Synthesis, cytotoxicity and anti-cancer activity of new alkynyl-gold(i) complexes†

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Alkynyl(triphenylphosphine)gold(i) complexes carrying variously substituted propargylic amines have been synthesized and fully characterized in solution and solid state. High levels of toxicity (i.e. micromolar range) were recognized for a series of cancer cell lines with particular emphasis on HT29, IGROV1, HL60 and I407. In particular the lead compound 3ab was identified as the most active compound in all cell lines (IC50: 1.7–7.9 μM).

Introduction

The class of metallodrugs based on gold complexes continues to gain credit within the scientific community due to their consolidated multiple pharmacological activities.1 In particular, seminal discoveries focusing on the treatment of symptoms of rheumatoid arthritis2 were expanded by applications of gold complexes as antimalarial agents3 and most recently also as anticancer drugs.4

The high toxicity that some gold(i), gold(III)5 and gold nanoparticles6/nanorods7 have shown against several tumour cell lines inspired the development of a number of structurally different organometallic species with chemical permutations both in the metal oxidation state and in the organic counterparts.8

In particular, the organic frameworks constituting the prodrug system proved to be actively involved in determining the overall toxicity of the species.9 In this direction, soft ligands such as phosphines, thiolates (class-I polymeric thiolates, class-II monomeric thiolates)2 or σ-donating nitrogen heterocyclic carbenes (NHCs)10 have been employed in gold complexes featuring anticancer activity.

Mechanistically, although, the real target of gold-based organometallic species is still under debate, recent investigations have unveiled that the observed cytotoxicity is mediated by their ability to alter mitochondrial functions through peculiar interactions with Se-containing enzymes TrxR.11 This class of enzymes is involved in the defence against oxidative damage and in redox signalling. A growing number of transcription factors including NF-kappaB or the Ref-1-dependent AP1 require thioredoxin reduction for DNA binding.12 Moreover, recent studies have shown that the anti-proliferative properties of gold(i) and gold(III) adducts may include their interaction with DNA: as a matter of fact, rapid inhibition of DNA synthesis was observed for gold(I) complexes containing AMPP, dppe or ADPP ligands.13

Very recently, Ott and coworkers documented on the synthesis, characterization and pharmacological investigation of a new family of mononuclear [alkynyl(triphenylphosphine)gold(i)] complexes of general structure PPh3Au=CCH2XR (X: O, N) with important anti-proliferative activity (micromolar range) in breast adenocarcinoma and colon carcinoma cells.14 Shortly after, the same team described the remarkable biological properties of binuclear gold(i) alkynyl analogs featuring bidentate phosphines as tethering units (Fig. 1).15 These studies emphasized also thioredoxin reductase (TrxR)16 as a plausible biological target of the pharmacologically active gold(i) species.17

The propargylic sidearm proved to contribute substantially to the overall pharmacological activity of the title species, therefore, careful modulation of this unit could lead to interesting perspectives in developing more selective and potent candidates for anticancer drugs.

In this regard, we present our recent investigation dealing with the documentation of novel [alkynyl(triphenylphosphine)-gold(i)] complexes comprising relatively unexplored propargylic amine derivatives as organic ligands. The possibility to create chemical diversity by means of readily accessible propargylic

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amine derivatives enabled a survey of several structural aspects such as nitrogen basicity and electronic/steric factors.

Results and discussion

Synthesis and characterization

In order to assess the effective role of the alkynyl sidearm in the biological spectrum of the gold species, a range of propargylic mono- and diamine derivatives 2a–f and mono/binuclear phosphinogold(i) complexes (i.e. PPh₃AuCl 1a and [PPh₃(CH₂)₃PPh₃][AuCl]₂ 1b), were selected as the key building blocks (Chart 1).

In particular, by reacting an equimolar amount of gold(I) chloride complexes 1a,b and the desired terminal alkyne under basic conditions (KOH, MeOH/EtOH), the corresponding alkynyl-gold complexes 3 were isolated in moderate to good yields (60–92%). Further purification was commonly carried out either by recrystallization or through flash chromatography on silica gel (Scheme 1).

Complexes 3 were obtained as white (pale brown in the case of 3ad) air stable solids featuring remarkable solubility in common organic solvents. They were fully characterized both in solution (NMR, IR, LC-MS) and in the solid state (3ab and 3ac).

In particular, NMR spectroscopy (CDCl₃, rt) was particularly diagnostic in monitoring the reaction course. As a matter of fact, the formation of adducts 3 caused the disappearance of the acetylenic C–H of the alkyne congeners (1H-NMR δ = 2.0–2.2 ppm), with the concomitant deshielding (∼0.15 ppm) of the propargylic methylene. Additionally, a marked down-shielding of the 31P-NMR signals in the final compounds 3a–e (39–42 ppm) occurred with respect to the congener 1a (32.9 ppm). In contrast, the 31P-NMR spectrum of the binuclear adduct 3bb displayed a shielded singlet (δ = 21.8 ppm) when compared with 1b (δ = 31.5 ppm). The presence of a single peak accounted for the formation of the C₂-symmetric adduct depicted in Scheme 1.

Solid state structure elucidation for complexes 3ab and 3ac was also carried out. In detail, crystals suitable for X-ray diffraction were collected through slow evaporation of EtOAc solutions of the corresponding species and the resulting structures are reported in Fig. 2. As expected for gold(i) complexes, Au adopts an almost linear coordination and the P–Au–C bond angles are very close to the ideal 180° [176.5(2) and 178.9(2)° for 3ab and 3ac, respectively]. The C≡C bond lengths of 1.183(7) and 1.194(7) Å are typical of terminal alkynyl gold(i) complexes. The crystal packing of 3ab is dominated by weak non-classical intermolecular C–H⋯O hydrogen bonds whereas in 3ac two phosphine phenyl rings in each molecule establish intermolecular π–π interactions with their symmetrically equivalent adjacent phenyl ligands generating infinite zig-zag chains along the c axis (Fig. S1 and S2†).
Biology

Cell lines included in the evaluation of toxicity profiles were malignant HT29, IGROV1, and HL60, and a non-malignant human epithelial intestinal cell line I407. IC$_{50}$ values of the drugs were calculated using Prisma, fitted by means of sigmoidal fit and listed in Table 1.

From the data collected in Table 1 some preliminary conclusions can be drawn. Within the portfolio of gold complexes in hand, 3ab was the more effective in inhibiting cell growth in all panel cell lines, auranofin, an antiarthritic gold(I) complex with antitumor activity, was included in the test panel for comparison.$^{19,20}$ Additionally, also compound 3ad showed some levels of cytotoxicity towards all the cell lines but the corresponding IC$_{50}$ values were constantly higher than that 3ab. In contrast, (+/-)-3aa, 3ac, 3bb, 3ae and 3af proved competent only on a few of the screened cell lines. In this scenario some peculiarities were also highlighted. In particular, complexes 3af and 3bb showed significant toxicity for IGROV1 and HL60 cells, respectively. Additionally, it is important to stress the lack of toxicity toward non-cancer cell lines shown by compounds 3bb, 3ae and 3af.

These bio-divergences clearly emphasised the role played by the presence of different moieties on the nitrogen atom in modulating the overall pharmacological properties of the gold complexes.

As mentioned above, inhibition of the seleno enzyme thioredoxin reductase (TrxR) is considered to be an important mechanism of the bioactivity of the gold(I) species.$^{21}$ In particular auranofin shows an high inhibitory effect both on cytosolic (TrxR1) and mitochondrial (TrxR2) isoforms of this enzyme.$^{22}$ Therefore, the potential of gold complexes to inhibit TrxR was studied on commercially available TrxR using the 2,6-dichloroindophenol (DCIP) reduction assay.

According to the results given in Table 2, complexes 3bb and 3af turned out to be effective inhibitors of TrxR showing an inhibitory potency close to that induced by auranofin with IC$_{50}$ values in the sub-micromolar range. The other complexes showed IC$_{50}$ values at least two orders of magnitude higher than the auranofin, whereas 3ab cannot be considered an inhibitor of this class of enzymes.

It can be pointed out that only binuclear compounds are able to inhibit TrxR at sub-micromolar concentration suggesting the presence of strong interaction with the enzyme. To highlight the interaction between compounds and TrxR we have evaluated the LC-MS spectra of the enzyme both in the presence of a binuclear compound (3bb) and in the presence of the mononuclear compound 3ab that does not inhibit the enzyme activity.

The results, reported in the ESI,† indicate that no covalent bond exists between TrxR and 3ab or 3bb (Fig. S3 and S4†).

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Of auranofin inhibition</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auranofin</td>
<td>100</td>
<td>0.018</td>
</tr>
<tr>
<td>3bb</td>
<td>73</td>
<td>0.354</td>
</tr>
<tr>
<td>3af</td>
<td>73</td>
<td>0.308</td>
</tr>
<tr>
<td>3ae</td>
<td>52</td>
<td>1.555</td>
</tr>
<tr>
<td>3ad</td>
<td>55</td>
<td>3.754</td>
</tr>
<tr>
<td>3aa</td>
<td>33</td>
<td>0.818</td>
</tr>
<tr>
<td>3ab</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

$^a$ First column: the inhibitory effect of different gold(I) complexes on TrxR is expressed as % of inhibition taking as reference the auranofin maximal inhibition. Second column: IC$_{50}$ values of the different gold(I) complexes and auranofin on TrxR activity.

Table 1 Half maximal inhibitory concentration (IC$_{50}$) of gold compounds in different cell lines after 24 h treatment (µM)$^a$

<table>
<thead>
<tr>
<th>Compound</th>
<th>HT29</th>
<th>IGROV1</th>
<th>HL60</th>
<th>I407</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auranofin</td>
<td>3.3 (1.8–6)</td>
<td>2.5 (0.4–15)</td>
<td>0.7 (0.3–1.6)</td>
<td>1.6 (0.9–2.8)</td>
</tr>
<tr>
<td>(+/-)-3aa</td>
<td>&gt;100</td>
<td>20 (10.06–39.20)</td>
<td>19.0 (7.43–50.69)</td>
<td>15.0 (9.14–24.65)</td>
</tr>
<tr>
<td>3ab</td>
<td>7.9 (5.39–11.59)</td>
<td>5.3 (3.87–7.43)</td>
<td>5.5 (4.69–6.69)</td>
<td>2.7 (1.19–6.18)</td>
</tr>
<tr>
<td>3ae</td>
<td>&gt;100</td>
<td>6.5 (4.12–10.31)</td>
<td>6.3 (5.05–7.94)</td>
<td>9.0 (6.55–12.83)</td>
</tr>
<tr>
<td>3ad</td>
<td>11.0 (8.97–15.01)</td>
<td>10.0 (6.40–17.84)</td>
<td>6.3 (5.05–7.94)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3af</td>
<td>&gt;100</td>
<td>7.7 (6.26–9.48)</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3bb</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.8 (0.28–2.40)</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

$^a$ 95% confidence intervals are reported in the brackets.
To gain some insight into the biological effects of these new derivatives, the most active compound toward all cell lines, 3ab was subjected to additional studies. In order to assess whether its effect was due to interference with cell cycle progression, DNA profiles of cultured cells were examined by flow cytometry and cell cycle analysis was performed by using the Multicycle Cycle Phoenix Flow system, and Modfit 5.0 software. Table 3 shows that the treatment with 3ab caused a marked accumulation of HT29, IGROV1 and I407 cells in the S phase, with respect to untreated cells.

In contrast, in HL60 treated cells, the growth arrest in the G0/G1 phase of the cell cycle was associated with a well distinguishable pre-G1 peak in DNA, suggestive of DNA fragmentation, characteristic of apoptosis (Fig. 3).

Interestingly, the treatment with the binuclear compounds did not induce any effect on the cell cycle, as reported in Table 4, where the cell cycle distribution of IGROV1 and HL60 in the presence of 3af and 3bb, respectively is shown.

### UV-Vis absorption titration analysis

Interactions between small molecules and DNA rank among the primary action mechanisms of cytotoxic activity. In order to compare the binding properties of the gold complexes with DNA, dissociation constants ($K_d$) were determined through inverse titration experiments. Two types of interactions can be devised by these experiments as we can argue by either an increase or a decrease of $\Delta A$ measured at 260 nm.

The increase of differential absorption of DNA in the presence of 3af, 3ab, 3ac, 3bb, and 3ac can be ascribed to a lower base stacking while the decreased differential absorbance observed for (+/-)-3aa suggests a higher compactness of DNA. No appreciable effect was observed for 3ad. In this regard, the differential spectra of 3af (A), (+/-)-3aa (B) and 3ad (C) are depicted in Fig. 4.

We then plot the differential absorbance at 260 nm for each molecule versus DNA concentration, as reported in Fig. 5. The estimation of the dissociation constant ($K_d$) for the complex formation as well as the limiting value for the $\Delta A_{260}$ was obtained fitting these data using a one-site saturation equation (Table 5).

The $K_d$ analysis confirms that 3ab interacts with DNA quite strongly ($K_d = 0.84 \pm 0.17 \mu M$) suggesting a partial explanation for its cellular toxicity. Among the other compounds, only 3af shows a strong interaction with DNA having a similar value of $K_d (1.54 \pm 0.58 \mu M)$. On the other hand, a decrease for the differential absorption spectra is observed for (+/-)-3aa. While the increase in the absorbance at 260 nm can be ascribed to a partial DNA denaturation, the decrease observed in the presence of compound 3aa, could be indicative of DNA supercoiling.

### Table 3 Cell cycle distribution of cell lines treated with 3ab

<table>
<thead>
<tr>
<th>Cell line</th>
<th>G0/G1 %</th>
<th>S %</th>
<th>G2/M %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT29</td>
<td>49.05</td>
<td>39.58</td>
<td>11.37</td>
</tr>
<tr>
<td>HT29 + 3ab (7.9 µM)</td>
<td>45.72</td>
<td>45.25</td>
<td>9.03</td>
</tr>
<tr>
<td>IGROV1</td>
<td>55.32</td>
<td>29.57</td>
<td>15.11</td>
</tr>
<tr>
<td>IGROV1 + 3ab (5.3 µM)</td>
<td>50.71</td>
<td>37.16</td>
<td>12.13</td>
</tr>
<tr>
<td>I407</td>
<td>70.38</td>
<td>23.62</td>
<td>6</td>
</tr>
<tr>
<td>I407 + 3ab (1.7 µM)</td>
<td>62.64</td>
<td>28.52</td>
<td>8.84</td>
</tr>
</tbody>
</table>

### Table 4 Cell cycle distribution of cell lines treated with 3af and 3bb

<table>
<thead>
<tr>
<th>Cell line</th>
<th>G0/G1 %</th>
<th>S %</th>
<th>G2/M %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGROV1</td>
<td>60</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>IGROV1 + 3af (7.7 µM)</td>
<td>64</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>HL60</td>
<td>48</td>
<td>14</td>
<td>38</td>
</tr>
<tr>
<td>HL60 + 3bb (0.8 µM)</td>
<td>50</td>
<td>17</td>
<td>33</td>
</tr>
</tbody>
</table>

Fig. 3 Effect of compound 3ab on the HL60 cell cycle. The cells were incubated for 24 h, (sx) with the vehicle (Ctrl), or (dx) with compound 3ab (3.3 µM), afterwards cell cycle distribution was determined by flow cytometry. Following treatment with 3ab, the cells are in the G0/G1 phase and a well detectable fraction of DNA is present as a sub-G1 peak (light blue peak).

Fig. 4 Differential absorption spectra of 3af (A), (+/-)-3aa (B) and 3ad (C) titrated with DNA.
Fluorescence titration of 15 µM ETBr bound to DNA with 3ab, up to 200 µM, does not show any appreciable change of the emission spectra of ETBr, suggesting that no intercalation of 3ab with DNA takes place (Fig. 6).

Conclusions

In conclusion, a new class of neutral [Au(i)]-alkynyl complexes based on monodentate or bidentate phosphine ligands has been developed and fully characterized both in the liquid and solid state. The gold(i)–Csp linkage was efficiently realized in high yields (60–92%) by condensing the gold-chloride congener with pre-functionalized terminal alkynes under convenient mild conditions (KOH, MeOH/EtOH).

Additionally, the biological activity of these organometallic species was comprehensively investigated and the data reported suggest that their cellular toxicity could be related to different mechanisms acting on different biological targets.

Compound 3ab showed a marked cytotoxicity on all cell lines tested, with IC50 values ranging from 1.7 µM for I407 to 7.9 µM for HT29 and caused cell cycle arrest in the S phase. Only in the HL60 cell line the growth arrest was in the G0/G1 phase of the cell cycle and it was associated with a well distinguishable pre-G1 peak that indicates DNA fragmentation that is characteristic of apoptosis. These effects on the cell cycle can be associated with an interaction of the molecule with DNA and this hypothesis is supported by the results of DNA titration where the dissociation constant of 3ab with salmon sperm DNA is in the sub-micromolar range. The real mechanism of 3ab–DNA interaction has not yet been fully elucidated, however, it cannot be attributed to an intercalation of the molecule into the DNA helix. Additionally, it should be mentioned that 3ab does not show any inhibitory effect on the thioredoxin reductase enzymatic activity.

On the other hand, the binuclear compounds (3bb and 3af) showed a cytotoxic effect only in HL60 (3bb: IC50 = 0.8 µM) and in IGROV1 (3af: IC50 = 7.7 µM) and they do not show any effect on the cell cycle (Table 4). This evidence suggests that the biological target of binuclear gold-species is not the DNA and they appear to act through the inhibition of thioredoxin reductase at sub-micromolar concentration (Table 2). However the mechanism of interaction of our alkynyl-gold(i) complexes with thioredoxin reductase is different as compared to auranofin and other gold compounds. In fact, while auranofin induces a mass shift in the mass spectra of this enzyme suggestive of protein binding of the gold-containing molecule, in our study no covalent adducts with the enzyme have been detected using LC/ESI-MS (see ESI†).

Moreover, inhibition of thioredoxin reductase is responsible for a decrease of the oxidative stress resistance and for alterations in redox signalling that are key factors for cell survival. Cancer cells are more resistant toward oxidative stress, for this reason compounds that are able to interfere with this phenomena, have a good chance to be anticancer drug candidates. Studies addressing the clarification and/or identification of additional biological targets as well as the development of structure–activity relationships are currently ongoing in our laboratories.

Experimental

Synthesis of the nitrogen containing [alkynylAu(i)] complexes 3

A solution of the desired alkyne (1.2 or 2.4 eq.) in reagent grade MeOH/EtOH (1:1 ratio, 0.05 M) was treated with the
desired gold(II) chloride precursor (1a, 1 eq.) and a solution of KOH (4 eq., 2 M in MeOH). The mixture was stirred in the dark until complete consumption of the alkene. The resulting solid was collected by filtration and washed with cooled MeOH. Pure material can be obtained via re-crystallization from a DCM : pentane solution, or flash chromatography.

(+/-) 3aa: white solid, purification via flash chromatography (cHex : AcOEt = 9 : 1–7 : 3); yield = 66%; 85–86 °C; IR = 2159 cm⁻¹ (C=C); 1H-NMR (400 MHz, CDCl₃): δ = 1.44 (d, J(H,H) = 6.4 Hz, 3H); 3.40 (d, J(H,H) = 17.6 Hz, 1H); 3.61 (d, J(H,H) = 2.0 Hz, 2H); 3.68 (d, J(H,H) = 17.6 Hz, 1H); 4.00 (q, J(H,H) = 6.4 Hz, 1H); 1H-NMR (100 MHz, CDCl₃): δ = 21.4, 21.6, 21.9, 24.6, 29.1, 31.1, 34.9, 40.9, 41.4, 77.6, 92.4, 127.8, 127.9, 128.9, 129.4, 130.4, 131.2, 131.3, 131.9, 132.2, 132.3, 133.4, 143.6; 31P-NMR (162 MHz, CDCl₃): δ = 21.8 ppm.

Cell cycle and cytotoxicity

Cell lines (HT29, IGROV1, HL60 and I407) were routinely cultured in RPMI 1640 medium (Lonza) supplemented with penicillin (Sigma-Aldrich) (100 U mL⁻¹), streptomycin (Sigma-Aldrich) (100 µg mL⁻¹), and 10% fetal bovine serum (Euroclone) in an environment of 5% CO₂, 37 °C and sub-cultured using a trypsin 0.25%–EDTA (Sigma-Aldrich) 0.02% solution. The cytotoxicity was determined with the MTT (3-(4,5-di- methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich) dye reduction assay.

Cells were plated in 96-well flat-bottom microplates at a density of 1 x 10⁴ cells per mL (100 µL per well), and 24 h later the test compounds were added, appropriately diluted with DMSO. The cells were exposed to various concentrations of the compounds (in a range 1 nM to 100 µM) for 24 h. The cytotoxicity was determined with the MTT (3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye reduction assay with minor modifications. Briefly, after incubation with the test compounds, MTT solution (0.2 mg mL⁻¹ in PBS) was added (100 µL per well). The plates were further incubated for 2 h at 37 °C, and the formazan crystals formed were dissolved by adding 100 µL per well of propanol. Optical densitometry was determined with a Wallac 1420 Victor2 Microplate Reader (Perkin Elmer) at 570 nm.

One hundred microliters of culture medium supplemented with the same amount of MTT solution and solvent was used as the blank solution. The IC₅₀ value was calculated according to the GraphPad Prism 5 software. All data are expressed as mean ± SD.

Cell cycle analysis

Cells were plated at an initial density of 10 000–20 000 cells per cm² in dish or flask, depending on the cell line. After 72 h of adhesion, the cells were treated with drugs at the concentration corresponding to the calculated IC₅₀, and after 24 h of treatment the effect was evaluated. Untreated and 24 h treated cells were detached, washed with PBS and the pellet was finally re-suspended in 0.01% Nonidet P-40 (Sigma-Aldrich), 10 µg per mL RNase (Sigma-Aldrich), 0.1% sodium citrate (Sigma-Aldrich), 50 µg per mL propidium iodide (PI) (Sigma-Aldrich), for 30 min at room temperature in the dark. Propidium iodide (PI) fluorescence was analyzed using a Beckman Coulter Epics XL-MCL flow cytometer and cell analysis was performed using the M cycle (Verity) and MODFIT 5.0 software.
**TrxR inhibition assay**

For this purpose, commercially available rat liver TrxR (Sigma-Aldrich) was used and diluted with distilled water to achieve a concentration of 0.05 U mL\(^{-1}\). The gold(i) complexes were freshly dissolved as stock solutions in DMSO. The reduction of the DCIP (2,6-dichloroindophenol) was followed spectrophotometrically at 600 nm using ε = 19.1 mM cm\(^{-1}\) using a Jasco V-550 spectrophotometer equipped with a stirring device and thermostatic control.

To each cuvette was added: 100 µL of enzyme solution, different concentrations of the compounds (ranging from 1 to 100 µM) or vehicle, 100 µM DCIP, 8 mM EDTA, 0.001% BSA in 20 mM potassium phosphate buffer pH 7.1 final volume.

The reaction was started by the addition of 2 mM NADPH. The absorbance at 500 nm was followed spectrophotometrically at 600 nm using DCIP (2,6-dichloroindophenol) was followed spectrophotometrically at 500 nm and emission spectra were recorded between 530 and 800 nm. Blank subtraction was applied.

**Competitive binding fluorescence studies**

Aliquots of stock solution of the gold complex 3ab dissolved in DMSO were added to solutions containing 15 µM calf thymus DNA (ctDNA) base pairs and 15 µM ethidium bromide (EtBr) in 15% DMSO 25 mM Tris-HCl buffer (pH 7.0) at 25 °C to give the final complex concentration ranging from 0 to 200 µM, according to the literature.\(^{22,24}\) Excitation wavelength was set at 500 nm and emission spectra were recorded between 530 and 800 nm. Blank subtraction was applied.

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**Notes and references**


CCDC 1045016 (3ab) and 1045017 (3ac) contain the supplementary crystallographic data for this paper.

PP3 and 2b were tested in the cell lines IGROV1 and HT29. Here, the alkyne proved to be inactive in both cases and the PPh3 furnished the following IC50 values: 47 μM (IGROV1) and 39 μM (HT29).


