

Synthesis and *in vitro* Antiproliferative Activity of Flavone and 6-Hydroxyflavone Oxime Ethers Derivatives

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Herein we report the synthesis of a series of *O*-alkyl oximes of flavone and 6-hydroxyflavone using a simple experimental protocol under solvent free conditions with yields up to 87%. Cytotoxicity of all compounds was evaluated against MDA-MB-231, PC-3, A-549 and MRC-5 cells. IC₅₀ values for two compounds were determined to be in the range 28.7-47.8 μM against all tested cell lines. Oxime ethers derivatives showed IC₅₀ values between 28.7 and 49.5 μM against MDA-MB-231, while the best activity was obtained for 6-hydroxyflavone with an IC₅₀ of 3.4 μM against this cell line. Compounds containing the substituent hydroxyl at the position six of flavone system displayed the best antiproliferative activity over MDA-MB-231 cells, being necessary this group to improve the sensibility on this type of cells. The antiproliferative activity of 6-hydroxyflavone is drastically diminished when the carbonyl group of flavone is changed by an oxime ether.

Keywords: synthesis, oxime ethers, flavone, 6-hydroxyflavone, antiproliferative activity

Introduction

Oxime ethers are useful and versatile compounds in organic synthesis, they are commonly easily prepared and relatively stable to moisture; thus, they can be stored under air during long periods.¹ Oxime is a pharmacophore group present in a variety of compounds with diverse biological activities, for instance, some α,β -unsaturated oximes are known as anticancer,² immunosuppressive,³ and antibacterial⁴ agents.

On the other hand, flavone scaffold is an important core in many compounds displaying a variety of pharmacological properties that depend on their substitution patterns. The wide range of biological activities of flavones has attracted great interest in the synthesis of derivatives with the objective of developing new therapeutic agents.⁵

Although oxime ether derivatives of flavones could display relevant biological activity, there are only a few reports about these kind of compounds, suggesting that these derivatives are not as promptly prepared. The first synthesis of a flavone oxime was reported in 1952 using

hydroxylamine, and 4-thionflavone or 4-thionflavone methiodide as precursors.⁶ Previous attempts using directly hydroxylamine and a flavone conducted to 3-*o*-hydroxyphenyl-5-phenylisooxazole instead of the oxime.⁷

Meshcheryakova *et al.*⁸ reported the synthesis of some flavone oxime ether derivatives using 4,4-dichloroflavone or 4-thionflavone and *O*-alkyl hydroxylamines, and the evaluation of their pharmacological activity revealed that they act on the central nervous system. Green *et al.*⁹ achieved the synthesis of 2-arylchromenone oximes using Ollis or Lawesson methods; and patented their use as inhibitors of protein kinases.

Schann *et al.*¹⁰ synthesized 2-heteroarylchromenone oximes as allosteric modulators of metabotropic glutamate receptors, via the synthesis of *O*-*t*-butyl oxime ethers employing microwave (MW) irradiation in methanol, and further deprotection. They also prepared some of these compounds directly with hydroxylamine under MW, with poor yields.

Therefore, this work was undertaken with the aim of extending the knowledge about the synthesis, characterization and applications of flavone oxime ethers.

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We prepared and evaluated the antiproliferative activity of twelve derivatives containing *O*-alkyl, *O*-benzyl and *O*-allyl moieties.

Results and Discussion

Chemistry

In order to obtain the target compounds by a previously optimized protocol, we carried out experiments using the model reaction between flavone (**1a**) and *O*-methylhydroxylamine hydrochloride (**2a**) in pyridine as a solvent and a base (Table 1, entry 1). Under these conditions, no evidence of the formation of compound **3a** was observed after 18 h, and the flavone was mostly recovered.

Then, we adapted procedures reported in the literature for analog structures using microwave irradiation. The reaction with *O*-methylhydroxylamine hydrochloride in methanol under microwave for 30 minutes (entry 2)¹⁰ did not give rise to compound **3a**; thus, we decided to perform a new attempt using the former conditions, adding pyridine as the base (entry 3). This procedure allowed us obtaining the desired oxime ether **3a** in a 19% according to ¹H nuclear magnetic resonance (NMR) spectra of the crude mixture, as a single isomer.

Due to the low reactivity displayed by flavone, we employed the conditions reported by Meshcheryakova *et al.*,⁸ that includes the transformation of flavone in 4,4-dichloroflavene (entry 4). The reaction was monitored by gas chromatography-mass spectrometry (GC-MS) and the dichloride intermediate was detected after the first step (a), but no evidence of the formation of **3a** was observed after the second one (b).

A fifth experiment (entry 5), employing 6 equivalents of both *O*-methylhydroxylamine hydrochloride and dry pyridine at 140 °C was carried out in order to displace the equilibrium towards the formation of oxime ether **3a**. After 2 h under these conditions, the starting material was not completely consumed, but the desired oxime ether was observed in 75% by ¹H NMR.

Guided by the previous result, and with the aim of finding the molar ratio of the reagents that allowed to obtain the highest yield of **3a**, we performed several experiments varying the equivalents of both **2a** and pyridine. Finally we found that conditions shown in entry 6, afforded the maximum amount of product (75% calculated by ¹H NMR, 54% isolated), with the lowest equivalents of starting materials.


Based on the results shown above, we synthesized a series of oxime ethers of flavone and 6-hydroxyflavone (**3b-l**) using the experimental conditions of entry 6. In order to synthesize oxime ethers **3c-f,i-l**, it was necessary to obtain the *O*-allyl and *O*-benzylhydroxylamines **5c-f** not commercially available, via Gabriel synthesis, and their hydrochlorides **2c-f** (Scheme 1).

The *E/Z* geometries of oxime ethers **3a-l**, were determined by 2D NOESY (nuclear Overhauser effect spectroscopy) experiments, which allowed to evidence correlations between hydrogens of *O*-CH₂ group and the heterocycle ring, showing that the *E* isomer was the most favored product.

Antiproliferative activity

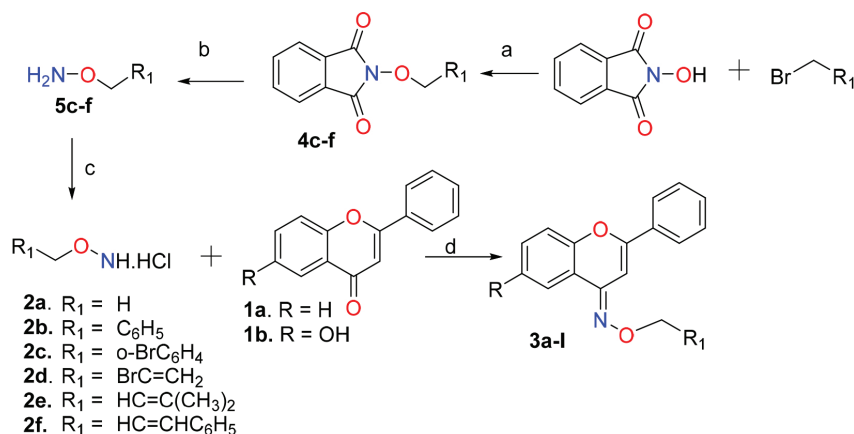
Cytotoxicity of all compounds was evaluated against MDA-MB-231, PC-3, A-549 and MRC-5 cells. Flavone (**1a**)

Table 1. Evaluation of reaction conditions to obtain compound **3a**



entry	Conditions	3a ^a / % (3a ^b / %)
1	2a (2.0 equiv.), Py, reflux, 18 h	0
2	2a (2.0 equiv.), MeOH, MW 150 W, 30 min	0
3 ^c	2a (2.0 equiv.), Py (2.0 equiv.), MeOH, MW 150 W, 30 min	5 (19)
4	(a) SOCl ₂ , Et ₃ N _(dry) , CHCl _{3(dry)} , reflux, 5 h; (b) 2a (1.2 equiv.), 1.5 h	0
5	2a (6.0 equiv.), Py _(dry) (6.0 equiv.), 140 °C (oil bath), 2.5 h	(75)
6 ^d	2a (2.5 equiv.), Py _(dry) (3.5 equiv.), 140 °C (oil bath), 2 h	54 (75)

^aIsolated yield; ^bcalculated yield by ¹H NMR; ^cmethod A; ^dmethod B.



Scheme 1. (a) K₂CO₃, DMSO, room temperature, 2-3 h (87-97%); (b) NH₂NH₂·H₂SO₄, K₂CO₃, Et₂O, H₂O, reflux, 5 h (76-90%); (c) HCl(g), Et₂O; (d) Py/Δ (54-87%).

and its oxime derivatives (**3a-f**) showed no relevant activity at the evaluated concentrations. IC₅₀ values for compounds **3h,i** presenting a hydroxyl and a benzyl group, were determined to be in the range of 28.7-47.8 μM for all tested cell lines. Compounds **3g-l** containing a hydroxyl group at sixth position, showed IC₅₀ between 28.7-49.5 μM against MDA-MB-231; however, the best activity was obtained for 6-hydroxyflavone (**1b**) with an IC₅₀ of 3.4 μM against the same cell line (Table 2). Previous studies have shown that 6-hydroxyflavone also exhibits activity over the leukemia cell lines HL-60 (IC₅₀ = 2.8 μM) and MOLT-4 (IC₅₀ = 6.3 μM).¹¹ It is important to emphasize that no antiproliferative activity was displayed by compound **1b** against healthy fibroblasts from lung (MRC-5).

On the other hand, the antitumor activity of baicalein, a naturally occurring flavonoid used in Chinese herbal

medicine, which contains hydroxyl groups at positions 5, 6 and 7, has motivated to develop studies aimed to verify the inhibitory effects and the mechanism involved in its antimetastatic effect against MDA-MB-231 cells. The IC₅₀ value of baicalein (59.5 μM),¹² higher than the IC₅₀ determined for 6-hydroxyflavone in this work over the same cell line, motivates our interest in continuing the study of this compound.

It is important to notice that despite the IC₅₀ values found in this work are not comparable with the reference drug vincristine (IC₅₀ = 0.008 μM against MDA-MB-231), the search for novel cytotoxic agents with selectivity for a particular cell line, and the comprehension of mechanisms of action, are necessary tasks towards the development of new drugs that allow to improve the existing anticancer therapies.

Table 2. *In vitro* IC₅₀ of compounds **1** and **3** against MDA-MB-231, PC-3, A-549 and MRC-5 cells

Compound	R	R ₁	IC ₅₀ / (μmol L ⁻¹)			
			MDA-MB-231	PC-3	A-549	MRC-5
1a	H	–	> 50	> 50	> 50	> 50
1b	OH	–	3.4 ± 0.1	> 50	> 50	> 50
3a	H	CH ₃	> 50	> 50	> 50	> 50
3b	H	CH ₂ C ₆ H ₅	> 50	> 50	> 50	> 50
3c	H	CH ₂ - <i>o</i> -BrC ₆ H ₄	> 50	> 50	> 50	> 50
3d	H	CH ₂ CBr=CH ₂	> 50	> 50	> 50	> 50
3e	H	CH ₂ -CH=C(CH ₃) ₂	> 50	> 50	> 50	> 50
3f	H	CH ₂ -CH=CHC ₆ H ₅	> 50	> 50	> 50	> 50
3g	OH	CH ₃	49.5 ± 0.8	> 50	> 50	> 50
3h	OH	CH ₂ C ₆ H ₅	28.7 ± 2.7	47.8 ± 0.6	40.2 ± 0.5	44.5 ± 0.7
3i	OH	CH ₂ - <i>o</i> -BrC ₆ H ₄	40.4 ± 1.7	43.6 ± 2.6	47.4 ± 0.9	39.8 ± 2.7
3j	OH	CH ₂ CBr=CH ₂	34.8 ± 2.2	> 50	> 50	39.7 ± 2.5
3k	OH	CH ₂ -CH=C(CH ₃) ₂	45.4 ± 0.3	> 50	> 50	> 50
3l	OH	CH ₂ -CH=CHC ₆ H ₅	38.7 ± 3.4	> 50	> 50	> 50

The IC₅₀ value was defined as the concentration of the compound which caused a 50% decrease of the cell viability.

Conclusions

The synthesis of twelve flavone and 6-hydroxyflavone oxime ether derivatives was achieved with reasonably good yields as a single isomer (*E*), using a simple experimental protocol. Changing the carbonyl group of flavone for an oxime ether did not increase the cytotoxic activity of the derivatives against the tested line cells. Hydroxyl group at the position six of flavone system is necessary to display activity against MDA-MB-231 since all compounds containing this substituent showed antiproliferative activity over this cell line. The antiproliferative activity of 6-hydroxyflavone is drastically diminished when the carbonyl group of flavone is changed by an oxime ether.

Experimental

Chemistry

¹H and ¹³C NMR spectra were acquired on a Bruker Avance spectrometer (300 and 75 MHz, respectively) in CDCl₃ or CD₃OD. Chemical shifts and configuration were assigned with the help of HSQC-edit (heteronuclear single quantum correlation), HMBC (heteronuclear multiple bond correlation), COSY (correlation spectroscopy) and NOESY experiments. High-resolution mass spectra (HRMS) were recorded on an Agilent 6520 q-TOF-MS instrument with orthogonal ESI (electrospray ionization). GC-MS analyses were performed on an Agilent 6850 series II gas chromatograph coupled to an Agilent 5975B VL mass spectrometer (electron ionization, 70 eV) equipped with split/splitless inlet (split relation 15:1, 260 °C), Agilent 6850 series automatic injector, and Agilent HP-5MS column (30 m × 0.25 mm × 0.25 μm); initial oven temperature 80 °C for 1 min, then a temperature ramp of 10 °C min⁻¹ to 320 °C (hold 3 min); total run time 28 min. Melting points were determined on a Thermo Fisher Scientific Rochford 9100 apparatus.

Synthesis of oxime ether derivatives **3a-l**

Method A (entry 3)

A solution of flavone **1a** (1.0 mmol), *O*-methyl hydroxylamine hydrochloride (2.0 mmol), and pyridine (2.0 mmol) in methanol (6.6 mL), was irradiated at 150 W for 30 minutes. The reaction mixture was treated with distilled water and extracted with dichloromethane. The organic phase was dried with Na₂SO₄, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography using silica gel and hexane:CH₂Cl₂ (4:1), yielding 5% of **3a**.

Method B (entry 6)

A mixture of flavone or 6-hydroxyflavone **1a-b** (1.0 mmol), an appropriate *O*-alkyl hydroxylamine hydrochloride (**2a-e**) (2.5 mmol), and dry pyridine (3.5 mmol), was stirred at 140 °C for 2-4 h. The reaction mixture was treated with distilled water and extracted with dichloromethane. The organic phase was dried with Na₂SO₄, filtered and concentrated under reduced pressure. The organic combined were purified by column chromatography (silica gel, hexane:CH₂Cl₂ 4:1 or CH₂Cl₂) to provide products **3a-l**.

2-Phenyl-4*H*-chromen-4-one *O*-methyloxime (**3a**)

Yield 54%; mp 57-58 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.05 (s, 3H, OCH₃), 7.08 (s, 1H, C=CH), 7.30-7.32 (m, 2H, H-6, 7 Ar), 7.42-7.49 (m, 4H, H-8, H-3', 4', 5' Ar), 7.89-7.92 (m, 2H, H-2', 6' Ar), 8.04 (dd, 1H, *J* 8.1, 1.5 Hz, H-5 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 62.06 (OCH₃), 93.81 (C=C), 117.53 (C-6 Ar), 118.37 (C-4a Ar), 123.01 (C-5 Ar), 124.65 (C-7 Ar), 125.71 (C-2', 6' Ar), 128.65 (C-8 Ar), 130.25 (C-4' Ar), 130.43 (C-3', 5' Ar), 132.89 (C-1' Ar), 143.84 (C-8a Ar), 151.91 (C=N), 155.23 (C=CH). Mass spectrum, *m/z* (relative intensity (*I*_{rel}), %): 251 [M]⁺ (72), 236 (15), 206 (100). Found, *m/z*: 252.1025 [M + H]⁺, C₁₆H₁₄NO₂. Calculated, *m/z*: 252.1025.

2-Phenyl-4*H*-chromen-4-one *O*-benzyloxime (**3b**)

Yield 70%; mp 69-70 °C; ¹H NMR (300 MHz, CDCl₃) δ 5.30 (s, 2H, OCH₂), 7.14 (s, 1H, C=CH), 7.21-7.37 (m, 8H, H-6, 7, 8, 2'', 3'', 4'', 5'', 6'' Ar), 7.39-7.52 (m, 3H, H-3', 4', 5' Ar), 7.87-7.91 (m, 2H, H-2', 6' Ar), 8.05 (dd, 1H, *J* 8.1, 1.5 Hz, H-5 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 76.22 (OCH₂), 94.07 (C=CH), 117.52 (C-6 Ar), 118.42 (C-4a Ar), 123.14 (C-7 Ar), 124.64 (C-5 Ar), 125.77 (C-2', 6' Ar), 127.75 (C-2'', 6'' Ar), 128.30 (C-3'', 5'' Ar), 128.38 (C-4'' Ar), 128.66 (C-8 Ar), 130.27 (C-4', Ar), 130.46 (C-3', 5' Ar), 132.88 (C-1' Ar), 138.30 (C-1'' Ar), 144.14 (C-8a Ar), 151.92 (C=N), 155.14 (C=CH). Mass spectrum, *m/z* (*I*_{rel}, %): 327 [M]⁺ (36), 236 (46), 206 (100). Found, *m/z*: 328.1337 [M + H]⁺, C₂₂H₁₈NO₂. Calculated, *m/z*: 328.1338.

2-Phenyl-4*H*-chromen-4-one *O*-(2-bromobenzyl)oxime (**3c**)

Yield 74%; mp 82-83 °C; ¹H NMR (300 MHz, CDCl₃) δ 5.37 (s, 2H, OCH₂), 7.16-7.36 (m, 6H, C=CH, H-6, 7 Ar, H-4'', 5'', 6'' Ar), 7.45-7.54 (m, 4H, H-8, 3', 4', 5' Ar), 7.60 (d, 1H, *J* 8.7 Hz, H-3'' Ar), 7.91-7.92 (m, 2H, H-2', 6' Ar), 8.03 (dd, 1H, *J* 7.5, 0.3 Hz, H-5 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 75.39 (OCH₂), 94.00 (C=CH), 117.50 (C-6 Ar), 118.30 (C-4a Ar), 122.84 (C-Br), 123.24 (C-5 Ar), 124.66 (C-7 Ar), 125.79 (C-2', 6' Ar), 127.30 (C-4''

Ar), 128.68 (C-5'' Ar), 128.94 (C-3', 5' Ar), 129.65 (C-6'' Ar), 130.32 (C-4' Ar), 130.53 (C-8 Ar), 132.55 (C-3'' Ar), 132.89 (C-1'), 137.94 (C-1'' Ar), 144.57 (C-8a Ar), 151.91 (C=N), 155.38 (C=CH). Mass spectrum, m/z (I_{rel} , %): 405 [M(⁷⁹Br)]⁺ (12), 236 (52), 206 (100). Found, m/z : 406.0446 [M + H]⁺, C₂₂H₁₇BrNO₂. Calculated, m/z : 406.0443.

2-Phenyl-4*H*-chromen-4-one *O*-(2-bromoallyl)oxime (**3d**)

Yield 72%; mp 79-80 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.83 (s, 2H, OCH₂), 5.67 (s, 1H, BrC=CH), 5.97 (s, 1H, BrC=CH), 7.16 (s, 1H, C=CH), 7.22-7.32 (m, 2H, H-6, 7 Ar), 7.45-7.49 (m, 4H, H-8, 3', 4', 5' Ar), 7.91-7.92 (m, 2H, H-2', 6' Ar), 8.04 (dd, 1H, J 7.8, 1.2 Hz, H-5 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 77.45 (OCH₂), 93.79 (C=CH), 117.49 (BrC=CH₂), 117.55 (C-6 Ar), 118.05 (C-4a Ar), 123.24 (C-5 Ar), 124.72 (C-7 Ar), 125.81 (C-2', 6' Ar), 128.71 (C-3', 5' Ar), 129.66 (BrC=CH₂), 130.42 (C-4' Ar), 130.68 (C-8 Ar), 132.79 (C-1' Ar), 144.92 (C-8a Ar), 151.96 (C=N), 155.31 (C=CH). Mass spectrum, m/z (I_{rel} , %): 355 [M(⁷⁹Br)]⁺ (13), 236 (36), 206 (100). Found, m/z : 356.0287 [M + H]⁺, C₁₈H₁₅BrNO₂. Calculated, m/z : 356.0286.

2-Phenyl-4*H*-chromen-4-one *O*-(3-methylbut-2-en-1-yl)oxime (**3e**)

Yield 64%; ¹H NMR (300 MHz, CDCl₃) δ 1.82 (s, 3H, CH₃), 1.84 (s, 3H, CH₃), 4.75 (d, 2H, J 7.0 Hz, OCH₂), 5.51 (t, 1H, J 7.0 Hz, HC=C(CH₃)₂), 7.10 (s, 1H, C=CH), 7.20-7.26 (td, 1H, J 7.2, 1.3 Hz, H-6 Ar), 7.28-7.32 (dd, 1H, J 8.0, 1.3 Hz, H-8 Ar), 7.40-7.54 (m, 4H, H-7, H-3', 4', 5' Ar), 7.86-7.96 (m, 2H, H-2', 6' Ar), 8.06 (dd, 1H, J 7.95, 1.3 Hz, H-5 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 18.28 (CH₃), 25.94 (CH₃), 70.91 (OCH₂), 94.14 (C=CH), 117.48 (C-8 Ar), 118.64 (C-4a Ar), 120.60 (HC=C(CH₃)₂), 123.06 (C-5 Ar), 124.57 (C-6 Ar), 125.72 (C-2', 6' Ar), 128.60 (C-3', 5' Ar), 130.16 (C-4' Ar), 130.30 (C-7 Ar), 132.97 (C-1' Ar), 137.57 (HC=C(CH₃)₂), 143.63 (C-8a Ar), 151.91 (C=N), 154.92 (C=CH). Found, m/z : 306.1494 [M + H]⁺, C₂₀H₂₀NO₂. Calculated, m/z : 306.1494.

2-Phenyl-4*H*-chromen-4-one *O*-cinnamyloxime (**3f**)

Yield 60%; mp 68-69 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.93 (d, 2H, J 6.2 Hz, OCH₂), 6.49-6.62 (dt, 1H, J 16.0, 6.2 Hz, HC=CHPh), 6.76 (d, 1H, J 16.0 Hz, HC=CHPh), 7.16 (s, 1H, C=CH), 7.22-7.56 (m, 11H, H-6, 7, 8, 3', 4', 5', 2'', 3'', 4'', 5'', 6'' Ar), 7.87-7.97 (m, 2H, H-2', 6' Ar), 8.09 (dd, 1H, J 7.9, 1.2 Hz, H-5 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 74.97 (OCH₂), 94.00 (C=CH), 117.55 (C-8 Ar), 118.44 (C-4a Ar), 123.14 (C-5 Ar), 124.68 (C-6 Ar), 125.76 (C-2', 6' Ar), 125.94 (HC=CHPh), 126.65 (C-2'', 6'' Ar), 127.76 (C-4'' Ar), 128.59 (C-3', 5' Ar), 128.68 (C-3'', 5''

Ar), 130.30 (C-4' Ar), 130.48 (C-7 Ar), 132.86 (C-1' Ar), 133.03 (HC=CHPh), 136.83 (C-1'' Ar), 144.04 (C-8a Ar), 151.92 (C=N), 155.14 (C=CH). Found, m/z : 354.1496 [M + H]⁺, C₂₀H₂₀NO₂. Calculated, m/z : 354.1494.

6-Hydroxy-2-phenyl-4*H*-chromen-4-one *O*-methyloxime (**3g**)

Yield 54%; mp 149-150 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.30 (s, 3H, OCH₃), 6.93 (dd, 1H, J 9.0, 2.9 Hz, H-7 Ar), 6.96 (s, 1H, C=CH), 7.19 (d, 1H, J 9.0 Hz, H-8 Ar), 7.31 (d, 1H, J 2.9 Hz, H-5 Ar), 7.40-7.50 (m, 3H, H-3', 4', 5' Ar), 7.70-7.80 (m, 2H, H-2', 6' Ar); ¹³C NMR (75 MHz, CDCl₃) δ 60.66 (OCH₃), 92.00 (C=CH), 106.50 (C-5 Ar), 118.30 (C-7 Ar), 118.40 (C-4a Ar), 118.69 (C-8 Ar), 125.18 (C-2', 6' Ar), 128.39 (C-3', 5' Ar), 129.92 (C-4' Ar), 132.78 (C-1' Ar), 143.92 (C-8a Ar), 145.54 (C-6 Ar), 154.23 (C=N), 155.17 (C=CH). Found, m/z : 268.0975 [M + H]⁺, C₁₆H₁₄NO₃. Calculated, m/z : 268.0974.

6-Hydroxy-2-phenyl-4*H*-chromen-4-one *O*-benzyloxime (**3h**)

Yield 58%; mp 150-151 °C; ¹H NMR (300 MHz, CDCl₃) δ 5.20 (s, 2H, OCH₂), 6.95 (dd, 1H, J 9.0, 3.0 Hz, H-7 Ar), 7.05 (s, 1H, C=CH), 7.18 (d, 1H, J 9.0 Hz, H-8 Ar), 7.28-7.35 (m, 6H, H-5, 2'', 3'', 4'', 5'', 6'' Ar), 7.37-7.45 (m, 3H, H-3', 4', 5' Ar), 7.81-7.84 (m, 2H, H-2', 6' Ar); ¹³C NMR (75 MHz, CDCl₃) δ 75.84 (OCH₂), 92.70 (C=CH), 106.96 (C-5 Ar), 118.37 (C-4a Ar), 118.62 (C-7 Ar), 119.08 (C-8 Ar), 125.67 (C-2', 6' Ar), 127.66 (C-2'', 6'' Ar), 128.08 (C-3'', 5'' Ar), 128.24 (C-4'' Ar), 128.53 (C-3', 5' Ar), 130.16 (C-4' Ar), 132.85 (C-1' Ar), 138.02 (C-1'' Ar), 145.12 (C-8a Ar), 145.85 (C-6 Ar), 153.86 (C=N), 156.54 (C=CH). Found, m/z : 344.1287 [M + H]⁺, C₂₂H₁₈NO₃. Calculated, m/z : 344.1287.

6-Hydroxy-2-phenyl-4*H*-chromen-4-one *O*-(2-bromobenzyl)oxime (**3i**)

Yield 76%; mp 123-124 °C; ¹H NMR (300 MHz, CDCl₃) δ 5.28 (s, 2H, OCH₂), 6.95 (dd, 1H, J 8.9, 3.0 Hz, H-7 Ar), 7.11 (s, 1H, C=CH), 7.15-7.26 (m, 2H, H-8, 4'' Ar), 7.28-7.39 (m, 2H, H-5, 5'' Ar), 7.41-7.48 (m, 3H, H-3', 4', 5' Ar), 7.51 (d, 1H, J 7.6 Hz, H-6'' Ar), 7.58 (d, 1H, J 7.6 Hz, H-3'' Ar), 7.81-7.91 (m, 2H, H-2', 6' Ar); ¹³C NMR (75 MHz, CDCl₃) δ 74.89 (OCH₂), 92.29 (C=CH), 106.73 (C-5 Ar), 118.32 (C-4a Ar), 118.39 (C-7 Ar), 118.93 (C-8 Ar), 122.52 (C-Br), 125.35 (C-2', 6' Ar), 127.16 (C-5''), 128.45 (C-3', 4', 5' Ar), 128.91 (C-4'' Ar), 129.66 (C-3'' Ar), 132.28 (C-6'' Ar), 132.78 (C-1' Ar), 137.64 (C-1'' Ar), 145.00 (C-8a Ar), 145.66 (C-6 Ar), 154.54 (C=N), 155.50 (C=CH). Found, m/z : 422.0398 [M + H]⁺, C₂₂H₁₇BrNO₃. Calculated, m/z : 422.0392.

6-Hydroxy-2-phenyl-4*H*-chromen-4-one *O*-(2-bromoallyl) oxime (**3j**)

Yield 69%; mp 129-130 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.76 (s, 2H, OCH₂), 5.61 (s, 1H, BrC=CH), 5.93 (s, 1H, BrC=CH), 6.96 (dd, 1H, *J* 9.0, 3.0 Hz, H-7 Ar), 7.07 (s, 1H, C=CH), 7.21 (d, 1H, *J* 9.0 Hz, H-8 Ar), 7.32 (d, 1H, *J* 3.0 Hz, H-5 Ar), 7.40-7.50 (m, 3H, H-3', 4', 5', Ar), 7.80-7.90 (m, 2H, H-2', 6' Ar); ¹³C NMR (75 MHz, CDCl₃) δ 77.14 (OCH₂), 92.53 (C=CH), 106.99 (C-5 Ar), 117.37 (BrC=CH₂), 118.14 (C-4a Ar), 118.64 (C-8 Ar), 119.30 (C-7 Ar), 125.62 (C-2', 6' Ar), 128.57 (C-3', 5' Ar), 129.42 (BrC=CH₂), 130.27 (C-4', Ar), 132.78 (C-1' Ar), 145.76 (C-6 Ar), 145.88 (C-8a Ar), 153.93 (C=N), 155.80 (C=CH). Found, *m/z*: 372.0235 [M + H]⁺, C₁₈H₁₅BrNO₃. Calculated, *m/z*: 372.0235.

6-Hydroxy-2-phenyl-4*H*-chromen-4-one *O*-(3-methylbut-2-en-1-yl)oxime (**3k**)

Yield 67%; mp 82-84 °C; ¹H NMR (300 MHz, CDCl₃) δ 1.77 (s, 3H, CH₃), 1.80 (s, 3H, CH₃), 4.74 (d, 2H, *J* 7.0 Hz, OCH₂), 5.56 (t, 1H, *J* 7.0 Hz, HC=C(CH₃)₂), 7.00 (dd, 1H, *J* 8.9, 2.8 Hz, H-7 Ar), 7.06 (s, 1H, C=CH), 7.19 (d, 1H, *J* 8.9 Hz, H-8 Ar), 7.43-7.51 (m, 4H, H-5, 3', 4', 5' Ar), 7.80-7.92 (m, 2H, H-2', 6' Ar); ¹³C NMR (75 MHz, CDCl₃) δ 18.30 (CH₃), 25.98 (CH₃), 70.71 (OCH₂), 93.24 (C=CH), 107.54 (C-5 Ar), 118.67 (C-4a Ar), 118.96 (C-7 Ar), 119.22 (C-8 Ar), 120.26 (HC=C(CH₃)₂), 125.36 (C-2', 6' Ar), 128.61 (C-3', 5' Ar), 130.22 (C-4' Ar), 132.89 (C-1' Ar), 138.08 (HC=C(CH₃)₂), 144.74 (C-8a Ar), 146.30 (C-6 Ar), 152.87 (C=N), 155.35 (C=CH). Found, *m/z*: 322.1445 [M + H]⁺, C₂₀H₂₀NO₃. Calculated, *m/z*: 322.1443.

6-Hydroxy-2-phenyl-4*H*-chromen-4-one *O*-cinnamyloxime (**3l**)

Yield 87%; mp 83-84 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.88 (d, 2H, *J* 6.0 Hz, OCH₂), 6.44-6.56 (dt, 1H, *J* 16.0, 6.0 Hz, HC=CHPh), 6.71 (d, 1H, *J* 16.0 Hz, HC=CHPh), 6.99 (dd, 1H, *J* 8.9, 2.9 Hz, C-7), 7.02 (d, 1H, *J* 8.9 Hz, C-8), 7.09 (s, 1H, C=CH), 7.24-7.55 (m, 9H, H-5, 3', 4', 5', 2'', 3'', 4'', 5'', 6'' Ar), 7.84-7.92 (m, 2H, H-2', 6' Ar); ¹³C NMR (75 MHz, CDCl₃) δ 74.83 (OCH₂), 93.12 (C=CH), 107.55 (C-5 Ar), 118.74 (C-4a Ar), 118.92 (C-7 Ar), 119.11 (C-8 Ar), 125.76 (HC=CHPh, C-2', 6' Ar), 126.62 (C-2'', 6'' Ar), 127.74 (C-4'' Ar), 128.56 (C-3', 5' Ar), 128.64 (C-3'', 5'' Ar), 130.24 (C-4' Ar), 132.91 (C-1' Ar), 133.10 (HC=CHPh), 136.75 (C-1'' Ar), 144.57 (C-8a Ar), 146.53 (C-6 Ar), 152.94 (C=N), 155.6 (C=CH). Found, *m/z*: 370.1444 [M + H]⁺, C₂₄H₂₀NO₃. Calculated, *m/z*: 370.1443.

Synthesis of 2-alkoxyisoindoline-1,3-diones

A solution of *N*-hydroxyphthalimide (1 mmol), an alkyl bromide (0.5 mmol), and K₂CO₃ (2 mmol) in DMSO (1 mL), was stirred at room temperature for 2-3 h. The reaction mixture was treated with distilled water (10 mL), filtered, washed with water and dried to provide products **3c-f**.

2-((2-Bromobenzyl)oxy)isoindoline-1,3-dione (**4c**)

Yield 92%; mp 159-160 °C; ¹H NMR (300 MHz, CDCl₃) δ 5.37 (s, 2H, OCH₂), 7.25 (td, 1H, *J* 7.5, 1.3 Hz, H-4' Ar), 7.37 (t, 1H, *J* 7.5 Hz, H-5' Ar), 7.56 (d, 1H, *J* 7.5 Hz, H-3' Ar), 7.66 (dd, 1H, *J* 7.5, 1.3 Hz, H-6' Ar), 7.72-7.79 (m, 2H, H-4, 7 Ar), 7.79-7.86 (m, 2H, H-5, 6 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 78.67 (OCH₂), 123.55 (C-5, 6 Ar), 124.55 (C-Br), 127.65 (C-5' Ar), 128.33 (C-3a, 1a Ar), 130.72 (C-4' Ar), 131.75 (C-6' Ar), 132.89 (C-3' Ar), 133.66 (C-1' Ar), 134.47 (C-4, 7 Ar), 163.38 (C=O). Mass spectrum, *m/z* (*I*_{rel}, %): 331 [M(⁷⁹Br)]⁺, 169 (100), 76 (22).

2-((2-Bromoallyl)oxy)isoindoline-1,3-dione (**4d**)

Yield 97%; mp 110-111 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.85 (s, 2H, OCH₂), 5.79 (s, 1H, BrC=CH), 6.16 (s, 1H, BrC=CH), 7.75-7.80 (m, 2H, H-4, 7 Ar), 7.85-7.89 (m, 2H, H-5, 6 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 81.08 (OCH₂), 122.82 (BrC=CH₂), 123.71 (C-5, 6 Ar), 124.66 (BrC=CH), 128.70 (C-3a, 1a Ar), 134.70 (C-4, 7 Ar), 163.28 (C=O). Mass spectrum, *m/z* (*I*_{rel}, %): 281 [M(⁷⁹Br)]⁺, 202 (100), 162 (18). Found, *m/z*: 281.9761 [M + H]⁺, C₁₁H₉BrNO₃. Calculated, *m/z*: 281.9766.

2-((3-Methylbut-2-en-1-yl)oxy)isoindoline-1,3-dione (**4e**)

Yield 78%; mp 95-96 °C; ¹H NMR (300 MHz, CDCl₃) δ 1.75 (s, 3H, CH₃), 1.79 (s, 3H, CH₃), 4.74 (d, 2H, *J* 7.6 Hz, OCH₂), 5.55 (t, 1H, *J* 7.6 Hz, HC=C), 7.71-7.81 (m, 2H, H-4, 7 Ar), 7.82-7.89 (m, 2H, H-5, 6 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 18.13 (CH₃), 25.97 (CH₃), 74.06 (OCH₂), 117.04 (HC=C(CH₃)₂), 123.45 (C-5, 6 Ar), 128.92 (C-3a, 1a Ar), 134.41 (C-4, 7 Ar), 143.72 (HC=C(CH₃)₂), 163.86 (C=O). Found, *m/z*: 232.0971 [M + H]⁺, C₁₃H₁₄NO₃. Calculated, *m/z*: 232.0974.

2-(Cinnamyloxy)isoindoline-1,3-dione (**4f**)

Yield 87%; mp 144-145 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.88 (d, 2H, *J* 7.0 Hz, OCH₂), 6.48 (dt, 1H, *J* 15.9, 7.0 Hz, HC=CHPh), 6.69 (d, 1H, *J* 15.9 Hz, HC=CHPh), 7.22-7.43 (m, 5H, H-2', 3', 4', 5', 6' Ar), 7.71-7.78 (m, 2H, H-4, 7 Ar), 7.78-7.86 (m, 2H, H-5, 6 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 78.67 (OCH₂), 122.01 (HC=CHPh),

123.56 (C-5, 6 Ar), 126.93 (C-4' Ar), 128.47 (C-2', 6' Ar), 128.64 (C-3', 5' Ar), 128.80 (C-3a, 1a Ar), 134.50 (C-4, 7 Ar), 135.77 (C-1' Ar), 137.53 (HC=C_HPh), 163.86 (C=O). Found, *m/z*: 302.0794 [M + Na]⁺, C₁₇H₁₃NaNO₃. Calculated, *m/z*: 302.0793.

Synthesis of *O*-alkylhydroxylamine hydrochlorides

To a stirred solution of hydrazine sulfate (1.2 mmol), K₂CO₃ (1.2 mmol) and water (2.0 mL) in THF (2.0 mL), was added the corresponding *O*-alkyl-*N*-hydroxyphthalimide (1.0 mmol), and the mixture was refluxed during 5 h, then distilled water (10 mL) was added. The resulting mixture was extracted with ethyl ether (3 × 10 mL), the organic phase was dried with sodium sulfate, filtrated and the solvent evaporated under reduced pressure. The residue was chromatographed by CC (column chromatography) using silica gel, Hex:AcOEt 7:3 to give the corresponding hydroxylamine. Subsequently the hydroxylamine was dissolved in dried ethyl ether and HCl(g) was bubbled to obtain the salt (**3c-f**).

O-(2-Bromobenzyl)hydroxylamine hydrochloride (**2c**)

Yield 78%; mp 98-99 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.76 (s, 2H, OCH₂), 7.18 (td, 1H, *J* 7.5, 1.5 Hz, H-5 Ar), 7.33 (t, 1H, *J* 7.5 Hz, H-4 Ar), 7.44 (dd, 1H, *J* 7.5, 1.5 Hz, H-6 Ar), 7.57 (d, 1H, *J* 7.5 Hz, H-3 Ar); ¹³C NMR (75 MHz, CDCl₃) δ 77.14 (OCH₂), 123.54 (C-Br), 127.35 (C-5 Ar), 129.31 (C-4 Ar), 129.95 (C-6 Ar), 132.74 (C-3 Ar), 136.90 (C=C_HO Ar). Mass spectrum, *m/z* (*I*_{rel}, %): 201 [M(⁷⁹Br)]⁺, 169 (100), 90 (25).

O-(2-Bromoallyl)hydroxylamine hydrochloride (**2d**)

Yield 87%; mp 153-154 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.30 (s, 2H, OCH₂), 5.70 (d, 1H, *J* 1.2 Hz, BrC=CH), 5.93 (d, 1H, *J* 1.2 Hz, BrC=CH); ¹³C NMR (75 MHz, CDCl₃) δ 79.60 (OCH₂), 119.05 (BrC=C_H), 129.71 (BrC=C_H). Mass spectrum, *m/z* (*I*_{rel}, %): 151 [M(⁷⁹Br)]⁺, 119 (61), 39 (100).

O-(3-Methylbut-2-en-1-yl)hydroxylamine hydrochloride (**2e**)

Yield 76%; mp 160-161 °C; ¹H NMR (300 MHz, acetone-*d*₆) δ 1.72 (s, 3H, CH₃), 1.74 (s, 3H, CH₃), 4.92 (d, 2H, *J* 7.4 Hz, OCH₂), 5.37 (t, *J* 7.4 Hz, 1H, HC=C(CH₃)₂); ¹³C NMR (75 MHz, acetone-*d*₆) δ 18.20 (CH₃), 25.72 (CH₃), 72.12 (OCH₂), 116.83 (HC=C(CH₃)₂), 143.12 (HC=C(CH₃)₂).

O-(3-Phenyl-2-propenyl)-1-hydroxylamine hydrochloride (**2f**)

Yield 90%; mp 176-177 °C; ¹H NMR (300 MHz, CDCl₃-CD₃OD) δ 4.72 (d, 2H, *J* 6.9 Hz, CH₂), 6.23-6.34

(dt, 1H, *J* 15.6, 6.9 Hz, HC=C_HPh), 6.83 (d, 1H, *J* 15.6 Hz, HC=C_HPh), 7.25-7.35 (m, 3H, H Ar), 7.40-7.45 (m, 2H, H Ar), ¹³C NMR (75 MHz, CDCl₃-CD₃OD) δ 75.74 (OCH₂), 119.76 (HC=C_HPh), 126.85 (Ar-2,6), 128.61 (Ar-3,5), 128.72 (Ar-5), 135.38(Ar-1), 138.53 (HC=C_HPh).

Antiproliferative activity

Cell lines and culture conditions

Cells were obtained from American Type Cell Culture (ATCC, Bethesda, BD). Tumor derived cells that originated in multiple tissue sites were maintained as follow: PC-3 (prostate adenocarcinoma), MRC-5 (fibroblasts derived from lung tissue) and A-549 (lung: NSCLC alveolar basal epithelial-squamous) in Dulbecco's modified Eagle's medium with high glucose (Lonza), supplemented with 10% (v/v) fetal bovine serum (Biowest), 2 mM L-glutamine, 5000 UI mL⁻¹ penicillin and 5 mg mL⁻¹ streptomycin.

MDA-MB-231 (breast adenocarcinoma) was grown in RPMI 1640 medium (Lonza) supplemented with 10% serum (Biowest) and 2 mM L-glutamine, 5000 UI mL⁻¹ penicillin and 5 mg mL⁻¹ streptomycin. Eight thousand cells were seeded in 96 well plates and grown in a 5% CO₂ atmosphere at 37 °C for 24 h before treatment. Cells were screened for mycoplasma contamination before each experiment, by means of DAPI staining (Invitrogen) and fluorescence microscopy camera (Motic CamPro 282A).

MTT assay

Cancer cells from breast (MDA-MB-231), prostate (PC-3) and lung (A-549) and healthy fibroblasts from lung (MRC-5) were seeded in a 96-well plate (5000 cells well⁻¹) for 24 h. Cells were then treated with concentrations in a range of 0 to 50 μM of compounds **1** and **3** (a compound was considered active if IC₅₀ ≤ 50 μM). Each compound at each concentration was tested as a triplicate. After 48 h of incubation, cells were washed and then 3,4,5-dimethylthiazolyl-2,2,5-diphenyltetrazolium bromide (MTT) was added for 4 h. Purple formazan crystals formed were then dissolved in DMSO and the plates were read under 570 nm. IC₅₀ values were determined by non-linear regression. Vincristine was used as a positive control at IC₅₀ determined by MTT assay under the same conditions described above.

Supplementary Information

Supplementary information is available free of charge at <http://jbcs.sbcs.org.br> as PDF file.

Acknowledgments

The authors thank Pontificia Universidad Javeriana (PUJ) and COLCIENCIAS for the financial support through the projects ID 5564 and 120365843351; J. E. D. thanks PUJ for the teaching assistantship.

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Submitted: February 13, 2017

Published online: July 5, 2017