

## Carbohydrate hydrolyzing enzymes inhibitory and free radical scavenging activities of substituted 2*H*-chromene-3-carboxylates, 2*H*-chromenyl Knoevenagel derivatives and 6*H*-chromenoquinolinones

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Three series of compounds namely ethyl 2-hydroxy-2-(trifluoromethyl)-2*H*-chromene-3-carboxylates **3a-q**, 2*H*-chromenyl Knoevenagel derivatives **7a-j** and 6*H*-chromenoquinolinones **9a-l** have been prepared. The synthesized compounds **3a-q**, **7a-j** and **9a-g** have been screened for anti-oxidant and anti-diabetic activity. Compound **3i** shows the most potent ABTS inhibitory activity when compared to trolox. Compounds **3b**, **3d**, **3j** and **3l** show  $\alpha$ -amylase inhibitory activity.

**Keywords:** 2*H*-Chromene-3-carboxylates, 2*H*-chromenyl Knoevenagel derivatives, 6*H*-chromenoquinolinones, salicylaldehydes, 4-chloro-3-formylcoumarin, anti-oxidant, anti-diabetic

Coumarins and their derivatives are important heterocyclic compounds<sup>1</sup> which exhibit various biological activities such as anti-cancer, anti-HIV, antiviral, antioxidant, antifungal, antibacterial, anticoagulant, anti-psoriatic and AChE inhibitors<sup>2</sup>. In addition, coumarins play an important role in laser technology, fluorescent indicators, optical brighteners and photosensitisers<sup>3</sup>. Our group focused research on feasible reactions of carbonyl compounds and salicylaldehydes with 3-oxobutanoates having chloro or trifluoro substituents. Condensation of salicylaldehydes with ethyl 4-chloro-3-oxobutanoate in the presence of piperidine in dry DCM provided 2*H*-chromenes<sup>4</sup> and, in turn, these derivatives were successfully converted to useful heterocyclic compounds<sup>5</sup>. However, the condensation of carbonyl compounds with ethyl 4,4,4-trifluoro-3-oxobutanoate in the presence of piperidine in dry DCM provided a series of (*E*)- $\alpha,\beta$ -unsaturated esters and ketones<sup>6</sup>. Further, we prepared various substituted chromenone derivatives and screened them for their DPPH, ABTS free radical scavenging,  $\alpha$ -glucosidase and amylase inhibitory activities<sup>7</sup>. The present manuscript describes the preparation of trifluoro substituted 2*H*-chromene-3-carboxylates, 2*H*-chromenyl Knoevenagel derivatives and 6*H*-chromenoquinolinones. Thus, synthesized compounds were screened for their DPPH, ABTS free radical scavenging,  $\alpha$ -glucosidase and amylase inhibitory activities.

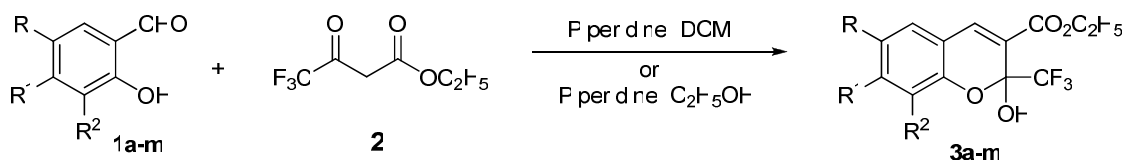
### Results and Discussion

**Schemes I-III** illustrate the preparation of trifluoro substituted 2*H*-chromene-3-carboxylates **3a-q**. Condensation of salicylaldehydes **1a-m** with ethyl 4,4,4-trifluoro-3-oxobutanoate **2** in the presence of piperidine in DCM under reflux conditions provided 2*H*-chromene-3-carboxylates **3a-m** (**Scheme I**, **Table I**).

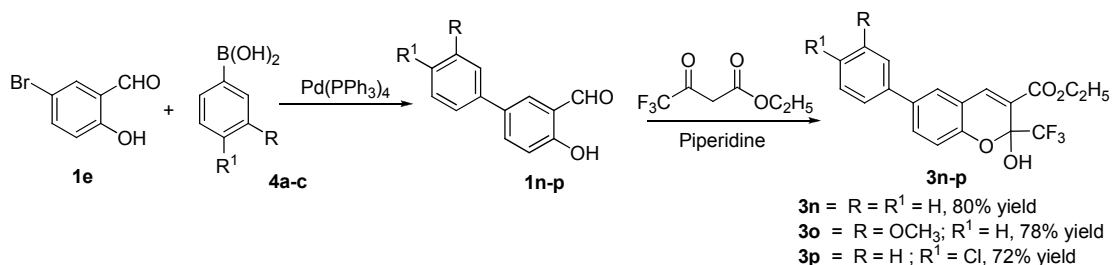
**Scheme II** illustrates the preparation of substituted coumarins **3n-p**. Suzuki coupling<sup>6</sup> of 5-bromosalicylaldehyde **1e** with boronic acids such as phenylboronic acid **4a**, 3-methoxyphenylboronic acid **4b** and 4-chlorophenylboronic acid **4c** in the presence of Pd(PPh<sub>3</sub>)<sub>4</sub> provided the corresponding salicylaldehydes **1n-p**. Condensation of substituted salicylaldehydes **1n-p** with ethyl 4,4,4-trifluoro-3-oxobutanoate **2** in the presence of piperidine in C<sub>2</sub>H<sub>5</sub>OH under reflux conditions provided the corresponding coumarins **3n-p**.

**Scheme III** illustrates the synthetic pathway to obtain pyranochromenone derivative **3q**. Condensation of 2,4-dihydroxybenzaldehyde **1i** and 2-methylbut-3-ene-2-ol in the presence of 5% citric acid containing ascorbic acid<sup>7</sup> afforded 7-hydroxy-2,2-dimethylchromancarbaldehyde **1q**. Condensation of **1q** with ethyl 4,4,4-trifluoro-3-oxobutanoate **2** afforded the target compound **3q** in 78% yield. Thus, synthesized compounds **3b-q** are new and well characterized by spectral data.

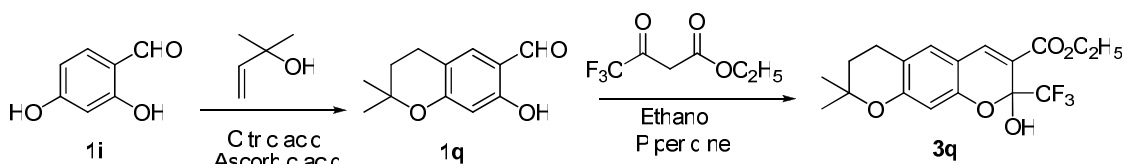
Having achieved the preparation of trifluoro substituted 2*H*-chromene-3-carboxylates **3a-q**, we next



Scheme I



Scheme II



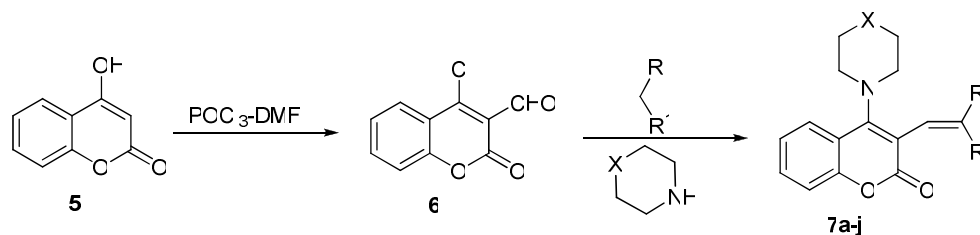
Scheme III

**Table I** — Preparation of trifluoro substituted 2*H*-chromenones **3a-m**

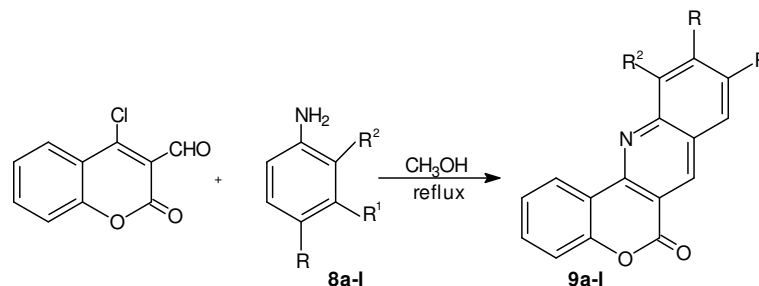
| S.No | R                                           | R <sup>1</sup> | R <sup>2</sup>                    | Product   | Yield (%) |
|------|---------------------------------------------|----------------|-----------------------------------|-----------|-----------|
| 1    | H <b>1a</b>                                 | H              | H                                 | <b>3a</b> | 87        |
| 2    | CH <sub>3</sub> <b>1b</b>                   | H              | H                                 | <b>3b</b> | 70        |
| 3    | C-(CH <sub>3</sub> ) <sub>3</sub> <b>1c</b> | H              | C-(CH <sub>3</sub> ) <sub>3</sub> | <b>3c</b> | 88        |
| 4    | Cl <b>1d</b>                                | H              | H                                 | <b>3d</b> | 89        |
| 5    | Br <b>1e</b>                                | H              | H                                 | <b>3e</b> | 91        |
| 6    | NO <sub>2</sub> <b>1f</b>                   | H              | H                                 | <b>3f</b> | 85        |
| 7    | Cl <b>1g</b>                                | H              | Cl                                | <b>3g</b> | 84        |
| 8    | Br <b>1h</b>                                | H              | Br                                | <b>3h</b> | 68        |
| 9    | H <b>1i</b>                                 | OH             | H                                 | <b>3i</b> | 83        |
| 10   | H <b>1j</b>                                 | O-Bn           | H                                 | <b>3j</b> | 86        |
| 11   | H <b>1k</b>                                 | O-allyl        | H                                 | <b>3k</b> | 65        |
| 12   | H <b>1l</b>                                 | O-prenyl       | H                                 | <b>3l</b> | 84        |
| 13   | Naphthyl <b>1m</b>                          | Naphthyl       | H                                 | <b>3m</b> | 86        |

prepared 4-substituted 2-oxo-2*H*-chromen-3-yl-methylene derivatives **7a-j** and 6*H*-chromenoquinolinones **9a-l** starting from 4-chloro-3-formylcoumarin **6**. Vilsmeier-Haack reaction of 4-hydroxycoumarin **5** in presence of POCl<sub>3</sub>-DMF provided 4-chloro-3-formylcoumarin **6**. The Knoevenagel condensation of 4-chloro-3-formylcoumarin **6** with diethyl malonate has been carried out in the presence of piperidine in CH<sub>2</sub>Cl<sub>2</sub> at RT. This provided diethyl 2-((2-oxo-4-(piperidin-1-

yl)-2*H*-chromen-3-yl)methylene)malonate **7a** (**Scheme IV**). During the reaction, we observed that when the chlorine is substituted with piperidine, it provided us **7a**. Under similar conditions, we have carried out the condensation reaction of **6** with various β-ketoesters such as ethyl 3-oxobutanoate, ethyl 3-oxo-3-phenylpropanoate, ethyl 4-chloro-3-oxobutanoate and ethyl 4,4,4-trifluoro-3-oxobutanoate. However, these experiments could not provide the corresponding Knoevenagel derivatives. Further, we have carried out the condensation reaction of **6** with ethyl 2-cyanoacetate and malononitrile. This provided the corresponding Knoevenagel derivatives **7b-c** in good yields by the nucleophilic substitution of chlorine with piperidine. Thereafter, we have chosen the various bases namely morpholine, piperazine, *N*-methylpiperazine and *N*-benzylpiperazine to study the feasibility of the condensation reaction and formation of the products. Accordingly, the condensation of 4-chloro-3-formylcoumarin **6** has been carried out with ethyl 2-cyanoacetate and malononitrile in presence of morpholine, piperazine, *N*-methylpiperazine and *N*-benzylpiperazine. This provided a series of 4-substituted Knoevenagel derivatives **7d-j** (**Scheme IV**). Thus, synthesized compounds **7b-j** are new and well characterized by spectral data (**Table II**).



Scheme IV



Scheme V

**Table II** — Preparation of 4-substituted Knoevenagel derivatives **7a-j**

| S.No | X                                                        | R                                             | R <sup>1</sup>                                | Product   | Yield (%) |
|------|----------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------|-----------|
| 1    | C                                                        | CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub> | CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub> | <b>7a</b> | 40        |
| 2    | C                                                        | CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub> | CN                                            | <b>7b</b> | 90        |
| 3    | C                                                        | CN                                            | CN                                            | <b>7c</b> | 60        |
| 4    | O                                                        | CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub> | CN                                            | <b>7d</b> | 62        |
| 5    | NH                                                       | CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub> | CN                                            | <b>7e</b> | 54        |
| 6    | <i>N</i> -CH <sub>3</sub>                                | CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub> | CN                                            | <b>7f</b> | 90        |
| 7    | <i>N</i> -CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub> | CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub> | CN                                            | <b>7g</b> | 70        |
| 8    | O                                                        | CN                                            | CN                                            | <b>7h</b> | 52        |
| 9    | <i>N</i> -CH <sub>3</sub>                                | CN                                            | CN                                            | <b>7i</b> | 72        |
| 10   | <i>N</i> -CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub> | CN                                            | CN                                            | <b>7j</b> | 52        |

**Table III** — Preparation of 6*H*-chromenoquinolinone derivatives **9a-l**

| S.No | R                | R <sup>1</sup>  | R <sup>2</sup>                    | Product   | Yield (%) |
|------|------------------|-----------------|-----------------------------------|-----------|-----------|
| 1    | H                | H               | H                                 | <b>9a</b> | 80        |
| 2    | H                | F               | H                                 | <b>9b</b> | 60        |
| 3    | F                | H               | H                                 | <b>9c</b> | 50        |
| 4    | Cl               | H               | H                                 | <b>9d</b> | 60        |
| 5    | Br               | H               | H                                 | <b>9e</b> | 50        |
| 6    | H                | CH <sub>3</sub> | CH <sub>3</sub>                   | <b>9f</b> | 70        |
| 7    | CH <sub>3</sub>  | CH <sub>3</sub> | H                                 | <b>9g</b> | 50        |
| 8    | CH <sub>3</sub>  | Br              | H                                 | <b>9h</b> | 60        |
| 9    | H                | H               | C <sub>2</sub> H <sub>5</sub>     | <b>9i</b> | 70        |
| 10   | H                | H               | CH(CH <sub>3</sub> ) <sub>2</sub> | <b>9j</b> | 60        |
| 11   | H                | CF <sub>3</sub> | H                                 | <b>9k</b> | 40        |
| 12   | OCH <sub>3</sub> | H               | H                                 | <b>9l</b> | 70        |

Having achieved the Knoevenagel derivatives **7a-j**, we have next, studied the condensation reaction of 4-chloro-3-formylcoumarin **6** with various aromatic amines (**Scheme V**). The initial condensation reaction has been carried out between 4-chloro-3-formylcoumarin **6** and aniline **8a** in the presence of methanol under reflux conditions without using any catalyst<sup>8</sup>. This provided 6*H*-chromenoquinolinone **9a** in very good yield. Further, the condensation reaction of **6** has been carried out with various aromatic anilines **8b-l**. The reactions proceeded smoothly and provide 6*H*-chromenoquinolinones **9b-l** in good yields. These compounds were well characterized by spectral data (**Table III**).

Thus synthesized compounds 2*H*-chromene-3-carboxylates **3a-q**, 2*H*-chromenyl Knoevenagel derivatives **7a-j** and 6*H*-chromenoquinolinones **9a-g** were screened for their DPPH radical scavenging activities described

by Walker and Everette method<sup>9</sup>. Percentage DPPH scavenging activity values of the compounds are presented in **Table IV-V**. Compound 2*H*-chromene-3-carboxylate **3q** and 2*H*-chromenyl Knoevenagel derivative **7j** displayed DPPH free radical scavenging activity at primary concentration of 100 µg/mL when compared to ascorbic acid. However the 2*H*-chromene-3-carboxylate compounds **3a-p**, 2*H*-chromenyl Knoevenagel derivatives **7a-i** and 6*H*-chromenoquinolinones **9a-g** could not display DPPH scavenging activity. Further, we have screened ABTS<sup>+</sup> scavenging activity for all the synthesized compounds. The percentage ABTS<sup>+</sup> scavenging activity of the compounds and their IC<sub>50</sub> values are presented in **Table IV-V**. The IC<sub>50</sub> ranged from 0.65–68.3 µM and the order of the compounds' potency in decreasing order was as **3i**

**Table IV** — DPPH, ABTS,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition activity profile of **3a-q**

| Entry | Compd                | % DPPH<br>(100 $\mu$ g/mL) | % ABTS<br>(50 $\mu$ g/mL) | ABTS<br>(IC50 $\mu$ M) | % AGi inhibition<br>(100 $\mu$ g/mL) | % $\alpha$ -Amylase<br>Inhibition<br>(400 $\mu$ g/mL) | $\alpha$ -Amylase<br>(IC50 $\mu$ M) |
|-------|----------------------|----------------------------|---------------------------|------------------------|--------------------------------------|-------------------------------------------------------|-------------------------------------|
| 1     | <b>3a</b>            | ND                         | 97.16 $\pm$ 0.0           | 19.44                  | ND                                   | 19.9 $\pm$ 0.3                                        | ---                                 |
| 2     | <b>3b</b>            | ND                         | 88.0 $\pm$ 4.3            | 37.7                   | ND                                   | 44.2 $\pm$ 0.3                                        | 1243                                |
| 3     | <b>3c</b>            | ND                         | 6.90 $\pm$ 2.58           | ---                    | ND                                   | 37.4 $\pm$ 1.0                                        | ---                                 |
| 4     | <b>3d</b>            | ND                         | 91.68 $\pm$ 0.9           | 15.2                   | ND                                   | 41.8 $\pm$ 1.7                                        | 1198                                |
| 5     | <b>3e</b>            | ND                         | 95.74 $\pm$ 0.3           | 68.3                   | ND                                   | 34.5 $\pm$ 2.4                                        | ---                                 |
| 6     | <b>3f</b>            | ND                         | 98.2 $\pm$ 0.7            | 6.88                   | ND                                   | 37.6 $\pm$ 1.4                                        | ---                                 |
| 7     | <b>3g</b>            | ND                         | 49.0 $\pm$ 0.86           | ---                    | ND                                   | 35.4 $\pm$ 3.8                                        | ---                                 |
| 8     | <b>3h</b>            | ND                         | 91.2 $\pm$ 0.3            | 10.31                  | ND                                   | 36.9 $\pm$ 0.3                                        | ---                                 |
| 9     | <b>3i</b>            | ND                         | 99.3 $\pm$ 0.3            | 0.65                   | ND                                   | 16.9 $\pm$ 2.4                                        | ---                                 |
| 10    | <b>3j</b>            | ND                         | 8.72 $\pm$ 0.57           | ---                    | ND                                   | 42.8 $\pm$ 0.3                                        | 341                                 |
| 11    | <b>3k</b>            | ND                         | 48.5 $\pm$ 18.9           | ---                    | 30.66                                | 30.3 $\pm$ 1.4                                        | ---                                 |
| 12    | <b>3l</b>            | ND                         | 78.3 $\pm$ 4.3            | 51                     | ND                                   | 38.9 $\pm$ 1.0                                        | 765                                 |
| 13    | <b>3m</b>            | ND                         | 99.8 $\pm$ 1.0            | 7.5                    | ND                                   | 34.0 $\pm$ 4.5                                        | ---                                 |
| 14    | <b>3n</b>            | ND                         | 81.8 $\pm$ 4.4            | 50.91                  | ND                                   | 35 $\pm$ 2.4                                          | ---                                 |
| 15    | <b>3o</b>            | ND                         | 6.90 $\pm$ 2.58           | ---                    | ND                                   | 37.4 $\pm$ 1.0                                        | ---                                 |
| 16    | <b>3p</b>            | ND                         | 16.02 $\pm$ 4.8           | ---                    | ND                                   | 36.4 $\pm$ 1.0                                        | ---                                 |
| 17    | <b>3q</b>            | 67.75                      | 39.15 $\pm$ 2.8           | ---                    | ND                                   | 23.8 $\pm$ 0.3                                        | ---                                 |
| 18    | <b>Ascorbic acid</b> | 90.69 $\pm$ 0.72           | ---                       | ---                    | ---                                  | ---                                                   | ---                                 |
| 19    | <b>Trolox</b>        | ---                        | 98.0 $\pm$ 0.6            | 2.4                    | ---                                  | ---                                                   | ---                                 |
| 20    | <b>Acarbose</b>      | ---                        | ---                       | ---                    | 42.65                                | 42.65 $\pm$ 1.0                                       | 38.7                                |

ND-Not detected

**Table V** — DPPH, ABTS scavenging,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition activities of **7a-j** and **9a-g**

| Entry | Compd                | % DPPH<br>(100 $\mu$ g/mL) | % ABTS<br>(50 $\mu$ g/mL)    | % $\alpha$ -Glucosidase inhibition<br>(100 $\mu$ g/mL) | % $\alpha$ -Amylase Inhibition<br>(400 $\mu$ g/mL) |
|-------|----------------------|----------------------------|------------------------------|--------------------------------------------------------|----------------------------------------------------|
| 1     | <b>7a</b>            | ND                         | 42.3 $\pm$ 0.0               | ND                                                     | ND                                                 |
| 2     | <b>7b</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 3     | <b>7c</b>            | ND                         | 21.2 $\pm$ 0.2               | ND                                                     | ND                                                 |
| 4     | <b>7d</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 5     | <b>7e</b>            | ND                         | 96.3 $\pm$ 0.3               | ND                                                     | ND                                                 |
| 6     | <b>7f</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 7     | <b>7g</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 8     | <b>7h</b>            | ND                         | 14.3 $\pm$ 0.3               | ND                                                     | ND                                                 |
| 9     | <b>7i</b>            | ND                         | 26.5 $\pm$ 0.3               | ND                                                     | ND                                                 |
| 10    | <b>7j</b>            | 26.5                       | 76.8 $\pm$ 0.57              | ND                                                     | ND                                                 |
| 11    | <b>9a</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 12    | <b>9b</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 13    | <b>9c</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 14    | <b>9d</b>            | ND                         | 14.5 $\pm$ 4.4               | ND                                                     | ND                                                 |
| 15    | <b>9e</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 16    | <b>9f</b>            | ND                         | 27.5 $\pm$ 4.88              | ND                                                     | ND                                                 |
| 17    | <b>9g</b>            | ND                         | ND                           | ND                                                     | ND                                                 |
| 18    | <b>Ascorbic acid</b> | 90.69 $\pm$ 0.72           | ---                          | ---                                                    | ---                                                |
| 19    | <b>Trolox</b>        | ---                        | 98.0 $\pm$ 0.6 (2.4 $\mu$ M) | ---                                                    | ---                                                |
| 20    | <b>Acarbose</b>      | ---                        | ---                          | 42.65                                                  | 42.65 $\pm$ 1.0                                    |

ND-Not detected

(0.65), **3f** (6.88), **3m** (7.5), **3h** (10.31), **3d** (15.2), **3a** (19.44), **3b** (37.7), **3n** (50.91), **3l** (51.0) and **3e** (68.3). Compound **3i** displayed more potent ABTS scavenging activity in the present series when compared to standard drug trolox (2.4  $\mu$ M).

The pancreatic  $\alpha$ -amylase and intestinal  $\alpha$ -glucosidase enzymes play an important role in digestion of dietary carbohydrate. Inhibition of these enzymes has become an important target in mitigating excursion in post prandial hyperglycaemia (PPHG)<sup>10</sup> in diabetic individuals.

The % inhibition and  $IC_{50}$  values of  $\alpha$ -glucosidase<sup>11</sup> and  $\alpha$ -amylase<sup>7d</sup> inhibitory activities for 2*H*-chromene-3-carboxylates **3a-q**, 2*H*-chromenyl Knoevenagel derivatives **7a-j** and 6*H*-chromenoquinolinones **9a-g** were tabulated in Table IV-V. Among the 2*H*-chromene-3-carboxylates **3a-q**, the compound **3k** (30.66%) displayed  $\alpha$ -glucosidase inhibitory activity when compared to standard drug acarbose (42.65%). However, compounds 2*H*-chromenyl Knoevenagel derivatives **7a-j** and 6*H*-chromenoquinolinones **9a-g** could not display any activity. The values of the  $\alpha$ -amylase inhibitory activity for 2*H*-chromene-3-carboxylates **3a-q**, 2*H*-chromenyl Knoevenagel derivatives **7a-j** and 6*H*-chromenoquinolinones **9a-g** are tabulated in Table IV-V. Among the 2*H*-chromene-3-carboxylates **3a-q**, the compounds **3b**, **3d**, **3j** and **3l** (341-1243  $\mu$ M) displayed  $\alpha$ -amylase inhibitory activity when compared to standard drug acarbose (38.72  $\mu$ M). However, compounds 2*H*-chromenyl Knoevenagel derivatives **7a-j** and 6*H*-chromenoquinolinones **9a-g** could not display  $\alpha$ -amylase inhibitory activity.

### Experimental Section

All the chemicals and reagents were purchased from Aldrich (Sigma-Aldrich, USA) and AVRA Chemicals Pvt. Ltd (Hyderabad, India) and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F<sub>254</sub> (mesh); spots were visualized under UV light. Column chromatographic separations were carried out over silica gel (60-120 mesh). Melting points were determined on a Mettler-Temp apparatus and are uncorrected. IR spectra were recorded with a Thermo Nicolet Nexus 670 spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker Avance 300 MHz spectrometers. Chemical shifts ( $\delta$ ) are quoted in parts per million and are referenced to tetramethylsilane (TMS) as internal standard. ESI-MS spectra were obtained on 7070H spectrometer operating at 70 eV using a direct inlet system.

### General experimental procedure for the preparation of 2-hydroxy-2-(trifluoromethyl)-2*H*-chromene-3-carboxylates **3a-q**

Piperidine (30 mol%) was added to a stirred solution of salicylaldehyde (**1a**, 1 mmol) and ethyl 4,4,4-trifluoro-3-oxobutanoate **2** (1 mmol) in ethanol (4 mL). The reaction mixture was refluxed for 5-6 h. After the completion of reaction (TLC), the reaction mixture was cooled to RT, the solvent was removed under reduced pressure and the obtained crude product was

purified by column chromatography using silica gel (60-120 mesh) with hexane/ethyl acetate (97:3) to afford ethyl 2-hydroxy-2-(trifluoromethyl)-2*H*-chromene-3-carboxylate **3a** as colorless solid. Similarly, the compounds **3b-q** were prepared from the corresponding salicylaldehydes **1b-q**.

### Spectral data

**Ethyl 2-hydroxy-2-(trifluoromethyl)-2*H*-chromene-3-carboxylate **3a**:** IR (KBr): 3297, 3081, 2977, 1953, 1686, 1634, 1607, 1574, 1489, 1460, 1397, 1372, 1348, 1294, 1263, 1225, 1170, 1125, 1098, 1039  $cm^{-1}$ ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  7.78 (s, 1H), 7.39 (td, 1H,  $J$  = 1.51, 7.54 Hz), 7.25 (dd, 1H,  $J$  = 1.51, 7.55 Hz), 7.05 (d, 2H,  $J$  = 7.55 Hz), 4.38 (q, 2H,  $J$  = 6.78 Hz), 1.41 (t, 3H,  $J$  = 7.17 Hz); ESI-MS:  $m/z$  311 [M+Na]<sup>+</sup>.

**Ethyl 2-hydroxy-6-methyl-2-(trifluoromethyl)-2*H*-chromene-3-carboxylate **3b**:** IR (KBr): 3547, 3491, 3264, 3064, 2924, 2854, 1676, 1580, 1509, 1464, 1381, 1353, 1225, 1182, 1149, 1102, 1043  $cm^{-1}$ ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  7.74 (s, 1H), 7.44 (s, 1H), 7.18 (dd, 1H,  $J$  = 8.61, 1.91 Hz), 7.04 (s, 1H), 6.93 (d, 1H,  $J$  = 8.61 Hz), 4.37 (q, 2H,  $J$  = 6.7 Hz), 2.29 (s, 3H), 1.39 (t, 3H,  $J$  = 6.7 Hz); ESI-MS:  $m/z$  327 [M+Na]<sup>+</sup>.

**Ethyl 6,8-di-*tert*-butyl-2-hydroxy-2-(trifluoromethyl)-2*H*-chromene-3-carboxylate **3c**:** IR (KBr): 3312, 2962, 2873, 1684, 1638, 1584, 1470, 1396, 1318, 1284, 1236, 1175, 1135, 1036, 1011  $cm^{-1}$ ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  7.76 (s, 1H), 7.44 (d, 1H), 7.38 (s, 1H), 7.09 (d, 1H,  $J$  = 2.26 Hz), 4.36 (q, 2H,  $J$  = 7.17 Hz), 1.42 (s, 9H), 1.39 (t, 3H,  $J$  = 7.17 Hz), 1.30 (s, 9H); ESI-MS:  $m/z$  423 [M+Na]<sup>+</sup>.

**Ethyl 6-chloro-2-hydroxy-2-(trifluoromethyl)-2*H*-chromene-3-carboxylate **3d**:** IR (KBr): 3404, 2922, 2853, 2835, 1686, 1635, 1570, 1480, 1377, 1287, 1184, 1128, 1051  $cm^{-1}$ ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  7.69 (s, 1H), 7.43 (s, 1H), 7.33 (dd, 1H,  $J$  = 2.15 Hz), 7.17 (d, 1H,  $J$  = 2.15 Hz), 6.99 (d, 1H,  $J$  = 9.33 Hz), 4.39 (q, 2H,  $J$  = 7.18 Hz), 1.40 (t, 3H,  $J$  = 7.18 Hz); ESI-MS:  $m/z$  345 [M+Na]<sup>+</sup>.

**Ethyl 6-bromo-2-hydroxy-2-(trifluoromethyl)-2*H*-chromene-3-carboxylate **3e**:** Solid, m.p. 98°C. IR (KBr): 3248, 2999, 2853, 1899, 1684, 1629, 1600, 1564, 1478, 1403, 1378, 1348, 1288, 1222, 1173, 1131  $cm^{-1}$ ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  7.69 (s, 1H), 7.47 (dd, 1H,  $J$  = 2.26, 8.30 Hz), 7.39 (d, 1H,  $J$  = 2.26 Hz), 6.94 (d, 1H,  $J$  = 9.06 Hz), 4.38 (q, 2H,  $J$  = 7.55), 1.40 (t, 3H,  $J$  = 7.55 Hz); ESI-MS:  $m/z$  349 [M-OH]<sup>+</sup>, 389 [M+Na]<sup>+</sup>.

**Ethyl 2-hydroxy-6-nitro-2-(trifluoromethyl)-2*H*-chromene-3-carboxylate **3f**:** Solid, m.p. 95°C. IR (KBr): 3309, 3086, 2857, 1691, 1620, 1580, 1481, 1398, 1376,

1227, 1178, 1102, 1026  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.29 (d, 2H,  $J = 8.99$  Hz), 7.83 (s, 1H), 7.18 (d, 1H,  $J = 8.99$  Hz), 4.42 (q, 2H,  $J = 6.99$  Hz), 1.43 (t, 3H,  $J = 6.99$  Hz); ESI-MS:  $m/z$  332  $[\text{M-H}]^+$ .

**Ethyl 6,8-dichloro-2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3g:** Solid, m.p.125°C. IR (KBr): 3297, 2985, 2940, 1698, 1633, 1562, 1457, 1393, 1372, 1225, 1164, 1085  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.68 (s, 1H), 7.49 (s, 1H), 7.44 (d, 1H,  $J = 2.26$  Hz), 7.18 (d, 1H,  $J = 2.26$  Hz), 4.40 (q, 2H,  $J = 7.55$  Hz), 1.41 (t, 3H,  $J = 7.55$  Hz); ESI-MS:  $m/z$  340  $[\text{M-OH}]^+$ .

**Ethyl 6,8-dibromo-2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3h:** IR (KBr): 3338, 2927, 2940, 1689, 1633, 1554, 1450, 1397, 1345, 1285, 1180  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.73 (d, 1H,  $J = 2.26$  Hz), 7.66 (s, 1H), 7.49 (s, 1H), 7.36 (d, 1H,  $J = 2.26$  Hz), 4.40 (q, 2H,  $J = 6.79$  Hz), 1.41 (t, 3H,  $J = 6.79$  Hz); ESI-MS:  $m/z$  429  $[\text{M-OH}]^+$ .

**Ethyl 2,7-dihydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3i:** Solid, m.p.120°C. IR (KBr): 3547, 3491, 3264, 3064, 2924, 2854, 1676, 1580, 1509, 1464, 1381, 1353, 1318, 1225, 1182, 1149, 1102, 1043  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.74 (s, 1H), 7.65 (s, 1H), 7.15 (d, 1H,  $J = 8.30$  Hz), 6.54 (s, 1H), 6.51 (d, 1H,  $J = 2.26$  Hz), 4.36 (q, 2H,  $J = 7.55$  Hz), 1.40 (t, 3H,  $J = 7.55$  Hz); ESI-MS:  $m/z$  327  $[\text{M+Na}]^+$ .

**Ethyl 7-(benzyloxy)-2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3j:** Solid, m.p.97°C. (KBr): 3260, 3069, 2924, 2856, 1673, 1615, 1569, 1506, 1435, 1378, 1286, 1170  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.74 (s, 1H), 7.62 (s, 1H), 7.44-7.32 (m, 5H), 7.17 (d, 1H,  $J = 9.06$  Hz), 6.66 (s, 1H), 5.08 (d, 2H,  $J = 4.53$  Hz), 4.35 (q, 2H,  $J = 6.79$  Hz), 1.38 (t, 3H,  $J = 6.79$  Hz); ESI-MS:  $m/z$  377  $[\text{M-OH}]^+$ , 417  $[\text{M+Na}]^+$ .

**Ethyl 7-(allyloxy)-2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3k:** Solid, m.p.110°C. IR (KBr): 3303, 2922, 2852, 1678, 1615, 1566, 1505, 1440, 1398, 1335, 1291, 1220, 1181, 1097  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.73 (s, 1H), 7.54 (s, 1H), 7.15 (d, 1H,  $J = 8.40$  Hz), 6.58 (s, 1H), 6.07-5.98 (m, 1H), 5.42 (d, 1H,  $J = 18.33$  Hz), 5.32 (d, 1H,  $J = 9.93$  Hz), 4.57-4.53 (m, 2H), 4.35 (q, 2H,  $J = 6.87$  Hz), 1.38 (t, 3H,  $J = 6.87$  Hz); ESI-MS: 327  $[\text{M-Na}]^+$ .

**Ethyl 2-hydroxy-7-((3-methylbut-2-en-1-yl)oxy)-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3l:** Liquid. IR (KBr): 3375, 2980, 2928, 1678, 1614, 1565, 1503, 1440, 1378, 1287, 1178, 1096  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.73 (s, 1H), 7.58 (s, 1H), 7.15 (d, 1H,  $J = 8.80$  Hz), 6.84 (s, 1H), 6.58 (dd, 1H,  $J = 6.84$  Hz), 5.45 (t, 1H,  $J = 6.84$  Hz), 4.56 (q, 2H,  $J = 6.84$

Hz), 4.35 (q, 2H,  $J = 6.84$  Hz), 1.58 (s, 3H), 1.57 (s, 3H), 1.4 (q, 3H,  $J = 6.84$  Hz); ESI-MS: 355  $[\text{M-OH}]^+$ .

**Ethyl 3-hydroxy-3-(trifluoromethyl)-3H-benzo[f]-chromene-2-carboxylate 3m:** Solid, m.p.100-102°C. IR (KBr): 3409, 2924, 2854, 1678, 1630, 1571, 1514, 1465, 1378, 1258, 1204, 1174, 1117, 1026  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.51 (s, 1H), 8.07 (d, 1H,  $J = 8.36$  Hz), 7.90 (d, 1H,  $J = 8.36$  Hz), 7.82 (d, 1H,  $J = 8.36$  Hz), 7.67 (s, 1H), 7.62 (t, 1H,  $J = 6.97$  Hz), 7.52-7.40 (m, 1H), 4.42 (q, 2H,  $J = 6.97$  Hz), 1.46 (t, 3H,  $J = 6.97$  Hz); ESI-MS: 361  $[\text{M+Na}]^+$ .

**Ethyl 2-hydroxy-6-phenyl-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3n:** Solid, m.p.126-28°C. IR (KBr): 3379, 2924, 2853, 1904, 1682, 1633, 1579, 1481, 1450, 1259, 1187, 1137, 1101, 1039  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.84 (s, 1H), 7.61 (dd, 1H,  $J = 2.00$ , 7.99 Hz), 7.52-7.35 (m, 6H), 7.12 (d, 1H,  $J = 8.99$  Hz), 4.39 (q, 2H,  $J = 6.99$  Hz), 1.40 (t, 3H,  $J = 6.99$  Hz); ESI-MS: 346  $[\text{M-OH}]^+$ .

**Ethyl 2-hydroxy-6-(3-methoxyphenyl)-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3o:** Solid, m.p. 75-77°C. IR (KBr): 3312, 2922, 2852, 1684, 1583, 1481, 1376, 1284, 1225, 1174, 1031  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.85 (s, 1H), 7.60 (dd, 1H,  $J = 2.26$ , 7.28 Hz), 7.54 (s, 1H), 7.48 (d, 1H,  $J = 2.66$  Hz), 7.32 (q, 1H,  $J = 2.26$  Hz), 7.14-7.02 (m, 2H), 6.90 (m, 1H), 4.40 (q, 2H,  $J = 6.79$  Hz), 3.87 (s, 3H), 1.42 (t, 3H,  $J = 6.79$  Hz); ESI-MS: 417  $[\text{M+Na}]^+$ .

**Ethyl 6-(4-chlorophenyl)-2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate 3p:** Liquid. IR (KBr): 3315, 2922, 2853, 1684, 1633, 1581, 1481, 1396, 1347, 1260, 1236, 1203, 1175, 1137, 1094  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.84 (s, 1H), 7.58 (dd, 1H,  $J = 2.26$ , 7.28 Hz), 7.52-7.30 (m, 4H), 7.10 (d, 1H,  $J = 8.30$  Hz), 4.40 (q, 2H,  $J = 7.55$ ), 1.42 (t, 3H,  $J = 7.55$  Hz); ESI-MS: 421  $[\text{M+Na}]^+$ .

**Ethyl 2-hydroxy-8,8-dimethyl-2-(trifluoromethyl)-2,6,7,8-tetrahydropyrano[3,2-g]chromene-3-carboxylate 3q:** Solid, m.p.120-22°C. IR (KBr): 3422, 2978, 2931, 1679, 1569, 1443, 1330, 1229, 1173, 1030  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.73 (s, 1H), 7.58 (s, 1H), 6.98 (s, 1H), 6.49 (s, 1H), 4.39 (q, 2H,  $J = 6.98$  Hz), 2.73 (t, 2H,  $J = 6.98$  Hz), 1.83 (t, 2H,  $J = 6.98$  Hz), 1.42 (t, 3H,  $J = 6.98$  Hz), 1.36 (s, 6H); ESI-MS: 355  $[\text{M-OH}]^+$ .

#### General experimental procedure for the preparation of 4-chloro-2-oxo-2H-chromene-3-carbaldehyde 6

*N,N*-Dimethylformamide (6 mmol) was added to a stirred solution of 4-hydroxycoumarin (1 mmol) and phosphorous oxytrichloride (6 mmol) at 0°C. The

reaction mixture was brought to RT and heated at 60-70°C for 4 hr. After the completion of the reaction (TLC), the reaction mixture was brought to RT and ice pieces were added. The reaction mixture was extracted with chloroform (3 × 20 mL) and the organic layer was washed with water (3 × 20 mL). The organic layer was separated and dried over anhyd. Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed under reduced pressure and the obtained residue was subjected to column chromatography over silica gel (60-120 mesh) using hexane-ethyl acetate as eluent to afford 4-chloro-2-oxo-2H-chromene-3-carbaldehyde **6** in 90% yield.

#### General experimental procedure for the preparation of 4-(*tert*-amino)-3-vinyl substituted 2H-chromene-2-ones 7a-j

Ethyl cyanoacetate or malononitrile (1 mmol) was added to a stirred solution of 4-chlorocoumarin-3-carbaldehyde (1 mmol) in ethanol (4 mL) at 10°C followed by amines (2.5 mmol). The reaction was slowly brought to RT and stirring was continued for 1-4 h. After completion of the reaction (TLC), the solid obtained was filtered and washed with hexane to remove impurities and if necessary, subjected to column chromatography over silica gel (60-120 mesh), using hexane-ethyl acetate to afford the pure product **7a**.

#### Spectral data

**Diethyl 2-((2-oxo-4-(piperidin-1-yl)-2H-chromen-3-yl)methylene)malonate 7a:** Pale yellow solid, m.p. 232-35°C. IR (KBr): 2854, 1713, 1525, 1218 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub> + DMSO-*d*<sub>6</sub>): δ 7.87 (d, 1H, *J* = 6.43 Hz), 7.54 (s, 1H), 7.34 (m, 1H), 7.23 (m, 1H), 7.15 (m, 1H), 4.20 (m, 4H), 3.5 (m, 4H), 1.89 (s, 6H), 1.45 (m, 3H), 1.35 (m, 3H); ESI-MS: *m/z* 400 [M+H]<sup>+</sup>.

**(E)-Ethyl 2-cyano-3-(2-oxo-4-(piperidin-1-yl)-2H-chromen-3-yl)acrylate 7b:** Yellow solid, m.p. 168-70°C. IR (KBr): 2850, 2222, 1711, 1586, 1288, 1226, 1102, 756 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.28 (s, 1H), 7.79 (d, 1H, *J* = 8.30 Hz), 7.54 (m, 1H), 7.27 (m, 2H), 4.35 (q, 2H, *J* = 6.79 Hz), 3.63 (m, 4H), 3.46 (m, 2H), 1.83 (m, 4H), 1.37 (t, 3H, *J* = 6.79 Hz); ESI-MS: *m/z* 353.1 [M+H]<sup>+</sup>.

**2-((2-Oxo-4-(piperidin-1-yl) - 2H-chromen-3-yl)methylene)malononitrile 7c:** Pale yellow solid, m.p. 188-90°C. IR (KBr): 2851, 2228, 1715, 1520, 1115, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 9.11 (m, 2H), 7.56 (t, 1H, *J* = 7.55 Hz), 7.33 (t, 2H, *J* = 8.3 Hz), 3.28 (t, 4H, *J* = 5.28 Hz), 1.92 (s, 4H), 1.80 (d, 2H, *J* = 6.43 Hz); ESI-MS: *m/z* 323.6 [M+NH<sub>4</sub>]<sup>+</sup>.

**(E)-Ethyl 2-cyano-3-(4-morpholino-2-oxo-2H-chromen-3-yl)acrylate 7d:** Yellow solid, m.p. 183-85°C. IR (KBr): 2224, 1707, 1588, 515, 1108, 756 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.37 (s, 1H), 7.62 (d, 1H, *J* = 8.3 Hz), 7.56 (t, 1H, *J* = 7.55 Hz), 7.45 (m, 1H), 7.25 (m, 1H), 4.45 (q, 2H, *J* = 7.54 Hz), 3.85 (m, 4H), 3.65 (m, 4H), 1.48 (t, 3H, *J* = 8.3 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 197.68, 192.44, 186.82, 161.92, 153.69, 152.26, 145.19, 133.03, 125.85, 123.78, 118.62, 115.23, 105.60, 93.27, 62.51, 54.31, 26.86, 23.76, 14.19; ESI-MS: *m/z* 355.2 [M+H]<sup>+</sup>.

**(E)-Ethyl 2-cyano-3-(2-oxo-4-(piperazin-1-yl)-2H-chromen-3-yl)acrylate 7e:** Yellow solid, m.p. 210-13°C. IR (KBr): 2923, 1710, 1604, 1220, 772 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.16 (s, 1H), 7.83 (d, 1H, *J* = 8.3 Hz), 7.71 (m, 1H), 7.57 (m, 1H), 7.30 (m, 1H), 4.22 (q, 2H, *J* = 7.54 Hz), 3.64 (m, 4H), 3.64 (m, 4H), 1.94 (t, 3H, *J* = 5.25 Hz); ESI-MS: *m/z* 354 [M+H]<sup>+</sup>.

**(E)-Ethyl 2-cyano-3-(4-(4-methylpiperazin-1-yl)-2-oxo-2H-chromen-3-yl)acrylate 7f:** Yellow solid, m.p. 213-15°C. IR (KBr): 2938, 2230, 1712, 1513, 1118, 761 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.26 (s, 1H), 7.76 (dd, 1H, *J* = 8.3, 1.5 Hz), 7.56 (t, 1H, *J* = 9.06 Hz), 7.29 (m, 2H), 4.33 (q, 2H, *J* = 6.71), 3.66-3.72 (m, 4H), 2.63 (m, 4H), 1.37 (t, 3H, *J* = 6.72 Hz); ESI-MS: *m/z* 368.2 [M+H]<sup>+</sup>.

**(E)-Ethyl 3-(4-(4-benzylpiperazin-1-yl)-2-oxo-2H-chromen-3-yl)-2-cyanoacrylate 7g:** Yellow solid, m.p. 235-38°C. IR (KBr): 2924, 2227, 1718, 1519, 750 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.27 (s, 1H), 7.76 (dt, 1H, *J* = 8.3 Hz), 7.55 (m, 1H), 7.29 (m, 7H), 4.33 (q, 2H, *J* = 7.55 Hz), 3.68 (t, 4H, *J* = 5.28 Hz), 3.61 (q, 4H, *J* = 7.55 Hz), 2.67 (t, 2H, *J* = 5.28 Hz), 1.37 (t, 3H, *J* = 6.79 Hz); ESI-MS: *m/z* 444 [M+H]<sup>+</sup>.

**2-((4-Morpholino-2-oxo-2H-chromen-3-yl)methylene)malononitrile 7h:** Orange solid, m.p. 193-95°C. IR (KBr): 2861, 2218, 1715, 1510, 1110, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.88 (s, 1H), 7.77 (d, 1H, *J* = 7.93 Hz), 7.67 (t, 1H, *J* = 7.53 Hz), 7.34 (m, 2H), 3.85 (t, 4H, *J* = 5.09 Hz), 3.92 (t, 4H, *J* = 5.09 Hz); ESI-MS: *m/z* 308.2 [M+H]<sup>+</sup>.

**2-((4-(4-Methylpiperazin-1-yl)-2-oxo-2H-chromen-3-yl)methylene)malononitrile 7i:** Orange solid, m.p. 222-25°C. IR (KBr): 2793, 2221, 1708, 1513, 1133, 766 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.85 (s, 1H), 7.73 (dd, 1H, *J* = 1.5, 8.3 Hz), 7.58 (m, 1H), 7.35 (d, 1H, *J* = 7.55 Hz), 7.28 (m, 1H), 3.84 (t, 4H, *J* = 4.53 Hz), 2.63 (t, 4H, *J* = 4.53 Hz), 2.40 (s, 3H); ESI-MS: *m/z* 343.1 [M+Na]<sup>+</sup>.

**2-((4-(4-Benzylpiperazin-1-yl)-2-oxo-2H-chromen-3-yl)methylene)malononitrile 7j:** Yellow solid, m.p.

245–47°C. IR (KBr): 2924, 2219, 1712, 1514, 1137, 751  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.86 (s, 1H), 7.70 (t, 1H,  $J = 6.79$  Hz), 7.57 (m, 1H), 7.29 (m, 7H), 3.86 (t, 4H,  $J = 5.28$  Hz), 3.61 (s, 2H), 2.67 (t, 4H,  $J = 5.27$  Hz); ESI-MS:  $m/z$  419.2  $[\text{M}+\text{Na}]^+$ .

#### General procedure for the preparation of 6H-chromeno[4,3-b]quinolin-6-ones 9a-l

Aniline (2.5 mmol) was added to a stirred solution of 4-chloro-2-oxo-2H-chromene-3-carbaldehyde (**6**, 1 mmol) in methanol (4 mL). The reaction mixture was refluxed for 4–8 h. The reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and the residue was subjected to column chromatography over silica gel (60–120 mesh) using hexane-ethyl acetate as eluent to afford the 6H-benzopyrano-[4,3-b]quinolin-6-one **9a**.

#### Spectral data

**6H-Chromeno[4,3-b]quinolin-6-one 9a**: Colorless amorphous solid, m.p. 227–30°C. IR (KBr): 3054, 2921, 2851, 1740, 1652, 945  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.25 (s, 1H), 8.80 (dd, 1H,  $J = 8.30, 1.55$  Hz), 8.24 (d, 1H,  $J = 8.3$  Hz), 8.03 (d, 1H,  $J = 8.3$  Hz), 7.91–7.96 (m, 1H), 7.58–7.68 (m, 2H), 7.39–7.48 (m, 2H);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 160.3, 158.4, 152.2, 146.5, 140.6, 138.5, 132.9, 131.9, 128, 126.9, 126.8, 124.7, 124.5, 119, 116, 115; ESI-MS:  $m/z$  248  $[\text{M}+\text{H}]^+$ .

**10-Fluoro-6H-chromeno[4,3-b]quinolin-6-one 9b**: White fluffy solid, m.p. 233–35°C. IR (KBr): 3054, 2851, 2921, 1740, 1652, 945  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.25 (s, 1H), 8.77 (dd, 1H,  $J = 8.3, 1.5$  Hz), 8.03–8.08 (m, 1H), 7.85 (d, 1H,  $J = 7.54$  Hz), 7.60–7.66 (m, 1H), 7.40–7.48 (m, 3H); ESI-MS:  $m/z$  266  $[\text{M}+\text{H}]^+$ .

**9-Fluoro-6H-chromeno[4,3-b]quinolin-6-one 9c**: Pale yellow fluffy solid, m.p. 223–25°C. IR (KBr): 3431, 3064, 2924, 1732, 1608  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.13 (s, 1H), 8.73 (dd, 1H,  $J = 8.3, 1.56$  Hz), 8.16 (d, 1H,  $J = 9.0$  Hz), 7.98 (d, 1H,  $J = 2.26$  Hz), 7.82 (d, 1H,  $J = 7.55$ ), 7.59–7.64 (m, 1H), 7.40–7.47 (m, 2H); ESI-MS:  $m/z$  266  $[\text{M}+\text{H}]^+$ .

**9-Chloro-6H-chromeno[4,3-b]quinolin-6-one 9d**: Colorless fluffy solid, m.p. 243–45°C. IR (KBr): 2924, 2853, 1742, 1603, 755  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.13 (s, 1H), 8.78 (dd, 1H,  $J = 8.3$  Hz), 8.18 (d, 1H,  $J = 7.55$  Hz), 7.96 (d, 1H,  $J = 8.23$  Hz), 7.81 (dd, 1H,  $J = 1.5, 7.56$  Hz), 7.58–7.62 (m, 1H), 7.37–7.48 (m, 2H); ESI-MS:  $m/z$  320  $[\text{M}+\text{K}]^+$ .

**9-Bromo-6H-chromeno[4,3-b]quinolin-6-one 9e**: Solid, m.p. 248–50°C. IR (KBr): 3441, 3064, 1741, 1606, 560  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.27 (s, 1H), 8.68 (dd, 1H,  $J = 7.7, 9.44$  Hz), 8.08 (d, 1H,  $J = 2.05$  Hz), 7.86 (d, 1H,  $J = 9.06$  Hz), 7.44 (dd, 1H,  $J = 2.05, 7.5$  Hz), 7.35–7.39 (m, 1H), 7.25–7.32 (m, 2H); ESI-MS:  $m/z$  365  $[\text{M}+\text{K}]^+$ .

**10,11-Dimethyl-6H-chromeno[4,3-b]quinolin-6-one 9f**: Pale brown solid, m.p. 238–40°C. IR (KBr): 3437, 2854, 1728, 1607, 744  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.09 (s, 1H), 8.80 (d, 1H,  $J = 7.61$  Hz), 7.75 (d, 1H,  $J = 8.61$  Hz), 7.55 (t, 1H,  $J = 7.65$  Hz), 7.37–7.44 (m, 3H), 2.87 (s, 3H), 2.57 (s, 3H); ESI-MS:  $m/z$  276  $[\text{M}+\text{H}]^+$ .

**9,10-Dimethyl-6H-chromeno[4,3-b]quinolin-6-one 9g**: Pale yellow solid, m.p. 237–40°C. IR (KBr): 2922, 2853, 1739, 1602, 772  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.05 (s, 1H), 8.73 (d, 1H,  $J = 6.92$  Hz), 7.97 (s, 1H), 7.90 (s, 1H), 7.54 (t, 1H,  $J = 6.92$  Hz), 7.37–7.43 (m, 2H), 2.84 (s, 3H), 2.58 (s, 3H); ESI-MS:  $m/z$  276  $[\text{M}+\text{H}]^+$ .

**10-Bromo-9-methyl-6H-chromeno[4, 3-b]quinolin-6-one 9h**: Pale brown fluffy solid, m.p. 263–65°C. IR (KBr): 2923, 2855, 1738, 1607  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.08 (s, 1H), 8.68 (d, 1H,  $J = 8.30$  Hz), 8.46 (s, 1H), 7.80 (s, 1H), 7.57 (t, 1H,  $J = 7.35$  Hz), 7.38–7.48 (m, 2H), 2.57 (s, 3H); ESI-MS:  $m/z$  340  $[\text{M}+\text{H}]^+$ .

**11-Ethyl-6H-chromeno[4,3-b]quinolin-6-one 9i**: Pale brown solid, m.p. 227–30°C. IR (KBr): 3443, 3060, 2864, 1730, 1606  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.20 (s, 1H), 8.81 (d, 1H,  $J = 7.53$  Hz), 7.87 (d, 1H,  $J = 7.93$  Hz), 7.77 (d, 1H,  $J = 7.55$  Hz), 7.58–7.63 (m, 2H), 7.43–7.49 (m, 2H), 3.46 (q, 2H,  $J = 7.36$  Hz), 1.5 (t, 3H,  $J = 7.36$  Hz); ESI-MS:  $m/z$  276  $[\text{M}+\text{H}]^+$ .

**9-Isopropyl-6H-chromeno[4,3-b]quinolin-6-one 9j**: Colorless fluffy solid, m.p. 235–28°C. IR (KBr): 3455, 3057, 2922, 1742, 1601  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.19 (s, 1H), 8.78 (d, 1H,  $J = 7.35$  Hz), 8.18 (dd, 1H,  $J = 8.3, 2.26$  Hz), 7.80–7.85 (m, 1H), 7.57–7.62 (m, 2H), 7.39–7.45 (m, 2H), 1.51 (s, 3H), 1.41 (s, 3H), 1.39 (s, 1H);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  160.87, 151.96, 149.53, 147.63, 148.27, 146.18, 139.80, 133.02, 131.46, 128.86, 126.84, 124.55, 124.29, 119.20, 116.73, 115.06, 33.55, 29.21, 23.13; ESI-MS:  $m/z$  290  $[\text{M}+\text{H}]^+$ .

**10-(Trifluoromethyl)-6H-chromeno[4, 3-b]quinolin-6-one 9k**: White fluffy solid, m.p. 267–70°C. IR (KBr): 3066, 2851, 1743, 1607, 1128  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.29 (s, 1H), 8.79 (d, 1H,  $J = 7.72$  Hz), 8.57 (s, 1H), 8.16 (d, 1H,  $J = 8.83$  Hz), 7.80 (d, 1H,  $J = 8.83$  Hz), 7.63 (t, 1H,  $J = 6.62$  Hz), 7.41–7.48 (m, 2H); ESI-MS:  $m/z$  316  $[\text{M}+\text{H}]^+$ .

**9-Methoxy-6H-chromeno[4,3-b]quinolin-6-one 9I:**

Pale brown fluffy solid, m.p. 230-32°C. IR (KBr): 2921, 2822, 1734, 1623, 1219  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.05 (s, 1H), 8.73 (d, 1H,  $J = 8.3$  Hz), 8.54 (s, 1H), 8.14 (d, 1H,  $J = 6.54$  Hz), 7.83 (d, 1H,  $J = 8.33$  Hz), 7.63-7.70 (m, 1H), 7.37-7.45 (m, 1H), 3.98 (s, 3H); ESI-MS:  $m/z$  278  $[\text{M}+\text{H}]^+$ .

**DPPH free radical scavenging activity**

Assay for the scavenging of stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was carried out<sup>7d</sup>. Briefly, in a 96-well micro plate, 25  $\mu\text{L}$  of test sample dissolved in DMSO (1 mg/mL), 100  $\mu\text{L}$  of 0.1 M tris-HCl buffer ( $\text{pH}$  7.4) and 125  $\mu\text{L}$  of 0.5 mM DPPH solution dissolved in absolute ethanol were added. The reaction mixture was shaken well and incubated in the dark for 30 min and read at 517 nm spectrophotometrically (Spectra Max plus 384, Molecular Devices Corporation, Sunnyvale, CA, USA). Percentage of DPPH scavenging was calculated as  $(1 - B/A) \times 100$  where A represents absorbance of control without test samples, and B represents absorbance in presence of test samples.

**ABTS<sup>+</sup> free radical scavenging assay**

Scavenging of the ABTS<sup>+</sup> [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] cation was performed as described by Walker and Everette<sup>9</sup> with suitable modifications. Briefly, 100 mL stock solution of ABTS<sup>+</sup> (0.5 mM) was prepared by addition of 1 mL potassium persulfate (6.89 mM PBS,  $\text{pH}$  8.0). The mixture was stored in the dark for 16 h. Test compounds were dissolved in DMSO (5 mg/mL). Primary screening was carried out by mixing 10  $\mu\text{L}$  of test compounds in 100  $\mu\text{L}$  of methanol followed by 190  $\mu\text{L}$  of ABTS<sup>+</sup> in a 96-well microplate. Absorbance of decolorized ABTS<sup>+</sup> was measured at 734 nm after 15 min incubation in the dark on a BioTek<sup>synergy4</sup> multi-mode microplate reader. For each test sample, a separate blank sample (devoid of ABTS<sup>+</sup>) was used for background subtraction. The percentage of ABTS<sup>+</sup> scavenging was calculated applying following formula: % ABTS<sup>+</sup> scavenging =  $[(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}})/\text{Absorbance}_{\text{control}} \times 100]$ . Various serial dilutions of active compounds were prepared and tested for determination of  $\text{SC}_{50}$  values. Suitable regression analysis was applied for calculation of  $\text{SC}_{50}$ .

 **$\alpha$ -Glucosidase inhibitory assay**

$\alpha$ -Glucosidase inhibitory activity was determined as per our earlier reported method<sup>11</sup>. Rat intestinal acetone

powder in normal saline (100:1; w/v) was sonicated properly and the supernatant was used as a source of crude intestinal  $\alpha$ -glucosidase after centrifugation. In brief, 10  $\mu\text{L}$  of test samples (5 mg/mL DMSO solution) were reconstituted in 100  $\mu\text{L}$  of 100 mM-phosphate buffer ( $\text{pH}$  6.8) in 96-well microplate and incubated with 50  $\mu\text{L}$  of crude intestinal  $\alpha$ -glucosidase for 5 min before 50  $\mu\text{L}$  substrate (5 mM, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside prepared in same buffer) was added. Release of *p*-nitrophenol was measured after 15 min incubation at 405 nm spectrophotometrically (Spectra-Maxplus<sup>384</sup>, Molecular Devices Corporation, Sunnyvale, CA, USA) and 5 min after incubation with substrate. Individual blanks for test samples were prepared to correct background absorbance where substrate was replaced with 50  $\mu\text{L}$  of buffer. Control sample contained 10  $\mu\text{L}$  DMSO in place of test samples. Percentage of enzyme inhibition was calculated as  $1 - [B]/[A] \times 100$  where [A] represents absorbance of control without test samples, and [B] represents absorbance in presence of test samples.

 **$\alpha$ -Amylase inhibitory assay**

The assay method was adapted as per our earlier and modified method<sup>7d</sup> accordingly to suite microplate reading. In brief, 40  $\mu\text{L}$  of test samples (5 mg/mL DMSO) were reconstituted in 160  $\mu\text{L}$  of 20 mM phosphate buffer ( $\text{pH}$  6.9) containing 6.7 mM sodium chloride in 2 mL Eppendorf tubes and incubated with 200  $\mu\text{L}$  of porcine pancreatic  $\alpha$ -amylase (4 U/mL prepared in ice cold distilled water) for 5 min. The reaction was started by the addition of 400  $\mu\text{L}$  soluble potato starch solution (0.5% w/v in 20 mM phosphate buffer,  $\text{pH}$  6.9). Exactly 3 min after incubation, 400  $\mu\text{L}$  DNS color reagent (1.0 g of 3,5-dinitrosalicylic acid, 30 g of sodium potassium tartrate and 20 mL of 2 N NaOH made upto a final volume of 100 mL in distilled water) was added. Closed tubes were placed in a water bath (85-90°C) for 10 min to develop color and cooled thereafter. 50  $\mu\text{L}$  of reaction mixtures was diluted with 175  $\mu\text{L}$  of distilled water in a 96-well microplate.  $\alpha$ -Amylase activity was determined by measuring the absorbance of the mixture at 540 nm spectrophotometrically as above. Individual blanks were prepared to correct for the background absorbance due to test samples. In this case, DNS solution was added in tubes before addition of substrate. The percentage inhibition of enzyme activity was calculated as above. All the tests were run in triplicate for this assay.

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## References

- 1 *Coumarins: Biology, Applications and Mode of Action*, edited by O'Kennedy R & Thomas R D (Wiley, Chichester, UK), **1997**; (b) Murray R D H, Mendez J & Brown S A, *The Natural Coumarins* (Wiley, New York), **1982**.
- 2 Maxwell A, *Mol Microbiol*, 9, **1993**, 681; (b) Musicki B, Periers A M, Laurin P, Ferroud D, Benedetti Y, Lachaud S, Chatreaux F, Haesslein J L, Lltis A, Pierre C, Khider J, Tessol N, Airault M, Demasse J, Dupuis-Hamelin C, Lassaigne P, Bonnefoy A, Vicat P & Klich M, *Bioorg Med Chem Lett*, 10, **2000**, 1695; (c) Chimichi S, Boccalini M, Cosimelli B, Viola G, Vedaldi D & Dall'Acqua F, *Tetrahedron Lett*, 43, **2002**, 7473; (d) Yoakim C, Bonneau P R, De'ziel R, Doyon L, Duan J, Guse I, Landry S, Malenfant E, Naud J, Ogilvie W W, O'Meara J A, Plante R, Simoneau B, Thavonekham B, Bös M & Cordingley M G, *Bioorg Med Chem Lett*, 14, **2004**, 739; (e) Uchiumi F, Hatano T, Ito H, Yoshida T & Tanuma S, *Antiviral Res*, 58, **2003**, 89; (f) Yu D, Suzuki M, Xie L, Morris-Natschke S L & Lee K H, *Med Res Rev*, 23, **2003**, 322; (g) Jeddy A S & Gleason B L, *Ann Pharmacother*, 37, **2003**, 1502; (h) Pineo G & Hull R D, *Hematol Oncol Clin North Am*, 17, **2003**, 201; (i) Rollinger J M, Hornick A, Langer T, Stuppner H & Prast H, *J Med Chem*, 47, **2004**, 6248; (j) Hariprasad V, Talele T T & Kulkarni V M, *Pharm Pharmacol Commun*, 4, **1998**, 365.
- 3 Sharma V K, Saharo P D, Sharma N, Rastogi R C, Ghoshal S K & Mohan D, *Spectrochim Acta A*, 59, **2003**, 1161.
- 4 Raju B C, Saidachary G, Ashok Kumar J & Sridhar B, *Helv Chim Acta*, 94, **2011**, 248.
- 5 Raju B C, Saidachary G & Ashok Kumar J, *Tetrahedron*, 68, **2012**, 6289; (b) Raju B C, Prasad K V, Saidachary G & Sridhar B, *Org Lett*, 16, **2014**, 420; (c) Saidachary G, Prasad K V, Divya D, Singh A, Ramesh U, Sridhar B & Raju B C, *Eur J Med Chem*, 76, **2014**, 460; (d) Saidachary G, Prasad K V, Sairam M & Raju B C, *Tetrahedron Lett*, 55, **2014**, 4753.
- 6 Raju B C & Suman P, *Chem Eur J*, 16, **2010**, 11840.
- 7 Raju B C, Tiwari A K, Kumar J A, Ali A Z, Agawane S B, Saidachary G & Madhusudana K, *Bioorg Med Chem*, 18, **2010**, 358; (b) Kumar J A, Tiwari A K, Ali A Z, Rao R R & Raju B C, *J Heterocycl Chem*, 48, **2011**, 1251; (c) Kumar J A, Tiwari A K, Saidachary G, Kumar D A, Ali A Z, Sridhar B & Raju B C, *Medicinal Chemistry*, 9, **2013**, 806; (d) Kumar J A, Tiwari A K, Saidachary G, Kishor C, Kumar D A, Ali A Z, Sridhar B, Addlagatta A & Raju B C, *Med Chem Res*, 23, **2014**, 2821.
- 8 Naveen M, Rambabu D, Mohan Rao R, Suresh Kumar Ch, Kalle A M, Ramakrishna G, Malla Reddy C, Basaveswara Rao M V & Pal M, *Bioorg Med Chem*, 20, **2012**, 759.
- 9 Walker R B & Everette J D, *J Agri Food Chem*, 57, **2009**, 1156.
- 10 Tiwari A K & Rao J M, *Curr Sci*, 83, **2002**, 30.
- 11 Chinthala Y, Kumar D A, Safaraz A, Singh S P, Arigari N K, Gupta N, Satya K V N S, Kumar, J K, Khan F, Tiwari A K & Paramjit G, *Eur J Med Chem*, 70, **2013**, 308.