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Synthetic and antimicrobial studies on new gold(I) complexes of imidazolidin-2-ylidenes

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Six new 1,3-diorganylimidazolidin-2-ylidene (NHC) gold(I) complexes of the type $[Au(NHC)_2]^+$ (1–6), were synthesized by reacting $[AuCl(PPh)_3]$ with 1,3-dimesitylimidazolidin-2-ylidene or bis(1,3-dialkylimidazolidin-2-ylidene). The complexes 1–6 were fully characterized by elemental analyses and spectroscopic data. The placement of mesityl or para-substituted benzyl groups on the nitrogen atoms of the ring of the complexes leads to the particularly active antibacterial agents evaluated in this work. It is worth noting that the *p*-methoxybenzyl derivative (2) inhibited the growth of *Pseudomona aeruginosa, Staphylococcus epidermidis, Staphylococcus aureus* and *Enterococcus faecalis* with minimum inhibitory concentration (MIC) values of 3.12 μg ml^{-1}, 6.25 μg ml^{-1}, 3.12 μg ml^{-1} and 3.12 μg ml^{-1} respectively. In contrast, the analogous *p*-dimethylaminobenzyl derivative (3) is effective only against *Escherichia coli* (MIC = 3.12 μg ml^{-1}).

KEYWORDS: *N*-heterocarbene-gold(I) complexes; antimicrobial activity; minimum inhibitory concentration

INTRODUCTION

The reported activity of two-coordinate gold(I) complexes with AuSP, AuNP, AuPP and AuSS cores, i.e. $[Au(L)(PPh_3)]$ (HL = mercaptonicotinic acid), $[Au(SATg)(PEt_3)]$ ($SATg^- = 2,3,4,6$-tetra-*o*-acetyl-1-thio-β-D-glycopyranosato-*S*), $[Au(L)(PPh_3)]$ (HL = pyrazole, imidazole), [Au(dppey)2]Cl (dppey = *cis*-$Ph_2P(CH{=}CH)PPh_2$), as potent antibacterial agents[1–7] prompted us to prepare suitable gold derivatives of the *N*-heterocyclic carbenes (NHCs), 1,3-diorganylimidazolidin-2-ylidenes (**1–6**). Furthermore, because a C-derivative is expected to be a softer donor than a similar *N*-derivative, its interaction with gold(I), a very soft acceptor, is expected to be favoured. Consequently, it may be envisaged that the rather strong bond between the metal and the C_2 of NHC[8,9] should prevent a rapid reaction of these complexes with the related biological thio compounds, allowing them to reach the cell targets.[7]

In fact, we recently described azolinium salts and the NHC complexes of rhodium(I) and ruthenium(II), some of which proved to be effective at inhibiting the growth of Gram-positive bacteria and found that, generally, hydrophobic substituents on the ring nitrogen made those compounds more effective.[10–13]

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EXPERIMENTAL

Methods and materials

All syntheses were carried out under an oxygen-free argon or nitrogen atmosphere using predried solvents and standard Schlenk techniques. The complex $[Au(PPh_3)Cl]$[14] and 1,3-diorganylimidazolidin-2-ylidene or their dimers were prepared according to literature methods.[12,15] IR spectra were recorded as KBr pellets in the range 4000–400 cm^{-1} on an ATI UNICAM systems 2000 Fourier transform spectrometer. ^{1}H NMR spectra (300.131 MHz) and ^{13}C NMR spectra (75.5 MHz) were recorded on a Bruker AM 300 WB FT spectrometer with δ referenced to residual solvent $CDCl_3$. Microanalyses were performed by TÜBITAK (Ankara).

General procedure for the preparation of gold–carbene complexes 1–6

A solution of 1,3-dimesitylimidazolidin-2-ylidene (1.6 mmol) or the corresponding bis(1,3-dialkylimidazolidin-2-ylidene)

(0.80 mmol) and [AuCl(PPh_3)] (0.75 mmol) in toluene (20 ml) were heated for 2 h under reflux. The resulting solution was cooled to room temperature and *n*-hexane (30 ml) was added to obtain a creamy solid. Recrystallization from CH_2Cl_2–Et_2O at 20 °C, gave colourless crystals of 1–6. The crystals were filtered off, washed with Et_2O (3 × 15 ml) and dried under vacuum. The reported yields are based on [Au(PPh_3)Cl] and relevant physical data are compiled in Tables 1–3.

Antimicrobial evaluation

Six selected synthesized compounds were screened for their *in vitro* antimicrobial activities against *Escherichia coli* (ATCC 25922), *Staphylococcus epidermidis* (ATCC 12228),

Table 1. Physical measurement of gold(I) carbene complexes

Compound	M.p. (°C)	Yield (%)	Micro Analysis, found (calc.) (%)		
			C	H	N
1	325–326	73	59.51 (59.68)	6.25 (6.16)	6.71 (6.63)
2	244–245	90	53.60 (53.49)	5.32 (5.16)	6.49 (6.57)
3	247–248	91	55.93 (55.72)	6.01 (6.19)	12.09 (12.38)
4	273–274	88	61.19 (61.29)	6.65 (6.71)	6.23 (6.21)
5	164–165	70	48.23 (48.41)	6.61 (6.82)	8.44 (8.69)
6	148–149	85	34.71 (34.68)	5.87 (5.82)	11.61 (11.55)

Table 2. IR and ^{1}H NMR spectroscopic data for compounds 1–6[a]

Compound	IR ν(NCN) (cm^{-1})	^{1}H NMR	
		Ring 4,5-CH_2	Other
1	1495	3.9 (s, 8H)	6.8 [s, 8H, 2,4,6-$(CH_3)_3C_6H_2$]; 1.8 and 2.4 [s, 36 H, 2,4,6-$(CH_3)_3C_6H_2$]
2	1513	3.7 (s, 8H)	6.9 and 7.2 [d, *J* 8.7 Hz, 16H, $CH_2C_6H_4OCH_3$-*p*]; 4.8 [s, 8H, $CH_2C_6H_4OCH_3$-*p*]; 3.8 [s, 12H, $CH_2C_6H_4OCH_3$-*p*]
3	1525	3.5 (s, 8H)	7.1 and 6.6 [d, *J* 8.7 Hz, 16H, $CH_2C_6H_4N(CH_3)_2$-*p*]; 4.6 [s, 8H, $CH_2C_6H_4N(CH_3)_2$-*p*]; 2.9 [s, 24 H, $CH_2C_6H_4N(CH_3)_2$-*p*]
4	1497	3.3 (s, 8H)	6.7 [s, 8H, 2,4,6-$(CH_3)_3C_6H_2CH_2$]; 2.2 and 2.3 [s, 36H, 2,4,6-$(CH_3)_3C_6H_2CH_2$]; 4.8 [s, 8H, 2,4,6-$(CH_3)_3C_6H_2CH_2$]
5	1456	3.7 (s, 8H)	4.50 [qu, *J* 8.2 Hz, 4H, NC*H* (Cp)], 2.6–1.6 [m, 32H, CH_2 (Cp)]
6	1519	3.7 (s, 8H)	3.6 [q, *J* 7.23 Hz, 8H, CH_2CH_3]; 1.2 [t, *J* 7.23 Hz, 12H, CH_2CH_3]

[a] s: singlet, d: doublet, qu: quintet, t: triplet.

Table 3. ^{1}C NMR spectroscopic data for compounds 1–6[a]

Compound	M═C	Ring 4,5-CH_2	Other
1	206.8	51.7	138.8, 135.8, 134.3, 129.7, [2,4,6-$(CH_3)_3C_6H_2$]; 21.5 and 17.7 [2,4,6-$(CH_3)_3C_6H_2$]
2	204.2	49.3	160.0, 129.4, 128.6, 114.8 [$CH_2C_6H_4OCH_3$-*p*]; 55.8 [$CH_2C_6H_4OCH_3$-*p*]; 54.0 [$CH_2C_6H_4OCH_3$-*p*]
3	203.7	49.0	150.9, 129.3, 122.4, 112.9 [$CH_2C_6H_4N(CH_3)_2$], 54.3 [$CH_2C_6H_4N(CH_3)_2$], 40.9 [$CH_2C_6H_4N(CH_3)_2$]
4	204.9	47.9	138.8, 137.9, 130.1, 127.5 [2,4,6-$(CH_3)_3C_6H_2CH_2$]; 21.3 and 20.9 [2,4,6-$(CH_3)_3C_6H_2CH_2$]; 48.9 [2,4,6-$(CH_3)_3C_6H_2CH_2$]
5	203.9	44.9	62.0, 61.9, 30.2, 24.2 [Cp carbon atoms]
6	204.2	48.9	45.5 [CH_2CH_3]; 14.4 [CH_2CH_3]

[a] δ ppm relative to Me_4Si in $CDCl_3$.

Staphylococcus aureus (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Enterobacter cloacae* (ATCC 13047), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10239) according to the serial dilution method.[10,16] Ampicillin and flucytosine were used as standard drugs for comparison. The results of the screening studies are given in Table 4.

Twofold serial dilutions of the compounds and reference drugs tested were prepared in Tryptic soy agar (Merck) for bacteria and Sabouraud dextrose agar (Difco) for yeast. The compounds (16.0 mg) were dissolved in dimethylsulfoxide (DMSO, 1 ml) and the solution was diluted with distilled water (9 ml). Further progressive double dilutions with melted media were performed to obtain the required concentrations of 1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.12 μg ml^{-1}. Petri dishes were inoculated with (1–5) × 10^6 cfu ml^{-1} and agar plates containing bacteria were incubated at 37 °C for 24 h and those containing yeast at 30 °C for 48 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the compound tested that yielded no visible growth on the plate. DMSO had no effect on the microorganisms in the concentrations studied.

RESULTS AND DISCUSSION

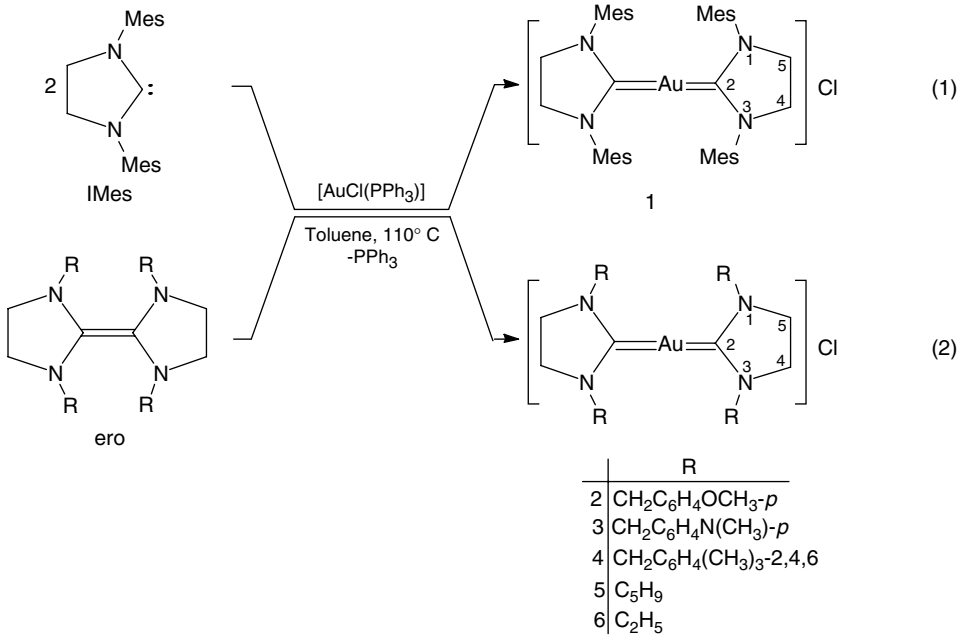
Chemistry

The complexes 1–6 studied in this work can be prepared by one of the following synthetic methods (Scheme 1):

Table 4. MIC of the compounds tested

	MIC (μg ml^{-1})						
Compound	*E. coli* (ATCC 25922)	*S. epidermidis* (ATCC 12228)	*S. aureus* (ATCC 29213)	*E. faecalis* (ATCC 29212)	*E. cloacae* (ATCC 13047)	*P. aeruginosa* (ATCC 27853)	*C. albicans* (ATCC 10239)
Ampicillin	<3.12	<3.12	<3.12	6.25	<3.12	25	—
Flucytosine	—	—	—	—	—	—	6.25
Mmi[a]	400	6.25	3.12	3.12	1600	3.12	200
1	800	800	3.12	800	3.12	1600	800
2	1600	6.25	3.12	3.12	1600	3.12	200
3	3.12	>1600	>1600	>1600	>1600	>1600	>1600
4	200	>1600	200	>1600	100	>1600	>1600
5	1600	>1600	50	800	12.5	>1600	1600
6	400	>1600	50	>1600	800	>1600	>1600

[a] Ref. 10 mmi = [1,3-(dimesitylmethylimidazolinium)] chloride.



Scheme 1. Synthesis of cationic gold(I)–NHC complexes used for antimicrobial studies..

(1) interaction of [AuCl(PPh_3)] with the free carbene IMes;[14] (2) C═C bond cleavage reaction of the appropriate electron-rich olefin (ero)[8] by [AuCl(PPh_3)] in boiling toluene. The method of choice depends on the availability of the carbene precursor. Thus, the free carbenes with an unhindered substituent on nitrogen atoms spontaneously dimerize to give eros.[9] However, the latter behave as nucleophilic carbenes to displace neutral and anionic ligands.[8]

All gold complexes described in this paper are white or cream solids and are stable in air and light. The analytical (Table l) and spectral data (Tables 2 and 3) are in good agreement with the proposed molecular formula.

The IR spectra of compounds 1–6 showed the absorption band due to NCN in the expected region. The coupling of the gold atom with the imidazolidin-2-ylidenes was further supported by the NMR spectra. The ^{1}H NMR spectra of the complexes showed peaks characteristic of the protons of the imidazolidine ring and the substituents on the nitrogen atoms. The existence of the metal–carbene bond is unequivocally confirmed by the ^{13}C NMR. Thus, C_2 carbon atoms gave singlet resonances that appear at characteristically low field (204–207 ppm) as in the corresponding gold(I) derivatives,[17] without noticeable influence of the nitrogen-substituents.

Antimicrobial activity

The results of MIC evaluation of 1–6 together with the [1,3-(dimesitylmethylimidazolinium)] chloride (mmi) are compiled in Table 4. Ampicillin and flucytosine were used as standard drugs for comparison.

Similar to mmi,[10] the new gold complexes, particularly 1–3, showed remarkable selective and effective activities against Gram-positive and Gram-negative bacteria. However, among the gold complexes tested, only **2** showed modest activity against the fungi and the yeast *C. albicans*. In contrast to mmi, the derived gold complex **4** did not effectively inhibit the growth of the bacteria tested in this study. Such a reduction in activity cannot simply be related to a reduction in the lipophilicity, since the three methyl residues on **4** strongly increased its lipophilicity compared with **2**.

In view of the results, it appears that substituted benzyls on nitrogen atoms and attachment of a gold atom to C_2 greatly influenced the antimicrobial activity. The variations of this factor can lead to properties ranging from almost inactive products, e.g. **4**, to products with strong biological activities, such as **2** and **3**. In this case, **2** the most active (with broad spectrum) product showing that the *p*-OMe substituent is most suitable for enhancing the antimicrobial properties required. It is also worth noting that **2** is the only gold(I) compound toxic to *P. aeruginosa* (MIC = 3.12 μg ml^{-1}), reported so far. Furthermore, **3** is effective only against Gram-negative *E. coli* (MIC = 3.12 μg ml^{-1}).

The mechanism of the actibacterial action is unclear at this time; owing to the strong metal–ligand bond,[18,19] no relevant dissociation equilibria under physiological conditions are to be expected for 1–6. This observed property is in contrast to the presently accepted view, which states that 'the magnitude of intensity of the antibacterial activities is strongly related to the rate of ligand exchange reactions of the gold(I) complexes'.[6] It is evident that, for the present case, a different mechanism is operational.

These studies, however, provide useful information about the biological activity of gold-containing compounds, with the possibility that this activity could become even more pronounced when more potent ligands are coupled with gold atoms. Furthermore, it would be interesting to extend the study of biological activities to antiarthritic and antitumour activities and to other $[Au(NHC)_2]^+$ complexes.

CONCLUSIONS

The affinity of gold(I) for the NHCs present in 1–6 is shown by the displacement reactions in Scheme 1, and *in vitro* antimicrobial activities of the new complexes are reported. Although the mechanism of antibacterial action is not known at present, it is clear from this work that the nature of the nitrogen substituent of the NHC ligand has a profound effect on the antimicrobial activity and selectivity of the resulting gold complexes. The best spectra of antimicrobial activity were observed with compounds **1**, **2** and **3**. The different para-substituted benzyls on nitrogen atoms characterizing these compounds are clearly responsible for their different activities against Gram-positive and Gram-negative bacteria.

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