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Synthesis, spectroscopic characterization, electrochemical behavior and computational analysis of mixed diamine ligand gold(III) complexes: antiproliferative and in vitro cytotoxic evaluations against human cancer cell lines

Said S. Al-Jaroudi · M. Monim-ul-Mehboob · Muhammad Altaf · Abdulaziz A. Al-Saadi · Mohammed I. M. Wazeer · Saleh Altuwaijri · Anvarhusein A. Isab

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Abstract The gold(III) complexes of the type $[(DACH)Au(en)]Cl_3$, 1,2-Diaminocyclohexane ethylenediamine gold(III) chloride [where 1,2-DACH = *cis*-, *trans*-1,2- and *S,S*-1,2diaminocyclohexane and en = ethylenediamine] have been synthesized and characterized using various analytical and spectroscopic techniques including elemental analysis, UV–Vis and FTIR spectra; and solution as well as solid-state NMR measurements. The solid-state ^{13}C NMR shows that 1,2-diaminocyclohexane (1,2-DACH) and ethylenediamine (en) are strongly bound to the gold(III) center via N donor atoms. The stability of the mixed diamine ligand gold(III) was determined by 1H and ^{13}C NMR spectra. Their electrochemical behavior was studied by cyclic voltammetry. The structural details and relative stabilities of the four possible isomers of the complexes were also reported at the B3LYP/LANL2DZ level of theory. The coordination sphere of these complexes around gold(III) center adopts distorted square planar geometry. The

computational study also demonstrates that *trans*-conformations is slightly more stable than the *cis*-conformations. The antiproliferative effects and cytotoxic properties of the mixed diamine ligand gold(III) complexes were evaluated in vitro on human gastric SGC7901 and prostate PC3 cancer cells using MTT assay. The antiproliferative study of the gold(III) complexes on PC3 and SGC7901 cells indicate that complex **1** is the most effective antiproliferative agent among mixed ligand based gold(III) complexes **1–3**. The IC_{50} data reveal that the in vitro cytotoxicity of complexes **1** and **3** against SGC7901 cancer cells are fairly better than that of cisplatin.

Keywords Gold(III) complex · 1,2-Diaminocyclohexane (1,2-DACH) · Ethylenediamine (en) · Antiproliferative effect · In vitro cytotoxicity · Gastric cancer (SGC7901) · Prostate cancer (PC3)

Introduction

The development of new metallodrugs with a pharmacological activity different from platinum drugs is one of the major goals of modern bioinorganic and bio-organometallic medicinal chemistry research (Janković et al. 2012; Arsenijević et al. 2013; Kouroulis et al. 2009; Altaf et al. 2014; Hartinger and Dyson 2009). Among these non-platinum anticancer drugs, gold complexes have recently gained significant attention as a class of compounds with different

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pharmacodynamic and kinetic properties than cisplatin with strong cell growth inhibiting effects (Kouroulis et al. 2009; Altaf et al. 2014). The cell growth inhibiting effects, in many cases, could be related to anti-mitochondrial effects that make the gold complexes interesting (Janković et al. 2012; Arsenijević et al. 2013; Kouroulis et al. 2009).

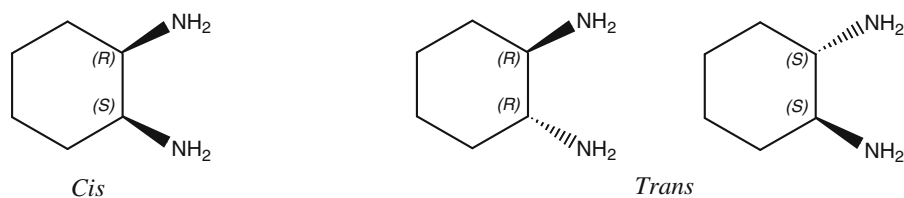
Oxaliplatin, the so-called third generation of platinum (II) complex was synthesized as the most promising drug molecule in order to overcome the cross-resistance experienced by cisplatin (Graham et al. 2004). It bears a 1,2-diaminocyclohexane (1,2-DACH) ligand and oxalate as a leaving group. The bulky chiral ligand, *1R,2R*-diaminocyclohexane (*1R,2R*-DACH), contributes to high cytotoxicity against cisplatin-resistant cell lines. It is possibly due to the steric hindrance effect of the 1,2-DACH-platinum–DNA adducts (Misset et al. 2000; Zdravski et al. 2002). In the same line, several substituted 1,2-DACH complexes have been evaluated for their cytotoxicity (Chaney 1995; Hoesehele et al. 1994). Furthermore, a great number of Pt(II) complexes containing *1R,2R*-DACH moiety have been synthesized and tested for anticancer activities against a panel of human cancer lines. A few of them have entered preclinical and clinical trials (Yu et al. 2006, 2008). Thus, it can be deduced that *1R,2R*-DACH is an effective carrier group in designing 1,2-DACH-type platinum(II) derivatives. Moreover, in search for better platinum(II) compounds, a wide variety of carrying ligands and leaving groups have been screened. Vicinal diamines, and particularly 1,2-DACH, appear to be useful carrying ligands (Monti et al. 2005; Berger et al. 2007).

Gold(III) complexes, which are isoelectronic and isostructural to platinum(II) complexes, hold promise as possible anticancer agents (Chaves et al. 2014; Cutillas et al. 2013). Surprisingly, only a few reports exist in the literature unfolding the cytotoxic properties and the in vivo anticancer effects of gold(III) complexes (van Rijt and Sadler 2009; Ronconi et al. 2006). Gold(III) complexes having the same square-planar geometries as cisplatin (Zou et al. 2013; Cattaruzza et al. 2011), gold(III) complexes currently became the subject of profound anti-cancer research and hold great potential to enter clinical trials since some of them are highly cytotoxic to solid cancer tumors in vitro and in vivo while causing minimal systemic toxicity (Ronconi et al. 2010; Sun and Che 2009). In general, gold(III) complexes are not very stable under physiological

conditions due to their high reduction potential and fast hydrolysis rate. Therefore, the selection of a suitable ligand to enhance the stability became a challenge in the design of gold(III) complexes. The Au(III) is most likely coordinated by at least two chelating nitrogen donors which lower the reduction potential of gold(III) center and by this means stabilize the complex (Giovagnini et al. 2005; Casini et al. 2008) and facilitated extensive pharmacological investigation, both in vitro and in vivo (Tiekink 2008; Casini et al. 2009).

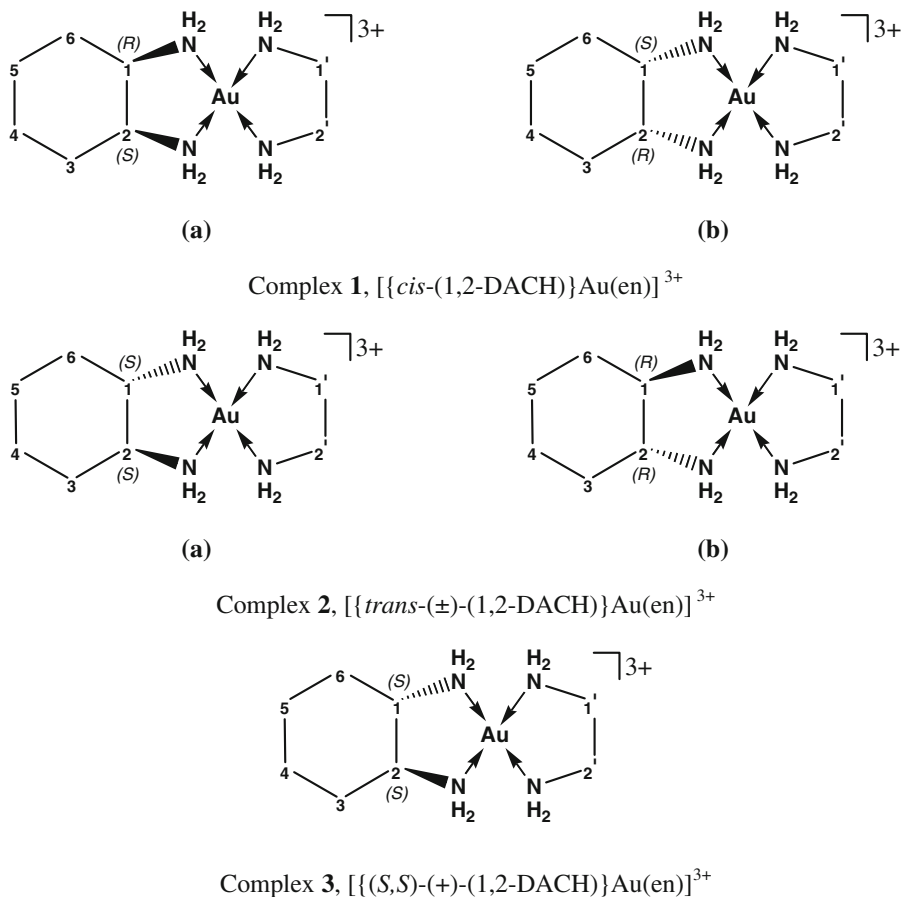
1,2-DACH ligand has structurally two asymmetric carbon centers, thus, 1,2-DACH can exist as three isomeric forms which includes two enantiomers (*1R,2R*-DACH) or (*trans*-1,2-DACH), (*1S,2S*-DACH) or (*trans*-1,2-DACH) and one diastereoisomer (*1R,2S*-DACH) or (*cis*-1,2-DACH). Since 1,2-DACH is chiral, the significance of stereochemical issues has been addressed by a number of investigators which affect the cytotoxicity of complexes containing 1,2-DACH (Kidani et al. 1977; Kemp et al. 2007). In spite of conflicting views (Gulloti et al. 1984; Noji et al. 1981; Pasini et al. 1982), the consensus is that the (*R,R*) isomer is generally more active than the (*S,S*) isomer (Burchenal et al. 1977; Bruck et al. 1984), although activity has also been demonstrated with the (*R,S*) isomer (Vollano et al. 1987). With regard to the stereochemistry of the complexes, Pt(II)(*1R,2R*-DACH) and Pt(II)(*1S,2S*-DACH) complexes have a higher anticancer activity than Pt(*1R,2S*-DACH) complex (Johnson et al. 1989; Al-Sarraf et al. 1987). However, the analogous gold(III) compound, [Au(en)₂]Cl₃ has been reported to have higher anticancer activity than gold(III) (*1R,2R*-DACH) (*trans*-1,2-DACH) and gold(III) (*1S,2S*-DACH) (*trans*-DACH) (Isab et al. 2011; Monim-ul-Mehboob et al. 2013; Al-Maythality et al. 2009; Al-Jaroudi et al. 2013, 2014).

As in the case of the parent cisplatin, the anticancer activity of platinum(II)-1,2-DACH is accompanied by the toxicity. The emergence of resistance, and low water solubility that can affect the pharmacokinetics, are additional features that must be improved in the pursuit for a more effective analog (Hanessian and Wang 1993). As a continuation of our interest in the synthesis of gold(III) complexes and to better understand the chemical and physical behavior of biologically relevant (1,2-DACH) gold(III) (en) complexes, the chiral isomers [*cis*-(1,2-DACH)Au(en)]Cl₃ (**1**), [*trans*-(±)-(1,2-DACH)Au(en)]Cl₃ (**2**) and [(*S,S*)-(+)-(1,2-DACH)Au(en)]Cl₃ (**3**), have been synthesized



Scheme 1 Structures of *cis*- and *trans*- isomers of 1,2-diaminocyclohexane (1,2-DACH)

Scheme 2 Possible structures of complexes **1**, **2** and **3**



and fully characterized by Elemental Analysis, NMR measurements, FTIR and UV–Vis spectroscopic techniques. Scheme 1 illustrates the structures of the ligands used in this study and Scheme 2 shows the structures of the complexes reported. Their cytotoxicity has been tested in vitro against human gastric SGC7901 and prostate PC3 cancer cell lines. To the best of our knowledge, this is the first work for synthesizing stable and highly water soluble gold(III) complexes based on mixed diamine ligands containing cyclohexane-1,2-diamine (1,2-DACH) and ethylenediamine (en).

Experimental

Chemicals, cell lines and cell cultures

Sodium tetrachloroaurate(III) dihydrate NaAuCl₄·2H₂O and ethylenediamine (en) were purchased from Sigma-Aldrich. *cis*-1,2-diaminocyclohexane *cis*-1,2-DACH, *trans*-(±)-diaminocyclohexane *trans*-(±)-DACH and (*S,S*)-(+)-diaminocyclohexane (*S,S*)-(+)-1,2-DACH were purchased from Aldrich. Absolute C₂H₅OH, CH₃OH, D₂O and DMSO-*d*₆ were obtained from Fluka Chemicals Co. All other reagents as well

as solvents were obtained from Aldrich Chemical Co., and used as received.

Human gastric SGC7901 cancer and prostate PC3 cancer cell lines were provided by American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal calf serum (FCS), penicillin (100 kU L^{-1}) and streptomycin (0.1 g L^{-1}) at 37°C in a 5 % CO_2 –95 % air atmosphere. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) was purchased from Sigma Chemical Co, St. Louis, MO, USA.

Synthesis of Au(III) complexes

Mixed ligand gold(III) chloride compounds namely *cis*-1,2-diaminocyclohexane ethylenediamine gold(III) chloride, $[(\text{en})\text{Au}\{\text{cis}\text{-}(1,2\text{-DACH})\}]\text{Cl}_3$ **1**; *trans*-(\pm)-1,2-diaminocyclohexane ethylenediamine gold(III) chloride, $[(\text{en})\text{Au}\{\text{trans}\text{-}(\pm)\text{-}(1,2\text{-DACH})\}]\text{Cl}_3$ **2**; and (*S,S*)-(+)-1,2-diaminocyclohexane ethylenediamine gold(III) chloride $[(\text{en})\text{Au}\{(S,S)\text{-}(+)\text{-}(1,2\text{-DACH})\}]\text{Cl}_3$ **3**; were synthesized by using one mole equivalent of Sodium aurate dihydrate $\text{NaAuCl}_4 \cdot 2\text{H}_2\text{O}$ with one mole of ethylenediamine (en) and one mole equivalent of *cis*-(1,2-DACH) or (*trans*-(\pm)-(1,2-DACH) or (*S,S*)-(+)-(1,2-DACH) respectively according to modification of the synthesis in the literature (Al-Maythaly et al. 2009; Al-Jaroudi et al. 2013; Zhu et al. 2006).

Sodium tetrachloroaurate dihydrate $\text{NaAuCl}_4 \cdot 2\text{H}_2\text{O}$, 398 mg (1.0 mmol) was dissolved in minimum volume i.e. 10 mL of absolute ethanol at ambient temperature. In a separate beaker, 1,2-diaminocyclohexane (1,2-DACH), 114 mg (1.0 mmol) was dissolved in minimum volume i.e. 10 mL of absolute ethanol at ambient temperature. Both solutions were mixed dropwise and stirred for a half hour. Finally, a clear solution was obtained and filtered. In a separate beaker, ethylenediamine (en), 120 mg (1.0 mmol) is dissolved in minimum volume i.e. 10 mL of absolute ethanol at ambient temperature. The addition of en solution is added drop wise to the above filtered solution. Upon stirring for overnight, the white precipitate of $[(\text{en})\text{Au}(1,2\text{-DACH})]\text{Cl}_3$ was obtained. The product was isolated, dissolved in 2 mL of water and filtered through Celite pad to remove NaCl. Addition of 100 mL of cold CH_3OH to the filtrate and a white precipitate was obtained filtered and washed cold CH_3OH . The solid product was dried under reduced pressure with P_2O_5 .

Table 1 Melting point (MP) and CHN analysis of gold(III) complexes **1**, **2** and **3**

Complex	MP ($^\circ\text{C}$)	Found (calculated) %		
		H	C	N
(1)	161–163	6.57 (6.64)	22.26 (22.59)	13.05 (13.17)
(2)	175–178	6.59 (6.64)	22.32 (22.59)	13.01 (13.17)
(3)	176–178	6.60 (6.64)	22.48 (22.59)	13.03 (13.17)

Table 2 λ_{max} values derived from UV–Vis spectra for Au(III) complexes **1**, **2** and **3**

Complex	λ_{max} (nm)
$\text{NaAuCl}_4 \cdot 2\text{H}_2\text{O}$	293
(1)	335
(2)	338
(3)	339

The yield of the compounds **1**, **2** and **3** was in the range of 75–80 %. Melting points and elemental analysis for complexes are presented in Table 1. The complexes prepared in the present study were characterized by FTIR and NMR measurements. The density functional calculations (DFC) studies based hybrid B3LYP is also performed to optimize the structures of gold(III) complexes. All the data support the formation of the desired $[(1,2\text{-DACH})\text{Au}(\text{en})]\text{Cl}_3$ complexes.

Electronic spectra

Electronic spectra were obtained for the gold(III) complexes using Lambda 200, Perkin-Elmer UV–Vis spectrometer. UV–Vis spectroscopy was used to determine the stability of the complexes in a physiological buffer (40 mM phosphate, 4 mM NaCl, pH 7.4). Electronic spectra were recorded on freshly prepared of each complex in buffer solution at room temperature. Then, their electronic spectra were monitored over 7 days at 37°C . The resulting UV–Vis absorption data are shown in Table 2.

Mid and far-IR studies

The solid-state FTIR spectra of the free ligands (1,2-DACH and en) and their corresponding mixed ligand

Table 3 Mid-FTIR frequencies, $\nu(\text{cm}^{-1})$ for the mixed ligand Au(III) complexes **1**, **2** and **3**

Complex	$\nu(\text{N-H})$	ν_{shift}	$\nu(\text{C-N})$	ν_{shift}	Refs.
en	3,393 w		1,033 m		Wadt and Hay (1985b)
[(en)AuCl ₂]Cl	3,422 br	29	1,045 m	12	Wadt and Hay (1985b)
<i>cis</i> -(1,2-(DACH))	3,356 m, 3,286 m		1,092 s		Hartinger and Dyson (2009)
[{ <i>cis</i> -(1,2-DACH)}AuCl ₂]Cl	3,414 w	93	1,183 s	91	Hartinger and Dyson (2009)
(1)	3,395 br	74 ^a , 2 ^b	1,182 w	90 ^a , 149 ^b	This work
<i>trans</i> -(\pm)-(1,2-DACH)	3,348 m, 3,271 m, 3,183 m		1,082 m		Hartinger and Dyson (2009)
[{ <i>trans</i> -(\pm)-(1,2-DACH)}AuCl ₂]Cl		137, 149, 201	1,175 m	93	Hartinger and Dyson (2009)
(2)	3,432 br	168 ^a , 39 ^b	1,180 m	93 ^a , 147 ^b	This work
(<i>S,S</i>)-(+)-(1,2-DACH)	3,340 m, 3,252 m, 3,167 m		1,082 m		Hartinger and Dyson (2009)
[{ <i>S,S</i> -(+)-(1,2-DACH)}AuCl ₂]Cl			1,171 m	89	Hartinger and Dyson (2009)
(3)	3,386 br	132 ^a , -7 ^b	1,180 m	98 ^a , 147 ^b	This work

^a With respect with (DACH)

^b With respect to (en)

Table 4 Far-FTIR frequencies, $\nu(\text{cm}^{-1})$ for complexes **1**, **2**, and **3**

Complex	Au-Cl	Au-N	Refs.
NaAuCl ₄ ·2H ₂ O	365	–	This work
[(en)AuCl ₂]Cl	–	391, 474	Wadt and Hay (1985c)
[<i>cis</i> -(1,2-DACH)AuCl ₂]Cl	352, 367	437	Hartinger and Dyson (2009)
(1)	–	326, 417	This work
[(<i>trans</i> -(\pm)-(1,2-DACH)AuCl ₂]Cl	353, 365	437	Hartinger and Dyson (2009)
(2)	–	391, 442	This work
[(<i>S,S</i>)-(+)-(1,2-DACH)AuCl ₂]Cl	353, 366	395, 436	Hartinger and Dyson (2009)
(3)	–	376, 440	This work

gold(III) complexes were recorded on a Perkin-Elmer FTIR 180 spectrophotometer using KBr pellets over the range 4,000–400 cm^{-1} . The selected mid-FTIR frequencies of free ligands and corresponding mixed ligands gold(III) complexes are given in Table 3. Far-infrared spectra were recorded for complexes **(1)**, **(2)** and **(3)** at 4 cm^{-1} resolution at room temperature as cesium chloride (CsCl) disks on a Nicolet 6700 FTIR with far-FTIR beam splitter. The selected far-FTIR data for free ligands and their corresponding mixed ligand gold(III) complexes are given in Table 4.

Solution NMR measurements

All NMR measurements were carried out on a Jeol JNM-LA 500 NMR spectrophotometer at 298 K. The ¹H NMR spectra were recorded at a frequency of 500.00 MHz. The ¹³C NMR spectra were obtained at a frequency of 125.65 MHz with ¹H broadband decoupling. The spectral conditions were: 32 k data points, 0.967 s acquisition time, 1.00 s pulse delay and 45 pulse angle. The chemical shifts are referenced to 1,4-dioxane as an internal standard in ¹³C NMR measurements. The

Table 5 ^1H NMR chemical shifts of free ligands and complexes **1**, **2** and **3** in D_2O

Compound	^1H (δ in ppm)						Refs.
	H1, H2	H3, H6 (eq)	H3, H6 (ax)	H4, H5 (eq)	H4, H5 (ax)	H1', H2'	
en	–	–	–	–	–	3.2, s	This work
<i>cis</i> -(1,2-DACH)	2.23, m	1.85, m	1.69, m	1.28, m	1.12, m	–	Hartertinger and Dyson (2009)
(1)	3.61, m	1.96, m	1.77, m	1.59, m	1.41, m	3.16, s	This work
<i>trans</i> -(\pm)-(1,2-DACH)	2.25, m	1.85, m	1.68, m	1.28, m	1.11, m	–	Hartertinger and Dyson (2009)
(2)	3.05, m	2.11, m	1.54, m	1.48, m	1.10, m	3.14, s	This work
(<i>S,S</i>)-(+)-(1,2-DACH)	2.24, m	1.85, m	1.69, m	1.28, m	1.11, m	–	Hartertinger and Dyson (2009)
(3)	3.08, m	2.19, m	1.63, m	1.54, m	1.19, m	3.17, s	This work

Table 6 Solution state ^{13}C NMR chemical shifts of free ligands and complexes **1**, **2** and **3** in D_2O

Compound	^{13}C (δ in ppm)			
	C1, C2	C3, C6	C4, C5	C1', C2'
en	–	–	–	37.67
<i>cis</i> -(1,2-DACH)	58.2	35.26	26.36	–
(1)	61.74	26.13	20.64	50.39
<i>trans</i> -(\pm)-(1,2-DACH)	58.46	35.55	26.63	–
(2)	64.59	32.95	24.12	50.63
(<i>S,S</i>)-(+)-(1,2-DACH)	58.27	35.32	26.43	–
(3)	64.44	32.84	24.02	50.48

^1H and ^{13}C NMR chemical shifts are given in Tables 5 and 6, respectively, according to Scheme 2.

Solid state NMR studies

Solid-state ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer at ambient temperature of 298 K. Samples were packed into 4 mm zirconium oxide (ZrO) rotors. Cross polarization (CP) and high power (HP) decoupling were employed. Pulse delay of 7.0 s and a contact time of 5.0 ms were used in the CPMAS experiments. The magic angle spinning (MAS) rates were maintained at 4 and 8 kHz. ^{13}C chemical shifts were referenced to tetramethylsilane (TMS) by setting the high frequency isotropic peak of solid adamantane to 38.56 ppm. The solid-state NMR data are given in Table 7.

Stability determination of the gold(III) complexes

The stability of complexes **(1)**, **(2)** and **(3)** were tested in water as well as in a mixture of solvents i.e. DMSO/water

(2/1 in v/v ratio) by ^1H and ^{13}C NMR measurements. To investigate the structural stability of the complexes, NMR spectra of the complexes dissolved in D_2O ; and in mixed $\text{DMSO-}d_6/\text{D}_2\text{O}$ (2/1 in v/v ratio) solution were obtained just after dissolution, 24 h and 1 week at room temperature in mixed $\text{DMSO-}d_6/\text{D}_2\text{O}$ and at 37 °C in D_2O . At least 20 mg of complexes **(1)**, **(2)** and **(3)** in 1 mL D_2O at 37 °C; and in 1 mL $\text{DMSO-}d_6/\text{D}_2\text{O}$ (2/1: v/v) at room temperature were subjected to ^1H and ^{13}C NMR measurements and followed by their spectral analysis. Immediately after dissolution of complexes **(1–3)** in the respective solvents and duplicate samples were then stored at room temperature and 37 °C, respectively, and analyzed again after 24 h and 1 week in order to determine stability of complexes. Since the complexes were not used beyond a week after dissolution, so NMR measurements were limited to one week.

Electrochemistry

The electrochemical experiments were performed at room temperature using a potentiostat (SP-300, Bio-Logic Science Instruments) controlled by EC-Lab v10.34 software package. The electrochemical experiments were performed at room temperature. All the measurements were performed on solutions de-aerated by bubbling ultra-pure nitrogen for 15 min. The values of reduction potential here reported were measured against a saturated calomel electrode (SCE). The cyclic voltammetry of the compounds **1**, **2** and **3** were measured at scan rate of 50 mV/s on a reference buffer (40 mM phosphate, 4 mM NaCl, pH 7.4) using platinum as working electrode and graphite as a counter electrode with a concentration of 1.0 mM at room temperature. Ferrocene was used as pseudo reference to

Table 7 Solid-state ^{13}C NMR chemical shifts of free ligands and corresponding complexes **1**, **2** and **3**

Complex	^{13}C (δ in ppm)				
	C1, C2	C3, C6,	C4, C5	C1', C2'	Refs.
[<i>cis</i> -(1,2-DACH)}AuCl ₂]Cl (1)	66.20, 65.35 64.32	30.98 28.85	27.02, 22.12 28.85, 22.94	– 54.3	Hartinger and Dyson (2009) This work
[<i>trans</i> -(\pm)-(1,2-DACH)AuCl ₂]Cl (2)	69.6 69.60, 65.45	37.37 36.63	27.99 27.53	– 54.08	Hartinger and Dyson (2009) This work
[(<i>S,S</i>)-(+)-(1,2-DACH)AuCl ₂]Cl (3)	70.21 67.1	37.86 36.19	29.16 27.65	– 54.18	Hartinger and Dyson (2009) This work

calibrate the working electrode. The couple $\text{Fe}^{\text{III/II}}$ formal potential of ferrocene occur at $E^{\circ} = +0.44$ V (vs SCE) in 0.1 M Bu_4NPF_6 solution in CH_3CN solvent which is similar to the report value under the same experimental condition (Hans et al. 1999). Conversion to values vs ENH was obtained upon adding +0.24 V to the corresponding SCE values.

Computational studies

The structures of the $[(1,2\text{-DACH})\text{Au}(\text{en})]^{3+}$ complexes in their four possible conformations (*cis*-S,R; *cis*-R,S; *trans*-S,S and *trans*-R,R) were optimized without any geometrical constrains using GAUSSIAN09 program (Frisch et al. 2009). The hybrid B3LYP density functional (the three-parameter Becke functional with correlation from the Lee–Yang–Parr functional) (Becke 1988; Lee et al. 1988) with the Los Alamos National Laboratory-2 double- ζ (LANL2DZ) basis set (Wadt and Hay 1985a, b, c) was employed in this study. We report results for some gold(III)-based complexes at this level of calculations (Al-Maythaly et al. 2009), giving decent results that are consistent with our experimental finding. Moreover, the stationary points have been confirmed by frequency calculation. Selected bond lengths and bond angles are given in Table 8 for the four molecular conformations, while Table 9 compares the relative stabilities based on the calculated energies of the optimized minimum structures.

MTT assay for antiproliferative effects of $[(1,2\text{-DACH})\text{Au}(\text{en})]\text{Cl}_3$ complexes (**1–3**) on PC3 and SCG7901 cancer cells

An MTT assay was used to obtain the number of living cells in the sample. Human gastric cancer SGC7901

Table 8 Selected bond lengths and bond angles of the complex $[(1,2\text{-DACH})\text{Au}(\text{en})]^{3+}$ in its four possible conformations

	1(a)	1(b)	2(a)	2(b)
Bond lengths (Å)				
Au–N ₁	2.147	2.14	2.145	2.146
Au–N ₂	2.147	2.132	2.145	2.146
Au–N ₃	2.131	2.118	2.134	2.135
Au–N ₄	2.132	2.127	2.134	2.135
N ₁ –C	1.538	1.54	1.539	1.539
N ₂ –C	1.538	1.541	1.539	1.539
N ₃ –C	1.544	1.559	1.545	1.544
N ₄ –C	1.545	1.552	1.545	1.544
Bond angles (°)				
N ₁ –Au–N ₃	177.7	179.5	179.4	179.5
N ₂ –Au–N ₄	177.6	179.4	179.4	179.5
N ₁ –Au–N ₂	81.8	82.1	82	81.9
N ₂ –Au–N ₃	100.5	98.3	98.6	98.6
N ₃ –Au–N ₄	77.2	81.7	80.9	80.9
N ₄ –Au–N ₁	100.6	97.9	98.5	98.6
Au–N ₁ –C	109.3	109.1	109	109
Au–N ₂ –C	109.1	109.1	109	109
Au–N ₃ –C	111.4	109.5	110.7	110.8
Au–N ₄ –C	111.8	109.4	110.7	110.8

and prostate cancer PC3 cells were seeded on 96-well plates at a predetermined optimal cell density, i.e. ca 6,000 cells/100 μL per well in 96-well plates, to ensure exponential growth in the duration of the assay. After 24 h pre-incubation, the growth medium was replaced with the experimental medium containing the appropriate drug, using one of gold(III) complexes **1**, **2** and **3** or a control using water. Six duplicate wells were set up for each sample, and cells untreated with drug served as a control. In one set of culture plates,

Table 9 Relative energies of the four possible conformations of the complexes **1** and **2**

Conformation	Relative energy (kcal/mol)
1(a)	3.61
1(b)	3.1
2(a)	0
2(b)	0.11

human gastric cancer SGC7901 and human prostate PC3 cells were treated with 10 μ M complexes **1**, **2** and **3** as the drug and the control (water) for 24, 48 and 72 h. In other sets, the compounds **1**, **2** and **3** with different concentration, i.e. 10, 20 and 30 μ M, were employed to determine the growth inhibitory effect for both PC3 and SGC7901 cells separately. After incubation, 10 μ L MTT (6 g/L, Sigma) was added to each well and the incubation was continued for 4 h at 37 $^{\circ}$ C. After removal of the medium, MTT stabilization solution [dimethylsulfoxide (DMSO): ethanol ($\text{C}_2\text{H}_5\text{OH}$) = 1:1 in v/v ratio] was added to each well, and shaken for 10 min until all crystals were dissolved. Then, the optical density was detected in a micro plate reader at 550 nm wavelength using an Enzyme-Linked Immuno-Sorbent Assay (ELISA) reader. After being treated with gold(III) complexes **1**, **2** and **3**, the cell viability was examined by MTT assay. Each assay was performed in triplicate. An MTT assay for the inhibitory effect has been used for complexes **1**, **2** and **3** against PC3 and SGC7901 cells. These cells were treated with various concentrations of complexes **1**, **2** and **3** for 24–72 h. All results are shown in Figs. 1, 2, 3, 4, 5, 6 and 7.

In vitro cytotoxic assay for PC3 and SGC7901 cancer cells

Human prostate PC3 and gastric SGC7901 cells were used in this study. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal calf serum (FCS), penicillin (100 kU L^{-1}) and streptomycin (0.1 g L^{-1}) at 37 $^{\circ}$ C in a 5 % CO_2 –95 % air atmosphere. Human gastric SGC7901 cells and prostate PC3 were incubated with these compounds at fixed concentrations or with water as a control to assess the inhibitory effect on cell growth. The standard MTT assay has been used to assess the inhibitory effect on cell growth. The cell survival versus drug concentration is plotted. Cytotoxicity was

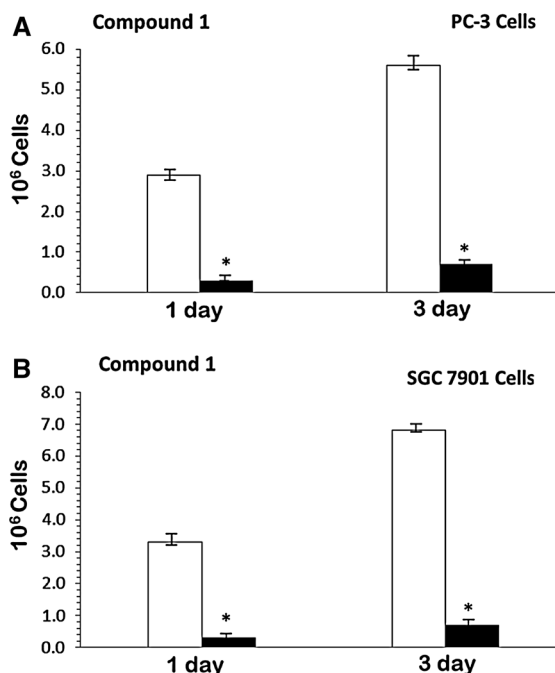


Fig. 1 Time dependent antiproliferative effect of 10 μ M complex **1** on **a** PC3 and **b** SGC7901 cells for 24 and 72 h using MTT. Results were expressed as the mean, SD. * P < 0.05

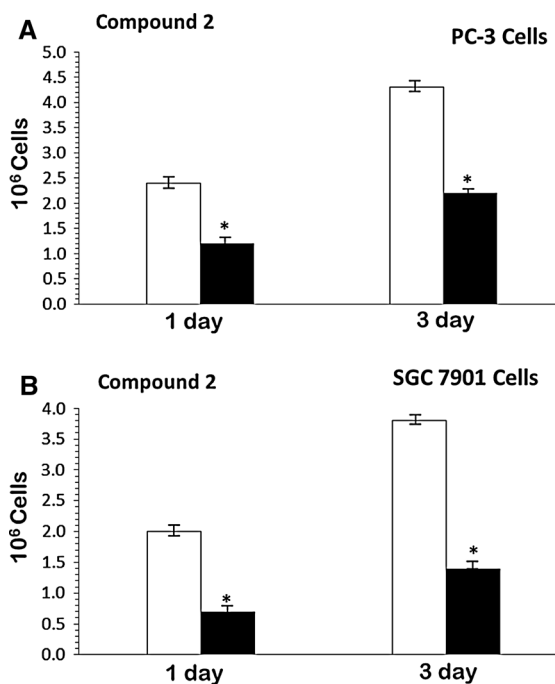


Fig. 2 Time dependent antiproliferative effects of 10 μ M complex **2** on **a** PC3 and **b** SGC7901 cells for 24 and 72 h using MTT. Results were expressed as the mean, SD. * P < 0.05

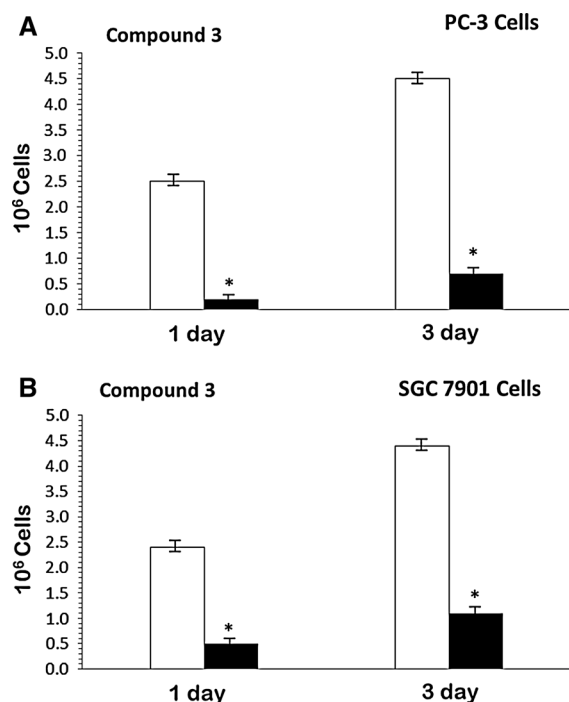


Fig. 3 Time dependent antiproliferative effects of 10 μ M complex **3** on **a** PC3 and **b** SGC7901 cells for 24 and 72 h using MTT. Results were expressed as the mean, SD. * $P < 0.05$

evaluated in vitro with reference to the IC_{50} value. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound to inhibit biological or biochemical functions. According to the FDA, IC_{50} represents the concentration of a drug/compound/complex that is required for 50 % inhibition in vitro. It is evaluated from the survival curves as the concentration needed for a 50 % reduction of survival. IC_{50} values are expressed in μ M. The IC_{50} values were calculated from dose–response curves obtained in replicate experiments. The IC_{50} data are presented in Table 10.

Results and discussion

UV–Vis spectra

The λ_{max} values obtained from UV–Vis spectra for the complexes studied are shown in Table 2. The gold(III) complexes (**1**), (**2**) and (**3**) exhibit, in a reference buffered phosphate solution, intense absorptions in the range 335–339 nm, which are assigned as ligand-to-metal

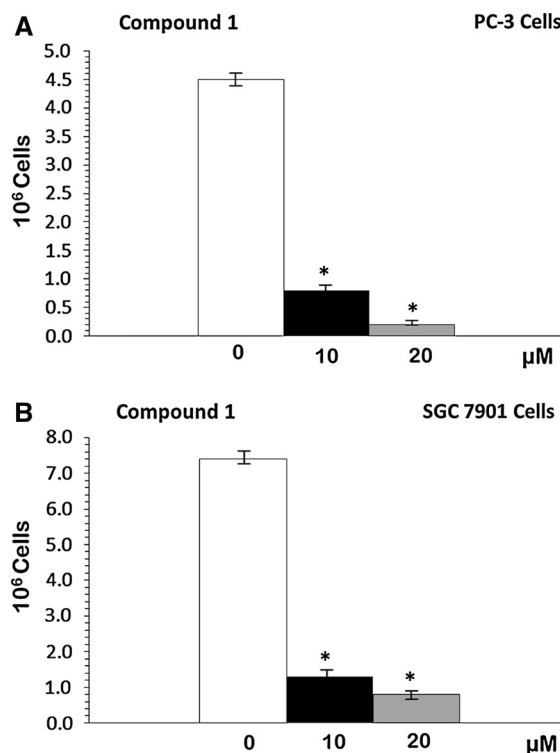


Fig. 4 Concentration dependent antiproliferative effects of complex **1** on **a** PC3 and **b** SGC7901 cells for 24 h. using MTT. Results were expressed as the mean, SD. * $P < 0.05$

charge-transfer (LMCT) transitions characteristically associated to the gold(III) center (Kimura et al. 1991). These absorption bands were previously assigned to $NH^+ - Au(III)$ charge-transfer bands (Kimura et al. 1991). It is worth-mentioning that these spectral features appear only at relatively high pH values ($pH > 6-7$) at which the deprotonation of ligand has fully occurred. According to crystal field theory for d^8 complexes the lowest unoccupied molecular orbital (LUMO) orbital is $d_{x^2-y^2}$, so ligand to metal charge transfer could be due to $p_{\sigma} \rightarrow d_{x^2-y^2}$ transition (Haruko et al. 1967).

The electronic spectra of compounds **1**, **2** and **3** were monitored at 37 $^{\circ}C$ over 3 days after mixing in the buffer solution. The electronic spectra for compounds **1**, **2** and **3** at just after mixing; and after 3 days are illustrated in Fig. 8. It is apparently observed that the transitions remain relatively unmodified over a period of 3 days. Such observations show a substantial evidence for the stability of these compounds **1**, **2** and **3** under the conditions of solution state. Nevertheless, a slight decrease in intensity of the characteristic bands was noticed with time without significant

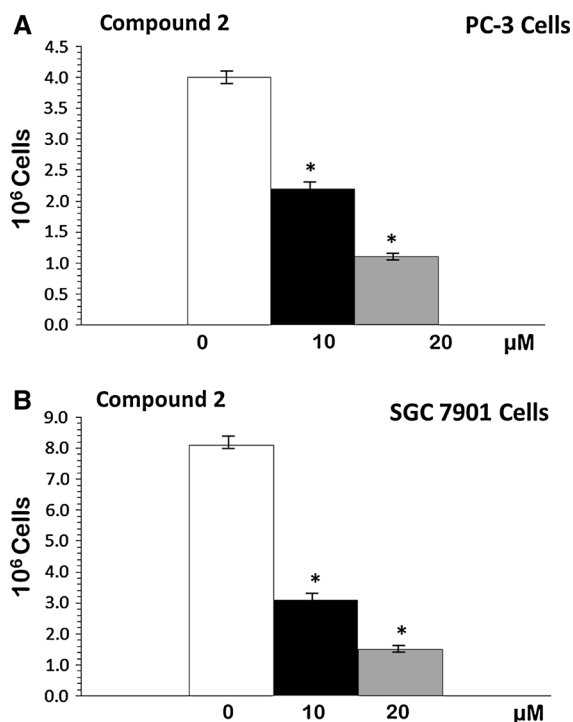


Fig. 5 Concentration dependent antiproliferative effects of complex **2** on **a** PC3 and **b** SGC7901 cells for 24 h. using MTT. Results were expressed as the mean, SD. * $P < 0.05$

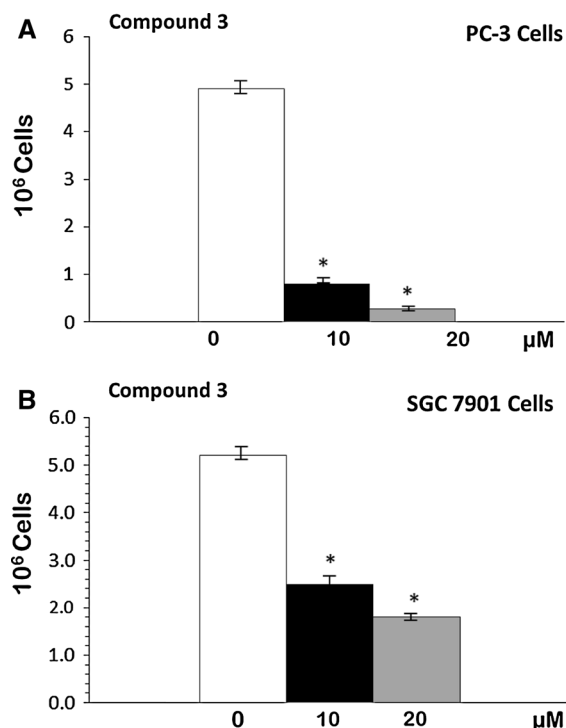


Fig. 6 Concentration dependent antiproliferative effects of complex **3** on **a** PC3 and **b** SGC7901 cells for 24 h. using MTT. Results were expressed as the mean, SD. * $P < 0.05$

modifications in shape of spectra. Further, such observation indicates that the gold center in these compounds remains in the +3 oxidation state. The minor spectral changes that are generally observed within the first hours may be ascribed either to dissociation of the amine ligands from the gold(III) complex or to partial reduction of gold(III) to metallic gold. In general, however, loss of spectral intensity is lower than 10 % of the original intensity within the observation period of 7 days which indicates high stability of these compounds in the buffer.

It is a possible proposition that compounds **1**, **2** and **3** would be stable enough in the physiological environment to undergo the necessary reactions/interactions required for bioactivity, without decomposition.

Mid and Far-FTIR spectroscopic characterization

The most significant bands recorded in the FTIR spectra of the ligand, [(1,2-DACH)Au(en)]Cl₃ complexes have been reported in Tables 3 and 4. It is noted that N–H stretching vibrations of complexes (**1–3**) exhibit, in the range 3,386–3,432 cm^{−1}, blue shifting

compared with the amino group of the corresponding free ligands. This is most likely due to stronger H-bonding interactions in the free ligands as compared to two coordinated amino- :NH₂ groups of 1,2-diaminocyclohexane (1,2-DAH) via donor N atoms, leading to formation of five member chelate with gold(III) center in corresponding compounds (**1–3**). The coordination of amino- :NH₂ with Au(III) center via nitrogen donor atom and formation of Au–N bond can be supported by the presence of a ν(Au–N) band at 417–442 cm^{−1} in the Far-FTIR (Beck et al. 1967). The C–N stretching bands also showed a significant shift to higher wave number, indicating a shorter C–N bond in the compound than in the free ligand. Moreover, there was no signal observed at 352 and 367 cm^{−1} corresponding to the symmetric and asymmetric stretching of the Cl–Au–Cl bonds in [(1,2-DACH)AuCl₂]⁺ type compounds, indicating the absence of the mono-(1,2-DACH)gold(III) chloride compound (Al-Maythality et al. 2009). The [(1,2-DACH)Au(en)]Cl₃ complexes **1–3** show N–H stretching frequencies generally lower in comparison with [(1,2-DACH)AuCl₂]Cl complexes (Table 3), most probably due to stronger hydrogen

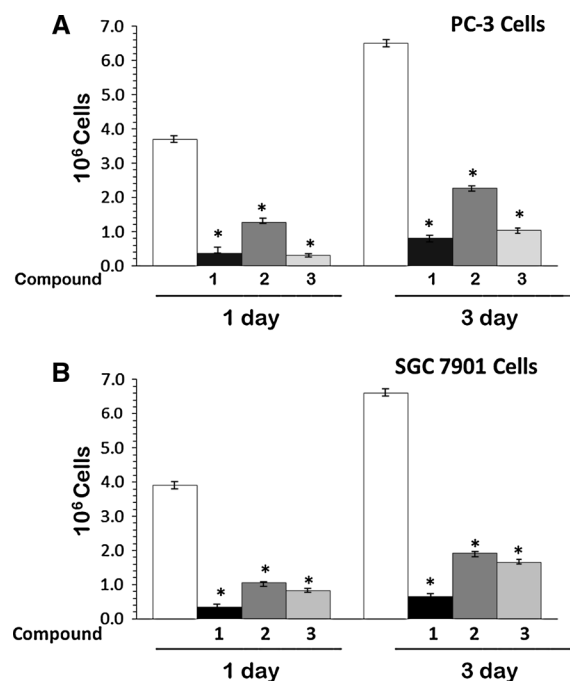


Fig. 7 Comparative time dependent antiproliferative effects of 10 μ M complexes **1**, **2** and **3** on **a** PC3 and **b** SGC7901 cells for 24 and 72 h using MTT assay. Results were expressed as the mean, SD. * $P < 0.05$

Table 10 In vitro cytotoxicity data of the complexes **1**, **2** and **3** after the exposure of 72 h towards human cancer SGC7901 and PC3 cell lines

Complex	IC ₅₀ (μ M) ^a	
	SGC7901	PC3
Cisplatin	7.3 \pm 0.5	1.1 \pm 0.1
(1)	5.5 \pm 0.2	4.8 \pm 0.1
(2)	7.9 \pm 0.2	8.9 \pm 0.1
(3)	5.8 \pm 0.2	6.1 \pm 0.1

^a Concentration of sample required to reduce the cell growth of tumor cell line by 50 %

bonding interactions with the chloride anions in the [(DACH)Au(en)]Cl₃ complexes. Furthermore the Au–N stretching frequencies are consistent with weaker Au–N bond strength in complexes (**1–3**) compared to [(1,2-DACH)AuCl₂]Cl complexes.

Solution-state NMR characterization

All ¹H NMR spectra supported the structures of the synthesized complexes as indicated by the integration of

the signals of C–H protons connected to the amino groups of the (1,2-DACH) and (en). For example, the ratio of the protons attached to amino group in both (1,2-DACH) and (en) for complex (**3**) is 1:2 as illustrated in Fig. 9. Its ¹³C NMR spectrum is also confirmed the complex's structure as shown in Fig. 10. The ¹H and ¹³C NMR chemical shifts of compounds (**1–3**) along with their corresponding free ligands are listed in Tables 5 and 6, respectively. In the ¹H and ¹³C NMR spectra of complexes (**1**), (**2**) and (**3**), one half of the total expected number of signals were noticed because of the C₂ symmetry of the 1,2-diamino-cyclohexane ring, which is considered as a rigid conformer that allowed, for instance, to distinguish equatorial H3 and H6 from axial H3 and H6 at room temperature. The signals of C–H protons connected to the amino groups for both (1,2-DACH) and (en) occur at 3.05–3.61 ppm, shifting downfield compared with the corresponding signals at 2.23–2.65 ppm in the free diamine ligands. The significant downfield shift was observed at 3.62 ppm for (**1**) complex with respect to the free *cis*-1,2-DACH ligand at 2.23 ppm. This can be attributed to the donation of nitrogen lone pairs to the gold center that causes de-shielding of the proton(s) next to the bonding nitrogen. On the other hand, ¹³C NMR downfield shift was observed only for the carbon next to the bonding nitrogen and the others carbons in the complex for (1,2-DACH) showed upfield shift presumably due to γ shielding effect. For instance, chemical shift of C3 and C4 for complex (**1**) observed at 26.13 and 20.64 ppm, respectively, whereas, for free diamine ligand it occurs at 35.26 and 26.36 ppm. It is also worth-mentioning that complexes (**1–3**), even though they have the same skeleton of (1,2-DACH) and (en), their carbon chemical shifts were not the same due to a different stereochemistry upon coordination.

Solid-state NMR characterization

As listed in Table 7, solid state NMR spectrum of complex (**3**) showed equivalency in the chemical shifts of carbon atoms (C1, C2), (C3, C6), (C4, C6) and (C1, C2) where two sets of peaks were observed, whereas, a similar behavior was not observed for carbon atoms of (DACH) in complexes (**1**) and (**3**). This indicates that these complexes (**1**) and (**3**) in the solid state lack C₂ symmetry, due to packing effect. In contrast, all synthesized complexes (**1**), (**2**) and (**3**) showed C₂ symmetry in the solution state as indicated earlier by solution ¹H and ¹³C NMR.

Fig. 8 UV-Vis spectra of complexes **1**, **2** and **3**, followed by dissolution in the buffer solution at 37 °C (a) just after mixing and (b) after 7 days

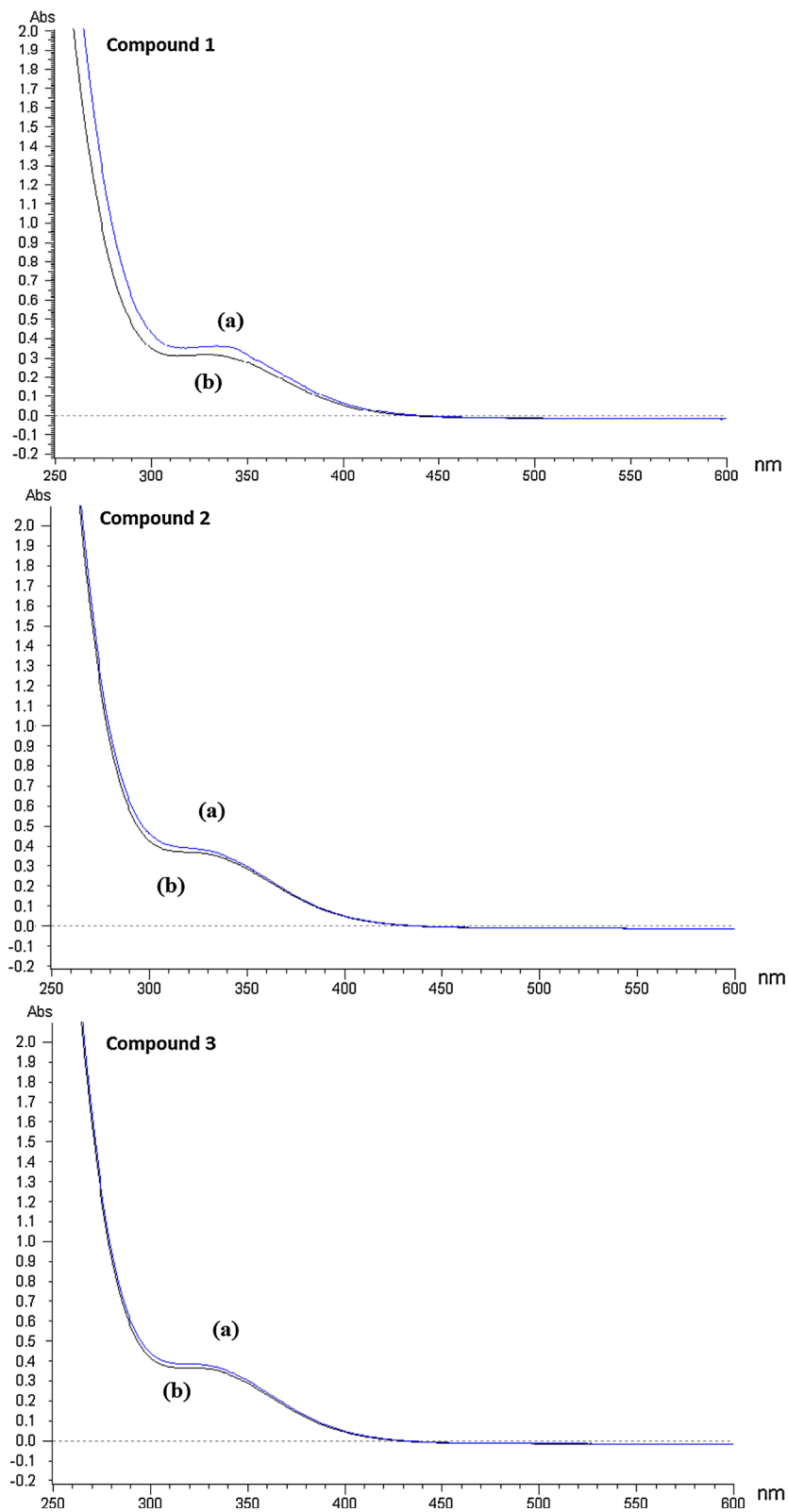


Fig. 9 Solution state ^1H NMR spectrum of $[(S,S)\text{-(+)-(1,2-DACH)}]\text{Au(en)}\text{Cl}_3$ complex

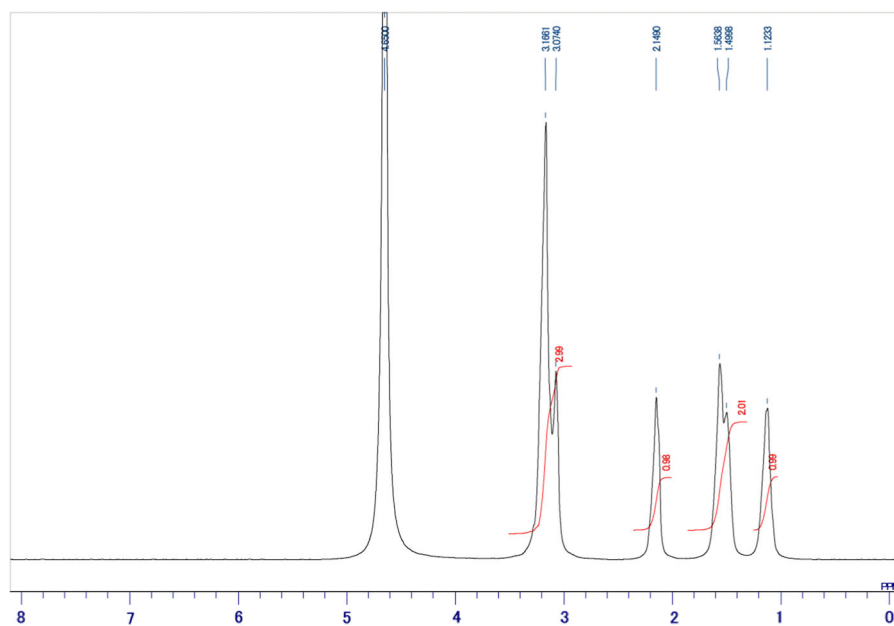
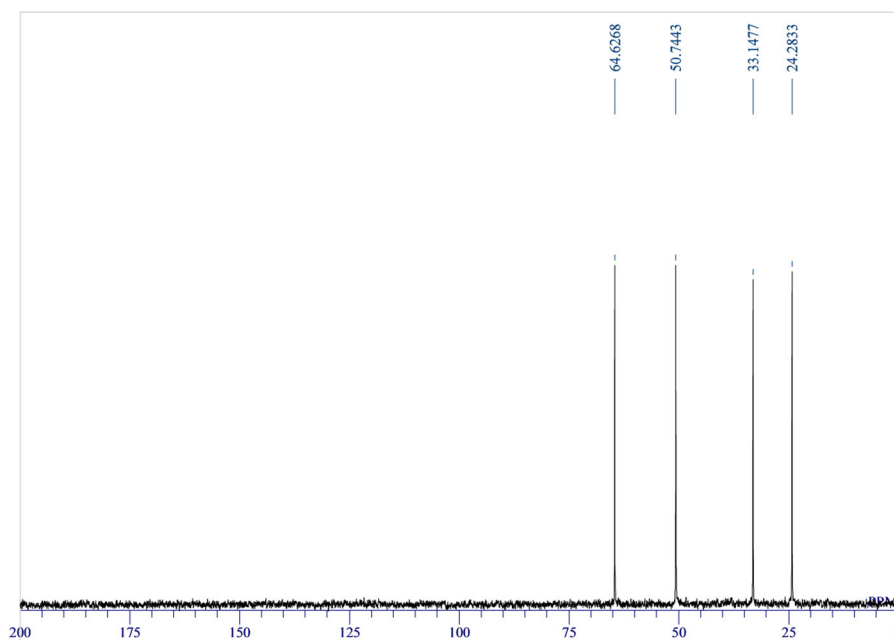


Fig. 10 Solution state $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of $[(S,S)\text{-(+)-(1,2-DACH)}]\text{Au(en)}\text{Cl}_3$ complex



Compared to solution chemical shifts, significant de-shielding in solid state is observed with similarity in chemical shift trends among all complexes **1–3** as given in Table 7, which is a clear indication of stability of the structural similarity in solid state as well as in solution state.

Computational analysis

The optimized structures of the $[(1,2\text{-DACH})\text{Au(en)}]^{3+}$ complexes as obtained from the B3LYP/LANL2DZ level of calculations are shown in Fig. 11. Selected quantitative structural parameters are also

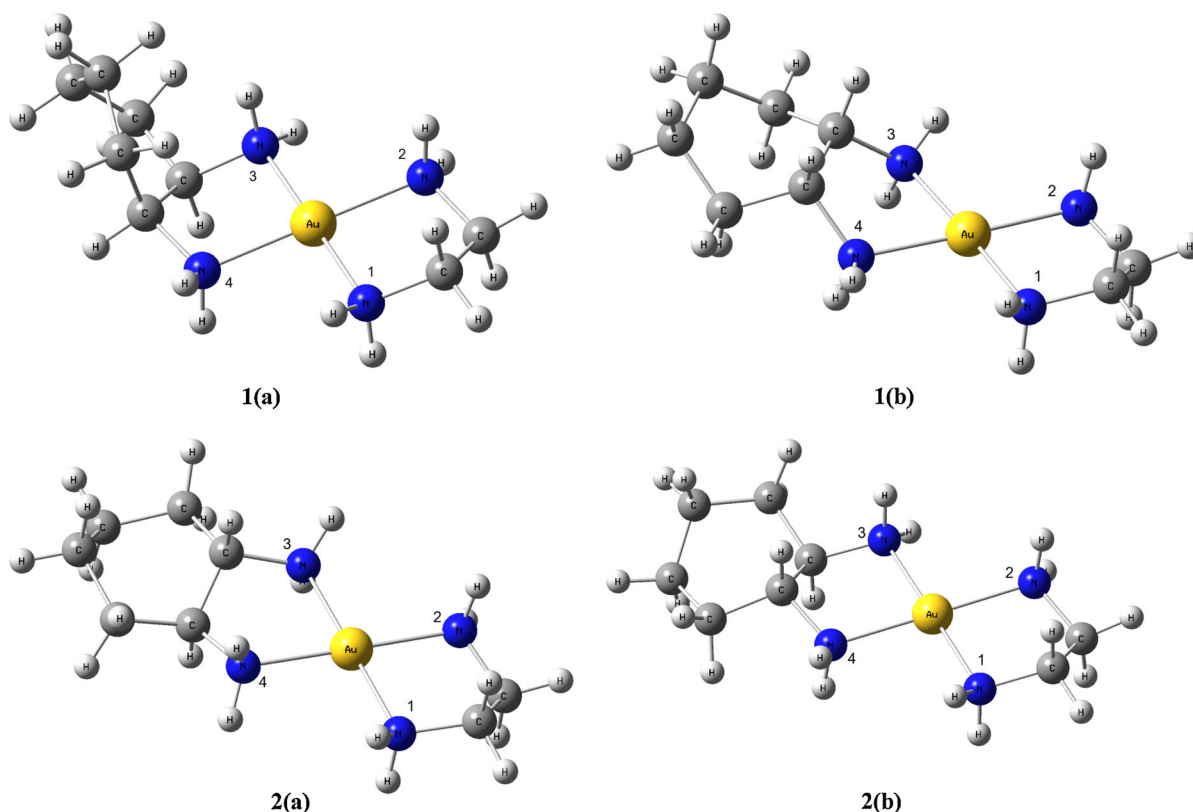


Fig. 11 Optimized geometries of **1(a)**, **1(b)**, **2(a)** and **2(b)**, obtained at the B3LYP/LanL2DZ level of theory using GAUSSIAN09

listed in Table 8. The complexes show a distorted square planar geometry structure around the gold(III) center. The N–Au–N angles in most of the conformations are within less than a degree from the perfect square planar geometry. The Au–N was predicted to be in the range of 2.12–2.15 Å for both (1,2-DACH) and (en) bidentate diamine ligands. The C–N bond length shows a significant increase by ca. 0.1 Å when it is compared with the same type of bonds in normal amines (Allen et al. 1987).

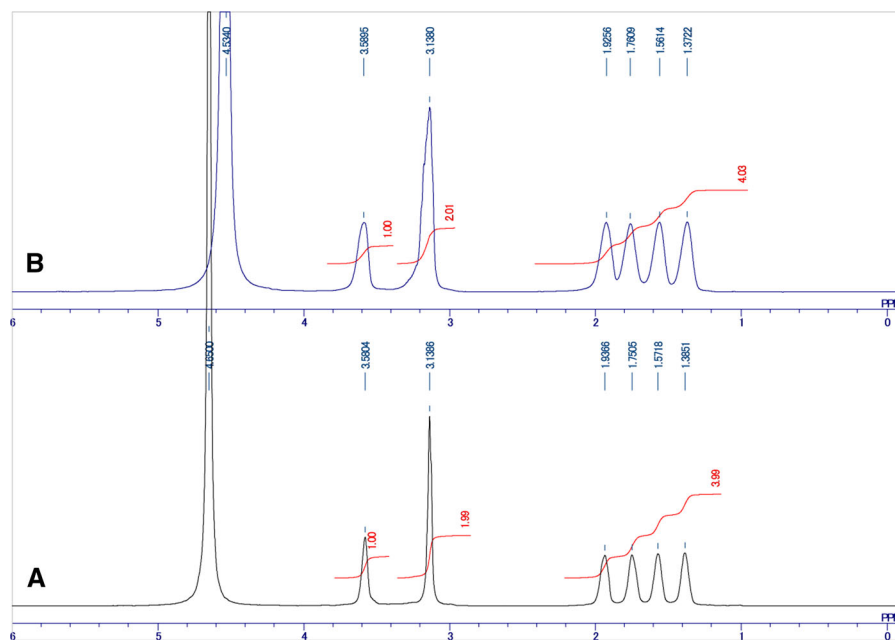
The four coordinated nitrogen atoms (two N from 1,2-DACH and two N from en) are predicted to adopt a sp^3 type of hybridization as it can easily be concluded by viewing the calculated bond angles (Table 8). From the computed energetics of the four structures of the complexes **1** and **2** (Table 9), the *trans*-conformations are more preferable compared to the *cis*-conformations with more than 3.5 kcal/mol difference. The most possible explanation of this energy variation is the ring configuration of the 1,2-DACH ligand, where the methylene (CH_2) units experience more steric

repulsion in the *cis* form in comparison to that in the *trans* form.

Stability determination of mixed diamine ligand gold(III) compounds

NMR spectra of the complexes were obtained upon immediate dissolution to serve as reference spectra and later at 24 h and after 7 days at 37 °C in D_2O and at room temperature in mixed $\text{DMSO-}d_6/\text{D}_2\text{O}$ in order to determine their stability. In general, all complexes showed high stability in D_2O as well as in mixed $\text{DMSO-}d_6/\text{D}_2\text{O}$ and their NMR profiles remained unchanged over the span of 7 days. For example, Figs. 12 and 13 illustrated, respectively, the ^1H and ^{13}C NMR profiles of the compound (**1**) at just after mixing and after 7 days. Whereas, these compounds in mixed $\text{DMSO-}d_6/\text{D}_2\text{O}$ solvent system were slightly less stable at the experimental conditions, in which, minor dissociation of ethylenediamine (en) out of the gold complexes was observed in 24 h. On the other

Fig. 12 Solution state ^1H NMR spectrum of [$\{cis-(+)-(1,2\text{-DACH})\}\text{Au(en)}\}\text{Cl}_3$ complex in D_2O **a** just after mixing and **b** after 7 days



hand, no dissociation was observed for (1,2-DACH). Among all synthesized complexes, the maximum dissociation for ethylenediamine (en) after 7 days was experienced for compound (**3**) with 25 %. ^1H and ^{13}C NMR profiles of compound (**3**) in $\text{DMSO-}d_6/\text{D}_2\text{O}$ at just after mixing and after 7 days as shown in Figs. **14** and **15** respectively. ^1H and ^{13}C NMR of compound (**3**) spectra after 7 days in $\text{DMSO-}d_6/\text{D}_2\text{O}$ showed extra peak at 3.07 and 37.24 ppm as shown in Figs. **14b** and **15b**, respectively, corresponding to the free (en) atoms. It is clearly concluded that the bond between gold(III) and (1,2-DACH) is stronger than the bond between gold(III) and (en) in these complexes (**1–3**), suggesting that ethylenediamine (en) could be a better leaving group.

Electrochemical behavior of mixed diamine ligand gold(III) complexes

The electrochemical behavior of compounds (**1**), (**2**) and (**3**) was investigated in a physiological environment through cyclic voltammetry (CV). The cyclic voltammetric curves of the complexes (**1**), (**2**) and (**3**) are shown in Fig. **16**. Table **11** summarizes the cyclic voltammetric data of all the studied compounds. The reduction potential values vs. NHE for the reduction processes exhibited by the complexes (**1**), (**2**) and (**3**), in a reference phosphate buffer solution, were in the

range (+0.46)–(+0.51) V. Cyclic voltammetric data indicated that *trans*-1,2-DACH conformer is slightly more stable than the *cis*-1,2-DACH conformer of the complexes which is also corroborated by UV–Visible spectral studies. Gold(III) complexes (**1**), (**2**) and (**3**) show one irreversible reduction process in which the controlled potential coulometry involves three electrons per mole. The occurrence of Au(III)/Au(0) reduction is visually indicated by the appearance of a thin gold layer deposited on the platinum electrode surface after exhaustive electrolysis (E_w , -0.7 V). In general, cyclic voltammetric results suggest that these compounds are quite stable under the physiological conditions.

The stability of the gold(III) compounds in the reference phosphate buffer was also checked after the addition of stoichiometric amounts of the biologically important reducing agent sodium ascorbate. It was observed that all complexes were quickly and almost completely reduced in 60 min.

Antiproliferative effects of gold(III) complexes (**1–3**) on prostate (PC3) and gastric (SCG7901) cancer cells

Modern oncologic or anticancer studies aim towards designing newer compounds showing enhanced anti-proliferative potential and not as much of associated

Fig. 13 Solution state $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of $[\{cis-(+)-(1,2\text{-DACH})\}\text{Au(en)}]\text{Cl}_3$ complex in D_2O **a** just after mixing and **b** after 7 days

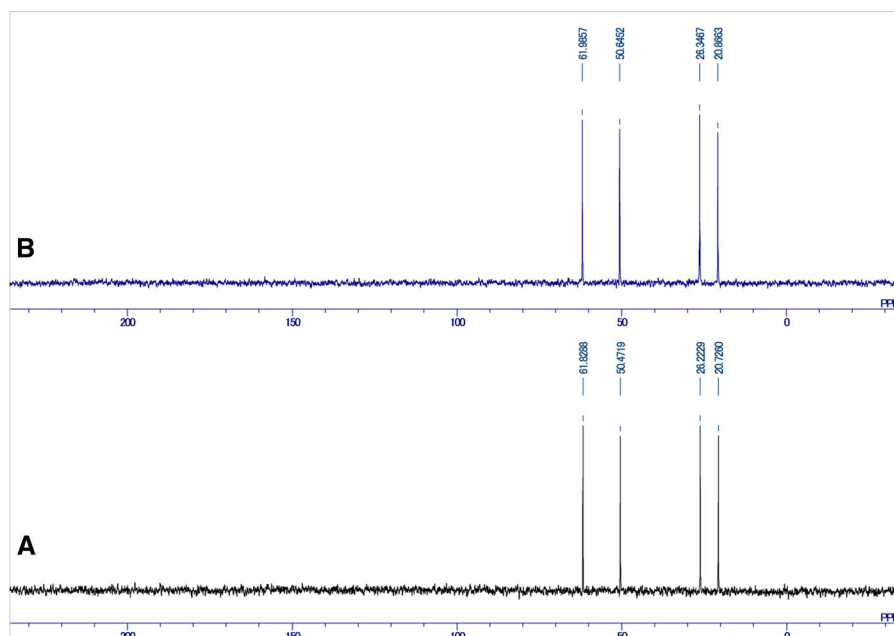
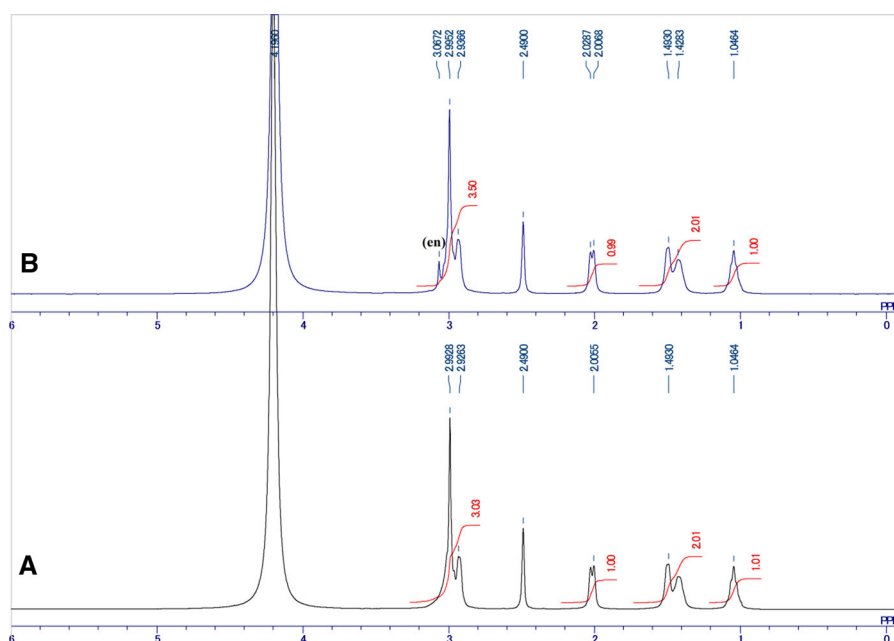


Fig. 14 Solution state ^1H NMR spectrum of $[\{(S,S')-(+)-(1,2\text{-DACH})\}\text{Au(en)}]\text{Cl}_3$ complex in D_2O **a** just after mixing and **b** after 7 days

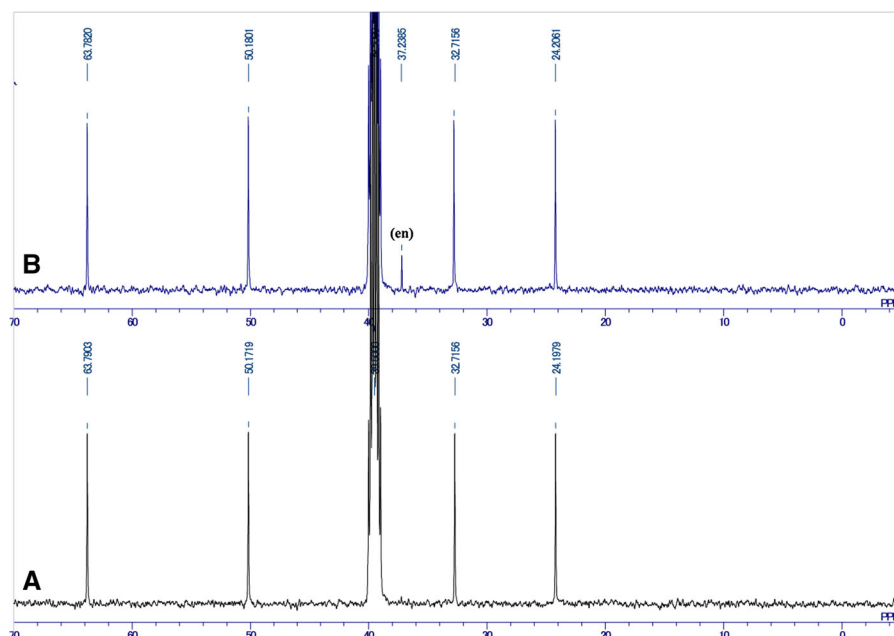


toxicity than cisplatin. In this connection, gold(III) complexes with various ligands including Au–N, Au–S or Au–C bonds are being extensively developed and investigated for their bioactivities as antiproliferative agents (Ott and Gust 2007; Ahmed et al. 2012). In this work, a new series of gold(III) complexes (**1–3**) containing mixed ethylenediamine (en) and 1,2-

DACH ligands are being evaluated for antiproliferation against PC3 and SGC7901 cancer cell lines.

Figures 1, 2 and 3 illustrated time dependent antiproliferative effects of complexes **1**, **2** and **3** respectively. In the time dependent, the growth inhibition on PC3 and SGC7901 cancer cells was studied using fixed concentration i.e. 10 μM . It is clearly

Fig. 15 Solution state $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of $[(S,S)\text{-(+)-(1,2-DACH)}]\text{Au(en)}]\text{Cl}_3$ complex in D_2O **a** just after mixing and **b** after 7 days



evident from Figs. 1, 2 and 3 that time dependent antiproliferative effects of complexes **1**, **2** and **3** on PC3 cancer cells are much better than those on SGC7901 cancer cells. Complexes (**1**) and (**3**) showed better cell inhibition against PC3 cell line than complex (**2**) as shown in Figs. 1, 2 and 3. However, Complex (**1**) showed much better cell inhibition against SGC7901 cancer cell line than complexes (**2**) and (**3**) as shown in Figs. 1, 2 and 3. Gold(III) complexes (**1**) and (**3**) demonstrated a comparable cell inhibition; against PC3 cell line as shown in Figs. 1 and 3, whether the complexes exposure time was 24 or 72 h. All the gold(III) complexes showed lower cell inhibition against both cancer cell lines for 72 h exposure time compared to 24 h as shown in Figs. 1, 2 and 3.

As we know, the cell growth inhibition also depends on concentration of the drug. So, we made concentration dependent cell growth inhibition study of gold(III) complexes (**1–3**) against human prostate PC3 and gastric SGC7901 cancer cells by using 10 and 20 μM concentrations. The results were according to the expectation that the cell inhibition was augmented with the increase in concentration of the complexes **1**, **2** and **3** as shown in Figs. 4, 5 and 6 respectively. It is generally observed from Figs. 4, 5 and 6 that concentration dependent antiproliferative effects of complexes **1**, **2** and **3** on PC3 cancer cells are superior to those on SGC7901 cancer cells. In the concentration

dependent cell growth inhibition study at two concentrations (10 and 20 μM), complex (**1**) showed much better cell inhibition against SGC7901 cancer cell line than complexes (**3**) and (**2**) as shown in Figs. 4, 5 and 6, whereas, complexes (**1**) and (**3**) showed better cell inhibition against PC3 cell line than complex (**2**), as shown in Figs. 4, 5 and 6. Gold(III) complexes (**1**) and (**3**) demonstrated a comparable cell inhibition; against PC3 cell line at 20 μM concentration as shown in Figs. 4 and 6 respectively.

Figure 7 illustrated the comparison of time dependent antiproliferative effects of 10 μM complexes **1**, **2** and **3** on both PC3 and SGC7901 cancer cells for 24 and 72 h. It has been observed that the order of time dependent antiproliferative effect is complex **1** > complex **3** > complex **2** for both PC3 and SGC7901 cancer cells. Such comparative study leads to conclusion that complex **1** is the most effective antiproliferative agent among mixed ligand based gold(III) complexes **1–3**.

Even though the exact mechanisms on antiproliferation of $[(\text{DACH})\text{Au(en)}]\text{Cl}_3$ type complexes on PC3 and SGC 7901 cancer cell lines remains vague. The significantly diminished renal toxicity of ethylenediamine complex of gold(III) could be attributed to their different anti-proliferative mechanism of action and selective sparing of the proximal tubular epithelial cells (Ahmed et al. 2012).

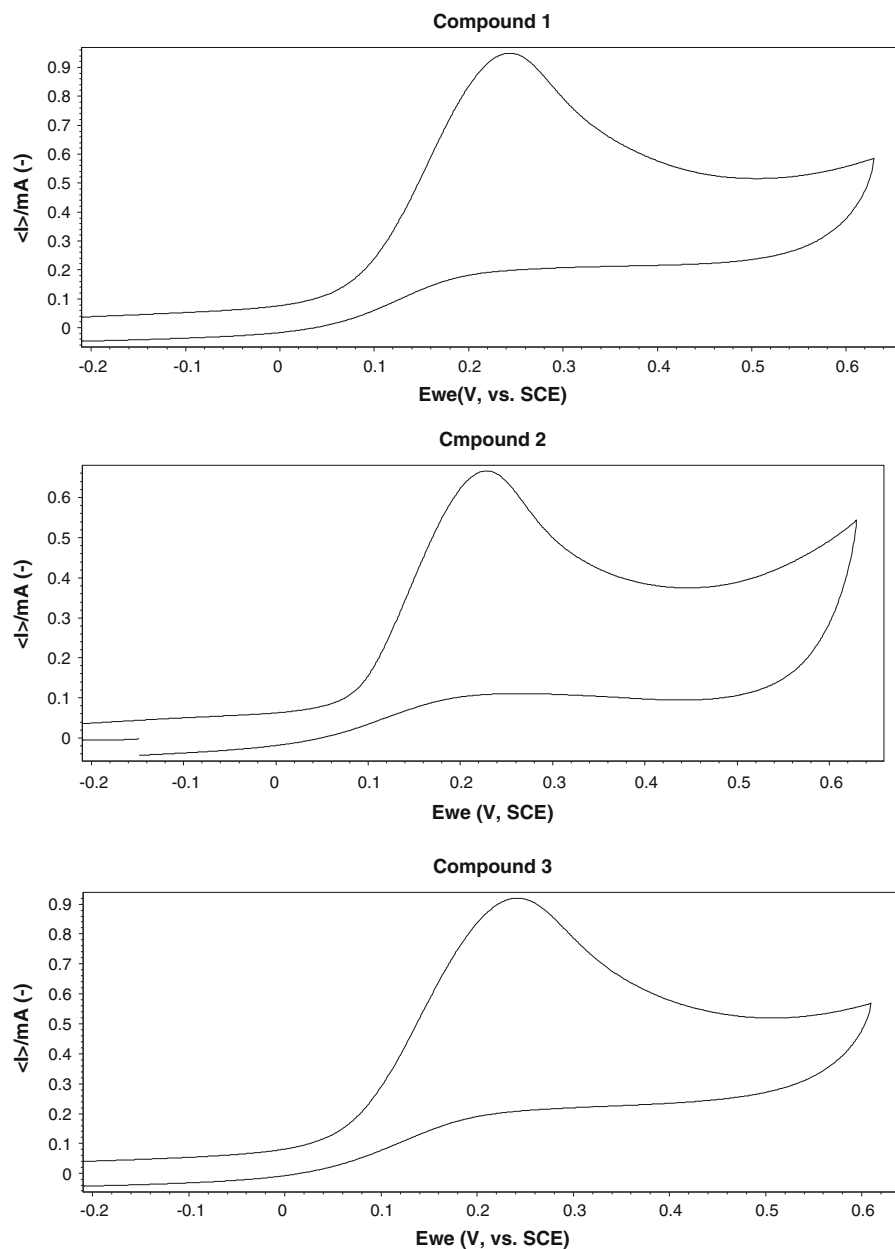


Fig. 16 Cyclic voltammograms of complexes **1**, **2** and **3** in the phosphate buffer at platinum electrode

Table 11 Peak Potential values vs. ENH for reduction of gold(III) complexes

Complex	E_p (V)
(1)	0.49
(2)	0.46
(3)	0.51

Most gold(III) compounds display reduced affinity for DNA and it seems reasonable that DNA is neither the primary nor the exclusive target for most gold(III) complexes. Recent studies have proposed a different mode of action for these compounds, in most of the cases, induce apoptosis was the mode of cell death (Vivek et al. 2009; Niemeyer 2001; Pellegrino et al. 2005). Their mechanism although not precisely

delineated. However, the mechanisms associated with the inhibitory effects of complexes (**1–3**) on the proliferation of rapidly dividing cancer cells may be comprised of a cumulative impact on the induction of cell cycle blockage (Taates et al. 2008), interruption of the cell mitotic cycle (Taates et al. 2008), apoptosis (programmed cell death) (Takemura et al. 2013) and necrosis (premature cell death) (Hayashi et al. 2014).

In vitro cytotoxicity of gold(III) complexes (1–3**) on prostate (PC3) and gastric (SGC7901) cancer cells**

Milovanovic et al. have studied the cytotoxicity studies of $[\text{Au}(\text{en})\text{Cl}_2]^+$ and $[\text{Au}(\text{SMC})\text{Cl}_2]^+$ where SMC = S-methyl-L-cysteine and $[\text{Au}(\text{DMSO})_2\text{Cl}_2]^+$ (DMSO = dimethyl sulphoxide). They concluded that gold(III) complexes are much faster to react with nucleophiles compare to Pt(II) complexes. They also demonstrated that gold(III) complexes exhibit relevant cytotoxic properties when tested on chronic lymphocytic leukemia cells (CLL). This conclusion indicates that gold(III) complexes have good potential for the treatment of cancer. In addition $[\text{Au}(\text{en})\text{Cl}_2]^+$ complex shows cytotoxicity profiles comparable to cisplatin (Milovanović et al. 2010). In this study, a new series of gold(III) complexes (**1–3**) is developed by replacing two monodentate Cl^- ligands with bidentate 1,2-DACH (1,2-diaminocyclohexane) ligand and subjected to in vitro cytotoxic evaluation against PC3 and SGC7901 cancer cell lines.

The in vitro cytotoxic effect of mixed ligand gold(III) diamine complexes against androgen-resistant prostate PC3 and human gastric SGC7901 cancer cells were studied using MTT assay. The in vitro cytotoxic activity depends on the exposure time and the concentration of complexes. For that reason, we used different concentrations and a 3-day exposure protocol to determine the IC_{50} values for all three complexes. The in vitro cytotoxicity in terms of IC_{50} values of cisplatin for PC3 and SGC7901 cells was included for a comparison.

The IC_{50} data for the Au(III) complexes (**1, 2 and 3**) showed in vitro cytotoxicity in a wide range of 1.1–8.9 μM for PC3 cells, as given in Table 10. It can clearly evident from IC_{50} data for PC3 cancer cells that complex (**1**) showed ca. 100 % better potent than complexes (**2**) and ca. 50 % better (**3**) respectively. It is easily concluded that complexes (**1**) is relatively

more effective cytotoxic agent than complexes (**2**) and (**3**). For PC3 cancer cells, the order of in vitro cytotoxicity in terms of IC_{50} values is cisplatin (1.1 μM) > complex **1** (4.8 μM) > complex **3** (6.1 μM) > complex **2** (8.9 μM) as it is known that lower the IC_{50} value, higher the in vitro cytotoxicity. All three complexes showed the lower potency *vis-a-vis* cisplatin.

According to IC_{50} data, $[\text{Au}(\text{en})_2]\text{Cl}_3$ with ethylenediamine ligand is a more prospective anti-cancer agent against prostate cancer PC3 cells (Monim-ul-Mehboob et al. 2013). The dose dependent studies showed that $[\text{Au}(\text{en})_2]\text{Cl}_3$ was found to execute a powerful and promising cytotoxic effect on PC3 cells which is comparable to that of cis-platin (Monim-ul-Mehboob et al. 2013). For PC3 cells, $[\text{Au}(\text{en})_2]\text{Cl}_3$ was recognized as effective cytotoxic agent as cis-platin while $[(\text{en})\text{AuCl}_2]\text{Cl}$ showed almost 7–9 fold lower cytotoxicity as compared to cis-platin (Isab et al. 2011; Monim-ul-Mehboob et al. 2013).

The IC_{50} data for the Au(III) complexes (**1, 2 and 3**) showed in vitro cytotoxicity in the range of 5.5–7.9 μM for SGC7901 cells, as given in Table 10. It can apparent from IC_{50} data for SGC7901 cancer cells that complex (**1**) showed comparable in vitro cytotoxicity to complex (**3**). Both complexes **2** and **3** are reasonably better cytotoxic agent than complex **3**. For SGC7901 cancer cells, the order of in vitro cytotoxicity in terms of IC_{50} values is complex **1** (5.5 μM) > complex **3** (5.8 μM) > cisplatin (7.3 μM) > complex **2** (7.9 μM). It is worth-mentioning that the in vitro cytotoxicity of both complexes **1** and **3** are fairly better than that of cisplatin.

For SGC7901 cells, $[\text{Au}(\text{en})_2]\text{Cl}_3$ show slightly lower cytotoxicity with respect to cis-platin (Monim-ul-Mehboob et al. 2013) whereas $[(\text{en})\text{AuCl}_2]\text{Cl}$ almost two fold more cytotoxic than cis-platin. $[(\text{en})\text{AuCl}_2]\text{Cl}$ may be potential anti-cancer agents for cis-platin resistant SGC7910 cells (Isab et al. 2011; Monim-ul-Mehboob et al. 2013). An independent assessment of $[\text{Au}(\text{en})_2]\text{Cl}_3$ and its derivatives reveals an interesting feature that SGC7901 gastric cancer cells exhibit 7–8 fold intrinsic resistance relative to the PC3 cancer cells with respect to cisplatin. On the contrary, the $[\text{Au}(\text{en})_2]\text{Cl}_3$ type complexes may have the potential to overcome mechanisms inducing resistance to cisplatin, particularly in the gastric cancer SGC7901 cells (Isab et al. 2011; Monim-ul-Mehboob et al. 2013). Nevertheless, only twofold or

less resistance to the $[\text{Au}(\text{en})_2]\text{Cl}_3$ type complexes was observed for PC3. This suggests that the intrinsic factors regulating cellular sensitivity to cisplatin and $[\text{Au}(\text{en})_2]\text{Cl}_3$ are different for PC3 and SGC-7901 cells. The factors affecting sensitivity of PC3 and SGC-7901 towards cisplatin cells are analogous in the $[\text{Au}(\text{en})_2]\text{Cl}_3$ type complexes (Isab et al. 2011; Monim-ul-Mehboob et al. 2013).

These in vitro cytotoxicity results reveal that gold(III) complexes containing ethylenediamine and 1,2-diaminocyclohexane ligands are better anticancer agents than $[\text{Au}(1,2\text{-DACH})\text{Cl}_2]\text{Cl}$, $[\text{Au}(1,2\text{-DACH})_2]\text{Cl}_3$; and $[\text{Au}(\text{en})_2]\text{Cl}_3$ and its derivative complexes against gastric SGC7901 cancer cell line (Isab et al. 2011; Monim-ul-Mehboob et al. 2013; Al-Maythality et al. 2009; Al-Jaroudi et al. 2013, 2014). According to IC_{50} data, gold(III) complexes (**1**) and (**3**) were more effective than $[\text{Au}(1,2\text{-DACH})\text{Cl}_2]\text{Cl}$ against prostate PC3 cancer cells (Al-Jaroudi et al. 2013). There is no doubt that present study is helpful for further exploiting and defining the potential role of gold(III) complexes in combat against prostate and gastric cancers.

Conclusion

The gold(III) complexes (**1–3**) containing ethylenediamine (en) and diaminocyclohexane (1,2-DACH) were characterized using elemental analyzer, solution- and solid- NMR measurements, UV–Vis, Mid- and Far-FTIR spectroscopic methods. The CHN analysis data support the formation of the mixed en and 1,2-DACH ligands gold(III) complexes (**1–3**) with general formula $[(1,2\text{-DACH})\text{Au}(\text{en})]\text{Cl}_3$. The spectroscopic methods and NMR measurements confirm the formation of gold(III) complexes containing bidentate en and 1,2-DACH ligands via N-donor atoms. The computational studies corroborate spectroscopic data of gold(III) complexes. The computational studies also demonstrate that *trans*-(1,2-DACH)-gold(III)-(en) isomer is slightly more stable than the *cis*-(1,2-DACH)-gold(III)-(en) isomer. The coordination sphere of these complexes around gold(III) center adopts distorted square planar geometry. According to antiproliferative effects of gold(III) complexes (**1–3**) on prostate (PC3) and gastric (SGC7901) cancer cells, the order of time dependent antiproliferative effect is complex **1** with *cis*-configuration > complex **3** with *(1S,2S)*(+)- configuration > complex **2** with *trans*-

configuration for both PC3 and SGC7901 cancer cells. Such comparative study leads to conclusion that complex **1** with *cis*-configuration of 1,2-DACH is the most promising antiproliferative agent among mixed ligand based gold(III) complexes **1–3**. The inhibitory effect of complexes **1–3** on the proliferation of rapidly dividing cells may be attributed to the induction of cell cycle blockage, interruption of the cell mitotic cycle, programmed cell death (apoptosis) or premature cell death (necrosis). The in vitro cytotoxicity results reveal that mixed diamine ligand gold(III) complexes are better anticancer agents than previously reported $[\text{Au}(1,2\text{-DACH})\text{Cl}_2]\text{Cl}$, $[\text{Au}(1,2\text{-DACH})_2]\text{Cl}_3$; and $[\text{Au}(\text{en})_2]\text{Cl}_3$ and its derivative complexes against gastric SGC7901 cancer cell line. Moreover, gold(III) complexes (**1**) and (**3**) were more effective than $[\text{Au}(1,2\text{-DACH})\text{Cl}_2]\text{Cl}$ against prostate PC3 cancer cells. There is no doubt that present study is helpful for further exploiting and defining the potential role of gold(III) complexes in the treatment of human prostate and gastric cancers. In short, $[(\text{cis-}1,2\text{-DACH})\text{Au}(\text{en})]\text{Cl}_3$ might be a potential chemopreventative and chemotherapeutic agent against human gastric SGC7901 cancer cells. Therefore, we would like to recommend the in vivo anticancer evaluation of complex **1** for further exploration of its anticancer activity.

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