Synthesis and structure elucidation of polyphenols containing the *N′*-methyleneformohydrazide scaffold as aurora kinase inhibitors

Short title: Examination of *N′*-methyleneformohydrazide-containing polyphenols

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Introduction

Aurora kinases play important roles in mitosis and cell division.[1] Three kinases family has been known as aurora A (aurA), B (aurB), and C (aurC). They have conserved catalytic domains, but their function and localization are not the same.[2-4] AurA localizes to spindle poles in mitosis and is essential to centrosome maturation and separation. AurB localizes to the microtubules interlinking at the spindle and is required for phosphorylation of histone H3.[5,6] Unlike aurA and aurB, aurC has not been studied well. It was found aurA and aurB were overexpressed in colon and breast tumors.[7,8] Other researches revealed aurA was overexpressed in gastric, ovarian, and pancreatic tumors.[9-12] Similar results were found from aurB.[5] Because their overexpression associates with tumorigenesis, they are targeted for cancer therapy.[2,13,14] Of many possible ways to inhibit the enzymatic activities of aurora kinases, one is to use small compounds as inhibitors. Several effective inhibitors have been known as follows: *N*-[(3*Z*)-2-oxo-3-[phenyl-[4-(piperidin-1-ylmethyl)anilino]methylidene]-1*H*-indol-5-yl]ethanesulfonamide (named as hesperadine, Suppl. Fig. 1A), *N*-(2-chlorophenyl)-4-[[2-[[4-[2-(4-ethyl-1-piperazinyl)-2-oxoethyl]phenyl]amino]-5-fluoro-4-pyrimidinyl]amino]-benzamide (named as TC-S7010, Suppl. Fig. 1B), *N*-[4-[[6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-yl]amino]phenyl]benzamide (named as ZM447439, Suppl. Fig. 1C), and *N*-[4-[4-(4-methylpiperazin-1-yl)-6-[(5-methyl-1*H*-pyrazol-3-yl)amino]pyrimidin-2-yl]sulfanylphenyl]cyclopropanecarboxamide (named as VX-680, Suppl. Fig. 1D).[13-16] Besides, it has been reported flavones [Fig. 1A] inhibit aurB.[17] Benzochalcones bearing pyrazoline moieties [Fig. 1B] show the inhibitory effects on aurora kinases.[18] Chromenylchalcones [Fig. 1C] bind to aurA based on their *in silico* docking study.[19] They all belong to polyphenols. We designed and synthesized 33 polyphenols containing the *N′*-methyleneformohydrazide scaffold based on moieties contained in Figs. 1A, B, and C as listed in Table 1. To confirm whether they show the inhibitory effects on aurA, *in*

vitro kinase assay was performed. Their half-maximal inhibitory concentration (IC_{50}) ranged between 1.14 and 98.93 μM. We report here the assignments of their ^{1}H and ^{13}C NMR data and high-resolution mass spectral data. These data can help us design a series of compounds containing novel aurora kinase inhibitor pharmacophores and identify newly synthesized polyphenols containing the *N′*-methyleneformohydrazide scaffold.

Experimental

General synthetic route

A synthetic scheme outlining the preparation of the polyphenol derivatives containing the *N′*-methyleneformohydrazide scaffold (i.e., derivatives **1**–**33**) is shown in Scheme 1. For the preparation of derivatives **1**–**20**, the intermediate chromone aldehydes **II** were synthesized from substituted 2-hydroxy acetophenones under Vilsmeier reaction conditions. Substituted 2-hydroxy acetophenone (**I**) was treated with an excess of phosphorous oxychloride and anhydrous *N*,*N*-dimethylformamide to afford chromone aldehyde (**II**), which was subsequently treated with benzohydrazide to give the corresponding derivatives **1**–**20**. Resveratrol derived aldehyde **III** was also employed under the same reaction conditions to obtain the desired compounds **21**–**26** and **29**–**30**.[20] Typical synthetic procedures for the preparation of derivatives **9** and **22** are described as follows.

Preparation of (*E*)-*N′*-((6-chloro-4-oxo-4*H*-chromen-3-yl)methylene)-3-hydroxybenzo hydrazide (**9**)

A mixture of anhydrous *N*,*N*-dimethylformamide (15 mL, 193 mmol) and phosphoryl chloride (1.5 mL, 23 mmol) was heated at 60–65 °C for 1.5 h, after which time 2-hydroxy-5-chloroacetophenone (5 mmol, 850 mg) was added, and the resulting mixture stirred at the same temperature for a further 2 h. The reaction progress was monitored by TLC. After

reaching completion, the reaction mixture was cooled to room temperature and poured into ice-water (400 mL), then neutralized using a saturated $NaHCO_3$ solution. This resulted in precipitation of the crude 6-chloro-3-formylchromone product, which was subsequently filtered then purified by recrystallization in ethanol (Yield: 80%; m.p: 166–168 °C). The purified 6-chloro-3-formylchromone (60 mg, 0.3 mmol) was then dissolved in ethanol (10 mL) containing *m*-hydroxybenzohydrazide (45 mg, 0.3 mmol). A catalytic amount of glacial acetic acid was added, and the resulting reaction mixture was heated under reflux at 85 °C for 5 h. After this time, the reaction mixture was cooled to room temperature, the solvent was removed under vacuum, and the resulting residue was purified by column chromatography to give the analytically pure compound **9** (Yield: 77%; m.p: 196–202 °C).

Preparation of (*E*)-*N*′-(2,4-dimethoxy-6-(4-methoxystyryl)benzylidene)-3-methoxybenzo hydrazide (**22**)

(*E*)-2-(4-methoxystyryl)-4,6-dimethoxybenzaldehyde (90 mg, 0.3 mmol) and *m*-anisic hydrazide (48 mg, 0.3 mmol) were dissolved in ethanol (10 mL).[20] To this solution was added a catalytic amount of glacial acetic acid, and the reaction mixture was heated under reflux at 85 °C for 7 h. After this time, the mixture was cooled to room temperature, the crude product was precipitated, and the resulting solid was filtered prior to purification by recrystallization in methanol (Yield: 72%; m.p: 160–164 °C).

NMR spectroscopy

The synthesized polyphenols containing the *N*′-methyleneformohydrazide scaffold were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) to a concentration of ~50 mM and transferred into 2.5 mm NMR tubes. All NMR data were collected using a Bruker AVANCE 400 spectrometer system (9.4 T; Bruker, Karlsruhe, Germany) equipped with a 5-mm inverse

broadband probehead at room temperature, and the chemical shifts were referenced to TMS (0 ppm). ^{1}H NMR experiments were performed using the following parameters: relaxation delay = 1 s, 90° pulse for 11.8 µs, 5500 Hz spectral width, 32 k data points, and 0.34 Hz/point digital resolution. The equivalent parameters for the ^{13}C NMR and DEPT experiments were 3 s, 15.0 µs, 21000 Hz, 64 k, and 0.64 Hz/point, respectively. Two-dimensional COSY, NOESY, HMQC, and HMBC experiments were acquired with data points of 2 k × 256 ($t_2 \times t_1$). The long-range coupling time for HMBC was 70 ms, and the mixing time for NOESY was 1.5 s. Processing and analysis of the NMR data were carried out as previously reported.[21]

General analytical procedures

To confirm the structures of the *N′*-methyleneformohydrazide-containing polyphenols, ultra-performance liquid chromatography-hybrid quadrupole-time-of-flight mass spectrometry (UPLC-TOFMS) was carried out using a Waters ACQUITY UPLC system (Waters, Milford, MA) with the help of Prof. Choong Hwan Lee at Konkuk University, Korea.[22] All high-resolution mass spectrometry (HR/MS) data were collected as $(M-H)^-$ ions.

***In vitro* aurora A kinase assay**

The substrate used *in vitro* human aurora A kinase assay was 200µM LeuArgArgAlaSerLeuGly and the concentration of ATP was 500 cpm/pmol. The detailed procedure followed to the method provided by EMD Millipore's KinaseProfiler service assay protocol (EMD Millipore Corporation, Billerica, MA).[23] TC-S7010 [Suppl. Fig. 1B] was used as a control. The statistical significance was analyzed using Student's t-test. All experiments were performed in triplicate

Results and Discussion

All 33 derivatives containing the *N*′-methyleneformohydrazide scaffold are listed in Table 1. As mentioned previously, these derivatives can be divided into a number of groups. For example, in the structures of derivatives **1**–**17**, the 4*H*-chromenyl group is attached to the *N*-methylene functionality and the carbonyl group bears a substituted phenyl group. Similarly, in derivatives **18**–**20**, the 4*H*-chromenyl group is attached to the *N*-methylene moiety, but the carbonyl group bears a substituted pyridine unit. Furthermore, in derivatives **21**–**26**, both the *N*-methylene and carbonyl groups bear substituted phenyl groups, while in derivatives **27**–**30**, a phenyl group is attached to the *N*-methylene moiety and the carbonyl group bears a substituted pyridine group. Finally, in derivatives **31**–**33**, methyl and phenyl groups are attached to the *N*-methylene functionality, while a substituted phenyl group is found on the carbonyl group. Of the 33 derivatives, the procedure for assigning the structure of derivative **22**, i.e., (*E*)-*N*′-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)-3-methoxybenzohydrazide [Fig. 2], is described here as an example.

A total of 24 signals were observed in the ^{13}C NMR spectrum of **22**, of which 5 were present at ~160 ppm. Four of these peaks (with the exception of the signal at 162.6 ppm) showed long-range couplings with methoxy protons in the HMBC spectrum, allowing the ^{13}C signal at 162.6 ppm to be assigned to the amide carbon, C-1a. In addition, as the two ^{13}C signals at 114.1 and 128.4 ppm exhibited double the intensities of the neighboring signals, they were assigned to the C-3″/C-5″ and C-2″/C-6″ atoms, respectively. The two proton signals at 6.95 and 7.69 ppm were assigned to the H-3″/H-5″ and H2″/H-6″ atoms, respectively. In addition, the ^{13}C peaks at 130.3 and 159.0 ppm exhibiting $^{3}J_{CH}$ couplings with H-3″/H-5″ and H2″/H-6″, respectively, were assigned as C-1″ and C-4″, respectively. The $^{3}J_{CH}$ coupling of C-4″ to a methoxy proton at 3.78 ppm allowed this to be assigned as the

4″-OCH_3 proton, while the ^{13}C peak at 55.1 ppm was assigned to the 4″-OCH_3 carbon atom based on HMQC results. Furthermore, due to the large coupling constants of the two proton signals at 7.20 and 8.51 ppm (i.e., 16.3 Hz), these signals were assigned to the double bond protons of the styryl group. Their corresponding carbon atoms were observed at 130.4 and 126.1 ppm, respectively. As a $^3J_{CH}$ coupling between the former carbon atom and the H2″/H-6″ atoms was observed by HMBC, this carbon atom was assigned as C-1″a. We also observed three correlating proton signals at 7.15, 7.44, and 7.56 ppm in the COSY spectrum, where the signal at 7.56 ppm exhibited a $^3J_{CH}$ coupling with C-1a, and so it was assigned as H-6. In addition, the carbon atom resonating at 117.4 ppm that was attached directly to the proton resonating at 7.15 ppm exhibited a $^3J_{CH}$ coupling to H-6, and so the proton signal at 7.15 ppm was assigned to the H-4 atom. The remaining proton signal at 7.44 ppm was assigned to the H-5 atom, while the carbon signal at 159.2 ppm exhibiting a $^3J_{CH}$ coupling with H-5 was assigned to the C-3 atom. Furthermore, the methoxy proton signal at 3.85 ppm showed a $^3J_{CH}$ coupling with the C-3 atom, and it was assigned as 3-OCH_3. Moreover, the two carbon signals at 112.7 and 134.9 ppm that exhibited $^3J_{CH}$ couplings with H-6 and H-5 were labelled C-2 and C-1, respectively. As expected, the most deshielded proton signal at 11.74 ppm was assigned to the NH group, which also exhibited a $^3J_{CH}$ coupling to the carbon C-1′a signal at 145.1 ppm. The signals corresponding to the C-2′ and C-6′ atoms (at 139.0 and 160.3 ppm) were assigned as such due to their $^3J_{CH}$ couplings with the H-1′a signal at 8.86 ppm. We also observed the COSY correlation of the two protons resonating at 7.00 and 6.58 ppm. Since the former was long-range coupled to the C-1″b signal (126.1 ppm) with $^3J_{CH}$, it was labeled as H-3′. As a result, the proton signal at 6.58 ppm was assigned to the H-5′ atom. Furthermore, as the methoxy proton signal at 3.87 ppm exhibited a $^3J_{CH}$ coupling with the C-6′ signal, it was assigned as 6′-OCH_3. Thus, of the four methoxy protons present, the final proton signal at 3.89 ppm was assigned to the 4′-OCH_3 group. The carbon signal at

161.1 ppm exhibiting a $^{3}J_{CH}$ coupling with this methoxy proton was labeled C-4′. Finally, the carbon signal at 112.8 ppm that exhibited $^{3}J_{CH}$ couplings with H-3′ and H-5′ was assigned to the C-1′ atom. The nOe cross peaks among H-1″b/H-6″, H-5″/4″-OCH_3, H-3′/H-1″a, H-1″b/H-1′a, NH/H-1′a, NH/H-2, and H-2/3-OCH_3 were observed in the NOESY spectrum. The key correlations obtained from the COSY, NOESY, and HMBC spectra of derivative **22** are shown in Fig. 3.

Similar procedures were employed to assign the NMR data of the other derivatives, and the complete assignments of the ^{1}H and ^{13}C NMR data for all derivatives containing the *N*′-methyleneformohydrazide scaffold (see Table 1) are listed in Tables 2 and 3, respectively. To confirm the structures of the various derivatives, HR/MS was carried out, and the calculated molecular masses are listed in Table 1 along with the experimental HR/MS results. All ^{1}H NMR, ^{13}C NMR, and HR/MS spectra are provided in the Supplementary Materials.

The IC_{50} values of 33 derivatives for aurora A kinase ranged from 1.14 to 98.93 μM as listed in Table 1 and they were plotted with error bars [Suppl. Fig. 2]. The IC_{50} value of TC-S7010 [Suppl. Fig. 1B] used as a control was 0.25 μM. Derivative **6**, (*E*)-4-fluoro-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide showed the best inhibitory effect and derivative **21**, (*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)benzohydrazide did the worst effect. We could therefore conclude that the analytical data provided herein allows us to identify the novel *N*′-methyleneformohydrazide-containing derivatives, and *in vitro* kinase assay data on aurora A help us design compounds containing novel aurora kinase inhibitor pharmacophores.

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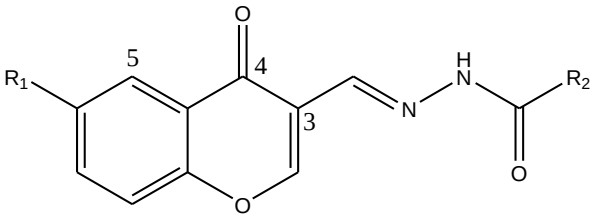
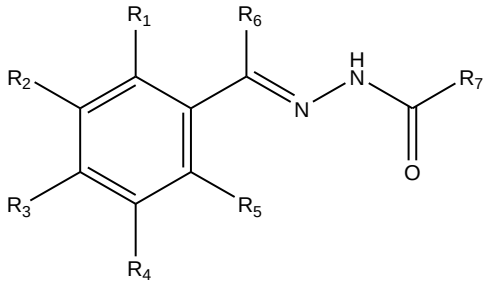
Table 1. Structures, names, HR/MS data, and IC_{50} values of polyphenol derivatives (**1**–**33**) containing the *N*′-methyleneformohydrazide scaffold.



derivative	R_1	R_2	mass (calcd./found)	name	IC_{50}/μM
1	H	Phenyl	291.0770/291.0773	(*E*)-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	2.96
2	H	3-Methoxyphenyl	321.0875/321.0861	(*E*)-3-methoxy-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	2.25
3	H	4-Methoxyphenyl	321.0875/321.0886	(*E*)-4-methoxy-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	2.33
4	H	3-Hydroxyphenyl	307.0719/307.0728	(*E*)-3-hydroxy-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	2.01
5	H	3-Boromophenyl	368.9875/368.9871	(*E*)-3-bromo-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	9.44
6	H	4-Fluorophenyl	309.0675/309.0664	(*E*)-4-fluoro-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	1.14
7	Cl	Phenyl	325.0380/325.0373	(*E*)-*N*'-((6-chloro-4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	11.36
8	Cl	3-Methoxyphenyl	355.0486/355.0479	(*E*)-*N*'-((6-chloro-4-oxo-4*H*-chromen-3-yl)methylene)-3-methoxybenzohydrazide	5.11
9	Cl	3-Hydroxyphenyl	341.0329/341.0313	(*E*)-*N*'-((6-chloro-4-oxo-4*H*-chromen-3-yl)methylene)-3-hydroxybenzohydrazide	1.35

10	Cl	3-Boromophenyl	402.9485/402.9488	(*E*)-3-bromo-*N*'-((6-chloro-4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	16.34
11	Cl	4-Fluorophenyl	343.0286/343.0258	(*E*)-*N*'-((6-chloro-4-oxo-4*H*-chromen-3-yl)methylene)-4-fluorobenzohydrazide	13.28
12	Br	Phenyl	368.9875/368.9876	(*E*)-*N*'-((6-bromo-4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	11.91
13	Br	3-Methoxyphenyl	398.9980/398.9986	(*E*)-*N*'-((6-bromo-4-oxo-4*H*-chromen-3-yl)methylene)-3-methoxybenzohydrazide	2.93
14	Br	3-Hydroxyphenyl	384.9824/384.9816	(*E*)-*N*'-((6-bromo-4-oxo-4*H*-chromen-3-yl)methylene)-3-hydroxybenzohydrazide	1.32
15	Br	3-Boromophenyl	446.8980/446.8990	(*E*)-3-bromo-*N*'-((6-bromo-4-oxo-4*H*-chromen-3-yl)methylene)benzohydrazide	13.89
16	Br	3-Fluorophenyl	386.9781/386.9756	(*E*)-*N*'-((6-bromo-4-oxo-4*H*-chromen-3-yl)methylene)-3-fluorobenzohydrazide	14.83
17	Br	4-Fluorophenyl	386.9781/386.9762	(*E*)-*N*'-((6-bromo-4-oxo-4*H*-chromen-3-yl)methylene)-4-fluorobenzohydrazide	7.98
18	H	3-Pyridinyl	292.0722/292.0712	(*E*)-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)nicotinohydrazide	2.26
19	H	4-Pyridinyl	292.0722/292.0705	(*E*)-*N*'-((4-oxo-4*H*-chromen-3-yl)methylene)isonicotinohydrazide	1.19
20	Cl	3-Pyridinyl	326.0332/326.0304	(*E*)-*N*'-((6-chloro-4-oxo-4*H*-chromen-3-yl)methylene)nicotinohydrazide	2.72



derivative	R_1	R_2	R_3	R_4	R_5	R_6	R_7	mass (calcd./found)	name	IC_{50}/μM
21	OCH_3	H	OCH_3	H	4-Methoxystyryl	H	Phenyl	415.1658/415.1658	(*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)benzohydrazide	98.93
22	OCH_3	H	OCH_3	H	4-Methoxystyryl	H	3-Methoxyphenyl	445.1763/445.1748	(*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)-3-methoxybenzohydrazide	84.16
23	OCH_3	H	OCH_3	H	4-Methoxystyryl	H	4-Methoxyphenyl	445.1763/445.1765	(*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)-4-methoxybenzohydrazide	96.18
24	OCH_3	H	OCH_3	H	4-Methoxystyryl	H	3-Hydroxyphenyl	432.1685/431.1608	(*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)-3-hydroxybenzohydrazide	64.89
25	OCH_3	H	OCH_3	H	4-Methoxystyryl	H	3-Boromophenyl	493.0763/493.0737	(*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)-3-bromobenzohydrazide	82.64
26	OCH_3	H	OCH_3	H	4-Methoxystyryl	H	4-Fluorophenyl	433.1564/433.1534	(*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)-4-fluorobenzohydrazide	98.52
27	H	H	OCH_3	OCH_3	H	H	4-Pyridinyl	284.1035/284.1039	(*E*)-*N*'-(3,4-dimethoxybenzylidene)isonicotinohydrazide	25.29

28	H	OCH_3	H	OCH_3	H	H	4-Pyridinyl	284.1035/284.1037	(*E*)-*N*'-(3,5-dimethoxybenzylidene)isonicotinohydrazide	29.14
29	OCH_3	H	OCH_3	H	4-Methoxystyryl	H	4-Pyridinyl	416.1610/416.1622	(*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)isonicotinohydrazide	73.69
30	OCH_3	H	OCH_3	H	4-Methoxystyryl	H	3-Pyridinyl	416.1610/416.1607	(*E*)-*N*'-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)nicotinohydrazide	89.53
31	H	H	OCH_3	H	H	CH_3	Phenyl	267.1134/267.1134	(*E*)-*N*'-(1-(4-methoxyphenyl)ethylidene)benzohydrazide	34.84
32	H	H	OCH_3	H	H	CH_3	3-Methoxyphenyl	297.1239/297.1234	(*E*)-3-methoxy-*N*'-(1-(4-methoxyphenyl)ethylidene)benzohydrazide	31.15
33	H	H	H	H	OH	CH_3	Phenyl	253.0977/253.0956	(*E*)-*N*'-(1-(2-hydroxyphenyl)ethylidene)benzohydrazide	30.18

Table 2. ^{1}H NMR chemical shifts of the polyphenol derivatives (**1–33**) containing the *N′*-methyleneformohydrazide scaffold. DMSO-d_6 was used as the NMR solvent. Multiplicity and coupling constants are given in parentheses; d and s represent doublet and singlet peaks, respectively. The units of the chemical shifts and coupling constants are ppm and Hz, respectively

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
H2	7.93 (dd, 8.0, 2.1)	7.46 (s)	7.93 (d, 8.8)	7.32 (s)	8.11 (s)	8.00 (dd, 8.8, 5.5)	7.92 (dd, 7.7, 1.7)	7.45 (s)	7.31 (s)	8.10 (s)
H3	7.53 (ddd, 8.0, 7.2, 1.9)	-	7.05 (d, 8.8)	-	-	7.35 (dd, 8.8, 8.6)	7.52 (ddd, 7.7, 7.2, 1.5)	-	-	-
H4	7.59 (dd, 7.2, 2.1)	7.16 (d, 8.1)	-	6.98 (d, 7.9)	7.79 (d, 7.9)	-	7.59 (dd, 7.2, 1.7)	7.15 (d, 8.1)	6.98 (d, 8.2)	7.79 (d, 7.9)
H5	7.53 (ddd, 8.0, 7.2, 1.9)	7.43 (dd, 8.1, 7.8)	7.05 (d, 8.8)	7.31 (dd, 7.9, 7.5)	7.49 (dd, 7.9, 7.8)	7.35 (dd, 8.8, 8.6)	7.52 (ddd, 7.7, 7.2, 1.5)	7.43 (dd, 8.1, 7.8)	7.31 (dd, 8.2, 7.4)	7.49 (dd, 7.9, 7.8)
H6	7.93 (dd, 8.0, 2.1)	7.51 (d, 7.8)	7.93 (d, 8.8)	7.35 (d, 7.5)	7.93 (d, 7.8)	8.00 (dd, 8.8, 5.5)	7.92 (dd, 7.7, 1.7)	7.50 (d, 7.8)	7.34 (d, 7.4)	7.92 (d, 7.8)
H1’a	8.64 (s)	8.64 (s)	8.61 (s)	8.62 (s)	8.62 (s)	8.62 (s)	8.59 (s)	8.61 (s)	8.59 (s)	8.58 (s)
H2’	8.84 (s)	8.83 (s)	8.81 (s)	8.86 (s)	8.84 (s)	8.84 (s)	8.85 (s)	8.83 (s)	8.84 (s)	8.85 (s)
H5’	8.13 (dd, 8.0, 1.7)	8.13 (dd, 8.0, 1.3)	8.13 (dd, 7.9, 1.6)	8.13 (dd, 7.9, 1.8)	8.12 (dd, 7.9, 1.7)	8.12 (dd, 8.0, 1.9)	8.02 (d, 2.6)	8.03 (d, 2.6)	8.03 (d, 2.5)	8.02 (d, 2.6)
H6’	7.55 (ddd, 8.0, 7.1, 1.2)	7.54 (ddd, 8.0, 7.1, 1.1)	7.55 (ddd, 7.9, 7.2, 1.0)	7.54(ddd, 7.9, 7.0, 1.1)	7.54 (ddd, 7.9, 7.0, 0.7)	7.54 (ddd, 8.0, 7.4, 1.0)	-	-	-	-
H7’	7.85 (ddd, 8.5, 7.1, 1.7)	7.85 (ddd, 8.4, 7.1, 1.3)	7.85 (ddd, 8.5, 7.2, 1.6)	7.85 (ddd, 8.4, 7.0, 1.8)	7.85 (ddd, 8.4, 7.0, 1.7)	7.85 (ddd, 8.4, 7.4, 1.9)	7.87 (dd, 9.0, 2.6)	7.88 (d, 9.0, 2.6)	7.87 (dd, 9.0, 2.5)	7.87 (dd, 9.0, 2.6)
H8’	7.72 (dd, 8.5, 1.2)	7.71 (dd, 8.4, 1.1)	7.72 (dd, 8.5, 1.0)	7.71 (dd, 8.4, 1.1)	7.71 (dd, 8.4, 0.7)	7.71 (dd, 8.4, 1.0)	7.77 (d, 9.0)	7.78 (d, 9.0)	7.78 (d, 9.0)	7.78 (d, 9.0)
3-OH	-	-	-	9.78 (s)	-	-	-	-	9.78 (s)	-
3-OCH_3	-	3.83 (s)	-	-	-	-	-	3.83 (s)	-	-
4-OCH_3	-	-	3.83 (s)	-	-	-	-	-	-	-
NH	11.96 (s)	11.91 (s)	11.83 (s)	11.88 (s)	12.01 (s)	11.97 (s)	11.97 (s)	11.91 (s)	11.90 (s)	11.97 (s)

Position	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
H2	8.00 (dd, 8.9, 5.7)	7.91 (dd, 7.7, 1.4)	7.45 (s)	7.30 (s)	8.10 (s)	7.71 (d, 10.2)	7.99 (dd, 8.8, 6.1)	9.07 (d, 2.5)	7.84 (dd, 6.0, 1.6)	9.07 (d, 2.5)
H3	7.36 (dd, 8.9, 8.6)	7.52 (ddd, 7.7, 7.4, 1.0)	-	-	-	-	7.35 (dd, 8.8, 8.7)	-	8.78 (dd, 6.0, 1.4)	-
H4	-	7.59 (dd, 7.4, 1.4)	7.15 (d, 8.0)	6.98 (d, 8.0)	7.79 (d, 8.0)	7.44 (dd, 8.6, 4.4)	-	8.76 (dd, 4.8, 1.7)	-	8.76 (dd, 4.8, 1.7)
H5	7.36 (dd, 8.9, 8.6)	7.52 (ddd, 7.7, 7.4, 1.0)	7.43 (dd, 8.0, 7.8)	7.29 (dd, 8.0, 7.4)	7.49 (dd, 8.0, 7.9)	7.57 (dd, 8.2, 5.7)	7.35 (dd, 8.8, 8.7)	7.56 (dd, 8.0, 4.8)	8.78 (dd, 6.0, 1.4)	7.56 (dd, 8.1, 4.8)
H6	8.00 (dd, 8.9, 5.7)	7.91 (dd, 7.7, 1.4)	7.50 (d, 7.8)	7.34 (d, 7.4)	7.92 (d, 7.9)	7.77 (d, 7.7)	7.99 (dd, 8.8, 6.1)	8.26 (ddd, 8.0, 2.5, 1.7)	7.84 (dd, 6.0, 1.6)	8.26 (ddd, 8.1, 2.5, 1.7)
H1'a	8.59 (s)	8.59 (s)	8.60 (s)	8.58 (s)	8.58 (s)	8.59 (s)	8.57 (s)	8.62 (s)	8.64 (s)	8.64 (s)
H2'	8.85 (s)	8.86 (s)	8.85 (s)	8.83 (s)	8.85 (s)	8.83 (s)	8.84 (s)	8.85 (s)	8.85 (s)	8.87 (s)
H5'	8.04 (d, 2.4)	8.16 (d, 2.1)	8.17 (d, 2.5)	8.16 (d, 2.4)	8.16 (d, 2.5)	8.15 (d, 2.4)	8.15 (d, 2.5)	8.12 (dd, 7.9, 1.8)	8.12 (dd, 7.9, 1.4)	8.04 (d, 2.6)
H6'	-	-	-	-	-	-	-	7.55(ddd, 7.9, 7.2, 1.1)	7.54(ddd, 7.9, 7.0, 0.9)	-
H7'	7.88 (dd, 8.9, 2.4)	7.99 (dd, 8.9, 2.1)	7.99 (dd, 8.9, 2.5)	7.99 (dd, 8.9, 2.4)	7.99 (dd, 8.9, 2.5)	7.98 (dd, 8.9, 2.4)	7.97 (dd, 8.9, 2.5)	7.85 (ddd, 8.4, 7.2, 1.8)	7.85 (ddd, 8.4, 7.0, 1.4)	7.89 (dd, 9.0, 2.6)
H8'	7.79 (d, 8.9)	7.71 (d, 8.9)	7.72 (d, 8.9)	7.71 (d, 8.9)	7.71 (d, 8.9)	7.70 (d, 8.9)	7.70 (d, 8.9)	7.71 (dd, 8.4, 1.1)	7.72 (dd, 8.4, 0.9)	7.79 (d, 9.0)
3-OH	-	-	-	9.79 (s)	-	-	-	-	-	-
3-OCH_3	-	-	3.83 (s)	-	-	-	-	-	-	-
NH	11.98 (s)	11.97 (s)	11.92 (s)	11.90 (s)	12.01 (s)	11.98 (s)	11.97 (s)	12.10 (s)	12.16 (s)	12.12 (s)

Position	21	22	23	24	25	26	27	28	29	30	31	32	33
H2	7.98 (dd, 7.5, 1.7)	7.52 (s)	7.97 (d, 7.8)	7.36 (s)	8.17 (s)	8.06 (dd, 8.9, 5.7)	7.81 (dd, 4.5, 1.5)	7.82 (dd, 4.3, 1.9)	7.89 (dd, 4.4, 1.6)	9.12 (d, 2.4)	7.89 (dd, 8.2, 1.3)	7.41 (s)	7.95 (dd, 7.8, 1.6)
H3	7.53 (ddd, 7.5, 7.1, 1.6)	-	7.06 (d, 7.8)	-	-	7.36 (dd, 8.9, 8.7)	8.78 (dd, 4.5, 1.3)	8.79 (dd, 4.3, 1.8)	8.79 (dd, 4.4, 1.6)	-	7.51 (ddd, 8.2, 7.2, 1.0)	-	7.55 (ddd, 7.8, 7.4, 1.6)
H4	7.59 (dd, 7.1, 1.7)	7.15 (d, 8.2)	-	6.98 (d, 7.8)	7.79 (d, 8.0)	-	-	-	-	8.76 (dd, 4.9, 1.7)	7.57 (dd, 7.2, 1.3)	7.14 (d, 7.8)	7.63 (dd, 7.4, 1.6)
H5	7.53 (ddd, 7.5, 7.1, 1.6)	7.44 (dd, 8.2, 7.8)	7.06 (d, 7.8)	7.31 (dd, 7.8, 7.4)	7.50 (dd, 8.0, 7.9)	7.36 (dd, 8.9, 8.7)	8.78 (dd, 4.5, 1.3)	8.79 (dd, 4.3, 1.8)	8.79 (dd, 4.4, 1.6)	7.56 (dd, 8.0, 4.9)	7.51 (ddd, 8.2, 7.2, 1.0)	7.43 (dd, 7.8, 7.5)	7.55 (ddd, 7.8, 7.4, 1.6)
H6	7.98 (dd, 7.5, 1.7)	7.56 (d, 7.8)	7.97 (d, 7.8)	7.40 (d, 7.4)	7.98 (d, 7.9)	8.06 (dd, 8.9, 5.7)	7.81 (dd, 4.5, 1.5)	7.82 (dd, 4.3, 1.9)	7.89 (dd, 4.4, 1.6)	8.31 (ddd, 8.0, 2.4, 1.7)	7.89 (dd, 8.2, 1.3)	7.47 (d, 7.5)	7.95 (dd, 7.8, 1.6)
H1'a	8.86 (s)	8.86 (s)	8.83 (s)	8.84 (s)	8.86 (s)	8.85 (s)	8.39 (s)	8.40 (s)	8.88 (s)	8.85 (s)	-	-	-
H2'	-	-	-	-	-	-	7.36 (d, 1.8)	6.90 (d, 2.1)	-	-	7.82 (d, 7.9)	7.81 (d, 8.1)	-
H3'	7.00 (d, 1.8)	7.00 (d, 2.0)	6.99 (d, 2.2)	6.99 (d, 2.0)	7.00 (d, 2.3)	7.00 (d, 2.0)	-	-	7.00 (d, 2.2)	7.00 (d, 2.5)	6.99 (d, 7.9)	6.99 (d, 8.1)	6.93 (d, 7.1)
H4'	-	-	-	-	-	-	-	6.59 (d, 2.1)	-	-	-	-	7.31 (dd, 8.3, 7.1)
H5'	6.58 (d, 1.8)	6.58 (d, 2.0)	6.58 (d, 2.2)	6.57 (d, 2.0)	6.58 (d, 2.3)	6.58 (d, 2.0)	7.03 (d, 8.3)	-	6.58 (d, 2.2)	6.58 (d, 2.5)	6.99 (d, 7.9)	6.99 (d, 8.1)	6.90 (dd, 8.3, 7.6)
H6'	-	-	-	-	-	-	7.23 (dd, 8.3, 1.8)	6.90 (d, 2.1)	-	-	7.82 (d, 7.9)	7.81 (d, 8.1)	7.64 (d, 7.6)
H1"a	7.20 (d, 16.3)	7.20 (d, 16.3)	7.19 (d, 16.4)	7.20 (d, 16.3)	7.20 (d, 16.2)	7.20 (d, 16.2)	-	-	7.20 (d, 16.3)	7.20 (d, 16.3)	-	-	-
H1"b	8.51 (d, 16.3)	8.51 (d, 16.3)	8.51 (d, 16.4)	8.50 (d, 16.3)	8.51 (d, 16.2)	8.51 (d, 16.2)	-	-	8.49 (d, 16.3)	8.50 (d, 16.3)	-	-	-
H2"	7.69 (d, 8.4)	7.69 (d, 8.5)	7.68 (d, 7.2)	7.68 (d, 8.4)	7.68 (d, 8.6)	7.69 (d, 8.0)	-	-	7.68 (d, 8.7)	7.69 (d, 8.5)	-	-	-
H3"	6.95 (d, 8.4)	6.95 (d, 8.5)	6.94 (d, 7.2)	6.94 (d, 8.4)	6.95 (d, 8.6)	6.95 (d, 8.0)	-	-	6.95 (d, 8.7)	6.95 (d, 8.5)	-	-	-
H5"	6.95 (d, 8.4)	6.95 (d, 8.5)	6.94 (d, 7.2)	6.94 (d, 8.4)	6.95 (d, 8.6)	6.95 (d, 8.0)	-	-	6.95 (d, 8.7)	6.95 (d, 8.5)	-	-	-

H6”	7.69 (d, 8.4)	7.69 (d, 8.5)	7.68 (d, 7.2)	7.68 (d, 8.4)	7.68 (d, 8.6)	7.69 (d, 8.0)	-	-	7.68 (d, 8.7)	7.69 (d, 8.5)	-	-	-
C1’a-CH_3	-	-	-	-	-	-	-	-	-	-	2.33 (s)	2.33 (s)	2.49 (s)
3-OH	-	-	-	9.72 (s)	-	-	-	-	-	-	-	-	-
3-OCH_3	-	3.85 (s)	-	-	-	-	-	-	-	-	-	3.83 (s)	-
4-OCH_3	-	-	3.84 (s)	-	-	-	-	-	-	-	-	-	-
2’-OH	-	-	-	-	-	-	-	-	-	-	-	-	13.36 (s)
3’-OCH_3	-	-	-	-	-	-	3.82 (s)	3.80 (s)	-	-	-	-	-
4’-OCH_3	3.89 (s)	3.89 (s)	3.89 (s)	3.89 (s)	3.89 (s)	3.89 (s)	3.81 (s)	-	3.90 (s)	3.89 (s)	3.80 (s)	3.80 (s)	-
5’-OCH_3	-	-	-	-	-	-	-	3.80 (s)	-	-	-	-	-
6’-OCH_3	3.87 (s)	3.87 (s)	3.87 (s)	3.86 (s)	3.87 (s)	3.87 (s)	-	-	3.87 (s)	3.87 (s)	-	-	-
4”-OCH_3	3.78 (s)	3.78 (s)	3.79 (s)	3.78 (s)	3.78 (s)	3.78 (s)	-	-	3.78 (s)	3.78 (s)	-	-	-
NH	11.78 (s)	11.74 (s)	11.65 (s)	11.70 (s)	11.85 (s)	11.79 (s)	11.95 (s)	12.07 (s)	11.99 (s)	11.93 (s)	10.69 (s)	10.67 (s)	11.32 (s)

Table 3. ^{13}C NMR chemical shifts (DMSO-d_6) of the polyphenol derivatives (**1–33**) containing the *N′*-methyleneformohydrazide scaffold

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
C1a	163.0	162.7	162.3	163.1	161.4	162.0	163.1	162.7	163.1	161.4	162.1	163.2	162.8	163.2	161.3	161.5	162.0	161.6	161.5	161.6
C1	133.2	134.5	125.17	134.6	135.3	129.6	133.1	134.5	134.5	135.2	129.5	133.1	134.5	134.5	135.2	135.4	129.5	128.9	140.2	128.9
C2	127.7	112.8	129.6	114.6	130.2	130.4	127.7	112.8	114.6	130.2	130.5	127.7	112.8	114.6	130.1	114.4	130.4	148.6	121.5	148.6
C3	128.5	159.2	113.7	157.5	121.8	115.5	128.5	159.2	157.5	121.8	115.6	128.6	159.3	157.5	121.7	163.1	115.5	-	150.4	-
C4	131.9	117.7	162.0	118.84	134.6	163.0	131.9	117.7	118.9	134.6	163.1	132.0	117.7	118.9	134.6	118.8	162.9	152.4	-	152.4
C5	128.5	129.7	113.7	129.6	130.8	115.5	128.5	129.6	129.6	130.8	115.6	128.6	129.7	129.6	130.7	130.7	115.5	123.7	150.4	123.7
C6	127.7	119.9	129.6	118.2	126.9	130.4	127.7	119.9	118.2	126.9	130.5	127.7	119.9	118.2	126.8	123.8	130.4	135.5	121.5	135.5
C1'a	140.4	140.5	139.6	140.3	141.0	140.5	140.0	140.0	139.8	140.6	140.2	140.1	140.1	139.9	140.5	140.5	140.1	141.1	141.7	140.7
C2'	154.6	154.6	154.3	154.5	154.7	154.6	154.9	154.7	154.8	154.9	155.0	154.9	154.9	154.83	154.8	154.8	154.9	154.8	154.9	155.1
C3'	118.4	118.4	118.5	118.5	118.2	118.3	118.5	118.4	118.5	118.3	118.5	118.60	118.6	118.64	118.5	118.5	118.53	118.2	118.1	118.3
C4'	175.1	175.1	175.1	175.1	175.0	175.1	174.1	174.0	174.1	174.0	174.1	174.0	174.0	174.0	173.8	173.8	173.9	175.1	175.0	174.1
C5'	125.2	125.2	125.23	125.2	125.2	125.2	124.2	124.1	124.2	124.2	124.2	127.3	127.3	127.3	127.3	127.2	127.3	125.2	125.2	124.2
C6'	126.1	126.1	126.0	126.1	126.1	126.1	130.6	130.5	130.5	130.6	130.6	124.9	124.9	124.9	124.8	124.8	124.9	126.2	126.2	130.6
C7'	134.7	134.7	134.6	134.7	134.7	134.7	134.6	134.5	134.5	134.5	134.6	137.3	137.3	137.3	137.2	137.2	137.3	134.8	134.8	134.6
C8'	118.7	118.7	118.7	118.76	118.7	118.8	121.3	121.2	121.3	121.3	121.3	121.5	121.5	121.5	121.4	121.4	121.5	118.8	118.8	121.3
C9'	155.8	155.8	155.8	155.8	155.8	155.8	154.4	154.3	154.4	154.4	154.4	154.8	154.8	154.80	154.7	154.7	154.8	155.8	155.8	154.4
C10'	123.3	123.3	123.3	123.4	123.3	123.4	124.5	124.5	124.5	124.5	124.5	118.56	118.5	118.55	118.4	118.4	118.51	123.4	123.3	124.5
3-OCH_3	-	55.4	-	-	-	-	-	55.3	-	-	-	-	55.4	-	-	-	-	-	-	-
4-OCH_3	-	-	55.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

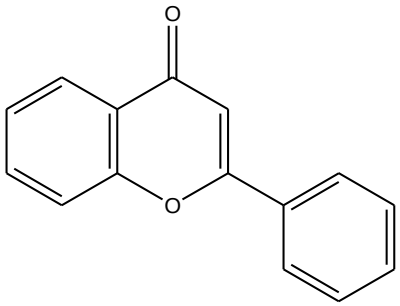
Position	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
C1a	162.9	162.6	162.4	163.0	161.3	161.8	161.5	161.7	161.5	161.4	163.6	163.3	164.4
C1	133.6	134.9	125.6	135.0	135.7	130.0	140.6	140.4	140.6	129.3	134.2	135.6	133.0
C2	127.7	112.7	129.6	114.6	130.18	130.4	121.5	121.5	121.6	148.7	127.8	113.0	128.1
C3	128.4	159.2	113.6	157.4	121.7	115.3	150.3	150.3	150.2	-	128.3	159.1	128.5
C4	131.5	117.4	161.8	118.5	134.3	162.8	-	-	-	152.1	131.3	117.1	131.9
C5	128.4	129.5	113.6	129.4	130.6	115.3	150.3	150.3	150.2	123.5	128.3	129.5	128.5
C6	127.7	119.9	129.6	118.2	126.9	130.4	121.5	121.5	121.6	135.4	127.8	119.9	128.1
C1’a	145.1	145.1	144.4	145.0	145.6	145.1	149.3	148.9	146.3	145.7	155.8	156.1	158.2
C1’	112.7	112.8	112.9	112.8	112.5	112.7	126.7	136.0	112.4	112.5	130.5	130.4	119.4
C2’	138.9	139.0	138.8	138.9	139.1	139.0	108.4	105.0	139.2	139.1	127.9	128.0	158.7
C3’	102.5	102.6	102.5	102.5	102.6	102.6	149.1	160.7	102.7	102.6	113.7	113.7	117.3
C4’	161.0	161.1	160.9	161.0	161.2	161.1	151.0	102.6	161.3	161.2	160.4	160.4	131.2
C5’	97.5	97.5	97.5	97.5	97.5	97.5	111.5	160.7	97.5	97.5	113.7	113.7	118.5
C6’	160.3	160.3	160.2	160.3	160.4	160.3	122.2	105.0	160.5	160.4	127.9	128.0	128.4
C1”a	130.33	130.4	130.31	130.30	130.4	130.31	-	-	130.5	130.4	-	-	-
C1”b	126.1	126.1	126.1	126.1	126.1	126.1	-	-	126.0	126.1	-	-	-
C1”	130.27	130.3	130.26	130.29	130.24	130.27	-	-	130.2	130.2	-	-	-
C2"	128.4	128.4	128.4	128.4	128.4	128.4	-	-	128.4	128.4	-	-	-
C3"	114.1	114.1	114.1	114.1	114.1	114.1	-	-	114.1	114.1	-	-	-
C4"	159.0	159.0	159.0	159.0	159.0	159.0	-	-	159.0	159.0	-	-	-
C5"	114.1	114.1	114.1	114.1	114.1	114.1	-	-	114.1	114.1	-	-	-
C6"	128.4	128.4	128.4	128.4	128.4	128.4	-	-	128.4	128.4	-	-	-
C1’a-CH_3	-	-	-	-	-	-	-	-	-	-	14.4	14.5	14.0
3-OCH_3	-	55.4	-	-	-	-	-	-	-	-	-	55.3	-
4-OCH_3	-	-	55.4	-	-	-	-	-	-	-	-	-	-
3’-OCH_3	-	-	-	-	-	-	55.5	55.3	-	-	-	-	-

4’-OCH_3	55.4	55.3	55.3	55.4	55.4	55.4	55.6	-	55.4	55.4	55.2	55.2	-
5’-OCH_3	-	-	-	-	-	-	-	55.3	-	-	-	-	-
6’-OCH_3	56.0	56.0	56.0	56.0	56.0	56.0	-	-	56.0	56.0	-	-	-
4”-OCH_3	55.1	55.1	55.1	55.1	55.1	55.1	-	-	55.1	55.1	-	-	-

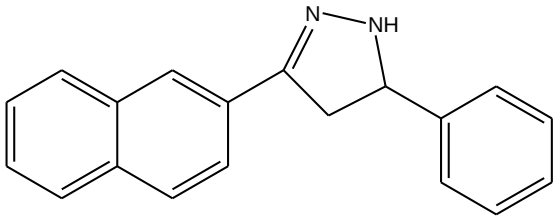
Figure Captions

Figure 1.

(A)



(B)



(C)

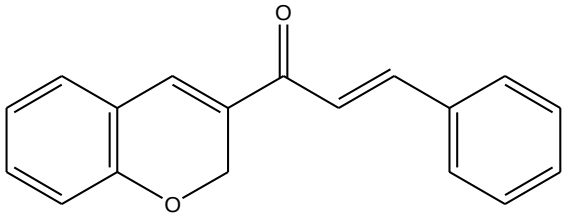


Figure 1. General structures of (A) flavone, (B) benzochalcone bearing pyrazoline moiety, and (C) chromenylchalcone.

Figure 2.

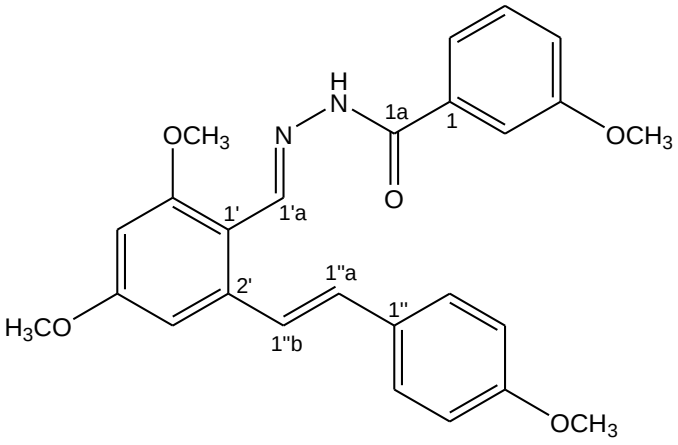


Figure 2. Structure of derivative **22**, (*E*)-*N*′-((*E*)-2-((*E*)-4-methoxystyryl)-4,6-dimethoxybenzylidene)-3-methoxybenzohydrazide.

Figure 3.

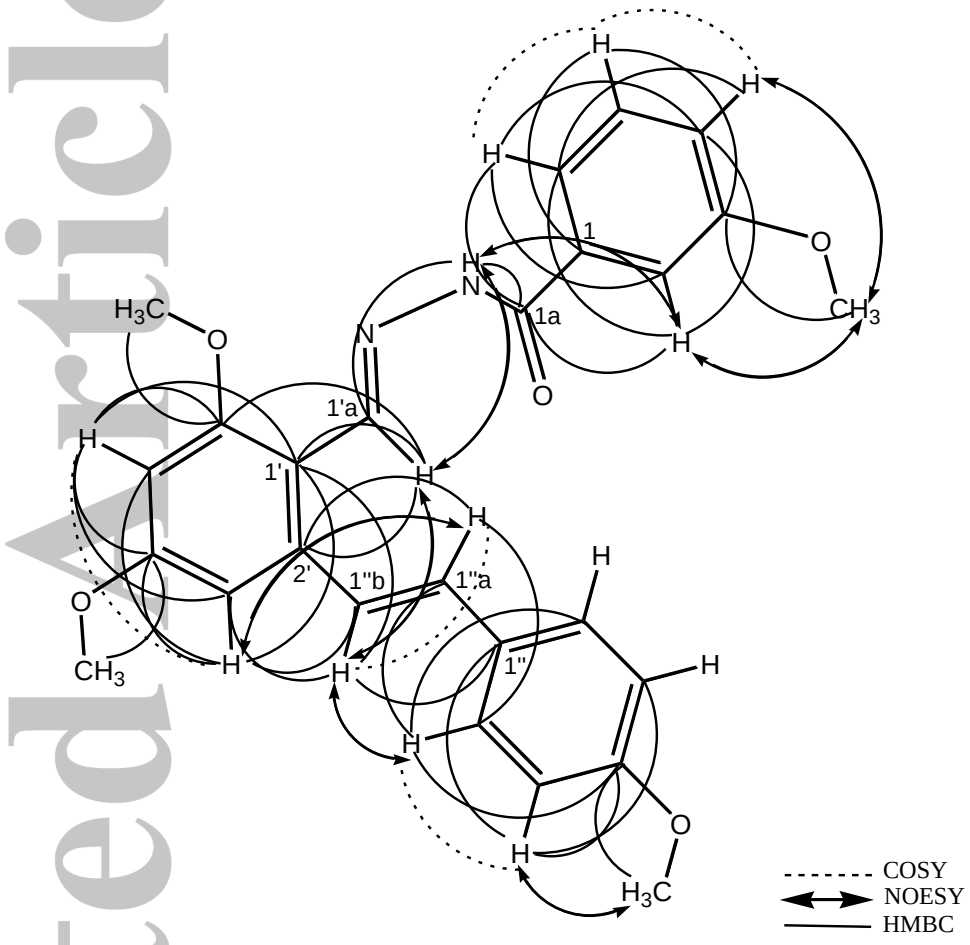
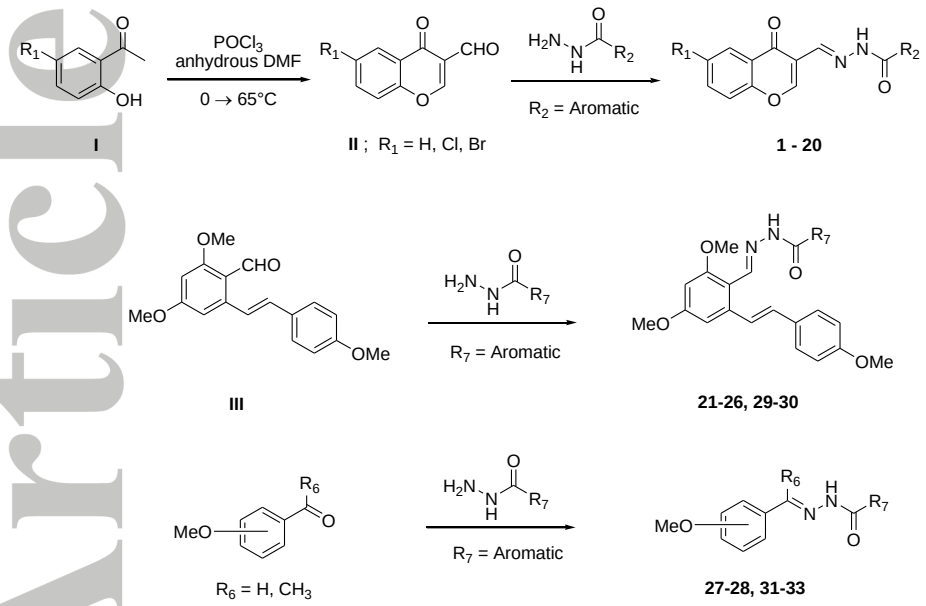


Figure 3. Key correlations obtained from the COSY, NOESY, and HMBC spectra of derivative **22**.

Scheme 1.



Scheme 1. Synthetic route to the various polyphenols containing the *N′*-methyleneformohydrazide scaffold (**1**–**33**).

Synthesis and structure elucidation of polyphenols containing the *N′*-methyleneformohydrazide scaffold as aurora kinase inhibitors

Dongsoo Koh, Yearam Jung, Seunghyun Ahn, Kenneth Hun Mok, Soon Young Shin, Yoongho Lim*

We designed polyphenols containing the *N′*-methyleneformohydrazide scaffold and synthesized 33 derivatives. To confirm whether they show the inhibitory effects on aurA, *in vitro* kinase assay was performed, and their half-maximal inhibitory concentration ranged between 1.14 and 98.93 μM. We report here the assignments of their ^{1}H and ^{13}C NMR data and high-resolution mass spectral data. These data can help us design a series of compounds containing novel aurora kinase inhibitor pharmacophores and identify newly synthesized polyphenols containing the *N′*-methyleneformohydrazide scaffold.