Bioorganic & Medicinal Chemistry 20 (2012) 1624–1638

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Substrate specificity of acetoxy derivatives of coumarins and quinolones towards Calreticulin mediated transacetylation: Investigations on antiplatelet function

Abha Kathuria [a], Nivedita Priya [b], Karam Chand [a], Prabhjot Singh [b], Anjali Gupta [a], Sarah Jalal [a], Shilpi Gupta [a], Hanumantharao G. Raj [b,c,*], Sunil K. Sharma [a,*]

[a] Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi 110 007, India
[b] Department of Biochemistry, V.P. Chest Institute, University of Delhi, Delhi 110 007, India
[c] 201, Skylark Jewel, Jagdish Nagar, Bangaluru 560075, India

ARTICLE INFO

Article history:
Received 16 August 2011
Revised 8 November 2011
Accepted 9 November 2011
Available online 16 November 2011

Keywords:
N-Acetylaminocoumarins
Quinolin-2-ones
Platelet CRTAase
Antiplatelet agents
Cyclooxygenase-1 (Cox-1)
Thromboxane A2 (TxA2)

ABSTRACT

Calreticulin transacetylase (CRTAase) is known to catalyze the transfer of acetyl group from polyphenolic acetates (PA) to certain receptor proteins (RP), thus modulating their activity. Herein, we studied for the first time the substrate specificity of CRTAase towards *N*-acetylamino derivatives of coumarins and quinolones. This study is endowed with antiplatelet action by virtue of causing CRTAase catalyzed activation of platelet Nitric Oxide Synthase (NOS) by way of acetylation leading to the inhibition of ADP/Arachidonic acid (AA)-dependent platelet aggregation. Among all the *N*-acetylamino/acetoxy coumarins and quinolones screened, 7-*N*-acetylamino-4-methylcoumarin (7-AAMC, **17**) was found to be the superior substrate to platelet CRTAase and emerged as the most promising antiplatelet agent both in vitro and in vivo. Further it caused the inhibition of cyclooxygenase-1 (Cox-1) resulting in the down regulation of thromboxane A2 (TxA2), modulation of tissue factor and the inhibition of platelet aggregation. It was also found effective in the inhibition of LPS induced pro-thrombotic conditions.

1. Introduction

The essential role played by platelets in the pathogenesis of arterial thrombosis is well established: platelets represent a major target of therapeutic interventions aiming at decreasing the incidence and severity of cardiovascular accidents in patients. Platelets adhere to damaged endothelium by a process mediated by the interaction between membrane receptors and various ligands present in the endothelium and sub endothelium. The platelet integrin GPs Ia/IIIb and Ib/IX bind collagen and von Willebrand factor (vWF), respectively. Adhesion results in platelet activation, which in turn leads to recruitment of other platelets to the site of injury. Agonists such as thrombin, thromboxane A2, and adenosine diphosphate (ADP) are produced or released locally, causing a conformational change in membrane GPIIb/IIIa receptor, which then mediates the final obligatory steps in platelet aggregation and becoming a functional receptor for adhesive molecules such as fibronectin, vitronectin, and vWF, the last of which may mediate platelet aggregation under high shear rate conditions.[1,2]

Numerous antiplatelet agents have been developed based on their ability to block the receptors responsible for platelet activation. However, there are three families of agents that inhibit platelet function, with proven clinical efficacy: (i) Cyclooxygenase inhibitors, such as aspirin; (ii) ADP receptor antagonists, such as the thienopyridine derivatives, for example ticlopidine and clopidogrel; (iii) glycoprotein (GP) IIb/IIIa (or integrin aIIbb3) antagonists. All these drugs are used during coronary interventions and in the medical management of acute coronary syndromes, while only aspirin and the thienopyridine derivatives are used in long-term prevention of cardiovascular events.[3]

While minimizing ischemic recurrences, an intensified antiplatelet regimen also invariably leads to severe side-effects, for example gastrointestinal toxicity due to aspirin include nausea, vomiting, dyspepsia, heartburn, gastrointestinal ulceration, etc.[4] Also, in recent years, the issue of resistance to antiplatelet agents, in particular aspirin and thienopyridines, has been highlighted in the medical literature.[5–8] Despite the fact that the currently available drugs suffer from some drawbacks and are prone to resistance, they have a good risk-to-benefit ratio which justify the unceasing search for agents that can further improve the clinical outcome of patients with atherosclerosis through greater efficacy and/or safety. New platelet targets for potential antithrombotic drugs include several receptors and effectors that are important for platelet function. Further, the agents causing the inhibition of cyclooxygenase catalyzed TxA2 synthesis would also lead to the inhibition of platelet aggregation. The enzyme NOS generates NO, which is essential for vascular function. NOS is involved in vessel

* Corresponding author. Tel.: +91 11 27666555.
E-mail address: sk.sharma90@gmail.com (S.K. Sharma).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.11.016

dilatation, inhibition of platelet and leukocyte adhesion, and inhibition of proliferation and migration of vascular smooth muscle cells.[9] Earlier reports from our group have established the activation of platelet NOS by certain class of polyphenolic acetates (PAs) by way of acetylation of NOS mediated by CRTAase.[10] Accordingly, PAs were found to be effective in the inhibition of ADP induced platelet aggregation.

In the present investigation, efforts have been made to compare the specificities of *N*-acetylamino and acetoxy derivatives of coumarins and quinolones for CRTAase mediated activation of NOS and also to delineate the structure–activity relationship (SAR) with reference to the effect of alkyl group at C-3 position, and substitution of C-4 methyl by trifloromethyl group of the coumarin/quinolone moiety.

2. Results

In earlier reports we elucidated the role of acetoxy groups on the benzenoid ring of chromones, coumarins, xanthones, flavones, and quinolones, in facilitating the acetylation of receptor proteins catalyzed by CRTAase. Also, we studied the factors, such as the proximity of the acetoxy group to the oxygen/nitrogen heteroatom, the role of carbonyl group on the benzopyran nucleus, and the effect of substituents on these molecules in controlling the protein acetylation.[11–14] However, *N*-acetylaminocoumarins, and quinolones have never been screened for CRTAase activity. In lieu of the above, efforts have been made to compare for the first time the specificities of heterocyclic compounds viz. *N*-acetylaminocoumarins/quinolones for CRTAase mediated activation of NOS and other enzymes. We have evaluated the specificity of CRTAase towards a series of 6/7-acetoxyquinolones and its consequent effect on the enhancement of NO levels in platelets and inhibitory effect on ADP/AA induced platelet aggregation. Also, C-6/C-8 acetyl-7-acetoxycoumarins (**48**–**51**) were included in this study to evaluate the effect of *C*-acetyl group on the benzenoid ring.

The results clearly demonstrated that specificity of the *N*-acetylamino/acetoxy derivatives for CRTAase is in the order: *N*-acetylaminocoumarins > acetoxyquinolones > *N*-acetylamino quinolones > acetylcoumarins, and among all the compounds screened 7-*N*-acetylamino-4-methylcoumarin (7-AAMC, **17**) was found to be the best substrate to platelet CRTAase compared to the other *N*-acetylamino/acetoxy polyphenolic derivatives resulting in significant inhibition of ADP induced platelet aggregation.

The leading compound, 7-AAMC, when administered to the rats, was found to be effective in inhibition of Cox-1 activity and eventually inhibition of thromboxane. This compound was taken further for detailed study, that is in vivo LPS mediated thrombotic prone conditions and the levels of iNOS and tissue factor (TF) were measured and found to be inhibited in the case of compound treated rats.

2.1. Chemistry

2.1.1. Synthesis of 7-*N*-acetylamino derivatives of coumarin (17–21)

The synthesis of 7-amino-4-methyl derivatives of coumarin was carried out by following the procedure given by Atkins and Bliss.[15] Herein, urethane protected *m*-aminophenol (3-hydroxyphenylurethane, **1**) was made to react with alkylated ethyl acetoacetate (**2**–**5**) or 4,4,4-trifluoroethyl acetoacetate (**6**) in the presence of 70% H_2SO_4–C_2H_5OH to obtain quantitatively 3-alkyl-7-carbethoxy-4-methyl/trifluoromethylcoumarin (**7**–**11**) via Pechmann condensation. The earlier published literature procedure from our group was followed for synthesizing alkylated ethyl acetoacetate (2-alkyl ethylbutanoate) from ethyl acetoacetate and alkyl bromide.[13] The deprotection of corresponding 7-carbethoxy-4-methyl/fluorocoumarin (**7**–**11**) was carried out with a 1:1 mixture of concentrated suphuric acid and acetic acid to yield 3-alkyl-7-amino-4-methyl/trifluoromethylcoumarin (**12**–**16**) which was then acetylated using acetic anhydride and catalytic amount of *N*,*N*-dimethyl amino pyridine (DMAP) in THF to yield corresponding *N*-acetyl coumarin **17**–**21** (Scheme 1).

2.1.2. Synthesis of *N*-(2-oxo-1,2-dihydroquinolin-7-yl) acetamide (25–27)

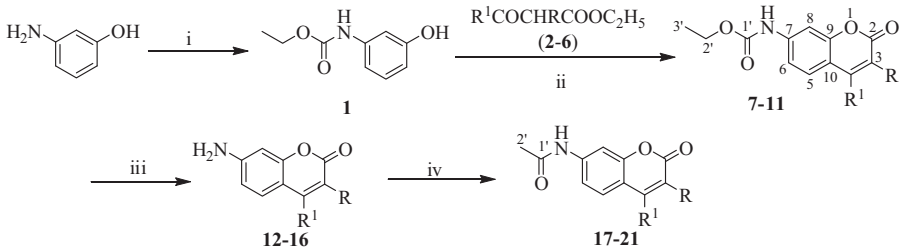
Synthesis of *N*-(2-oxo-(4-methyl/trifloromethyl)-1,2-dihydro quinolin-7-yl)acetamide (**25**–**27**) was carried out from 1,3-diaminobenzene and substituted ethyl acetoacetate (**2**,**3** and **6**) by following Conrad Limpach synthesis,[16] the compound so obtained (**22**–**24**) was then subjected to acetylation (Scheme 2).

2.1.3. Synthesis of acetoxy derivatives of quinolin-2(1*H*)-ones (38–42)

Acetoxy derivatives of quinolin-2-ones were synthesized by following the method reported earlier from our group.[14] Anisidines were first reacted with substituted ethyl acetoacetate (**2** and **6**) via Knorr reaction to give methoxy quinolones (**28**–**32**). Subsequently demethylation, was carried out using a mixture of hydrobromic acid and acetic acid, the corresponding hydroxy quinolones (**33**–**37**) so obtained were then acetylated with acetic anhydride in acetic acid to yield acetoxyquinolones **38**–**42** (Scheme 3).

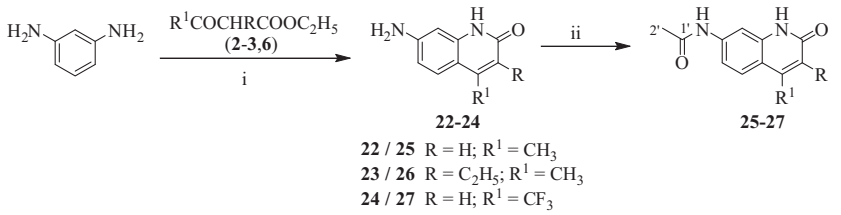
2.1.4. Synthesis of 6/8-acetyl-7-acetoxycoumarins (49–52)

7-Acetoxy-4-methylcoumarin (**43**) obtained by following the literature procedure,[17] was subjected to Fries migration[18] and a mixture of 8-acetyl- (**44**–**45**) and 6-acetyl- (**46**–**47**) 7-hydroxycoumarins in 9:1 ratio was obtained. The mixture was separated through column chromatography and the resulting compounds

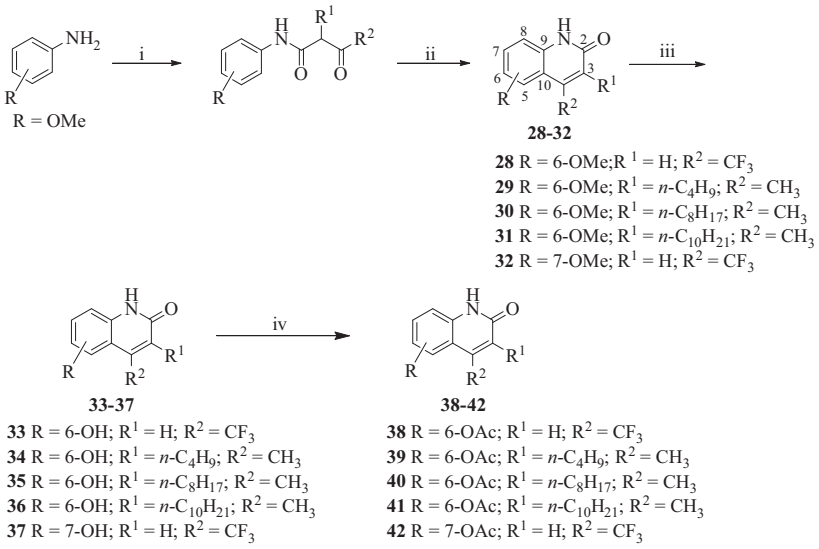


2 / 7 / 12 / 17 R = H; $R^1 = CH_3$
3 / 8 / 13 / 18 R = C_2H_5; $R^1 = CH_3$
4 / 9 / 14 / 19 R = *n*-C_6H_{13}; $R^1 = CH_3$
5 / 10 / 15 / 20 R = *n*-$C_{10}H_{21}$; $R^1 = CH_3$
6 / 11 / 16 / 21 R = H; $R^1 = CF_3$

Scheme 1. Synthesis of 7-aminocoumarin derivatives. Reagents and conditions: (i) C_2H_5OCOCl, Et_2O, rt; (ii) H_2SO_4–C_2H_5OH (7:3), rt; (iii) H_2SO_4–CH_3COOH (1:1), reflux; (iv) $(CH_3CO)_2O$, DMAP, THF, rt.



Scheme 2. Synthesis of 7-amino derivatives of quinolones. Reagents and conditions: (i) 150 °C; (ii) $(CH_3CO)_2O$, CH_3COOH.



Scheme 3. Synthesis of acetoxy derivatives of quinolones. Reagents and conditions: (i) $R^2COCHR^1COOC_2H_5$, 150 °C; (ii) H_2SO_4, 100 °C; (iii) HBr–CH_3COOH (7:3), 120 °C; (iv) $(CH_3CO)_2O$, THF, rt.

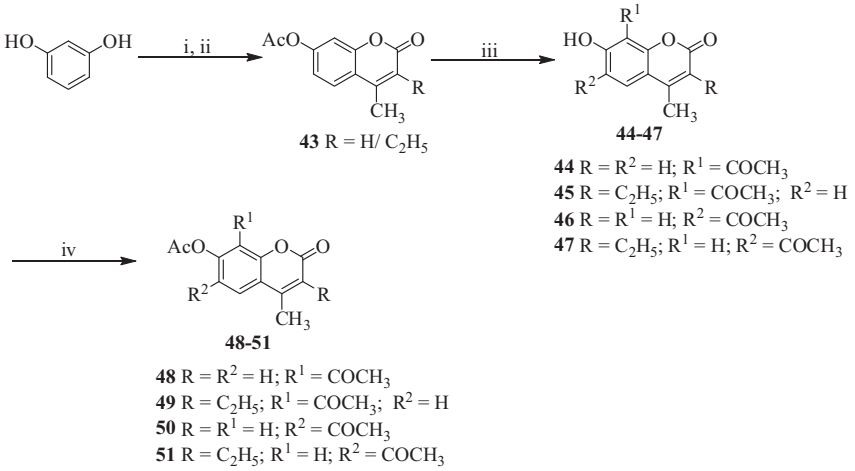
were then acetylated using acetic anhydride and catalytic amount of DMAP to yield the title compounds (Scheme 4).

All the compounds were fully characterized on the basis of their physical and spectral data, and of total forty six coumarin and quinolone derivatives synthesized, twenty five, that is, **8**–**10**, **18**–**20**, **23**, **26**, **29**–**31**, **34**–**36**, **38**–**42**, **45**, and **47**–**51** are novel and reported for the first time. Though the compounds **1**, **7**, **11**–**17**, **21**–**22**, **24**–**25**, **27**–**28**, **32**–**33**, **37**, **44**, **a**nd **46** are known in literature, however their complete spectral data is not reported. Herein, we have reported the spectral data for all these compounds in the experimental section.

2.2. Biological activity

2.2.1. CRTAase activities

The results illustrated in Figure 1 revealed the differential specificity of platelet CRTAase to a number of *N*-acetylamino/acetoxy derivatives of coumarins and quinolones. The specificity of these compounds towards platelet CRTAase was found to be in the order: *N*-acetylaminocoumarins > acetoxyquinolones > *N*-acetylaminoquinolones > acetylcoumarins. It is evident from the results (Fig. 1) that by incorporating an alkyl group at C-3 position of coumarins and quinolones resulted in drastic reduction of CRTA-



Scheme 4. Synthesis of acetoxy derivatives of acetylcoumarins. Reagents and conditions: (i) $CH_3COCHRCOOC_2H_5$, concd H_2SO_4, rt; (ii) $(CH_3CO)_2O$, DMAP, THF, rt; (iii) $AlCl_3$, 125–170 °C; (iv) $(CH_3CO)_2O$, DMAP, THF, rt.

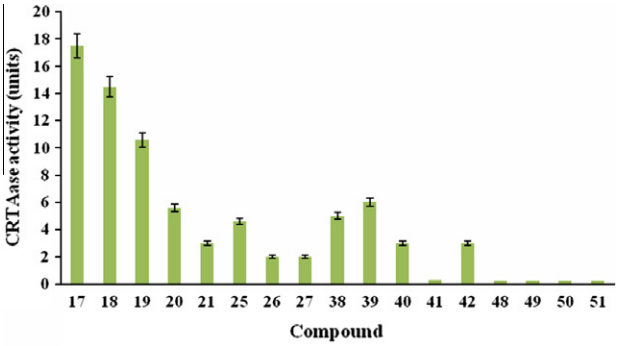


Figure 1. The effect of test compounds on platelet CRTAase. CRTAase activity was assayed in platelet lysate using test compounds (50 μM). The unit of CRTAase was expressed in terms of % inhibition of GST under the experimental conditions.

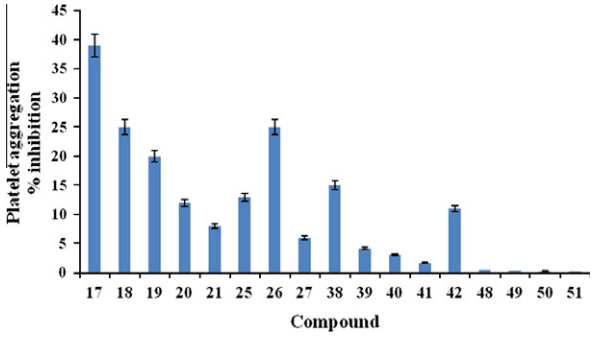


Figure 3. Antiplatelet activity of test compounds in vitro. PRP was incubated with the test compound (100 μM) for 10 min at 37 °C followed by the addition of ADP (15 μM) and platelet aggregation was monitored by aggregometry. Values are mean ± SEM of five observations.

ase activity, the activity decreases with increase in the size of alkyl chain. Also a decline in platelet CRTAase activity was observed on substituting the C-4 methyl by trifluoromethyl group, that is in compounds **21**, **27**, **38**, and **42**. Further the presence of acetyl group at C-6/C-8 position on the benzenoid ring in coumarins **48**–**51** resulted in poor inhibition of platelet aggregation or no inhibition at all.

2.2.2. Enhancement of intracellular nitric oxide (NO) levels

2.2.2.1. Measurement of NO level by flow cytometry. The influence of *N*-acetylamino or acetoxy derivatives of coumarins/quinolones on the NO level in platelets has been described in Figure 2. Platelets were incubated with *N*-acetylamino/acetoxy coumarins and quinolones followed by the measurement of NO levels by flow cytometry. It is evident from the results that compound **17** (7-AAMC) profoundly enhanced NO level in platelets as compared to other acetoxy derivatives. The structural modification of *N*-acetylaminocoumarins was found to influence their effect on NO production in platelets in tune with the specificity of platelet CRTAase to these compounds (Fig. 2).

2.2.2.2. Effect of acetoxy quinolones and coumarins on platelet aggregation. *2.2.2.2.1. In vitro platelet aggregation.* In vitro antiplatelet activity of the test compounds is depicted in Figure 3, among all the compounds tested, compound **17** was found to be most effective in causing the inhibition of ADP and AA induced platelet aggregation. This compound at concentration 25–250 μM showed a definite trend of dose dependent inhibition of ADP/AA induced platelet aggregation. The IC_{50} value in vitro for ADP and AA induced platelet aggregation is 145 ± 2.4 and 77 ± 3.0 μM, respectively (Table 1).

PRP was incubated with the test compound (25–250 μM) for 10 min at 37 °C followed by the addition of ADP (15 μM)/AA (0.5 mM) and platelet aggregation was monitored by aggregometry. Values are mean ± SEM of five observations. IC_{50} value was determined from a dose–response plot.

2.2.2.2.2. In vivo platelet aggregation. 7-AAMC was found to effectively inhibit both ADP as well as AA induced platelet aggregation in vivo like aspirin as per the dose dependent curve (Fig. 4). The IC_{50} values of 7-AAMC in case of ADP and AA induced platelet aggregation was found to be 125.3 ± 1.4 μmol/kg (27.2 ± 0.30 mg/kg) and 55 ± 1.5 μmol/kg (12 ± 0.41 mg/kg), respectively. The optimum dose of 7-AAMC in the case of ADP as an inducer was found to be 160.2 ± 1.7 μmol/kg (34.76 ± 0.35 mg/kg), which had exhibited maximum inhibitory effect, as evident from the dose

Table 1
In vitro IC_{50} value of 7-AAMC for ADP and AA

Compound	IC_{50} values (μM)	
	ADP	AA
7-AAMC	145 ± 2.4	77 ± 3.0
ASA	Nil	80 ± 2.5

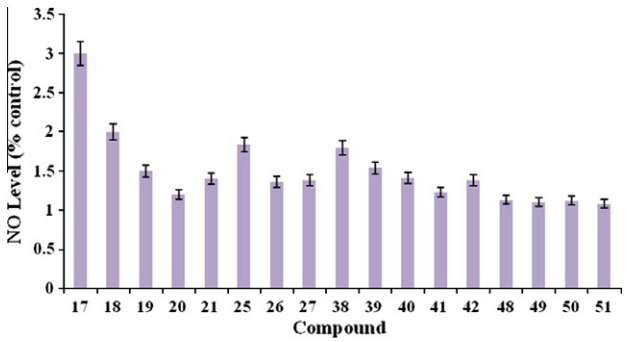


Figure 2. Effect of test compounds on platelet NOS activity. Human platelets were incubated with test compounds (100 μM) along with L-arginine (100 μM) and DCFH-DA (2 μM) for 30 min at 37 °C followed by the measurement of DCF fluorescence.

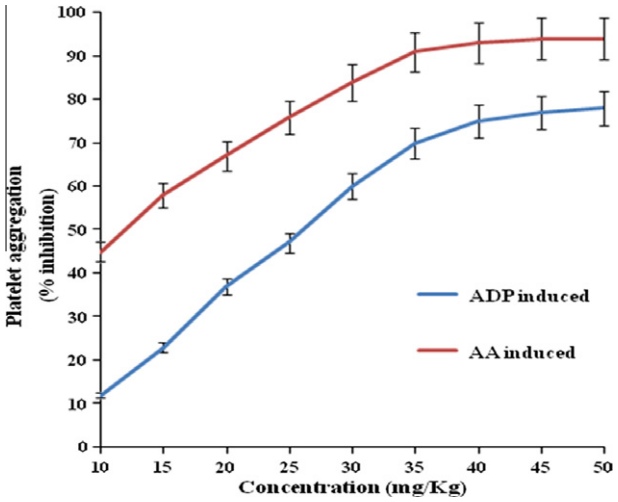


Figure 4. Dose-dependent inhibition of platelet aggregation by 7-AAMC in in vivo. Rats were administered with 7-AAMC, a single dose po (10–50) mg/kg, sacrificed after 24 h, blood was drawn and ADP/AA induced platelet aggregation was measured. Values are mean ± SEM of five observations. For all doses of 7-AAMC (p <0.005) when compared between ADP and ASA.

dependent curve. This effective concentration of 7-AAMC was used in studies related to the investigation of the mechanism of the anti-platelet activity. The inhibition of ADP induced platelet aggregation in in vivo of rats administered with ASA po (125.3 μmol/kg, IC_{50} of 7-AAMC in case of ADP) was found to be 52% while ASA when administered at a dose of 50.6 μmol/kg (IC_{50} of 7-AAMC in case of AA) showed 55% of inhibition of AA induced platelet aggregation. Evidently, both 7-AAMC and ASA were found to effectively inhibit AA induced platelet aggregation to a greater extent as compared with that of ADP.

2.2.3. Cox-1 activity assay

The effect of 7-AAMC on the Cox-1 activity in vivo is shown in Figure 5. Three groups of rats were treated with 7-AAMC, and ASA as against control po, sacrificed after 24 h and the Cox-1 activity was measured by ELISA method. 7-AAMC was found to effectively inhibit Cox-1 activity like aspirin by approximately 3.34-folds.

2.2.4. Modulation of TxB2 metabolite

PRP assayed for AA induced platelet aggregation were bioassayed for TxB2 concentration by ELISA method. In control samples TxB2 concentration was 85.4 pg/mg protein and dropped to 36.2 pg/mg (0.42-folds of control) and 33.6 pg/mg (0.39-folds of control) protein in ASA and 7-AAMC treated samples, respectively (Fig. 6).

2.2.5. Modulation of tissue factor (TF) level

The administration of LPS to rats resulted in the enhancement of tissue factor level upto 6.3 ± 0.33 folds in PBMCs (peripheral blood mononuclear cell). The Prior treatment of rats with 7-AAMC/ASA led to the remarkable decrease in LPS induced elevation of TF upto 1.93 ± 0.31 and 4.95 ± 0.25 folds, respectively (Fig. 7).

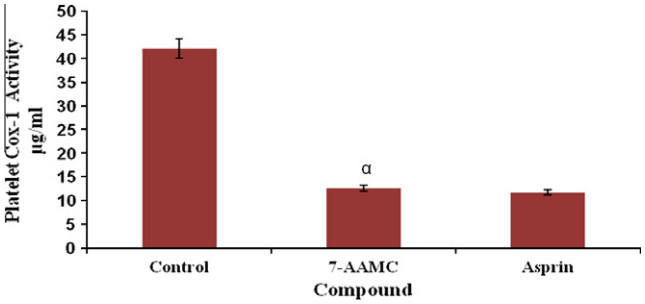


Figure 5. Effect of 7-AAMC on inhibition of Cox-1 activity in platelets. ASA/7-AAMC were administered to rats po (160.2 μmol/kg) and sacrificed after 24 h, blood was drawn and PRP prepared. Platelet lysate was bioassayed for Cox-1 activity. Values are mean ± SEM of five observations. $^{\alpha}(p < 0.001)$ compared to the Cox-1 activity of control.

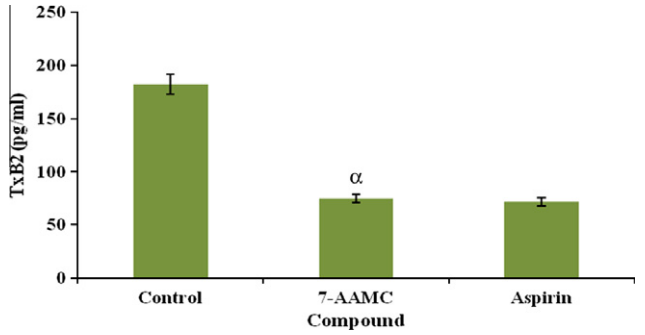


Figure 6. Effect of 7-AAMC on inhibition of TxB2 levels. ASA/7-AAMC were administered to rats po (160.2 μmol/kg), sacrificed after 24 h and blood was drawn. PRP prepared were assessed for AA induced platelet aggregation for 10 mins. Plasma was used to analyze TxB2 level. $^{\alpha}(p < 0.01)$ as compared to the control group.

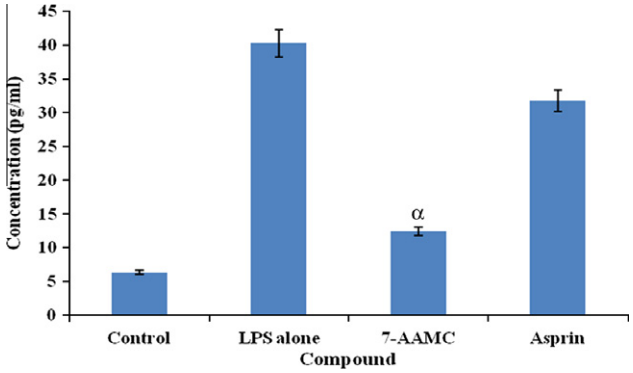


Figure 7. Tissue factor measurement in PBMCs. Rats were administered test compounds (160.2 μmol/kg) po for 5 days. A group of rats treated with 7-AAMC/aspirin were administered with LPS ip (2.5 mg/kg b.w.) 6 h before sacrificing. Another group of rats were administered LPS alone. Lymphomonocytes were isolated from blood and TF expression was assayed by ELISA. Control rats were treated accordingly. $^{\alpha}p < 0.05$ compared between 7-AAMC and ASA treated groups. $^{\alpha}p < 0.001$ compared between 7-AAMC and LPS alone treated groups.

2.2.6. Modification of LPS induced iNOS expression by 7-AAMC

Immunodetection of LPS-induced iNOS expressions were performed in PBMCs by western blotting. Figure 8 clearly depicted that the samples treated with LPS alone have intense iNOS expression (lane 3) whereas there was a diminished expression of iNOS in LPS plus 7-AAMC treated samples (lane 5). The ASA treated lane showed much higher iNOS expression, compared to 7-AAMC.

3. Discussion

Cardiovascular diseases such as myocardial infarction, unstable angina, and deep vein thrombosis greatly contribute to the mortality in the developed world. For the treatment of such heart conditions there is a greater need for the application of anti-platelet drugs.[19] Theoretically, anti-platelet agents can be developed that target each step in the platelet activation or inhibition mechanisms. Numerous new anti-platelet agents were developed based on their inhibitory effects on platelet activation. Nonetheless, the number of anti-platelet agents ready for clinical trials is still insufficient, and deleterious side-effects are also associated with most of the existing agents. Therefore, the search for an ideal anti-platelet agent is going on worldwide. Our earlier work convincingly established that Calreticulin, an important Ca^{2+} binding protein of lumen of endoplasmic reticulum, mediate the transfer of acetyl group from polyphenolic (PAs) to target protein such as NOS.[20–22] The protein acetyl transferase function of Calreticulin utilizing PAs as the acetyl group donors was termed CRTAase.[23] For the past many years our group and others have studied the versatility of wide variety of biologically relevant heterocyclic compounds viz. coumarins, biscoumarins, chromones, flavones, isoflavones, and xanthones towards CRTAase mediated acetylation of functional proteins leading to expression of biological and pharmacological effects.[11–13,23–27] The results suggested that the specificity of these polyphenolic acetates for CRTAase is in the order: acetoxycoumarins = acetoxychromones > acetoxyflavones = acetoxyxanthones. Further investigations on the specificity for Calreticulin transacetylase (CRTAase) with respect to the number and positions of acetoxy groups on the benzenoid ring of coumarin revealed that acetoxy groups in proximity to the oxygen heteroatom (at C-7 and C-8 positions) demonstrate a high degree of specificity to

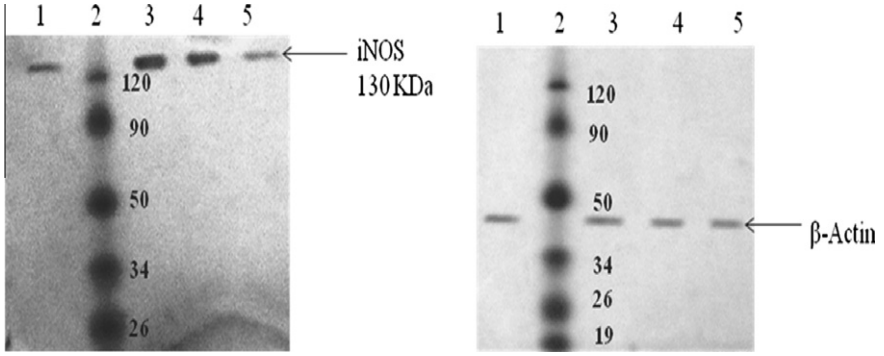


Figure 8. Inhibition of iNOS expression in PBMC of rat administered po with 7-AAMC. Rats were administered test compounds (160.2 μmol/kg) po for five days. A group of rats (placebo/compound treated) were administered LPS ip (2.5 mg/kg b.w.) 6 h before sacrificing. Lymphomonocytes were isolated from blood and iNOS expression was assayed by placebo administered rats were treated accordingly. Western blot was carried out using anti-iNOS antibody (1:1000, v/v). Lane 1: Control; lane 2: Prestained marker; lane 3: LPS; lane 4: ASA+LPS; lane 5: 7-AAMC+LPS.

CRTAase.[11] The carbonyl group on the pyran nucleus of PAs was found absolutely essential as the polyphenolic acetates (PAs) devoid of carbonyl group at C-2/C-4 position were found to support negligible activity of CRTAase, when used as the substrate.[27] It has also been observed that the acetoxy phenylcoumarins (having a phenyl ring instead of a methyl group at the C-4 position, yielded reduction of CRTAase activity while acetoxy dihydrocoumarin, had no profound effect on the CRTAase activity.[27] We have also studied the influence of an alkyl group at the C-3 position of the acetoxycoumarins on the CRTAase activity. The increase in size of the alkyl group at the C-3 position of coumarin nucleus resulted in the reduction of CRTAase activity and related effects.[13] In our previous studies we reported that acetoxy coumarins and quinolones are endowed with antiplatelet action by virtue of causing CRTAase catalyzed activation of platelet Nitric Oxide Synthase (NOS) by way of acetylation leading to the inhibition of ADP/AA-dependent platelet aggregation.

Herein we sought to correlate for the first time the ability of amino containing heterocyclic compounds viz. *N*-acetylaminocoumarins/quinolones to inhibit platelet aggregation with special reference to the specificity of platelet CRTAase to these derivatives and NOS activation. The results presented in the Figure 1 revealed that the acetoxy derivatives of coumarins and quinolones are the substrates for platelet CRTAase to varying degrees. Among all the *O*/*N*-acetyl derivatives screened, 7-AAMC (**17**) was found to be the most suitable substrate to platelet CRTAase that activated platelet NOS to a greater extent compared to the other compounds resulting in inhibition of ADP/AA induced platelet aggregation. These results depict a structural–activity relation where the affinity for various *N*-acetylamino/acetoxy derivatives is in the order: *N*-acetylaminocoumarins > acetoxyquinolones > *N*-acetylaminoquinolones > acetylcoumarins. The results show that the *N*-acetylamino functionality on the benzenoid ring of coumarins plays a crucial role in enhancing its specificity of platelet CRTAase, however it does not have any significance in case of quinolones. It is the position of acetoxy group on the benzenoid ring of quinolones that dictates their specificity for platelet CRTAase. Further C-6/C-8 acetyl derivatives of coumarins do not have much effect on the CRTAase activity. It is thus evident from the results (Fig. 1) that the addition of alkyl group at C-3 position of coumarins and quinolones results in drastic reduction of CRTAase activity, and this effect increases with the size of alkyl chain. Also, the replacement of C-4 methyl group by triflormethyl group resulted in the significant decline of CRTAase activity of platelets.

The ability of 7-AAMC (**17**) to inhibit platelet aggregation can be correlated to the enhancement of NO levels in blood platelets. The enhanced NO formation in blood vessels is known to regulate the vascular functions.[26] The blunting of Cox-1 activity and eventually TxA2, an AA metabolite acting as an endogenous platelet activator, intensifies the extent of inhibition of platelet aggregation. It is noteworthy that 7-AAMC, inhibited the Cox-1 activity even better than aspirin which is so far considered to be the best antiplatelet inhibitor. While its deacetylated analog, that is 7-amino-4-methylcoumarin (**12**), the synthetic precursor of 7-AAMC was totally ineffective for the inhibition of platelet aggregation and the similar pattern was observed for Cox-1 activity too. These observations highlighted the crucial role of *N*-acetylamino group of coumarin for activation of platelet NOS through acetylation leading to the antiplatelet action of acetylamino derivatives of coumarins. The expression of LPS induced iNOS was measured in isolated rat PBMCs. In these models, the increased iNOS production correlates with the vascular injury.[28] TF expression is also induced in circulating monocytes by bacterial LPS and pro-inflammatory cytokines. Binding of factor VII to the extracellular domain of TF catalyzes the generation of factor Xa and IXa, triggering thrombin generation.[29] 7-AAMC treated samples showed apparent decrease in LPS induced TF expression in PBMCs. However, the toxicity of 7-AAMC if any, has to be ascertained before they are chosen for antiplatelet therapy. The results documented in this manuscript have projected for the first time the antiplatelet action of a 7-AAMC implicating the cardinal role of CRTAase in the mechanism of action.

4. Conclusion

Earlier studies from our laboratory for the first time focused the attention on the versatility of polyphenolic acetates from the point of view of CRTAase mediated acetylation of functional proteins leading to the expression of biological and pharmacological effects. We have already demonstrated the ability of acetoxy derivatives of quinolones as the possible antiplatelet agents. In the present study we have taken the derivatives of coumarins, aminocoumarins and quinolones for the analysis of their antiplatelet activity. Among which 7-AAMC stood prominent and was found to overcome the thrombotic prone conditions effectively when compared to aspirin viz., inhibition of iNOS and TF. Thus, 7-AAMC could be further studied to evaluate the potentiality as an antiplatelet agent.

5. Experimental section

5.1. Chemistry

5.1.1. General

All the solvents were dried and distilled prior to their use. Reactions were monitored by precoated TLC plates (Merck Silica Gel

$60F_{254}$); the spots were visualized either by UV light, or by spraying with 5% alcoholic $FeCl_3$ solution. Silica gel (100–200 mesh) was used for column chromatography. Melting points were recorded in capillaries in suphuric acid bath and Buchi M-560 melting point apparatus. Infrared spectra were recorded on Perkin-Elmer FT-IR model 9 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on Bruker AC-400 and Jeol-400 (400, 100.6 MHz) NMR spectrometer and Avance-300 (300, 75.5 MHz) spectrometer using TMS as internal standard. The chemical shift values are on δ scale and the coupling constant values (J) are in Hz. The ESI MS of the known compounds and the HRMS of novel compounds were recorded on Agilent-6210 ES-TOF, JEOL JMX-SX-102A, Bruker Compass Data Analysis 4.0 (micrOTOF-Q II 10262) and Waters LCT Micromass-KC455. All the compounds synthesized were either crystallized or purified through column chromatography using different solvents, the purity of all the compounds obtained was greater than 95%.

5.1.2. General procedure for synthesis of 3-hydroxyphenylurethane (1)

Ethyl chloroformate (10.0 g, 92 mmol) was added in one portion to a stirred suspension of *m*-aminophenol (10.0 g, 92 mmol) in 400 mL of anhydrous diethyl ether. A white precipitate (amine hydrochloride) formed immediately. The reaction mixture was stirred for 2 h at room temperature. The hydrochloride was removed by filtration. The filtrate was then evaporated to give grey coloured solid. Further crystallization from petroleum ether (200 mL) gave upon cooling (0 °C) a white solid (7.0 g, 84%). Melting point = 91–92 °C (Literature value = 91–95 °C)[15]; UV (acetonitrile) λ_{max}: 279 and 285 nm; IR (KBr) ν_{max}: 3302.57 (NH), 3050.98, 2987.92, 1698.61 (NHCOO), 1624.18, 1558.07, 1474.68, 1251.02, 1099.55, 864.31 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.30 (t, 3H, J = 7.0 Hz, H-3′), 4.22 (q, 2H, J = 7.0 Hz, H-2′), 6.56–6.64 (m, 2H, H-4 and H-6), 6.77 (s, 1H, H-2), 6.93 (br s, 1H, OH), 7.12 (t, 1H, J = 8.1 Hz, H-5), 7.37 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.43 (C-3′), 61.68 (C-2′), 106.01, 110.47 and 110.76 (C-2, C-4 and C-6), 129.93 (C-5), 138.84 (C-1), 154.12 (C-3), 156.95 (C-1′). ESI MS: calcd for $C_9H_{11}NO_3$ $[M]^{+\cdot}$ 181, found 181.

5.1.3. General procedure for the synthesis of 7-carbethoxy aminocoumarins (7–11)

A solution of 3-hydroxyphenylurethane **1** (7.0 g) and un/substituted ethyl acetoacetate (**2**–**6**, 1.2 equiv) suspended in 88 mL of 70% ethanolic H_2SO_4 was stirred at room temperature for 4–6 h. The product formation was monitored by TLC. On completion of the reaction the clear yellow solution was poured into 400 mL of ice cold water, giving a voluminous brown crystalline precipitate. The solid was filtered and then crystallized from ethanol to give 7-carbethoxy aminocoumarin (**7**–**11**).

5.1.3.1. 7-Carbethoxyamino-4-methylcoumarin (7). The title compound (**7**) was prepared from ethyl acetoacetate (**2**) and 3-hydroxy phenylurethane (**1**) following the general procedure as light brown colour crystals in 83% yield. Melting point = 185–186 °C (Literature value = 186–188 °C)[15]; UV (acetonitrile) λ_{max}: 292 and 324 nm; IR (KBr) ν_{max}: 3289.27 (NH), 2976.16, 1730.12 (COO), 1701.80 (NHCOO), 1626.71, 1529.44, 1409.61, 1092.54, 1008.02, 862.66 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.27 (t, 3H, J = 6.9 Hz, H-3′), 2.38 (s, 3H, C-4 CH_3), 4.17 (q, 2H, J = 6.9 Hz, H-2′), 6.22 (s, 1H, H-3), 7.40 (d, 1H, J = 8.7 Hz, H-6), 7.54 (s, 1H, H-8), 7.67 (d, 1H, J = 8.7 Hz, H-5), 10.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 14.36 (C-3′), 17.92 (C-4 CH_3), 60.68 (C-2′), 104.37, 111.78, 114.24 and 114.74 (C-3, C-6, C-8 and C-10), 125.91 (C-5), 142.82 (C-7), 153.18 and 153.32 (C-4 and C-9), 153.80 (C-1′), 160.05 (C-2); ESI MS: calcd for $C_{13}H_{13}NO_4$ $[M]^{+\cdot}$ 247, found 247.

5.1.3.2. 7-Carbethoxyamino-3-ethyl-4-methylcoumarin (8). The title compound (**8**) was prepared from ethyl 2-acetylbutanoate (**3**) and 3-hydroxyphenylurethane (**1**) by following the general procedure and was obtained as brown colour crystals in 42% yield. Melting point = 158–160 °C; UV (acetonitrile) λ_{max}: 295 and 324 nm; IR (KBr) ν_{max}: 3287.54 (NH), 2923.38, 2854.63, 1729.65 (COO), 1682.13 (NHCOO), 1623.45, 1539.28, 1458.34, 1235.06, 1013.77, 863.25 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.12 (t, 3H, J = 7.6 Hz, $-CH_2CH_3$), 1.30 (t, 3H, J = 7.2 Hz, H-3′), 2.38 (s, 3H, C-4 CH_3), 2.65 (q, 2H, J = 7.6 Hz, $-CH_2CH_3$), 4.23 (q, 2H, J = 7.0 Hz, H-2′), 7.12 (br s, 1H, NH), 7.36 (d, 1H, J = 8.4 Hz, H-6), 7.41 (d, 1H, J = 2.0 Hz, H-8), 7.49 (d, 1H, J = 8.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 13.18 (C-3′), 14.56 and 14.59 ($-CH_2CH_3$ and C-4 CH_3), 20.97 ($-CH_2CH_3$), 61.73 (C-2′), 105.72, 114.43 and 116.231 (C-6, C-8 and C-10), 125.22 (C-3), 126.12 (C-5), 140.53 (C-7), 145.72 (C-4) 152.90 (C-9), 153.31 (C-1′), 162.05 (C-2); HRMS: calcd for $C_{15}H_{17}NO_4$ $[M+H]^+$ 276.1158, found 276.1225.

5.1.3.3. 7-Carbethoxyamino-3-hexyl-4-methylcoumarin (9). The title compound (**9**) was prepared from ethyl 2-acetyloctanoate (**4**) and 3-hydroxy phenylurethane (**1**) by following the general procedure and was obtained as brown colour crystals in 31% yield. Melting point = 138–140 °C; UV (acetonitrile) λ_{max}: 295 and 324 nm; IR (KBr) ν_{max}: 3257.82 (NH), 3103.62, 2924.17, 1733.34 (COO), 1682.12 (NHCOO), 1612.72, 1537.51, 1459.71, 1081.78, 876.20 cm^{-1}; ^1H NMR (Acetone-d_6, 400 MHz): δ 0.85 (br s, 3H, $-CH_2(CH_2)_4CH_3$), 1.23 (t, 3H, J = 7.6 Hz, H-3′), 1.29–1.49 (m, 8H, $-CH_2(CH_2)_4CH_3$), 2.39 (s, 3H, C-4 CH_3), 2.58 (t, 2H, J = 7.8 Hz, $-CH_2(CH_2)_4CH_3$), 4.15 (q, 2H, J = 6.8 Hz, H-2′), 7.42 (dd, 1H, J = 2.4 and 8.8 Hz, H-6), 7.59 (d, 1H, J = 2.4 Hz, H-8) 7.62 (d, 1H, J = 8.8 Hz, H-5), 8.99 (br s, 1H, NH); ^{13}C NMR (Acetone-d_6, 100.6 MHz): 13.51 (C-3′), 13.98 ($-CH_2(CH_2)_4CH_3$ and C-4 CH_3), 22.46, 27.26, 28.63, 29.21, 31.60 ($-(CH_2)_5CH_3$), 60.79 (C-2′), 104.63, 114.15 and 115.52 (C-6, C-8 and C-10), 124.20 (C-3), 125.50 (C-5), 141.85 (C-7), 145.86 (C-4), 153.06 (C-9), 154.06 (C-1′), 160.96 (C-2); HRMS: calcd for $C_{19}H_{25}NO_4$ $[M+H]^+$ 332.1856, found 332.1851.

5.1.3.4. 7-Carbethoxyamino-3-decyl-4-methylcoumarin (10). The title compound (**10**) was prepared from ethyl 2-acetyldodecanoate (**5**) and 3-hydroxyphenylurethane (**1**) following the general procedure and was obtained as brown colour crystals in 28% yield. Melting point = 128–130 °C; UV (acetonitrile) λ_{max}: 295 and 324 nm; IR (KBr) ν_{max}: 3259.95 (NH), 3102.62, 2923.44, 1732.62 (COO), 1683.01 (NHCO), 1609.53, 1537.96, 1231.90, 1081.86, 875.82 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 0.83 (br s, 3H, $-CH_2(CH_2)_8CH_3$), 1.21–1.39 (m, 19H, H-3′ and $-CH_2(CH_2)_8CH_3$), 2.34 (s, 3H, C-4 CH_3), 2.50 (br s, 2H, $-CH_2(CH_2)_4CH_3$), 4.14 (q, 2H, J = 6.8 Hz, H-2′), 7.36 (d, 1H, J = 8.1 Hz, H-6), 7.50 (s, 1H, H-8), 7.65 (d, 1H, J = 8.7 Hz, H-5), 10.06 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): 13.92 (C-3′), 14.41 and 14.44 ($-CH_2(CH_2)_8CH_3$ and C-4 CH_3), 22.09, 23.21, 26.83, 28.16, 28.70, 28.86, 28.98, 29.63, 31.28 ($-(CH_2)_9CH_3$), 60.59 (C-2′), 104.10, 114.17 and 114.80 (C-6, C-8 and C-10), 123.19 (C-3), 125.72 (C-5), 141.74 (C-7), 146.43 (C-4), 152.24 (C-9), 153.32 (C-1′), 160.82 (C-2); HRMS: calcd for $C_{23}H_{33}NO_4$ $[M+H]^+$ 388.2443, found 388.2482.

5.1.3.5. 7-Carbethoxyamino-4-trifluoromethylcoumarin (11). The title compound (**11**) was prepared from 3-hydroxyphenylurethane (**1**) and ethyl 4,4,4-trifluoromethyl acetoacetate (**6**) by following the general procedure as colourless crystals in 30% yield. Melting point = 170 °C decomposed (Literature

value = 172 °C)[30]; UV (acetonitrile) λ_{max}: 336 nm; IR (KBr) ν_{max}: 3315.20 (NH), 3082.02, 2987.55, 1744.42 (COO), 1721.96 (NHCOO), 1622.16, 1591.88, 1482.06, 1285.01, 1074.13, 867.22 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz): δ 1.33 (t, 3H, J = 7.2 Hz, H-3′), 4.26 (q, 2H, J = 7.1 Hz, H-2), 6.61 (s, 1H, H-3), 7.48 (dd, 1H, J = 1.5 and 9.0 Hz, H-6), 7.58 (d, 1H, J = 9.0 Hz, H-5), 7.76 (d, 1H, J = 1.5 Hz, H-8), 9.53 (s, 1H, NH); ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 75.5 MHz): δ 14.12 (C-3′), 60.83 (C-2′), 105.49, 107.48, 112.12 and 115.07 (C-3, C-6, C-8 and C-10), 122.99 (C-5), 125.10 (d, J = 32.4 Hz, –CF_3), 141.13–140.70 (q, J = 275.5 Hz, C-4), 143.92 (C-7), 153.16 (C-9), 155.00 (C-1′), 158.97 (C-2); ESI MS: calcd for $C_{13}H_{10}F_3NO_4$ $[M]^{+\cdot}$ 301, found 301.

5.1.4. General procedure for the synthesis of 7-aminocoumarins (12–16)

7-Carbethoxy aminocoumarins **7**–**11** (5.0 g) were refluxed for 4 h in a mixture of concentrated H_2SO_4 and glacial acetic acid (1:1, 10 ml). On cooling a yellow precipitate was deposited. The mixture was poured over 100 mL of ice cold water and allowed to stand overnight. The resulting suspension was made slightly alkaline with 50% aqueous NaOH under cold conditions. The brown precipitate formed was then filtered and washed with ice cold water (3 × 50 mL). Crystallization from ethanol yielded light brown coloured crystals.

5.1.4.1. 7-Amino-4-methylcoumarin (12). The title compound (**12**) was obtained as brown colour crystals in 72% yield by following the general procedure. Melting point = 220–222 °C (Literature value = 220–224 °C)[15]; UV (acetonitrile) λ_{max}: 296 and 326 nm; IR (KBr) ν_{max}: 3412.00, 3300.14 (NH_2), 3113.42, 2924.94, 1681.89 (COO), 1619.66, 1407.59, 1261.96, 1076.10, 868.00 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 2.28 (s, 3H, C-4 CH_3), 5.88 (s, 1H, H-3), 6.09 (br s, 2H, NH_2), 6.39 (s, 1H, H-8), 6.53–6.54 (m, 1H, H-6), 7.38 (d, 1H, J = 8.8 Hz, H-5); ^{13}C NMR (Acetone-d_6, 100.6 MHz): δ 18.57 (C-4 CH_3), 99.06 (C-8), 108.02, 109.39 and 111.714 (C-3, C-6 and C-10), 126.75 (C-5), 153.62, 154.29 and 156.00 (C-4, C-7 and C-9), 161.28 (C-2); ESI MS: calcd for $C_{10}H_9NO_2$ $[M+H]^+$ 176, found 176.

5.1.4.2. 7-Amino-3-ethyl-4-methylcoumarin (13). The title compound (**13**) was obtained as brown colour crystals in 41% yield by following the general procedure. Melting point = 206–208 °C[31]; UV (acetonitrile) λ_{max}: 326 nm; IR (KBr) ν_{max}: 3454.05, 3356.11 (NH_2), 3241.13, 3075.38, 2959.43, 1677.62 (COO), 1643.77, 1450.78, 1260.50, 1061.28, 857.59 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.23 (br s, 3H, –CH_2CH_3), 2.32 (s, 3H, C-4 CH_3), 2.48 (m, 2H, –CH_2CH_3), 7.34–7.63 (m, 3H, H-5, H-6 and H-8); ^{13}C NMR (Acetone-d_6, 100 MHz): δ 13.45 and 14.95 (–CH_2CH_3 and C-4 CH_3), 20.78 (–CH_2CH_3), 99.99 (C-8), 114.69 and 115.33 (C-6 and C-10), 124.94 (C-3), 126.26 (C-5), 146.74 (C-4), 152.76 and 153.85 (C-7 and C-9), 161.19 (C-2); ESI MS: calcd for $C_{12}H_{13}NO_2$ $[M]^{+\cdot}$ 203, found 203.

5.1.4.3. 7-Amino-3-hexyl-4-methylcoumarin (14)[32]. The title compound (**14**) was obtained as brown colour crystals in 26% yield by following the general procedure. Melting point = 198–200 °C; UV (acetonitrile) λ_{max}: 348 nm; IR (KBr) ν_{max}: 3447.78, 3356.61 (NH_2), 3245.31, 2953.29, 2923.26, 1678.16 (COO), 1458.82, 1258.38, 1073.04, 857.62 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 0.81 (br s, 3H, –$CH_2(CH_2)_4CH_3$), 1.23–1.34 (m, 8H, –$CH_2(CH_2)_4CH_3$), 2.24 (s, 3H, C-4 CH_3), 2.42 (br s, 2H, –$CH_2(CH_2)_4CH_3$), 5.90 (br s, 2H, NH_2), 6.34 (s, 1H, H-8), 6.51 (d, 1H, J = 8.4 Hz, H-6), 7.36 (d, 1H, J = 8.4 Hz, H-5); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 14.49 and 14.88 (–$CH_2(CH_2)_4CH_3$ and C-4 CH_3), 22.62, 27.19, 28.97, 29.21, 31.69 (–$(CH_2)_5CH_3$), 98.94 (C-8), 109.96 and 111.75 (C-6 and C-10), 119.34 (C-3), 126.51 (C-5), 147.80 (C-4), 152.45 and 154.32 (C-7 and C-9), 161.90 (C-2); HRMS: calcd for $C_{16}H_{21}NO_2$ $[M+H]^+$ 260.1572, found 260.1652.

5.1.4.4. 7-Amino-3-decyl-4-methylcoumarin (15)[32]. The title compound (**15**) was obtained as brown colour crystals in 30% yield by following the general procedure. Melting point = 172–174 °C; UV (acetonitrile) λ_{max}: 339 nm; IR (KBr) ν_{max}: 3451.51, 3358.51 (NH_2), 3246.34, 3091.60, 2956.00, 1676.82 (COO), 1458.40, 1263.87, 1072.48, 857.50 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 0.85 (br s, 3H, –$CH_2(CH_2)_8CH_3$), 1.23–1.39 (m, 16H, –$CH_2(CH_2)_8CH_3$), 2.28 (s, 3H, C-4 CH_3), 2.45–2.47 (m, 2H, –$CH_2(CH_2)_8CH_3$), 5.93 (br s, 2H, NH_2), 6.39 (s, 1H, H-8), 6.56 (d, 1H, J = 8.4 Hz, H-6), 7.40 (d, 1H, J = 8.4 Hz, H-5); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 13.86 and 14.27 (–$CH_2(CH_2)_8CH_3$ and C-4 CH_3), 22.02, 22.02, 26.58, 28.37, 28.63, 28.84, 28.92, 28.92, 31.22 (–$(CH_2)_9CH_3$), 98.39 (C-8), 109.41 and 111.17 (C-6 and C-10), 118.80 (C-3), 126.89 (C-5), 147.17 (C-4), 151.85 and 153.74 (C-7 and C-9), 161.37 (C-2); HRMS: calcd for $C_{20}H_{29}NO_2$ $[M+Na]^+$ 338.2096, found 338.2071.

5.1.4.5. 7-Amino-4-trifluoromethylcoumarin (16). The title compound (**16**) was obtained as light brown colour crystals in 46% by following the general procedure. Melting point = 225–227 °C. (Literature value = 222 °C)[30]; UV (acetonitrile) λ_{max}: 336 nm; IR (KBr) ν_{max}: 3455.49, 3362.91 (NH_2), 3092.60, 2710.67, 1710.36 (COO), 1451.57, 1222.50, 998.15, 852.77 cm^{-1}; ^1H NMR (Methanol-d_4, 400 MHz): δ 6.36 (s, 1H, H-3), 6.54 (d, 1H, J = 2.2 Hz, H-8), 6.64 (dd, 1H, J = 2.3 and 8.7 Hz, H-6), 7.37–7.41 (m, 1H, H-5); ^{13}C NMR (Methanol-d_4, 100.6 MHz): δ 100.64 (C-8), 104.04, 108.54 and 113.51 (C-3, C-6 and C-10), 123.50 (C-5), 127.19 (d, J = 277.2 Hz, –CF_3), 142.70–143.69 (q, J = 32.4 Hz, C-4), 155.62 and 158.35 (C-7 and C-9), 162.26 (C-2); ESI MS: calcd for $C_{10}H_6F_3NO_2$ $[M]^{+\cdot}$ 229, found 229.

5.1.5. General procedure for the synthesis of 7-*N*-acetylamino coumarins (17–21)

A mixture of 7-aminocoumarin (500 mg, **12**–**16**) and DMAP (10–20 mg) was dissolved in minimum amount of THF. To this acetic anhydride was added and the resultant mixture was stirred at room temperature for 24 h. On completion of the reaction 50 mL ice cold water was added. The crude off-white solid was then filtered, washed with water and dried. The crude solid so obtained was crystallized with ethanol to give 7-*N*-acetylaminocoumarin derivatives.

5.1.5.1. 7-*N*-Acetylamino-4-methylcoumarin (17). The title compound (**17**) was obtained as colourless crystals in 72% yield by following the general procedure. Melting point = 200–204 °C[33]; UV (acetonitrile) λ_{max}: 325 nm; IR (Nujol) ν_{max}: 3299.12 (NH), 3112.77, 2922.62, 1715.58 (COO), 1682.19 (NHCO), 1619.76, 1587.24, 1463.26, 1262.01, 1076.43, 868.84 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.10 (s, 3H, H-2′), 2.39 (s, 3H, C-4 CH_3), 6.25 (s, 1H, H-3), 7.45 (d, 1H, J = 8.4 Hz, H-6), 7.70 (d, 1H, J = 8.7 Hz, H-5), 7.75 (s, 1H, H-8), 10.38 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 17.87 (C-4 CH_3), 24.11 (C-2′), 105.30, 112.04, 114.72 and 114.89 (C-3, C-6, C-8 and C-10), 125.73 (C-5), 142.53 (C-7), 152.97 and 153.61 (C-4 and C-9), 159.95 (C-2), 168.99 (C-1′); ESI MS: calcd for $C_{12}H_{11}NO_3$ $[M+H]^+$ 218, found 218.

5.1.5.2. 7-*N*-Acetylamino-3-ethyl-4-methylcoumarin (18). The title compound (**18**) was obtained as off-white colour crystals in 67% yield by following the general procedure. Melting point = 180–182 °C; UV (acetonitrile) λ_{max}: 296 and 323 nm; IR (KBr) ν_{max}: 3289.04 (NH), 3109.24, 2974.59, 1716.33 (COO), 1689.51 (NHCO), 1618.41, 1585.07, 1427.01, 1250.93, 1089.60,

864.15 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.08 (t, 3H, J = 7.5 Hz, $-CH_2CH_3$), 2.38 (s, 3H, H-2′), 2.45 (s, 3H, C-4 CH_3), 2.58 (br s, 2H, $-CH_2CH_3$), 7.23 (d, 1H, J = 7.8 Hz, H-6), 7.34–7.41 (m, 1H, H-5), 7.53 (s, 1H, H-8), 10.05 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 12.87 and 14.14 ($-CH_2CH_3$ and C-4 CH_3), 20.21 ($-CH_2CH_3$), 24.11 (C-2′), 105.08, 114.89 and 115.35 (C-6, C-8 and C-10), 124.70 (C-3), 125.64 (C-5), 141.48 (C-7), 146.13 (C-4), 152.04 (C-9), 160.64 (C-2), 168.93 (C-1′); HRMS: calcd for $C_{14}H_{15}NO_3$ $[M+H]^+$ 246.1085, found 246.1125.

5.1.5.3. 7-*N*-Acetylamino-3-hexyl-4-methylcoumarin (19). The title compound (**19**) was obtained as light brown colour crystals in 60% yield by following the general procedure. Melting point = 160 °C; UV (acetonitrile) λ_{max}: 326 nm; IR (Nujol) ν_{max}: 3297.88 (NH), 3113.01, 2924.71, 1730.94 (COO), 1674.98 (NHCO), 1614.95, 1590.20, 1462.44, 1265.21, 1099.24, 860.80 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.88 (br s, 3H, $-CH_2(CH_2)_4CH_3$), 1.25–1.52 (m, 8H, $-CH_2(CH_2)_4CH_3$), 2.25 (s, 3H, H-2′), 2.39 (s, 3H, C-4 CH_3), 2.64 (t, 2H, J = 7.6 Hz, $-CH_2(CH_2)_4CH_3$), 7.54 (d, 1H, J = 8.7 Hz, H-6), 7.64 (s, 1H, H-8), 7.84 (d, 1H, J = 8.4 Hz, H-5), 8.35 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 13.87 and 14.42 ($-CH_2(CH_2)_4CH_3$ and C-4 CH_3), 21.99, 24.09, 26.81, 28.09, 28.59, 31.04 (C-2′, $-(CH_2)_5CH_3$), 105.09, 114.90 and 115.34 (C-6, C-8 and C-10), 123.49 (C-3), 125.64 (C-5), 141.17 (C-7), 146.38 (C-4), 152.04 (C-9), 160.80 (C-2), 168.92 (C-1′); HRMS: calcd for $C_{18}H_{23}NO_3$ $[M+H]^+$ 302.1678, found 302.1746.

5.1.5.4. 7-*N*-Acetylamino-3-decyl-4-methylcoumarin (20). The title compound (**20**) was obtained as light yellow colour crystals in 60% yield by following the general procedure. Melting point = 148–150 °C; UV (acetonitrile) λ_{max}: 296 and 326 nm; IR (Nujol) ν_{max}: 3321.03 (NH), 3192.27, 3107.29, 2922.50, 1732.71 (COO), 1673.95 (NHCO), 1624.77, 1614.53, 1536.35, 1256.52, 1083.00, 871.14 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.87 (br s, 3H, $-CH_2(CH_2)_8CH_3$), 1.25–1.49 (m, 16H, $-CH_2(CH_2)_8CH_3$), 2.25 (s, 3H, H-2′), 2.40 (s, 3H, C-4 CH_3), 2.64 (br s, 2H, $-CH_2(CH_2)_4CH_3$), 7.54 (d, 1H, J = 9.3 Hz, H-6), 7.64 (br s, 1H, H-5), 7.82 (s, 1H, H-8), 8.37 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 13.87 and 14.41 ($-CH_2(CH_2)_8CH_3$ and C-4 CH_3), 20.99 (C-2′), 22.03, 24.10, 26.82, 28.10, 28.64, 28.81, 28.92, 28.92, 31.23 ($-(CH_2)_9CH_3$), 105.12, 114.91 and 115.35 (C-6, C-8 and C-10), 123.50 (C-3), 125.58 (C-5), 141.48 (C-7), 146.33 (C-4), 152.06 (C-9), 160.80 (C-2), 168.91 (C-1′); HRMS: calcd for $C_{22}H_{31}NO_3$ $[M+H]^+$ 358.2337, found 358.2377.

5.1.5.5. 7-*N*-Acetylamino-4-trifluoromethylcoumarin (21). The title compound (**21**) was obtained as brown colour crystals in 76% yield by following the general procedure. Melting point = 178–180 °C (Literature value = 227 °C)[30]; UV (acetonitrile) λ_{max}: 337 nm; IR (KBr) ν_{max}: 3337.75 (NH), 3071.84, 2925.07, 1712.25 (COO), 1621.47 (NHCO), 1586.99, 1515.30, 1240.08, 1023.68, 858.46 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.14 (s, 3H, H-2′), 6.89 (s, 1H, H-3), 7.53–7.67 (m, 2H, H-5 and H-6), 7.91 (s, 1H, H-8), 10.54 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 24.19 (C-2′), 105.91, 107.98, 114.20 and 115.77 (C-3, C-6, C-8 and C-10), 123.49 (C-5), 125.35 (d, J = 276.3 Hz, $-CF_3$), 138.98–139.82 (q, J = 31.7 Hz, C-4), 143.49 (C-7), 154.72 (C-9) 158.61 (C-2), 169.39 (C-1′); ESI MS: calcd for $C_{12}H_8F_3NO_3$ $[M+H]^+$ 272, found 272.

5.1.6. General procedure for the synthesis of 7-aminoquinolin-2(1*H*)-one (22–24)

1,3-Diaminobenzene (1.0 g, 9.3 mmol), was added to substituted ethyl acetoacetates (1.2 equiv, **2**–**3**, **6**) and the mixture was refluxed for 20 h. It was then poured on ice (100 g) and the precipitate was filtered. The product was obtained through column chromatography using silica gel (100–200 mesh) in methanol/chloroform (1:99).

5.1.6.1. 7-Amino-4-methylquinolin-2(1*H*)-one (22). The title compound (**22**) was obtained as yellow solid in 65% yield by following the above general procedure. Melting point: 271–272 °C (Literature value = 271 °C)[34]; UV (MeOH) λ_{max}: 335 and 352 nm; IR (KBr) ν_{max}: 3423.29, 3307.93 (NH_2), 2920.17, 1656.23 (NHCO), 1553.03, 1474.45, 1259.63, 1069.35, 914.48, 839.04, 689.74 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.28 (s, 3H, C-4 CH_3), 5.74 (br s, 2H, NH_2), 5.96 (s, 1H, H-3), 6.37 (s, 1H, H-8), 6.46 (d, 1H, J = 8.4 Hz, H-6), 7.34 (d, 1H, J = 8.7 Hz, H-5), 11.18 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 18.55 (C-4 CH_3), 96.85, 110.48, 110.55 and 114.67 (C-3, C-6, C-8 and C-10), 125.67 (C-5), 140.81 (C-9), 148.13 (C-4), 151.15 (C-7), 162.55 (C-2); ESI MS: calcd for $C_{10}H_{10}N_2O$ $[M]^+$ 174, found 174.

5.1.6.2. 7-Amino-3-ethyl-4-methylquinolin-2(1*H*)-one (23). The title compound (**23**) was obtained as yellow solid in 65% yield by following the above general procedure. Melting point: 280–281 °C; UV (MeOH) λ_{max}: 336 and 349 nm; IR (KBr) ν_{max}: 3459.26, 3363.28 (NH_2), 2962.89, 1624.72 (NHCO), 1555.37, 1417.38, 1333.30, 1263.33, 1058.13, 879.67, 781.94, 690.30 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.00 (t, 3H, J = 7.4 Hz, $-CH_2CH_3$), 2.30 (s, 3H, C-4 CH_3), 2.56 (q, 2H, J = 7.4 Hz, $-CH_2CH_3$), 5.60 (br s, 2H, NH_2), 6.3 (s, 1H, H-8), 6.47 (dd, 1H, J = 1.2 and 8.4 Hz, H-6), 7.38 (d, 1H, J = 8.7 Hz, H-5), 11.19 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 13.73 and 14.30 ($-CH_2CH_3$ and C-4 CH_3), 19.37 ($-CH_2CH_3$), 96.78, 110.53 and 110.95 (C-6, C-8 and C-10), 125.44 (C-3), 126.17 (C-5), 139.05 (C-9), 141.99 (C-4), 150.05 (C-7), 162.14 (C-2). HRMS: calcd for $C_{12}H_{14}N_2O$ $[M+Na]^+$ 225.1004, found 225.0998.

5.1.6.3. 7-Amino-4-(trifluoromethyl)quinolin-2(1*H*)-one (24). The title compound (**24**) was obtained as yellow solid in 70% yield by following the above general procedure. Melting point: 274 °C (Literature value = 274 °C)[35]; UV (MeOH) λ_{max}: 352 nm; IR (KBr) ν_{max}: 3365.74, 3239.82 (NH_2), 2930.56, 1647.95 (NHCO), 1554.42, 1478.31, 1282.65, 1259.44, 1175.51, 1130.41, 969.84, 868.46, 659.01 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 6.06 (br s, 2H, NH_2), 6.34, 6.37 (2 × s, 2H, H-3 and H-8), 6.47 (d, 1H, J = 8.4 Hz, H-6), 7.26 (d, 1H, J = 6.9 Hz, H-5), 11.73 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 96.67, 103.46 (C-6 and C-8), 111.79 and 113.42 (C-3 and C-10), 121.02 (C-5), 125.08 (C-9), 136.53–137.33 ($-CF_3$), 142.11 (C-4), 152.07 (C-7), 160.87 (C-2); HRMS: calcd for $C_{10}H_7F_3N_2O$ $[M+H]^+$ 229.0583, found 229.0582.

5.1.7. General procedure for the synthesis of *N*-(2-oxo-1,2-dihydroquinolin-7-yl)acetamides (25–27)

7-Aminoquinolin-2(1*H*)-ones (1.0 g, **22**–**24**) were added to a solution of acetic anhydride and acetic acid (1:4, 10 ml). The mixture was refluxed for 6 h and poured on ice. The precipitate was filtered and washed with water and ether to yield 7-*N*-acetylquinolin-2-ones (**25**–**27**).[36]

5.1.7.1. *N*-(4-Methyl-2-oxo-1,2-dihydroquinolin-7-yl) acetamide (25). The title compound (**25**) was obtained as brown crystals in 60% yield by following the general procedure. Melting point: 300 °C (Literature value = 300 °C)[34]; UV (MeOH) λ_{max}: 331 and 347 nm; IR (KBr) ν_{max}: 3417.52 (NH), 3130.05, 1673.47 (NHC-OCH_3), 1644.18 (NHCO), 1574.00, 1453.17, 1281.91, 1074.76, 987.98, 812.16, 683.74 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.09 (s, 3H, H-2′), 2.38 (s, 3H, C-4 CH_3), 6.26 (s, 1H, H-3), 7.30 (d, 1H, J = 8.7 Hz, H-6), 7.62 (d, 1H, J = 8.7 Hz, H-5), 7.99 (s, 1H, H-8), 10.197, 11.53 (2 × br s, 2H, 2 × N*H*CO); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 18.44 (C-4 CH_3), 24.18 (C-2′), 104.37 and 113.48 (C-

6 and C-8), 115.46 and 118.89 (C-3 and C-10), 125.31 (C-5), 139.51 (C-9), 141.05 (C-4), 147.74 (C-7), 162.14 (C-2), 168.85 (C-1′); ESI MS: calcd for $C_{12}H_{12}N_2O_2$ $[M]^{+\cdot}$ 216, found 216.

5.1.7.2. *N*-(3-Ethyl-4-methyl-2-oxo-1,2-dihydroquinolin-7-yl) acetamide (26). The title compound (**26**) was obtained as brown crystals in 60% yield by following the general procedure. Melting point: 305–306 °C; UV (MeOH) λ_{max}: 332 and 343 nm; IR (KBr) ν_{max}: 3448.26 (NH), 3191.35, 2964.96, 1680.38 ($NHCOCH_3$), 1641.46 (NHCO), 1585.52, 1458.61, 1267.91, 1099.42, 956.61, 808.78, 622.31 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.02 (t, 3H, J = 7.2 Hz, $-CH_2CH_3$), 2.08 (s, 3H, H-2′), 2.37 (s, 3H, C-4 CH_3), 2.61 (q, 2H, J = 6.9 Hz, $-CH_2CH_3$), 7.29 (d, 1H, J = 8.7 Hz, H-6), 7.63 (d, 1H, J = 9.0 Hz, H-5), 7.73 (s, 1H, H-8), 10.12, 11.51 (2 × br s, 2H, 2 × NHCO); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 13.40 and 14.37 ($-CH_2CH_3$ and C-4 CH_3), 19.56 ($-CH_2CH_3$), 24.16 (C-2′), 104.06, 113.36 and 115.90 (C-6, C-8 and C-10), 125.08 (C-3), 130.52 (C-5), 137.81 (C-9), 140.01 (C-4), 141.38 (C-7), 161.79 (C-2), 168.67 (C-1′); HRMS: calcd for $C_{14}H_{16}N_2O_2$ $[M+H]^+$ 245.1245, found 245.1285.

5.1.7.3. *N*-(2-Oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-7-yl)acetamide (27). The title compound (**27**) was obtained as brown crystals in 90% yield by following the general procedure. Melting point: >300 °C[35]; UV (MeOH) λ_{max}: 345 nm; IR (KBr) ν_{max}: 3291.66 (NH), 3107.16, 2926.93, 1682.41 ($NHCOCH_3$), 1658.36 (NHCO), 1612.70, 1586.81, 1468.85, 1278.43, 1250.44, 1011.56, 869.86, 654.62 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.09 (s, 3H, H-2′), 6.79 (s, 1H, H-3), 7.35 (d, 1H, J = 9.0 Hz, H-6), 7.62 (d, 1H, J = 8.7 Hz, H-5), 7.98 (s, 1H, H-8); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 24.16 (C-2′), 104.71, 108.61 (C-6 and C-8), 114.62 (C-3), 119.16 (C-10), 120.76 (C-5), 124.84 (C-9), 136.11–136.93 ($-CF_3$), 140.86 (C-4), 142.06 (C-7), 160.41 (C-2), 169.12 (C-1′); HRMS: calcd for $C_{12}H_9F_3N_2O_2$ $[M+Na]^+$ 293.0508, found 293.0508.

5.1.8. Synthesis of methoxy quinolin-2(1*H*)-ones (28–29)

Anisidine (2 g, 16.0 mmol) was added dropwise to substituted ethyl acetoacetate (3 mL, 16.0 mmol), the mixture was refluxed for 12 h. The mixture was then cooled and poured on sodium carbonate solution. The compound was then extracted with 50 ml ethyl acetate and the solvent was evaporated. 70% Sulphuric acid (5 mL) was added to it and the solution was heated at 95 °C for 6 h. It was then poured on crushed ice (100 g) and the precipitate was filtered and washed with water and ether. The crude product so obtained was purified by column chromatography.

5.1.8.1. 6-Methoxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (28)[37]. The product was obtained through column chromatography in ethyl acetate/petroleum ether (1:49). The title compound (**28**) was obtained as yellow solid in 70% yield by following the general procedure. Melting point: 270 °C; UV (MeOH) λ_{max}: 329 and 351 nm; IR (KBr) ν_{max}: 2925.60, 2854.60, 1617.99 (NHCO), 1560.66, 1522.43, 1496.43, 1420.29, 1385.36, 1279.82, 1255.71, 1227.75, 1193.02, 1152.09, 1102.61, 1029.68, 947.35, 917.65, 845.40, 822.41, 747.90, 725.71 and 623.49 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 3.72 (s, 3H, $-OCH_3$), 7.13 (s, 1H, H-3), 7.48–7.82 (m, 2H, H-5 and H-7), 7.95 (br s, 1H, H-8) and 12.14 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 55.53 ($-OCH_3$), 99.12, 100.01 (C-7 and C-5), 114.68 (C-3), 122.18 and 123.69 (C-8 and C-9), 130.67 (C-10), 143.96 (C-4), 144.06–145.09 ($-CF_3$), 157.89 (C-6) and 161.55 (C-2); HRMS: calcd for $C_{11}H_8F_3NO_2$ $[M]^{+\cdot}$ 243.1819, found 243.5345.

5.1.8.2. 3-Butyl-6-methoxy-4-methylquinolin-2(1*H*)-one (29). The title compound (**29**) was obtained through column chromatography in ethyl acetate/petroleum ether (1:4). It was obtained as grey solid in 70% yield following the general procedure. Melting point: 173–176 °C; UV (acetonitrile) λ_{max}: 210, 237, 239 and 349 nm; IR (KBr) ν_{max}: 2952.38, 2928.73, 2857.02, 1654.13 (NHCO), 1624.22 (NHCO), 1505.90, 1223.80, 1460.16, 1413.99, 1036.53, 825.91, 635.93 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 7.2 Hz, $-CH_2(CH_2)_2CH_3$), 1.36–1.45 (m, 4H, $-CH_2(CH_2)_2CH_3$), 2.36 (s, 3H, C-4 CH_3), 2.82 (t, 2H, J = 7.3 Hz, $-CH_2(CH_2)_4CH_3$), 3.76 (s, 3H, $-OCH_3$), 6.98–7.00 (m, 2H, H-5 and H-7), 7.25 (d, 1H, J = 9.5 Hz, H-8), 12.34 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 14.06 ($-CH_2(CH_2)_2CH_3$), 15.16 (C-4 CH_3), 22.94, 26.80, 31.29 ($-(CH_2)_3CH_3$), 55.65 ($-OCH_3$), 106.48 and 117.30 (C-5 and C-7), 117.69 and 121.66 (C-8 and C-10), 131.42 (C-3), 132.04 (C-9), 142.31 (C-4), 154.77 (C-6), 163.49 (C-2); HRMS: calcd for $C_{15}H_{20}NO_2$ $[M+H]^+$ 246.1449, found 246.1489.

5.1.8.3. 6-Methoxy-4-methyl-3-octylquinolin-2(1*H*)-one (30). The title compound (**30**) was obtained through column chromatography in ethyl acetate/petroleum ether (1:9). It was obtained as white solid in 65% yield following the general procedure. Melting point: 118–120 °C; UV (acetonitrile) λ_{max}: 212, 237 and 351 nm; IR (KBr) ν_{max}: 2925.61, 2849.06, 1654.00 (NHCO), 1623.85 (NHCO), 1506.12, 1417.76, 1277.61, 1208.23, 1040.08, 855.67, 642.98 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.77 (t, 3H, J = 6.6 Hz, $-CH_2(CH_2)_6CH_3$), 1.17–1.47 (m, 12H, $-CH_2(CH_2)_6CH_3$), 2.35 (s, 3H, C-4 CH_3), 2.70 (t, 2H, J = 7.3 Hz, $-CH_2(CH_2)_6CH_3$), 3.75 (s, 3H, $-OCH_3$), 6.96–6.98 (m, 2H, H-5 and H-7), 7.24–7.26 (m, 1H, H-8), 12.43 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 14.10 ($-CH_2(CH_2)_6CH_3$), 15.18 (C-4 CH_3), 22.65, 27.09, 29.11, 29.31, 29.55, 29.88, 31.91 ($-(CH_2)_7CH_3$), 55.65 ($-OCH_3$), 106.50, 117.33 (C-5 and C-7), 117.67 and 121.67 (C-8 and C-10), 131.46 (C-3), 132.10 (C-9), 142.27 (C-4), 154.78 (C-6), 163.49 (C-2); HRMS: calcd for $C_{19}H_{27}NO_2$ $[M+H]^+$ 302.2075, found 302.2115.

5.1.8.4. 3-Decyl-6-Methoxy-4-methylquinolin-2(1*H*)-one (31). The title compound (**31**) was obtained through column chromatography in ethyl acetate/petroleum ether (1:9). It was obtained as grey solid in 65% yield following the general procedure. Melting point: 112–114 °C; UV (acetonitrile) λ_{max}: 213, 238, 274 and 350 nm; IR (KBr) ν_{max}: 2924.60, 2854.04, 1652.28 (NHCO), 1623.77 (NHCO), 1504.18, 1460.98, 1223.22, 1039.48, 932.58, 636.69 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, J = 6.6 Hz, $-CH_2(CH_2)_8CH_3$), 1.25–1.59 (m, 16H, $-CH_2(CH_2)_8CH_3$), 2.45 (s, 3H, C-4 CH_3), 2.81 (t, 2H, J = 7.6 Hz, $-CH_2(CH_2)_8CH_3$), 3.85 (s, 3H, $-OCH_3$), 7.06–7.09 (m, 2H, H-5 and H-7), 7.36 (d, 1H, J = 9.5 Hz, H-8), 12.60 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 14.09 ($-CH_2(CH_2)_8CH_3$), 15.18 (C-4 CH_3), 22.66, 27.08, 29.34, 29.60, 29.65, 29.65, 29.74, 29.87, 31.89 ($-(CH_2)_9CH_3$), 55.63 ($-OCH_3$), 106.44, 117.35 (C-5 and C-7), 117.67 (C-8), 121.65 (C-10), 131.47 (C-3), 132.09 (C-9), 142.25 (C-4), 154.75 (C-6), 163.52 (C-2); HRMS: calcd for $C_{21}H_{31}NO_2$ $[M+Na]^+$ 352.2252, found 352.2247.

5.1.8.5. 7-Methoxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (32). The title compound (**32**) was obtained through column chromatography in ethyl acetate/petroleum ether (1:49). It was obtained by as yellow solid in 80% yield following the general procedure. Melting point: 250–252 °C, (Literature value = 252 °C)[30]; UV (MeOH) λ_{max}: 337 nm; IR (KBr) ν_{max}: 2977.72, 2921.33, 2855.32, 1629.65 (NHCO), 1522.80, 1483.44, 1458.58, 1400.34, 1376.35, 1326.42, 1298.56, 1271.62, 1223.69, 1186.71, 1138.76, 1122.76, 1036.98, 1013.18, 970.18, 915.63, 895.30, 839.82, 810.73, 711.90, 669.89 and 616.21 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 3.84 (s, 3H, $-OCH_3$), 6.77 (s, 1H, H-3), 6.92–6.96 (m, 2H, H-6 and H-8), 7.61 (d, 1H, J = 8.4 Hz, H-5) and 12.20 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 55.54 ($-OCH_3$), 99.79, 107.01 (C-6 and C-8), 112.00 (C-3), 118.13 (C-10), 124.37 (C-5), 125.64 (C-9), 136.23–136.64 ($-CF_3$), 141.75 (C-4), 160.38 (C-7) and 161.63

(C-2); HRMS: calcd for $C_{11}H_8F_3NO_2$ $[M+H]^+$ 244.1819, found 244.3801.

5.1.9. Synthesis of hydroxy quinolin-2(1*H*)-one (33–37)

Methoxy quinolin-2(1*H*)-ones (**28**–**32**) was dissolved in a solution of hydrobromic acid/acetic acid (7:3). The mixture was refluxed for 72 h and then poured on ice. The precipitate was then filtered and washed with water and ether.

5.1.9.1. 6-Hydroxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (33)[37]. The title compound (**33**) was obtained as yellow solid in 70% yield by following the general procedure. Melting point: 268 °C; UV (MeOH) λ_{max}: 332 and 354 nm; IR (KBr) ν_{max}: 3587.74 (OH), 3105.12, 2992.77, 2812.77, 2655.46, 1610.31 (NHCO), 1568.30, 1529.48, 1499.70, 1459.35, 1387.53, 1295.46, 1260.18, 1233.69, 1154.25, 1105.03, 950.44, 926.72, 857.70, 832.05, 732.62, 721.92 and 625.53 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 7.04 (s, 1H, H-3), 7.19–7.34 (m, 2H, H-5 and H-7), 7.95 (d, 1H, J = 6.6 Hz, H-8), 10.29 (br s, 1H, OH), 11.96 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 99.54 and 102.94 (C-5 and C-7), 119.95 (C-3), 122.69 and 123.45 (C-8 and C-9), 130.70 (C-10), 143.04 (C-4), 144.02–144.48 (–*C*F_3), 156.27 (C-6), 161.11 (C-2); HRMS: calcd for $C_{10}H_6F_3NO_2$ $[M+H]^+$ 230.0423, found 230.0424.

5.1.9.2. 3-Butyl-6-hydroxy-4-methylquinolin-2(1*H*)-one (34). The title compound (**34**) was obtained as white solid in 80% yield following the general procedure. Melting point: 196–198 °C; UV (acetonitrile) λ_{max}: 274, 284 and 350 nm; IR (KBr) ν_{max}: 3203.29 (OH), 2954.34, 2929.36, 1647.69 (NHCO), 1623.66 (NHCO), 1505.05, 1280.15, 1196.18, 877.85, 713.34, 642.61 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 0.77 (t, 3H, J = 6.9 Hz, –$CH_2(CH_2)_2CH_3$), 1.21–1.24 (m 4H, –$CH_2(CH_2)_2CH_3$), 2.21 (s, 3H, C-4 CH_3), 2.40–2.47 (m, 2H, –$CH_2(CH_2)_2CH_3$), 6.88 (dd, 1H, J = 2.2 and 8.4 Hz, H-7), 6.91 (s, 1H, H-5), 7.03 (d, 1H, J = 8.8 Hz, H-8), 9.07 (br s, 1H, OH), 11.31 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 13.94 (–$CH_2(CH_2)_2CH_3$), 14.80 (C-4 CH_3), 22.45, 26.27, 30.85 (–$(CH_2)_3CH_3$), 108.55, 116.14 (C-5 and C-7), 118.31 and 121.00 (C-8 and C-10), 130.38 (C-9), 131.41 (C-3), 141.01 (C-4), 152.01 (C-6), 161.04 (C-2); HRMS: calcd for $C_{14}H_{17}NO_2$ $[M+H]^+$ 232.1293, found 232.1332.

5.1.9.3. 6-Hydroxy-4-methyl-3-octylquinolin-2(1*H*)-one (35). The title compound (**35**) was obtained as white solid in 80% yield following the general procedure. Melting point: 205–207 °C; UV (acetonitrile) λ_{max}: 207, 235, 274 and 351 nm; IR (KBr) ν_{max}: 3265.87 (OH), 2956.23, 2926.20, 2854.98, 1647.99 (NHCO), 1623.38 (NHCO), 1503.76, 1276.79, 1205.13, 859.03, 642.02 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 0.71 (t, 3H, J = 5.4 Hz, –$CH_2(CH_2)_6CH_3$), 1.09–1.27 (m, 12H, –$CH_2(CH_2)_6CH_3$), 2.20 (s, 3H, C-4 CH_3), 2.47 (t, 2H, J = 6.6 Hz, –$CH_2(CH_2)_6CH_3$), 6.82 (dd, 1H, J = 1.4 and 8.8 Hz, H-7), 6.91 (d, 1H, J = 1.4 Hz, H-5), 7.02 (d, 1H, J = 8.8 Hz, H-8), 9.22 (br s, 1H, OH), 11.31 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 13.98 (–$CH_2(CH_2)_6CH_3$), 14.80 (C-4 CH_3), 22.16, 26.54, 28.65, 28.78, 29.01, 29.35, 31.36 (–$(CH_2)_7CH_3$), 108.54, 116.14 (C-5 and C-7), 118.29 and 121.00 (C-8 and C-10), 130.38 (C-9), 131.44 (C-3), 140.98 (C-4), 152.02 (C-6), 161.06 (C-2); HRMS: calcd for $C_{18}H_{25}NO_2$ $[M+H]^+$ 288.1919, found 288.1958.

5.1.9.4. 3-Decyl-6-hydroxy-4-methylquinolin-2(1*H*)-one (36). The title compound (**36**) was obtained as white solid in 80% yield, following the general procedure. Melting point: 209–211 °C; UV (acetonitrile) λ_{max}: 210, 235, 276 and 352 nm; IR (KBr) ν_{max}: 3213.80 (OH), 2959.73, 2925.88, 2852.54, 1646.53 (NHCO), 1623.06 (NHCO), 1505.04, 1279.36, 1205.61, 880.57, 642.15 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 0.81 (s, 3H, J = 6.6 Hz, –$CH_2(CH_2)_8CH_3$), 1.19–1.37 (m, 16H, –$CH_2(CH_2)_8CH_3$), 2.30 (s, 3H, C-4 CH_3), 2.57 (t, 2H, J = 7.3 Hz, –$CH_2(CH_2)_8CH_3$), 6.91 (dd, 1H, J = 2.2 and J = 8.8 Hz, H-7), 7.00 (d, 1H, J = 2.2 Hz, H-5), 7.11 (d, 1H, J = 8.8 Hz, H-8), 9.28 (br s, 1H, OH), 11.40 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 13.99 (–$CH_2(CH_2)_8CH_3$), 14.81 (C-4 CH_3), 22.17, 26.56, 28.66, 28.79, 29.09, 29.09, 29.13, 29.35, 31.70 (–$(CH_2)_9CH_3$), 108.53 and 116.12 (C-5 and C-7), 118.28 and 120.98 (C-8 and C-10), 130.39 (C-9), 131.44 (C-3), 140.92 (C-4), 152.01 (C-6), 161.04 (C-2); HRMS: calcd for $C_{20}H_{29}NO_2$ $[M+Na]^+$ 338.2096, found 338.2091.

5.1.9.5. 7-Hydroxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (37). The title compound (**37**) was obtained as yellow solid in 70% yield by following the general procedure. Melting point: >300 °C (Literature mp = >300 °C)[30]; UV (MeOH) λ_{max}: 338 nm; IR (KBr) ν_{max}: 3421.52 (OH), 3092.43, 2928.06, 1665.48 (NH*CO*), 1624.06 (NH*CO*), 1550.55, 1474.27, 1435.70, 1417.71, 1367.57, 1292.79, 1265.70, 1230.75, 1205.67, 1159.83, 1140.13, 1020.97, 976.95, 873.58, 842.56, 818.90, 723.79 and 661.73 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 6.68 (s, 1H, H-3), 6.76–6.82 (m, 2H, H-5 and H-8), 7.53 (dd, 1H, J = 2.1 and 8.7 Hz, H-6), 10.57 (br s, 1H, OH) and 12.08 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 100.70 and 106.05 (C-6 and C-8), 112.89 (C-3), 117.04 (C-10), 120.80 (C-5), 124.44 (C-9), 125.75 (C-4), 136.34–136.75 (–*C*F_3), 141.86 (C-7) and 160.45 (C-2); HRMS: calcd for $C_{10}H_6F_3NO_2$ $[M+H]^+$ 230.0423, found 230.0428.

5.1.10. Synthesis of 2-Oxo-1,2-dihydro-6/7-yl acetate (38–42)

Hydroxy quinolin-2(1*H*)-ones (**33**–**37**) were added to a solution of acetic anhydride and acetic acid (1:4). The mixture was refluxed for 6 h and poured on ice. The precipitate was filtered and washed with water and ether.

5.1.10.1. 2-Oxo-4-(trifluoromethyl)-1,2-dihydro-6-yl acetate (38). The title compound (**38**) was obtained as white crystals in 90% yield by following the general procedure. Melting point: 296 °C; UV (MeOH) λ_{max}: 284 and 317 nm; IR (KBr) ν_{max}: 3435.52, 2935.89, 1781.96 (–OCO), 1607.63 (NHCO), 1574.73, 1509.30, 1474.64, 1368.73, 1277.44, 1175.13, 1138.89, 1097.65, 1067.80, 1053.62, 1011.83, 950.61, 931.82, 895.53, 839.20, 731.04 and 669.78 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.37 (s, 3H, –OCO*C*H_3), 7.81 (dd, 1H, J = 2.1 and 9.0 Hz, H-7), 7.98 (d, 1H, J = 2.1 Hz, H-5), 8.01 (s, 1H, H-3), 8.29 (br s, 1H, J = 9.3 Hz, H-8); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 20.88 (–OCO*C*H_3), 110.36, 113.19 (C-7 and C-5), 119.30 (C-3), 123.53 (C-8), 128.06 (C-9), 131.20 (C-10), 146.23–147.18 (–*C*F_3), 150.65 (C-4), 155.69 (C-6), 168.24 (C-2) and 169.05 (–*O*COCH_3); HRMS: calcd for $C_{12}H_8F_3NO_3$ $[M+H]^+$ 272.0456, found 271.9899.

5.1.11. 3-Butyl-4-methyl-2-oxo-1,2-dihydroquinolin-6-yl acetate (39)

The title compound (**39**) was obtained as white crystals in 83% yield following the general procedure. Melting point: 180–182 °C; UV (acetonitrile) λ_{max}: 270, 279, 334 and 336 nm; IR (KBr) ν_{max}: 2954.84, 2929.81, 2861.11, 1761.82 (–OCO), 1662.99 (NHCO), 1503.03, 1206.71, 1180.61, 921.86 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.95 (t, 3H, J = 6.9 Hz, –$CH_2(CH_2)_2CH_3$), 1.41–1.54 (m, 4H, –$CH_2(CH_2)_2CH_3$), 2.31 (s, 3H, C-4 CH_3), 2.43 (s, 3H, –OCO*C*H_3), 2.79 (t, 2H, J = 7.6 Hz, –$CH_2(CH_2)_2CH_3$), 7.16 (dd, 1H, J = 2.2 and 8.8 Hz, H-7), 7.37–7.39 (m, 2H, H-5 and H-8), 12.39 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 14.02 (–$CH_2(CH_2)_2CH_3$), 15.11 (C-4 CH_3), 21.07 (–OCO*C*H_3), 22.89, 26.72, 31.17 (–$(CH_2)_3CH_3$), 116.47, 116.99 (C-5 and C-7), 121.57 and 123.02 (C-8 and C-10), 132.53 (C-3), 134.65 (C-9), 142.41 (C-4), 145.31 (C-6), 163.81 (C-2), 169.87 (–*O*CO*C*H_3); HRMS: calcd for $C_{16}H_{19}NO_3$ $[M+Na]^+$ 296.1263, found 296.1257.

5.1.11.1. 4-Methyl-3-octyl-2-oxo-1,2-dihydroquinolin-6-yl acetate (40). The title compound (**40**) was obtained as white crystals in 80% yield following the general procedure. Melting point: 139–141 °C; UV (acetonitrile) λ_{max}: 203, 231 and 333 nm; IR (KBr) ν_{max}: 2923.00, 2852.55, 1759.66 (–OCO), 1659.74 (NHCO), 1504.25, 1212.45, 1183.04, 919.92 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.84–0.86 (m, 3H, $-CH_2(CH_2)_6CH_3$), 1.26–1.53 (m, 12H, $-CH_2(CH_2)_6CH_3$), 2.31 (s, 3H, C-4 CH_3), 2.42 (s, 3H, $OCOCH_3$), 2.77 (t, 2H, J = 7.3 Hz, $-CH_2(CH_2)_2CH_3$), 7.15 (d, 1H, J = 8.1 Hz, H-7), 7.37–7.40 (m, 2H, H-5 and H-8), 12.49 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 14.09 ($-CH_2(CH_2)_6CH_3$), 15.12 (C-4 CH_3), 21.07 ($OCOCH_3$), 22.63, 27.00, 28.98, 29.28, 29.50, 29.82, 31.88 ($-(CH_2)_7CH_3$), 116.46, 117.01 (C-5 and C-7), 121.56 and 122.99 (C-8 and C-10), 132.56 (C-3), 134.66 (C-9), 142.39 (C-4), 145.29 (C-6), 163.82 (C-2), 169.86 ($OCOCH_3$); HRMS: calcd for $C_{20}H_{27}NO_3$ $[M+H]^+$ 330.2024, found 330.2064.

5.1.11.2. 3-Decyl-4-methyl-2-oxo-1,2-dihydroquinolin-6-yl acetate (41). The title compound (**41**) was obtained as white crystals in 80% yield following the general procedure. Melting point: 130–132 °C; UV (acetonitrile) λ_{max}: 269, 279, 333 and 348 nm; IR (KBr) ν_{max}: 2922.31, 2851.35, 1760.48 (–OCO), 1660.86 (NHCO), 1505.10, 1217.86, 1012.71, 927.50, 629.66 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.92 (t, 3H, J = 6.9 Hz, $-CH_2(CH_2)_8CH_3$), 1.32–1.60 (m, 16H, $-CH_2(CH_2)_8CH_3$), 2.38 (s, 3H, C-4 CH_3), 2.49 (s, 3H, $-OCOCH_3$), 2.85 (t, 2H, J = 7.7 Hz, $-CH_2(CH_2)_8CH_3$), 7.23 (dd, 1H, J = 2.2 and 8.8 Hz, H-7), 7.44–7.48 (m, 2H, H-5 and H-8), 12.64 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 14.09 ($-CH_2(CH_2)_8CH_3$), 15.11 (C-4 CH_3), 21.06 ($-OCOCH_3$), 22.65, 26.99, 28.12, 28.29, 29.32, 29.56, 29.63, 29.83, 31.87 ($-(CH_2)_9CH_3$), 116.44, 117.05 (C-5 and C-7), 121.56 and 122.99 (C-8 and C-10), 132.54 (C-3), 134.67 (C-9), 142.38 (C-4), 145.28 (C-6), 163.85 (C-2), 169.86 ($-OCOCH_3$); HRMS: calcd for $C_{22}H_{31}NO_3$ $[M+Na]^+$ 380.2202, found 380.2196.

5.1.11.3. 2-Oxo-4-(trifluoromethyl)-1,2-dihydro-7-yl acetate (42). The title compound (**42**) was obtained as white crystals in 90% yield by following the general procedure. Melting point: 190 °C; UV (MeOH) λ_{max}: 336 and 275 nm; IR (KBr) ν_{max}: 3432.60, 2925.24, 2851.40, 1766.92 (–OCO), 1683.55 (NHCO), 1617.88, 1567.98, 1521.40, 1422.24, 1373.73, 1331.68, 1289.58, 1267.15, 1207.14, 1188.00, 1166.51, 1137.41, 1020.35, 976.43, 924.25, 884.45, 856.51, 825.17, 728.52 and 653.95 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.32 (s, 3H, $-OCOCH_3$), 6.97 (s, 1H, H-3), 7.12 (dd, 1H, J = 1.8 and 8.7 Hz, H-6), 7.19 (d, 1H, J = 1.8 Hz, H-8) and 7.73 (d, 1H, J = 8.7 Hz, H-5); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 20.98 ($-OCOCH_3$), 108.94, 110.91 (C-6 and C-8), 117.40 (C-3), 121.45 (C-10), 124.27 (C-5), 125.72 (C-9), 136.00–136.42 (CF_3), 140.84 (C-4), 152.56 (C-7), 160.15 (C-2) and 168.84 ($-OCOCH_3$); HRMS: calcd for $C_{12}H_8F_3NO_3$ $[M]^{+\cdot}$ 271.1920, found 271.3652.

5.1.12. Synthesis of 8/6-acetyl-7-hydroxycoumarin (44–47)

7-Acetoxy-4-methylcoumarin[17] (2 g, 9.2 mmol) and anhydrous aluminium chloride (4.5 g, 34 mmol) were taken in a round bottom flask fitted with a reflux condenser. The temperature of the reaction mixture was raised quickly to 125 °C and then slowly over a period of 2 h to 170 °C. On completion of the reaction, crushed ice was added to the reaction mixture followed by the acidification using dilute hydrochloric acid. The crude product was filtered, washed with water followed by ether and then subjected to column chromatography (petroleum ether–ethyl acetate)

5.1.12.1. 8-Acetyl-7-hydroxy-4-methylcoumarin (44). The title compound **44** was obtained as light yellow solid in 70% yield by column chromatography using ethyl acetate–petroleum ether (1:20) as an eluent; Melting point: 162–163 °C (Literature mp = 162–163 °C)[18]; UV (acetonitrile) λ_{max}: 211, 308 and 350 nm; IR (Nujol) ν_{max}: 2920.95, 2853.95, 1738.43 ($COCH_3$), 1732.03 (CO), 1610.85, 1463.28, 1371.81, 1234.08, 1087.60, 1059.53, 878.03, 658.62 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.36 (s, 3H, C-4 CH_3), 2.86 (s, 3H, $-COCH_3$), 6.08 (s, 1H, H-3), 6.82 (d, 1H, J = 8.8 Hz, H-6), 7.60 (d, 1H, J = 8.8 Hz, H-5), 13.48 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 19.08 (C-4 CH_3), 33.79 ($-COCH_3$), 109.09 (C-8), 110.89, 111.73 and 114.96 (C-3, C-6 and C-10), 131.19 (C-5), 152.95 (C-9), 154.98 (C-4), 159.19 (C-7), 166.47 (C-2), 204.21 ($COCH_3$). ESI MS: calcd for $C_{12}H_{10}O_4$ $[M]^{+\cdot}$ 218, found 218.

5.1.12.2. 8-Acetyl-3-ethyl-7-hydroxy-4-methylcoumarin (45). The title compound **45** was obtained as light yellow solid in 70% yield by column chromatography using ethyl acetate–petroleum ether (1:20) as an eluent. Melting point: 171–172 °C; UV (acetonitrile) λ_{max}: 240, 273, 305 and 350 nm; IR (KBr) ν_{max}: 2970.32, 2935.77, 1717.83 (CO), 1614.92, 1370.28, 1215.39, 1092.50, 834.73, 647.36, 469.32 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.09 (t, 3H, J = 7.3 Hz, $-CH_2CH_3$), 2.33 (s, 3H, C-4 CH_3), 2.59 (q, 2H, J = 7.3 Hz, $-CH_2CH_3$), 2.86 (s, 3H, $-COCH_3$), 6.78 (d, 1H, J = 8.7 Hz, H-6), 7.60 (d, 1H, J = 9.5 Hz, H-5), 13.34 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 12.90 ($-CH_2CH_3$), 14.77 (C-4 CH_3), 20.57 ($-CH_2CH_3$), 33.82 ($-COCH_3$), 108.88 (C-8), 112.46 and 114.62 (C-6 and C-10), 124.14 (C-3), 131.23 (C-5), 146.03 (C-4), 153.32 (C-9), 160.07 (C-7), 165.34 (C-2), 204.24 ($-COCH_3$); HRMS: calcd for $C_{14}H_{14}O_4$ $[M+H]^+$ 247.0926, found 247.0965.

5.1.12.3. 6-Acetyl-7-hydroxy-4-methylcoumarin (46). The title compound **46** was obtained as light yellow solid in 70% yield by column chromatography using ethyl acetate–petroleum ether (1:10) as an eluent. Melting point: 210–211 °C (Literature value = 211 °C)[38]; UV (acetonitrile) λ_{max}: 257, 297 and 342 nm; IR (KBr) ν_{max}: 3438.76 (OH), 2357.39, 1738.11 (CO), 1614.66, 1369.57, 1308.01, 1233.59, 1059.21, 876.65, 657.39, 455.93 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.42 (s, 3H, C-4 CH_3), 2.68 (s, 3H, $-COCH_3$), 6.15 (s, 1H, H-3), 6.82 (s, 1H, H-8), 7.94 (s, 1H, H-5), 12.61 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 18.57 (C-4 CH_3), 26.63 ($-COCH_3$), 105.39 (C-8), 112.86 and 112.99 (C-3 and C-10), 117.07 (C-6), 128.12 (C-5), 151.62 (C-4), 158.71 (C-9), 159.88 (C-7), 165.31 (C-2), 203.30 ($-COCH_3$); ESI MS: calcd for $C_{12}H_{10}O_4$ $[M+H]^+$ 219, found 219.

5.1.12.4. 6-Acetyl-3-ethyl-7-hydroxy-4-methylcoumarin (47). The title compound **47** was obtained as light yellow solid in 70% yield by column chromatography using ethyl acetate–petroleum ether (1:10) as an eluent. Melting point: 144–146 °C; UV (acetonitrile) λ_{max}: 210, 230, 256, 305 and 341 nm; IR (KBr) ν_{max}: 3413.02 (OH), 3057.27, 2973.31, 1721.02 (CO), 1646.41, 1386.40, 1254.88, 1167.11, 1058.49, 930.50, 805.41, 599.82 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.10 (t, 3H, J = 7.3 Hz, $-CH_2CH_3$), 2.38 (s, 3H, C-4 CH_3), 2.61 (q, 2H, J = 7.3 Hz, $-CH_2CH_3$), 2.67 (s, 3H, $-COCH_3$), 6.74 (s, 1H, H-8), 7.91 (s, 1H, H-5), 12.50 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 13.03 ($-CH_2CH_3$), 14.77 (C-4 CH_3), 20.86 ($-CH_2CH_3$), 26.57 ($-COCH_3$), 104.72 (C-8), 113.52 and 116.92 (C-6 and C-10), 125.99 (C-3), 127.88 (C-5), 144.75 (C-4), 157.27 (C-9), 160.65 (C-7), 164.16 (C-2), 203.38 ($-COCH_3$); HRMS: calcd for $C_{14}H_{14}O_4$ $[M+Na]^+$ 269.0790, found 269.0784.

5.1.13. Synthesis of 7-acetoxy derivative of acetyl coumarin (48–51)

A mixture of 7-hydroxycoumarin (500 mg, **44**–**47**) and DMAP (10–20 mg) were dissolved in minimum amount of THF. To this acetic anhydride (1.0 equiv) was added and the resultant mixture was stirred at room temperature for 24 h. On completion of the reaction 50 mL ice cold water was added. The crude off-white solid

was then filtered, washed with water and dried. The crude solid so obtained was crystallized with ethanol to give 7-acetoxycoumarin derivatives.

5.1.13.1. 7-Acetoxy-8-acetyl-4-methylcoumarin (48). The title compound **48** was obtained as light yellow solid in 98% yield; Melting point: 188–190 °C; UV (acetonitrile) λ_{max}: 212, 274 and 313 nm; IR (KBr) ν_{max}: 3082.40, 2925.76, 1769.48 (–OCO), 1740.26 ($COCH_3$), 1698.32 (CO), 1596.00, 1381.65, 1199.64, 1054.96, 864.42, 593.44 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.29 (s, 3H, –OCOCH_3), 2.45 (s, 3H, C-4 CH_3), 2.68 (s, 3H, –COCH_3), 6.31 (s, 1H, H-3), 7.09 (d, 1H, J = 8.0 Hz, H-6), 7.68 (d, 1H, J = 8.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 18.96 (C-4 CH_3), 20.86 (–OCOCH_3), 32.04 (–COCH_3), 111.11 and 114.71 (C-3 and C-6), 118.12 (C-10), 123.31 (C-8), 126.60 (C-5), 149.43 (C-7), 151.05 (C-4), 151.92 (C-9), 159.06 (C-2), 168.87 (–OCOCH$_3$), 197.87 (–$COCH_3$); HRMS: calcd for $C_{14}H_{12}O_5$ $[M+H]^+$ 261.0718, found 261.0757.

5.1.13.2. 7-Acetoxy-8-acetyl-3-ethyl-4-methylcoumarin (49). The title compound **49** was obtained as light yellow solid in 98% yield; Melting point: 155–157 °C; UV (Acetonitrile) λ_{max}: 276, 2849 and 314 nm; IR (KBr) ν_{max}: 3085.89, 2982.69, 1778.16 (–OCO), 1717.48 ($COCH_3$), 1701.29 (CO), 1595.10, 1371.81, 1185.78, 1093.23, 1054.47, 891.60, 590.73 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.09 (t, 3H, J = 7.3 Hz, –CH_2CH_3), 2.28 (s, 3H, –OCOCH_3), 2.42 (s, 3H, C-4 CH_3), 2.66–2.72 (m, 5H, –CH_2CH_3 and –COCH_3), 6.06 (d, 1H, J = 8.7 Hz, H-6), 7.67 (d, 1H, J = 8.8 Hz, H-5), ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 12.94 (–CH_2CH_3), 14.81 (C-4 CH_3), 20.84 (–CH_2CH_3), 21.03 (–OCOCH_3), 32.07 (–COCH_3), 118.80 and 118.91 (C-6 and C-10), 122.84 (C-3), 126.51 (C-8), 127.98 (C-5), 145.06 (C-4), 148.29 (C-7), 149.54 (C-9), 159.95 (C-2), 167.97 (–OCOCH$_3$), 198.12 (–$COCH_3$); HRMS: calcd for $C_{16}H_{16}O_5$ $[M+Na]^+$ 311.0895, found 311.0890.

5.1.13.3. 7-Acetoxy-6-acetyl-4-methylcoumarin (50). The title compound **50** was obtained as light yellow solid in 98% yield; Melting point: 169–171 °C; UV (acetonitrile) λ_{max}: 207, 251 and 313 nm; IR (KBr) ν_{max}: 3091.97, 2925.08, 1756.51 (–OCO), 1685.61 ($COCH_3$), 1631.92 (CO), 1385.77, 1268.96, 1195.18, 1138.24, 1051.53, 915.02, 572.94 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.40 (s, 3H, –OCOCH_3), 2.49 (s, 3H, C-4 CH_3), 2.61 (s, 3H, –COCH_3), 6.32 (s, 1H, H-3), 7.11 (s, 1H, H-8), 8.08 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 18.72 (C-4 CH_3), 21.21 (–OCOCH_3), 29.65 (–COCH_3), 112.51 (C-3), 115.38 (C-8), 117.88 (C-10), 127.33 (C-5 and C-6), 151.66, 151.76 (C-4 and C-7), 156.22 (C-9), 159.50 (C-2), 168.77 (–OCOCH$_3$), 195.64 (–$COCH_3$); HRMS: calcd for $C_{14}H_{12}O_5$ $[M+K]^+$ 299.0322, found 299.0316.

5.1.13.4. 7-Acetoxy-6-acetyl-3-ethyl-4-methylcoumarin (51). The title compound **51** was obtained as light yellow solid in 98% yield; Melting point: 128–130 °C; UV (acetonitrile) λ_{max}: 250 and 312 nm; IR (KBr) ν_{max}: 3422.06, 2973.74, 1761.00 (–OCO), 1723.04 ($COCH_3$), 1682.15 (CO), 1618.65, 1365.45, 1198.62, 1151.04, 1057.74, 909.97, 780.07, 532.01 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.16 (t, 3H, J = 7.6 Hz, –CH_2CH_3), 2.39 (s, 3H, –OCOCH_3), 2.46 (s, 3H, C-4 CH_3), 2.61 (s, 3H, –COCH_3), 2.69 (q, 2H, -CH_2CH_3), 7.07 (s, 1H, H-8), 8.09 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 12.95 (–CH_2CH_3), 14.66 (C-4 CH_3), 21.12 (–CH_2CH_3), 21.22 (–OCOCH_3), 29.65 (–COCH_3), 112.04 (C-8), 118.62 (C-10), 127.05 (C-3), 127.24 (C-5), 128.70 (C-6), 144.76 (C-4), 150.72 (C-7), 154.77 (C-9), 160.46 (C-2), 168.91 (–OCOCH$_3$), 195.86 (–$COCH_3$); HRMS: calcd for $C_{16}H_{16}O_5$ $[M+H]^+$ 289.1031, found 289.1071.

5.2. Biological section

5.2.1. Assay of platelet CRTAase

The assay mixture consisted of 0.25 M potassium phosphate buffer (pH 6.5), washed platelet lysate (20 μg protein), test compound (50 μM) added in 10 μL of methanol, to make up the final volume of 0.8 mL. The contents of the tube, scaled up as per the requirement were preincubated at 37 °C for various periods. The aliquots were removed periodically into a spectrophotometer cuvette containing CDNB and GSH to make up their concentration (1 mM) in a total volume of 1 mL and the progress of the GST activity was followed at 340 nm using Cary spectrophotometer (Cary Bio100). Reactions wherein substrates replaced with vehicle alone served as controls. The unit of CRTAase was expressed in terms of % inhibition of GST under the experimental conditions.

5.2.2. Blood collection from human volunteers and preparation of platelet rich plasma (PRP)

Blood from healthy volunteers (n = 45; age, 27 ± 1.2 yrs) were taken for this study after full explanation to them about the details of the experiment and taking their consent. Approval of the Ethical Committee of Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India, was obtained in the meeting held on 31-8-2007 for this study and certificate was issued on October 3rd 2007. 9 mL of venous blood was collected with 1.0 mL of 3.8% trisodium citrate from healthy human volunteers after overnight fasting and had abstained from medication including aspirin, paracetamol and alcohol. The citrated blood was used for the preparation of PRP. Platelet count was determined in PRP using electronic cell counter, SYSMEX Model FA 20 and were adjusted to 250×10^6/mL with Platelet-poor plasma (PPP).

5.2.3. Aggregometry

PRP (500 μL) was pipetted into siliconized glass cuvettes and kept at 37 °C for 2 min in the aggregometer. The test compounds dissolved in methanol (100 μM), were added in the reaction cuvette to analyse the platelet aggregation. Individual samples were then incubated at 37 °C for 10 min. After incubation period, platelet aggregation was induced by the addition of ADP (15 μM)/AA (1 mM) and assessed by using a Platelet Aggregation Profiler (BIODATA CORPORATION, Model PAP-4) and the results were expressed as the maximum percentage of light transmittance change (% max) from the baseline at the end of the recording time, using PPP as a reference. Platelet aggregation curves were recorded for 6 minutes and analysed according to internationally established standards. 7-AAMC (**17**), the lead compound was carried for in depth study where various concentrations (25–250 μM) were used to obtain the concentration response curves.

5.2.4. Measurement of NOS activity

PRP was incubated with test compounds (100 μM) for 10 min and the activity was triggered by the addition of ADP (15 μM) for 5 min. Platelets were paletted down and were washed twice with PBS and resuspended in the standard buffer (137 mM NaCl, 2.8 mM KCl, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.4 mM Na_2HPO_4, 0.35% BSA, 10 mM HEPES, 5.5 mM glucose, pH 7.4) containing 1 μL of DCF-DA (dissolved in CH_3OH) in a total volume of 1 mL to make the final concentration 2 μM and kept at 37 °C for 30 min and then the reaction was stopped by placing the tubes containing the reaction mixture at ice. One set of PRP aliquots were preincubated with *L*-NAME (100 μM)), inhibitor of NOS for 30 min before the addition of test compounds and subjected to NOS assay. Briefly NO was measured in cells labelled with the NO fluorescent dye DCFH-DA. The median fluorescence was measured using the FITC detector (525 nm).

5.2.5. Administration of test compound to the rats

Male Sprague Dawley rats (weight 200–250 g) housed in mesh cages maintained at 25 °C and illuminated at 12:12 h light/dark cycles. This study was conducted in the Department of Biochemistry, V.P. Chest Institute, University of Delhi, Delhi, India. All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Ethics Committee (IAEC). A known amount of the 7-AAMC/ASA was suspended in appropriate volume of normal saline, sonicated for 30 sec and the preparation was administered to the rats (3–4 per group) orally.

5.2.6. Assessment of platelet aggregation

The doses of 7-AAMC were taken at a range, (10–50) mg/kg or (46–230 μmol/kg), administered to the rats orally once and were sacrificed after 24 h, blood samples were taken by cardiac puncture followed by the measurement of ADP/AA induced platelet aggregation and IC_{50} was determined. The observed IC_{50} concentration of 7-AAMC was compared with same concentration of ASA for antiplatelet activity. The optimum concentration (concentration below saturating dose with maximum inhibitory effect) obtained from the dose response curve was used for in depth study of 7-AAMC as an antiplatelet candidate drug.

5.2.7. Assay of Cox-1 activity

Three groups of animals were taken Group 1: control; Group 2: 7-AAMC; Group 3: ASA. Control animals received orally, saline alone. The animals were separately administered 7-AAMC /ASA (160.2 μmol/kg each) po and were sacrificed after 24 h. Platelets lysate were prepared as mentioned above and assessed for Cox-1 activity. The assay was carried out using Cox-1 ELISA kit (*Cayman Chemical*), according to the manufacturer's protocol. Briefly, 50 μL of each lysed samples were added to the wells, the enzymatic reaction was initiated by adding 100 μM *N*,*N*,*N*,*N*-tetramethyl-*p*-phenylenediamine (TMPD) and 100 μM AA (saturating condition) in assay buffer. Inhibitors were added to the incubation reaction at different time intervals before the addition of TMPD and AA. The enzyme activity was measured by ELISA reader, monitoring the appearance of oxidised TMPD at 590 nm.

5.2.8. Analysis of TxB2 in rat platelets

PRP (250×10^6 per mL of platelets) of rats, administered with 7-AAMC/ASA (160.2 μmol/kg) was incubated with AA for 10 min at 37 °C. Platelets were settled down by centrifugation and plasma was taken for the estimation of TxB2 levels. TxB2 was bioassayed by ELISA method using *Cayman's Chemicals* as per manufacturer's instruction.

5.2.9. Effect of 7-AAMC (17) under thrombotic prone condition: LPS administration to rats

Rats were administered daily 7-AAMC/ASA (160.2 μmol/kg) for 5 days. The timing was chosen as per the preliminary time dependent studies carried on 7-AAMC at a concentration of 160.2 μmol/kg in order to withstand the LPS insult, and it was observed that at least 5 days were required by 7-AAMC to obtain full inhibitory activity on pro-inflammatory condition such as expression of TF/iNOS (data not shown). On the day 5, the last dose of the indicated drug was administered followed by an intra peritoneal injection of LPS, at a dose of 2.5 mg/kg and the animals were sacrificed after 6 h. PBMCs were taken for the assessment of TF and iNOS.

5.2.10. Analysis of tissue factor

The lymphocytes were prepared from rat blood by density gradient using histopack and lysed in 100 μL of lysis buffer for 5 min at 4 °C and centrifuged at 2500*g* for 10 min. The supernatant obtained was used for the assay of TF, by ELISA method using *Assay Pro kit* as per the manufacturers' instructions.

5.2.11. Immunodetection of iNOS

Lysed Lymphocytes were used for western blotting method to detect the LPS induced iNOS expression. The protein concentration in the supernatant was determined by the Bradford method (Bio-Rad) and equal amount of proteins (30 μg) were denatured and separated on SDS–PAGE for western blotting. After electrophoresis, the separated protein on the gel was transblotted to a nitro cellulose membrane (*Axiva*) at 300 mA for 2.5 h at 4 °C. Membrane was kept in blocking reagent (5% blotto) to block the nonspecific sites. The primary antibody was diluted (1: 1000) in Tris–buffer saline (TBS) containing 1% BSA and was incubated with the blot for 1 h at 4 °C with moderate agitation. The membrane was extensively washed with 0.05% Tween-20 detergent in TBS, (TBST), followed by incubation with HRP conjugated secondary antibody (Sigma Chemical Co.) for same time duration at room temperature. The membrane was washed extensively with TBST/TBS and the transblotted protein bands were visualized by treating with diaminobenzidene (DAB) and hydrogen peroxide.

5.2.12. Statistical analysis

Calculations and statistics were performed using the Graph Pad Prism 3.02 software. The one-way analysis of variance (ANOVA) tests followed by the post-hoc Turkey multiple comparisons test and Student's tests were used. Data were expressed as mean ± standard error. *P* values less than 0.05 ($P < 0.05$) were considered to be statistically significant.

Acknowledgements

Financial support from the DU-DST Purse grant, University of Delhi and Defense Research and Development Organization (DRDO) is gratefully acknowledged. We are also thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi for awarding SRF to A. Kathuria, N. Priya, K. Chand, A. Gupta and S. Gupta.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.11.016.

References and notes

1. O' Brien, J. R. *Lancet* **1990**, *335*, 711.
2. Pande, A. K.; Sethi, K. K. *Heart Views* **2000**, *1*, 258.
3. Cattaneo, M. *Hematol. J.* **2004**, *5*, S170.
4. Törüner, M. *Anatol. J. Cardiol.* **2007**, *7*, 27.
5. Cattaneo, M. *Arterioscler. Thromb. Vasc. Biol.* **2004**, *24*, 1980.
6. Patrono, C. *J. Thromb. Haemost.* **2003**, *1*, 1710.
7. Hankey, G. J.; Eikelboom, J. W. *Lancet* **2006**, *367*, 606.
8. Campbell, C. L.; Steinhubl, S. R. *J. Thromb. Haemost.* **2005**, *3*, 665.
9. Loscalzo, J.; Welch, G. *Prog. Cardiovasc. Dis.* **1995**, *38*, 87.
10. Bansal, S.; Gaspari, M.; Raj, H. G.; Kumar, A.; Cuda, G.; Verheji, E.; Tyagi, Y. K.; Ponan, P.; Rastogi, R. C.; Parmar, V. S. *Appl. Biochem. Biotechnol.* **2008**, *144*, 37.
11. Singh, I.; Kohli, E.; Raj, H. G.; Gyanda, K.; Jain, S. K.; Tyagi, Y. K.; Gupta, G.; Kumari, R.; Kumar, A.; Pal, G.; Prasad, A. K.; Rastogi, R. C.; Olsen, C. E.; Jain, S. C.; Parmar, V. *Bioorg. Med. Chem.* **2002**, *10*, 4103.
12. Raj, H. G.; Kohli, E.; Goswami, R.; Goel, S.; Rastogi, R. C.; Jain, S. C.; Wengel, J.; Olsen, C. E.; Parmar, V. S. *Bioorg. Med. Chem.* **2001**, *9*, 1085.
13. Kathuria, A.; Gupta, A.; Priya, N.; Singh, P.; Raj, H. G.; Prasad, A. K.; Parmar, V. S.; Sharma, S. K. *Bioorg. Med. Chem.* **2009**, *17*, 1550.
14. Priya, N.; Gupta, A.; Chand, K.; Singh, P.; Kathuria, A.; Raj, H. G.; Parmar, V. S.; Sharma, S. K. *Bioorg. Med. Chem.* **2010**, *18*, 4085.
15. Atkins, R. L.; Bliss, D. E. *J. Org. Chem.* **1978**, *43*, 1975.
16. Kaslow, C. E.; Cook, D. J. *J. Am. Chem. Soc.* **1945**, *67*, 1969.
17. Pechmann, H. V.; Duisberg, C. *Chem. Ber.* **1883**, *16*, 2119.
18. Russell, A.; Frye, J. R. *Org. Synth. Coll.* **1955**, *3*, 281; **1941**, *21*, 22.
19. Hennekens, C. H.; Dyken, M. L.; Fuster, V. *Circulation*, **1997**, *96*, 2751; *Appl. No.*, **1998**, 016011.

20. Seema; Kumari, R.; Gupta, G.; Saluja, D.; Kumar, A.; Goel, S.; Tyagi, Y. K.; Gulati, R.; Vinocha, A.; Murlidhar, K.; Dwarkanath, B. K.; Rastogi, R. C.; Parmar, V. S.; Patkar, S. A.; Raj, H. G. *Cell. Biochem. Biophys.* **2007**, *47*, 53.
21. Kohli, E.; Gaspari, M.; Raj, H. G.; Parmar, V. S.; Sharma, S. K.; Van der Greef, J.; Kumari, R.; Gupta, G.; Seema; Khurana, P.; Tyagi, Y. K.; Watterson, A. C.; Olsen, C. E. *Biochim. Biophys. Acta* **2004**, *1698*, 55.
22. Singh, P.; Ponnan, P.; Krishnan, S.; Tyagi, T. K.; Priya, N.; Bansal, S.; Scumaci, D.; Gaspari, M.; Cuda, G.; Joshi, P.; Gambhir, J. K.; Saluja, D.; Prasad, A. K.; Saso, L.; Rastogi, R. C.; Parmar, V. S.; Raj, H. G. *J. Biochem.* **2010**, *147*, 625.
23. Raj, H. G.; Kumari, R.; Seema; Gupta, G.; Kumar, R.; Saluja, D.; Muralidhar, K. M.; Kumar, A.; Dwarkanath, B. S.; Rastogi, R. C.; Prasad, A. K.; Patkar, S. A.; Watterson, A. C.; Parmar, V. S. *Pure Appl. Chem.* **2006**, *78*, 985.
24. Raj, H. G.; Parmar, V. S.; Jain, S. C.; Goel, S.; Singh, A.; Gupta, K.; Rohil, V.; Tyagi, Y. K.; Jha, H. N.; Olsen, C. E.; Wengel, J. *Bioorg. Med. Chem.* **1998**, *6*, 1895.
25. Raj, H. G.; Parmar, V. S.; Jain, S. C.; Goel, S.; Singh, A.; Tyagi, Y. K.; Jha, H. N.; Olsen, C. E.; Wengel, J. *Bioorg. Med. Chem.* **1999**, *7*, 369.
26. Kumar, A.; Singh, B. K.; Sharma, N. K.; Gyanda, K.; Jain, S. K.; Tyagi, Y. K.; Baghel, A. S.; Pandey, M.; Sharma, S. K.; Prasad, A. K.; Jain, S. C.; Rastogi, R. C.; Raj, H. G.; Watterson, A. C.; Parmar, V. S. *Eur. J. Med. Chem.* **2007**, *42*, 447.
27. Kumar, A.; Singh, B. K.; Tyagi, R.; Jain, S. C.; Sharma, S. K.; Prasad, A. K.; Raj, H. G.; Rastogi, R. C.; Watterson, A. C.; Parmar, V. S. *Bioorg. Med. Chem.* **2005**, *13*, 4300.
28. Loscalzo, J. *Thromb. Vasc. Biol.* **2001**, *21*, 1259.
29. Toschi, V.; Gallo, R.; Lettino, M.; Fallon, J. T.; Gertz, S. D.; Fernandez-Ortiz, A.; Chesebro, J. H.; Bradimon, L.; Nemerson, Y.; Fuster, V.; Bradimon, J. J. *Circulation* **1997**, *95*, 594.
30. Bisell, E. R.; Mitchell, A. R.; Smith, R. E. *J. Org. Chem.* **1980**, *45*, 2283.
31. Dzyubenko, M. I.; Vodotyka, G. S.; Maslov, V. V.; Nikitchenko, V. M. *Optikai Spektroskopiya* **1975**, *39*, 310; *Chem. Abstr.* **1975**, *83*, 211135.
32. Kathuria, A.; Jalal, S.; Tiwari, R.; Shirazi, A. N.; Gupta, S.; Kumar, S.; Parang, K.; Sharma, S. K. *Bioorg. Med. Chem.* **2011** (communicated).
33. Yang, S.-P.; Han, L.-J.; Wang, D.-Q.; Ding, T.-Z. *Acta Crystallogr., Sect. E* **2006**, *62*, o5196.
34. Jayashree, B. S.; Thomas, S.; Nayak, Y. *Med. Chem. Res.* **2010**, *19*, 193.
35. Uray, G.; Strohmeier, G. A.; Fabian, W. M. F. *Proceedings of ECSOC-3 and proceedings of ECSOC-4,* September 1–30, **1999** and **2000**, 521; *Chem. Abstr.* **2000**, *134*, 366519.
36. Wallingford, V. H.; Homeyer, A. H. U.S. Patent 2,358,768, 1944; *Chem. Abstr.* **1944**, *39*, 12041.
37. Zhi, L.; Tegley, C.; Pio, B.; Arjan van Oeveren, C.; Motamedi, M.; Martinborough, E.; West, S.; Higuchi, R.; Hamann, L.; Farmer, L. PCT Int. Appl. 2001, 356 pp; *Chem. Abstr.* **2001**, *134*, 207727.
38. Ramesh, P.; Das, A. T.; Mohandass, P.; Nagasathya, R. *Ind. J. Chem.* **2008**, *47B*, 1447.