same activity as **8**; assuming that the electronic nature of the substituents is of minor importance. This was evidenced by observing compounds **6** and **7** where the methyl group was combined with chloro or diethyl-amino groups, respectively, resulting in comparable activities (IC_{50} 0.468 and 0.495 mM, respectively). However, replacement of the 6-methyl group in **8** with the bulkier phenyl function, compound **9**, led to abolishment of activity. On the other hand, compounds with 4-oxo-3,4-dihydro pyrimidine system **5** ($R^2 = CH_3$) and **10** (R^2 = OH) showed moderate potency with IC_{50} values 0.700 and 0.816 mM, respectively.

Considering the tri-substituted derivatives **12–17**, compound **12** (IC_{50} 0.700 mM), having an additional chloro-ethyl group at

position-5 of the pyrimidine ring of **6**, exhibited weaker potency than compound **6** (IC_{50} 0.468 mM) and this may be attributed to the increased bulk on the pyrimidine**.** Amazing enough, the presence of the phenyl ring although in a different position in this tri-substituted pattern was beneficial to the activity. The presence of adjacent aryl and cyano or carboxamidine substituent on the pyrimidine ring of the hybrids **13**–**17** (Table 1, Fig. 3) resulted in high vasorelaxant activity (IC_{50} 0.421–0.595 mM). Compound **13** showed activity similar to its 4-oxopyrimidinyl congener **15** (IC_{50} 0.595 and 0.547 mM, respectively). Introduction of a *p*-diethylamino group on the phenyl ring of **15**, compound **17** resulted in enhancement of activity (IC_{50} 0.421 mM). It is noteworthy to point out that enhancing the hydrophilicity of compounds **13** and **15** through replacement of the cyano substituent with carboxamidine led to improvement of activity (**13** versus **14** and **15** versus **16**). The overall results of compounds **5**–**17** denoted that the presence of substituted pyrimidine moiety linked to a coumarin core through NH spacer is befit to promising vasorelaxant activity. Moreover, both hydrophobic and steric effects of the substituents at the pyrimidine moiety might play a significant role in influencing the activity.

Secondly, from the IC_{50} values presented in Table 2 (Fig. 4), modest to high activity was observed for the chalcone intermediates **22a**–**g** and few of their cyclization products **23a**–**g**. Concerning the chalcones, the most potent compounds **22b** (Ar = thiophene) and **22e** (Ar = *p*-trifluoromethyl phenyl) displayed higher activity (IC_{50} 0.371 and 0.374 mM, respectively) than that of the precursor compound **21** (IC_{50} 0.479 mM) and the standard prazocin (IC_{50} 0.487 mM). This was in agreement with previous reports on the importance of the chalcone system for this activity.[42–44] Compounds **22a** (Ar = 5-methyl furanyl, IC_{50} = 0.566 mM) and **22c** (Ar = phenyl, IC_{50} = 0.526 mM) showed good activity comparable to **22b**. Introduction of methyl-thio and nitro groups in position 4 of the phenyl ring of compound **22c** reduced the activity, **22f** (IC_{50} 0.705 mM) and **22g** (IC_{50} 0.700 mM), respectively; indicating that the hydrophobic and/or electronic effects of the aryl substituent at the coumarin chalcone system might play a significant role in influencing their vasorelaxant activity. Unexpectedly, compound **22d** bearing *p*-tolyl substituent was found to be inactive.

Regarding the overall activity of the new hybrids **23a**–**g**, few of the compounds possessed relaxation activity. The most active compound **23b** (Ar = thiophene) presented relatively fair good vasorelaxant potency (IC_{50} 0.650 mM) but lower than the precursor chalcone **22b** and prazocin. The activities decreased according to **23c** > **23d** > **23e** > **23f** and the least active compound was **23a**. Unexpectedly, compound **23g** (Ar = *p*-nitrophenyl) failed to produce any activity. These results implied that the cyclization of chalcones **22a**–**g** to the corresponding 2-amino-6-substituted pyrimidine hybrids reduced activity, yet it could be suggested that the presence of such pyrimidine moieties at the coumarin core might improve the pharmacokinetic profile of the molecules.

In general, it could be concluded that the activity of the first group of hybrids, pyrimidinylamino coumarins **5**–**17** was superior to that of the second one; pyrimidinyl coumarins **23a**–**g** and the most active compounds were among the first group and the chalcones, especially **8**, **17** (IC_{50} 0.411, IC_{50} 0.421 mM) and **22b** , **22e** (IC_{50} 0.371, 0.374 mM) , respectively.

2.3. QSAR studies

In an attempt to correlate the vasorelaxant activity with the physiochemical properties of the synthesized coumarin derivatives, QSAR study was undertaken. Descriptors of the molecular modeling software, Molecular Operating Environment (MOE version 2008.10.2),[57] were used. For the purpose of multiple linear regression analysis, vasorelaxant activity data (IC_{50} mM, Tables 1 and 2) of the compounds were converted to negative logarithm in micromolar units (pIC_{50}) as presented in Table 3. The most relevant descriptors derived for modeling the activity of the training set of the coumarin derivatives were calculated as shown in Table 3. Generally, five to one compounds to descriptors ratio needed to generate a reliable model using multiple linear regressions. The best derived QSAR model for the 20 coumarin derivatives was presented by the following estimated tetra-parametric equation with correlation coefficient (r^2) = 0.75638 and root mean square error (RMSE) = 0.06134.

$$pIC_{50} = -2.75773 + 0.23607 \log S + 0.18580\ S \log P + 0.00207\,MW + 0.00123\,TPSA$$

From the equation positive correlation of log*S* (log solubility in water), *S*log*P* (log octanol/water partition coefficient), TPSA (topological polar surface area) and MW (molecular weight) with the vasorelaxant activity was observed. The high coefficient value of log*S* and the comparatively lower value of *S*log*P* suggested that the increase in water solubility while retaining good lipophilic nature of the compounds would lead to enhancement of activity. In other words, keeping the proper balance between the aqueous solubility and the hydrophobic character of the overall molecule is the

Table 3
The molecular descriptor values, experimental and predicted activity values of the studied compounds

Compound No	log*S*	*S*log*P*	TPSA	Weight	Exp. pIC_{50} (μM)	Predicted pIC_{50} (μM)	Residual values (RES)
3	−2.4919	1.2010	52.32	161.1600	−2.7259	−2.7235	−0.0024
4	−2.5169	0.8391	88.14	203.2020	−2.6212	−2.6401	0.0189
5	−4.0240	1.4203	79.79	269.2600	−2.8451	−2.7851	−0.0600
6	−5.2549	3.1142	64.11	287.7060	−2.6703	−2.7594	0.0891
7	−5.1024	3.3070	67.35	324.3840	−2.6946	−2.6147	−0.0799
8	−4.5227	2.7692	64.11	267.2880	−2.6138	−2.6926	0.0788
13	−6.6742	3.2733	113.92	355.3570	−2.7745	−2.8607	0.0862
14	−5.3636	2.4332	148.41	388.3800	−2.6703	−2.5939	−0.0764
16	−4.8609	1.1914	138.07	389.3600	−2.6684	−2.6998	0.0314
17	−6.7533	3.2976	106.82	427.4640	−2.6243	−2.7376	0.1133
21	−3.1337	1.8300	52.60	218.2080	−2.6803	−2.6477	−0.0326
22a	−5.4650	3.4249	61.63	310.3008	−2.7528	−2.7890	0.0362
22c	−5.4000	3.5235	52.60	306.3121	−2.7210	−2.7807	0.0597
22e	−6.4566	4.8538	52.60	374.3100	−2.5729	−2.6588	0.0859
22f	−6.4214	4.2454	52.60	352.4036	−2.8482	−2.7671	−0.0511
22g	−6.1902	3.4317	104.41	351.3096	−2.8451	−2.8318	−0.0133
23b	−6.4598	3.3951	87.33	351.3860	−2.8129	−2.8287	0.0158
23c	−6.8372	3.3336	87.33	345.3580	−2.9031	−2.9386	0.0355
23d	−7.3111	3.642	87.33	359.3850	−2.9694	−2.9659	−0.0035
23f	−7.8586	4.0555	87.33	391.4510	−3.0577	−2.9557	−0.1020

key for maintaining high activity. This was in good agreement with the obtained experimental data, where one of the most active compounds; **8** (IC_{50} 0.411 mM) possessed considerable balance between log*S* value (−4.5227) and *S*log*P* value (2.7692). On the other hand, compound **23f** which showed apparent drop in log*S* value (−7.8586) and high *S*log*P* value (4.0555), proved to be the least active one (IC_{50} 1.1412 mM).

3. Conclusion

Two groups of novel pyrimidine coumarin hybrids, 6-(substituted pyrimidin-2-yl)aminocoumarins **5**–**17** and 8-(6-aryl-2-aminopyrimidin-4-yl)-7-methoxy coumarins **23a**–**g** were synthesized as potential vasodilators. The effect of these compounds and the coumarin intermediates **3**, **4**, **21** and **22a**–**g** on nor-epinephrine induced contracture in thoracic rat aortic rings was investigated using prazocin as reference drug. In general, it could be concluded that the activity of the first group of hybrids, pyrimidinylamino coumarins **5**–**17** was superior to that of the second one; pyrimidinylcoumarins **23a**–**g**. Considering the fact that the intermediate chalcones **22a**–**g** possessed higher activity over their cyclized pyrimidinylcoumarins **23a**–**g** and observing the relatively flexible structures of these chalcones compared to their corresponding pyrimidines suggested that flexibility is a structural feature required to facilitate the binding of the appropriate functional groups to the biotarget. This assumption could be supported by observing the influence of the –NH-spacer of the pyrimidinylamino derivatives **5**–**17** that enabled the pyrimidine ring to adopt different conformations allowing potential binding groups to take their proper orientation in space. Nevertheless, this assumption should be carefully considered and this will be the aim of a future work for the development and optimization of coumarin–pyrimidine hybrids.

Furthermore, the result of the QSAR studies performed made clear that the increase in aqueous solubility while retaining good hydrophobic character of the overall molecule is the key for maintaining high relaxation activity.

4. Experimental

4.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. TLC was monitored on FLUKA silica gel TLC aluminium cards (0.2 mm thickness) with fluorescent indicator 254 nm using chloroform/methanol (9:1) as eluent. Melting points were performed on Stuart SMP3 version 5 digital melting point apparatus and were uncorrected. Elemental microanalyses were performed at the micro-analytical center, Faculty of Science, Cairo University. NMR spectra were recorded on Varian mercury 300BB at 300 MHz for ^{1}H NMR, and at 75.45 MHz for ^{13}C NMR; using tetramethylsilane (TMS) as internal reference. Chemical shift values were given in ppm. Mass spectra were performed on Fennigan MAT, SSQ 7000 mass spectrophotometer at 70 eV. IR spectra were recorded on Bruker FT-IR spectrophotometer as potassium bromide disc.

4.1.1. 6-Nitro-2*H*-chromen-2-one (2)

Compound **2** was prepared according to the literature procedure[46–48] (mp 181 °C, as reported).

4.1.2. 6-Amino-2*H*-chromen-2-one (3)

Compound **3** was prepared according to the literature procedure[46–48] (mp 164 °C, as reported).

4.1.3. 1-(2-Oxo-2*H*-chromen-6-yl) guanidine (4)

A mixture of 6-aminocoumarin **3** (2.00 g; 12.40 mmol), cyanamide (1.00 g; 24.80 mmol) and ethanolic hydrochloric acid (50 ml) was refluxed for 24 h. The reaction mixture was evaporated till dryness. The residue was dissolved in dimethyl formamide (DMF), and poured on water to give a clear solution. The solution was neutralized with sodium bicarbonate and the product was filtered. The dried product was crystallized from DMF.

Mp 226–228 °C; yield: 57%. IR ν_{max}/cm^{-1}: 3395–3159 (NHs, NH_2), 3051 (CH aromatic), 1725 (C=O), 1632 (NH). ^{1}H NMR (DMSO-d_6): δ: 5.2–5.5 (s, br, 4H, NHs, NH_2, D_2O exch.), 6.38 (d, 1H, CH-3, J = 9.0 Hz), 7.02 (d, 1H, CH-7, J = 9.0 Hz), 7.08 (s, 1H, CH-5), 7.21 (d, 1H, CH-8, J = 9.0 Hz), 7.94 (d, 1H, CH-4, J = 9.0 Hz). MS, *m*/*z*: 203 [M$^+$]. Anal. Calcd for $C_{10}H_9N_3O_2{\cdot}H_2O$ (221.08): C, 54.29; H, 5.01; N, 19.00. Found: C, 54.72; H, 4.73; N, 20.00.

4.1.4. 6-(3,4-Dihydro-6-methyl-4-oxopyrimidin-2-ylamino)-2*H*-chromen-2-one (5)

6-Guanidocoumarin **4** (1.00 g; 4.90 mmol) and ethyl acetoacetate (1.30 g; 1.26 ml; 9.80 mmol) were heated under reflux in dry DMF (10 ml) for 24 h. The reaction mixture was poured on water; the solid was filtered, dried and crystallized from DMF/water.

Mp >300 °C; yield: 45%. IR ν_{max}/cm^{-1}: 3429–3336 (NHs), 3047 (CH aromatic), 2920, 2910 (CH aliphatic), 1730 (C=O), 1662 (C=O), 1626 (NH). ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 3H, CH_3), 5.75 (s, 1H, CH-5 pyrimidine), 6.45 (d, 1H, CH-3, J = 9.5 Hz), 7.33 (d, 1H, CH-7, J = 9.0 Hz), 7.78 (d, 1H, CH-8, J = 9.0 Hz), 8.00 (s, 1H, CH-5), 8.04 (d, 1H, CH-4, J = 9.2 Hz), 9.2 (s, br, 2H, NHs, D_2O exch.). MS, *m*/*z*: 269 [M$^+$]. Anal. Calcd for $C_{14}H_{11}N_3O_3$ (269.26): C, 62.45; H, 4.09; N, 15.61. Found: C, 62.37; H, 4.32; N, 15.63.

4.1.5. 6-(4-Chloro-6-methylpyrimidin-2-ylamino)-2*H*-chromen-2-one (6)

Compound **5** (1.50 g; 5.50 mmol) was refluxed in phosphorous oxychloride (10 ml) for 5 h. Excess phosphorous oxychloride was distilled under reduced pressure and the oily residue was triturated with ice-water and neutralized with ammonium hydroxide. The solid was filtered, left to dry and recrystallized from DMF/water.

Mp >300 °C; yield: 57%. IR ν_{max}/cm^{-1}: 3410 (NH), 3116 (CH aromatic), 2950, 2910 (CH aliphatic), 1720 (C=O), 1620 (NH). ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 6.44 (d, 1H, CH-3, J = 9.3 Hz), 7.29 (d, 1H, CH-7, J = 8.5 Hz), 7.48 (s, 1H, CH-5), 7.83 (d, 1H, CH-8, J = 8.7 Hz), 7.87 (s, 1H, CH-5 pyrimidine), 7.94 (d, 1H, CH-4, J = 9.0 Hz), 9.7 (s, br, 1H, NH, D_2O exch.). Anal. Calcd for $C_{14}H_{10}ClN_3O_2{\cdot}H_2O$(305.06): C, 55.00; H, 3.96; N, 13.74. Found: C, 55.07; H, 4.03; N, 14.92.

4.1.6. 6-[4-(*N*,*N*-Diethylamino)-6-methylpyrimidin-2-ylamino]-2*H*-chromen-2-one (7)

A solution of compound **6** (1.00 g; 3.50 mmol) and diethyl amine (0.51 g; 0.72 ml; 7.00 mmol) in dry DMF was heated at 100 °C for 6 h. The mixture was evaporated till dryness under reduced pressure, triturated with water and filtered. The crude product was dried and crystallized from DMF/ethanol.

Mp 248–250 °C; yield: 30%. IR ν_{max}/cm^{-1}: 3402 (NH), 3093 (CH aromatic), 2970, 2927 (CH aliphatic), 1720 (C=O), 1620 (NH). ^{1}H NMR (DMSO-d_6): δ 1.18 (t, 6H, 2 CH_2CH_3, J = 7.0 Hz), 2.72 (s, 3H, CH_3), 3.33 (m, 4H, 2 CH_2CH_3, J = 7.0 Hz), 6.44 (d, 1H, CH-3, J = 9.3 Hz), 7.28 (d, 1H, CH-7, J = 8.7 Hz), 7.84 (s, 1H, CH-5), 7.85 (d,1H, CH-8, J = 8.7 Hz), 7.94 (s, 1H, CH-5 pyrimidine), 7.97 (d, 1H, CH-4, J = 9.0 Hz), 9.3 (s, br, 1H, NH, D_2O exch.). Anal. Calcd for $C_{18}H_{20}N_4O_2$ (324.39): C, 66.65; H, 6.21; N, 17.27. Found: C, 66.52; H, 5.98; N, 16.30.

4.1.7. 6-(4,6-Dimethylpyrimidin-2-ylamino)-2*H*-chromen-2-one (8)

Compound **8** was prepared from **4** (1.00 g; 4.90 mmol) and acetyl acetone (0.98 g; 1.00 ml; 9.80 mmol) applying the same procedure for compound **5**.

Mp 231–233 °C; yield: 40%. IR ν_{max}/cm^{-1}: 3300 (NH), 3043 (CH aromatic), 2950, 2920 (CH aliphatic), 1697 (C=O), 1616 (NH). ^1H NMR (DMSO-d_6): δ 2.33 (s, 6H, 2 CH_3), 6.42 (d, 1H, CH-3, J = 9.0 Hz), 6.65 (s, 1H, CH-5), 7.34 (d, 1H, CH-7, J = 9.0 Hz), 7.90 (d, 1H, CH-8, J = 9.0 Hz), 8.04 (d, 1H, CH-4, J = 9.0 Hz), 8.21 (s, 1H, CH-5 pyrimidine), 9.66 (s, 1H, NH, D_2O exch.). Anal. Calcd for $C_{15}H_{13}N_3O_2$ (267.29): C, 67.41; H, 4.87; N, 15.73. Found: C, 67.31; H, 4.78; N, 15.34.

4.1.8. 6-(6-Methyl-4-phenylpyrimidin-2-ylamino)-2*H*-chromen-2-one (9)

6-Guanidocoumarin **4** (1.00 g; 4.90 mmol) and benzoyl acetone (1.00 g; 9.80 mmol) were heated in mineral oil at 200 °C for 2 h. The reaction mixture was poured onto petroleum ether; the solid was filtered, left to dry and crystallized from DMF/water.

Mp >300 °C; yield: 40%. IR ν_{max}/cm^{-1}: 3350 (NH), 3099 (CH aromatic), 2924, 2872 (CH aliphatic), 1714 (C=O), 1618 (NH). ^1H NMR (DMSO-d_6): δ 2.07 (s, 3H, CH_3), 6.43 (d, 1H, CH-3, J = 9.0 Hz), 7.30–7.37 (m, 3H, CH-5, CH-7 and CH-8), 7.60–7.94 (m, 6H, Ar H and CH-5 pyrimidine), 8.2 (d, 1H, CH-4, J = 9.0 Hz), 9.30 (s, 1H, NH, D_2O exch.). Anal. Calcd for $C_{20}H_{15}N_3O_2{\cdot}2H_2O$(365.14): C, 65.74; H, 5.24; N, 11.50. Found: C, 66.00; H, 5.00; N, 12.26.

4.1.9. 6-Hydroxy-2-(2-oxo-2*H*-chromen-6-ylamino) pyrimidin-4(3*H*)-one (10)

Compound **10** was prepared from **4** (1.00 g; 4.90 mmol) and diethyl malonate (1.56 g; 1.50 ml; 9.80 mmol) adopting the same method for compound **9**.

Mp 265–266 °C; yield: 38%. IR ν_{max}/cm^{-1}: 3327 (OH), 3184 (NHs), 3080 (CH aromatic), 1716 (C=O), 1681 (C=O), 1622 (NH). ^1H NMR (DMSO-d_6): δ 6.36 (d, 1H, CH-3, J = 9.0 Hz), 7.06–7.41 (m, 3H, CH-5, CH-7 and CH-8), 7.56 (s, 1H, CH-5 pyrimidine), 7.89 (d, 1H, CH-4, J = 9.0 Hz), 9.70–10.20 (s, br, 3H, NHs and OH, D_2O exch.). MS, m/z: 271 [M^+]. Anal. Calcd for $C_{13}H_9N_3O_4$ (271.23): C, 57.56; H, 3.32; N, 15.50. Found: C, 57.79; H, 3.56; N, 15.37.

4.1.10. 6-(4, 6-Dichloropyrimidin-2-ylamino)-2*H*-chromen-2-one (11)

Compound **11** was prepared from **10** using the same method adopted for compound **6**.

Mp >300; yield: 52%. IR ν_{max}/cm^{-1}: 3307 (NH), 3095 (CH aromatic), 1716 (C=O), 1624 (NH). ^1H NMR (DMSO-d_6): δ 6.45 (d, 1H, CH-3, J = 9.2 Hz), 7.30 (d, 1H, CH-7, J = 9.0 Hz), 7.41 (s, 1H, CH-5), 7.85 (d,1H, CH-8, J = 9.0 Hz), 7.94 (d, 1H, CH-4, J = 9.0 Hz), 8.05 (s, 1H, CH-5 pyrimidine), 9.50 (s, 1H, NH, D_2O exch.). Anal. Calcd for $C_{13}H_7Cl_2N_3O_2$ (306.99): C, 50.67; H, 2.29; N, 13.64. Found: C, 50.70; H, 2.40; N, 13.55.

4.1.11. 6-[4-Chloro-5-(2-chloroethyl)-6-methylpyrimidin-2-ylamino]-2*H*-chromen-2-one (12)

6-Guanidocoumarin **4** (1.00 g; 4.90 mmol) and 2-acetylbutyrolactone (0.75 g; 0.65 ml; 5.90 mmol) were refluxed in phosphorous oxychloride (10 ml) for 5 h. The reaction mixture was evaporated under reduced pressure and the oily residue was triturated with ice-water and neutralized with ammonium hydroxide. The solid was filtered, dried and crystallized from DMF/water.

Mp >300 °C; yield: 35%. IR ν_{max}/cm^{-1}: 3383 (NH), 3053 (CH aromatic), 2920, 2850 (CH aliphatic), 1732 (C=O), 1620 (NH). ^1H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 2.89 (t, 2H, CH_2CH_2Cl, J = 6.5 Hz), 3.95 (t, 2H, CH_2CH_2Cl, J = 6.5 Hz), 6.54 (d, 1H, CH-3, J = 9.0 Hz), 7.77 (d, 1H, CH-7, J = 9.0 Hz), 7.87 (s, 1H, CH-5), 8.06 (d, 1H, CH-8, J = 9.0 Hz), 8.30 (d, 1H, CH-4, J = 9.0 Hz), 10.05 (s, 1H, NH, D_2O exch.). MS, m/z: 351 [M^++1]. Anal. Calcd for $C_{16}H_{13}Cl_2N_3O_2$ (350.21): C, 54.87; H, 3.72; N, 12.00. Found: C, 55.08; H, 3.96; N, 11.80.

4.1.12. 4-Amino-2-(2-oxo-2*H*-chromen-6-ylamino)-6-phenylpyrimidine-5-carbonitrile (13)

6-Guanidocoumarin **4** (1.00 g; 4.90 mmol) and benzylidene malononitrile (1.50 g; 5.90 mmol) were heated under reflux in dry DMF (10 ml) for 24 h. The reaction mixture was poured onto water; the solid was filtered, left to dry and crystallized from DMF/water. Mp 213–215 °C; yield: 58%. IR ν_{max}/cm^{-1}: 3334 br (NH, NH_2), 3064 (CH aromatic), 2204 (C≡N), 1716 (C=O), 1624 (NH). ^1H NMR (DMSO-d_6): δ 6.48 (d, 1H, CH-3, J = 9.3 Hz), 7.32 (d, 1H, CH-7, J = 9.0 Hz), 7.54–7.84 (m, 6H, Ar H and CH-8), 7.94 (s, 1H, CH-5), 8.02 (d, 1H, CH-4, J = 9.3 Hz), 10.02 (s, br, 3H, NH, NH_2, D_2O exch.). MS, m/z: 355 [M^+]. Anal. Calcd for $C_{20}H_{13}N_5O_2$ (355.36): C, 67.62; H, 3.67; N, 19.72. Found: C, 67.38; H, 4.05; N, 19.43.

4.1.13. 4-Amino-*N*-hydroxy-2-(2-oxo-2*H*-chromen-6-ylamino)-6-phenylpyrimidine-5-carboxamidine (14)

A mixture of compound **13** (1.00 g; 2.80 mmol), hydroxylamine hydrochloride (0.20 g; 3.00 mmol) and sodium bicarbonate (0.32 g; 3.00 mmol) in dry DMF (10 ml) was heated at 100 °C for 24 h. The mixture was concentrated, poured onto water and filtered. The crude product was dried and recrystallized from DMF/ethanol.

Mp 249–251 °C; yield: 27%. IR ν_{max}/cm^{-1}: 3348 br (OH, NHs, NH_2s), 3085 (CH aromatic), 1716 (C=O), 1620 (NHs, NH_2s). ^1H NMR (DMSO-d_6): δ 6.52 (d, 1H, CH-3, J = 9.0 Hz), 7.24–7.27 (m, 5H, Ar H), 7.32 (d, 1H, CH-7, J = 9.0 Hz), 7.55 (s, 1H, CH-5), 7.84 (d, 1H, CH-8, J = 9.0 Hz), 7.99 (d, 1H, CH-4, J = 9.0 Hz), 10.1 (s, br, 6H, NH, NH_2s and OH, exch.). ^{13}C NMR: δ 116.22–136.72 (aromatic Cs), 144.38 (C-4 chromene), 148.76 (C-4, pyrimidine), 160.08 (C=O, chromene), 162.24 (C of amidine group), 164.84 (C-6, pyrimidine), 169.12 (C-2, pyrimidine). Anal. Calcd for $C_{20}H_{16}N_6O_3$ (388.38): C, 61.85; H, 4.15; N, 21.64. Found: C, 61.68; H, 4.28; N, 21.33.

4.1.14. 4-Oxo-2-(2-oxo-2*H*-chromen-6-ylamino)-6-phenyl-3, 4-dihydropyrimidine-5-carbonitrile (15)

Compound **15** was prepared from **4** (1.00 g; 4.90 mmol) and ethyl benzylidene cyanoacetate (1.20 g; 5.90 mmol) applying the same procedure as compound **13**.

Mp 255–257 °C; yield: 64%. IR ν_{max}/cm^{-1}: 3350–3296 (NHs), 3088 (CH aromatic), 2200 (C≡N), 1737 (C=O), 1681 (C=O), 1656 (NH). ^1H NMR (DMSO-d_6): δ 6.43 (d, 1H, CH-3, J = 9.6 Hz), 7.29 (d, 1H, CH-7, J = 9.0 Hz), 7.45–7.79 (m, 5H, Ar H), 7.89 (d, 1H, CH-8, J = 9.0 Hz), 7.93 (s, 1H, CH-5), 8.05 (d, 1H, CH-4, J = 9.4 Hz), 11.00 (s, br, 2H, NHs). ^{13}C NMR: δ 86.28 (C-5, pyrimidine), 116.29–136.99 (aromatic Cs and C≡N), 144.15 (C-6, chromene), 149.12 (C-4, chromene), 159.60 (C=O, chromene), 159.94 (C-6, pyrimidine), 162.22 (C-2, pyrimidine), 169.77 (C=O, pyrimidine). MS, m/z: 356 [M^+]. Anal. Calcd for $C_{20}H_{12}N_4O_3$ (356.34): C, 67.42; H, 3.37; N, 15.73. Found: C, 67.85; H, 3.85; N, 15.71.

4.1.15. *N*-Hydroxy-4-oxo-2-(2-oxo-2*H*-chromen-6-ylamino)-6-phenyl-3,4-dihydro pyrimidine-5-carboxamidine (16)

A mixture of compound **15** (1.00 g; 2.80 mmol), hydroxylamine hydrochloride (0.20 g; 3.00 mmol) and sodium bicarbonate (0.32 g; 3.00 mmol) in dry DMF was heated at 100 °C for 24 h. The mixture was concentrated, poured over ice-water and the precipitate formed was collected and filtered. The dry crude product was recrystallized from DMF/ethanol.

Mp 290–292 °C; yield: 32%. IR ν_{max}/cm^{-1}: 3356 br. (OH, NHs, NH_2), 3086 (CH aromatic), 1715 (C=O), 1612 (NH). ^1H NMR

(DMSO-d_6): δ 6.45 (d, 1H, CH-3, J = 9.3 Hz), 7.33 (d, 1H, CH-7, J = 9.0 Hz), 7.48–7.65 (m, 6H, CH-5, Ar H), 7.83 (d, 1H, CH-8, J = 9.0 Hz), 7.97 (d, 1H, CH-4, J = 9.3 Hz), 9.8 (s, br, 5H, 2 NHs, NH_2 and OH, D_2O exch.). Anal. Calcd for $C_{20}H_{15}N_5O_4$ (389.36): C, 61.69; H, 3.88; N, 17.99. Found: C, 61.48; H, 4.09; N, 17.96.

4.1.16. 6-[4-(*N*,*N*-Diethylamino) phenyl]-4-oxo-2-(2-oxo-2*H*-chromen-6-ylamino)-3,4-dihydropyrimidine-5-carbonitrile (17)

Compound **17** was prepared from **4** (1.00 g; 4.90 mmol) and ethyl 4-(*N*,*N*-diethylamino)benzylidene cyanoacetate (1.60 g; 5.90 mmol) adopting the same method used for compound **13**.

Mp 201–202 °C; yield: 87%. IR ν_{max}/cm^{-1}: 3400 (NHs), 3050 (CH aromatic), 2972, 2870 (CH aliphatic), 2208 (C≡N), 1718 br (C=Os), 1610 (NH). ^{1}H NMR (DMSO-d_6): δ 1.27 (t, 6H, 2 CH_2CH_3, J = 7.0 Hz), 3.51 (q, 4H, 2CH_2CH_3, J = 7.2 Hz), 6.45 (d, 1H, CH-3, J = 9.2 Hz), 6.76 (d, 2H, CH-3 phenyl and CH-5 phenyl, J = 8.2 Hz), 6.84 (d, 2H, CH-2 phenyl and CH-6 phenyl, J = 8.2 Hz,) 7.24 (d, 1H, CH-7, J = 9 Hz), 7.31 (s, 1H, CH-5), 7.65 (d, 1H, CH-8, J = 9 Hz), 7.95 (d, 1H, CH-4, J = 9.3 Hz), 9.61 (s, 2H, NHs, D_2O exch.). Anal. Calcd for $C_{24}H_{21}N_5O_3$ (427.47): C, 67.45; H, 4.92; N, 16.39. Found: C, 67.40; H, 5.19; N, 14.84.

4.1.17. 7-Acetoxy-2*H*-chromen-2-one (19)

Compound **19** was prepared according to the literature procedure[51] (mp 147 °C, as reported).

4.1.18. 8-Acetyl-7-hydroxy-2*H*-chromen-2-one (20)

Compound **20** was prepared according to the literature procedure[51] (mp 167 °C, as reported).

4.1.19. 8-Acetyl-7-methoxy-2*H*-chromen-2-one (21)

Compound **21** was prepared according to the literature procedure[52–54] (mp 123 °C, as reported).

4.1.20. General procedure for synthesis of 7-methoxy-8-[arylacryloyl]-2*H*-chromen-2-one (22a–g)

To an ice cooled solution of 8-acetyl-7-methoxy-2*H*-chromen-2-one **21** (2.00 g, 9.16 mmol) and the appropriate aldehyde (9.16 mmol) in ethanol (20 ml); a cooled solution of 10% sodium hydroxide (2 ml) was added dropwise. The reaction mixture was maintained at 0 °C for 1 h, and then stirred at room temperature for 24 h. The reaction mixture was poured on ice-water and neutralized with acetic acid. The solid was filtered and washed several times with cold water. The dried product was recrystallized from acetone.

4.1.20.1. 7-Methoxy-8-[3-(5-methylfuran-2-yl)acryloyl]-2*H*-chromen-2-one (22a).

Mp 130–132 °C; yield: 75%. IR ν_{max}/cm^{-1}: 3037 (CH aromatic), 2993, 2852 (CH aliphatic), 1703 (C=O$_s$). ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.30 (m, 2H, CH-3 and CH-4 furan), 6.59 (d, 1H, COC*H*=CH, J = 15.9 Hz), 6.92 (d, 1H, CH-3 furan, J = 3.0 Hz), 7.04 (d, 1H, COCH=C*H*, J = 15.9 Hz), 7.18 (d, 1H, CH-6 J = 8.7), 7.81 (d, 1H, CH-5, J = 8.7 Hz), 8.06 (d, 1H, CH-4 J = 9.3 Hz). MS, m/z 310: [M$^+$]. Anal. Calcd for $C_{18}H_{14}O_5$ (310.30): C, 69.67; H, 4.55. Found: C, 69.37; H, 4.50.

4.1.20.2. 7-Methoxy-8-[3-(thiophen-2-yl)acryloyl]-2*H*-chromen-2-one (22b).

Mp 180–182 °C; yield: 78%. IR ν_{max}/cm^{-1}: 3093 (CH aromatic), 2970, 2843 (CH aliphatic), 1732 (C=O), 1703 (C=O). ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 6.31 (d, 1H, CH-3, J = 9.6 Hz), 6.74 (d, 1H, COC*H*=CH, J = 15.9 Hz), 7.15–7.22 (m, 2H, CH-6 and CH-4 thiophene), 7.51–7.58 (m, 2H, COCH=C*H* and CH-3 thiophene), 7.79–7.83 (m, 2H, CH-5 and CH-5 thiophene), 8.03 (d, 1H, CH-4, J = 9.6 Hz). MS, m/z: 312 [M$^+$]. Anal. Calcd for $C_{14}H_{17}O_4S$ (312.34): C, 65.37; H, 3.87. Found: C, 65.36; H, 3.78.

4.1.20.3. 7-Methoxy-8-[3-phenylacryloyl]-2*H*-chromen-2-one (22c).

Mp 186–187 °C; yield: 80%. IR ν_{max}/cm^{-1}: 3050 (CH aromatic), 2916, 2847 (CH aliphatic), 1732 (C=O), 1659 (C=O). ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 6.29 (d, 1H, CH-3, J = 9.6 Hz), 7.10 (d, 1H, COC*H*=CH, J = 15.9 Hz), 7.18 (d, 1H, CH-6, J = 9.0 Hz), 7.33–7.46 (m, 4H, CH-3 phenyl, CH-4 phenyl, CH-5 phenyl, COCH=C*H*), 7.71 (m, 2H, CH-2 phenyl, CH-6 phenyl, J = 8.l Hz), 7.79 (d ,1H, CH-5, J = 9.0 Hz), 8.31 (d, 1H, CH-4, J = 9.6 Hz). MS, m/z: 306 [M$^+$+1]. Anal. Calcd for $C_{20}H_{16}O_4{\cdot}0.5H_2O$ (313.55): C, 72.78; H, 4.82. Found: C, 73.27; H, 5.20.

4.1.20.4. 7-Methoxy-8-(3-*p*-tolylacryloyl)-2*H*-chromen-2-one (22d).

Mp 137–138 °C; yield: 80%. IR ν_{max}/cm^{-1}: 3034 (CH aromatic), 2927, 2843 (CH aliphatic), 1732 (C=O), 1703 (C=O). ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.31 (d, 1H, CH-3, J = 9.6 Hz), 7.06 (d, 1H, COC*H*=CH, J = 16.2 Hz), 7.20–7.24 (m, 3H, CH-6, CH-3 phenyl, CH-5 phenyl), 7.29 (d, 1H, COCH=C*H*, J = 16.2 Hz), 7.60 (d, 2H, CH-2 phenyl, CH-6 phenyl, J = 8.1 Hz), 7.80 (d, 1H, CH-5, J = 8.4 Hz), 8.04 (d, 1H, CH-4, J = 9.6 Hz). MS, m/z: 320 [M$^+$]. Anal. Calcd for $C_{20}H_{16}O_4{\cdot}H_2O$ (338.35): C, 72.94; H, 5.20. Found: C, 72.69; H, 4.99.

4.1.20.5. 7-Methoxy-8-{3-[4-(trifluoromethyl)phenyl]acryloyl}-2*H*-chromen-2-one (22e).

Mp 206–208 °C; yield: 79%. IR ν_{max}/cm^{-1}: 3050 (CH aromatic), 2976, 2846 (CH aliphatic), 1732 (C=O$_s$). ^{1}H NMR (DMSO-d_6): δ 3.89 (s, 3H, OCH_3), 6.30 (d, 1H, CH-3, J = 9.3 Hz), 7.19–7.29 (m, 2H, CH-6 and COC*H*=CH), 7.44 (d, 1H, COCH=C*H*, J = 16.2 Hz), 7.71 (d, 2H, CH-3 phenyl, CH-5 phenyl, J = 8.4 Hz), 7.80 (d, 1H, CH-5, J = 8.7 Hz), 7.93 (d, 2H, CH-2 phenyl, CH-6 phenyl, J = 8.4 Hz), 8.01 (d, 1H, CH-4, J = 9.3 Hz). ^{13}C NMR (DMSO-d_6): δ 56.50 (OCH_3), 108.59–137.96 (aromatic Cs), 143.52 (C-4), 143.93 (CH=C*H*-phenyl), 151.10 (C-8), 158.85 (C-7), 159.17 (C=O, chromene), 191.14 (C=O). MS, m/z: 374 [M$^+$]. Anal. Calcd for $C_{20}H_{13}F_3O_4$ (374.31): C, 64.18; H, 3.50. Found: C, 64.00; H, 3.60.

4.1.20.6. 7-Methoxy-8-{3-[4-(methylthio) phenyl]acryloyl}-2*H*-chromen-2-one (22f).

Mp 201–203 °C; yield: 85%. IR ν_{max}/cm^{-1}: 3039 (CH aromatic), 2974, 2846 (CH aliphatic), 1722 (C=O), 1697 (C=O). ^{1}H NMR (DMSO-d_6): δ 2.50 (s, 3H, SCH_3), 3.87 (s, 3H, OCH_3), 6.29 (d, 1H, CH-3, J = 9.6 Hz), 7.05 (d, 1H, COC*H*=CH, J = 16.2 Hz), 7.18 (d, 1H, CH-6, J = 9.0 Hz), 7.26–7.35 (m, 3H, COCH=C*H*, CH-2 phenyl, CH-6 phenyl), 7.63 (d, 2H, CH-3 phenyl, CH-5 phenyl, J = 8.7 Hz), 7.79 (d ,1H, CH-5, J = 9.0 Hz), 8.01 (d, 1H, CH-4, J = 9.6 Hz). ^{13}C NMR (DMSO-d_6): δ 14.04 (SCH_3), 56.50 (OCH_3), 108.64–130.37 (aromatic Cs), 144.20 (C-4), 145.79 (CH=C*H*-phenyl), 151.06 (C-8), 158.79 (C-7), 159.47 (C=O, chromene), 191.42 (C=O). MS, m/z: 352 [M$^+$]. Anal. Calcd for $C_{20}H_{16}O_4S$ (352.40): C, 68.16; H, 4.58. Found: C, 68.09; H, 4.40.

4.1.20.7. 7-Methoxy-8-[3-(4-nitrophenyl)acryloyl]-2*H*-chromen-2-one (22g).

Mp 152–154 °C; yield: 65%. IR ν_{max}/cm^{-1}: 3039 (CH aromatic), 2976, 2846 (CH aliphatic), 1716 (C=O$_{(s)}$), 1508, 1346 (NO_2). ^{1}H NMR (C*D*Cl$_3$): δ 4.08 (s, 3H, OCH_3), 6.17 (d, 1H, CH-3, J = 9.6 Hz), 6.70 (d, 1H, CH-6, J = 8.7 Hz), 7.12 (d, 1H, COC*H*=CH, J = 12.6 Hz), 7.62 (d, 1H, CH-5, J = 8.7 Hz), 7.96 (d, 2H, CH-2 phenyl, CH-6 phenyl, J = 8.4 Hz), 8.04 (d, 1H, COCH=C*H*, J = 12.6 Hz), 8.18 (d, 1H, CH-4, J = 9.6 Hz), 8.30 (d, 2H, CH-3 phenyl, CH-5 phenyl, J = 8.4 Hz). MS, m/z: 351 [M$^+$]. Anal. Calcd for $C_{19}H_{13}NO_6$ (351.31): C, 64.96; H, 3.73; N, 3.99. Found: C, 64.76; H, 3.53; N, 4.20.

4.1.21. General procedure for synthesis of 8-[2-amino-6-(substituted) pyrimidin-4-yl]-7-methoxy-2*H*-chromen-2-one (23a–g)

To a solution of the appropriate chalcone **22a**–**g** (2.50 mmol) in absolute ethanol (50 ml), guanidine hydrochloride (0.27 g,

2.80 mmol) and 10 drops of hydrochloric acid were added. The mixture was heated under reflux for 8 h. The solution was filtered while hot and left to cool. The solid separated was filtered out, left to dry and recrystallized from ethanol.

4.1.21.1. 8-[2-Amino-6-(5-methylfuran-2-yl)pyrimidin-4-yl]-7-methoxy-2*H*-chromen-2-one (23a). Mp 194–196 °C; yield: 40%. IR ν_{max}/cm^{-1}: 3433 br (NH_2), 2947, 2920 (CH aliphatic), 1736 (C=O), 1604 (NH_2). ^1H NMR ($CDCl_3$): δ 1.57 (s, 2H, NH_2, D_2O exch.), 2.36 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.10 (d, 1H, CH-4 furan, J = 3.3 Hz), 6.25 (d, 1H, CH-3, J = 9.3 Hz), 6.57 (d, 1H, CH-3 furan, J = 3.3 Hz), 6.91 (d, 1H, CH-6, J = 8.7 Hz), 7.03 (s, 1H, CH-5 pyrimidine), 7.48 (d, 1H, CH-5, J = 8.7 Hz), 7.63 (d, 1H, CH-4, J = 9.6 Hz). MS, m/z: 349 [M^+]. Anal. Calcd for $C_{19}H_{15}N_3O_4$ (349.34): C, 65.32; H, 4.33; N, 12.03. Found: C, 65.40; H, 4.40; N, 12.29.

4.1.21.2. 8-[2-Amino-6-(thiophen-2-yl)pyrimidin-4-yl]-7-methoxy-2*H*-chromen-2-one (23b). Mp 173–175 °C; yield: 52%. IR ν_{max}/cm^{-1}: 3283 (NH_2), 3101 (CH aromatic), 2947, 2850 (CH aliphatic), 1728 (C=O), 1604 (NH_2). ^1H NMR ($CDCl_3$): δ 1.70 (s, 2H, NH_2, D_2O exch.), 3.86 (s, 3H, OCH_3), 6.22 (d, 1H, CH-3, J = 9.6 Hz), 6.92 (d, 1H, CH-6, J = 8.7 Hz), 7.02–7.43 (m, 4H, CH-5 pyrimidine and CHs thiophene), 7.51 (d, 1H, CH-5, J = 8.7 Hz), 7.64 (d, 1H, CH-4, J = 9.6 Hz). Anal. Calcd for $C_{18}H_{13}N_3O_3S$ (351.38): C, 61.53; H, 3.73; N, 11.96. Found: C, 61.60; H, 3.80; N, 12.22.

4.1.21.3. 8-(2-Amino-6-phenylpyrimidin-4-yl)-7-methoxy-2*H*-chromen-2-one (23c). Mp 186–188 °C; yield: 55%. IR ν_{max}/cm^{-1}: 3444 (NH_2), 3086 (CH aromatic), 2985, 2947 (CH aliphatic), 1728 (C=O), 1604 (NH_2). ^1H NMR ($CDCl_3$): δ 1.62 (s, 2H, NH_2, D_2O exch.), 3.88 (s, 3H, OCH_3), 6.25 (d, 1H, CH-3, J = 9.6 Hz), 6.93 (d, 1H, CH-6, J = 8.7 Hz), 7.01 (s, 1H, CH-5 pyrimidine), 7.27–7.54 (m, 6H, CH-5 and Ar. H), 7.65 (d, 1H, CH-4, J = 9.6 Hz). MS, m/z: 345 [M^+]. Anal. Calcd for $C_{20}H_{15}N_3O_3$ (345.35): C, 69.56; H, 4.38; N, 12.17. Found: C, 69.70; H, 4.40; N, 12.54.

4.1.21.4. 8-(2-Amino-6-*p*-tolylpyrimidin-4-yl)-7-methoxy-2*H*-chromen-2-one (23d). Mp 181–183 °C; yield: 68%. IR ν_{max}/cm^{-1}: 3429 (NH_2), 3028 (CH aromatic), 2947, 2916 (CH aliphatic), 1728 (C=O), 1600 (NH_2). ^1H NMR ($CDCl_3$): δ 1.59 (s, 2H, NH_2, D_2O exch.), 2.37 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.25 (d, 1H, CH-3, J = 9.6 Hz), 6.93 (d, 1H, CH-6, J = 8.7 Hz), 7.02 (s, 1H, CH-5 pyrimidine), 7.17 (d, 2H, CH-3 phenyl, CH-5 phenyl, J = 8.1 Hz), 7.40 (d, 2H, CH-2 phenyl, CH-6 phenyl, J = 8.1 Hz), 7.51 (d, 1H, CH-5, J = 8.7 Hz), 7.64 (d, 1H, CH-4, J = 9.6 Hz). MS, m/z: 359 [M^+]. Anal. Calcd for $C_{21}H_{17}N_3O_3$ (359.38): C, 70.18; H, 4.77; N, 11.69. Found: C, 69.88; H, 4.69; N, 11.97.

4.1.21.5. 8-{2-Amino-6-[4-(trifluoromethyl) phenyl] pyrimidin-4-yl}-7-methoxy-2*H*-chromen-2-one (23e). Mp 233–235 °C; yield: 58%. IR ν_{max}/cm^{-1}: 3442, 3414 (NH_2), 3068 (CH aromatic), 2951, 2922 (CH aliphatic), 1732 (C=O), 1604 (NH_2). ^1H NMR ($CDCl_3$): δ 1.60 (s, 2H, NH_2, D_2O exch.), 3.91 (s, 3H, OCH_3), 6.26 (d, 1H, CH-3, J = 9.6 Hz), 6.94 (d, 1H, CH-6, J = 8.7 Hz), 7.05 (d, 2H, CH-3 phenyl, CH-5 phenyl, J = 8.3 Hz), 7.37 (d, 2H, CH-2 phenyl, CH-6 phenyl, J = 8.3 Hz), 7.52 (d, 1H, CH-5, J = 8.4 Hz), 7.64 (s, 1H, CH-5 pyrimidine), 7.65 (d, 1H, CH-4, J = 9.6 Hz). ^{13}C NMR ($CDCl_3$): δ 56.48 (OCH_3). 107.89 (C-5 pyrimidine), 113.04 -130.03 (aromatic Cs+CF_3), 138.00 (C-4), 142.90 (C-8), 143.78 (C-7), 152.00 (C=O chromene, C-4 pyrimidine), 159.49 (C-6 pyrimidine), 159.70 (C-2 pyrimidine). MS, m/z: 414 [M^++1]. Anal. Calcd for $C_{21}H_{14}F_3N_3O_3$ (413.35): C, 61.02; H, 3.41; N, 10.17. Found: C, 61.31; H, 3.80; N, 9.82.

4.1.21.6. 8-{2-Amino-6-[4-(methylthio)phenyl]pyrimidin-4-yl}-7-methoxy-2*H*-chromen-2-one (23f). Mp 206–208 °C; yield: 60%. IR ν_{max}/cm^{-1}: 3429 (NH_2), 3043 (CH aromatic), 2981, 2916 (CH aliphatic), 1720 (C=O), 1585 (NH_2). ^1H NMR ($CDCl_3$): δ 1.64 (s, 2H, NH_2, D_2O exch.), 2.50 (s, 3H, SCH_3), 3.89 (s, 3H, OCH_3), 6.25 (d, 1H, CH-3, J = 9.6 Hz), 6.92 (d, 1H, CH-6, J = 8.7 Hz), 7.01 (d, 2H, CH-3 phenyl, CH-5 phenyl, J = 8.4 Hz),7.23 (d, 2H, CH-2 phenyl, CH-6 phenyl, J = 8.4 Hz), 7.42 (d, 1H, CH-5, J = 8.4 Hz), 7.63 (s, 1H, CH-5 pyrimidine) 7.66 (d, 1H, CH-4, J = 9.6 Hz). MS, m/z: 391 [M^+]. Anal. Calcd for $C_{21}H_{17}N_3O_3S$ (391.44): C, 64.43; H, 4.38; N, 10.73. Found: C, 64.20; H, 4.50; N, 10.41.

4.1.21.7. 8-[2-Amino-6-(4-nitrophenyl)pyrimidin-4-yl]-7-methoxy-2*H*-chromen-2-one (23g). Mp 190–192 °C; yield: 60%. IR ν_{max}/cm^{-1}: 3390 (NH_2), 3080 (CH aromatic), 2920, 2850 (CH aliphatic), 1712 (C=O), 1600 (NH_2). ^1H NMR (DMSO-d_6): δ 1.36 (s, 2H, NH_2, D_2O exch.), 4.04 (s, 3H, OCH_3), 6.18 (d, 1H, CH-3, J = 9.6 Hz), 6.93 (d, 1H, CH-6, J = 8.7 Hz), 7.43 (d, 1H, CH-5, J = 8.7 Hz), 7.74 (s, 1H, CH-5 pyrimidine), 7.87 (d, 1H, CH-4, J = 9.6 Hz), 8.05 (d, 2H, CH-2 phenyl, CH-6 phenyl, J = 8.5 Hz), 8.21 (d, 2H, CH-3 phenyl, CH-5 phenyl, J = 8.5 Hz). MS, m/z: 391 [M^++1]. Anal. Calcd for $C_{20}H_{14}N_4O_5$ (390.35): C, 61.54; H, 3.62; N, 14.35. Found: C, 61.20; H, 3.27; N, 14.03.

4.2. Vasorelaxant procedure

The in vitro study was performed at the Pharmacology Department, the National Research Center, Giza, Egypt, after approval from the Ethics committee of the center and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85–23, revised 1985).

The vasodilatation activity screening procedure was carried out according to the standard reported techniques[55,56] by testing the effects of the synthesized compounds **3**–**17**, **22a**–**g**, **23a**–**g** on isolated thoracic aortic rings of male Wister rats (250–350 g). After light ether anesthesia, the rats were sacrificed by cervical dislocation. The aortas were immediately excised, freed of extraneous tissues and prepared for isometric tension recording. Aorta was cut into (3–5 mm width) rings and each ring was placed in a vertical chamber '10 ml jacketed automatic multi-chamber organ bath system (Model No. ML870B6/C, Panlab, Spain)' filled with Krebs solution composed of (in mM): NaCl, 118.0; KCl, 4.7; $NaHCO_3$, 25.0; $CaCl_2$, 1.8; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11.0 and oxygenated with carbogen gas (95% O_2/5% CO_2) at 37 ± 0.5 °C. Each aortic ring was mounted between two stainless steel hooks passed through its lumen. The lower hook was fixed between two plates, while the upper one was attached to a force displacement transducer (Model No. MLT0201, Panlab, Spain) connected to an amplifier (Power Lab, AD Instruments Pty. Ltd) which is connected to a computer. The Chart for windows (v 3.4) software was used to record and elaborate data.

Preparations were stabilized fewer than 2 g resting tension during 2 h and then the contracture response to nor-epinephrine hydrochloride (10^{-6} M) was measured before and after exposure to increasing concentrations of the tested synthesized compounds. The tested compounds **3**–**17**, **21**, **22a**–**g**, **23a**–**g** were dissolved in dimethylsulfoxide (DMSO) as stock solution (10 ml of 0.01 M). Control experiments were performed in the presence of DMSO alone, at the same concentrations as those used with the derivatives tested, which demonstrated that the solvent did not affect the contractile response of isolated aorta. The observed vasodilatation activity screening data were reported (Tables 1 and 2) and the potency (IC_{50}, concentration necessary for 50% reduction of maximal nor-epinephrine hydrochloride induced contracture) was determined by the best fit line technique.

4.3. Computational method

All the computational works were performed on Molecular Operating Environment software (MOE version 2008.10.2).[57]

The structures of 20 compounds used as training set were sketched using molecular builder of MOE and each structure was subjected to energy minimization up to 0.01 Kcal/mol Å using the MMFF94x force field. Optimization methods were used followed by conformational search of each energy-minimized structure. The most stable conformer of each structure was selected and saved into database to generate the common descriptors. QuaSAR descriptor module of MOE was used to calculate about 180 descriptors for each molecule. The probability density functions used are Gaussian. The RMSD tolerance was set to 0.5 Å. Regression analysis was performed using pIC_{50} as dependent factor and the calculated physicochemical descriptors as predictable variables. In this study, the pool of descriptors was optimized using principal components analysis (PCA). The optimization started with the reduction in the number of molecular descriptors by the determination of the highly inter-correlated descriptor pairs and only one from each pair was selected; then the descriptors with insignificant variance through the data set were also rejected. QSAR model was then constructed after ensuring reasonable correlation of vasorelaxant activity with the individual descriptors and minimum inter-correlation among the descriptors used in the derived model. The quality of the model was assessed using the statistical parameter r^2.

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Supplementary data

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