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Schiff bases of 3-formylchromone as thymidine phosphorylase inhibitors

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ABSTRACT

3-Formylchromone (**1**), 3-methyl-7-hydroxychromone (**2**) and Schiff bases of 3-formylchromone **3–19** have been synthesized and their anti-thymidine phosphorylase inhibitory activity was evaluated. Compounds **1–19** showed a varying degree of thymidine phosphorylase inhibition with IC_{50} values 19.77 ± 3.25 to 480.21 ± 2.34 μM. Their activity was compared with the standard 7-deazaxanthine (IC_{50} = 39.28 ± 0.76 μM). Compound **12** showed an excellent thymidine phosphorylase inhibitory activity with an IC_{50} value of 19.77 ± 3.25 μM, better than the standard. Compound **4** also showed an excellent inhibitory activity (IC_{50} = 40.29 ± 4.56 μM). The parent 3-formylchromone (**1**) and 3-methyl-7-hydroxychromone (**2**) were found to be inactive. The structures of the compounds were elucidated by using spectroscopic techniques, including ^{1}H NMR, EI MS, IR, UV and elemental analysis.

1. Introduction

Thymidine phosphorylase (EC 2.4.2.4) is an angiogenic factor that exerts its angiogenic effect by stimulating the endothelial cell migration. This has been identified as a potential target in the development of anti-cancer drugs.[1] Thymidine phosphorylase catalyses the hydrolysis (reversible phosphorylation) of thymidine into thymine and 2-deoxyribose-1-phosphate. This enzyme also has a transferase activity, whereby 2-deoxyribose is transferred from the 2′-deoxynucleoside to another base.[2] This enzyme is normally expressed in blood platelets and human placenta, and was found to be produced by different cell types in culture, such as human foreskin fibroblasts and vascular smooth muscle cells. Abnormally the level of thymidine phosphorylase is highly expressed in various pathological disorders including pancreatic, gastric carcinoma, colon carcinoma, uterine sarcoma, uterine leiomuoma, renal carcinoma, breast and lung cancers, astrocytic tumors, cervical intraepithelial neoplasia, gastric carcinoma, carcinomas of the stomach, colon, ovary and bladder, Kaposi sarcoma, atherosclerosis, and in various inflammatory diseases.[3] Anti-thymidine phosphorylase is a form of targeted therapy that uses drugs or other substances to stop tumors from inducing the formation of new blood vessels. Identification of effective inhibitors of thymidine phosphorylase is, therefore, a need for the treatment of various types of neoplastic and non-neoplastic diseases.

Several potent thymidine phosphorylase inhibitors have been reported ranging from micro to nano-molar activities.[4–11] The best well known example is 5-chloro-6-[1-(2-iminopyrrolidinyl) methyl] uracil hydrochloride (thymidine phosphorylase inhibitor) with IC_{50} value of 35 nm.[4] In the subsequent paper from same group (Matsushita et al.) showed that thymidine phosphorylase inhibitor suppresses tumor growth in xenograft nude mice, increased apoptotic index and suppress liver metastasis of KB/TP cells.[5] Based on these studies, thymidine phosphorylase inhibitor was suggested for combination therapy with trifluorothymidine (TFT), an anti-tumor agent that is rapidly inactivated by thymidine phosphorylase.[12] The success of TAS-102 (A combination of TPI and TFT) in phase 1 preclinical trials have encourage us and others to synthesize and evaluate non-nucleoside based compounds for thymidine phosphorylase inhibition that may provide better lead in drug design against thymidine phosphorylase.

The chromone moiety forms an important component of pharmacophores of a number of biologically active molecules of synthetic as well as natural origin. Many of them have useful medicinal applications. Consequently, chromone chemistry continues to draw considerable attention of synthetic organic and medicinal chemists.[13] Chromones are widely distributed in nature, especially in the plant kingdom, and a wide spectrum of useful properties of biological importance are associated with them.[14] Chromone derivatives have been of special interest to organic synthesis due to their biological activities including antimycobacterial, antifungal, anticonvulsant, antimicrobial and mushroom tyrosinase inhibition activities.[15–17] These derivatives also serve as intermediates to many products of fine chemical industries such as pharmaceuticals, agrochemicals and dyestuffs.[18]

As a part of our lead discovery program, we screened a library of 3-formylchromone and its derivatives for their thymidine

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phosphorylase inhibitory activity. Herein we report 3-formylchromone and its derivatives as a new class of potential thymidine phosphorylase inhibitors.

2. Results and discussion

2.1. Chemistry

3-Formylchromone was prepared by the Vilsmeier–Haack synthesis (Scheme 1).[19] Schiff bases of 3-formylchromone were prepared by a condensation reaction of 3-formylchromones (**1**) with a variety of aromatic and aliphatic amines in ethanol (Scheme 2).

The preparation of Schiff's bases was carried out by stirring the mixture of 3-formyl chromone (**1**) (1 mmol) with substituted aromatic and aliphatic amines (1 mmol) in ethanol. The progress of reaction was monitored by TLC. The resulting product was recrystallized from ethanol.

3-Methyl-7-hydroxychromone (**2**) was prepared by reacting resorcinol with ethyl acetoacetate in the presence of phosphorous pentoxide in refluxing ethanol (Scheme 3). In a typical reaction, to resorcinol (4.5 mmol) in dry ethanol (10 ml), ethyl acetoacetate (4.5 mmol) and phosphorus pentoxide (4.5 mmol) was added slowly with intensive stirring with heating. The solid was filtered off and filtrate was evaporated to afford crude 3-methyl-7-hydroxychromone (**2**). The crude solid product was recrystallized from ethanol.

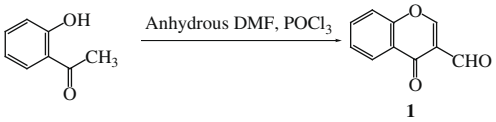
The structures of the synthesized compounds were determined by using spectroscopic techniques which include ^{1}H NMR, EI MS, IR and UV spectroscopy. Elemental analysis results were also found to be satisfactory.

2.2. Biology

3-Formylchromone (**1**), 3-methyl-7-hydroxychromone (**2**) and Schiff's bases of 3-formylchromone **3**–**19** were synthesized and randomly screened for their thymidine phosphorylase inhibitory potential, according to literature protocol.[20]

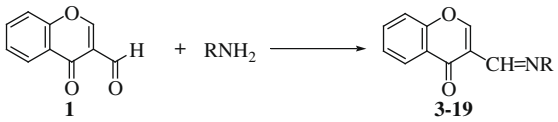
Compounds **1–19** demonstrated a varying degree of thymidine phosphorylase inhibitory activity with IC_{50} values in the range of 19–480 μM, if compared with standard drug 7-deazaxanthine having an IC_{50} value of 39.28 ± 0.76 μM. Compound **12** (IC_{50} = 19.77 ± 3.25 μM) was found to be the most active member of the series, more potent than the standard. Compound **4** also showed an excellent anti-thymidine phosphorylase activity with an IC_{50} = 40.29 ± 4.56 μM. Compounds **9**, **10**, **16** and **17** showed IC_{50} values greater than 100 μM, therefore considered to be inactive. Remaining compounds **1, 2, 7, 8, 11, 13–15, 18** and **19** were found to be largely inactive against the enzyme (Table 1).

Limited SAR study suggests that the activity of compounds largely depends upon the substitution on aromatic ring. Compound **12** with the *para* methyl and *meta* methoxy substitutions on phenyl ring showed a highest degree of thymidine phosphorylase inhibitory activity, whereas compound **4** with the *ortho* and *para* dichloro substitutions on phenyl moiety showed an activity similar to the standard. However, parent compound 3-formylchromone (**1**) and chromone **2** found to be completely inactive. The activity pattern suggest that inhibitory potential of 3-formylchromone (**1**) enhances when it converts into Schiff bases. Compound **13** which is



1

Scheme 1.



1 **3-19**

Scheme 2.

related to compound **12** without a *para*-methyl substitution on aromatic ring, was found to be completely inactive suggesting that a *para*-methyl substitution is essential for activity. Structurally similar compound **6** in which a *meta*-methyl group was present instead of a *meta*-methoxy group and absence of *para*-methyl group on the aromatic ring found to be about 3.5 time less active (IC_{50} = 67.32 ± 3.56 μM) than compound **12** suggesting that methoxy residue on aromatic ring at particular position plays a vital role in thymidine phosphorylase inhibitory activity. Comparison of activity of compounds **4** and **5** demonstrated that the replacement of an *ortho* chloro group with carbonyl phenyl moiety results in a sharp decline in activity.

In conclusion, varyingly substituted Schiff's bases **3**–**19** along with parent 3-formyl chromone (**1**) and 3-methyl-7-hydroxychromone (**2**) were synthesized and screened for their thymidine phosphorylase inhibitory potential. It was found that compounds **4** and **12** posses excellent activities, where compound **12** showed a better inhibitory activity than the standard 7-deazaxanthine, whereas, compound **4** exhibited an activity comparable to the standard. Apparently substitution on the aromatic ring from amine part might be responsible for the thymidine phosphorylase inhibitory activity. Compounds **4** and **12,** most active compounds of this series, can therefore serve as lead compounds for further studies.

2.3. Determination of IC_{50} assay

The concentration of test compounds that inhibited the enzyme activity by 50% (IC_{50} values) were determined by monitoring the effect of increasing concentrations of compounds in the assay described below. The IC_{50} values were calculated from inhibition data by using EZ-Fit Enzyme Kinetic Program (Perrella Scientific Inc., Amherst, USA).

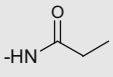
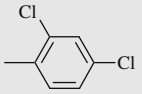
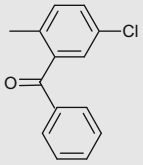
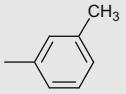
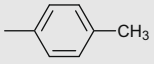
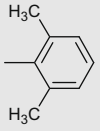
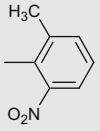
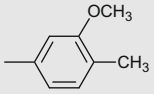
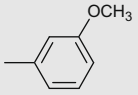
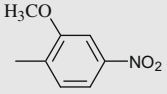
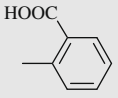
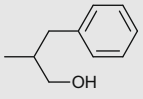
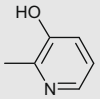
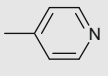
2.4. Thymidine phosphorylase assay protocol

TP/PD-ECGF (*E. coli* thymidine phosphorylase (Sigma T6632) activity was determined by measuring the absorbance at 290 nm spectrophotometrically. The activity was performed as per manufacturer instruction. The original method described by Krenitsky (Krenitsky et al., 1979)[20] was modified. Briefly, total reaction mixture of 200 μl contained 145 μl of potassium phosphate buffer (pH 7.4), 30 μl of enzyme (*E. coli* thymidine phosphorylase (Sigma T6632) at concentration 0.05 and 0.002 U, respectively, were incubated with 5 μl of test materials for 10 min at 25 °C in microplate reader. After incubation, pre read at 290 nm was taken to deduce the absorbance of substrate particles. Substrate (20 μl, 1.5 mM) was dissolved in potassium phosphate buffer was immediately added to plate and continuously read after 10, 20, and 30 min in microplate reader. All assays were performed in triplicate.

3. Experimental

Melting points were determined on a Büchi 434 melting point apparatus and were uncorrected. NMR was performed on a Bruker AM 300, 400 and 500 MHz, respectively. CHN analysis was performed on a Carlo Erba Strumentazion-Mod-1106, Italy. Ultraviolet (UV) spectra were recorded on Perkin–Elmer Lambda-5 UV–vis spectrometer in MeOH. Infrared (IR) spectra were recorded on JASCO

Table 1
Thymidine phosphorylase inhibitory activities of 3-formylchromone (**1**) and its Schiff bases **3**–**19**

Compound No.	R^1	IC_{50} ± SEM[a] (μM)
3		145.56 ± 3.25
4		40.29 ± 4.56
5		75.93 ± 1.28
6		67.32 ± 3.56
7		N.A.[b]
8		N.A.[b]
9		480.21 ± 2.34
10	$-(-CH_2)_2-$	149.78 ± 5.21
11	$-C(CH_3)_3$	N.A.[b]
12		19.77 ± 3.25
13		N.A.[b]
14		N.A.[b]
15		N.A.[b]
16		472.60 ± 3.76
17		340.01 ± 2.78
18		N.A.[b]
19		N.A.[b]
–	7-Deazaxanthine[c]	39.28 ± 0.76

[a] SEM is the standard error of the mean.
[b] N.A.—Not active.
[c] 7-Deazaxanthine standard inhibitor for thymidine phosphorylase.

IR-A-302 Spectrometer as KBr disc. Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel glass plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm or by iodine vapors.

3.1. Procedure for the synthesis of 3-formylchromones (1)

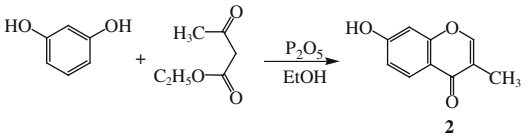
To the dry dimethylformamide (12.32 ml) in a three necked flask, $POCl_3$ (49 mmol) was added slowly with intensive stirring at 50 °C. Heating and stirring was continued for 2 h at 45–55 °C. The solution of 2-hydroxyacetophenone (10 mmol) in DMF (3.57 ml) was then slowly added under stirring at 50 °C. The stirring was continued for 2 h, at 55–60 °C. After cooling, the mixture was kept over night at room temperature and diluted slowly by adding crushed ice (300 g) and stirred again for 6 h. The crystals were filtered off and recrystallized from alcohol.

Yield: 82%; mp = 142 °C; R_f: 0.56 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 203.6 ($\log\varepsilon = 4.41$) nm; IR (KBr): ν_{max} 3057.4 (br, ArCH), 768.1, 1697 (C=O) cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ: 10.18 (s, 1H, CHO), 8.30 (s, 1H, H-2), 8.14 (d, $J_{5,6}$ = 7.7 Hz, 1H, H-5), 7.61 (d, $J_{8,7}$ = 7.6 Hz, 1H, H-8), 7.57 (t, $J_{7,6}$ = $J_{7,8}$ = 7.6 Hz, 1H, H-7), 7.47 (t, $J_{6,7}$ = $J_{6,5}$ = 7.5 Hz, 1H, H-6); EI MS: *m/z* (rel. abund.%) 174 (M^+, 2.3), 145 (100), 120 (31), 104 (53), 92 (31); Anal. Calcd for $C_{10}H_6O_3$: (174) Found: C, 68.96; H, 3.48; Requires: C, 68.97; H, 3.47.

3.2. Procedure for the synthesis of 3-methyl-7-hydroxychromone (2)

3-Methyl-7-hydroxychromone (**2**) was prepared by reacting resorcinol with ethyl acetoacetate in the presence of phosphorous pentaoxide in refluxing ethanol (Scheme 3). In a typical reaction, to resorcinol (4.5 mmol) in dry ethanol (10 ml), ethyl acetoacetate (4.5 mmol) and phosphorus pentaoxide (4.5 mmol) was added into slowly with intensive stirring with heating. Refluxing and stirring was continued for 2 h for completion of reaction, the progress of reaction was monitored by TLC. The solid was filtered off and filtrate was evaporated to afford solid crude 3-methyl-7-hydroxychromone (**2**). The crude product was recrystallized from alcohol.

Yield = 82%; mp = 142 °C; R_f = 0.48 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 322.4 ($\log\varepsilon = 4.8$) nm; IR (KBr): ν_{max} 1721.5, 3424.2 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.62 (d, $J_{5,6}$ = 8.7 Hz, 1H, H-5), 6.81 (dd, $J_{6,8}$ = 2.3 Hz, $J_{6,5}$ = 8.7 Hz, 1H, H-6), 6.70 (s, 1H, H-8), 6.10 (s, 1H, H-2), 2.41 (s, 3H, CH_3), EI MS: *m/z* (rel. abund.%) 176 (M^+, 90.67), 148 (100), 120 (16.66), 105 (3.22), 91 (28.22); Anal. Calcd for $C_{10}H_8O_3$: (176) Found: C, 68.19; H, 4.56. Requires: C, 68.18; H, 4.58.



Scheme 3.

4. General procedure for the synthesis of Schiff bases of 3-formylchromones 3–19

The preparation of Schiff bases was carried out by stirring the mixture of 3-formylchromone (**1**) (1 mmol) with substituted aromatic and aliphatic amines (1 mmol) in ethanol 2–4 h at 50–100 °C. The progress of reaction was monitored by TLC. The resulting products were recrystallized from alcohol to afford the title compounds **3–19**.

4.1. *N*′-[-(4-Oxo-4*H*-chromen-3-yl) methylidene] propanohydrazide (3)

Yield = 46%; mp = 278 °C; R_f = 0.42 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 200.1 ($\log\varepsilon$ = 4.15) nm; IR (KBr): ν_{max} 3447.8 (NH), 3050 (ArCH), 2924 (CH_3), 2862 (CH_2), 1703 (C=O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ: 10.43 (s, 1H, N–H), 8.29 (s, 1H, H-2), 8.18 (d, $J_{5,6}$ = 8.4 Hz, 1H, H-5), 7.81 (td, $J_{7,5}$ = 1.5 Hz, $J_{7,6}$ = $J_{7,8}$ = 8.4 Hz, 1H, H-7), 7.64 (d, $J_{8,7}$ = 8.4 Hz, 1H, H-8), 7.52 (td, $J_{6,8}$ = 1.5, $J_{6,5}$ = $J_{6,7}$ = 8.4 Hz, 1H, H-6), 4.50 (s, 1H, CH=N), 2.31–2.36 (m, 2H, CH_2), 1.20 (t, $J_{3',2'}$ = 7.6 Hz, 3H, CH_3); EI MS: *m/z* (rel. abund.%) 244 (M^+, 12), 188 (12), 172 (100), 120 (93), 92 (26), 92 (31); Anal. Calcd for $C_{13}H_{12}N_2O_3$: (244) [Found: C, 69.14; H, 5.37; N, 5.77. Requires: C, 69.12; H, 5.39; N, 5.76].

4.2. 3-{[(2, 4-Dichlorophenyl)imino]methyl}-4*H*-chromen-4-one (4)

Yield = 52%; mp = 132 °C; R_f = 0.48 (EtOAc:Hex, 3:7); UV (MeOH); λ_{max} 207 ($\log\varepsilon$ = 4.19) nm; IR (KBr): ν_{max} 1647.1 (C=O), 1594 (Ar) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ: 8.70 (s, 1H, H-2), 7.71 (s, 1H, H-3′), 7.40–7.90 (m, 4H, H-5,6,7,8), 7.03 (d, $J_{5',6'}$ = 7.5 Hz, 1H, H-5′), 6.78 (d, $J_{6',5'}$ = 7.5 Hz, 1H, H-6′), 5.90 (s, 1H, CH=N); EI MS: *m/z* (rel. abund.%) 316.8 (M^+, 48.92), 281.8 (62.60), 213 (3.97), 172 (100), 146 (24.53), 120.9 (47.83), 91.9 (31.69); Anal. Calcd for $C_{16}H_9Cl_2NO_2$: (387) [Found: C, 60.42; H, 2.83; N, 22.87. Requires: C, 60.40; H, 2.85; N, 22.89].

4.3. 3-{[(2-Benzoyl-4-chlorophenyl) imino] methyl}-4*H*-chromen-4-one (5)

Yield = 76%; mp = 138 °C; R_f = 0.52 (EtOAc:Hex, 3:7); UV (MeOH); λ_{max} 229.4 ($\log\varepsilon$ = 4.0) nm; IR (KBr): ν_{max} 3066 (br, CH), 2920 (CH), 1645.3 (C=O), 1291.1 (CN) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.51 (s, 1H, H-2), 8.29 (dd, $J_{5,7}$ = 1.6, $J_{5,6}$ = 7.9 Hz, 1H, H-5), 7.93 (t, $J_{7,8}$ = $J_{7,6}$ = 7.3 Hz, 1H, H-7), 7.81 (d, $J_{6',5'}$ = 7.3 Hz, 1H, H-6′), 7.71–7.78 (m, 5H, H-8,3′,2″,4″,6″), 7.44–7.48 (m, 3H, Ar-H-6,3″,5″), 7.36 (d, $J_{5',6'}$ = 7.3 Hz, 1H, H-5′), 6.91 (s, 1H, CH=N); EI MS: *m/z* (rel. abund.%) 386 (M^+, 27), 309 (8), 120 (31), 281 (3), 229 (31), 151 (22), 104 (53), 77 (100); Anal. Calcd for $C_{23}H_{14}ClNO_3$: (387) [Found: C, 71.22; H, 3.65; N, 3.62. Requires: C, 71.23; H, 3.64; N, 3.61].

4.4. 3-{[(3-Methylphenyl) imino] methyl}-4*H*-chromen-4-one (6)

Yield = 60%; mp = 116 °C; R_f = 0.46 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 193 ($\log\varepsilon$ = 4.2) nm; IR (KBr): ν_{max} 1646.4 (C=O), 1602.5 (C=N), 3064.9 (Ar C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.50 (s, 1H, H-2), 7.71 (dd, $J_{5,7}$ = 1.5 Hz, $J_{5,6}$ = 8.4 Hz, 1H, H-5), 7.49 (t, $J_{7,6}$ = $J_{7,8}$ = 8.4 Hz, 1H, H-7), 7.31 (d, $J_{6',5'}$ = 7.2 Hz, 1H, H-6′), 7.28 (s, 1H, H-2′), 7.11 (dd, $J_{5',6'}$ = 0.9 Hz, $J_{5',4'}$ = 5.8 Hz, 1H, H-5′), 7.04 (d, $J_{4',5'}$ = 6.2 Hz, 1H, H-4′), 6.91 (d, $J_{8,7}$ = 8.4 Hz, 1H, H-8), 6.80 (t, $J_{6,5}$ = $J_{6,7}$ = 8.4 Hz, 1H, H-6), 5.71 (s, 1H, CH=N), 2.30 (s, 3H, CH_3); EI MS: *m/z* (rel. abund.%) 262 (M^+, 10.1), 145 (60.9), 106 (100), 91 (34); Anal. Calcd for $C_{17}H_{13}NO_2$: (263) [Found: C, 77.54; H, 4.96; N, 5.34. Requires: C, 77.55; H, 4.98; N, 5.32].

4.5. 3-{[(4-Methylphenyl)imino]methyl}-4*H*-chromen-4-one (7)

Yield = 52%; mp: 118 °C; R_f = 0.38 (EtOAc/Hex, 5:5); UV (MeOH); λ_{max} 387.4 ($\log\varepsilon$ = 4.8) nm; IR (KBr): ν_{max} 3066 (Aromatic CH), 2920 (CH), 1645.3 (C=O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ: 8.50 (s, 1H, H-2), 7.42–7.53 (m, 4H, H-5,6,7, 8), 7.07 (d, $J_{2',3'}$ = $J_{6',5'}$ = 7.2 Hz, 2H, H-2′,6′), 7.00 (d, $J_{3',2'}$ = $J_{5',6'}$ = 7.2 Hz, 2H, H-3′,5′), 6.9 (s, 1H, CH=N), 2.35 (s, 3H, CH_3); EI MS: *m/z* (rel. abund.%) 263 (M^+, 100), 248 (6.68), 172 (80.17), 146 (86.26), 120 (29.57), 105 (60.21), 91 (82.82); Anal. Calcd for $C_{17}H_{13}NO_2$: (263) [Found: C, 77.54; H, 4.96; N, 5.33. Requires: C, 77.55; H, 4.98; N, 5.32].

4.6. 3-{[(2,6-Dimethylphenyl)imino]methyl}-4*H*-chromen-4-one (8)

Yield = 53%; mp = 84 °C; R_f = 0.61(EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 203.2 ($\log\varepsilon$ = 4.2) nm; IR (KBr): ν_{max} 3066 (ArCH), 2920 (Aliphatic CH), 1645.3 (C=O), 1291.1 (C–N) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ: 8.31 (s, 1H, H-2), 8.18 (d, $J_{5,6}$ = 8.4 Hz, 1H, H-5) 7.81 (td, $J_{7,5}$ = 1.4 Hz, $J_{7,6}$ = $J_{7,8}$ = 8.4 Hz, 1H, H-7), 7.64 (d, $J_{8,7}$ = 8.4 Hz, 1H, H-8), 7.52 (t, $J_{6,5}$ = $J_{6,7}$ = 8.4 Hz, 1H, H-6), 7.07 (d, $J_{3',4'}$ = $J_{5',4'}$ = 8.5 Hz, 2H, H-3′, 5′), 6.56 (t, $J_{4',3'}$ = $J_{4',5'}$ = 8.5 Hz, 1H, H-4′), 4.50 (s, 1H, CH=N), 2.35 (s, 6H, $2CH_3$); EI MS: *m/z* (rel. abund.%) 276.9 (M^+, 64.62), 261.8 (22.85), 172 (57.44), 145.9 (40.40), 120 (100), 105 (67.30); Anal. Calcd for $C_{18}H_{15}NO_2$: (277) [Found: C, 77.95; H, 5.46; N, 5.06. Requires: C, 77.96; H, 5.45; N, 5.05].

4.7. 3-{[(2-Methyl-6-nitrophenyl) imino] methyl}-4*H*-chromen-4-one (9)

Yield = 63%; mp = 145 °C; R_f = 0.46 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 216 ($\log\varepsilon$ = 4.24) nm; IR (KBr): ν_{max} 1697 (C=O), 3050 (ArCH), 2924.1 (CH_3) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ: 8.19 (s, 1H, H-2), 7.81 (dd, $J_{5,7}$ = 1.9, $J_{5,6}$ = 8.2 Hz, 1H, H-5), 7.62 (d, $J_{5',4'}$ = 8.5 Hz, 1H, H-5′), 7.47 (dt, $J_{6,8}$ = 1.5, $J_{6,5}$ = $J_{6,7}$ = 8.2 Hz, 1H, H-6), 7.28 (d, $J_{8,7}$ = 7.1 Hz, 1H, H-8), 6.95 (dd, $J_{3',5'}$ = 2.3, $J_{3',4'}$ = 7.1 Hz, 1H, H-3′), 6.93 (s, 1H, CH=N), 6.51 (t, $J_{7,6}$ = $J_{7,8}$ = 7.1 Hz, 1H, H-7), 6.30 (d, $J_{4',3'}$ = 6.2 Hz, 1H, H-4′); EI MS: *m/z* (rel. abund.%) 292 (M^+, 13), 172 (100), 248 (6), 145 (9), 120 (14), 105 (5), 92 (32); Anal. Calcd for $C_{17}H_{12}N_2O_4$: (308) [Found: C, 66.22; H, 3.93; N, 9.08. Requires: C, 66.23; H, 3.92; N, 9.09].

4.8. 3-{[(2-{[-(4-Oxo-4*H*-chromen-3-yl)methylidene]amino} ethyl)imino] methyl}-4*H*-chromen-4-one (10)

Yield = 23%; mp = 188 °C; R_f = 0.35 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 353 ($\log\varepsilon$ = 4.12) nm; IR (KBr): ν_{max} 1646.1 (C=O), 1677.1 (C=N), 3073.7 (Ar C=C) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ: 8.20 (s, 2H, H-2/2′), 7.76 (dd, $J_{5,7}$ = $J_{5',7'}$ = 3.1 Hz, $J_{5,6}$ = $J_{5',6'}$ = 7.6 Hz, 2H, H-5/5′), 7.36 (t, $J_{7,6}$ = $J_{7,8'}$ = $J_{7',6'}$ = $J_{7',8'}$ = 7.6 Hz, 2H, H-7/7′), 6.98 (d, $J_{8,7}$ = $J_{8',7'}$ = 8.1 Hz, 2H, H-8/8′), 6.83 (t, $J_{6,5}$ = $J_{6,7}$ = $J_{6',5'}$ = $J_{6',7'}$ = 7.4 Hz, 2H, H-6/6′), 6.80 (s, 2H, CH=N), 3.89 (s, 4H, (-CH_2CH_2); EI MS: *m/z* (rel. abund.%) 372 (M^+, 8.65), 252 (2.49), 216 (80.41), 187 (97.81), 121 (80.22); Anal. Calcd for $C_{22}H_{16}N_2O_4$: (372) [Found: C, 70.94; H, 4.34; N, 7.50. Requires: C, 70.96; H, 4.33; N, 7.52].

4.9. 3-[Bis(*tert*-butylamino)methyl]-4*H*-chromen-4-one (11)

Yield = 46%; mp = 138 °C; R_f = 0.45 (EtOAc/Hex, 5:5); UV (MeOH); λ_{max} 202.6 ($\log\varepsilon$ = 4.3); IR (KBr): ν_{max} 1641.1 (C=N),

2972 (Ar CH), 3074 (ArCH) cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ: 8.30 (s, 1H H-2), 7.98 (dd, $J_{5,7}$ = 1.0 Hz, $J_{5,6}$ = 7.6 Hz, 1H, H-5), 7.55 (td, $J_{7,5}$ = 1.5, $J_{7,6}$ = $J_{7,8}$ = 7.8 Hz, 1H, H-7), 7.29 (d, $J_{8,7}$ = 7.8 Hz, 1H, H-8), 7.11 (t, $J_{6,8}$ = 3.1, $J_{6,7}$ = $J_{6,5}$ = 7.8 Hz, 1H, H-6), 5.80 (s, 1H, CH=N), 1.54 (s, 9H, $3CH_3$); EI MS: *m/z* (rel. abund.%) 229 (M^+, 16), 200 (47), 172 (75), 121 (96); Anal. Calcd for $C_{14}H_{15}NO_2$: (229) [Found: C, 73.32; H, 6.58; N, 6.12. Requires: C, 73.34; H, 6.59; N, 6.11].

4.10. 3-{[(3-Methoxy-4-methylphenyl) imino] methyl}-4*H*-chromen-4-one (12)

Yield = 72%; mp = 86 °C; R_f = 0.42 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 307.6 ($\log\varepsilon$ = 4.5) nm; IR (KBr): ν_{max} 1648.5 (C=O), 909.7 (ArCH) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ: 8.50 (s, 1H, H-2), 7.42–7.90 (m, 4H, H-5,6,7,8), 7.03 (d, $J_{5',6'}$ = 7.5 Hz, 1H, H-5′), 6.93 (s, 1H, H-2′), 6.72 (d, $J_{6',5'}$ = 7.5 Hz, 1H, H-6′), 6.14 (s, 1H, CH=N), 3.7 (s, 3H, OCH_3), 1.27 (s, 3H, CH_3); EI MS: *m/z* (rel. abund.%) 292 (M^+, 100), 276 (41.09), 188 (18.29), 172 (57.53), 145.8 (55.69), 120.8 (54.16), 90.9 (11.32); Anal. Calcd for $C_{18}H_{15}NO_3$: (293) [Found: C, 73.70; H, 5.16; N, 4.80. Requires: C, 73.71; H, 5.15; N, 4.78].

4.11. 3-{[(3-Methoxyphenyl)imino]methyl}-4*H*-chromen-4-one (13)

Yield = 43%; mp = 178 °C; R_f = 0.35 (EtOAc/Hex, 5:5); UV (MeOH); λ_{max} 204 ($\log\varepsilon$ = 4.5) nm; IR (KBr): ν_{max} 1205.3 (O-CH_3 stretch), 908.9 (ArCH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.82 (s, 1H, H-2), 7.97 (dd $J_{5,7}$ = 1.5 Hz, $J_{5,6}$ = 8.8 Hz, 1H, H-5), 7.52 (t, $J_{7,6}$ = $J_{7,8}$ = 7.5 Hz, 1H, H-7), 7.28 (d, $J_{6',5'}$ = 7.2 Hz, 1H, H-6′), 7.09 (d, $J_{8,7}$ = 8.3 Hz, 1H, H-8), 6.92 (t, $J_{6,5}$ = $J_{6,7}$ = 7.5 Hz, 1H, H-6), 6.78 (s, 1H, H-2′), 6.75 (t, $J_{5',4'}$ = $J_{5',6'}$ = 8.3 Hz, 1H, H-5′), 6.65–6.73 (m, 1H, H-4′), 6.51 (s, 1H, CH=N), 4.01 (s, 3H, OCH_3); EI MS: *m/z* (rel. abund.%) 278 (M^+, 52.6), 264 (4), 250 (43.85), 120 (77); Anal. Calcd for $C_{17}H_{13}NO_3$: (279) [Found: C, 73.14; H, 4.66; N, 5.03. Requires: C, 73.11; H, 4.69; N, 5.02].

4.12. 3-{[(2-Methoxy-4-nitrophenyl) imino] methyl}-4*H*-chromen-4-one (14)

Yield = 63%; mp = 268 °C; R_f = 0.46 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 217.2 ($\log\varepsilon$ = 5.0) nm; IR (KBr): ν_{max} 1655.5 (C=O), 1589.7 (C=N), 3100.2 (ArCH) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.50 (s, 1H, H-2), 8.21 (d, $J_{5,6}$ = 8.1 Hz, 1H, H-5), 7.72 (d, $J_{5',6'}$ = 8.5 Hz, 1H, H-5′), 7.70 (t, $J_{7,6}$ = $J_{7,8}$ = 7.5 Hz, 1H, H-7), 7.61 (s, 1H, H-3′), 7.43 (d, $J_{8,7}$ = 8.1 Hz, 1H, H-8), 7.42 (s, 1H, CH=N), 7.40 (t, $J_{6,5}$ = $J_{6,7}$ = 8.1 Hz, 1H, H-6), 6.61 (d, $J_{6',5'}$ = 8.5 Hz, 1H, H-6′), 3.90 (s, 3H, OCH_3); EI MS: *m/z* (rel. abund.%) 323 (M^+, 9.39), 308 (41.6), 262 (19.87), 234 (3.68), 172 (100), 120 (60.01), 91 (60); Anal. Calcd for $C_{17}H_{12}N_2O_5$: (324) [Found: C, 62.98; H, 3.71; N, 8.62. Requires: C, 62.96; H, 3.73; N, 8.64].

4.13. 2-{[-(4-Oxo-4*H*-chromen-3-yl) methylidene] amino}benzoic acid (15)

Yield = 64%; mp = 96 °C; R_f = 0.42 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 232.1 ($\log\varepsilon$ = 4.3) nm; IR (KBr): ν_{max} 1646.4 (C=O), 1680.1 (C=N), 3065.9 (ArCH) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.51 (s, 1H, H-2), 8.28 (d, $J_{5,6}$ = 7.6 Hz, 1H, H-5), 8.23 (d, $J_{3',4'}$ = 7.3 Hz, 1H, H-3′), 7.80 (dd, $J_{5',6'}$ = 1.3 Hz, $J_{5',4'}$ = 7.6 Hz, 1H, H-5′), 7.49 (t, $J_{7,8}$ = $J_{7,6}$ = 8.5 Hz, 1H, H-7), 7.41 (t, $J_{6,5}$ = $J_{6,7}$ = 7.2 Hz, 1H, H-6), 7.30 (t, $J_{4',3'}$ = $J_{4',5'}$ = 8.3 Hz, 1H, H-4′), 7.01 (d, $J_{8,7}$ = 7.2 Hz, 1H, H-8), 6.63 (s, 1H, CH=N), 6.61 (d, $J_{6',5'}$ = 7.6 Hz, 2H, H-6′); EI MS: *m/z* (rel. abund.%) 292 (M^+, 22.04), 188 (1.05), 172 (58.29), 120 (96.35), 91.9 (100); Anal. Calcd for $C_{17}H_{11}NO_4$: (293) [Found: C, 69.73; H, 3.80; N, 4.75. Requires: C, 69.62; H, 3.78; N, 4.78].

4.14. 3-{[(1-Benzyl-2-hydroxyethyl)imino]methyl}-4*H*-chromen-4-one (16)

Yield = 63%; mp = 110 °C; R_f = 0.52 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 206.8 ($\log\varepsilon$ = 4.12) nm; IR (KBr): ν_{max} 3066 (ArCH), 2920 (CH), 1645.3 (C=O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ: 8.01 (s, 1H, H-2), 7.50–8.24 (m, 4H, ArH), 7.02–7.40 (m, 4H, H-5,6,7,8), 6.56 (s, 1H, CH=N), 3.57–3.85 (m, 1H, CH_2CHCH_2–OH), 3.07 (d, $J_{1',2'}$ = 5.5 Hz, 2H, CH_2CHCH_2–OH), 2.91 (m, 2H, CH_2–$CHCH_2OH$), EI MS: *m/z* (rel. abund.%) 307 (M^+, 5.16), 276 (16.85), 215.9 (41.09), 172 (23.12), 159 (50.14), 145.9 (14.37), 120 (59.07), 105 (16.32); Anal. Calcd for $C_{19}H_{17}NO_3$: (307) [Found: C, 75.49; H, 5.57; N, 5.65. Requires: C, 74.25; H, 5.58; N, 4.56].

4.15. 3-{[(3-Hydroxy-2-pyridinyl)imino]methyl}-4*H*-chromen-4-one (17)

Yield = 62%; mp = 144 °C; R_f = 0.5 (EtOAc/Hex, 3:7); UV (MeOH); λ_{max} 209.4 ($\log\varepsilon$ = 4.3) nm; IR (KBr): ν_{max} 1645.3 (C=O), 1291.1 (C–N), 3066 (ArCH) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.30 (s, 1H, OH), 8.63 (d, $J_{6',5'}$ = 7.2 Hz, 1H, H-6′), 8.30 (s, 1H, H-2), 7.49 (t, $J_{7,8}$ = $J_{7,6}$ = 8.1 Hz, 1H, H-7), 7.37 (d, $J_{4',5'}$ = 7.2 Hz, 1H, H-4′), 7.22 (d, $J_{5,6}$ = 7.5 Hz, 1H, H-5), 7.09 (t, $J_{6,5}$ = $J_{6,7}$ = 7.5 Hz, 1H, H-6), 7.04 (d, $J_{8,7}$ = 8.1 Hz, 1H, H-8), 6.95 (t, $J_{5',6'}$ = $J_{5',4'}$ = 7.2 Hz, 1H, H-5′), 6.93 (s, 1H, CH=N); EI MS: *m/z* (rel. abund.%) 265 (M^+, 28), 262 (7), 248 (3), 237 (24), 210 (7), 172 (48), 146 (4), 120 (30), 95 (88), 77 (16); Anal. Calcd for $C_{15}H_{10}N_2O_3$: (265) [Found: C, 67.69; H, 4.0; N, 10.30. Requires: C, 67.67; H, 3.79; N, 10.52].

4.16. 3-[(4-Pyridinylimino) methyl]-4*H*-chromen-4-one (18)

Yield = 60%; mp = 140 °C; R_f = 0.34 (EtOAc/Hex, 5:5); UV (MeOH); λ_{max} 201.9 ($\log\varepsilon$ = 4.4) nm; IR (KBr): ν_{max} 1645.3 (C=O), 1284.4 (C–N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.68 (s, 1H, CH=N), 8.52 (s, 1H, H-2), 8.17 (s, 1H, H-2′), 7.91 (d, $J_{4',5'}$ = 7.2 Hz, 1H, H-4′), 7.72 (t, $J_{7,8}$ = $J_{7,6}$ = 7.5 Hz, 1H, H-7), 7.58 (t, $J_{5',4'}$ = $J_{5',6'}$ = 7.2 Hz, 1H, H-5′), 7.47 (d, $J_{6',5'}$ = 7.2 Hz, 1H, H-6′), 7.22 (d, $J_{5,6}$ = 7.5 Hz, 1H, H-5), 7.12 (t, $J_{6,5}$ = $J_{6,7}$ = 7.5 Hz, 1H, H-6), 7.02 (d, $J_{8,7}$ = 8.4 Hz, 1H, H-8); EI MS: *m/z* (rel. abund.%) 249 (M^+, 62), 172 (55), 146 (33), 120 (38); Anal. Calcd for $C_{15}H_{10}N_2O_2$: (249) [Found: C, 71.93; H, 4.04; N, 11.18. Requires: C, 71.99; H, 4.03; N, 11.19].

4.17. 3-[(3-Pyridinylimino) methyl]-4*H*-chromen-4-one (19)

Yield = 62%; mp = 140 °C; R_f = 0.35 (EtOAc/Hex, 5:5); UV (MeOH); λ_{max} 201.4 ($\log\varepsilon$ = 4.3) nm; IR (KBr): ν_{max} 1645.3 (C=O), 1291.1 (C-N), 3066 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.68 (s, 1H, H-1′), 8.50 (s, 1H, H-2), 7.98 (d, $J_{3',2'}$= $J_{5',6'}$ = 7.3 Hz, 2H, H-3′,5′), 7.72 (t, $J_{7,8}$ = $J_{7,6}$ = 7.5 Hz, 1H, H-7), 7.37 (d, $J_{2',3'}$= $J_{6',5'}$ = 7.3 Hz, 2H, H-2',6′), 7.22 (d, $J_{5,6}$ = 7.5 Hz, 1H, H-5), 7.12 (t, $J_{6,5}$ = $J_{6,7}$ = 7.5 Hz, 1H, H-6), 7.02 (d, $J_{8,7}$ = 8.4 Hz, 1H, H-8), EI MS: *m/z* (rel. abund.%) 249 (M^+, 62), 172 (55), 146 (33), 120 (38); Anal. Calcd for $C_{15}H_{10}N_2O_2$: (249) [Found: C, 71.93; H, 4.04; N, 11.18; Requires: C, 71.99; H, 4.03; N, 11.19].

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