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Gold(I)-Catalyzed Synthesis of 4*H*-Benzo[*d*][1,3]oxazines and Biological Evaluation of Activity in Breast Cancer Cells

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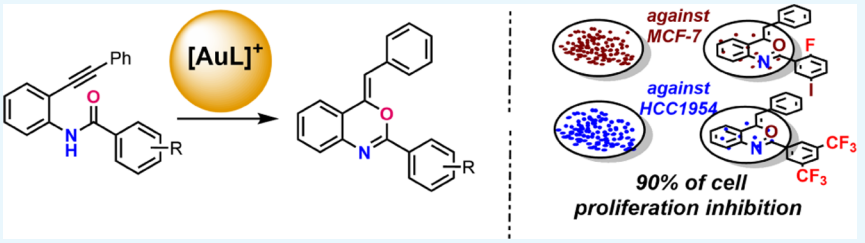

ABSTRACT: The first gold(I)-catalyzed cycloisomerization procedure applied to the synthesis of substituted 4*H*-benzo[*d*][1,3]-oxazines has been developed starting from *N*-(2-alkynyl)aryl benzamides. The chemoselective oxygen cyclization via the 6-*exo*-dig pathway yielded the observed heterocycles in modest to good chemical yields under very mild reaction conditions. The obtained oxazines were assayed on the breast cancer (BC)-derived cell lines MCF-7 and HCC1954 with differential biological activity. The newly synthesized 4*H*-benzo[*d*][1,3]oxazine compounds showed several degrees of cell proliferation inhibition with a remarkable effect for those compounds having a substituted aryl at C-2 of the molecules. The 4*H*-benzo[*d*][1,3]oxazines showed an IC_{50} ranking from 3.1 to 95 μM in MCF-7 and HCC1954 cells. These compounds represent potential drug candidates for BC treatment. However, additional assays are needed to elucidate their complete effect over the cellular and molecular hallmarks of cancer.

INTRODUCTION

Oxazines[1] are a class of heterocyclic compounds broadly studied in chemistry. In specific, 4*H*-benzo[*d*][1,3]oxazines have been extensively used in different fields. Their importance can be found in a broad applicability since this core can be found in heat-resistant and electronic materials,[2] naturally occurring active compounds,[3] and biologically important molecules[4] such as pharmaceuticals, agrochemicals,[5] anxiolytics, anticonvulsants,[6] fungicides, or anti-inflammatories[7] among others. Representative examples of the benzo[*d*][1,3]oxazine nucleus is established by etifoxine, a potent GABA receptor inhibitor, or by efavirenz, which is an efficient inhibitor of reverse transcriptase against HIV-1 mutant strain[8] (Figure 1).

Regarding diseases that cause great mortality, 4*H*-benzo[*d*]-[1,3]oxazines were successfully used as human leucocyte elastase and C1r serine protease inhibitors.[9] Finally, in the context of this work, they have been used as progesterone receptor agonist and DNA-binding antitumor agents.[10] We strongly considered this antitumor activity to design, postulate, and explore a family of highly substituted 4*H*-benzo[*d*][1,3]-oxazines in the biological assays of activity against MCF7 and HCC1954 breast cancer (BC) cell lines, which have been previously used as models for several compounds testing for cancer treatment.[11,12] BC is one of the most frequent and deathly pathologies worldwide, women from 45 to 55 years old being the most vulnerable population. In 2020, 684,996 deaths were registered.[11,13,14] Notably, there is a great difference in 5 year overall survival between developed and underdeveloped countries with 80% of the population versus 40%, respectively.[15] MCF7 cells have been used as a model for BC[16,17] since 1973,[18] and several compounds have been used to evaluate their potential in cancer treatment.[19,20]

Regarding the synthesis of the new 4*H*-benzo[*d*][1,3]-oxazines, several procedures have been developed for accessing this core (Figure 2).

Some of the more representatives include metal-catalyzed procedures with Pd,[21−23] Cu,[24] and Fe;[25] also, different metal-

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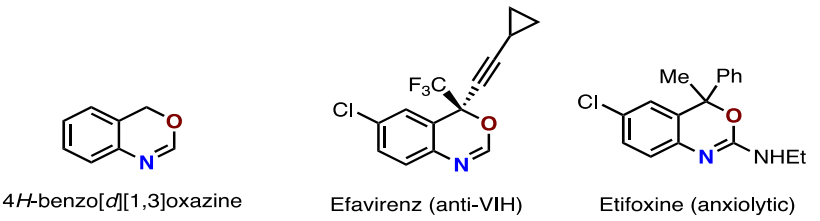

Figure 1. 4*H*-Benzo[*d*][1,3]oxazine core and examples of relevance.

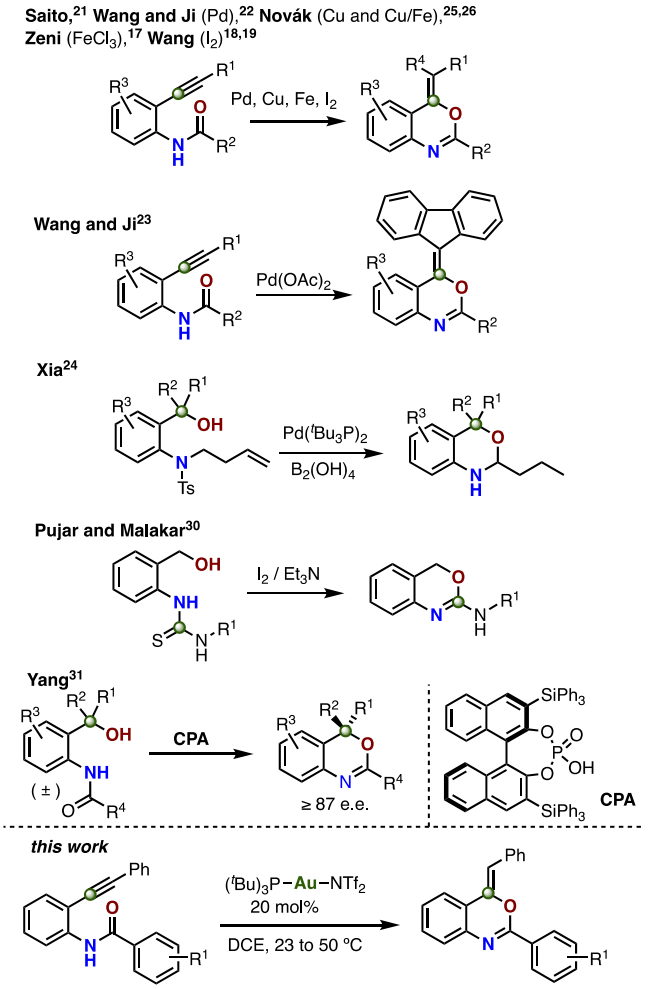

Figure 2. Described procedures for the synthesis of 4*H*-benzo[*d*]-[1,3]oxazines and our developed protocol.

free-catalyzed protocols using I_2[26,27] or chiral phosphoric acids[28] have been reported. All the aforementioned methods involve the use of high temperatures, potentially toxic reagents or starting materials, and general nonmild conditions. According to our research group interest,[29] herein, we present our gold(I)-catalyzed approach of 4*H*-benzo[*d*][1,3]oxazines using very mild reaction conditions. To the best of our knowledge, this is the first procedure using gold(I) catalysis applied to the synthesis of benzo[*d*][1,3]oxazines[30] (Figure 2).

RESULTS AND DISCUSSION

Organic Synthesis. The starting material synthesis of the *N*-(2-alkynyl)aryl benzamides **5**−**15** had taken place by two different routes (A and B) using the amide formation bond and the Sonogashira alkynylation as main tools (Figure 3).

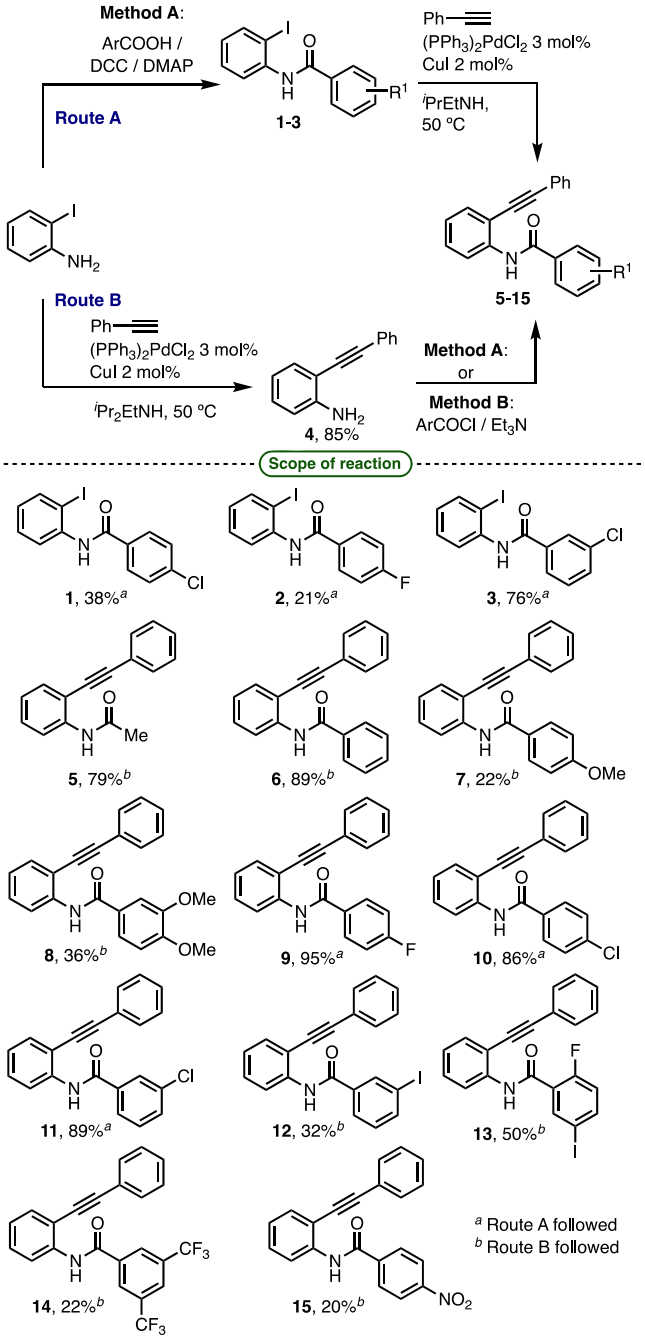

Figure 3. Routes for the synthesis of *N*-(2-alkynyl)aryl benzamides **5**−**15**.

In the *N*-(2-alkynyl)aryl benzamide synthesis, route A started with the amide formation on 2-iodoaniline. The use of different substituted benzoic acids in the presence of dimethyl amino-pyridine (DMAP) and dicyclohexyl carbodiimide (DCC)

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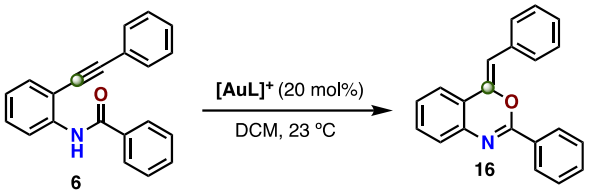
(method A) produced 2-iodobenzamides **1**−**3** in low to good yields (21−76%). The following Sonogashira alkynylation using phenyl acetylene under catalytic conditions of $(Ph_3P)_2PdCl_2$ and CuI led to the formation of *N*-(2-alkynyl)aryl benzamides **5**−**15**. On the other hand, route B started with the Sonogashira alkynylation on 2-iodoaniline with phenyl acetylene to yield **4** in 85%. Next, amide formation using method A or the corresponding benzoyl chloride derivatives in the presence of triethylamine (method B) gave rise to the desired benzamide in modest to good yields (20−95%). The electron-donating (**5**−**8**) and electron-attracting groups (**9**−**15**) were perfectly tolerated in the procedure, generating a great variety of precursors to be assayed in gold(I) catalysis.

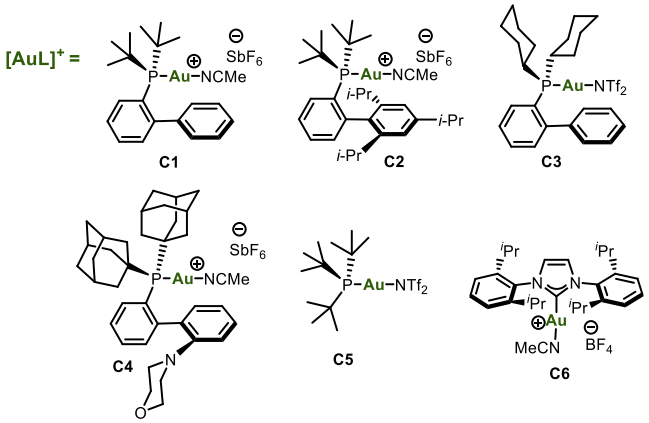
After having the *N*-(2-alkynyl)aryl benzamides produced, we proceeded to test and optimize our hypothesis on the gold(I)-catalyzed synthesis of 4*H*-benzo[*d*][1,3]oxazines. Accordingly, several cationic gold(I) complexes were assayed to determine the best yield (Table 1).

Table 1. Optimization of the Gold(I)-Catalyzed Synthesis of 4*H*-Benzo[*d*][1,3]oxazine 16[a]



entry	catalyst	time (h)	yield (%)[b]
1	**C1**	24	92[c]
2	**C2**	20	66
3	**C3**	17	61
4	**C4**	23	60
5	**C5**	24	95
6	**C6**	21	90

[a]Reaction conditions: all the reactions were carried out using 0.1 mmol of **6** and 20 mol % gold(I) catalyst at 23 °C in DCM (0.1 M), without a nitrogen atmosphere. [b]Yields were determined using mesitylene as an internal standard. [c]Isolated yields.

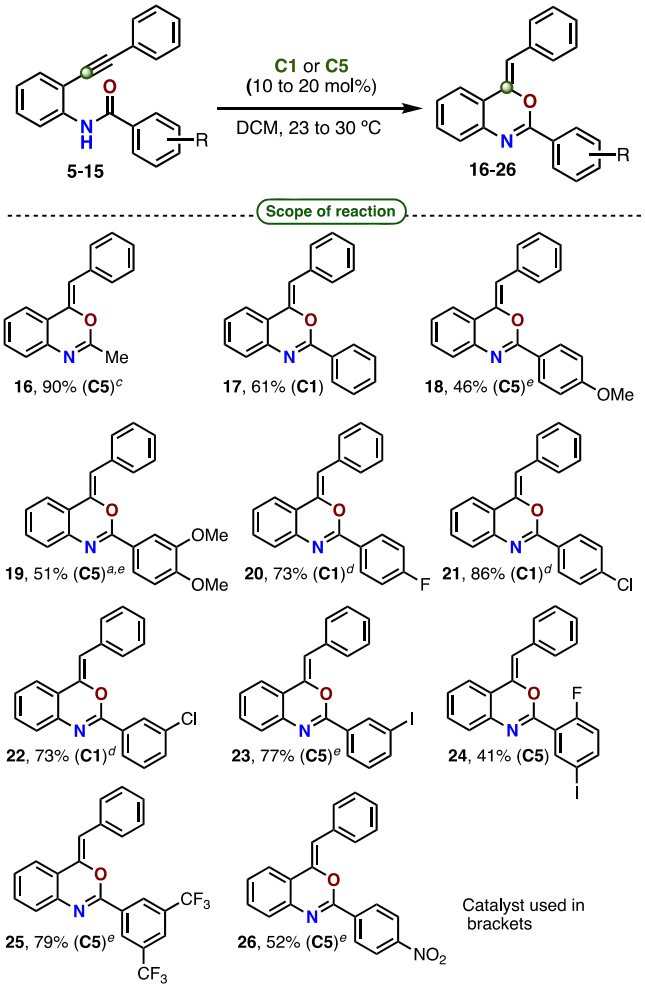


The optimization was carried out using *N*-(2-alkynyl)aryl benzamide **6** as a model. In such a way, we started by testing the cationic catalyst **C1** (Echavarren's catalyst)[31] using increasing amounts of the catalyst starting from 5 to 15 mol %; however, the full consumption of the starting material was achieved with 20 mol % catalytic charge obtaining the desired benzoxazine **16** in an excellent yield of 92% (entry 1). Accordingly, we decided to test **C2**−**C5** in this catalytic amount. Next, the ^{t}BuXPhos-based[32] gold(I) catalyst **C2** was tested, obtaining a moderate 66% yield of the desired product (entry 2). The following catalyst tested which contained the cyclohexyl JohnPhos-based[33] gold(I) catalyst **C3** yielded the expected compound in 61%. On the other hand, cationic gold(I) catalyst **C3** containing MorDalphos[34] as phosphine gave a similar 60% yield. Also, the use of gold(I) complex **C5** containing Fu's[35] phosphine gave rise to **16** in an excellent 95% yield. Finally, cationic carbene IPr-based[36] gold(I) catalyst **C6** led to the formation of the desired 4*H*-benzo[*d*][1,3]oxazine **16** in good 90% yield. After this optimization, the catalysts **C1** and **C5** turned out to be the most efficient and were used in the following cycloisomerization reactions.

With the optimized conditions, we proceeded to carry out the gold(I)-catalyzed cycloisomerization reaction to test the scope of this protocol (Table 2).

According to our optimization table, catalysts **C1** and **C5** were the most efficient; thereby, we decided to test both when a cyclization reaction showed a complex profile. The obtained

Table 2. Scope of the Gold(I)-Catalyzed Synthesis of a Family of Highly Substituted 4*H*-Benzo[*d*][1,3]oxazines[a,b]



[a]Reaction conditions: unless otherwise indicated, all the reactions were carried out using 20 mol % gold(I) catalyst at 23 °C in DCM (0.1 M), without a nitrogen atmosphere. [b]Isolated yields reported. [c]3 mol % catalyst used. [d]10 mol % catalyst used. [e]Reaction heated at 30 °C.

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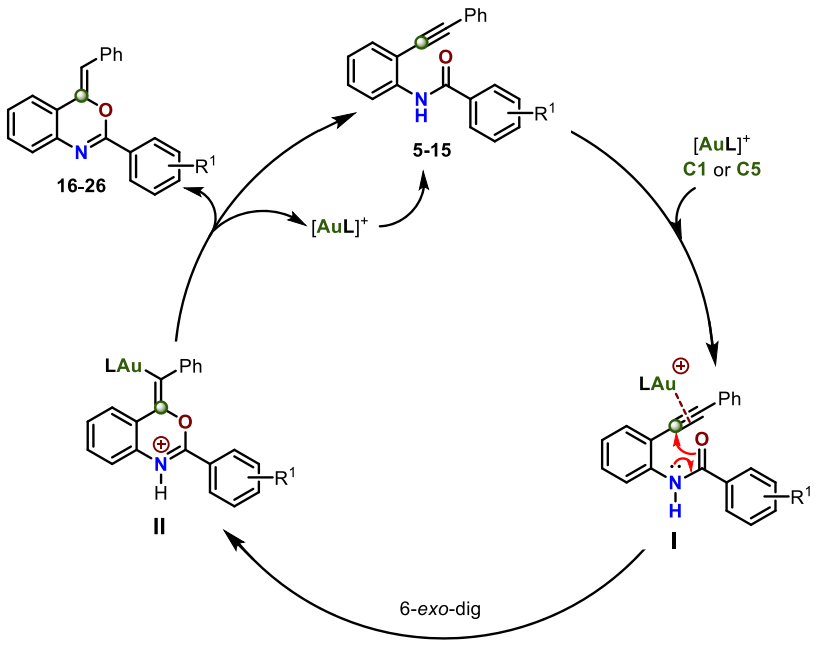

Figure 4. Plausible reaction mechanism of the gold(I)-catalyzed synthesis of 4*H*-benzo[*d*][1,3]oxazines.

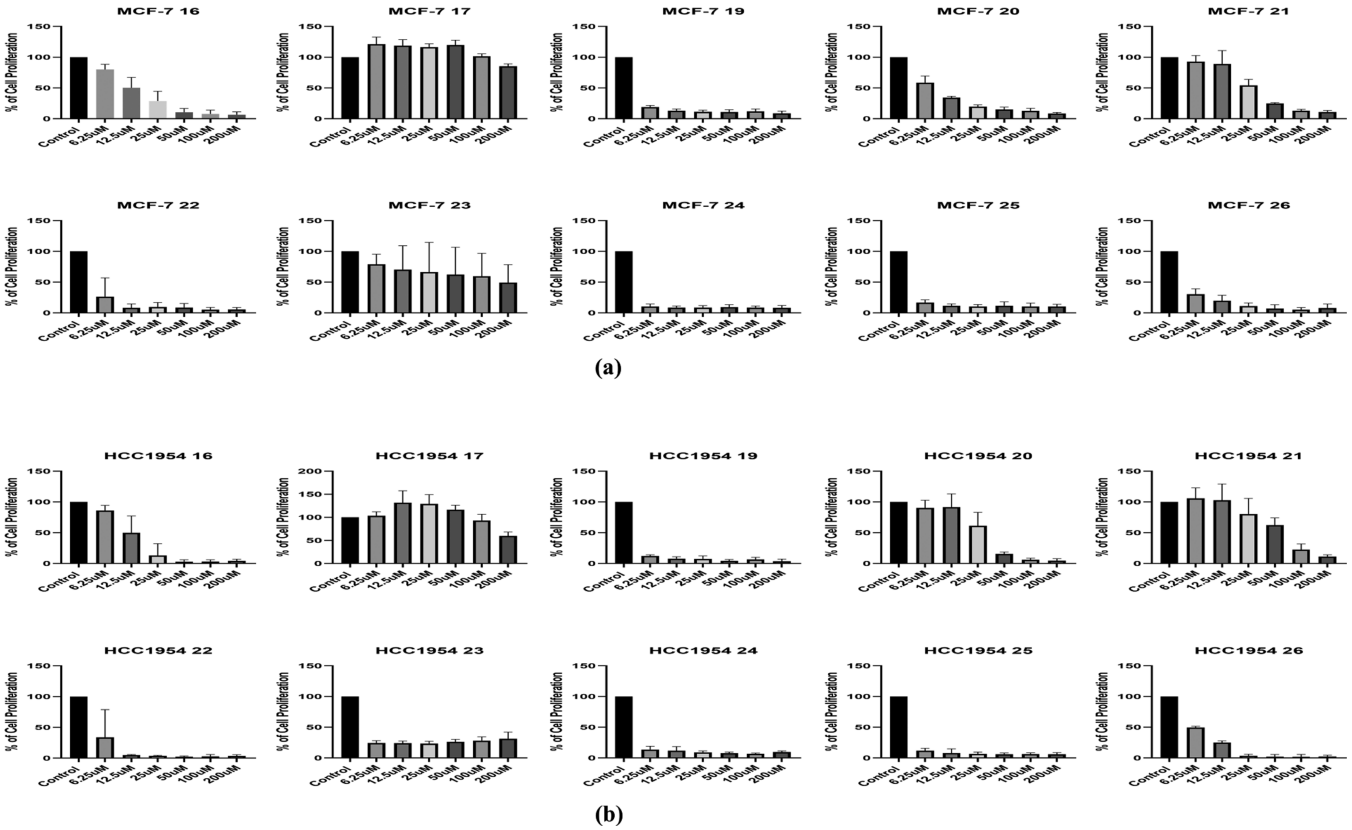

Figure 5. Differential effect of the 4*H*-benzo[*d*][1,3]oxazine compounds **16**−**26** in the proliferation of MCF-7 and HCC1954 cell lines. (a) MCF-7 cells were treated with increasing doses of compounds **16**−**26**. (b) HCC1954 cells were treated with increasing doses of compounds **16**−**26**. Control cells were cells treated with DMSO.

oxazines were designed to consistently have a benzylidene group at C-4; then, the most relevant variations were present in the aryl group at C-2. In such a way, the gold(I)-catalyzed cycloisomerization of the starting *N*-(2-alkynyl)aryl benzamides **5**−**15** allowed the formation of highly substituted 4-benzyliden-2-aryl-4*H*-benzo[*d*][1,3]oxazines **16**−**26**. This procedure tolerated the methyl group (**16**) with an excellent yield of 90% and the phenyl ring (**17**) at 61%. Also, electron-rich aryls containing one or two methoxy groups (**18** and **19**) yielded the corresponding oxazines in 46 and 51%, respectively. Interestingly, these reactions needed soft heating at 30 °C to complete the starting material consumption. Other examples containing

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electron-attracting groups in the aryl at C-2 such as fluorine (**20**), chlorine (**21** and **22**), iodine (**23**), fluorine and iodine (**24**), trifluoromethyl (**25**), or the nitro group (**26**) could be successfully obtained, generally with good yields (73−86%); only two of these examples gave rise to modest 41 and 52% yields. In this set of electron-attracting derivatives, the aryls with iodine, trifluoromethyl, and nitro groups were heated at 30 °C to complete the reaction.

It is important to highlight that the reactions to obtain the family of the synthesized oxazines were carried out under very mild conditions such as room temperature or 30 °C, without the use of an inert atmosphere and under operationally easy to handle conditions since they just needed the mixture of the starting material and the gold(I) catalyst in dry DCM. These characteristics represent a significant improvement regarding the previously described metal-catalyzed procedures, by considering that they required heating at 70 °C or more and a nitrogen atmosphere and that the palladium catalyst or the phosphines used had to be sometimes manipulated in a glovebox.

Finally, according to several reports on the gold(I) chemistry,[37,38] it is possible to propose the following reaction mechanism (Figure 4).

The mechanism starts with the coordination of the cationic gold(I) complexes **C1** or **C5** to the *N*-(2-alkynyl)aryl benzamides **5**−**15** to get the intermediate **I**. The following chemoselective attack of the oxygen of amide to the internal carbon of the triple bond led to the formation of the vinylidene gold(I) benzoxazonium **II** via stereoselective 6-*exo*-dig cyclization; certainly, this explains the exclusive formation of the Z-isomer in the obtained products. The final protodeauration gives rise to the observed 4-benzyliden-2-aryl-4*H*-benzo[*d*][1,3]-oxazines **16**−**26** with the concomitant regeneration of the catalyst, which continues with another cycle.

Biological Evaluation in BC. The new 4*H*-benzo[*d*][1,3]-oxazines presented a remarkable effect on cell proliferation inhibition with important difference between MCF-7 and HCC1954 response to the compounds (Figure 5a,b) that could be attributable to the molecular background of cells, while the former is Erb-B2 receptor tyrosine kinase 2 (HER)+/−, estrogen receptor (ER)+, and progesterone receptor (PR)+ and the latter is HER+, ER−, PR−.[39−41] The proliferation inhibition in MCF-7 was as follows: 24, 25, 19, 18, 22, 21, 16 and 20. It should be noted that compounds **23** and **17** did not have effects on cell proliferation inhibition. In contrast, while compounds 24, 25, 19, 18, 22, and 20 showed a statistically significant effect from the concentration of 6.25 μM, compounds **16** and **21** presented effects at 12.5 and 25 μM, respectively, in MCF-7 cells (Figure 5a). In contrast, it must be noted that in HCC1954 cells, the 4*H*-benzo[*d*][1,3]oxazines presented different effects, specifically with compound **23** which showed 70% proliferation inhibition from 6.25 μM in HCC1954, while in MCF-7, a null effect was recorded (Figure 5a,b). The most potent effect of 4*H*-benzo[*d*][1,3]oxazines in HCC1954 cells was as follows: 25, 19, 24, 20, 23, 22, 16, 18, 21, and 17. Another difference was that in HCC1954 cells, all the compounds showed a stronger effect compared to that of MCF-7; therefore, it seems that HCC1954 is more susceptible to 4*H*-benzo[*d*][1,3]oxazines than MCF-7, Table 3 and Figure S2. The substituents in the aryl at C-2 of 4*H*-benzo[*d*][1,3]oxazines seem to be important in achieving cell proliferation inhibition since it can be noticed that compounds **17** and **23** are the simplest in regard to this structural feature (Table 2). The benzoxazines have been reported as promising

Table 3. IC_{50} of 4*H*-Benzo[*d*][1,3]oxazines in BC Cells

compound	MCF7 (μM)	HCC1954 (μM)
16	12.20	12.09
17	95.82	87.37
19	3.485	3.375
20	7.172	27.65
21	24.92	47.28
22	4.189	5.190
23		3.114
24	3.408	3.275
25	3.529	3.373
26	4.148	6.280

inhibitors of cell proliferation with IC_{50} ranking from 1 to 200 μM. Mbaba reported an IC_{50} of 11 μM in HCC70 cells,[42] while Bollu reported 1.1−41.5 μM in MDA-MB-231 cells.[43] It should be noted that different compounds were tested in different cell lines. In contrast, de Brito et al. tested benzoxazines in MCF-7 cells, showing an IC_{50} of 21.8 and 28.8 μM for two different oxazines.[44] In our present work, the IC_{50} ranked from 3.1 to 95 μM with astounding difference with compound **23** showing effects in HCC1954 but not in MCF-7 cells, Figure S1 (see the Supporting Information). The observed different effect could be explained based on the cells' molecular context that finally results in cellular responses.[45] Expression difference of ER, PR, and HER2 could account for this singular specific effect. ER and PR can regulate gene transcription either by directly binding to DNA response elements directly or indirectly via other transcription factors such as induction and coregulator recruiting[46] and noncoding RNA regulation.[47] In addition, ER and PR could interact with several proteins and regulate cell signaling pathways through nongenomic mechanisms.[48,49] The molecular and cellular mechanism underlying the effect of 4*H*-benzo[*d*][1,3]oxazines is under study in our research group.

CONCLUSIONS

In summary, we developed the first gold(I)-catalyzed cycloisomerization protocol of *N*-(2-alkynyl)aryl benzamides, which was applied to the synthesis of substituted 4-benzyliden-2-aryl-4*H*-benzo[*d*][1,3]oxazines **16**−**26** in modest to excellent yields. The developed procedure took place under very mild reaction conditions such as room temperature or heating at 30 °C and without the use of an inert atmosphere. These characteristics represent important advantages over the previously described metal-catalyzed procedures that are usually carried out under stronger heating and argon atmosphere conditions. MCF-7 and HCC1954 BC cells presented different effects to 4*H*-benzo[*d*]-[1,3]oxazines, remarkably with compound **23**, which elicited 70% proliferation inhibition in HCC1954 versus a null effect on MCF-7 cells. Stronger to weaker compound effects on MCF-7 cells were as follows: 24, 25, 19, 18, 22, 21, 16, and 20. Compounds **23** and **17** recorded a null effect. In HCC1954 cells, the effect of the compounds was as follows: 25, 19, 24, 20, 23, 22, 16, 18, 21, and 17. This suggests that the HCC1954 cell line is more susceptible to 4*H*-benzo[*d*][1,3]oxazines than MCF-7 cells. Additionally, it could be speculated that the substituents in the aryl at C-2 of 4*H*-benzo[*d*][1,3]oxazines is important in achieving cell proliferation inhibition; nevertheless, further experiments are needed to validate our hypothesis.

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EXPERIMENTAL SECTION

General Methods. All reactions were carried out under an inert atmosphere using dry solvents and anhydrous conditions and were capped with a rubber septum unless otherwise mentioned. Reactions were followed by thin-layer chromatography (0.25 mm Merck silica gel plates 60F-254) using UV light as the visualizing agent. Flash column chromatography employed silica gel (40−60 μm, 230−400 mesh) purchased from Sigma-Aldrich. The new compounds were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, and high-resolution mass spectra (HR-MS). The corresponding copies for ^{1}H and ^{13}C NMR spectra are provided. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Advance III (500 MHz) spectrometer. All ^{1}H NMR data were reported in δ units, parts per million (ppm) and were calibrated relative to the signals for residual chloroform (7.26 ppm) in deuterochloroform ($CDCl_3$). The ^{13}C NMR data reported were obtained with ^{1}H decoupling unless otherwise stated. The following abbreviations explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, br = broad, and m = multiplet. Infrared (IR) spectra were recorded using a PerkinElmer system 2000 FT-IR spectrometer. HR-MS was performed on a Bruker Daltonics ESI-QTOF-MS maXis impact using ESI-TOF (electrospray ionization−time of flight).

Synthesis. *Method A. Acylation of 2-(Phenylethynyl)-aniline.*[51] A 25 mL oven-dried round-bottom flask equipped with a magnetic stir bar was charged with 2-(phenylethynyl)-aniline (0.1 g, 0.517 mmol, 1 equiv) in DCE (4 mL). Then, DIPEA (0.15 mL, 4 equiv) at 0 °C was added. After dissolving and obtaining a homogeneous mixture, the corresponding acyl chloride (0.12 mL, 2 equiv) was added and stirred at 23 °C for 5 h. The completion of the reaction was determined by TLC analysis. To quench the reaction, H_2O (30 mL) was added. The aqueous phase was extracted with DCM (3 × 25 mL), dried over Na_2SO_4, filtrated, and finally concentrated under reduced pressure. The crude products were purified by flash chromatography on silica gel with an EtOAc/hexanes system to obtain the desired products.

Method B. Amidation of 2-Iodoanilines. A 25 mL oven-dried round-bottom flask equipped with a magnetic stir bar was charged with 2-iodoaniline (0.5 g, 2.283 mmol, 1 equiv) or 2-(phenylethynyl)aniline (0.1 g, 0.518 mmol, 1 equiv) in DCM (4 mL). Next, the corresponding benzoic acids (1.553 mmol, 3 equiv) were added and stirred at 23 °C until a homogeneous mixture was obtained. Afterward, DCC (1.554 mmol, 3 equiv) and DMAP (0.517 mmol, 1 equiv) were added at 23 °C for 24 h. The completion of the reaction was determined by TLC analysis. The aqueous phase was extracted with DCM (3 × 25 mL); the organic phase was dried over Na_2SO_4, filtrated, and concentrated at reduced pressure. The crude products were purified by flash chromatography on silica gel with an EtOAc/hexanes system to obtain the desired products.

Sonogashira Alkynylation Procedure.[52] A 25 mL oven-dried round-bottom flask equipped with a magnetic stir bar was charged with 2-iodoaniline (0.500 g, 2.283 mmol, 1 equiv) or 2-iodobenzamides (0.100 g, 0.0280 mmol, 1 equiv) in 15 mL of ^{i}PrEtNH and stirred for 10 min at 50 °C. Then, CuI (0.0056 g, 3 mol %) and $(Ph_3P)_2PdCl_2$ (0.0084 g, 3 mol %) were added for 10 min while maintaining the temperature. Subsequently, phenylacetylene (0.336 mL, 1.2 equiv) was added dropwise. The mixture was stirred at 50 °C for 3 h. The completion of the reaction was determined by TLC analysis. Afterward, the reaction was cooled until room temperature and quenched with H_2O (30 mL). The aqueous phase was extracted with DCM (3 × 25 mL), collected, dried over Na_2SO_4, filtrated, and concentrated at reduced pressure. The crude products were purified by flash chromatography on silica gel with an EtOAc/hexanes system to obtain the desired products.

Procedure for Gold(I) Catalysis. Although our optimization showed that generally, the cycloisomerization proceeded with 20 mol % catalyst, some indicated examples needed 3 or 10 mol % only.

General Procedure for Gold(I)-Catalyzed Synthesis of 4*H*-Benzo[*d*][1,3]oxazine. A 25 mL oven-dried round-bottom flask equipped with a magnetic stir bar was charged with the corresponding *N*-(2-alkynyl)aryl benzamides (1 equiv) in anhydrous DCM (2 mL) and stirred at 23 or 30 °C. Then, gold(I) catalyst **C1** or **C5** (3 or 10 or 20 mol %) was added, without a nitrogen atmosphere. The completion of the reaction was determined by TLC analysis. The reaction was allowed to reach room temperature and quenched by adding three drops of Et_3N and H_2O (30 mL). The aqueous phase was extracted with DCM (3 × 25 mL), then dried over Na_2SO_4, filtered, and concentrated at reduced pressure. The crude products were purified by flash chromatography on silica gel with an EtOAc/hexanes system to obtain the desired product.

Examples in Figure 3. *4-Chloro-N-(2-iodophenyl)-benzamide* ***1***. The following compound was obtained according to Method B, using 2-iodoaniline (0.5 g, 2.2835 mmol, 1 equiv) as a starting material and 4-chlorobenzoic acid (1.0687 g, 6.8507 mmol, 3 equiv). The crude material was purified by flash column chromatography over silica gel with the system 1% EtOAc/hexane to afford the product **1** (310 mg, 38%) as a white solid. mp = 143−145 °C. IR (neat) ν/cm^{-1}: 3262 (s), 2927 (w), 1647 (s), 1522 (s), 1307 (m), 1019 (m). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.42 (dt, J = 8.4, 1.7 Hz, 1H), 8.22 (s, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.82 (dd, J = 8.1, 1.6 Hz, 1H), 7.50 (dd, J = 8.4, 1.9 Hz, 2H), 7.41 (t, J = 7.8 Hz, 1H), 6.90 (t, J = 7.7 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.9, 138.9, 138.7, 138.5, 133.4, 129.6, 129.9, 128.7, 126.4, 121.9, 90.6. HRMS (ESI+) m/z: calcd for $C_{13}H_{10}ClINO$ $[M + H]^+$, 357.9496; found, 357.9524.

4-Fluoro-N-(2-iodophenyl)benzamide ***2***. The following compound was obtained according to Method B, using 2-iodoaniline (0.5 g, 2.2835 mmol, 1 equiv) as a starting material and 4-fluorobenzoic acid (0.9592 g, 6.8507 mmol, 3 equiv). The crude material was purified by flash column chromatography over silica gel with the system 1% EtOAc/hexane to afford the product **2** (160 mg, 21%) as a white solid. mp = 127−130 °C. IR (neat) ν/cm^{-1}: 3221 (m), 3163 (m), 1645 (s), 1496 (s), 1232 (s). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.42 (d, J = 8.4 Hz, 1H), 8.21 (s, 1H), 7.98 (dd, J = 8.6, 5.3 Hz, 2H), 7.82 (d, J = 8.0 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.20 (t, J = 8.4 Hz, 2H), 6.92−6.85 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.0 (d, J = 254 Hz), 164.1, 138.7, 138.0, 130.5 (d, J = 3 Hz), 129.4 (d, J = 9 Hz), 129.3, 126.0, 121.6, 115.9 (d, J = 19 Hz), 90.2. HRMS (ESI+) m/z: calcd for $C_{13}H_{10}FINO$ $[M + H]^+$, 341.9791; found, 341.9811.

3-Chloro-N-(2-iodophenyl)benzamide ***3***. The following compound was obtained according to Method B, using 2-iodoaniline (0.5 g, 2.2835 mmol, 1 equiv) as a starting material and 3-chlorobenzoic acid (1.0687 g, 6.8507 mmol, 3 equiv). The crude material was purified by flash column chromatography over silica gel with the system 2% EtOAc/hexane to afford the product **3** (624 mg, 76%) as a white solid. mp = 123−125 °C. IR (neat) ν/cm^{-1}: 3281 (m), 2929 (m), 1651 (s), 1530 (s), 1272 (s), 1128 (s). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (d, J = 8.0

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Hz, 1H), 7.79 (s, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.44 (d, J = 7.1 Hz, 1H), 7.29 (d, J = 8.0 Hz, 3H), 7.08 (d, J = 7.9 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.1, 141.7, 140.5, 135.8, 134.6, 132.4, 130.2, 129.9, 129.6, 129.5, 129.2, 127.2, 98.7. HRMS (ESI+) m/z: calcd for $C_{13}H_{10}ClINO$ $[M + H]^+$, 357.9496; found, 357.9512.

2-(Phenylethynyl)aniline ***4***. The following compound was obtained according to the Sonogashira Alkynylation Procedure, using 2-iodoaniline (0.500 g, 2.283 mmol, 1 equiv) as a starting material and phenylacetylene (0.336 mL, 1.2 equiv). The crude material was purified by flash column chromatography over silica gel with the system 5% EtOAc/hexane to afford the product **4** (380 mg, 85%) as an orange solid. The spectroscopic data were consistent with those previously described in the literature.[21] ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58−7.51 (m, 2H), 7.41−7.30 (m, 4H), 7.15 (td, J = 7.8, 1.5 Hz, 1H), 6.79−6.72 (m, 2H), 4.40 (br s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 147.3, 132.2, 131.5, 129.7, 128.4, 128.2, 123.3, 118.3, 114.6, 108.2, 94.8, 85.8.

N-(2-(Phenylethynyl)phenyl)acetamide ***5***. Compound **5** was obtained according to Method A, using 2-(phenylethynyl)-aniline (0.1 g, 0.517 mmol, 1 equiv) as a starting material and acetyl chloride (0.07 mL, 2 equiv). The crude material was purified by flash column chromatography over silica gel with the system 10% EtOAc/hexane to afford the product **5** (111.6 mg, 79%) as a yellow solid. The spectroscopic data correlated with those described previously.[21] ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, J = 8.4 Hz, 1H), 7.98 (s, 1H), 7.57−7.52 (m, 2H), 7.50 (dd, J = 7.8, 1.6 Hz, 1H), 7.40 (p, J = 4.0 Hz, 3H), 7.35 (td, J = 7.8, 1.6 Hz, 1H), 7.07 (t, J = 7.6 Hz, 1H), 2.02 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.1, 138.9, 131.6, 131.5, 129.7, 128.9, 128.6, 123.4, 122.3, 119.3, 111.8, 96.4, 84.2, 25.0.

N-(2-(Phenylethynyl)phenyl)benzamide ***6***. Compound **6** was obtained according to Method A, using 2-(phenylethynyl)-aniline (0.1 g, 0.517 mmol, 1 equiv) as a starting material and benzoyl chloride (0.12 mL, 2 equiv). The crude material was purified by flash column chromatography over silica gel with the system 10% EtOAc/hexane to afford the product **6** (141.0 mg, 89%) as a yellow solid. The spectroscopic data corresponded to those described in the literature.[21] ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.96 (s, 1H), 8.64 (d, J = 8.3 Hz, 1H), 7.97 (dd, J = 7.6, 1.7 Hz, 2H), 7.60−7.52 (m, 4H), 7.49 (t, J = 7.6 Hz, 2H), 7.45−7.37 (m, 4H), 7.15−7.10 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.0, 139.1, 134.9, 132.0, 131.5, 131.4, 129.9, 129.0, 128.9, 128.6, 127.0, 123.5, 122.2, 119.1, 112.2, 97.0, 84.5.

4-Methoxy-N-(2-(phenylethynyl)phenyl)benzamide ***7***. The reaction was carried out according to Method B, using 2-(phenylethynyl)aniline (0.1 g, 0.518 mmol, 1 equiv) as a starting material and 4-methoxybenzoic acid (0.2362 g, 1.5536 mmol, 3 equiv). The crude material was purified by flash column chromatography over silica gel with the system 4% EtOAc/hexane to afford the product 7 (374 mg, 22%) as a white solid. The spectroscopic data were consistent with those previously described.[50] ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.87 (s, 1H), 8.61 (d, J = 8.4 Hz, 1H), 7.95−7.91 (m, 2H), 7.55 (tt, J = 7.6, 4.7, 2.0 Hz, 3H), 7.44−7.38 (m, 4H), 7.10 (t, J = 7.6 Hz, 1H), 6.99−6.95 (m, 2H), 3.88 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.6, 162.6, 139.3, 131.5, 131.4, 129.9, 128.9, 128.9, 128.6, 127.1, 123.3, 122.3, 119.0, 114.1, 112.0, 96.8, 84.6, 55.5.

3,4-Dimethoxy-N-(2-(phenylethynyl)phenyl)benzamide ***8***. It was obtained according to Method B, using 2-(phenylethynyl)aniline (0.1 g, 0.518 mmol, 1 equiv) as a starting material and 3,4-methoxybenzoic acid (0.2828 g, 1.5536 mmol, 3 equiv). The crude material was purified by flash column chromatography over silica gel with the system 5% EtOAc/hexane to afford the product **8** (672 mg, 36%) as a yellow solid. mp = 128−131 °C. IR (neat) ν/cm^{-1}: 3410 (m), 3323 (m), 2929 (s), 2850 (s), 1675 (m), 1626 (m), 1573 (m), 1507 (s), 1266 (m). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.88 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 7.57−7.51 (m, 5H), 7.40 (dd, J = 5.0, 1.9 Hz, 4H), 7.11 (dd, J = 8.4, 7.0 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 3.94 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.7, 152.2, 149.2, 139.3, 131.5, 131.4, 129.9, 129.0, 128.6, 127.5, 123.3, 122.2, 119.7, 119.0, 112.0, 110.2, 110.3, 96.7, 84.5, 56.1, 55.7. HRMS (ESI+) m/z: calcd for $C_{23}H_{20}NO_3$ $[M + H]^+$, 358.1443; found, 358.1467.

4-Fluoro-N-(2-(phenylethynyl)phenyl)benzamide ***9***. The following compound was obtained according to the Sonogashira Alkynylation Procedure, using 4-fluoro-*N*-(2-iodophenyl)-benzamide (0.08 g, 0.2346 mmol, 1 equiv) as a starting material and phenylacetylene (0.309 mL, 1.2 equiv). The crude material was purified by flash column chromatography over silica gel with the system 1% EtOAc/hexane to afford the product **9** (70 mg, 95%) as a light-brown solid. mp = 142−144 °C. IR (neat) ν/cm^{-1}: 3300 (s), 3061 (m), 2925 (m), 2440 (w), 2212 (w), 1652 (s), 1607 (s), 1505 (s), 1447 (s), 1226 (m). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.86 (s, 1H), 8.59 (d, J = 8.3 Hz, 1H), 7.99−7.95 (m, 2H), 7.54 (ddd, J = 9.8, 7.5, 2.7 Hz, 3H), 7.41 (tq, J = 8.3, 2.6 Hz, 4H), 7.15 (dt, J = 8.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.5, 139.8, 131.7, 131.5, 130.1, 129.6, 129.8, 129.5, 128.8, 123.8, 122.3, 119.9, 116.4, 116.7, 112.4, 97.9, 84.7. ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.1 (d, J = 258 Hz), 164.1, 139.0, 131.6, 131.5, 131.2 (d, J = 3 Hz), 130.1, 129.5 (d, J = 9 Hz), 129.2, 128.8, 123.8, 122.3, 119.2, 116.1 (d, J = 22 Hz), 112.4, 97.1, 84.5. HRMS (ESI+) m/z: calcd for $C_{21}H_{15}FNO$ $[M + H]^+$, 316.1138; found, 316.1161.

4-Chloro-N-(2-(phenylethynyl)phenyl)benzamide ***10***. The reaction was carried out according to the Sonogashira Alkynylation Procedure, using 4-chloro-*N*-(2-iodophenyl)-benzamide (0.1 g, 0.2801 mmol, 1 equiv) as a starting material and phenylacetylene (0.369 mL, 1.2 equiv). The crude material was purified by flash column chromatography over silica gel with the system 1% EtOAc/hexane to afford the product **10** (80 mg, 86%) as a light-brown solid. mp = 144−147 °C. IR (neat) ν/cm^{-1}: 3292 (m), 2925 (m), 2859 (m), 2214 (w), 1730 (m), 1649 (s), 1528 (s), 1447 (s), 1317 (m). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.87 (s, 1H), 8.58 (d, J = 8.3 Hz, 1H), 7.89 (d, J = 8.3 Hz, 2H), 7.55−7.51 (m, 3H), 7.46−7.39 (m, 6H), 7.39 (dt, J = 12.9, 8.0 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.4, 138.9, 138.9, 133.8, 131.7, 131.8, 130.4, 129.6, 129.3, 128.8, 128.5, 123.9, 122.5, 119.3, 112.7, 97.2, 84.5. HRMS (ESI+) m/z: calcd for $C_{21}H_{15}ClNO$ $[M + H]^+$, 332.0842; found, 332.0863.

3-Chloro-N-(2-(phenylethynyl)phenyl)benzamide ***11***. The following compound was obtained according to the Sonogashira Alkynylation Procedure, using 3-chloro-*N*-(2-(phenylethynyl)-phenyl)benzamide (0.1 g, 0.3020 mmol, 1 equiv) as a starting material and phenylacetylene (0.398 mL, 1.2 equiv). The crude material was purified by flash column chromatography over silica gel with the system 2% EtOAc/hexane to afford the product **11** (82 mg, 89%) as a white solid. mp = 145−147 °C. IR (neat) ν/cm^{-1}: 3292 (s), 2929 (s), 1726 (m), 1651 (s), 1524 (s), 1311 (m). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (s, 1H), 8.61 (d, J = 8.3 Hz, 1H), 7.96 (d, J = 2.0 Hz, 1H), 7.85 (dd, J = 7.6, 1.6 Hz, 1H), 7.60−7.52 (m, 4H), 7.46−7.38 (m, 5H), 7.14 (t, J = 7.6 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 163.6, 138.7, 136.7, 135.14, 132.1, 131.5, 131.4, 130.3, 129.9, 129.1,

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128.7, 127.1, 125.3, 123.8, 122.0, 119.1, 112.4, 97.3, 84.3. HRMS (ESI+) m/z: calcd for $C_{21}H_{15}ClNO$ $[M + H]^+$, 332.0842; found, 332.0865.

3-Iodo-N-(2-(phenylethynyl)phenyl)benzamide ***12***. The following compound was obtained according to Method B, using 2-(phenylethynyl)aniline (0.1 g, 0.518 mmol, 1 equiv) as a starting material and 3-iodobenzoic acid (0.3852 g, 1.5536 mmol, 3 equiv). The crude material was purified by flash column chromatography over silica gel with the system 2% EtOAc/hexane to afford the product **12** (71 mg, 32%) as a yellow solid. mp = 143−145 °C. IR (neat) ν/cm^{-1}: 3285 (m), 2957 (s), 2855 (s), 1728 (m), 1260 (m). ^1H NMR (500 MHz, $CDCl_3$): δ 8.87 (s, 1H), 8.60 (d, J = 8.3 Hz, 1H), 8.29 (d, J = 2.3 Hz, 1H), 7.96−7.93 (m, 1H), 7.90 (dd, J = 7.9, 1.5 Hz, 1H), 7.62−7.54 (m, 3H), 7.45−7.39 (m, 4H), 7.23 (d, J = 7.9 Hz, 1H), 7.14 (t, J = 7.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.8, 163.3, 142.5, 140.9, 138.9, 135.6, 131.4, 130.5, 130.0, 129.2, 129.0, 128.7, 126.4, 123.8, 119.0, 112.3, 93.8, 84.1. HRMS (ESI+) m/z: calcd for $C_{21}H_{15}INO$ $[M + H]^+$, 424.0198; found, 424.0224.

2-Fluoro-5-iodo-N-(2-(phenylethynyl)phenyl)benzamide ***13***. Compound **13** was obtained according to Method B, using 2-(phenylethynyl)aniline (0.1 g, 0.518 mmol, 1 equiv) as a starting material and 2-fluoro-5-iodobenzoic acid (0.4131 g, 1.5536 mmol, 3 equiv). The crude material was purified by flash column chromatography over silica gel with the system 10% EtOAc/hexane to afford the product **13** (115 mg, 50%) as a yellow solid. mp = 130−132 °C. IR (neat) ν/cm^{-1}: 3391 (s), 2927 (s), 1724 (m), 1683 (s), 1451 (m), 1266 (s), 753 (s). ^1H NMR (500 MHz, $CDCl_3$): δ 9.42 (d, J = 15.0 Hz, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.53 (dd, J = 7.5, 2.4 Hz, 1H), 7.81 (ddd, J = 8.4, 4.8, 2.4 Hz, 1H), 7.57 (td, J = 7.8, 2.6 Hz, 3H), 7.45−7.36 (m, 4H), 7.14 (t, J = 7.5 Hz, 1H), 6.96 (dd, J = 11.7, 8.6 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.1 (d, J = 253 Hz), 159.4, 142.4 (d, J = 9 Hz), 140.9, 138.8, 131.9, 131.4, 129.6, 128.7, 128.3, 123.9, 123.0 (d, J = 12 Hz), 122.3, 119.9, 118.3 (d, J = 26 Hz), 112.7, 96.6, 88.0, 83.9. HRMS (ESI+) m/z: calcd for $C_{21}H_{14}FINO$ $[M + H]^+$, 442.0104; found, 442.0141.

N-(2-(Phenylethynyl)phenyl)-3,5-bis(trifluoromethyl)-benzamide ***14***. The following compound was obtained according to Method B, using 2-(phenylethynyl)aniline (0.1 g, 0.518 mmol, 1 equiv) as a starting material and 3,5-bis(trifluoromethyl)benzoic acid (0.4008 g, 1.5536 mmol, 3 equiv). The crude material was purified by flash column chromatography over silica gel with the system 5% EtOAc/hexane to afford the product **14** (49 mg, 22%) as a yellow solid. mp = 140−144 °C. IR (neat) ν/cm^{-1}: 3281 (m), 2929 (m), 1651 (s), 1530 (s), 1272 (s), 1128 (s). ^1H NMR (500 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.61 (d, J = 8.4 Hz, 1H), 8.41 (s, 2H), 8.07 (s, 1H), 7.59 (dd, J = 7.8, 1.5 Hz, 1H), 7.56−7.50 (m, 2H), 7.48−7.36 (m, 4H), 7.19 (t, J = 7.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 162.3, 138.5, 137.4, 133.0 (d, J = 34 Hz), 132.1, 131.8, 130.4, 129.7, 129.1, 127.6 (d, J = 4 Hz), 124.9, 123.2 (d, J = 273 Hz), 122.0, 119.7, 113.1, 98.1, 84.1. HRMS (ESI+) m/z: calcd for $C_{23}H_{14}F_6NO$ $[M + H]^+$, 434.0980; found, 434.1005.

4-Nitro-N-(2-(phenylethynyl)phenyl)benzamide ***15***. The following compound was obtained according to Method A, using 2-(phenylethynyl)aniline (0.1 g, 0.517 mmol, 1 equiv) as a starting material and 4-nitrobenzoyl chloride (0.1920, 1.0357 mmol, 2 equiv). The crude material was purified by flash column chromatography over silica gel with the system 10% EtOAc/hexane to afford the product **15** (35 mg, 20%) as an orange solid. The spectroscopic data correspond to those already described in the literature.[21] ^1H NMR (500 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.58 (d, J = 8.3 Hz, 1H), 8.33 (d, J = 8.5 Hz, 2H), 8.11 (d, J = 8.5 Hz, 2H), 7.57 (dd, J = 7.7, 1.4 Hz, 1H), 7.55−7.50 (m, 2H), 7.48−7.39 (m, 4H), 7.18 (t, J = 7.6 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 162.8, 149.9, 140.3, 138.3, 131.3, 130.0, 129.4, 129.3, 128.8, 128.1, 124.3, 124.1, 121.9, 119.3, 112.6, 97.3, 84.1.

Examples in Table 2. *(Z)-4-Benzylidene-2-methyl-4H-benzo[d][1,3]oxazine* ***16***. The following compound was obtained according to the Procedure for Gold(I) Catalysis, using *N*-(2-(phenylethynyl)phenyl)acetamide (0.030 g, 0.1276 mmol, 1 equiv) as a starting material and gold(I) catalyst **C1** (0.0030 g, 0.0038 mmol, 3 mol %). The crude material was purified by flash column chromatography over silica gel with the system 5% EtOAc/hexane to afford the product **16** (28 mg, 90%) as a white solid. The spectroscopic data matched with those previously described in the literature.[21] ^1H NMR (500 MHz, $CDCl_3$): δ 8.38 (d, J = 8.3 Hz, 1H), 7.57 (d, J = 7.7 Hz, 1H), 7.51−7.41 (m, 5H), 7.37 (ddd, J = 8.4, 7.1, 1.3 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 6.64 (s, 1H), 2.09 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 139.6, 137.6, 134.0, 128.9, 128.6, 128.5, 125.0, 123.5, 120.2, 115.9, 111.4, 27.8.

(Z)-4-Benzylidene-2-phenyl-4H-benzo[d][1,3]oxazine ***17***. This compound was obtained according to the Procedure for Gold(I) Catalysis, using *N*-(2-(phenylethynyl)phenyl)-benzamide (0.030 g, 0.1009 mmol, 1 equiv) as a starting material and gold(I) catalyst **C5** (0.013 g, 0.0201 mmol, 20 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **17** (19 mg, 61%) as a yellow solid. The spectroscopic data matched with those previously described in the literature.[21] ^1H NMR (500 MHz, $CDCl_3$): δ 8.27 (d, J = 7.7 Hz, 2H), 7.74 (d, J = 7.7 Hz, 2H), 7.54 (dq, J = 20.5, 7.4 Hz, 5H), 7.43 (tt, J = 15.9, 7.7 Hz, 5H), 6.27 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 135.3, 131.8, 131.4, 129.3, 128.7, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 127.4, 126.6, 122.4, 121.9, 121.1.

(Z)-4-Benzylidene-2-(4-methoxyphenyl)-4H-benzo[d]-[1,3]-oxazine ***18***. This compound was obtained according to the Procedure for Gold(I) Catalysis, using 4-methoxy-*N*-(2-(phenylethynyl)phenyl)benzamide (0.026 g, 0.0794 mmol, 1 equiv) as a starting material and gold(I) catalyst **C5** (0.010 g, 0.0158 mmol, 20 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **18** (12 mg, 46%) as a white solid. mp = 95−98 °C. IR (neat) ν/cm^{-1}: 3072 (m), 2931 (s), 1675 (s), 1321 (s). ^1H NMR (500 MHz, $CDCl_3$): δ 7.68−7.63 (m, 3H), 7.58−7.55 (m, 1H), 7.34 (d, J = 7.3 Hz, 2H), 7.25−7.20 (m, 4H), 7.19−7.15 (m, 1H), 6.79 (s, 2H), 6.77 (s, 1H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.7, 163.4, 141.8, 138.7, 133.4, 133.2, 129.6, 128.6, 128.5, 127.9, 127.6, 124.3, 123.1, 121.1, 114.1, 109.1, 55.9. HRMS (ESI+) m/z: calcd for $C_{22}H_{18}NO_2$ $[M + H]^+$, 328.1338; found, 328.1366.

(Z)-4-Benzylidene-2-(4-fluorophenyl)-4H-benzo[d][1,3]-oxazine ***20***. The following compound was obtained according to the Procedure for Gold(I) Catalysis, using 4-fluoro-*N*-(2-(phenylethynyl)phenyl)benzamide (0.049 g, 0.1372 mmol, 1 equiv) as a starting material and gold(I) catalyst **C1** (0.0735 g, 0.0137 mmol, 10 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **20** (36 mg, 73%) as a yellow solid. mp = 130−132 °C. IR (neat) ν/cm^{-1}: 2929 (s), 2853 (m), 1588 (m), 1507 (m), 1221 (s), 766 (s). ^1H NMR (500 MHz, $CDCl_3$): δ 8.13 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 7.8 Hz, 2H), 7.58 (d, J = 7.8 Hz, 1H), 7.45−7.37 (m, 4H), 7.35−7.28 (m, 2H), 7.25−7.21 (m, 2H), 6.22 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ

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165.4 (d, J = 253 Hz), 154.5,145.6, 139.2, 135.0, 131.9, 130.9, 130.5 (d, J = 9 Hz), 129.8, 128.8 (d, J = 5 Hz), 128.3, 127.1, 122.3, 121.9, 116.9 (d, J = 23 Hz), 102.2. HRMS (ESI+) m/z: calcd for $C_{21}H_{15}FNO$ $[M + H]^+$, 316.1138; found, 316.1165.

(Z)-4-Benzylidene-2-(4-chlorophenyl)-4H-benzo[d][1,3]-oxazine ***21***. The following compound was obtained according to the Procedure for Gold(I) Catalysis, using (*Z*)-4-chloro-*N*-(2-(phenylethynyl)phenyl)benzamide (0.035 g, 0.1057 mmol, 1 equiv) as a starting material and gold(I) catalyst **C1** (0.0816 g, 0.0095 mmol, 10 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **21** (30 mg, 86%) as a yellow solid. mp = 143−145 °C. IR (neat) ν/cm^{-1}: 2929 (s), 2855 (m), 1679 (s), 1600 (s), 1256 (s). ^1H NMR (500 MHz, $CDCl_3$): δ 8.26−8.13 (m, 2H), 7.69 (d, J = 7.9 Hz, 2H), 7.59 (d, J = 7.9 Hz, 1H), 7.40 (t, J = 7.6 Hz, 2H), 7.35−7.27 (m, 2H), 7.24 (d, J = 7.1 Hz, 2H), 7.14 (t, J = 8.6 Hz, 2H), 6.22 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 154.3, 145.3, 138.8, 138.1, 135.6, 134.8, 131.5, 130.6, 130.0, 129.6, 129.4, 129.2, 129.1, 127.0, 122.0, 102.0. HRMS (ESI+) m/z: calcd for $C_{21}H_{15}ClNO$ $[M + H]^+$, 332.0842; found, 332.0869.

(Z)-4-Benzylidene-2-(3-chlorophenyl)-4H-benzo[d][1,3]-oxazine ***22***. The following compound was obtained according to the Procedure for Gold(I) Catalysis, using 3-chloro-*N*-(2-(phenylethynyl)phenyl)benzamideoxazine (0.030 g, 0.0906 mmol, 1 equiv) as a starting material and gold(I) catalyst **C1** (0.0699 g, 0.0090 mmol, 10 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **22** (22 mg, 73%) as an orange solid. mp = 94−97 °C. IR (neat) ν/cm^{-1}: 2923 (s), 1722 (m), 1317 (s), 749 (s). ^1H NMR (500 MHz, $CDCl_3$): δ 7.88−7.85 (m, 1H), 7.66−7.64 (m, 1H), 7.49−7.45 (m, 2H), 7.33−7.27 (m, 5H), 7.21−7.15 (m, 3H), 7.15−7.11 (m, 2H), 6.78 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.0, 141.2, 138.5, 137.2, 134.6, 132.8, 130.6, 129.9, 129.6, 128.8, 128.6, 128.5, 128.1, 125.0, 123.9, 121.2, 114.6, 110.4. HRMS (ESI+) m/z: calcd for $C_{21}H_{15}ClNO$ $[M + H]^+$, 332.0842; found, 332.0865.

(Z)-4-Benzylidene-2-(3-iodophenyl)-4H-benzo[d][1,3]-oxazine ***23***. The following compound was obtained according to the Procedure for Gold(I) Catalysis, using 3-iodo-*N*-(2-(phenylethynyl)phenyl)benzamide (0.022 g, 0.0520 mmol, 1 equiv) as a starting material and gold(I) catalyst **C5** (0.0070 g, 0.0104 mmol, 20 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **23** (16 mg, 73%) as a white solid. mp = 90−93 °C. IR (neat) ν/cm^{-1}: 2922 (s), 1684 (s), 1452 (s), 1318 (s). ^1H NMR (500 MHz, $CDCl_3$): δ 7.91 (d, J = 7.3 Hz, 1H), 7.78 (s, 1H), 7.64 (s, 2H), 7.56 (s, 1H), 7.35−7.29 (m, 3H), 7.25 (s, 1H), 7.19 (s, 2H), 7.11 (d, J = 7.3 Hz, 1H), 6.95 (s, 1H), 6.77 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.7, 141.5, 141.2, 139.4, 138.5, 137.3, 133.3, 130.1, 129.5, 128.9, 128.6, 128.1, 125.1, 124.0, 121.2, 114.8, 110.4, 93.9. HRMS (ESI+) m/z: calcd for $C_{21}H_{15}INO$ $[M + H]^+$, 424.0198; found, 424.0235.

(Z)-4-Benzylidene-2-(2-fluoro-5-iodophenyl)-4H-benzo-[d]-[1,3]oxazine ***24***. The following compound was obtained according to the Procedure for Gold(I) Catalysis, using 2-fluoro-5-iodo-*N*-(2-(phenylethynyl)phenyl)benzamide (0.096 g, 0.2176 mmol, 1 equiv) as a starting material and gold(I) catalyst **C5** (0.0295 g, 0.0435 mmol, 20 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **24** (39 mg, 41%) as a white solid. mp = 93−95 °C. IR (neat) ν/cm^{-1}: 3072 (m), 2931 (s), 1675 (s), 1321 (s). ^1H NMR (500 MHz, $CDCl_3$): δ 8.27 (d, J = 8.2 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.55−7.52 (m, 1H), 7.44 (ddd, J = 7.8, 4.8, 2.2 Hz, 1H), 7.40 (t, J = 7.8 Hz, 1H), 7.35 (d, J = 14.8 Hz, 1H), 7.23 (d, J = 7.5 Hz, 2H), 7.17 (t, J = 7.5 Hz, 2H), 7.12 (t, J = 7.3 Hz, 1H), 6.69 (s, 1H), 6.48 (t, J = 7.3 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.6, 163.8, 159.2 (d, J = 256 Hz), 141.8, 140.1, 139.1 (d, J = 2 Hz), 137.7, 132.3 (d, J = 9 Hz), 130.8, 128.8, 127.8, 125.1, 124.1, 120.6, 117.8 (d, J = 22 Hz), 115.1, 111.2, 86.3. HRMS (ESI+) m/z: calcd for $C_{21}H_{14}FINO$ $[M + H]^+$, 442.0104; found, 442.0139.

(Z)-4-Benzylidene-2-(3,5-bis(trifluoromethyl)phenyl)-4H-benzo[d][1,3]oxazine ***25***. The following compound was obtained according to the Procedure for Gold(I) Catalysis, using *N*-(2-(phenylethynyl)phenyl)-3,5-bis(trifluoromethyl)-benzamide (0.043 g, 0.0992 mmol, 1 equiv) and gold(I) catalyst **C5** (0.0135 g, 0.0198 mmol, 20 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **25** (33 mg, 79%) as a white solid. mp = 105−108 °C. IR (neat) ν/cm^{-1}: 2925 (m), 1732 (w), 1454 (w), 1140 (m). ^1H NMR (500 MHz, $CDCl_3$): δ 8.21 (d, J = 8.2 Hz, 1H), 7.87 (s, 2H), 7.72−7.66 (m, 2H), 7.46−7.41 (m, 1H), 7.38 (t, J = 7.5 Hz, 1H), 7.16 (d, J = 7.5 Hz, 2H), 7.12−7.04 (m, 3H), 6.80 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.4, 140.4, 138.5, 137.9, 132.9, 130.2 (d, J = 3 Hz), 129.6, 129.2, 128.9, 128.52, 125.8, 124.7, 122.1 (d, J = 273 Hz), 121.3, 115.1, 111.4. HRMS (ESI+) m/z: calcd for $C_{23}H_{14}F_6NO$ $[M + H]^+$, 434.0980; found, 434.1009.

(Z)-4-Benzylidene-2-(4-nitrophenyl)-4H-benzo[d][1,3]-oxazine ***26***. Compound **26** was obtained according to the Procedure for Gold(I) Catalysis, using 4-nitro-*N*-(2-(phenylethynyl)phenyl)benzamide (0.020 g, 0.0854 mmol, 1 equiv) as a starting material and gold(I) catalyst **C5** (0.0080 g, 0.0116 mmol, 20 mol %). The crude material was purified by flash column chromatography over silica gel with the system hexane to afford the product **26** (11 mg, 52%) as a red solid. The spectroscopic data matched with those previously described in the literature.[21] ^1H NMR (500 MHz, $CDCl_3$): δ 8.04−7.99 (m, 3H), 7.66 (t, J = 8.2 Hz, 3H), 7.37 (d, J = 8.2 Hz, 2H), 7.22 (d, J = 7.5 Hz, 2H), 7.13 (d, J = 7.5 Hz, 2H), 7.09 (t, J = 7.5 Hz, 1H), 6.79 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.4, 149.8, 141.2, 140.7, 138.5, 133.0, 131.2, 129.0, 128.8, 128.5, 125.5, 124.5, 123.5, 121.3, 114.9, 111.0.

Biological Assays on BC. *Cell Lines.* The tumor cell lines MCF-7 and HCC1954 were grown in Dulbecco's modified Eagle medium (Invitrogen Corporation, Carlsbad, CA, United States) enriched with 5% fetal bovine serum. Medium change and passage were achieved every 3 and 4 days, respectively. The MCF-7 and HCC1954 cell lines were generously provided by Professor V. Treviño from ITSM.

Cell Proliferation Analysis. The method for quantifying cell proliferation was carried out with the use of crystal violet dye in 1× phosphate-buffered saline (2.7 mM KCl, 1.8 mM KH_2PO_4, 136 mM NaCl, 10 mM Na_2HPO_4 pH 7.4). The treated cells were incubated in methanol for 15 min and washed two times with water. Cells were dyed with 0.1% crystal violet and washed three times with water. Crystal violet was recovered with 10% acid acetic to be analyzed in a microplate reader Multiskan GO spectrophotometer (Thermo Scientific, Ratastic, Finland).

■ ASSOCIATED CONTENT

Supporting Information

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Copies of ^{1}H and ^{13}C for compounds **1**−**26** and curves of dose−response of the compounds **16**−**26** (PDF)

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L.A.S.-Q., K.R.T.-C., N.M., D.B.P., M.L.-C., and R.O.-A.: organic synthesis, M.T.-J.: spectroscopic analysis, I.F.-S., J.G.A.-H., A.J.G.-L., R.G.-H., C.A.R.-H., and Y.L.-H.: biological evaluation, and J.A.L., L.C.-G., and C.R.S.-A.: analysis, discussion, and writing paper.

Notes

The authors declare no competing financial interest.

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DEDICATION

In memory of our colleague and friend **Kevin**.

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