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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of pyrazole-4-carbaldehyde derivatives for their antifungal activity

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Abstract A series of pyrazole-4-carbaldehyde containing coumarin derivatives (**9a–l**) were achieved starting from substituted 6-hydroxy-4-methyl coumarins in a facial manner by Vilsmeier–Haack Formylation reaction in good yields, and their chemical structures were determined by Fourier transform infrared, ^{1}H, ^{13}C-nuclear magnetic resonance and mass spectroscopic techniques. Compounds **9a–l** were docked into monoamine oxidase from *Aspergillus niger* and shown strong π-stacking interactions with the cage forming amino acids of **Trp430** and **Phe466**, and were also further evaluated against antifungal activity on *Aspergillus niger* by taking Clotrimazole as standard. Out of twelve synthesized compounds **9a**, **9b**, **9g**, and **9h** were showed good and **9c**, **9d**, **9e**, **9i**, **9j**, and **9k** were showed moderate antifungal activity.

Keywords Coumarin · Pyrazole · Vilsmeier–Haack formylation · Antifungal activity · *Aspergillus niger*

Abbreviations

^{1}H-NMR	Proton nuclear magnetic resonance
MAO	Mono amine oxidase
ADMET	Absorption, distribution, metabolism, excretion and toxicity

Introduction

Fungal infections are significant cause of mortality despite of advances in research, in the field of medicinal chemistry. Information incurred from literature it is evident that, Aspergilli is a foremost reason in immune compromised fungal infected patients (Denning et al. 1991; Denning et al. 2003; Denning 1998; Leger et al. 2000; Yu et al. 2005; Morya et al. 2009). Till date more than 600 plants have been reported for their antifungal properties with nitrogen, oxygen containing heterocycles, however many of them are not available therapeutically due to their carcinogenic and mutagenic properties. In spite of such properties five member ring heterocycles shows wide range of applications in medicinal chemistry. This group includes triazole, imidazole, thiadiazole, pyrazole, oxadiazole, thiazole etc. These are particularly most important in the area of antibacterial and antifungal agents. (Foroumadi et al. 2009; Barker 2006; Güzeldemirci and Küçükbasmaci 2010; Olender et al. 2009; Tanitame et al. 2004) Later hardly any of them were explored for their active components. But still functionalized nitrogen or oxygen containing heterocyclic moiety play a predominant role in medicinal chemistry as fungicides like scopoletin (Carpinella et al. 2005) (1) a hydroxyl coumarin, Angelicin (Kokil et al. 2010) (2) furano coumarin and Fenpyrazamine (Xinhua et al. 2008) (3) a pyrazole derivative. Nowadays, it is possible to make

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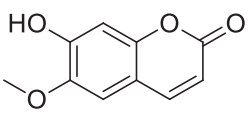
modifications of active chemical structures, in order to synthesize compounds with improved therapeutic activity and reduced toxicity.

Many fungicides contain the *azole* heterocyclic moiety in its chemical structure, which makes a basic structural unit of the drug. Aspergilli species outline a new therapeutic target



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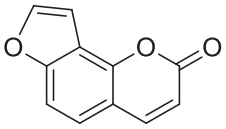

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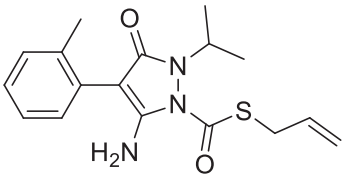

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One of such compound active towards fungal infections is pyrazole and its derivatives (Hameed 2013) pyrazole and substituted pyrazole derivatives are attained considerable interest in the field of medicinal chemistry. Slight changes in their structure offered degree of diversity due to its improved efficiency and less toxicity towards biological targets. Series of novel aryl pyrazoles were synthesized and tested for their activity against bacteria and fungi (Lv et al. 2010; Rahimizadeh et al. 2010; Aragade et al. 2009; Mostafa et al. 2011; Gupta et al. 2010; Pitucha et al. 2010, 2011). Antibacterial or antifungal are wide area covered phrases, include several drug targets in their activity.

From the details provided by (Atkin et al. 2008) it is clear that several regions of mono amine oxidase (MAO-N) protein play an important role, their specificity towards the activity of different amines. Among such sites ASN 336, MET 348, and ILE 246 are important towards the selectivity of chiral amines. In addition to this, it is also apparent that MAO-N shares the sequence similarity of 23 and 22% with human MAO-A and MAO-B, respectively. This sequence similarity is much more prominent at the core region around the flavin adenine dinucleotide (FAD) binding site and substrate active site. Several drug like molecules were identified and synthesized for the inhibition of human MAO-A and B. Such drug like molecules includes coumarins and substituted pyrazole derivatives. The sequence sharing and substrate similarity gave an extra advantage in the selection of MAO-N as drug target. The published information towards the human MAO gives an added advantage to streamline the selection of specific substrate towards MAO-N.

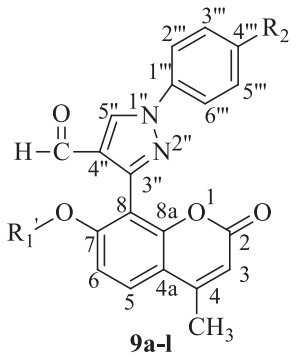
The target is selected based upon the structural requirement and substrate similarity to understand the mode of binding. MAO-N was selected as drug target for molecular docking studies based upon the substrate specificity and the mode of action against aspergillus. In recent years great interest was played for the development of novel pyrazole derivatives, which were found to possess MAO activity. (Fioravanti et al. 2007) due to its mortality rate in immune suppressed patients. As coumarins and pyrazoles comprise an array of biologically active compounds, it is of great interest to synthesize new heterocyclic compounds incorporating pyrazole moiety in coumarins which may exhibit better or different type of biological properties.

Among several targets in *Aspergillus niger* (AN), MAO create a fundamental interest in physiology and medicinal chemistry due to its role in metabolism of neurotransmitter molecules such as serotonin and dopamine. MAOs are flavo proteins which catalyze the transformation of amines into emines then further hydrolyzed to give aldehyde and ketone (Schilling and Lerch 1995). MAO-N shows closest structural and sequence similarity with human MAO's (Atkin et al. 2008). Because of their physiological and medicinal interest we have under taken synthesis, molecular modeling and in vitro biological activity of pyrazole-4-carbaldehyde containing coumarin derivatives for the inhibition of AN.

Experimental protocols

The crystal structure of MAO-N (PDB id: 2VVL) (Atkin et al. 2008) was downloaded from the protein data bank and Glide 5.6 (Glide 2005) was used for molecular modeling studies. The protein was preprocessed by applying missing bond orders in substrate structure and was energy minimized using protein preparation wizard. Energy minimization was terminated at the RMSD convergence of 0.30 Å. The grid was generated around the active site of enzyme by defining centroid of active site amino acids around the hydrophobic binding pocket. Receptor Vander Waals scaling for non polar atoms was set to 0.9 (Friesner et al. 2004) to make active site roomier. Ligands (Table 1) were built using maestro build panel and *LigPrep* application in Schrödinger was used to generate all possible protonation states at physiological pH of 7 ± 2. Ionizer in Liprep was used to generate all possible tautomers of the ligands. The low energy conformers of the ligands were produced with LigPrep using the MMFF94s force field (Halgren 1999). Ligands were

Table 1 Structural details and ADMET properties and dock scores of the molecules used in this study



9a-l

Mol	R_1	R_2	Dock score	Energy	MW	Donor	Acceptor	Log*P*	Log*S*	Zone of inhibition (mm)/PIDG (%)
9a	H	H	−9.69	−48.01	346.34	1	6.25	2.62	−4.86	46/43.75
9b	CH_3	H	−9.28	−45.85	360.37	0	6.25	3.04	−4.56	38/18.75
9c	C_2H_5	H	−9.55	−46.20	374.40	0	6.25	3.48	−5.13	30/−6.25
9d	C_3H_7	H	−9.59	−47.77	388.42	0	6.25	3.87	−5.55	30/−6.25
9e	C_4H_9	H	−10.19	−49.84	402.45	0	6.25	4.02	−5.17	30/−6.25
9f	C_5H_{11}	H	−7.67	−38.21	416.48	0	6.25	4.68	−6.29	22/−31.25
9g	H	OCH_3	−9.30	−48.97	376.37	1	7.00	2.71	−5.07	46/43.75
9h	CH_3	OCH_3	−8.54	−48.23	390.40	0	7.00	3.09	−4.71	36/12.50
9i	C_2H_5	OCH_3	−8.27	−46.63	404.42	0	7.00	3.54	−5.28	30/−6.25
9j	C_3H_7	OCH_3	−8.07	−45.61	418.45	0	7.00	3.93	−5.70	30/−6.25
9k	C_4H_9	OCH_3	−8.06	−46.95	432.48	0.00	7.00	4.32	−6.16	28/−12.50
9l	C_5H_{11}	OCH_3	−6.99	−30.45	446.50	0.00	7.00	4.72	−6.62	20/−37.50
Clotrimazole 1	–	–	−6.79	−26.72	344.84	0	1.50	5.40	−5.94	32
Actidione 1	–	–	−6.80	−32.71	281.35	1	5.70	1.44	−2.97	–

checked for their ADMET properties using QikProp application in Schrödinger suite. ADMET properties of molecules were (Table 1) observed in acceptable range of Lipinski rule of five, a required condition for drug likeness of newly synthesized molecules. These conformations were selected and docked into the grid generated using the standard precision docking mode (Halgren 1999). The ligands were docked into the active site flexibly with pyramidal nitrogen inversions and sample ring conformations. Each molecule was checked with at most of 1000 poses during docking run and the best was selected based upon the position and interaction with the active site. Post docking minimization was applied on the best pose to further enhance in the mode of binding and results were analyzed.

Chemistry

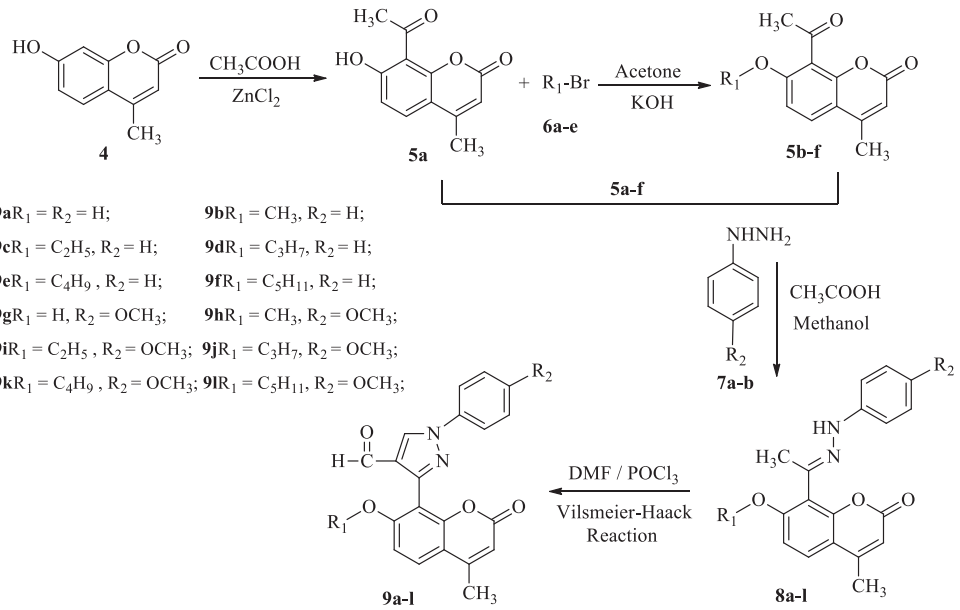
Instruments

Thin layer chromatographic (TLC) analysis was performed on pre-coated aluminum plates of silica gel 60 F_{254} MEARK. The infrared (IR) spectra of samples were recorded in the range of 4000–400 cm^{-1} by Bruker Optics, Germany TENSOR 27. ^{1}H-NMR spectra (400 MHz) and ^{13}C-NMR spectra (100 MHz) were recorded in chloroform ($CHCl_3$), dimethyl sulfoxide by employing tetramethylsilane as an internal standard on AV-III 400 MHz Spectrometer (Bruker, France). Melting points were measured in open capillary tubes on a Polmon Apparatus-90 melting point device. Scheme 1.

General procedure for synthesis

7-hydroxy-4-methyl-2H-chromen-2-one (**4**) was prepared (Desai and Mavani 1942) by the condensation of ethylacetoacetate and resorcinol in presence of con.H_2SO_4.

8-Acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one (**5a**) 7-Hydroxy-4-methyl-2H-chromen-2-one (**4**) (1 g, 0.005 mmol) was dissolved in 10 ml glacial acetic acid, and refluxed for 3 h in presence of fussed Zinc chloride (1.6 g), to yield a light cream color solid which was purified



Scheme 1 Synthesis of 3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(9a-l)**

on column chromatography to get 8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one (**5a**) (Sheikh et al. 2010).

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.67 (d, $J = 8.0$ Hz, 5-H), 6.91 (d, $J = 8.0$ Hz, 6-H), 6.17 (d, $J = 1.2$ Hz, 3-H), 2.97 (s, 2′-CH_3), 2.42 (d, $J = 1.2$ Hz, 4-CH_3).

8-Acetyl-7-methoxy-4-methyl-2*H*-chromen-2-one (**5b**) 8-Acetyl-7-hydroxy-4-methyl-2H-chromen-2-one (**5a**) (1 g, 0.004 mmol) and methyl bromide (**6a**) (0.4 ml) were dissolved in dry acetone (25 ml) and refluxed over anhydrous potassium carbonate for 3 h on water bath. The completion of the reaction was monitored by TLC. The acetone was removed under reduced pressure and crushed ice was added to the residue. The separated solid was filtered and washed with water to get 8-acetyl-7-methoxy-4-methyl-2H-chromen-2-one (**5b**) (El-Zahar and Abd 2009).

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.56 (d, $J = 8.0$ Hz, 5-H), 6.88 (d, $J = 8.0$ Hz, 6-H), 6.15 (d, $J = 1.2$ Hz, 3-H), 3.91 (s, OCH_3), 2.60 (s, 2″-CH_3), 2.40 (d, $J = 1.2$ Hz, 4-CH_3).

7-Hydroxy-4-methyl-8-(1-(2-phenylhydrazono) ethyl)-2*H*-chromen-2-one (**8a**) A mixture of 8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one (**5a**) (1 g, 0.004 mmol) and phenylhydrazine (**7a**) (0.5 ml, 0.004 mmol) in methanol (30 ml) containing few drops of glacial acetic acid was refluxed for 1 h on a water bath. Methanol was removed under reduced pressure and was added ice cold water. The separated solid was filtered, washed with water and dried. Then the solid was subjected to column chromatography and eluted with ethyl acetate and hexane (2:8) to get 7-hydroxy-4-methyl-8-(1-(2-phenylhydrazono) ethyl)-2H-chromen-2-one (**8a**).

IR (KBr): υ 3336 (N–H), 3100 (O–H), 1735 (C=O of coumarin), 1602 (C=C of coumarin) cm^{-1}

^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 12.07 (s, NH), 7.45 (d, $J = 8.0$ Hz, 5-H), 7.30–7.34 (m, 2″-H, 6″-H), 7.06 (d, $J = 8.0$ Hz, 6-H), 6.94–6.97 (m, 3″-H, 4″-H, 5″-H), 6.13 (d, $J = 1.2$ Hz, 3-H), 2.58 (s, 2′-CH_3), 2.41 (d, $J = 1.2$ Hz, 4-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 161.0 (C-1′), 160.1 (C-2), 153.3 (C-7), 152.6 (C-4), 143.6 (C-8a), 129.6 (C-3″, C-5″), 125.3 (C-1″), 121.4 (C-5), 113.8 (C-4″), 113.2 (C-2″, C-6″), 112.8 (C-8), 110.9 (C-4a), 110.7 (C-3), 110.5 (C-6), 19.1 (C-2′) and 17.0 (C-4-CH_3). Mass (ES): *m/z* 309 $[M + H]^+$. M.P: 190 °C, yield: 92%.

7-Methoxy-4-methyl-8-(1-(2-phenylhydrazono) ethyl)-2*H*-chromen-2-one (**8b**) IR (KBr): υ 3342 (N–H), 1741 (C=O of coumarin), 1613 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 12.04 (s, NH), 7.51 (d, $J = 8.2$ Hz, 5-H), 7.34–7.39 (m, 2‴-H, 6‴-H), 7.12 (d, $J = 8.2$ Hz, 6-H), 6.96–7.08 (m, 3‴-H, 4‴-H, 5‴-H), 6.20 (s, 3-H), 4.01 (s, 1′-CH_3) 2.62 (s, 2″-CH_3), 2.28 (s, 4-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 166.3 (C-1′), 160.9 (C-2), 159.4 (C-7), 153.8 (C-4), 152.1 (C-8a), 144.4 (C-1‴), 129.9 (C-3‴, C-5‴), 125.9 (C-5), 121.7 (C-4‴), 113.7 (C-2‴, C-6‴), 112.4 (C-8), 112.0 (C-4a), 110.4 (C-3), 109.4 (C-6), 53.1 (C-1′), 19.7 (C-2″)and 16.0 (C-4-CH3). Mass (ES): *m/z* 323 $[M + H]^+$. M.P: 187 °C, yield: 91%.

7-Ethoxy-4-methyl-8-(1-(2-phenylhydrazono)ethyl)-2*H*-chromen-2-one (**8c**) IR (KBr): υ 3338 (N–H), 1739 (C=O of coumarin), 1589 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 12.02 (s, NH), 7.49 (d, $J = 8.0$ Hz, 5-H), 7.32–7.37 (m, 2‴-H, 6‴-H), 7.11 (d, $J = 8.0$ Hz, 6-H), 6.95–7.10 (m, 3‴-H, 4‴-H, 5‴-H), 6.18 (d, $J = 1.2$ Hz,

3-H), 4.13 (q, $J = 7.2$ Hz, 1′-CH_3) 2.60 (s, 2″-CH_3), 2.31 (d, $J = 1.2$ Hz, 4-CH_3), 2.16 (t, $J = 7.2$ Hz, 2′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 165.9 (C-1″), 159.7 (C-2), 155.9 (C-7), 153.2 (C-4), 149.7 (C-8a), 143.9 (C-1‴), 128.7 (C-3‴, C-5‴), 125.1 (C-5), 122.1 (C-4‴), 113.9 (C-8), 112.9 (C-6), 112.7 (C-2‴, C-6‴), 110.6 (C-4a), 109.0 (C-3), 63.7 (C-1′), 20.1 (C-2″), 16.8 (C-4-CH_3) and 15.5 (C-2′). Mass (ES): *m/z* 337 $[M + H]^+$. M.P: 182 °C, yield: 91%.

4-Methyl-8-(1-(2-phenylhydrazono)ethyl)-7-propoxy-2*H*-chromen-2-one (**8d**) IR (KBr): υ 3328 (N–H), 1737 (C=O of coumarin), 1594 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 12.23 (s, NH), 7.42 (d, $J = 8.2$ Hz, 5-H), 7.31–7.37 (m, 2‴-H, 6‴-H), 7.14 (d, $J = 8.2$ Hz, 6-H), 6.98–7.12 (m, 3‴-H, 4‴-H, 5‴-H), 6.11 (s, 3-H), 4.34 (t, $J = 7.2$ Hz, 1′-CH_2) 2.61 (s, 2″-CH_3), 2.43 (s, 4-CH_3), 2.06 (m, 2′-CH_2), 1.90 (t, $J = 7.2$ Hz, 3′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 165.4 (C-1″), 160.7 (C-2), 159.5 (C-7), 153.7 (C-4), 149.2 (C-8a), 144.7 (C-1‴), 129.4 (C-3‴, C-5‴), 125.4 (C-5), 122.4 (C-4‴), 114.2 (C-8), 113.1 (C-6), 112.9 (C-2‴, C-6‴), 110.7 (C-4a), 109.3 (C-3), 64.2 (C-1′), 24.6 (C-2″), 20.8 (C-4-CH_3), 16.9 (C-2′) and 16.2 (C-3′). Mass (ES): *m/z* 351 $[M + H]^+$. M.P:168 °C, yield: 92%.

7-Butoxy-4-methyl-8-(1-(2-phenylhydrazono) ethyl)-2*H*-chromen-2-one (**8e**) IR (KBr): υ 3347 (N–H), 1712 (C=O of coumarin), 1582 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 13.01 (s, NH), 7.52 (d, $J = 8.2$ Hz, 5-H), 7.38–7.44 (m, 2‴-H, 6‴-H), 7.00 (d, $J = 8.2$ Hz, 6-H), 6.56–6.66 (m, 3‴-H, 4‴-H, 5‴-H), 6.24 (d, $J = 1.2$ Hz, 3-H), 4.41 (t, $J = 7.4$ Hz, 1′-CH_2) 2.77 (s, 2″-CH_3), 2.43 (d, $J = 1.2$ Hz, 4-CH_3), 2.02–2.09 (m, 2′-CH_2), 1.94–1.98 (m, 3′-CH_2), 1.20 (t, $J = 7.4$ Hz, 4′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 166.2 (C-1″), 161.2 (C-2), 159.8 (C-7), 153.3 (C-4), 151.2 (C-8a), 144.1 (C-1‴), 129.6 (C-3‴, C-5‴), 124.7 (C-5), 121.8 (C-4‴), 114.7 (C-8), 112.7 (C-6), 112.1 (C-2‴, C-6‴), 110.9 (C-4a), 110.1 (C-3), 64.9 (C-1′), 30.8 (C-2″), 21.4 (C-4-CH_3), 17.2 (C-2′), 16.8 (C-3′) and 14.9 (C-4′). Mass (ES): *m/z* 365 $[M + H]^+$. M.P:162 °C, yield: 93%.

4-Methyl-7-(pentyloxy)-8-(1-(2-phenylhydrazono) ethyl)-2*H*-chromen-2-one (**8f**) IR (KBr): υ 3350 (N–H), 1748 (C=O of coumarin), 1612 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 13.60 (s, NH), 7.12 (d, $J = 8.0$ Hz, 5-H), 6.94–6.99 (m, 2‴-H, 6‴-H), 6.72 (d, $J = 8.0$ Hz, 6-H), 6.12–6.18 (m, 3‴-H, 4‴-H, 5‴-H), 6.04 (s, 3-H), 4.25 (t, $J = 7.8$ Hz, 1′-CH_2) 2.82 (s, 2″-CH_3), 2.34 (s, 4-CH_3), 1.79–1.83 (m, 2′-CH_2), 1.33–1.43 (m, 3′-CH_2, 4′-CH_2), 1.01 (t, $J = 7.8$ Hz, 5′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 166.4 (C-1″), 160.2 (C-2), 158.7 (C-7), 153.4 (C-4), 151.9 (C-8a), 143.9 (C-1‴), 128.4 (C-3‴, C-5‴), 124.3 (C-5), 121.4 (C-4‴), 113.2 (C-8), 112.0 (C-6), 111.8 (C-2‴, C-6‴), 110.7 (C-4a), 109.3 (C-3), 65.4 (C-1′), 32.0 (C-2″), 27.7 (C-4-CH_3), 23.1 (C-2′), 19.3 (C-3′), 17.1 (C-4′)and 14.0 (C-5′). Mass (ES): *m/z* 379 $[M + H]^+$. M.P:147 °C, yield: 91%.

7-Hydroxy-8-(1-(2-(4-methoxyphenyl) hydrazono) ethyl)-4-methyl-2*H*-chromen-2-one (**8g**) IR (KBr): υ 3336 (N–H), 3102 (O–H), 1737 (C=O of coumarin), 1605 (C=C of coumarin) cm^{-1} ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 12.80 (s, NH), 7.66 (d, $J = 8.2$ Hz, 5-H), 7.44–7.50 (m, 2″-H, 6″-H), 7.22 (d, $J = 8.2$ Hz, 6-H), 6.99–7.12 (m, 3″-H, 5″-H), 6.23 (s, 3-H), 4.07 (s, 4″-OCH_3), 2.62 (s, 2′-CH_3), 2.32 (s, 4-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 167.2 (C-1′), 160.8 (C-2), 158.4 (C-7), 153.0 (C-4″), 151.2 (C-4), 149.7 (C-8a), 136.0 (C-1″), 132.1 (C-5), 119.7 (C-2″, C-6″), 117.0 (C-8), 116.2 (C-3″, C-5″), 114.7 (C-6), 113.1 (C-4a), 112.4 (C-3), 54.8 (C-4″-OCH_3), 22.2 (C-2′)and 16.3 (C-4-CH_3). Mass (ES): *m/z* 339 $[M + H]^+$. M.P: 200–202 °C, yield: 86%.

7-Methoxy-8-(1-(2-(4-methoxyphenyl) hydrazono) ethyl)-4-methyl-2*H*-chromen-2-one (**8h**) IR (KBr): υ 3341 (N-H), 1744 (C=O of coumarin), 1603 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 13.42 (s, NH), 7.41 (d, $J = 8.2$ Hz, 5-H), 7.31–7.37 (m, 2‴-H, 6‴-H), 7.26 (d, $J = 8.2$ Hz, 6-H), 6.98–7.11 (m, 3‴-H, 5‴-H), 6.22 (s, 3-H), 4.04 (s, 1′-CH_3), 3.85 (s, 4‴-OCH_3), 2.49 (s, 2″-CH_3), 2.27 (s, 4-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 166.1 (C-1″), 161.3 (C-2), 158.2 (C-7), 153.9 (C-4‴), 150.5 (C-4), 149.1 (C-8a), 135.8 (C-1‴), 132.7 (C-5), 119.4 (C-2‴, C-6‴), 117.2 (C-8), 116.7 (C-3‴, C-5‴), 114.5 (C-6), 113.4 (C-4a), 112.6 (C-3), 55.5 (C-1′), 55.3 (C-4‴-OCH_3), 20.8 (C-2″) and 17.2 (C-4-CH_3). Mass (ES): *m/z* 353 $[M + H]^+$. M.P:192 °C, yield: 86%.

7-Ethoxy-8-(1-(2-(4-methoxyphenyl) hydrazono) ethyl)-4-methyl-2*H*-chromen-2-one (**8i**) IR (KBr): υ 3334 (N–H), 1750 (C=O of coumarin), 1607 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 13.68 (s, NH), 7.42–7.52 (m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.20 (d, $J = 0.8$ Hz, 3-H), 4.14 (q, $J = 7.6$ Hz, 1′-CH_2), 3.79 (s, 4‴-OCH_3), 2.88 (s, 2″-CH_3), 2.00 (d, $J = 0.8$ Hz, 4-CH_3), 1.77 (t, $J = 7.6$ Hz, 2′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 167.7 (C-1″), 161.7 (C-2), 157.9 (C-7), 153.7 (C-4‴), 150.9 (C-4), 148.7 (C-8a), 135.3 (C-1‴), 132.1 (C-5), 119.7 (C-2‴, C-6‴), 117.3 (C-8), 116.4 (C-3‴, C-5‴), 114.2 (C-6), 113.2 (C-4a), 112.4 (C-3), 65.2 (C-1′), 54.6 (C-4‴-OCH_3), 20.2 (C-2″), 17.8 (C-4-CH_3) and 17.1 (C-2′). Mass (ES): *m/z* 367 $[M+H]^+$. M.P:177 °C, Yield: 89%.

8-(1-(2-(4-methoxyphenyl) hydrazono) ethyl)-4-methyl-7-propoxy-2H-chromen-2-one (**8j**) IR (KBr): υ 3329 (N–H), 1718 (C=O of coumarin), 1588 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 12.45 (s, NH), 7.27–7.39

(m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.22 (s, 3-H), 4.18 (t, $J = 7.6$ Hz, 1′-CH_2), 3.83 (s, 4‴-OCH_3), 2.69 (s, 2″-CH_3), 2.20 (s, 4-CH_3), 1.90–1.97 (m, 2′-CH_3), 1.52 (t, $J =$ 7.6 Hz, 3′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 167.1 (C-1″), 160.4 (C-2), 157.2 (C-7), 154.1 (C-4‴), 151.4 (C-4), 149.5 (C-8a), 134.7 (C-1‴), 132.0 (C-5), 120.1 (C-2‴, C-6‴), 117.6 (C-8), 116.8 (C-3‴, C-5‴), 115.1 (C-6), 113.2 (C-4a), 111.7 (C-3), 68.8 (C-1′), 55.2 (C-4‴-OCH_3), 21.5 (C-2″), 20.0 (C-4-CH_3), 17.2 (C-2′) and 16.8 (C-3′). Mass (ES): *m/z* 381 $[M+H]^+$. M.P: 170 °C, Yield: 88%.

7-butoxy-8-(1-(2-(4-methoxyphenyl) hydrazono) ethyl)-4-methyl-2H-chromen-2-one (**8k**) IR (KBr): υ 3332 (N–H), 1722 (C=O of coumarin), 1594 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 13.07 (s, NH), 7.19–7.29 (m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.21 (s, 3-H), 4.09 (t, $J = 7.2$ Hz, 1′-CH_2), 3.99 (s, 4‴-OCH_3), 2.71 (s, 2″-CH_3), 2.30 (s, 4-CH_3), 1.97–2.05 (m, 2′-CH_2), 1.88–1.93 (m, 3′-CH_2), 1.11 (t, $J = 7.2$ Hz, 4′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 166.9 (C-1″), 161.0 (C-2), 158.8 (C-7), 153.5 (C-4‴), 151.0 (C-4), 148.8 (C-8a), 135.2 (C-1‴), 132.5 (C-5), 120.0 (C-2‴, C-6‴), 116.8 (C-8), 116.0 (C-3‴, C-5‴), 114.9 (C-6), 113.2 (C-4a), 111.3 (C-3), 70.7 (C-1′), 54.8 (C-4‴-OCH_3), 29.6 (C-2″), 21.7 (C-4-CH_3), 18.9 (C-2′), 17.4 (C-3′) and 16.5 (C-4′). Mass (ES): *m/z* 395 $[M + H]^+$. M.P:166 °C, yield: 88%.

8-(1-(2-(4-Methoxyphenyl) hydrazono) ethyl)-4-methyl-7-(pentyloxy)-2*H*-chromen-2-one (**8l**) IR (KBr): υ 3348 (N–H), 1751 (C=O of coumarin), 1604 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 13.04 (s, NH), 7.15–7.27 (m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.20 (s, 3-H), 4.11 (t, $J = 7.6$ Hz, 1′-CH_2), 3.82 (s, 4‴-OCH_3), 2.89 (s, 2″-CH_3), 2.41 (s, 4-CH_3), 1.94–2.01 (m, 2′-CH_2), 1.66–1.72 (m, 3′-CH_2, 4′-CH_2), 0.98 (t, $J = 7.6$ Hz, 5′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 166.5 (C-1″), 160.2 (C-2), 157.3 (C-7), 153.2 (C-4‴), 151.7 (C-4), 147.6 (C-8a), 134.9 (C-1‴), 130.1 (C-5), 120.8 (C-2‴, C-6‴), 116.8 (C-8), 116.2 (C-3‴, C-5‴), 113.8 (C-6), 113.0 (C-4a), 110.6 (C-3), 71.0 (C-1′), 56.7 (C-4‴-OCH_3), 32.3 (C-2″), 30.1 (C-4-CH_3), 24.7 (C-2′), 20.8 (C-3′), 16.2 (C-4′) and 16.0 (C-5′). Mass (ES): *m/z* 409 $[M + H]^+$. M.P: 161 °C, yield: 90%.

3-(7-Hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**9a**) Dry *N*,*N*-dimethylformamide (4 ml) was cooled to 0 °C in a round bottom flask and $POCl_3$ (3 ml) was added slowly under stirring over 15–20 min, and stirring was continued for another 15 min at the same temperature. To this mixture 7-hydroxy-4-methyl-8-(1-(2-phenylhydrazono)ethyl)-2H-chromen-2-one (**8a**) (0.5 g; 0.001 mmol) was added as solid directly in small aliquots at a time, remove the ice bath, the resulting reaction mixture was, stirred at room temperature for 6 h, completion of reaction was monitored by TLC and poured into ice cold water. The solid separated on neutralization with $NaHCO_3$ was filtered, washed with water and purified on column chromatography with ethyl acetate: pet ether (15:85) to obtain 3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**9a**).

IR (KBr): υ 3150 (O–H), 2780 (C–H of aldehyde), 1724 (C=O of aldehyde), 1690 (C=O of coumarin), 1597 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 10.02 (s, CHO), 8.13 (s, 5′-H), 7.73 (d, $J = 8.0$ Hz, 5-H), 7.61–7.63 (m, 2″-H, 6″-H), 7.54–7.58 (m, 3″-H, 4″-H, 5″-H), 7.45 (d, $J = 8.0$ Hz, 6-H), 6.18 (s, 3-H), 2.47 (s, 4-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 187.0 (CHO), 160.7 (C-2), 158.6 (C-7), 152.5 (C-4), 148.5 (C-3′), 132.8 (C-8a), 128.9 (C-1″), 124.7 (C-5′), 121.0 (C-3″, C-5″), 116.6 (C-8), 116.0 (C-2″, C-6″), 115.6 (C-5), 113.1 (C-4″), 112.4 (C-4a), 110.1 (C-3), 106.5 (C-4′), 105.2 (C-6) and 19.2 (C-4-CH_3). Mass (ES): *m/z* 347 $[M + H]^+$. Anal. calcd for: C, 69.36; H, 4.07; N, 8.09%. Found: C, 69.03; H, 3.88; N, 8.40 %. M. P:168 °C, yield: 72%.

3-(7-Methoxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**9b**) IR (KBr): υ 2777 (C–H of aldehyde), 1731 (C=O of aldehyde), 1698 (C=O of coumarin), 1602 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 10.00 (s, CHO), 8.24 (s, 5″-H), 7.68 (d, $J = 8.2$ Hz, 5-H), 7.62–7.66 (m, 2‴-H, 6‴-H), 7.54–7.60 (m, 3‴-H, 4‴-H, 5‴-H), 7.47 (d, $J = 8.2$ Hz, 6-H), 6.24 (s, 3-H), 3.94 (s, 1′-CH_3), 2.39 (s, 4-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 189.8 (CHO), 159.6 (C-2), 157.4 (C-7), 150.7 (C-4), 149.2 (C-3″), 146.0 (C-8a), 138.2 (C-1‴), 130.8 (C-5″), 128.2 (C-3‴, C-5‴), 127.6 (C-8), 124.2 (C-2‴, C-6‴), 118.5 (C-5), 112.9 (C-4‴), 112.1 (C-4a), 110.8 (C-3), 105.7 (C-4″), 101.3 (C-6), 55.4 (C-1′) and 18.1 (C-4-CH_3). Mass (ES): *m/z* 361 $[M + H]^+$. Anal. calcd for: C, 69.99; H, 4.48; N, 7.77%. Found: C, 69.73; H, 4.32; N, 8.06%. M.P: 162 °C, yield: 72%.

3-(7-Ethoxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**9c**) IR (KBr): υ 2748 (C–H of aldehyde), 1718 (C=O of aldehyde), 1682 (C=O of coumarin), 1585 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 9.95 (s, CHO), 8.80 (s, 5″-H), 7.69 (d, $J = 8.4$ Hz, 5-H), 7.59–7.63 (m, 2‴-H, 6‴-H), 7.52–7.57 (m, 3‴-H, 4‴-H, 5‴-H), 7.48 (d, $J = 8.4$ Hz, 6-H), 6.21 (s, 3-H), 4.01 (q, $J = 7.6$ Hz, 1′-CH_2) 2.63 (s, 4-CH_3), 2.02 (t, $J =$ 7.6 Hz, 2′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 190.0 (CHO), 159.3 (C-2), 157.9 (C-7), 150.4 (C-4), 149.9 (C-3″), 146.2 (C-8a), 138.6 (C-1‴), 130.2 (C-5″), 127.9 (C-3‴, C-5‴), 126.3 (C-8), 124.8 (C-2‴, C-6‴), 118.9 (C-5), 113.2 (C-4‴), 112.8 (C-4a), 110.2 (C-3), 105.1 (C-4″), 101.9 (C-6), 62.2 (C-1'), 18.4 (C-4-CH_3) and 16.3 (C-2′). Mass (ES): *m/z* 375 $[M + H]^+$. Anal. calcd for: C, 70.58; H, 4.85; N,

7.48%. Found: C, 70.24; H, 4.72; N, 7.71%. M.P:157 °C, yield: 74%.

3-(4-Methyl-2-oxo-7-propoxy-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**9d**) IR (KBr): υ 2754 (C–H of aldehyde), 1714 (C=O of aldehyde), 1677 (C=O of coumarin), 1584 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 10.10 (s, CHO), 8.44 (s, 5″-H), 7.69 (d, $J = 7.8$ Hz, 5-H), 7.64–7.67 (m, 2‴-H, 6‴-H), 7.55–7.59 (m, 3‴-H, 4‴-H, 5‴-H), 7.42 (d, $J = 7.8$ Hz, 6-H), 6.10 (d, J = 0.8 Hz, 3-H), 4.33 (t, $J = 7.8$ Hz, 1′-CH_2), 2.41 (d, $J = 0.8$ Hz, 4-CH_3), 2.02 (m, 2′-CH_2), 1.83 (t, J = 7.8 Hz, 3′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 191.2 (CHO), 160.2 (C-2), 158.1 (C-7), 150.9 (C-4), 150.0 (C-3″), 146.9 (C-8a), 138.9 (C-1‴), 131.0 (C-5″), 127.6 (C-3‴,C-5‴), 126.5 (C-8), 124.9 (C-2‴, C-6‴), 118.1 (C-5), 113.1 (C-4‴), 112.2 (C-4a), 110.7 (C-3), 105.9 (C-4″), 102.2 (C-6), 64.9 (C-1′), 23.0 (C-4-CH_3), 19.2 (C-2′) and 14.6 (C-3′). Mass (ES): *m/z* 389 $[M + H]^+$. Anal. calcd for: C, 71.12; H, 5.19; N, 7.21%. Found: C, 70.85; H, 4.86; N, 7.39%. M.P:154 °C, yield: 78%.

3-(7-Butoxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**9e**) IR (KBr): υ 2762 (C–H of aldehyde), 1737 (C=O of aldehyde), 1699 (C=O of coumarin), 1593 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 9.89 (s, CHO), 8.68 (s, 5″-H), 7.63 (d, $J = 8.0$ Hz, 5-H), 7.58–7.61 (m, 2‴-H, 6‴-H), 7.54–7.57 (m, 3‴-H, 4‴-H, 5‴-H), 7.52 (d, $J = 8.0$ Hz, 6-H), 6.27 (s, 3-H), 4.39 (t, $J = 7.2$ Hz, 1′-CH_2) 2.40 (s, 4-CH_3), 1.95–1.97 (m, 2′-CH_2), 1.64–1.69 (m, 3′-CH_2), 1.16 (t, $J = 7.2$ Hz, 4′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 192.0 (CHO), 161.4 (C-2), 158.6 (C-7), 151.2 (C-4), 149.4 (C-3″), 146.4 (C-8a), 139.1 (C-1‴), 130.2 (C-5″), 128.1 (C-3‴, C-5‴), 126.9 (C-8), 124.2 (C-2‴, C-6‴), 118.3 (C-5), 112.8 (C-4‴), 111.0 (C-4a), 109.7 (C-3), 106.2 (C-4″), 102.1 (C-6), 65.8 (C-1′), 32.3 (C-4-CH_3), 20.1 (C-2′), 18.6 (C-3′) and 14.0 (C-4′). Mass (ES): *m/z* 403 $[M + H]^+$. Anal. calcd for: C, 71.63; H, 5.51; N, 6.96%. Found: C, 71.38; H, 5.16; N, 7.31%. M.P:148 °C, yield: 78%.

3-(4-Methyl-2-oxo-7-(pentyloxy)-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**9f**) IR (KBr): υ 2794 (C–H of aldehyde), 1743 (C=O of aldehyde), 1684 (C=O of coumarin), 1576 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 9.63 (s, CHO), 8.26 (s, 5″-H), 7.71 (d, $J = 8.2$ Hz, 5-H), 7.65–7.69 (m, 2‴-H, 6‴-H), 7.57–7.61 (m, 3‴-H, 4‴-H, 5‴-H), 7.55 (d, $J = 8.2$ Hz, 6-H), 6.20 (s, 3-H), 4.26 (t, $J = 7.8$ Hz, 1′-CH_2) 2.38 (s, 4-CH_3), 1.81–1.91 (m, 2′-CH_2), 1.34–1.43 (m, 3′-CH_2, 4′-CH_2), 0.92 (t, $J = 7.8$ Hz, 5′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 189.6 (CHO), 159.2 (C-2), 157.3 (C-7), 150.3 (C-4), 148.7 (C-3″), 145.8 (C-8a), 138.4 (C-1‴), 131.2 (C-5″), 128.7 (C-3‴, C-5‴), 126.5 (C-8), 124.0 (C-2‴, C-6‴), 117.9 (C-5), 113.1 (C-4‴), 111.8 (C-4a), 109.7 (C-3), 106.0 (C-4″), 101.4 (C-6), 66.1 (C-1′), 31.4 (C-4-CH_3), 28.9 (C-2′), 22.4 (C-3′), 20.4 (C-4′) and 13.8 (C-5′). Mass (ES): *m/z* 417 $[M + H]^+$. Anal. calcd for: C, 72.10; H, 5.81; N, 6.73%. Found: C, 71.88; H, 5.50; N, 7.13%. M.P:146 °C, yield: 82%.

3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde (**9g**) IR (KBr): υ 3175 (O–H), 2788 (C–H of aldehyde), 1724 (C=O of aldehyde), 1674 (C=O of coumarin), 1588 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 10.21 (s, CHO), 8.66 (s, 5′-H), 7.14–7.26 (m, 5-H, 6-H, 2″-H, 6″-H, 3″-H, 5″-H), 6.22 (d, $J = 0.8$ Hz, 3-H), 3.92 (s, 4″-OCH_3), 2.60 (d, $J = 0.8$ Hz, 4-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 190.2 (CHO), 161.0 (C-2), 160.2 (C-7), 158.7 (C-4″), 153.4 (C-4), 152.3 (C-3′), 147.6 (C-8a), 132.7 (C-1″), 130.5 (C-5′), 127.7 (C-5), 124.7 (C-8), 120.8 (C-3″, C-5″), 115.3 (C-4a), 114.7 (C-3), 112.5 (C-4′), 110.8 (C-2″, C-6″), 105.2 (C-6), 55.6 (C-4″-OCH_3) and 19.1 (C-4-CH_3). Mass (ES): *m/z* 377 $[M + H]^+$. Anal. calcd for: C, 67.02; H, 4.28; N, 7.44%. Found: C, 66.68; H, 4.16; N, 7.62%. M.P:180 °C, yield: 68%.

3-(7-Methoxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde (**9h**) IR (KBr): υ 2782 (C–H of aldehyde), 1735 (C=O of aldehyde), 1698 (C=O of coumarin), 1597 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 9.88 (s, CHO), 8.46 (s, 5″-H), 7.18–7.27 (m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.24 (s, 3-H), 4.03 (s, 1′-CH_3), 3.74 (s, 4‴-OCH_3), 2.12 (s, 4-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 190.3 (CHO), 161.2 (C-2), 160.4 (C-7), 158.4 (C-4‴), 153.9 (C-4), 151.7 (C-3″), 146.9 (C-8a), 133.0 (C-1‴), 130.6 (C-5″), 127.2 (C-5), 124.3 (C-8), 120.0 (C-3‴, C-5‴), 115.8 (C-4a), 114.2 (C-3), 112.5 (C-4″), 110.2 (C-2‴, C-6‴), 105.4 (C-6), 55.8 (C-1′), 52.7 (C-4‴-OCH_3) and 18.8 (C-4-CH_3). Mass (ES): *m/z* 391 $[M + H]^+$. Anal. calcd for: C, 67.69; H, 4.65; N, 7.18%. Found: C, 67.44; H, 4.49; N, 7.52%. M.P:181 °C, yield: 68%.

3-(7-Ethoxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde (**9i**) IR (KBr): υ 2793 (C–H of aldehyde), 1743 (C=O of aldehyde), 1678 (C=O of coumarin), 1594 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 10.06 (s, CHO), 8.62 (s, 5″-H), 7.21–7.34 (m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.27 (d, $J = 1.2$ Hz, 3-H), 4.07 (q, $J = 7.2$ Hz, 1′-CH_2), 3.84 (s, 4‴-OCH_3), 1.99 (d, $J = 1.2$ Hz, 4-CH_3), 1.76 (t, $J = 7.2$ Hz, 2′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 189.9 (CHO), 161.7 (C-2), 160.8 (C-7), 158.1 (C-4‴), 153.9 (C-4), 151.4 (C-3″), 146.7 (C-8a), 132.9 (C-1‴), 130.4 (C-5″), 127.3 (C-5), 124.1 (C-8), 120.7 (C-3‴, C-5‴), 115.4 (C-4a), 114.4 (C-3),

113.1 (C-4″), 110.4 (C-2‴, C-6‴), 104.9 (C-6), 64.5 (C-1′), 56.1 (C-4‴-OCH_3), 19.4 (C-4-CH_3) and 16.2 (C-2′). Mass (ES): *m/z* 405 $[M + H]^+$. Anal. calcd for: C, 68.31; H, 4.98; N, 6.93%. Found: C, 68.07; H, 4.81; N, 7.24%. M.P: 172 °C, yield: 71%.

1-(4-Methoxyphenyl)-3-(4-methyl-2-oxo-7-propoxy-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehyde (**9j**) IR (KBr): *υ* 2785 (C–H of aldehyde), 1741 (C=O of aldehyde), 1691 (C=O of coumarin), 1592 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): *δ* 10.11 (s, CHO), 8.68 (s, 5″-H), 7.16–7.29 (m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.33 (d, $J = 0.8$ Hz, 3-H), 4.02 (t, $J = 7.6$ Hz, 1′-CH_2), 3.77 (s, 4‴-OCH_3), 2.22 (d, $J = 0.8$ Hz, 4-CH_3), 1.87–1.91 (m, 2′-CH_2), 1.46 (t, $J = 7.6$ Hz, 3′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): *δ* 190.2 (CHO), 161.1 (C-2), 160.3 (C-7), 158.1 (C-4‴), 153.1 (C-4), 152.0 (C-3″), 147.1 (C-8a), 133.1 (C-1‴), 130.9 (C-5″), 127.7 (C-5), 124.1 (C-8), 120.1 (C-3‴, C-5‴), 115.9 (C-4a), 114.7 (C-3), 112.8 (C-4″), 109.2 (C-2‴, C-6‴), 104.7 (C-6), 65.3 (C-1′), 56.7 (C-4‴-OCH_3), 23.7 (C-4-CH_3), 19.8 (C-2′) and 16.4 (C-3′). Mass (ES): *m/z* 419 $[M + H]^+$. Anal. calcd for: C, 68.89; H, 5.30; N, 6.69%. Found: C, 68.56; H, 5.02; N, 6.98%. M.P: 164 °C, yield: 71%.

3-(7-Butoxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde (**9k**) IR (KBr): *υ* 2762 (C–H of aldehyde), 1726 (C=O of aldehyde), 1688 (C=O of coumarin), 1603 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): *δ* 10.01 (s, CHO), 8.57 (s, 5″-H), 7.13–7.26 (m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.21 (s, 3-H), 4.21 (t, $J = 7.4$ Hz, 1′-CH_2), 3.93 (s, 4‴-OCH_3), 2.37 (s, 4-CH_3) 1.98–2.03 (m, 2′-CH_2), 1.80–1.87 (m, 3′-CH_2), 0.97 (t, $J = 7.4$ Hz, 4′-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz): *δ* 189.1 (CHO), 161.0 (C-2), 160.2 (C-7), 158.7 (C-4‴), 153.0 (C-4), 152.3 (C-3″), 147.1 (C-8a), 132.7 (C-1‴), 131.0 (C-5″), 127.9 (C-5), 124.8 (C-8), 120.8 (C-3‴, C-5‴), 116.1 (C-4a), 114.3 (C-3), 113.0 (C-4″), 109.8 (C-2‴, C-6‴), 105.2 (C-6), 66.0 (C-1′), 55.4 (C-4‴-OCH_3), 32.8 (C-4-CH_3) 20.1 (C-2′), 18.6 (C-3′) and 14.8 (C-4′). Mass (ES): *m/z* 433 $[M + H]^+$. Anal. calcd for: C, 69.43; H, 5.59; N, 6.48%. Found: C, 69.14; H, 5.28; N, 6.76%. M.P:158 °C, yield: 74%.

1-(4-Methoxyphenyl)-3-(4-methyl-2-oxo-7-(pentyloxy)-2*H*-chromen-8-yl)-*1H*-pyrazole-4-carbaldehyde (**9l**) IR (KBr): *υ* 2777 (C–H of aldehyde), 1718 (C=O of aldehyde), 1671 (C=O of coumarin), 1587 (C=C of coumarin) cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz): *δ* 10.04 (s, CHO), 8.24 (s, 5″-H), 7.13–7.24 (m, 5-H, 6-H, 2‴-H, 6‴-H, 3‴-H, 5‴-H), 6.21 (d, $J = 0.8$ Hz, 3-H), 4.27 (t, $J = 8.0$ Hz, 1′-CH_2) 3.89 (s, 4‴-OCH_3), 2.24 (d, $J = 0.8$ Hz, 4-CH_3), 1.84–1.92 (m, 2′-CH_2), 1.45–1.56 (m, 3′-CH_2, 4′-CH_2), 0.94 (t, $J = 7.6$ Hz, 5′-CH_3). ^{13}C-NMR: ($CDCl_3$, 100 MHz): *δ* 190.0 (CHO), 161.4 (C-2), 159.7 (C-7), 157.9 (C-4‴), 153.4 (C-4), 151.9 (C-3″), 147.4 (C-8a), 132.4 (C-1‴), 130.8 (C-5″), 126.8 (C-5), 124.0 (C-8), 119.2 (C-3‴, C-5‴), 116.0 (C-4a), 114.5 (C-3), 113.2 (C-4″), 110.6 (C-2‴, C-6‴), 104.3 (C-6), 66.7 (C-1′), 55.0 (C-4‴-OCH_3), 31.9 (C-4-CH_3), 29.8 (C-2′), 23.3 (C-3′), 20.8 9C-4′) and 13.6 (C-5′). Mass (ES): *m/z* 447 $[M + H]^+$. Anal. calcd for: C, 69.94; H, 5.87; N, 6.27%. Found: C, 69.42; H, 5.61; N, 6.56%. M.P:144 °C, yield: 75%.

Testing of the biological activity

Medium used for organism growth

Czapek-Dox Agar was used as culture broth. Ingredients in g/l; Agar 15.0 g, $NaNO_3$ 2.0 g, K_2HPO_4 1.0 g, $MgSO_4$ $7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4$ $7H_2O$ 0.2 g, Sucrose 30.0 g and distilled water 1 l.

The pH of the medium, prepared from above ingredients is adjusted to 5.0–5.5. The medium was sterilized in the autoclave at 121 °C under pressure (15 lbs) for 15 min then cooled to 45–50 °C and poured in 20 ml volume in each sterilized Petridish and allowed to solidify.

Antifungal testing The antifungal activity screening was done by the paper disk method (Neppi 1964). After solidification of media, petriplates were inoculated with actively growing culture of AN. Filter paper disks of 5 mm diameter were dipped in the test solution of different concentration. After drying the disk, it was kept on czapek-dox agar in petriplates seeded with AN and screened for antifungal activity after incubated at 37 °C for 3–4 days.

Results and discussion

Chemical synthesis

8-Acetyl-7-hydroxy-4-methyl-2H-chromen-2-one (**5a**) and 8-acetyl-7-alkoxy-4-methyl-2H-chromen-2-one (**5b–f**) were condensed with phenyl hydrazines under reflux condition for 1 h on water bath in presence of methyl alcohol and glacial acetic acid to obtain phenylhydrazone analogs of coumarin (**8a–l**). These were further subjected to Vilsmeier–Haack formylation to obtain the novel pyrazole-4-carbaldehyde containing coumarin derivatives (**9a–l**). All the reactions were monitored by TLC and final compounds were purified by column chromatography with ethyl acetate: pet ether (15:85).

Synthesized compounds chemical structures were established on the basis of their FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopic techniques. In the IR spectra of

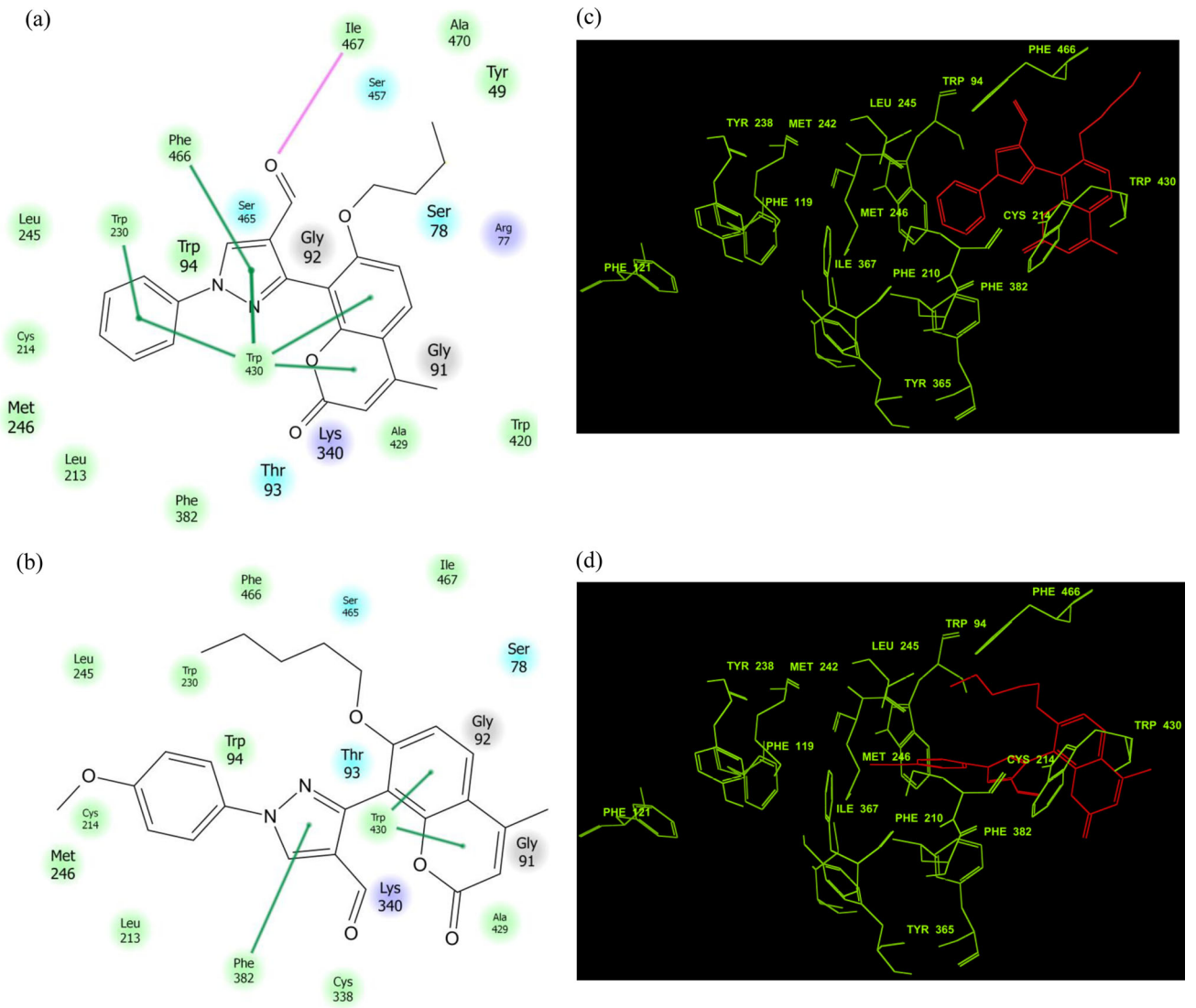


Fig. 1 **a** Docking interactions of molecule '**9e**' with the hydrophobic binding pocket of the protein. This is showing total 6 $\pi-\pi$ stacking interactions (Trp230, Trp430 (4) and Phe466) and one HBI with Ile467 in the protein. **b** Least dock score molecule '**9l**' showing π-stacking interactions with amino acid Phe382 and Trp430 of the protein. Solid (*green*) *line* represents π-stacking interactions and pink represents HBI with the backbone of amino acid. **c**, **d** Dock pose of the ligands **9e** (**c**) and **9l** (**d**) into the binding pocket of MAO-N protein

phenylhydrazone analogs of coumarin (**8a–l**) N–H absorption is identified in the range 3328–3350 cm^{-1}. Phenylhydrazone analogs of coumarin (**8a–l**) were further subjected to Vilsmeier-Haack formylation reaction to yield pyrazole compounds (**9a–l**) in good yields. In the IR spectra of final compounds (**9a–l**) C–H and C=O of aldehyde showed absorption in the range 2748–2794 and 1714–1743 cm^{-1}, respectively. ^{1}H-NMR and ^{13}C NMR recorded in $CDCl_3$ and chemical shifts (δ) expressed in PPM. Pyrazole ring-H and aldehyde-H appeared at 8.13–8.80 and 9.63–10.21 ppm, respectively as singlet. In ^{13}C-NMR aldehyde carbon atoms appeared at 187–192 ppm. The results between docking and antifungal activity studies were efficacy of present paper.

Molecular modeling

MAO-N is a flavo protein which contains non-covalently bonded FAD as a cofactor. The active site of MAO-N has a large hydrophobic cavity and extends to the surface of the protein. The hydrophobic nature of substrate cavity engendered the specificity of MAO-N towards hydrophobic and cyclic substrates (Alexeeva et al. 2002; Carr et al. 2005). Active site of MAO-N forms a large single cavity lined with hydrophobic residues-TRP94, PHE119, PHE121, PHE210, LUE213, CYS214, TYR238, MET242, LUE245, MET246, TYR365, ILE367, PHE382, TRP430, and PHE466 (Atkin et al. 2008). TRP430 and PHE466 formed a cage in MAO-N, which is perpendicular to the re-face of the

iso alloxazine ring. These are conserved aromatic residues in number of amine oxidases suggests that it is an important role in the function of the enzyme (Binda et al. 1999; Li et al. 2006). Due to its role in the function of an enzyme, molecular modeling studies were performed on MAO-N to emphasize this fact.

Docking results

All synthesized molecules were docked into the active site of MAO-N. The hydrophobic nature of substrate cavity engendered specificity of MAO-N towards hydrophobic and cyclic substrates. Hence, hydrophobic amino acids in the active site are showing $\pi-\pi$ stacking interactions with the molecule. Molecules are consistently showing stacking interactions with cage forming amino acids of Trp430 or Phe466. The other amino acids which are involved in stacking interactions are Trp230, Phe382 and Trp420.

Protein in its native conformation shows the HBI between the cofactor-FAD Thr93 and Ile467 of the protein. These HBI are later observed with the docked molecules of pyrazole aldehyde derivatives. Along with π-stacking interactions few molecules are showing hydrogen bond interaction (HBI) with close acceptor groups in amino acids of the protein. HBI are observed with 'O' atom of CHO group in the molecule with either of Thr93, Ser465, or Ile467 of the protein with the bond distance of 1.80 or 2.28 Å depends on the orientation of the molecule. The 'O' atom of CHO group in pyrazole derivatives are in close proximity to the amino acids Ser465 and Ile467 of the protein, but HBI are shown by few of them due to the steric hindrance developed at 'R_1' substitution. The substituted 'R_1' groups are enhancing the dock score, log*P* and log*S* values of the corresponding molecules. This trend was not observed with molecules of '**9f**' and '**9l**' where 'R_1' is substituted with '*n-pentyl*' group.

The deviation is much prominent in these two molecules and is because of long chain pentyl group, which further producing flip in the molecule of about 180° further changing the contribution of π-stacking interactions and HBI with the protein (Supplementary material). This flip was observed within the C–C bond between pyrazole and coumarin moiety. The same is evidently seen in the large decrease of PIDG of **9f** and **9l** which shown −31.25% with reference.

Among 12 derivatives of the pyrazole molecules the highest dock score shown by the molecules '**9e**'. The actual tendency is deviated in this molecule and this is expected to due to the combined effect of hydrogen bond contribution (Ser465) and $\pi-\pi$ stacking interactions with the cage forming amino acids. This is shown in the Fig. 1.

The decreasing order of the dock score was observed in all the other molecules with the increase of 'R_1' substitution except in '**9e**'. Whereas the decrease in dock scores is much evident in second series of molecules, with 'OCH_3' group at 'R_2' position. R_2 substitution in molecules showed significant changes in overall performance of the molecule e.g. '**9l**'. The observed least dock score resulting from two significant contributions like steric bulky group and 'OCH_3' at para position to phenyl as 'R_2' substitution. Hence, these results suggest that the unremitting increase in hydrophobicity is not the sole factor which governs the binding affinity of the molecule. Along with the hydrophobicity, a molecule with hydrogen bond donor species further enhances the binding proficiency of molecule towards the target.

Biological activity

After 3–4 days the petridishes were checked for growth inhibition zone. The presence of clear zone of growth inhibition around the paper disk indicates the inhibition of growth of organism due to the compound considered as to be active. If there is no clear zone of inhibition around the disk in the petridish indicates no activity of the sample. If partial zone of inhibition was observed that indicates the moderate inhibition of growth. The antifungal activity of the compounds tested is given in Table 1. The zone of inhibition was measured by scientific scale in mm and Percentage inhibition of diameter growth (PIDG) is calculated (Himratul-Aznita et al. 2011).

Conclusion

The compounds **9a**, **9b**, **9g**, and **9h** showed high activity where as compounds **9f** and **9l** exhibited poor activity against AN than the standard clotrimazole. The predicted docking score values does not match the experimental values on a number-by-number basis. However an interesting correlation between experimental studies and docking studies were observed. Results from ADMET, bond angles and dock scores of the molecules concluded that, the optimum length of the substitution is up to four carbons. The further increase in the hydrophobic nature decreases the bonding affinity of the molecule.

Conflict of interest The authors declare that they have no competing interests.

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