Supplementary Information

Species-Dependent Effects of 3-Hydroxychromone Derivatives on Biofilm Biomass and Planktonic Growth

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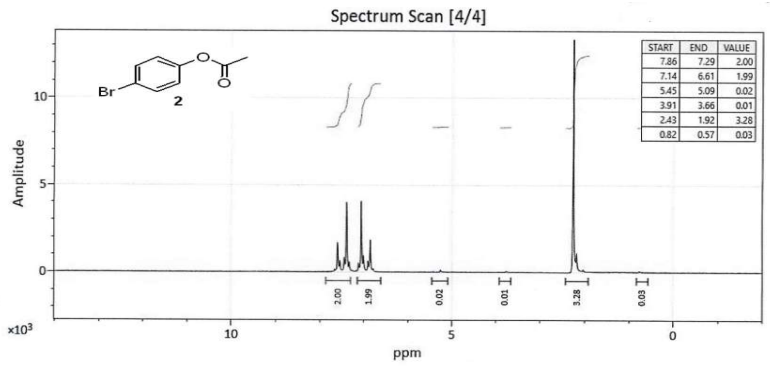
S1. Supplementary Method.

S1.1 Chemical synthesis

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used without further purification. TLC plates used for determining reaction progress were plastic sheets precoated with SiO_2 60Fm. Flash column chromatography was performed on silica gel (70-230 mesh). All ^1H NMR spectra were recorded at 47 or 400 MHz and all ^{13}C NMR spectra (when available) were recorded at 100 MHz at rt in either DMSO-*d6* or $CDCl_3$.

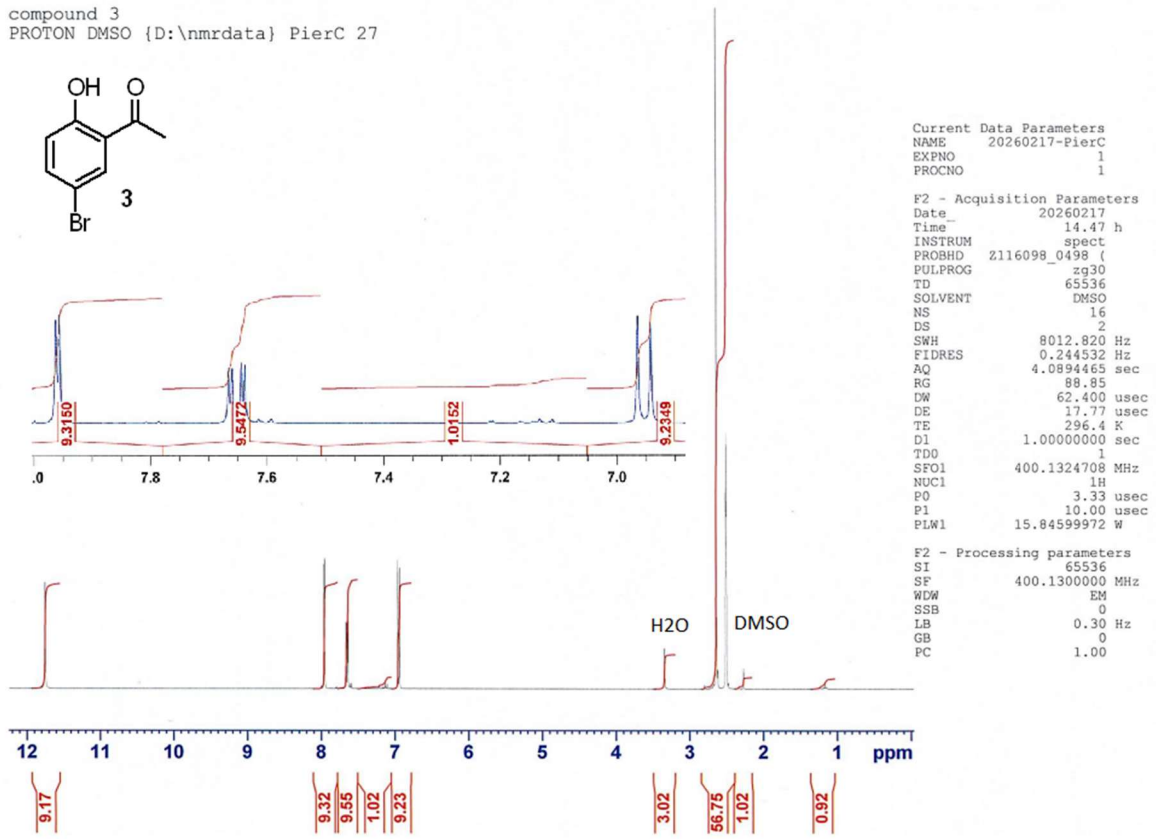
S1.2 4-Bromophenyl acetate, 2.

In a 500 mL round-bottom flask equipped with magnetic stir bar 4-bromophenol (compound **1**, 10.0 g; 57.8 mmol; 1.00 equiv.) was dissolved in 200 mL dichloromethane. Acetic anhydride (6.6 mL; 69.36 mmol; 1.2 equiv.) and triethylamine (12.1 mL; 86.7 mmol; 1.5 equiv.) were then added via syringe at room temperature. The mixture was left stirring for 1.0 hour at ambient temperature, then poured into a separating funnel containing 1M aqueous HCl. The layers were separated and the aqueous layer was further extracted with dichloromethane. The combined organics were washed with brine and dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography eluting with 30% ethyl acetate in hexanes to furnish 11.38 g of product as a pale yellow oil (52.9 mmol; 91.5% yield), though it can also be used as is, without purification, for the next step. ^1H NMR (47 MHz; $CDCl_3$): δ (ppm) 7.5 (d, 2 H, Ar-H); 7.0 (d, 2 H, Ar-H); 2.1 (s, 3 H, CH_3CO).



S1.3 5'-bromo-2'-hydroxy-acetophenone, 3.

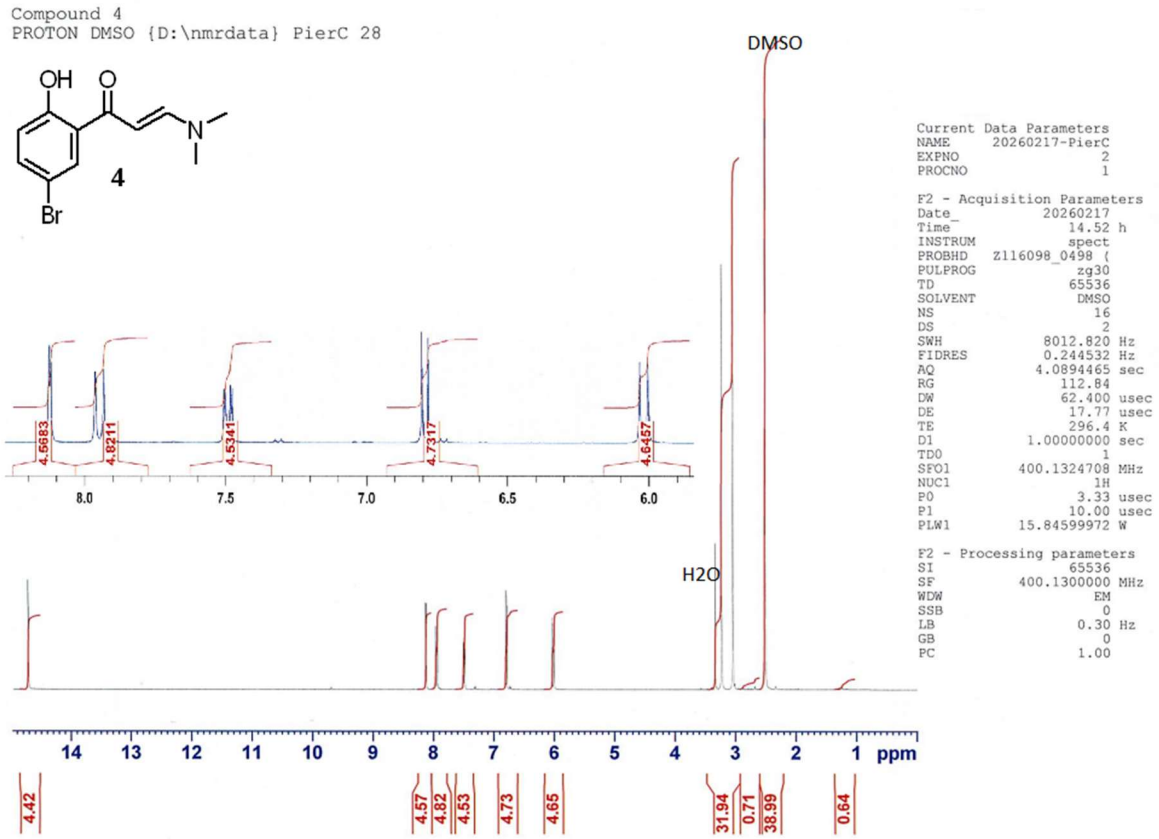
Acetate ester **2** (11.0 g; 51.2 mmol; 1.0 equiv.) was placed in a 250 mL round-bottom flask equipped with a large magnetic stir bar and treated at room temperature with aluminum chloride (20.48 g; 153.6 mmol; 3.0 equiv.) under argon atmosphere. The mixture was then placed in an oil bath, heated to 130 °C, and the resulting liquid mixture was stirred for a half hour. It was then cooled to 60-70 °C and poured into ice water. The product was extracted three times with ethyl acetate. The combined organic layers were washed with brine and dried over Na_2SO_4. The crude product was purified by column chromatography eluting with 45% ethyl acetate in hexanes to afford 8.37 g of desired acetophenone as a yellow solid (38.9 mmol; 76% yield). ^{1}H NMR (400 MHz; $CDCl_3$): δ (ppm) 12.2 (s, 1 H, C2'-OH); 7.96 (d, 1 H, J = 2.5 Hz, C6'H); 7.65 (dd, 1H, J = 2.5 and 5.6 Hz, C4'H); 6.96 (d, 1 H, J = 5.6 Hz, C3'H); 2.64 (s, 3 H, COCH3).



S1.4 1-(5'-bromo-2'-hydroxy-phenyl)-3-*N,N*-dimethylamino-prop-2-en-1-one, 4.

Acetophenone **3** (8.61 g; 40.0 mmol; 1.0 equiv.) was placed in a round-bottom flask and dissolved in 150 mL anhydrous 1,2-dimethoxyethane. Pyridinium *para*-toluenesulfonate was then added as a catalyst (1.14 g; 6.00 mmol; 0.15 equiv.) followed by *N,N*-dimethylformamide dimethylacetal (DMF-DMA) (5.3 mL; 40.0 mmol; 1.0 equiv.). The mixture was heated to reflux overnight, then cooled to ambient temperature. The mixture was poured into water and the precipitate was vacuum filtered on a Buchner funnel. The solid was air dried then placed under high vacuum. 8.72 g of desired enaminone **4** was obtained as a bright orange solid (80.7 % yield). ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm)): 14.72 (s, 1 H, OH); 8.12 (d, 1 H, J = 2.4

Hz, C6'H); 7.94 (d, 1 H, J = 11.8 Hz, vinyl H); 7.49 (dd, 1 H, J = 8.8 and 2.4 Hz, C4'H); 6.79 (d, 1 H, J = 8.8 Hz, C3'H); 6.02 (d, 1 H, J = 11.8 Hz, vinyl H); 3.23 (s, 3 H, N-CH3); 3.04 (s, 3 H, N-CH3).



S1.5 6-bromo-3-hydroxy-chromen-4-one, 6.

Crude enaminone **4** (8.72 g; 32.3 mmol; 1 equiv.) was dissolved in 350 mL dichloromethane. Meta-chloroperoxybenzoic acid (m-CPBA; ~70% w/w) (23.9 g; 96.9 mmol; 3.0 equiv.) was then added over 15 minutes, portion wise (NOTE: strong exotherm!!). The orange color of starting material is quickly converted to a pale yellow, and after 2 h a white flocculent precipitate starts to form. The mixture was left stirring vigorously over 36 hours. The mixture was further diluted with dichloromethane, then poured into 100 mL of 1 M aqueous NaOH solution. The layers were separated and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and brine. It was then dried (Na_2SO_4), filtered through a short plug of silica gel, which was washed with 40% ethyl acetate in hexanes, and the solvents were removed *in vacuo*. After trituration in hot ethyl acetate and filtration, 6-bromo-3-hydroxychromen-4-one **6** (4.69 g; 60.3% yield) was obtained as a pale yellow solid. (^{1}H NMR (400 MHz; $SO(CD_3)_2$): δ (ppm) 9.35 (br s, 1 H, OH); 8.28 (s, 1 H, H2); 8.18 (d, 1 H, J = 2.4 Hz; H5); 7.91 (dd, 1 H, J = 9.0 and 2.4 Hz; H7); 7.64 (d, 1 H, J = 9.0 Hz; H8). GC-MS m/z = 240 (100%); 242 (97%).

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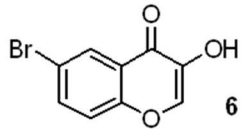

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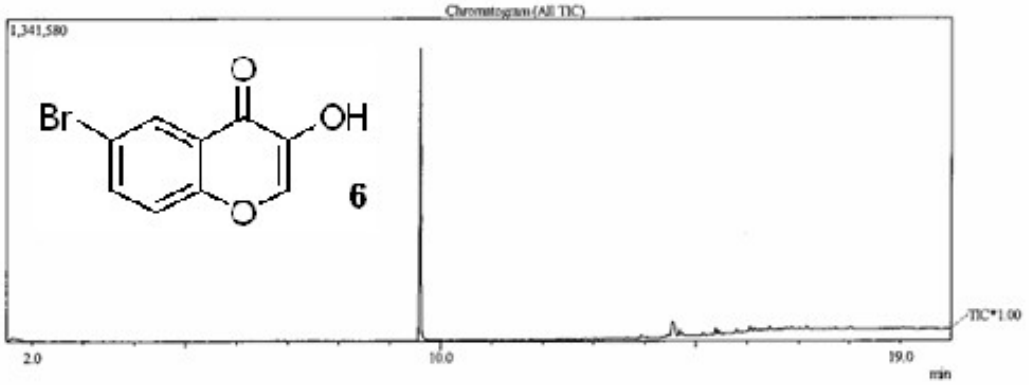

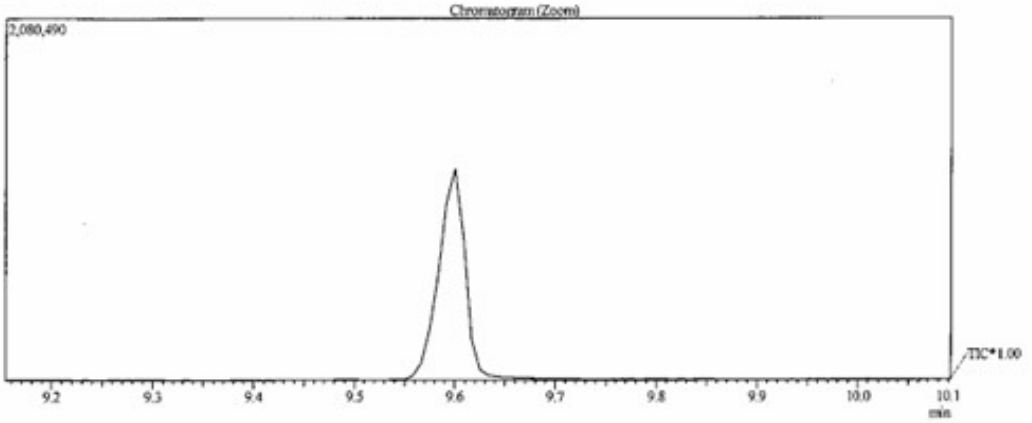

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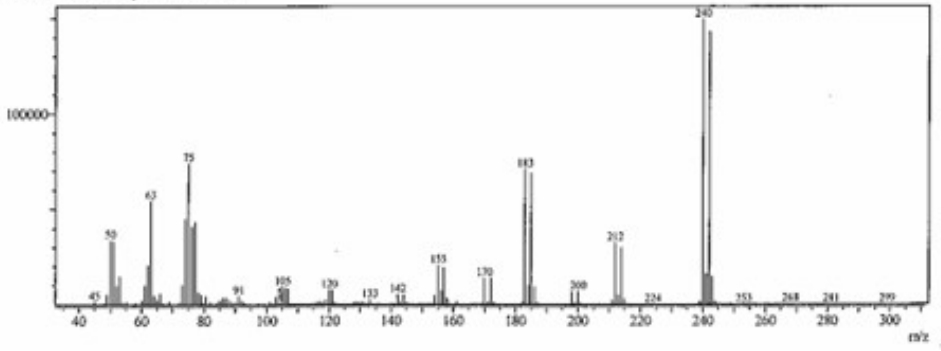
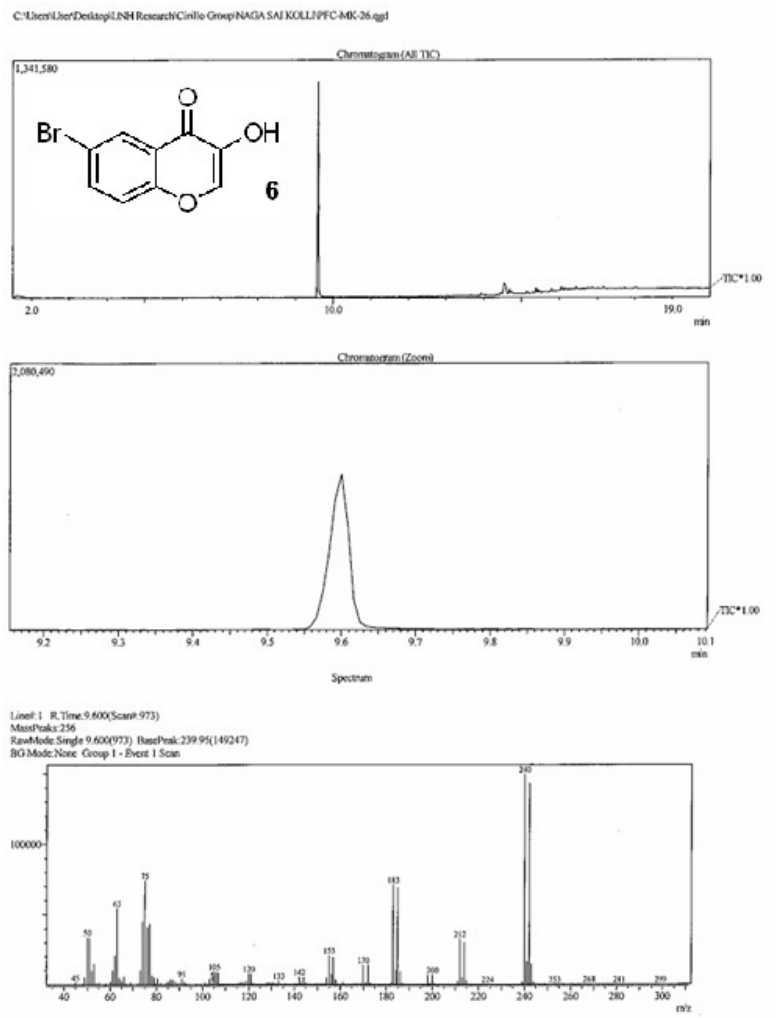
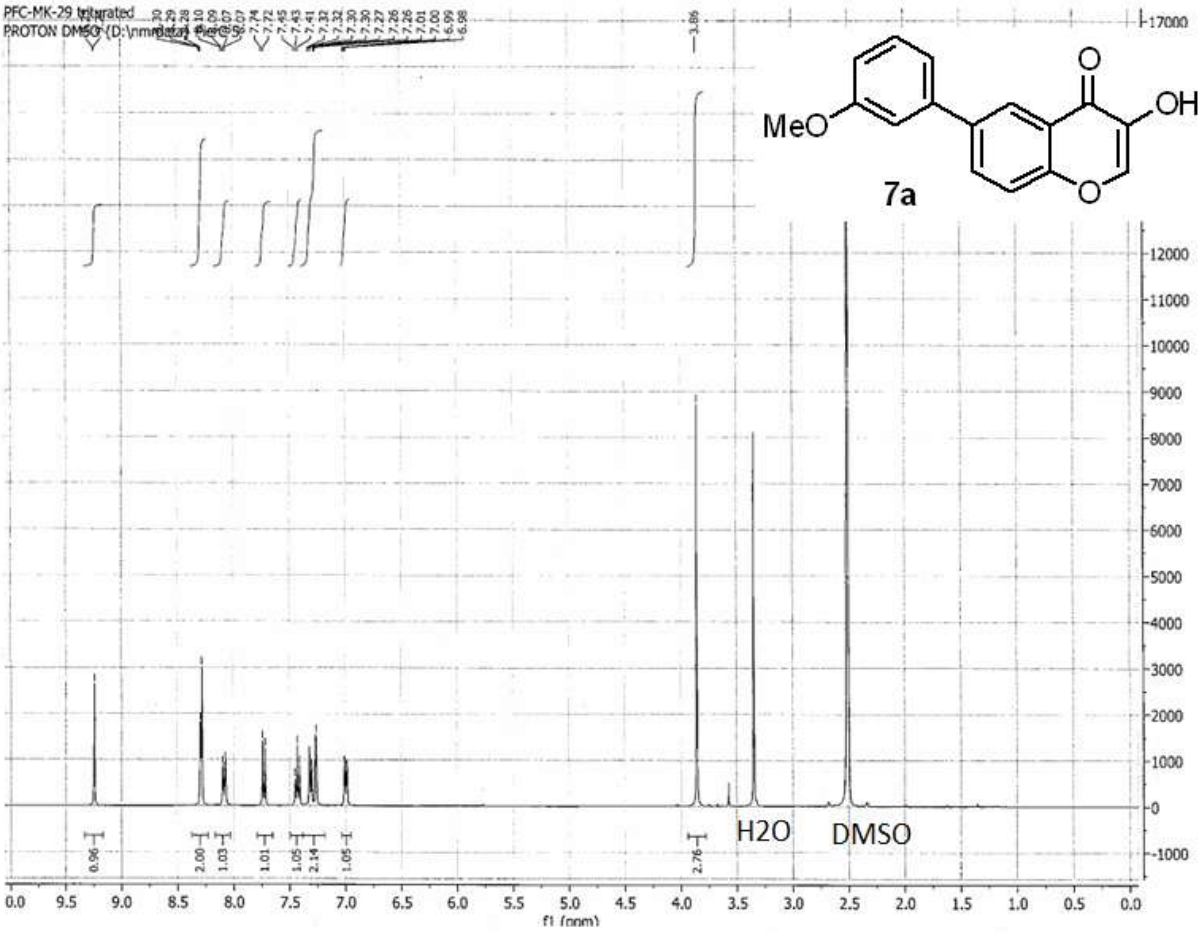




S1.6 6-(3-methoxyphenyl)-3-hydroxy-chromen-4-one, 7a.

Bromo-hydroxy-chromenone **6** (250 mg; 1.04 mmol; 1.0 equiv.) was dissolved in 2.5 mL 1,4-dioxane. Water (1.25 mL) was added, then 3-methoxyphenylboronic acid (188 mg; 1.24 mmol; 1.2 equiv.) and potassium carbonate (286 mg; 2.07 mmol; 2.0 equiv.). The mixture was purged with argon gas for 10 minutes then $Pd(dppf)_2Cl_2$ catalyst (37.9 mg; 0.05 mmol; 0.05 equiv.) was added. The mixture was heated at 120 ºC for 2 hours, then it was allowed to cool down and was quenched with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried with Na_2SO_4, decanted, and the solvent was removed *in vacuo*. The product was purified by column chromatography to afford a tan powder (196 mg; 70% yield). ^{1}H NMR (400 MHz; $SO(CD_3)_2$): δ (ppm) 9.22 (s, 1 H, OH); 8.27 (s, 1 H); 8.25 (s, 1 H); 8.05 (d, 1 H); 7.70 (d, 1 H); 7.42 (dd, 1 H); 7.30 (d, 1 H); 7.25 (s, 1 H); 7.00 (d, 1 H); 3.88 (s, 3 H, OCH_3). ^{13}C NMR (400 MHz; $SO(CD_3)_2$): δ (ppm) 173.2; 160.3; 155.3; 142.4; 141.3; 140.6; 136.9; 132.6; 130.7; 123.4; 122.7; 119.69; 119.66; 114.1; 112.8; 55.70 (O<u>C</u>H3).

GC-MS: m/z = 268 (100%).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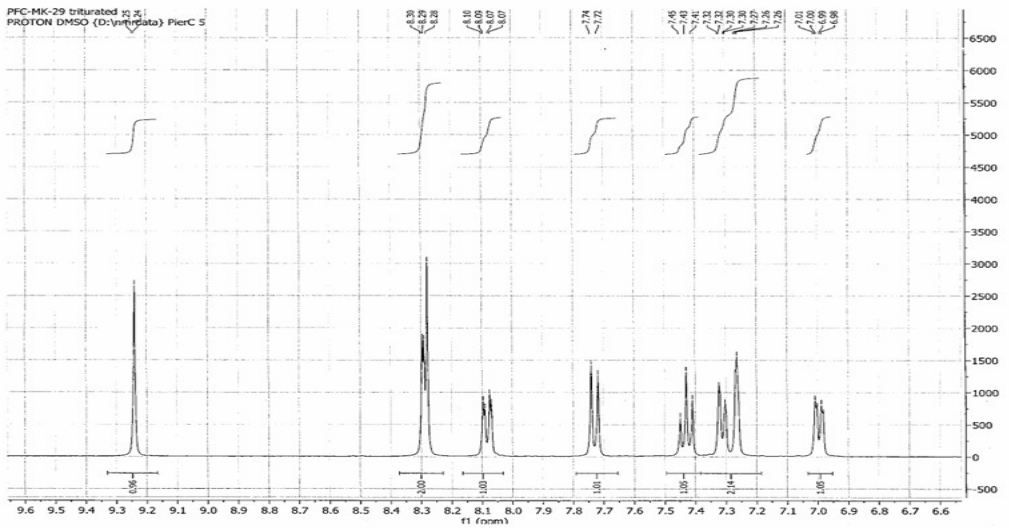
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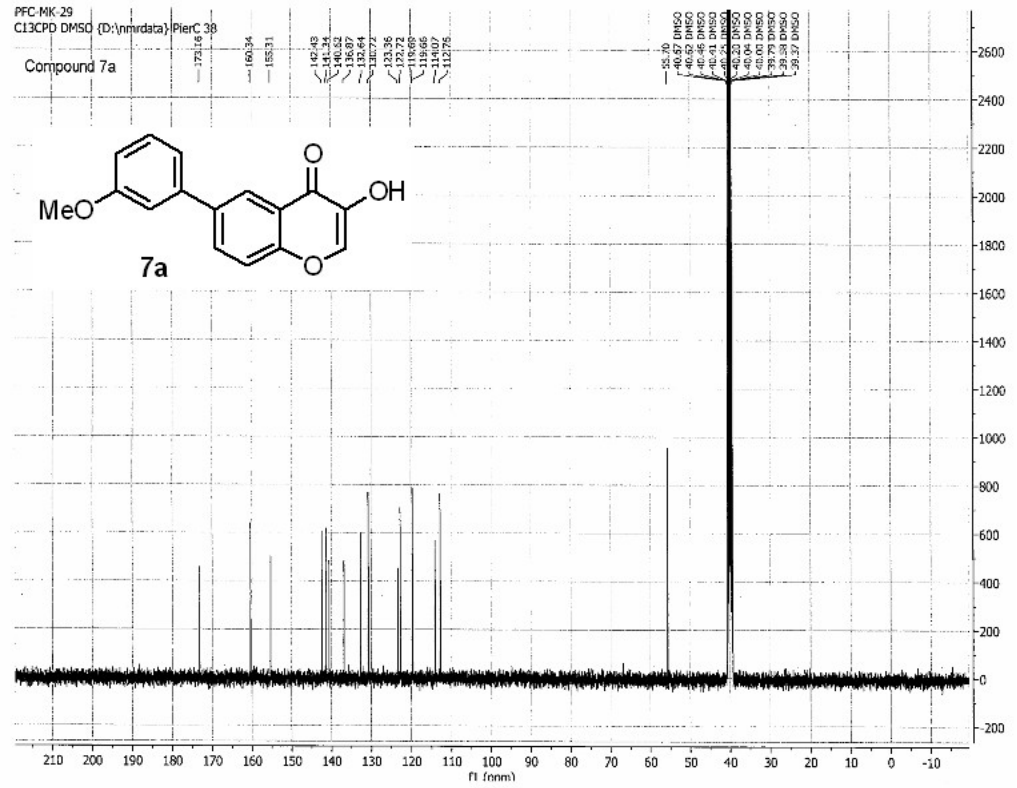
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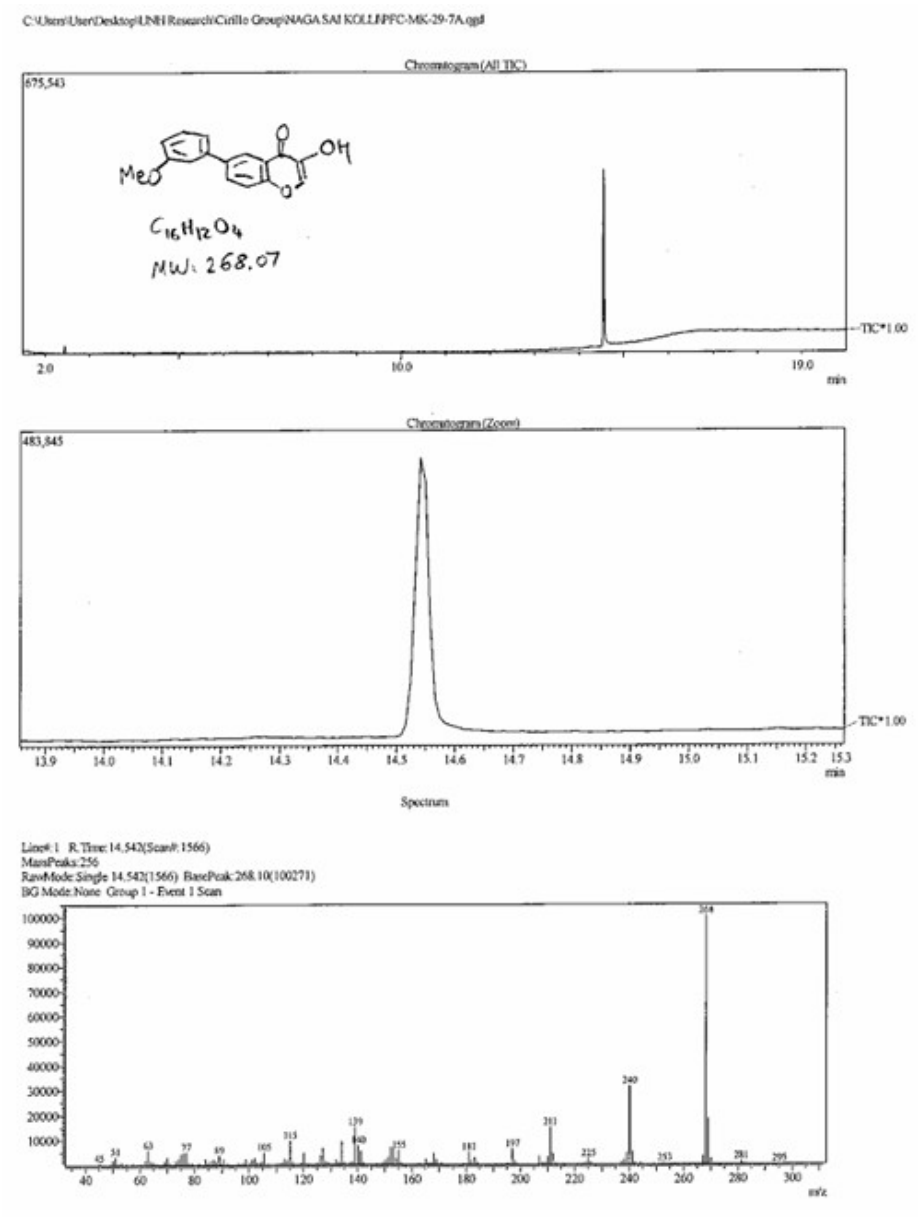
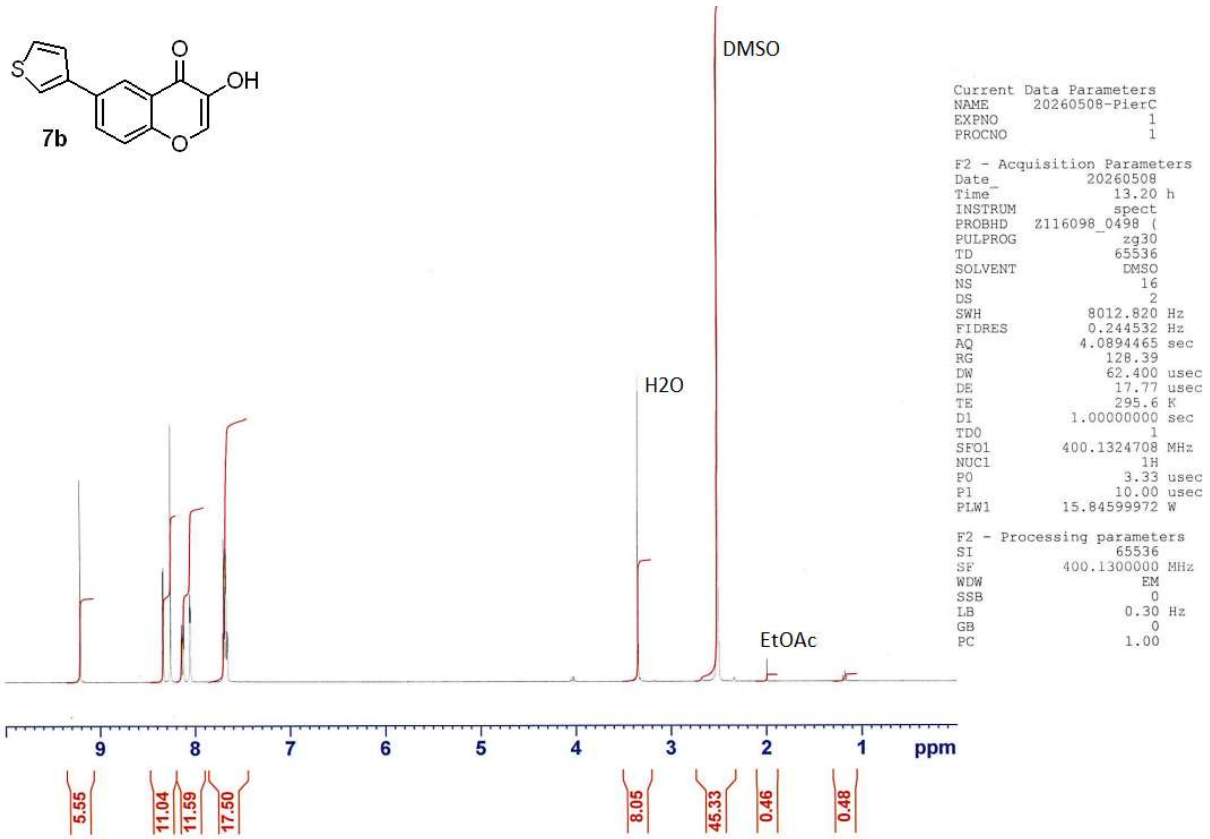


S1.7 6-(3-thiophenyl)-3-hydroxy-chromen-4-one, 7b.

Bromo-chromenone **6** (240 mg; 1.00 mmol; 1.0 equiv.) was dissolved in 25 mL 1,4-dioxane. Water (12 mL) was added, then 3-thienylboronic acid (154 mg; 1.20 mmol; 1.2 equiv.) and potassium carbonate (276 mg; 2.00 mmol; 2.0 equiv.). The mixture was purged with argon gas for 10 minutes, then $Pd(PPh_3)_2Cl_2$ catalyst (36 mg; 0.05 mmol; 0.05 equiv.) was added. The mixture was heated at 120 °C for 2 hours, during which time it turned dark brown, then it was allowed to cool and was quenched with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried with Na_2SO_4, decanted, and the solvent was removed *in vacuo*. The product was purified by column chromatography then triturated with dichloromethane to afford a yellow powder (153 mg; 63% yield). ^{1}H NMR (400 MHz;

$SO(CD_3)_2$) δ (ppm): 9.21 (br s, 1 H, OH); 8.33 (d, 1 H, J = 2.2 Hz); 8.26 (s, 1 H); 8.13 (dd, 1 H, J = 9.7 and 2.3 Hz); 8.05 (dd, 1 H, J = 2.8 and 1.4 Hz); 7.71-7.65 (m, 3 H). GC-MS: m/z = 244.

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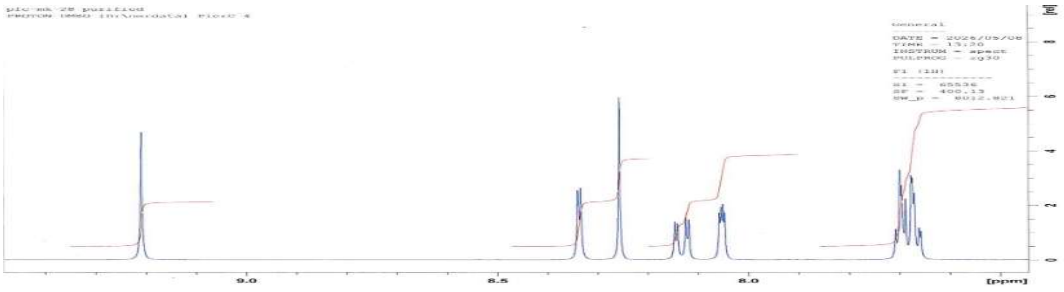
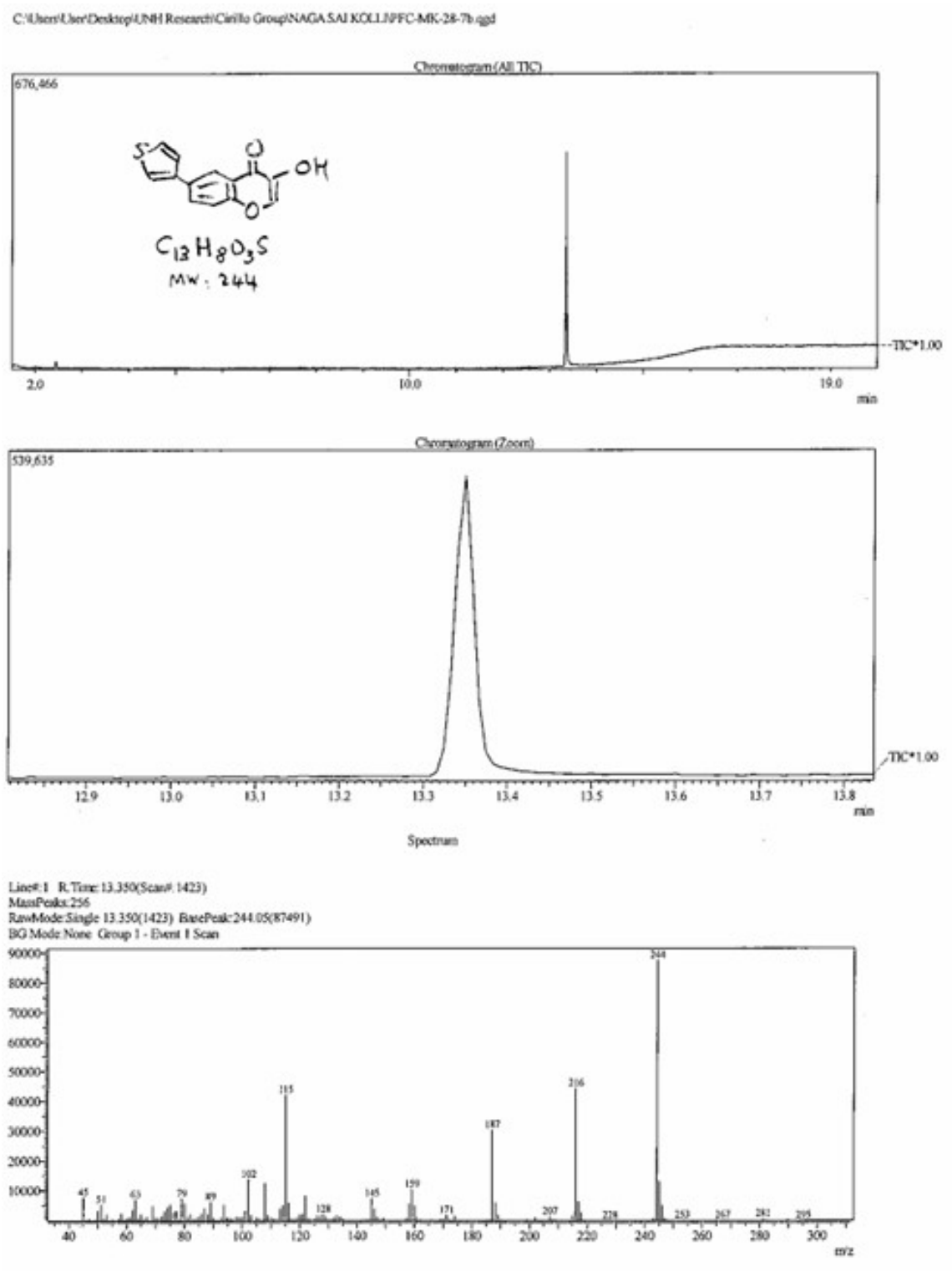
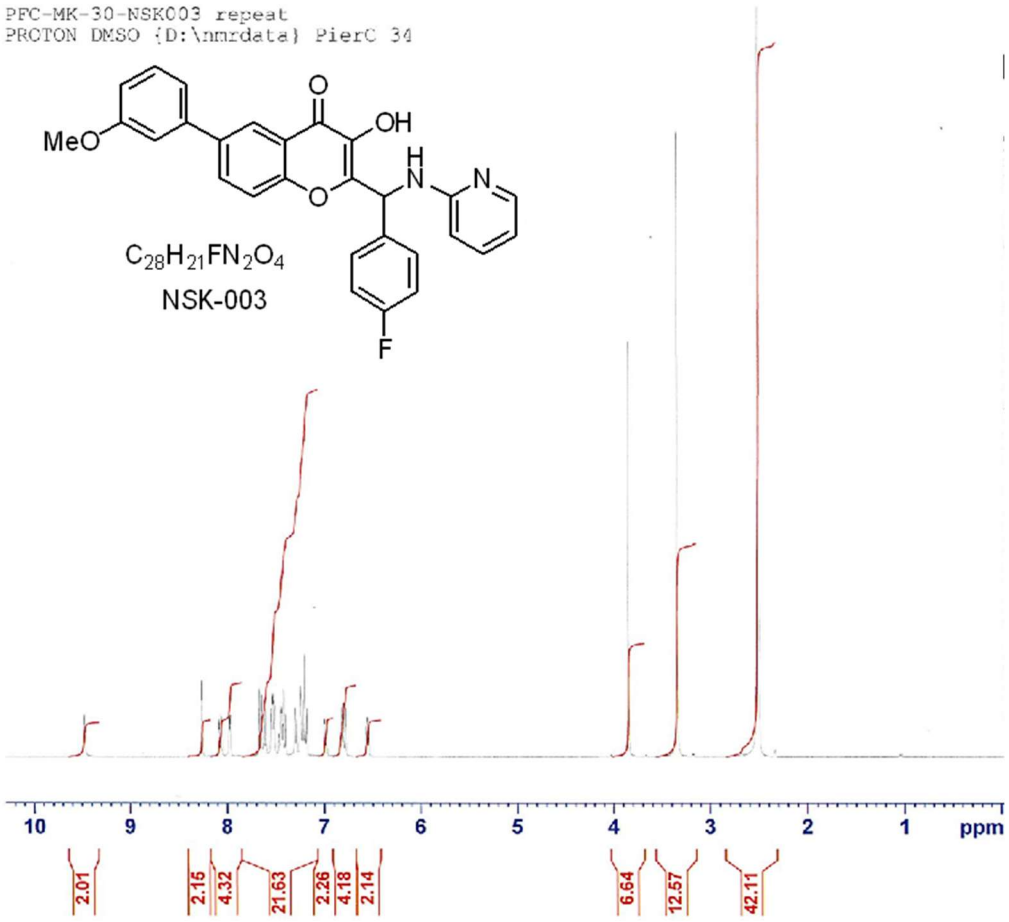




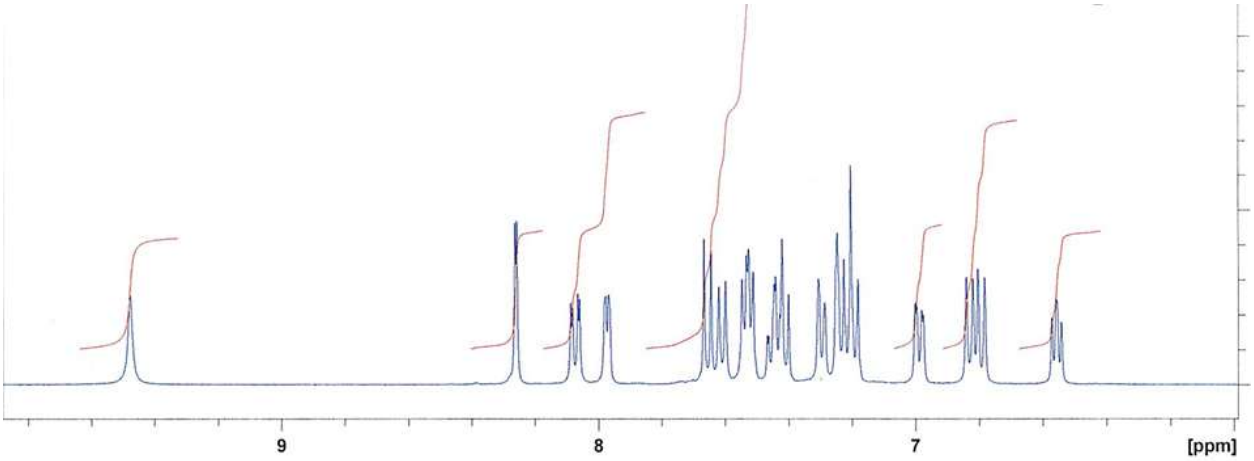
S2. Structural confirmation and molecular information.

S2.1 NSK 3. 4-Fluorobenzaldehyde (66 mg; 0.537 mmol; 1.2 equiv.) and 2-aminopyridine (51 mg; 53.7 mmol; 1.2 equiv.) were added to a 5 mL pear-shaped flask. Isopropanol (1.5 mL) was added, then once dissolution is completed, compound **7a** (120 mg; 0.447 mmol; 1.0 equiv.) was added. The resulting

suspension was heated to 85 ºC (oil bath) for 1 hour. When the reaction was completed as monitored by TLC, the mixture was removed from the heat, allowed to cool, then ether was added and the pure product precipitated as a tan solid, which was collected by vacuum filtration using a Hirsch funnel (38 mg; 31% yield). ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.43 (s, 1 H, OH); 8.27 (s, 1 H); 8.07 (d, 1 H); 7.98 (d, 1 H); 7.65 (d, 1 H); 7.62-7.50 (m overlap, 3 H); 7.50-7.40 (m overlap, 2 H); 7.30 (d, 1 H); 7.27-7.16 (m overlap, 3 H); 6.99 (d, 1 H); 6.85-6.78 (m overlap, 2 H); 6.55 (dd, 1 H); 3.81 (s, 3 H, OCH_3). ^{13}C NMR (100 MHz; $SO(CD_3)_2$) δ (ppm): 172.91; 160.35; 157.88; 154.57; 151.49; 147.82; 140.55; 138.37; 137.44; 136.93; 136.53; 136.50; 132.67; 130.72; 129.45; 129.37; 122.69; 122.64; 119.66; 119.46; 115.91; 115.70; 114.10; 113.33; 112.71; 109.76; 55.71; 50.67.



See expansion of region δ 9.4 to 6.6 ppm below (next page)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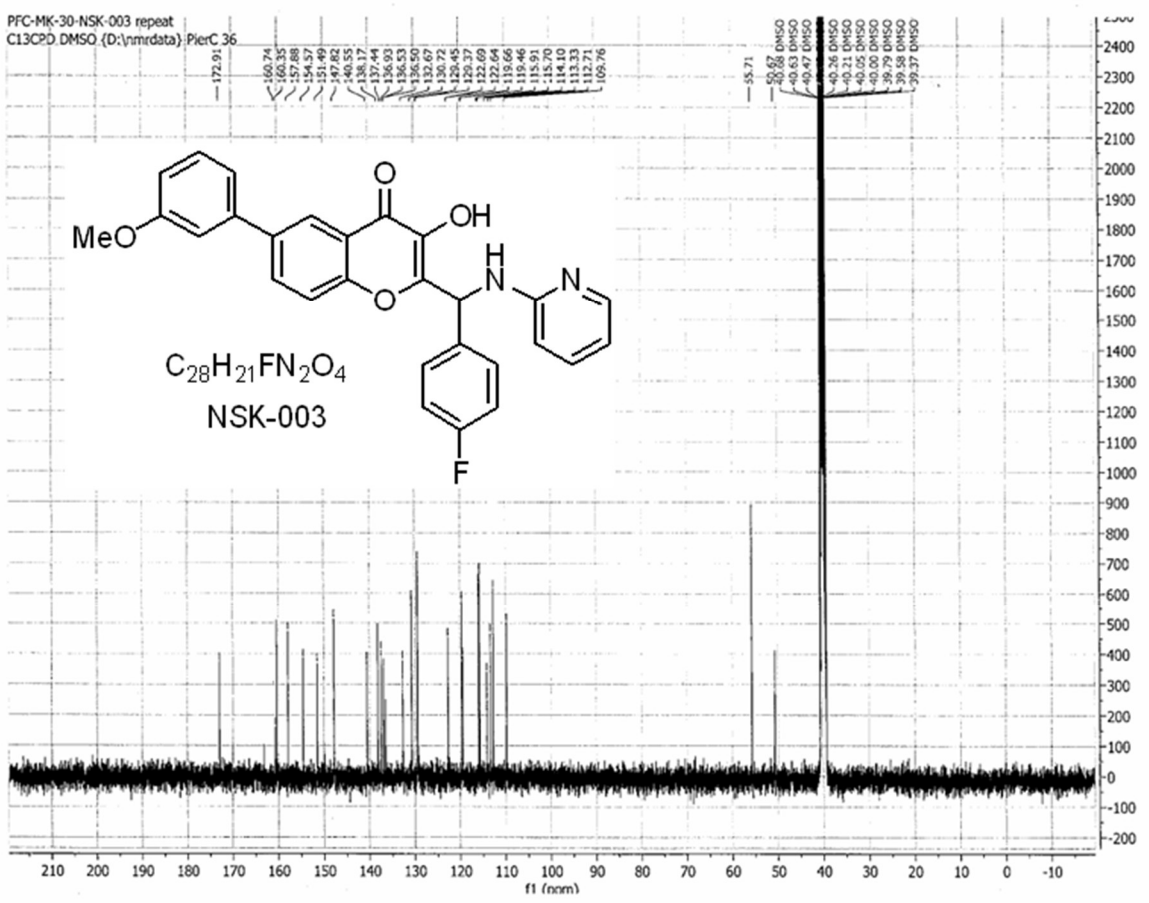
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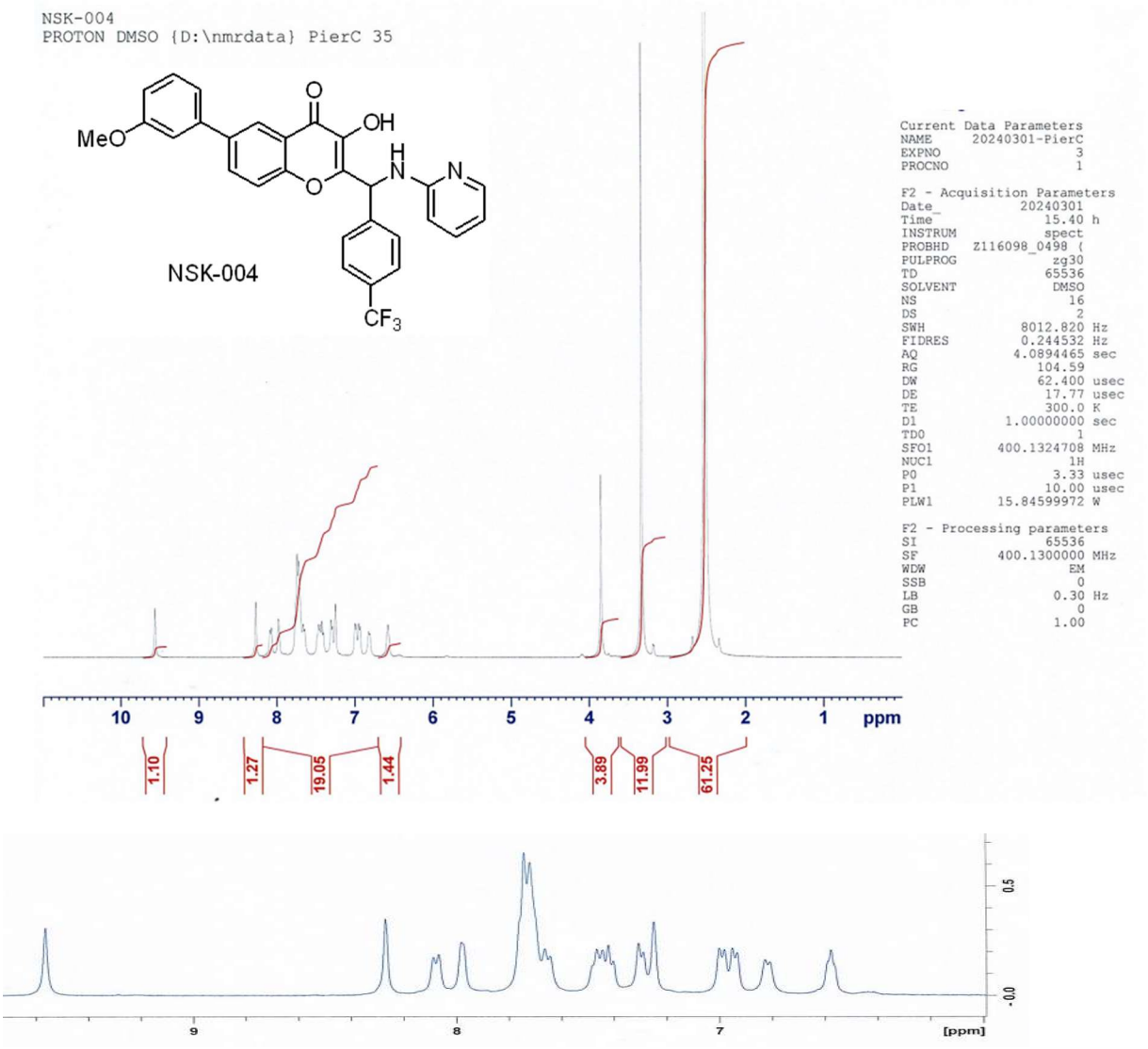
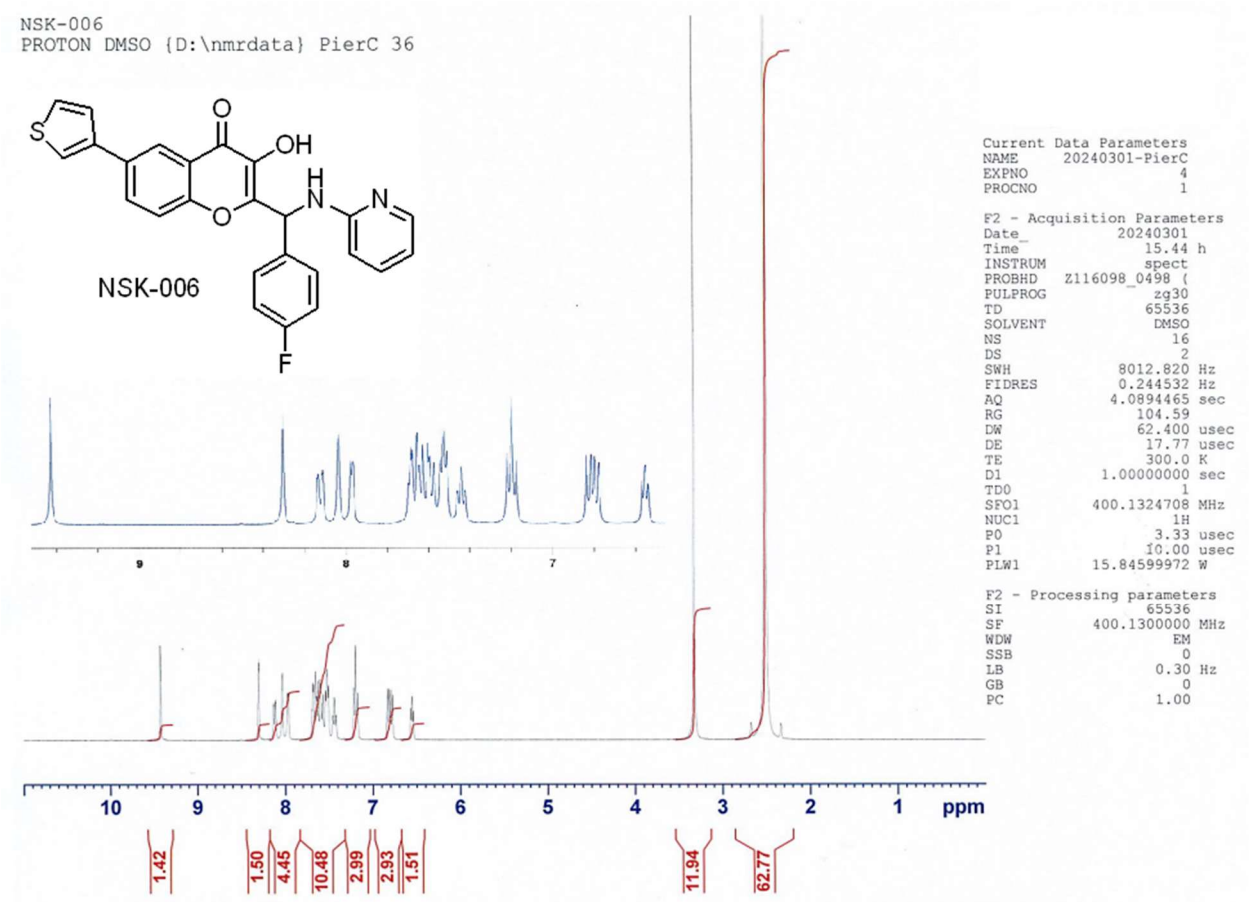
S2.2 NSK 4. Obtained from compound **7a**, 2-aminopyridine, and 4-trifluoromethylbenzaldehyde, following a similar procedure as described for NSK 3. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.57 (s, 1 H, OH); 8.27 (s, 1 H); 8.08 (d, 1 H); 7.99 (s, 1 H); 7.80-7.63 (m overlap, 5 H); 7.50-7.41 (m overlap, 2 H); 7.31 (d, 1 H); 7.25 (s, 1 H); 7.00 (d, 1 H); 6.96 (d, 1 H); 6.81 (d, 1 H); 6.59 (dd, 1 H); 3.84 (s, 3 H, OCH_3).



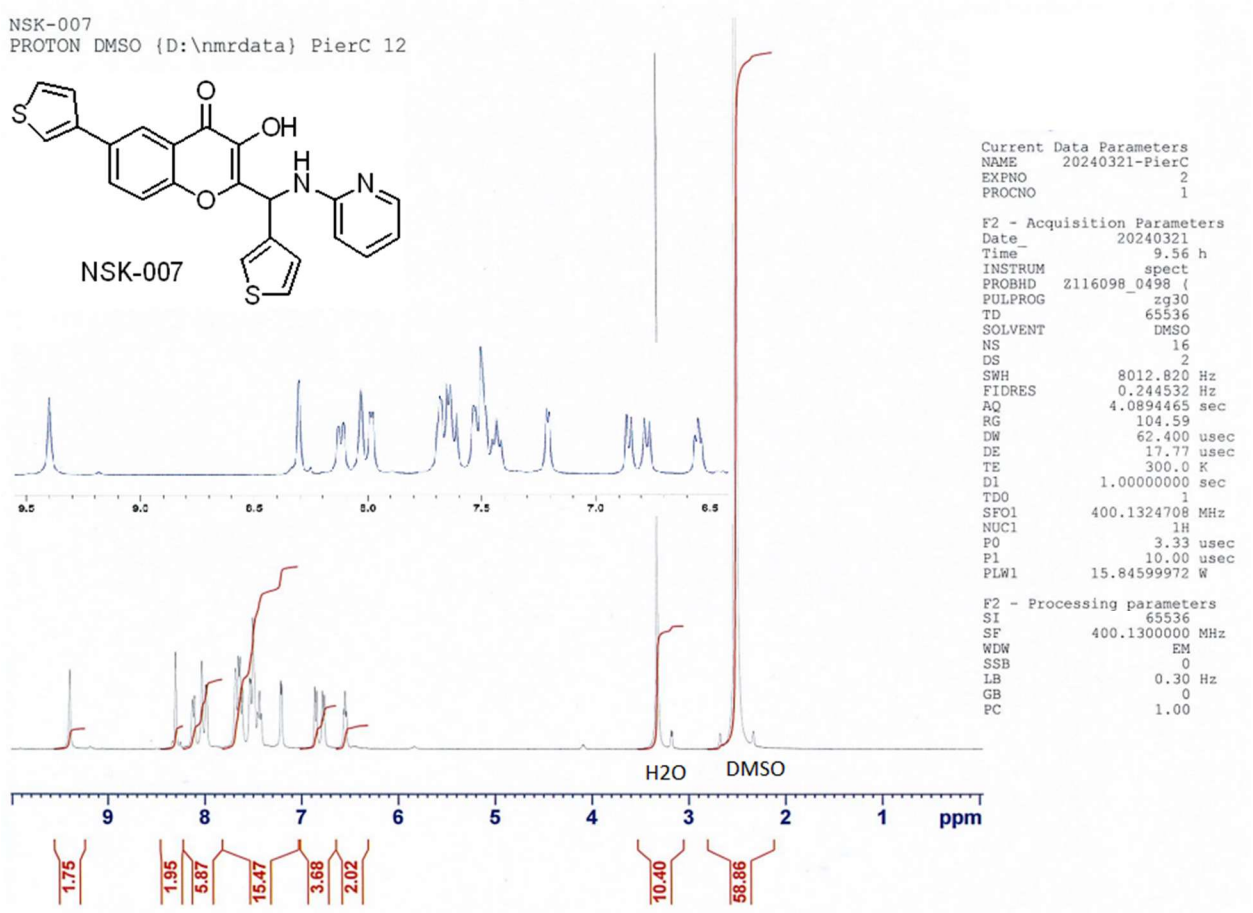
S2.3 NSK 6.

Obtained from compound **7b**, 2-aminopyridine, and 4-fluorobenzaldehyde, following a similar procedure as described for NSK 3. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.41 (s, 1 H, OH); 8.30 (s, 1 H); 8.12 (d, 1 H); 8.04 (s, 1 H); 7.97 (d, 1 H); 7.52-7.70 (overlapping m, 6 H); 7.49 (dd, 1 H); 7.19 (dd, 2 H); 6.80 (dd, 2 H); 6.55 (dd, 1 H).

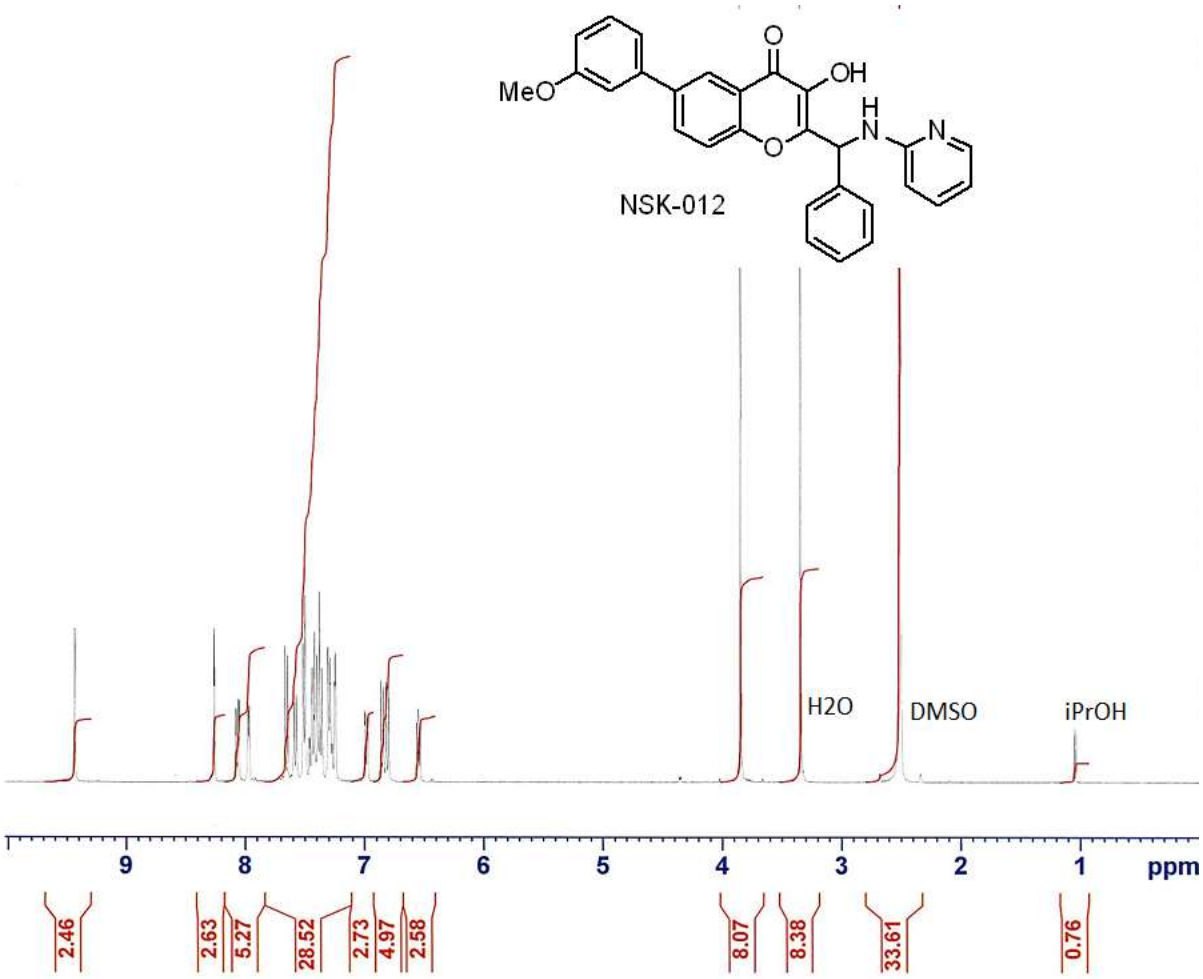


S2.4 NSK 7.

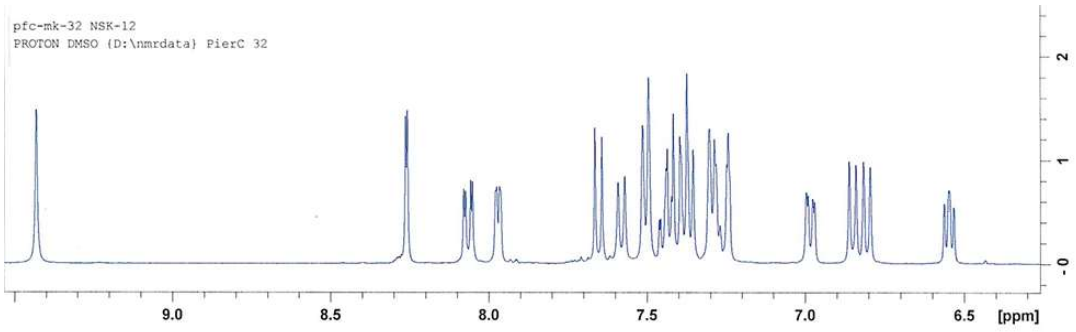
Obtained from compound **7b**, 2-aminopyridine, and 3-thiophene carbaldehyde, following a similar procedure as described for NSK 3. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.40 (s, 1 H, OH); 8.30 (s, 1 H); 8.12 (d, 1 H, J = 7.9 Hz); 8.03 (s, 1 H); 7.99 (d, 1 H, J = 4.0 Hz); 7.68-7.60 (m overlap, 3 H); 7.58-7.50 (m overlap, 2 H); 7.43 (dd, 1 H, J = 7.2 and 7.4 Hz); 7.21 (d, 1 H, J = 4.6 Hz); 6.85 (d, 1 H, J = 8.4 Hz); 6.77 (d, 1 H, J = 8.3 Hz); 6.55 (dd, 1 H, J = 5.7 and 5.8 Hz).

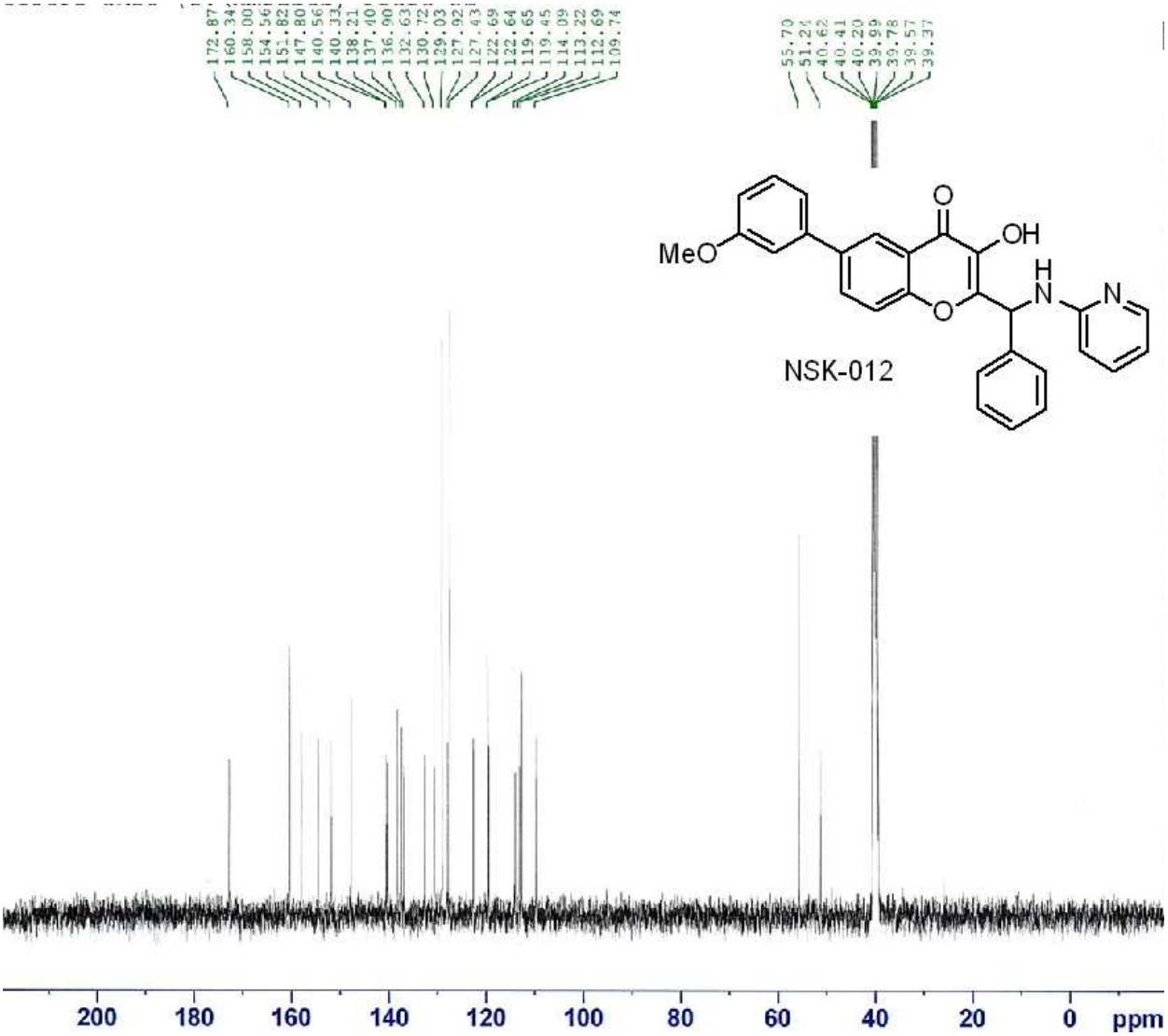


S2.5 NSK 12. Obtained from compound **7a**, 2-aminopyridine, and benzaldehyde, following a similar procedure as described for NSK 3. A light tan solid; 69% yield. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.42 (br s, 1 H, OH); 8.26 (d, 1 H, J = 2.0 Hz); 8.06 (d, 1 H, J = 7.1 Hz); 7.96 (d, 1 H, J = 4.7 Hz); 7.65 (d, 1 H, J = 8.8 Hz); 7.58 (d, 1 H, J = 7.4 Hz); 7.50 (d, 1 H, J = 7.4 Hz); 7.45-7.33 (m overlap, 6 H); 7.29 (d, 1 H, J = 6.2 Hz); 7.24 (s, 1 H); 6.98 (dd, 1 H, J = 8.0 and 1.6 Hz); 6.85 (d, 1 H, J = 8.3 Hz); 6.80 (d, 1 H, J = 8.2 Hz); 6.55 (dd, 1 H, J = 6.0 and 6.4 Hz); 3.83 (s, 3 H, OCH_3). ^{13}C NMR (100 MHz; $SO(CD_3)_2$) δ (ppm): 172.87; 160.34; 158.00; 154,56; 151.82; 147.80; 140.56; 140.33; 138.21; 137.40; 136.90; 132.63; 130.72; 129.03; 127.92; 127.43; 122.69; 122.64; 119.65; 119.45; 114.09; 113.22; 112.69; 109.74; 55.70; 51.24.



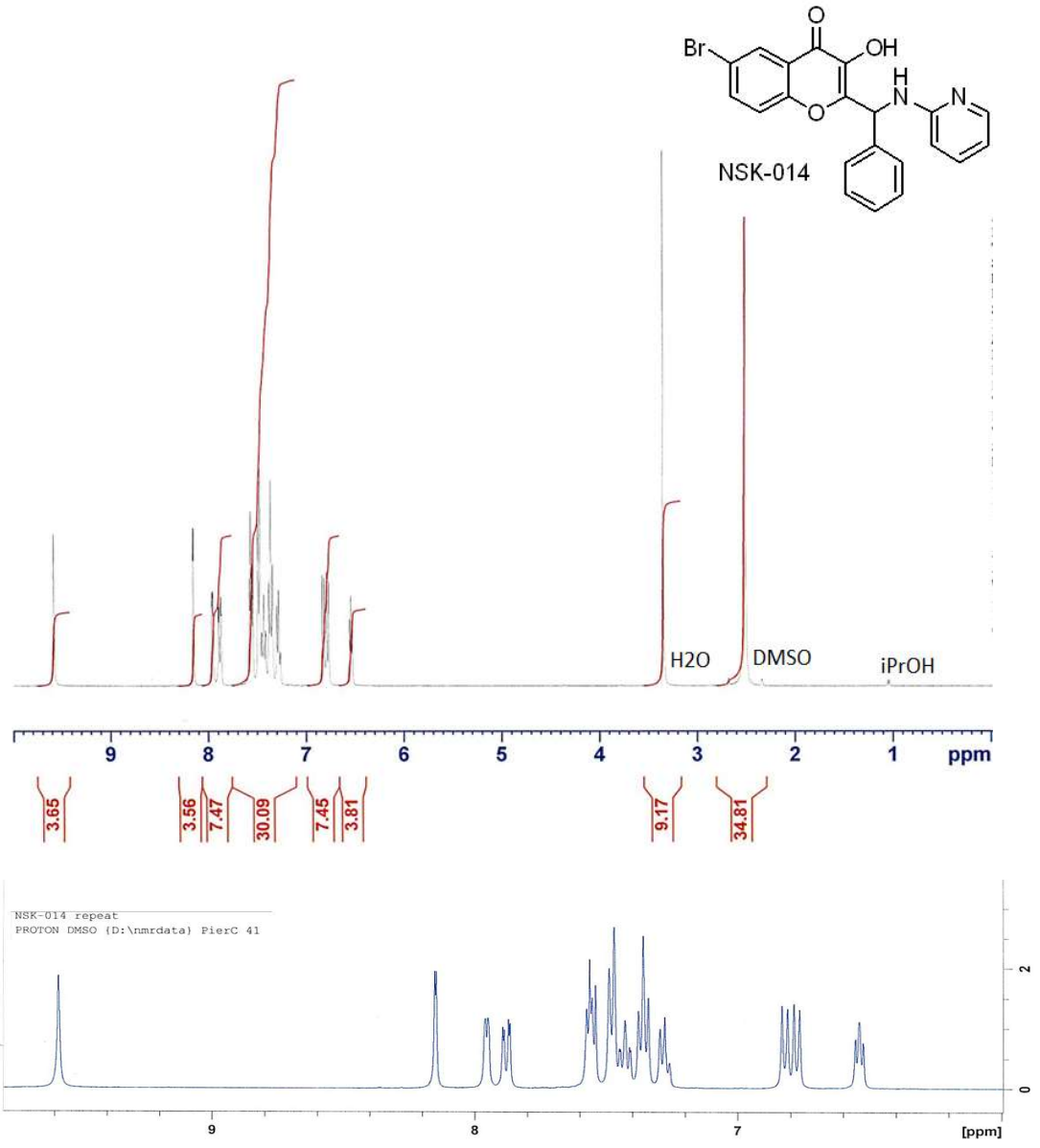
See expansion of region δ 9.5 to 6.5 ppm below (next page)





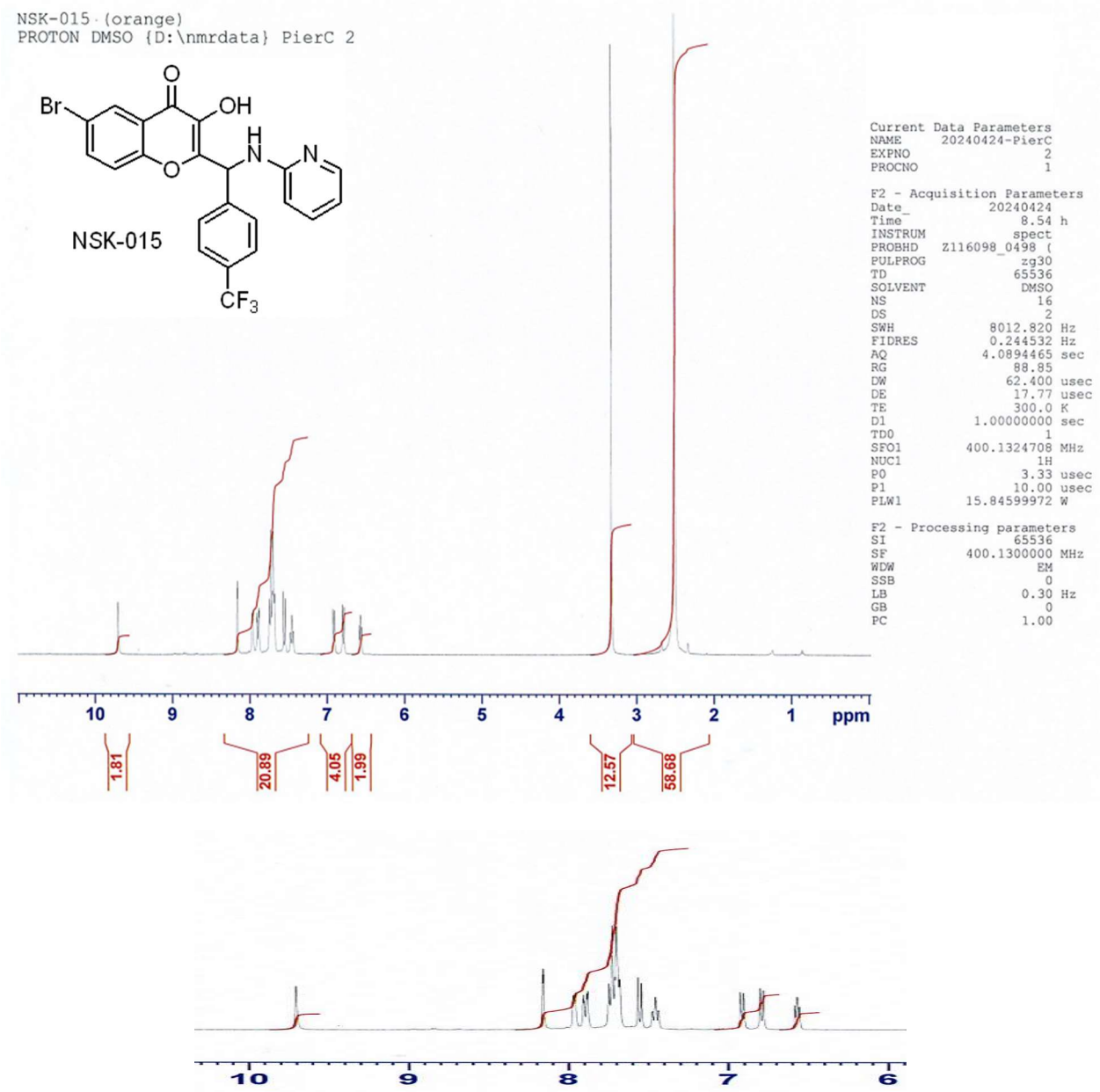
S2.6 NSK 14.

Obtained from compound **6**, 2-aminopyridine, and benzaldehyde, following a similar procedure as described for NSK 3. An off-white solid; 83% yield. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.56 (br s, 1 H, OH); 8.15 (d, 1 H, J = 2.0 Hz); 7.94 (d, 1 H, J = 5.0 Hz); 7.88 (dd, 1 H, J = 10.0 and 2.0 Hz); 7.56-7.51 (m overlap, 2 H), 7.49-7.46 (m overlap, 2 H); 7.44 (dd, 1 H, J = 10.0 and 8.5 Hz); 7.38-7.32 (m, 2 H); 7.28 (d, 1 H, J = 6.5 Hz); 6.82 (d, 1 H, J = 8.8 Hz); 6.77 (d, 1 H, J = 8.8 Hz); 6.53 (dd, 1 H, J = 7.5 and 7.5 Hz).



S2.7 NSK 15.

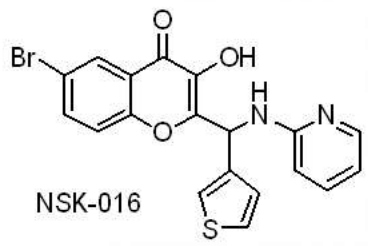
Obtained from compound **6**, 2-aminopyridine, and benzaldehyde, following a similar procedure as described for NSK 3. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.70 (s, 1 H); 8.18 (s, 1 H); 7.99 (s, 1 H); 7.89 (d, 1 H); 7.75-7.60 (m overlap, 5 H); 7.51 (d, 1 H); 7.42 (dd, 1 H); 6.91 (d, 1 H); 6.80 (d, 1 H); 6.50 (dd, 1 H).



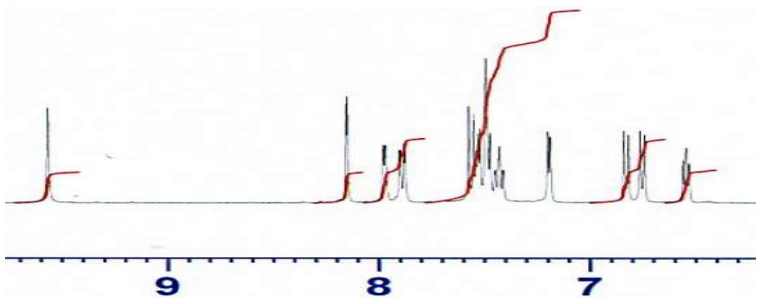
S2.8 NSK 16.

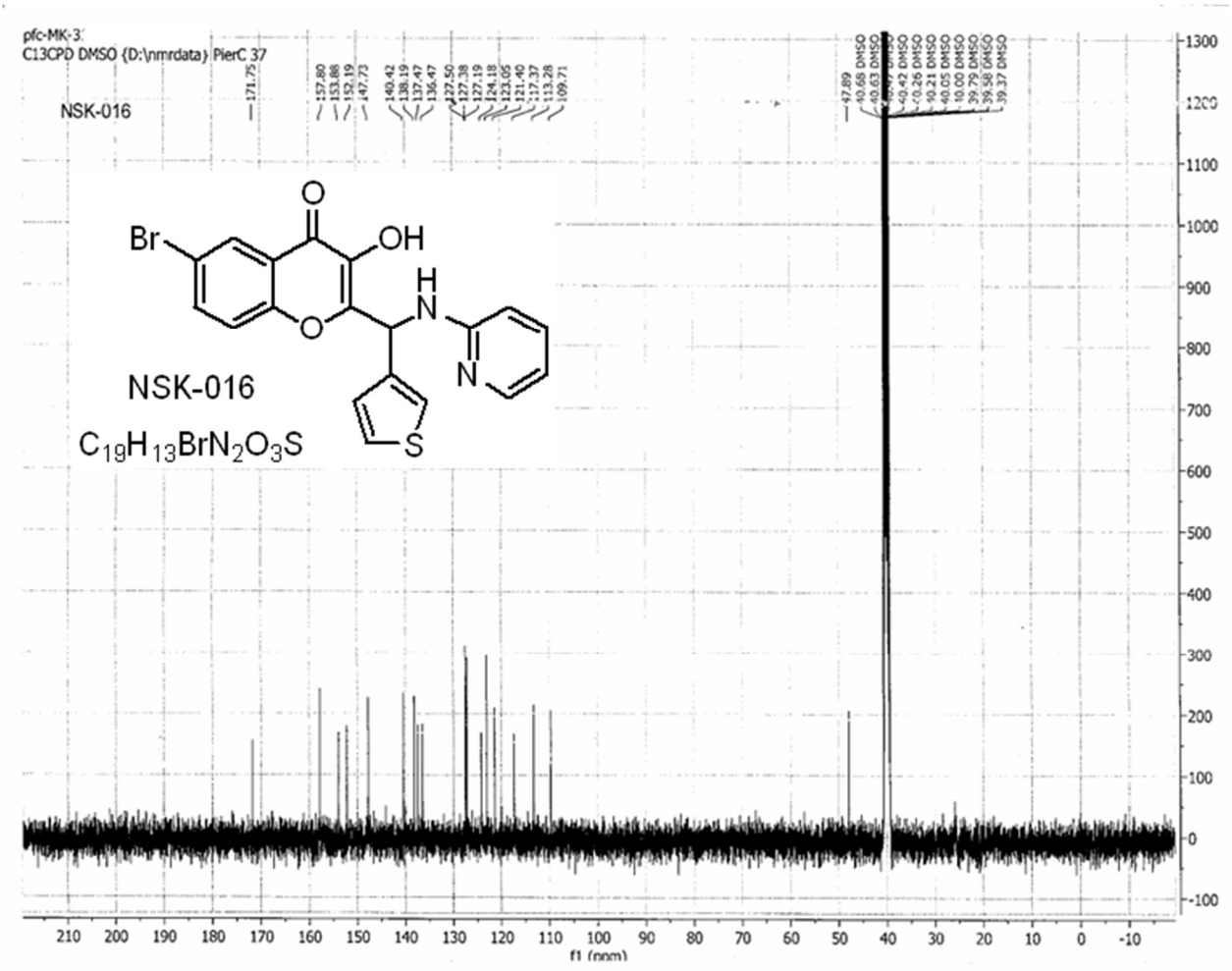
Obtained from compound **6**, 2-aminopyridine, and benzaldehyde, following a similar procedure as described for NSK 3. A light tan powder; 94% yield. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.55 (s, 1 H); 8.16 (s, 1 H); 8.00 (s, 1 H); 7.90 (d, 1 H); 7.50-7.30 (m overlap, 5 H); 7.19 (d, 1 H); 6.81 (d, 1 H); 6.75 (d, 1 H); 6.53 (m, 1 H). ^{13}C NMR (100 MHz; $SO(CD_3)_2$) δ (ppm): 171.75; 157.80; 153.88; 152.19; 147.73; 140.42; 138.19; 137.47; 136.47; 127.50; 127.38; 127.19; 124.18; 123.05; 121.40; 117.37; 113.28; 109.71; 47.89.

NSK-016
PROTON DMSO (D:\nmrdata) PierC 3



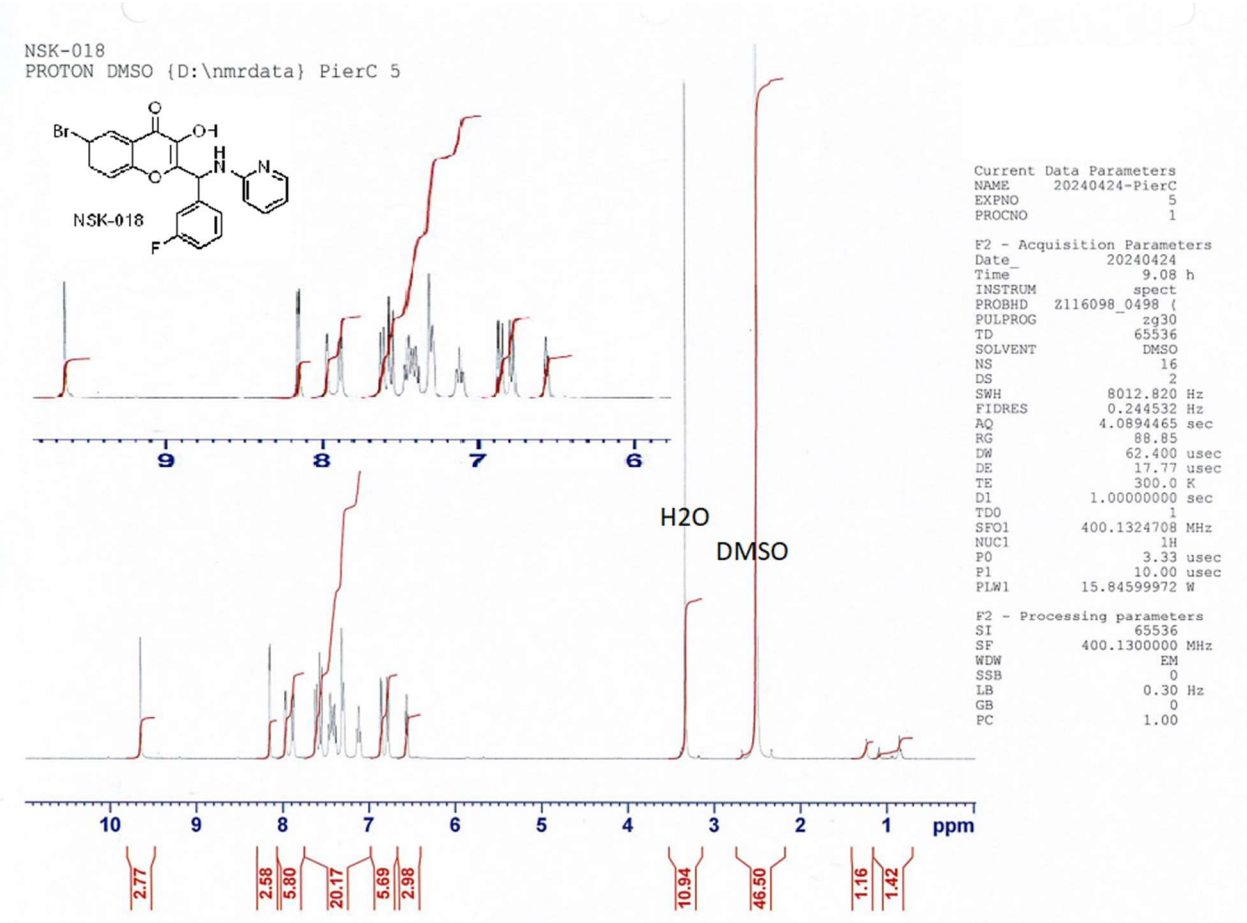
NSK-016




pfc-MK-3
C13CPD DMSO (D:\nmrdata) PierC 37
NSK-016
171.75
157.80
153.86
152.19
147.73
140.42
138.19
137.47
136.47
127.50
127.38
127.19
124.18
123.05
121.40
117.37
113.28
109.71
47.89
40.68 DMSO
40.63 DMSO
40.47 DMSO
40.26 DMSO
40.21 DMSO
40.05 DMSO
40.00 DMSO
39.79 DMSO
39.58 DMSO
39.37 DMSO
Br
O
OH
H
N
O
N
S
NSK-016
$C_{19}H_{13}BrN_2O_3S$
f1 (ppm)

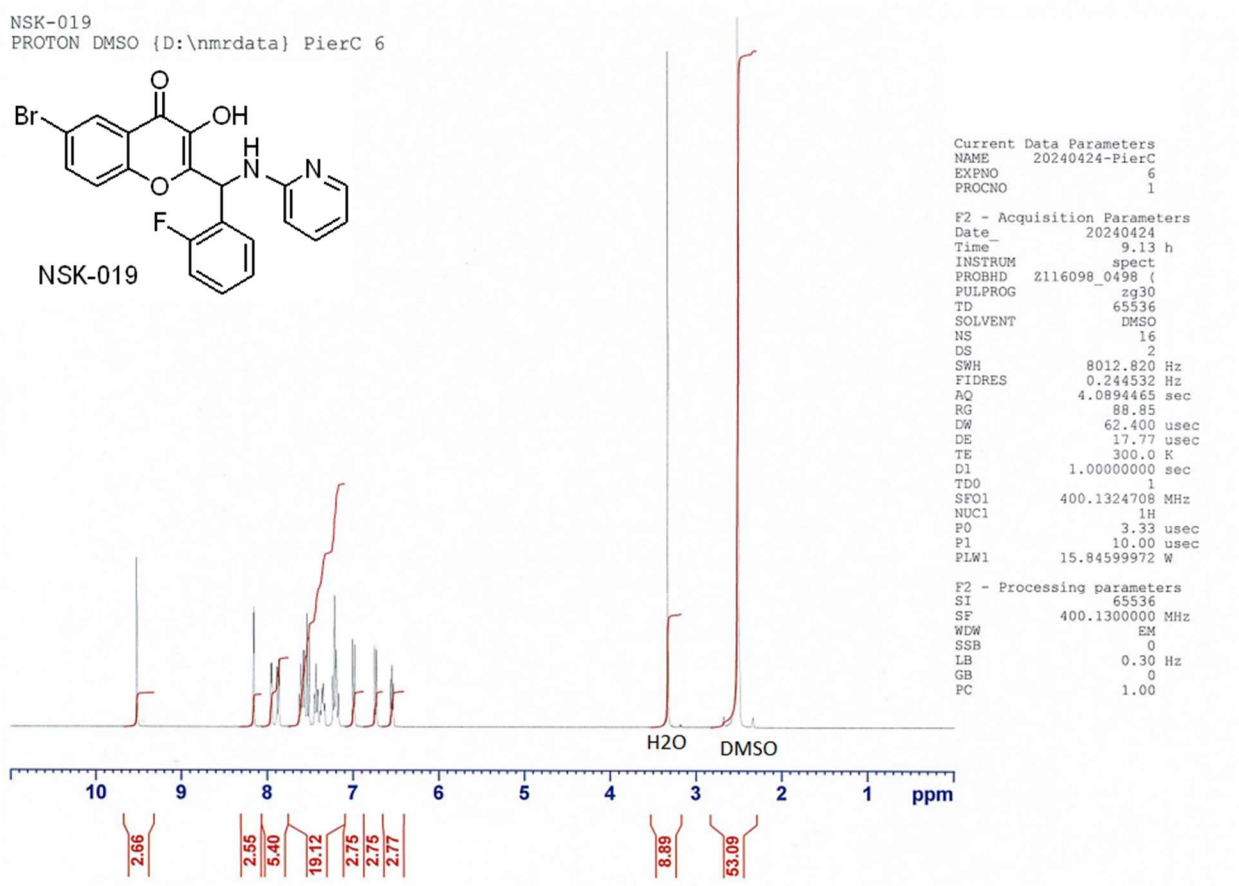
S2.9 NSK 18.

Obtained from compound **6**, 2-aminopyridine, and 3-fluorobenzaldehyde, following a similar procedure as described for NSK 3. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.62 (s, 1 H); 8.15 (s, 1 H); 7.98 (s, 1 H); 7.89 (d, 1 H); 7.61 (d, 1 H); 7.58 (d, 1 H); 7.50-7.40 (m overlap, 2 H); 7.31-7.25 (m overlap, 2 H); 7.10 (dd, 1 H); 6.85 (d, 1 H); 6.78 (d, 1 H); 6.55 (dd, 1 H).



S2.10 NSK 19.

Obtained from compound **6**, 2-aminopyridine, and 2-fluorobenzaldehyde, following a similar procedure as described for NSK 3. ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ (ppm): 9.51 (s. 1 H); 8.18 (s, 1 H); 7.96 (s, 1 H); 7.89 (d, 1 H); 7.65-7.52 (overlap m, 3 H); 7.46 (dd, 1 H); 7.39 (dd, 1 H); 7.27-7.15 (m, 2 H); 6.99 (d, 1 H); 6.73 (d, 1 H); 6.56 (dd, 1 H).



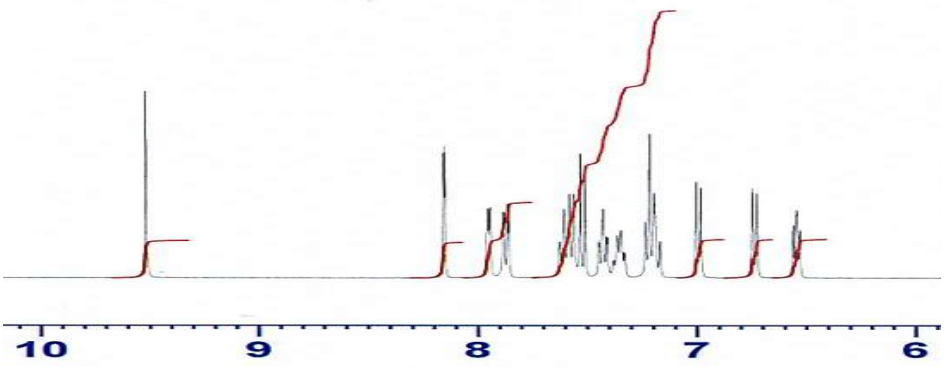


Table S1. Summary of compound concentrations, stock concentrations, and dilution series used for each microorganism and compound for growth-inhibition experiments.

Microorganism	Compounds	Compound Concentration in 250 µL Bacterial solutions (µg/mL)	Stock Concentrations in DMSO (mg/mL)	Volume of Stock Solutions added into 249.5 µL Bacterial Solutions for the Final Compound Concentrations (µL)
P. aeruginosa PAO1 & *S. aureus* ALC2085	NSK 3	200, 100, 80, 40, 20, 10, 1	100, 50, 40, 20, 5, 0.5	0.5
	NSK 4			
	NSK 6			
	NSK 7			
	NSK 12			
	NSK 14			
	NSK 15	40, 20, 10, 5, 2.5 1.25	20, 10, 5, 2.5, 1.25, 0.625	
	NSK 16			
	NSK 18			
	NSK 19			
P. aeruginosa PAO1	CIP			