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Gold(I) compounds with lansoprazole-type ligands: synthesis, characterization and anticancer properties *in vitro*†

M. Serratice,‡[a] B. Bertrand,‡[bc] E. F. J. Janssen,[b] E. Hemelt,[b] A. Zucca,[a] F. Cocco,[a] M. A. Cinellu*[a] and A. Casini*[b]

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A number of gold(I) complexes containing the proton pump inhibitor (PPI) lansoprazole and its reduced precursor 2-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methylthio)-1*H*-benzo[*d*]imidazole have been synthesized and their biological effects have been evaluated in human cancer and non-tumorigenic cells *in vitro*. The lansoprazole-based compounds appear to act through a V-H$^+$-ATPase-mediated mechanism.

Gold compounds have a long and important tradition in medicine, the so-called *Chrysotherapy* that derives its name from Chryses, a golden-haired heroine of Greek Mythology. Such a tradition dates back to the Chinese (2500 B.C.) and especially flourished in Europe during the late Middle Age and the Renaissance. In early times of modern pharmacology, gold compounds were widely used for the treatment of several diseases, especially as anti-infective and anti-tubercular agents. Nowadays, gold-based drugs are only used as a treatment for severe rheumatoid arthritis, because of their systemic toxicity and poor stability. Moreover, in recent years, gold compounds hold great promise as potential anticancer drugs.[1,2] In fact, a conspicuous number of coordination and organometallic gold complexes, with highly different chemical structures, were reported to manifest outstanding antiproliferative properties in cancer cells *in vitro*, and sometimes *in vivo*.[3–5]

With the aim of developing new gold-based cytotoxic agents, our groups have been particularly focusing on the study of gold(I) and gold(III) complexes with nitrogen donor ligands and on the investigation of their biological mechanisms of action.[6–8]

It is worth mentioning that resistance to cytotoxic agents is a major problem in treating cancer. The mechanisms underlying this phenomenon appear to profit of functions involved in the control of cell homeostasis. A mechanism of resistance may be the alteration of the tumour microenvironment *via* changes in the pH gradient between the extracellular environment and the cell cytoplasm.[9] In fact, the extracellular pH of solid tumours is significantly more acidic than that of normal tissues,[10] thus impairing the uptake of weakly basic chemotherapeutic drugs and reducing their anticancer effect. Therefore, a strategy to revert drug resistance is the use of agents that disrupt the pH gradient in tumours by inhibiting the function of pumps generating the pH gradient itself, such as vacuolar H$^+$-ATPases (V-H$^+$-ATPases).[11,12]

A class of V-H$^+$-ATPase inhibitors, called proton pump inhibitors (PPIs), have emerged as the drug class of choice for treating patients with peptic diseases.[13,14] These drugs inhibit gastric acid secretion by targeting the gastric acid pump, but they also directly inhibit V-H$^+$-ATPases. PPIs are in general protonable weak bases selectively accumulating in acidic spaces. Currently, there are five PPIs available for treating gastric and duodenal ulcer disease, namely omeprazole, pantoprazole, esomeprazole, rabeprazole and lansoprazole. They are all substituted benzimidazoles that behave as prodrugs that need to be activated *in vivo*.[13]

Lansoprazole (HL^2, Fig. 1) has a structure that combines a benzimidazole ring and a pyridine ring connected through a sulfinyl linkage. The compound's activation occurs by addition of two protons to the nitrogens on either side of the sulfinyl group. The second protonation on the benzimidazole ring causes rearrangement of the sulfinyl into a sulfenic acid or a sulfenamide. The latter can react with the cysteine molecules of the H$^+$-ATPase to form one or two disulfide bonds.[15]

PPI may revert chemoresistance and increase chemosensitivity of different human tumour cells. Recent findings from Fais *et al.* have shown that PPI pretreatment sensitized tumour cell lines to the effect of cisplatin, 5-fluorouracil and vinblastine.[16] PPI pretreatment was associated with the

[a]*Università degli Studi di Sassari, Dipartimento di Chimica e Farmacia, Via Vienna, 2, Sassari, I-07100, Italy. E-mail: cinellu@uniss.it; Fax: +39 079 229559*

[b]*Dept. of Pharmacokinetics, Toxicology and Targeting, Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands. E-mail: a.casini@rug.nl; Fax: +31 50 363 3274*

[c]*Institut de Chimie Moléculaire de l'Université de Bourgogne, UMR 6302 CNRS Université de Bourgogne, Dijon, France*

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‡ These two authors contributed equally to the work.

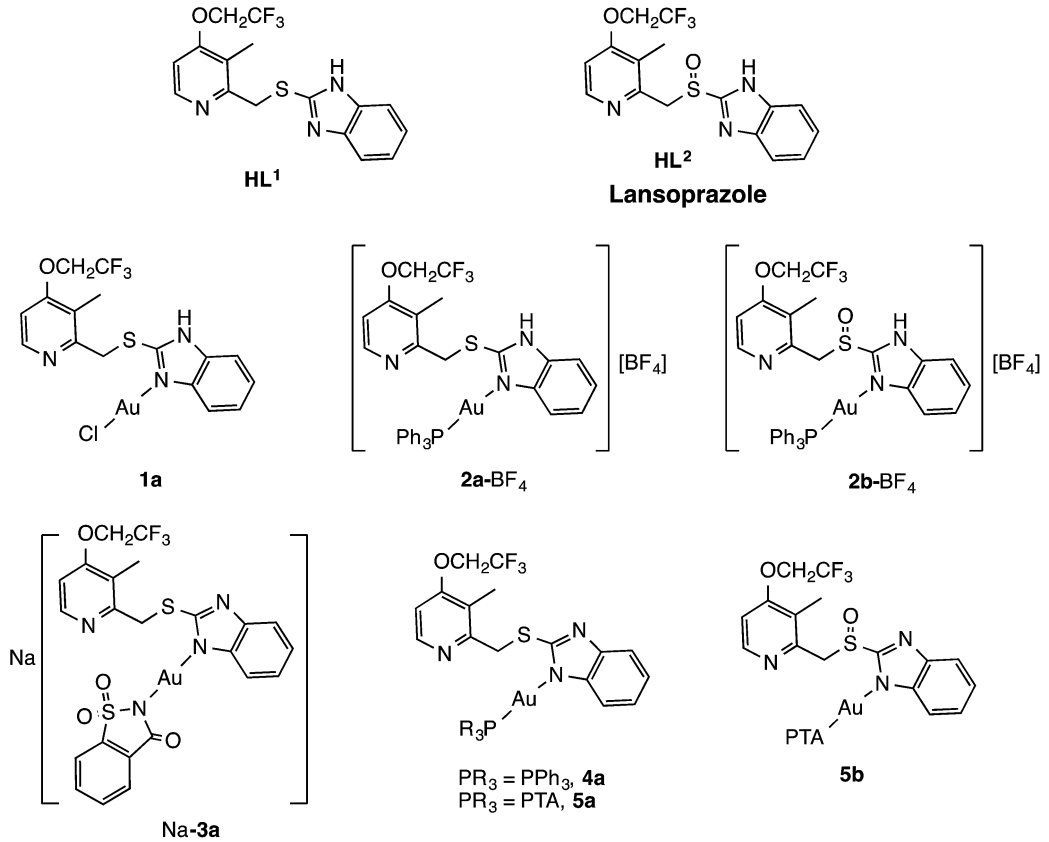


Fig. 1 Ligands HL^1 and lansoprazole (HL^2) and their mononuclear gold(I) complexes.

inhibition of V-H^+-ATPase activity and an increase of both extracellular pH and the pH of lysosomal organelles, consistent with a cytoplasmic retention of the cytotoxic drugs and targeting to the nucleus in the case of doxorubicin.[16] *In vivo* experiments showed that oral pretreatment with omeprazole induced a sensitivity of the human solid tumors to anticancer drugs.[16] Moreover, PPI pre-treatment of melanoma cells increased cellular uptake of *cisplatin*, as compared to untreated cells, in an acidic-depend manner.[17]

Interestingly, other studies described the advantages of combining lansoprazole with metal ions, such as Ni(II), Co(II), Mn(II), Cu(II) and Hg(II), as well as inner transition metals (Th(IV), Ce(IV), Nd(III) and Gd(III)), for therapeutic purposes as antibacterial or antifungal agents.[18,19] Recently, the pump inhibitor pantoprazole has also been linked to a Gd-based compound to enhance the accumulation of the resulting MRI contrast agent in the stomach and colon.[20]

Within this frame, we decided to explore the possibility to couple gold ions to lansoprazole-type ligands in order to achieve "bifunctional" metal compounds able to selectively target cancer cells. Thus, we report here on the synthesis and chemical characterization of a series of mono- and binuclear gold(I) compounds bearing lansoprazole-type ligands. The anti-proliferative properties of the compounds have been evaluated in cancer cell lines, including the human ovarian cancer A2780 cells and their *cisplatin* resistant variant A2780cisR, as well as in non-cancerous human embryonic kidney HEK-293T cells to assess compounds' selectivity. Moreover, preliminary results on the possible contribution of a V-H^+-ATPase-mediated mechanism in the compounds' biological mode of action were obtained.

Initially, lansoprazole (2-((3-methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl)methylsulfinyl)-1*H*-benzo[*d*]imidazole) (HL^2) was prepared by oxidation of 2-((3-methyl-4-(2,2,2-trifluoroethoxy) pyridin-2-yl)methylthio)-1*H*-benzo[*d*]imidazole (HL^1, Fig. 1) according to a recently reported method.[21]

Afterward, a variety of gold(I) complexes, both mono- (Fig. 1) and dinuclear (Fig. 2), have been prepared by reaction of the two ligands with the appropriate gold(I) precursor. The compounds have been characterized by ^1H and ^{31}P NMR, as well as IR spectroscopy and elemental analysis (see ESI† for details).

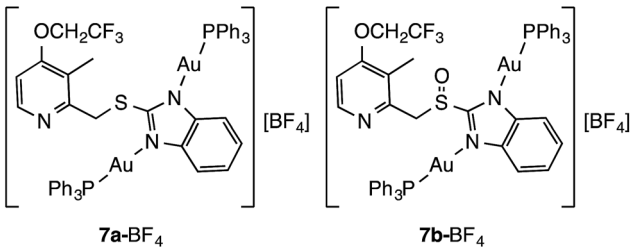


Fig. 2 Dinuclear complexes based on lansoprazole-type ligands.

Three of the mononuclear complexes, namely [Au(HL^1)Cl] (**1a**) and [Au(HL^n)(PPh$_3$)](BF$_4$) (n = 1, **2a**-BF$_4$; n = 2, **2b**-BF$_4$), contain the ligand – HL^1 or HL^2 – in its neutral form. The neutral complex **1a** was prepared by reaction of HL^1 with [Au(THT)Cl] (THT = tetrahydrothiophene). The two cationic complexes **2a**-BF$_4$ and **2b**-BF$_4$, both featuring triphenylphosphine as ancillary ligand, were obtained by reaction of the appropriate ligand with the solvento complex [Au(PPh$_3$)(solv)](BF$_4$), this was prepared *in situ* by reaction of [Au(PPh$_3$)Cl] with AgBF$_4$ in dichloromethane.

Of the three possible coordination sites, *i.e.* the sulfur atom, the pyridine and the imidazole nitrogen atom, the IR and ^{1}H NMR data suggest that the latter is likely coordinated to the Au-Cl fragment in **1a**. Indeed, its far IR spectrum shows the Au-Cl stretching vibration at 341 cm^{-1}, consistent with a Au-Cl *trans* to a nitrogen atom, and the ^{1}H NMR spectrum in CD_2Cl_2 shows two multiplets at δ 7.71 and 7.63 ppm, each integrating for 1 proton, attributed to H$^{6'}$ and H$^{3'}$ (numbering scheme in Experimental section†), respectively, which in the free ligand give rise to only one multiplet at 7.55 ppm, thus suggesting involvement of the iminic nitrogen of the imidazole ring in the coordination to AuCl.

In the case of **2a**-BF$_4$ and **2b**-BF$_4$, coordination of the AuPPh$_3$ fragment to a nitrogen atom is inferred by their ^{31}P{^{1}H} NMR spectrum, where a singlet resonance at δ 31.6 and 31.2 ppm, respectively, is consistent for linear gold(I) PPh$_3$ derivatives having a phosphorus *trans* to a nitrogen atom. The ^{1}H NMR spectra of the two cationic complexes are characterized by broad signals, which suggest a fluxionality process involving positional exchange of the (PPh$_3$)Au$^+$ group between the two iminic nitrogen coordination sites. The site-exchange mode is represented in Scheme 1. A similar behaviour was previously observed for analogous gold(I) complexes of 2-(2′-pyridyl)benzimidazole.[8] All signals are shifted upfield, as usually observed for PPh$_3$ complexes.

The neutral complexes **4a**, **5a** and **5b** and the anionic complex Na-**3a**, contain the ligand in its deprotonated form. The saccharinate complex Na-**3a** was obtained from reaction of HL^1 with Na[Au(sac)$_2$][22] (sacH = saccharine) in acetonitrile solution. The triphenylphosphine complex **4a** was synthesized by reaction of the solvento complex [Au(PPh$_3$)(solv)](BF$_4$) with HL^1 and KOH in acetone/MeCN/H$_2$O, while the PTA (PTA = 1,3,5-triaza-7-phosphaadamantane) complexes **5a** and **5b** have been obtained by reaction of [Au(PTA)Cl] with HL^1 and HL^2, respectively, and KOH in H$_2$O/MeCN solution.

The IR spectrum of Na-**3a** shows the C=O and the SO$_2$ stretching vibrations of the saccharinate ligand at 1689, 1290 and 1171 cm^{-1}, respectively. The proton spectrum in CD_2Cl_2 shows well resolved signals for both the L^1 and the saccharinate ligand in 1 : 1 molar ratio; most of the L^1 signals are slightly downfield shifted with respect to the free ligand and the H$^{3'}$ and H$^{6'}$ protons give rise to two multiplets, one partially overlapping with the H$^{4''}$ and H$^{5''}$ resonances of the saccharinate ligand at 7.76 ppm, the other at 7.67 ppm, thus suggesting coordination of the Au(sac) moiety to the deprotonated nitrogen atom of the imidazole ring. The ^{31}P{^{1}H} NMR spectra of **4a**, **5a** and **5b** show singlet resonances at, respectively, δ 32.2, −58.8 and −58.6 ppm consistent with linear gold(I) PPh$_3$ (**4a**) and PTA (**5a** and **5b**) derivatives having a phosphorus *trans* to a nitrogen atom.[8]

The binuclear complexes **7a**-BF$_4$ and **7b**-BF$_4$ (Fig. 2) were synthesized by reaction of the respective deprotonated ligand with 2 eq. of the solvento complex [(PPh$_3$)Au(solv)](BF$_4$) in dichloromethane. Their ^{1}H NMR spectra are characterized by well resolved signals with the correct L : PPh$_3$ integral ratio. In both cases, two signals are found in their ^{31}P{^{1}H} NMR spectrum at 31.2 and 33.1 (**7a**-BF$_4$) and 31.0 and 33.2 (**7b**-BF$_4$), attributable to the Au(PPh$_3$) coordinated respectively to the iminic and amidic nitrogen atom of the imidazole ring.

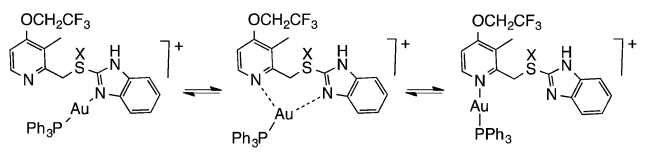
The new compounds were screened for their antiproliferative properties on human ovarian cancer cell lines sensitive (A2780) and *cisplatin* resistant (A2780cisR) as well as in human embryonic kidney HEK-293T cells using the classical MTT assay (see Experimental for details†) in comparison to the "free" ligands and *cisplatin* used as reference. A dose-dependent inhibition of cell growth was observed in all cell lines with IC$_{50}$ values ranging from *ca.* 0.7 to >70 μM after 72 hours incubation as depicted in Table 1.

Several conclusions can be drawn from the obtained results: all compounds elicit good antiproliferative properties, while the ligands are moderately toxic in the selected cell lines (the least

Table 1 Cell viability (IC$_{50}$ values) of gold(I) compounds and ligands HL^1 and lansoprazole (HL^2) in human ovarian carcinoma cell lines sensitive (A2780) or resistant to *cisplatin* (A2780cisR) and in HEK-293T cells after 72 h incubation

IC$_{50}$[a] (μM)			
Compound	A2780	A2780cisR	HEK-293T
HL^1	26.3 ± 8.5	66.8 ± 10.4	>70
Lansoprazole (HL^2)	45.6 ± 2.6	59.0 ± 15.2	>70
PPh$_3$	20.2 ± 4.0	16.0 ± 4.9	18.6 ± 2.6
PTA	>100	>100	>100
1a	10.9 ± 0.5	28.2 ± 2.4	19.9 ± 0.5
2a-BF$_4$	3.5 ± 0.3	4.2 ± 0.8	6.8 ± 1.0
2b-BF$_4$	1.1 ± 0.3	0.7 ± 0.1	5.3 ± 0.4
Na-**3a**	8.3 ± 2.1	13.4 ± 3.8	12.4 ± 0.5
4a	3.0 ± 0.5	2.8 ± 0.7	3.9 ± 0.5
5a	9.0 ± 0.8	10.3 ± 2.7	10.5 ± 1.2
5b	16.2 ± 1.1	13.2 ± 4.6	12.1 ± 0.5
7a-BF$_4$	1.0 ± 0.1	1.2 ± 0.3	2.7 ± 0.8
7b-BF$_4$	1.5 ± 0.3	0.9 ± 0.4	5.0 ± 0.9
Cisplatin	2.4 ± 0.6	35.0 ± 7.0	12.0 ± 1.9

[a] The reported values are the mean ± SD of at least three determinations.



Scheme 1 Proposed site-exchange mode of the (PPh$_3$)Au$^+$ group between the two iminic nitrogen coordination sites in **2a**-BF$_4$ and **2b**-BF$_4$.

toxic being PTA ligand with IC_{50} higher than 100 μM). Secondly, each gold complex shows similar cell growth inhibition in both A2780 and A2780cisR cells. Notably, most of the new compounds (made exception for **1a**) are more active than *cisplatin* towards the resistant cell line A2780cisR, indicating that different mechanisms of action take place with respect to classical DNA alkylating agents as platinum drugs. This is not surprising since gold-based compounds have been shown to mainly target proteins/enzymes in cancer cells.[23]

The most effective derivatives of the series, even more potent than *cisplatin*, are the mononuclear compound **2b**-BF_4 derivative of lansoprazole, and the binuclear complexes **7a**-BF_4 and **7b**-BF_4 (IC_{50} in the 1 μM range), all of them containing PPh_3 ligands and being positively charged in solution. The gold(I) derivatives bearing either the 1,3,5-triaza-7-phosphaadamantane (**5a** and **5b**) or the saccharine (Na-**3a**) ligands are *ca.* 10-fold less effective. In general, the activities of the lansoprazole gold(I) complexes are very similar to those containing the reduced HL^1 ligand, but with the same secondary ligand. However, compound **2a**-BF_4 is less potent in cancer cells than the lansoprazole derivative **2b**-BF_4. Moreover, compounds **2b**-BF_4 and **7b**-BF_4 show certain selectivity towards the cancer cells, being *ca.* 4-5-fold less effective in the non-cancerous HEK-293T cell line.

In order to assess if the various gold complexes may exert their biological effects through a V-H^+-ATPase-mediated mechanism, we evaluated the levels of intracellular ATP in A2780 cells treated with the compounds for 3 h as described in the Experimental section.† Interestingly, and in accordance with previously reported data for omeprazole and esomeprazole,[16] cells treated with lansoprazole present higher levels of intracellular ATP than untreated cells (4.5 ± 0.3 *vs.* 2.9 ± 0.3 pmol per μg protein, respectively), suggesting inhibition of the V-H^+-ATPase activity (Fig. 3). In addition, all the compounds based on lansoprazole also showed similar increase in ATP levels (following the order **2b**-BF_4 ≫ **7b**-BF_4 > **5b** ≈ lansoprazole, see Fig. 3), although it must be noted that for compound **5b** we had to reach 5 μM concentration to obtain a significant shift compared to controls. Conversely, ligand HL^1 and its derivatives did not show such an effect at any of the tested concentrations, supporting the idea that other molecular mechanisms may be at the basis of their antiproliferative properties. Specifically, gold(I) complexes with phosphine ligands have been often described as potent inhibitors of intracellular seleno-enzymes such as thioredoxin reductase.[24] A possible reason for this markedly different behaviour between the two classes of compounds is that the lack of the sulfinyl group in HL^1 does not allow activation of the ligand and its conversion into the sulfenamide responsible for V-H^+-ATPase inhibition.

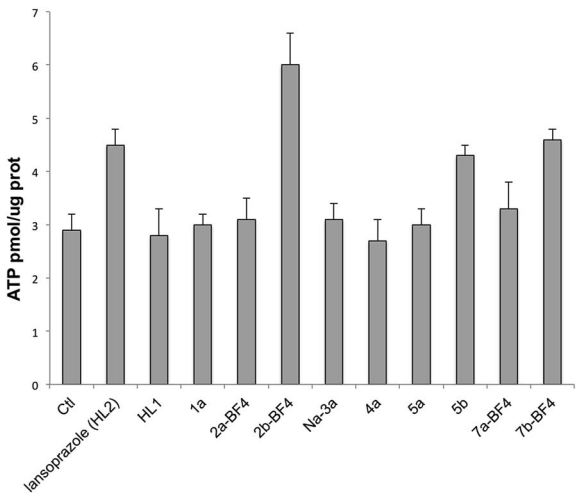


Fig. 3 Intracellular ATP levels in A2780 cells untreated (Ctl) and treated with fixed concentration of compounds (2 μM, and 5 μM for **5b**) for 3 hours at 37 °C. The difference in cellular ATP levels for HL^2 and **7b**-BF_4 treated cells with respect to controls was statistically significant ($p < 0.05$, unpaired Student's *t* test).

Conclusions

In summary, we have synthesized and characterized nine new gold(I) complexes bearing lansoprazole-type moieties. The series includes mononuclear complexes containing the neutral ligand (**1a**, **2a**-BF_4, and **2b**-BF_4), mononuclear compounds bearing the ligand in its deprotonated form (Na-**3a**, **4a**, **5a** and **5b**), as well as the cationic binuclear complexes (**7a**-BF_4 and **7b**-BF_4). The compounds have shown to possess enhanced antiproliferative properties in human ovarian cancer cells sensitive and resistant to *cisplatin* with respect to the "free" lansoprazole-type ligands.

Preliminary data on the lansoprazole-based complexes suggest that inhibition of V-H^+-ATPase activity may represent an important aspect of its mechanism of action compared to the case of gold(I) complexes containing the lansoprazole reduced precursor. Certainly, several differences in the compounds' structure may account for the observed diverse biological effects, including differences in transport mechanisms.

Although further studies are necessary to validate the mechanisms of action of these new complexes, we believe that our results hold promise to design novel cytotoxic metal compounds that can be activated *via* the microenvironmental acidification by cancerous tissues and whose biological targets may differ from those of classical Pt(II) chemotherapic agents.

Acknowledgements

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Notes and references

1 E. R. Tiekink, *Inflammopharmacology*, 2008, **16**, 138–142.

2 S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini and L. Messori, *Med. Res. Rev.*, 2010, **30**, 550–580.
3 B. Bertrand and A. Casini, *Dalton Trans.*, 2014, 4209–4219.
4 E. M. Nagy, L. Ronconi, C. Nardon and D. Fregona, *Mini-Rev. Med. Chem.*, 2012, **12**, 1216–1229.
5 L. Messori and A. Casini, *Curr. Top. Med. Chem.*, 2011, **11**, 2647–2660.
6 A. Casini, M. A. Cinellu, G. Minghetti, C. Gabbiani, M. Coronnello, E. Mini and L. Messori, *J. Med. Chem.*, 2006, **49**, 5524–5531.
7 M. A. Cinellu, L. Maiore, M. Manassero, A. Casini, M. Arca, H. H. Fiebig, G. Kelter, E. Michelucci, G. Pieraccini, C. Gabbiani and L. Messori, *ACS Med. Chem. Lett.*, 2010, **1**, 336–339.
8 M. Serratrice, M. A. Cinellu, L. Maiore, M. Pilo, A. Zucca, C. Gabbiani, A. Guerri, I. Landini, S. Nobili, E. Mini and L. Messori, *Inorg. Chem.*, 2012, **51**, 3161–3171.
9 S. Simon, D. Roy and M. Schindler, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 1128–1132.
10 I. F. Tannock and D. Rotin, *Cancer Res.*, 1989, **49**, 4373–4384.
11 T. Nishi and M. Forgac, *Nat. Rev. Mol. Cell Biol.*, 2002, **3**, 94–103.
12 A. De Milito and S. Fais, *Expert Opin. Pharmacother.*, 2005, **6**, 1049–1054.
13 J. Horn, *Clin. Ther.*, 2000, **22**, 266–280.
14 E. A. Lew, *Aliment. Pharmacol. Ther.*, 1999, **13**, 11–16.
15 J. M. Shin and N. Kim, *J. Neurogastroenterol Motil.*, 2013, **19**, 25–35.
16 F. Luciani, M. Spada, A. De Milito, A. Molinari, L. Rivoltini, A. Montinaro, M. Marra, L. Lugini, M. Logozzi, F. Lozupone, C. Federici, E. Lessi, G. Parmiani, G. Arancia, F. Belardelli and S. Fais, *J. Natl. Cancer Inst.*, 2004, **96**, 1702–1713.
17 C. Federici, F. Petrucci, S. Caimi, A. Cesolini, M. Logozzi, M. Borghi, S. D'Ilio, L. Lugini, N. Violante, T. Azzarito, C. Majorani, D. Brambilla and S. Fais, *PLoS One*, 2014, **9**, e88193.
18 S. Malik, S. Das, A. Singh and L. Mitu, *E-J. Chem.*, 2012, **9**, 1919–1928.
19 S. Verma, S. Shrivastva and R. Shrivastva, *J. Chem.*, 2013, **2013**, 309179.
20 G. M. Maharvi, A. E. Bharucha and A. H. Fauq, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 2808–2811.
21 S. Gangula, C. R. Elati, A. Neredla, S. R. Baddam, U. K. Neelam, R. Bandichhor and A. Dongamanti, *Org. Process Res. Dev.*, 2010, **14**, 229–233.
22 M. A. Cinellu, L. Maiore, A. Schier, H. Schmidbaur and D. Rossi, *Z. Naturforsch., B: J. Chem. Sci.*, 2008, **63**, 1027–1034.
23 A. de Almeida, B. L. Oliveira, J. D. G. Correia, G. Soveral and A. Casini, *Coord. Chem. Rev.*, 2013, **257**, 2689–2704.
24 A. Bindoli, M. P. Rigobello, G. Scutari, C. Gabbiani, A. Casini and L. Messori, *Coord. Chem. Rev.*, 2009, **253**, 1692–1707.

