RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Potent selective inhibition of MMP-14 by chloroauric acid and its inhibitory effect on cancer cell invasion

Yanyan Wang[1], Hezhen Lu[1], Dahai Yu[1], Jinrui Zhang[1], Weiguo Liang[1], Zhimin Zhang[2*], Xuexun Fang[1*]

1. Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, College of Life Science, Jilin University, 2699 Qianjin Street, Changchun, 130012, P. R. China

2. Department of Endodontics and Operative Dentistry, School of Stomatology, Jilin University, Changchun, 130021, P. R. China

*Corresponding author: Zhimin Zhang, Xuexun Fang

Tel.: +86-431-85155249;

Fax: +86-431-85155200.

E-mail address: fangxx@jlu.edu.cn

Abstract

Matrix metalloproteinases (MMPs) are a family of zinc-containing proteases with vital roles in extracellular matrix remodeling. The regulation of MMPs can modulate a number of cellular activities. The therapeutic potential of MMP inhibitors has been shown for diseases such as arthritis and cancer. This paper is the first to demonstrate that $HAuCl_4$ inhibits the activity of MMP-2 and MMP-13 as well as has a more specific inhibitory effect on MMP-14. The effect of $HAuCl_4$ on MMP-14 involves a non-competitive reversible inhibitory mechanism. Moreover, 0 μM–50 μM $HAuCl_4$ did not affect the cell viability of HT-1080 human fibrosarcoma cells. However, $HAuCl_4$ at these concentrations showed significant inhibitory effects on the invasion of the HT-1080 cells, thereby suggesting that $HAuCl_4$ may modulate tumor cell behaviors by inhibiting MMP-14. These findings provide initial clues to further elucidate the biological activity of $HAuCl_4$ and its potential therapeutic value for related diseases.

Keywords: MMPs; Chloroauric acid; Inhibition mechanism; Cell invasion

1 Introduction

Matrix metalloproteinases (MMPs) are a group of zinc-containing proteases that are mostly responsible for extracellular matrix (ECM) turnover [1]. MMPs can also regulate the synthesis and secretion of cytokines, growth factors, hormones, and cellular adhesive molecular receptors [2]. Consequently, MMPs are involved in various physiological and pathological processes, including embryo formation [3], organ development [4], wound healing [5], tissue remodeling [5], tumorigenesis [6], cancer progression [7], cardiovascular diseases [8], nervous system disease [9], arthritis [10], and respiratory disease [11], among others. To date, a number of MMP inhibitors have been developed for therapeutic purposes against diseases such as cancer and arthritis [12]. However, most MMP inhibitors (MMPIs) are organic compounds; only a few inorganic MMPIs have been reported [13]. We previously showed that trivalent aluminum salts have inhibitory effects on MMPs and can inhibit the migration and metastasis of tumors without affecting cell viability [14]. However, the inhibitory effects of trivalent Au compounds on MMPs and protease-related diseases have not yet been reported.

Except for the zero-valence state of colloidal gold, Au mainly exists in monovalent and trivalent states under physiological conditions. Chloroauric acid is an inorganic compound with a crystalline tetrahydrate that is known to contain $H_5O_2^+$, $AuCl_4$, and two water molecules [15]. The $AuCl_4^-$ anion has a square planar molecular geometry. The Au–Cl distances are approximately 2.28 Å, and other d8 complexes adopt similar structures, e.g., $[PtCl_4]^{2-}$ [15, 16]. Its release of the proton (H^+) gives chloroauric acid acidic characteristics. Chloroauric acid is a strong monoprotic conjugate acid. Although this acid forms aqueous solutions, such solutions are unstable because of the hydrolysis of the tetrachloridoaurate ion. Thus, Au in this acid can be restored to a low-valence state or even a zero-valence state [17]. However, this decomposition route is unlikely because HCl is a strong acid; Cl^-

ions are highly unlikely to combine with H^+ to form HCl in an aqueous solution. Gold that exists in different oxidation states has rich coordination chemistry [18]. Therefore, even subtle changes in the structure of these metal complexes can cause dramatic changes in their physicochemical and biological properties. Chloroauric acid is the precursor used in purifying gold by electrolysis [19] and in preparing gold nanoparticles (AuNPs) [20].

Au, Pt, and Ru are homologous metals. Gold and its complexes have demonstrated unique biological and medical properties [21]. The Au(III) complex showed selective anti-microbial activity against the Gram-positive bacteria (Bacillus cereus and Staphyloccocus aureus) being more toxic than its Au(I) analogue, while the free Au(0) is totally inactive [22]. Similarly, information on the use of Au(III) complexes as anti-HIV agents is scarce; only a few reports have been recently published [23, 24]. Gold complexes, with the anti-malaria drug chloroquine as the lead structure, were tested for their activity against Plasmodium falciparum. The activity of some Au(III) cyclometallated complexes was assayed against mammalian and parasitic cysteine proteases, which are involved in the life cycles of parasites, such as Schistosoma, Plasmodium, Trypanosoma brucei, Trypanosoma cruzi, and Leishmania [25–27].

Different types of Au(I) and Au(III) compounds possess effective antitumor activity in vitro and in vivo (in animals) [28]. Au(III) is isoelectronic with Pt(II), and tetracoordinate Au(III) complexes have the same square-planar geometries as cisplatin; therefore, the anticancer activity of Au(III) compounds has been investigated [29]. Previous studies suggested that in contrast to cisplatin, gold complexes target proteins but not DNA [30, 31]. Au(III) dithiocarbamates show anticancer activity, wherein their primary target is the proteasome [32]. Treatment of human breast tumor-bearing nude mice with a Au(III) dithiocarbamate complex causes significant inhibition of tumor growth, which is associated

with proteasome inhibition and massive apoptotic induction in vivo [28, 32]. Gold possesses two beta-emitting radioactive isotopes, namely, ^{198}Au and ^{199}Au, which are potentially suitable for therapeutic applications [33]. A number of Schiff base and thiosemicarbazonato complexes are prepared with ^{198}Au [33].

To date, the greatest concern is the study of disease treatment with nanometer gold complexes, especially for anticancer treatment [34]. The combined physical, chemical, optical, and electronic properties of gold nanoparticles (AuNPs) provide a new platform for imaging and diagnosis of cancer [35–40]. Therefore, drugs can be selectively provided [27, 41–43], treatment can target sensitive cells and tissues [44, 45], surgery can be supervised and guided, and chemotherapy can be specifically given to specific disease loci [46, 47]. However, other studies also found that nanometer gold can promote the rapid increase of NO in the blood. The released NO inside the cell may react with the superoxide anion (O_2^-) to generate peroxynitrite anion ($ONOO^-$), which has strong oxidation activity and greater destructive effects, thereby inducing a series of oxidative stress effects. This discovery warns against the application of nanometer gold as biological probes, drug carriers, and vehicles in cells or organisms. Furthermore, Au can affect the activation or nuclear translocation of transcription factors formed by the binding of NF-κB with DNA and can play an important role in the signal pathway [48]. An improved understanding of the physiological processes of gold compounds will provide a rational basis for their further development into novel anticancer drugs.

In this paper, we analyzed the inhibition effects of chloroauric acid on the enzyme activity of representative members of the MMP family. MMP-2, -13, and -14 belong to the gelatin enzymes, collagen-type enzymes, and membrane MMPs, respectively [49]. Given the specific degradation of different substrates, these MMPs can degrade different types of collagen and gelatin. MMPs participate

92 in multiple physiological or pathological processes that require ECM remodeling, and their excessive
93 expression is closely related to migration, invasion, and metastasis of tumor cell during tumor
94 progression [50–52]. The mechanism and type of inhibition were analyzed by enzyme kinetics.
95 Moreover, the influence of gold chloride acid on the cell viability, migration behavior, and invasive
96 ability of HT-1080 tumor cells was analyzed at the cytological level. Thus, the inhibition mechanism of
97 chloroauric acid on MMPs was inferred. This study is vital in exploring the significant role of gold
98 compounds in related diseases. The potential value of MMPs for the development of novel relevant
99 drugs was demonstrated.

100 **2 Materials and Methods**

101 **2.1 Materials and reagents**

102 DQ-gelatin was purchased from Invitrogen. The recombinant catalytic domains of human MMP-2,
103 -13, and -14 were expressed in *Escherichia coli*, purified, and refolded in our laboratory [53]. Gelatin
104 was obtained from Sigma. Other reagents and solvents used in experiments were of analytical or
105 reagent grade, as deemed appropriate.

106 **2.2 Enzyme activity assay**

107 An FLx800 fluorescence microplate reader (Bio-Tek) was used to measure the MMP activity.
108 DQ-gelatin was employed as the substrate. Kinetics assays were performed at 37 °C in 50 mM HEPES
109 buffer (pH 7.5) containing 0.2 M NaCl, 1 mM $CaCl_2$, 20 μM $ZnSO_4$, and 0.05% Brij-35. For the
110 inhibition assays, metal ions were incubated with an appropriate quantity of MMPs for 15 min to
111 ensure that equilibrium was reached before adding the fluorescent substrate. The extent of inhibition
112 was determined using the initial rates with and without the inhibitor [54]. The FLx800 fluorescence

microplate reader (Bio-Tek) was also used to measure the cathepsin activity, with z-Phe-Arg-AMC as the substrate. Cathepsin B: 25 mM MEs, 5 mM DTT, pH 6.0; Cathepsin L: 0.4 M NaOAc, 4 mM EDTA, 8 mM DTT, pH 5.5. For the enzyme inhibition assays, the activity data were fitted in a linearised curve using Origin version 7.5. The IC_{50} values were then determined from the curve accordingly.

2.3 Enzyme kinetics of chloroauric acid inhibition on MMP-14

To determine the inhibitory mechanism, we performed further kinetic analysis of the inhibition of chloroauric acid on MMP-14. Different quantities of enzyme (0.125 μg/ml−1 μg/ml) were added into the reaction mixture, and then the same substrate and inhibitor concentration (0.2 and 0.4 μM) were added. Under different concentrations of the enzyme and inhibitor, the reaction rate was reduced with gradient concentration-dependence as compared with the control group. All straight lines in the standardization plot passed through the zero point. Therefore, the inhibition of MMP-14 by chloroauric acid was initially determined as reversible inhibition.

To analyze the inhibitory type, we selected 1 μg/mL as the final concentration of the enzyme. The final concentrations of the inhibitor were selected as 0, 0.2, 0.4, and 0.6 μM, respectively, according to the detected $IC_{50} = 0.3$ μM during the inhibition of MMP-14 by chloroauric acid; substrates with different final concentrations (0.5 μg/ml−5 μg/ml) were added. The inhibition type was obtained by the Lineweaver−Burk and Dixon plots [55, 56].

2.4 Cell culture

The human fibrosarcoma carcinoma cell line HT-1080 was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) supplemented with penicillin

(100 units/ml) and streptomycin (100 µg/ml) at 37 °C with 5% CO_2.

2.5 Measurement of cell viability (MTT)

HT-1080 cells were seeded into 96-well plates at a density of 1×10^4 cells/well in 200 µl of DMEM containing 10% fetal bovine serum. At 24 h after seeding, the medium was removed, and the cells were incubated for 24 h with DMEM containing 10% FBS in the absence or presence of various concentrations of metal ions. Subsequently, 200 µl of DMEM and 20 µl of 5 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to each well. The plates were placed in an incubator at 37 °C in 5% CO_2 for 4 h. The medium was then replaced with 150 µl of DMSO, and the absorbance was recorded at 490 nm.

2.6 Matrigel invasion assay

The Boyden chamber technique (transwell analysis) was performed as previously described [57]. Cancer cells were treated with chloroauric acid or solvent (as control). Homogeneous single-cell suspensions (1×10^5 cells/well) were added to the upper chambers. The cells were allowed to invade the wells at 37 °C for 24 h in a CO_2 incubator. Migrated cells were stained with 0.1% crystal violet at room temperature for 10 min and examined by light microscopy. Quantification of migrated cells was performed according to published criteria.

3 Results and discussion

3.1 Chloroauric acid specifically suppressed the activity of MMP-14 in a dose-dependent manner

Our previous work on trivalent Al and $K_3[Fe(CN)_6]$ on MMP-16 revealed that these trivalent metal salts and their complexes all have significant effects on the activity of MMP-16 as well as the

migratory and invasive abilities of the cells [14]. The significant inhibition of MMPs by these inorganic trivalent metal salts is a new discovery in the field of MMP inhibitors. Chloroauric acid is a trivalent compound of gold. Thus, MMP-2, -13, and -14 were selected in our study to analyze whether chloroauric acid has inhibitory effects on MMPs. The average values of the three measurements are shown in Figs. 1a–1c. The results showed that with the increasing concentration, chloroauric acid had significant inhibitory effects on MMP-2, -13, and -14, with IC_{50} values in the micromolar and sub-micromolar range. The inhibition of MMP-14 was particularly remarkable. Control experiment with fluorogenic substrate in presence of $HAuCl_4$ alone were performed in the absence of the MMPs (data not shown).

To analyze whether chloroauric acid can specifically inhibit MMPs, we selected the members of cathepsin family (Cathepsin B and L) with different catalytic mechanisms from MMP family for the enzyme activity analysis. Cathepsins belong to the cysteine protease family. These enzymes have a cysteine in their catalytic sites that starts the proteolysis cascade reactions. Cathepsins have an important role in promoting the occurrence and development of tumors. Among the cathepsins, cathepsin B can promote the tumor progression through the degradation of the basement membrane as well as the ECM components and elements [58]. In addition, the proliferation and growth of tumor cells with the silenced cathepsin L gene are slower and the invasion is significantly reduced [59]. Chloroauric acid at a final concentration of 10 μM was found to have no obvious inhibitory effects on the two cathepsins (Table 1). A comparison of enzyme inhibition by chloroauric acid on three recombinant proteases (MMP-2, -13, and -14) and cathepsins (Cathepsin B and L) implies that chloroauric acid has inhibitory effects on three MMPs, with relative specificity for MMP-14 (Table 1).

Table 1 The IC_{50} of $HAuCl_4$ and $NaAuCl_4$ on MMPs (MMP-2, -13, and -14) and Cathepsin (Cathepsin

176 B and L).

Proteinases	IC_{50} (μM)		
	$HAuCl_4$	$NaAuCl_4$	NaCl
MMP-2	2.28	6	No
MMP-13	9.83	8	No
MMP-14	0.30	10	No
Cathepsin B	No	No	No
Cathepsin L	No	No	No

177

178 Note: No means no inhibition were observed to the activity of the corresponding enzyme with 10
179 μM metal complex.

180 **3.2 Inhibition mechanism and types of chloroauric acid on MMP-14**

181 Chloroauric acid possesses significant inhibitory effects on MMP-2, -13, and -14, especially for
182 MMP-14. By contrast, the effects on Cathepsin B and L are not significant. Therefore, the inhibition of
183 chloroauric acid on MMP-14 is specific to a certain degree although the specific inhibition mechanism
184 remains unclear. To evaluate the mechanism and type of inhibition, we performed further dynamic
185 analysis on the inhibition of MMP-14 by chloroauric acid. Different enzyme concentrations were added
186 into the test system, and then the same amount of substrate and inhibitor was added. Changes in the
187 enzyme activity were determined, and the curves of enzyme concentration and enzyme activity were
188 drawn (Fig. 2a). Under different concentrations of the enzyme (0.125 μg/ml−1 μg/ml) and inhibitor (0.2
189 and 0.4 μM), the reaction rate was reduced as compared with the control group, with a gradient
190 concentration dependent. All the straight lines in the plot passed through the zero point; therefore, the
191 inhibition of MMP-14 by chloroauric acid was initially determined as reversible inhibition.

In the following experiments, 1 μg/mL was selected as the final concentration of enzyme to analyze the inhibitory type; the respective final concentrations of inhibitors were 0, 0.2, 0.4, and 0.6 μM, according to the detected $IC_{50} = 0.3$ μM for the inhibition of MMP-14 by chloroauric acid; substrates with different final concentrations (0.5 μg/ml−5 μg/ml) were then added. A plot of the Michaelis−Menten equation is presented in Fig. 2b. K_m remained constant whereas V_{max} decreased with increasing inhibitor concentration. The plot of the Lineweaver−Burk equation is shown in Fig. 2c. With increasing concentration, K_m was constant but V_{max} decreased. Therefore, the observed inhibition is non-competitive. The Dixon plot equation was used to obtain Fig.2d. The two straight lines (representing substrate concentrations of 1 and 2 μg/ml) intersected at the second quadrant, with the position of intersection point as $-K_i$, which suggests non-competitive inhibition. Therefore, chloroauric acid noncompetitively inhibited MMP-14 within the concentration range of 0 μM−0.6 μM. That is, a competitive relationship does not exist between the respective effects of the substrate and chloroauric acid on MMP-14. The combination of MMP-14 with the substrate or chloroauric acid does not influence other combinations, but the formed compound cannot be degraded, thereby resulting in decreased enzyme activity. The binding site is the group beyond the active site. Its structure is not similar to that of the substrate, and the inhibition cannot be relieved by increasing the concentration of substrate.

A number of studies based on different physicochemical techniques suggest that the probable binding sites of Au(III) are N(1)/N(7) atoms of adenosine, N(7) or C(6)O of guanosine, N(3) of cytidine, and N(3) of thymidine, which are analogous to the possible binding sites of the isoelectronic Pt(II) ion [60]. Recent in vitro studies showed that the interactions of several Au(III) complexes with calf thymus DNA are weak, whereas significant binding to model proteins occurs [61]. This

phenomenon is supported by Fricker et al. [30], who demonstrated that a Au(III)-damp complex has a clear preference for S-donor ligands, such as glutathione and cysteine, with only limited reactivity against nucleosides and their bases. Therefore, a novel mechanism was proposed, wherein proteins containing exposed cysteine residues may be proper targets for that class of Au(III) complexes. Au(III) complexes also interact with bovine serum albumin [62]. These complexes make very stable adducts; once these adducts are formed, they are destroyed only by adding strong ligands for Au(III), such as cyanide [63]. Based on these findings, the selective modification of surface protein residues by Au(III) compounds could be the molecular basis for their biological effects. This hypothesis has prompted the search for novel gold–protein interactions in an attempt to identify possible targets responsible for the biological effects of gold compounds. The key proteins that are modified by Au(III) complexes and responsible for triggering apoptosis have yet to be identified [64].

MMP-14 is synthesized with a signal peptide (Signal), a prodomain (Pro) for latency, a catalytic domain (Catalytic) with catalytic zinc ion (Zn) for proteolytic activity, a linker-1 (L1), a hemopexin domain (Hpx), a linker-2 (L2), a transmembrane domain (TM), and a cytoplasmic tail (CP) [65]. Our results indicated that inhibition by chloroauric acid is achieved by interaction with a site other than the active center of MMP-14 within the catalytic domain of the enzyme. However, the exact location of the interaction remains to be identified.

3.3 Toxicity of chloroauric acid on HT-1080 cells by MTT assay

Based on the specific inhibitory effects of chloroauric acid on MMPs, especially MMP-14, the influence of chloroauric acid on the behavior of MMP-14-related tumor cells was analyzed. The MTT assay was used to determine the toxicity of chloroauric acid on HT-1080 cells. As shown in Fig. 3a,

chloroauric acid has certain influence to the viability of HT-1080 cells. Its IC_{50} was approximately 125 μM. No evident effects on the cell viability were observed when the chloroauric acid concentration was less than 100 μM. When the chloroauric acid concentration was less than 100 μM, increased cytotoxicity was notably absent despite the increasing concentration. However, the cell viability suddenly decreased when its concentration was greater than 100 μM. This trend may be attributed to the sudden increase of cell permeability when chloroauric acid enters into the cells, which eventually leads to cell death and decreased cell viability. According to the aforementioned results, 0 μM−50 μM was selected as the drug concentration range in the subsequent cell experiments to study its influence on the behavior of the tumor cells without affecting the cell vitality. We also performed the cell viability test of chloroauric acid and its sodium salt on a non-cancerous cell line, 3T3 cells, a established mouse embryo fibroblast cell line. The result is shown in Fig.3b. Our result revealed that chloroauric acid exhibited similar effect on the viability of HT-1080 cells and 3T3 cells. $NaAuCl_4$ showed similar effect on the viability of HT1080 cells and 3T3 cells compare to the chloroauric acid (Fig. 3c and 3d). However, it reduced the cell viability of HT-1080 cells at a much higher concentration compare to the chloroauric acid.

3.4 Chloroauric acid can effectively inhibit the invasion behaviors of HT-1080 cells

The Matrigel invasion assay is a common method to study tumor cells. Given their invasive nature, tumor cells can degrade matrix by secreting hydrolytic enzymes such as MMPs, thereby entering into the lower culture chamber through the micropores and then adhering to the bottom of the chamber. After staining, the invasive ability of tumor cells could be analyzed. An invasion assay was used to analyze the influences of chloroauric acid on the invasive ability of HT-1080 under conditions that do not affect the cell vitality. As shown in Fig. 4a and 4b, the number of invasion cells was significantly

257 decreased with increasing drug concentration. When the final concentration was 25 μM, the invasion
258 rate was <50% as compared with the control group. When the final concentration of chloroauric acid
259 was 50 μM, the invasion rate dropped to approximately 35%. Therefore, the aforementioned results
260 show that chloroauric acid has obvious inhibitory effects on the invasion of HT-1080 cells, and this
261 inhibition is concentration dependent. As MMP-14 is a key enzyme in controling the focal degradation
262 of ECM during cell migration [66], it is reasonable to speculate that chloroauric acid inhibits MMP-14
263 activity in the cell membrane surface and inhibits the ability of degradation of extracellular matrix,
264 which affect the cell migration and invasion.

265 Chloroauric acid has a specific inhibition effect on MMP-14 and can effectively inhibit the
266 invasion behaviors of HT-1080 cells. Further measurement of the pH value of cell culture media
267 containing the compound and the enzyme activity measurement system revealed that the pH value of
268 the reaction system is not changed in the applied concentrations of chloroauric acid. Therefore, the
269 inhibition of MMP-14 chloroauric acid, as well as the influence of chloroauric acid on cell migration
270 and invasion, was not influenced by the changing system pH. To analyze whether $AuCl_4^-$ is the active
271 group in the influences of chloroauric acid on HT-1080 cells, we selected the relatively stable sodium
272 salt of chloroauric acid for an enzymology study to determine the effects on the behavior of tumor cells.
273 The results show that both compounds had different inhibitory effects on proteases. $NaAuCl_4 \cdot 2H_2O$
274 exhibited an inhibitory effect on the MMP-2, -13, and -14 and did not suppress cathepsins, but its
275 inhibition of MMP-2, -13, and -14 was weaker than that of chloroauric acid, especially for MMP-14,
276 with IC_{50} values of 8, 6, and 10 μM, respectively (Table 1). In addition, its effects on the cytotoxicity
277 and behavior of HT-1080 cells were compared. The MTT assay (**Fig. 3c**) showed that $NaAuCl_4$ had
278 certain influence to the viability of HT-1080 cells, with an IC_{50} of approximately 350 μM. When

$NaAuCl_4$ was less than 250 μM, no evident effects on cell viability were observed. Compared with chloroauric acid (Fig. 3a and 3b), $NaAuCl_4$ had lower toxicity in HT-1080 cells (Fig. 3c) and 3T3 cells(Fig. 3d). Under conditions that did not affect cell vitality, $NaAuCl_4$ (12.5 μM−50 μM) had no obvious inhibitory effects on the migration and invasion of HT-1080 cells. On the contrary, results showed that $NaAuCl_4$ promoted these behaviors, as shown in **Fig. 4a and 4c.** The mechanism of this phenomena is unclear. Sodium ions are involved in various biological processes and are significant for the maintenance of cell vitality, which may eventually leads to the very different effects of the two compounds on tumor cell behaviors. Although we attempted to analyze the inhibitory effects of both compounds, further studies are still needed to clarify the effects of these two compounds on cell vitality.

In addition, based on the great structural variety of the ligands and the derived complexes, a unique mode of action or pharmacological profile for all Au(III) complexes is unlikely to exist. In particular, direct DNA damage, modification of the cell cycle, mitochondrial damage, such as thioredoxin reductase (TrxR) inhibition, proteasome inhibition, modulation of specific kinases, and other cellular processes, are affected by gold compounds; their interaction eventually triggers apoptosis, which seems to have a major role in the mechanism of action of gold compounds [21, 28]. Research on these topics is significant in understanding the roles of Au(III) and Au complexes in regulating the activity of MMPs.

4 Conclusions

In this paper, the results show that chloroauric acid have specific inhibitions to MMP-14, and the inhibition mechanisms are the non-competitive type, namely there is no competitive relationship

between the effects of the substrate and chloroauric acid on MMP-14. These results indicate that the inhibition are performed by combining the compounds with the non-active center of MMP-14 and changing its structure and conformation, which has not been reported so far. We have also found that chloroauric acid can affect the behaviors of tumor cell HT-1080, and in particular, chloroauric acid may play a role in tumor cell behaviors by inhibiting MMP-14. The research of thesis is very meaningful for the understanding of Au(III) and Au complexes in regulating the activity of MMPs.

References

1. Bellayr IH, Mu X, Li Y, *Future Med Chem*,2009, 1, 1095-1111.
2. Vargová V, Pytliak M, Mechírová V, *EXS*, 2012, 103, 1-33.
3. Bischof P, Campana A, *Baillieres Best Pract Res Clin Obstet Gynaecol*, 2000, 14, 801-814.
4. Srinivasan V, Spence DW, Moscovitch A, Pandi-Perumal SR, Trakht I, Brown GM, Cardinali DP. Cardinali, J Pineal Res, 2010, 48, 1-8.
5. Ganguly K, Sharma AV, Reiter RJ, Swarnakar S, *J Pineal Res*, 2010, 49, 130-140.
6. Sternlicht M D, Werb Z, Annu, *Rev. Cell Dev. Biol*, 2001, 17463-17516.
7. Ege blad M, Werb Z, *Nat. Rev. Cancer*, 2002, 2, 161-174.
8. Dilmé JF, Bellmunt S, Camacho M, Solà-Villà D, Romero JM, Escudero JR, Vila L, *Eur J Vasc Endovasc Surg*, 2014, 48, 374-381.
9. Milner JM, Cawston TE, *Curr. Drug Targets. Inflamm. Allergy*, 2005, 4, 363-375.
10. Burrage PS, Brinckerhoff CE, *Curr. Drug Targets*, 2007, 8, 293-303.

11. Murphy G, Nagase H, Nat. *Clin. Pract. Rheumatol*, 2008, 4, 128-135.

12. Murphy G, Nagase H, *Molecular Aspects of Medicine*, 2008, 29, 290-308.

13. Chaudhary AK, Pandya S, Ghosh K, Nadkarni A, *Mutation Research*, 2013, 753, 7-23.

14. Shen Y, Liu S, Jin F, Mu T, Li C, Jiang K, Tian W, Yu D, Zhang Y, Fang X, Biometals, 2012, 25, 541-551.

15. Williams, Jack Marvin, Peterson, Selmer Wiefred, *Journal of the American Chemical So*ciety, 1969, 91, 776-777. doi:10.1021/ja01031a062. ISSN 0002-7863.

16. Adam J. Bridgeman, *Inorganic Chemistry*, 2008, 47, 4817-4825

17. Ronconi L, Giovagnini L, Marzano C, Bettio F, Graziani R, Pilloni G, Fregona D, *Inorg. Chem*, 2005, 44, 1867-1881

18. Sadler PJ, *J Rheumatol Suppl*, 1982, 8, 71-78.

19. Lei Y, Chen H, Dai H, Zeng Z, Lin Y, Zhou F, Pang D, *Biosens Bioelectron*, 2008, 23, 1200-1207.

20. Smithies O, Lawrence M, Testen A, Horne LP, Wilder J, Altenburg M, Bleasdale B, Maeda N, Koklic T, *Langmuir*, 2014, 30,13394-13404

21. Maia, Deflon, Abram, *Future Med. Chem*, 2014, 6, 1515-1536.

22. Nakajima A. *World J. Microb. Biot*, 2003, 19, 369-374.

23. John Wiley, Sons, West Sussex, Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicine. Gielen M, Tiekink ERT (Eds). UK (2005).

24. Liu F, Anis R, Hwang E, Ovalle R, Varela-Ramírez A, Aguilera RJ, Contel M, *Molecules*, 2011, 16, 6701-6720 .

25. Maia PI, Fernandes AG, Silva JJ, Andricopulo AD, Lemos SS, Lang ES, Abram U, Deflon VM, *J Inorg. Biochem*, 2010,104, 1276-1282.

26. Navarro M, *Coord. Chem. Rev*, 2009, 253, 1619-1626.

27. Navarro M, Vasquez F, Sanchez-Delgado RA, Perez H, Sinou V, Schrevel J. *J. Med. Chem*, 2004, 47, 5204-5209.

28. Milacic V, Fregona D, Dou QP, *Histol Histopathol*, 2008, 23, 101-108

29. McKeage MJ, Maharaj L, Berners-Price SJ, *Coord Chem. Rev*, 2002, 232, 127-135.

30. Fricker SP, Skerjl R, Cameron BR, Mosi R, Zhu Y, Recent developments in gold drugs 2003; AnorMED Inc, Langley BC, V2Y 1N5, Canada. GOLD 2003.

31. Marcon G, Messori L, Orioli P, Cinellu MA, Minghetti G, *Eur. J. Biochem*, 2003, 270, 4655-4661.

32. Milacic V, Chen D, Ronconi L, Landis-Piwowar KR, Fregona D, Dou QP, *Cancer Res*, 2006, 66, 10478-10486.

33. Bottenus BN, Kan P, Jenkins T, Ballard B, Rold TL, Barnes C, Cutler C, Hoffman TJ, Green MA, Jurisson SS, *Nucl. Med. Biol*, 2010, 37, 41-49.

34. Che CM, Sun RW, *Chem Commun (Camb)*, 2011, 47, 9554-9560.

35. Dreaden EC, Austin LA, Mackey MA, El-Sayed MA, *Ther Deliv*, 2012,3, 457-478.

36. Wang H, Huff TB, Zweifel DA, He W, Low PS, Wei A, Cheng JX, *Proc. Natl Acad. Sci. USA*. 2005, 102, 15752-15756.

37. Zavaleta CL, Smith BR, Walton I, Doering W, Davis G, Shojaei B, Natan MJ, Gambhir SS, *Proc. Natl Acad. Sci. USA*, 2009, 106, 13511-13516.

38. Lu W, Huang Q, Ku G, Wen X, Zhou M, Guzatov D, Brecht P, Su R, Oraevsky A, Wang LV, Li C, *Biomaterials,* 2010, 31, 2617-2626.

39. Kim D, Jeong YY, Jon S, *ACS Nano*, 2010, 4, 3689-3696.

40. Von Maltzahn G, Centrone A, Park JH, Ramanathan R, Sailor MJ, Hatton TA, Bhatia SN, *Adv. Mater*, 2009, 21, 1-6.

41. Dreaden EC, Mwakwari SC, Sodji QH, Oyelere AK, El-Sayed MA, *Bioconjug. Chem*, 2009, 20, 2247-2253.

42. Giljohann DA, Seferos DS, Prigodich AE, Patel PC, Mirkin CA, *J. Am. Chem. Soc*, 2009, 131, 2072-2073.

43. Dhar S, Daniel WL, Giljohann DA, Mirkin CA, Lippard SJ, *J. Am. Chem. Soc*, 2009, 131, 14652-14653.

44. Hainfeld JF, Dilmanian FA, Zhong Z, Slatkin DN, Kalef-Ezra JA, Smilowitz HM, *Phys. Med. Biol*, 2010, 55, 3045-3046.

45. Chithrani DB, Jelveh S, Jalali F, van Prooijen M, Allen C, Bristow RG, Hill RP, Jaffray DA, *Radiat. Res*, 2010, 173, 719-728.

46. Qian XM, Peng XH, Ansari DO, Yin-Goen Q, Chen GZ, Shin DM, Yang L, Young AN, Wang MD, Nie S, *Nat. Biotechnol*, 2008, 26, 83-90.

47. Jung Y, Reif R, Zeng Y, Wang RK, *Nano Lett*, 2011, 11, 2938-2943

48. Lewis JB, Wataha JC, McCloud V, Lockwood PE, Messer RL, Tseng WY, *J Biomed Mater Res A*, 2005, 74, 474-481.

49. Amălinei C, Căruntu ID, Bălan RA, *Romanian Journal of Morphology and Embryology*, 2007, 48, 323-334.

50. Ardif G, Reboul P, Pelletier J P, Martel-Pelletier J, *Mod Rheumatol*, 2004, 14, 197-204.

51. Hashizume K, *J Reprod Dev*, 2007, 53, 1-11.

52. Weaver MA, *Clin Exp Metastasis*, 2006, 23, 97-105.

53. Shi X, Jin F, Wang H, Yang J, Wang Z, Fang X, *Chemical Research in Chinese Universities*, 2006, 22, 129-133.

54. Yang J, Shen Y, Hong Y, Jin F, Zhao S, Wang M, Shi X, Fang X, *Journal of Ethnopharmacology*, 2008, 117, 285-289.

55. Fang X, Zhan Y, Yang J, Yu D, *Journal of Molecular Catalysis B: Enzymatic*, 2014, 104, 1-7.

56. Zhang X, Shi L, Li X, Sheng Q, Yao L, Shen D, Lü ZR, Zhou HM, Park YD, Lee J, Zhang Q, *J Biosci Bioeng*, 2014, 117, 696-705.

57. Ji H, Wang J, Fang B, Fang X, Lu Z, *J Neurooncol*, 2011, 103, 445-451.

58. Johnson JL, George SJ, Newby AC, Jackson CL, *Proc. Natl. Acad. Sci USA*, 2005, 102, 15575-15580.

59. Kim YS, Joh TH, *Disorders Biomol Ther (Seoul)*, 2012, 20, 133-143.

60. Crooke ST, Mirabelli CK, *Am J Med*, 1983, 75, 109-113.

61. Marzano C, Bettio F, Baccichetti F, Trevisan A, Giovagnini L, Fregona D, *Chem. Biol. Interact*, 2004, 148, 37-48.

62. He XM, Carter DC, *Nature*, 1992, 358, 209-215.

63. Messori L, Marcon G, Orioli P, *Bioinorg. Chem. Appl*, 2003, 1, 177-187.

64. Chow KH, Sun RW, Lam JB, Li CK, Xu A, Ma DL, Abagyan R, Wang Y, Che CM, Cancer Res, 2010, 70, 329-337.

65. Yoshifumi Itoh, *IUBMB Life*, 2006, 58, 589-596.

66. Andrei V Chernov, Nor Eddine Sounni, Albert G Remacle, Alex Y Strongin, *The journal of biological chemistry*, 2009, 284, 12727-12734.

Figure captions

Fig. 1 a-c Dose-dependent inhibition effect of the chloroauric acid on MMP-2, -13, and -14

The enzyme was incubated with chloroauric acid at 37 °C for 15 min. The activity was then determined using an FLx800 fluorescence microplate reader (Bio-Tek). Enzyme reaction mixtures contained 0.2 μg of DQ-gelatin substrate and 1 μl of enzyme solution in 100 μl of 50 mM HEPES buffer (pH 7.5), with 0.2 M NaCl, 1 mM $CaCl_2$, 20 μM $ZnSO_4$, and 0.05% Brij-35 at 37 °C. Data are presented as the mean values ($n = 3$). The activity data were fitted in a linearised curve using Origin version 7.5. The IC_{50} values were then determined from the curve accordingly.

Fig. 2 Inhibition mechanism and mixed-type inhibition kinetics of chloroauric acid on MMP-14 activity

a: Plots of chloroauric acid concentrations (v) versus the concentration of MMP-14. Values of v were 0 μM (closed squares), 0.2 μM (closed circles), and 0.4 μM (closed triangles).

b: MMP-14 activity in the presence of different chloroauric acid and substrate concentrations.

c: Lineweaver–Burk plot. The chloroauric acid concentrations were 0 μM (closed squares), 0.2 μM (closed circles), 0.4 μM (closed triangles), and 0.6 μM (inverted closed triangles).

d: Dixon plots of reversible enzyme inhibitors. The substrate concentration for curves 1–4 were 0, 0.2, 0.4, and 0.6 μM, respectively.

Data were reported as mean ± SD and at least three separate experiments were performed.

Fig. 3 Chloroauric acid inhibits HT-1080 cell viability

a, c: An in vitro study was initiated by treating HT-1080 cells with increasing doses of chloroauric acid (0, 5, 10, 20, 40, 60, 80, 100 150, 200, and 250 μM) and $NaAuCl_4$ (0, 7, 15, 31, 62, 100 ,125, 200, 250, 350, and 500 μM) for 24 h.

b, d : An in vitro study was initiated by treating non-cancerous cells (3T3 cells) with increasing doses of chloroauric acid and $NaAuCl_4$ (0, 7.5, 15, 31, 62.5, 100 ,125, 200, and 250μM) for 24 h.

The viability of the chloroauric acid and $NaAuCl_4$ treated cells was measured using the MTT assay. Results were expressed as a percentage of control, which was considered as 100%. * indicates $P < 0.05$ compared with the controls. ** indicates $P < 0.01$ compared with the controls. Data were shown as means ± SD from three independent experiments.

Fig. 4 Chloroauric acid inhibits invasion in a dose-dependent manner in HT -1080 cell lines

Approximately 1×10^5 cells were seeded into 24-well plates with cell culture inserts, the cells were treated with different concentrations of chloroauric acid or $NaAuCl_4$ (0, 12.5, 25, and 50 μM) . After 24 h of incubation, the cells that invaded to the underside of the filters were visualized under a microscope (a), and the cell invasion ratio was calculated (b and c). The obtained values were calculated from the average total number of cells of three filters. Results are presented as the mean ± SD of three independent experiments. * indicates $P < 0.05$ compared with the controls. ** indicates $P < 0.01$ compared with the controls. Data were shown as means ± SD from three independent experiments.

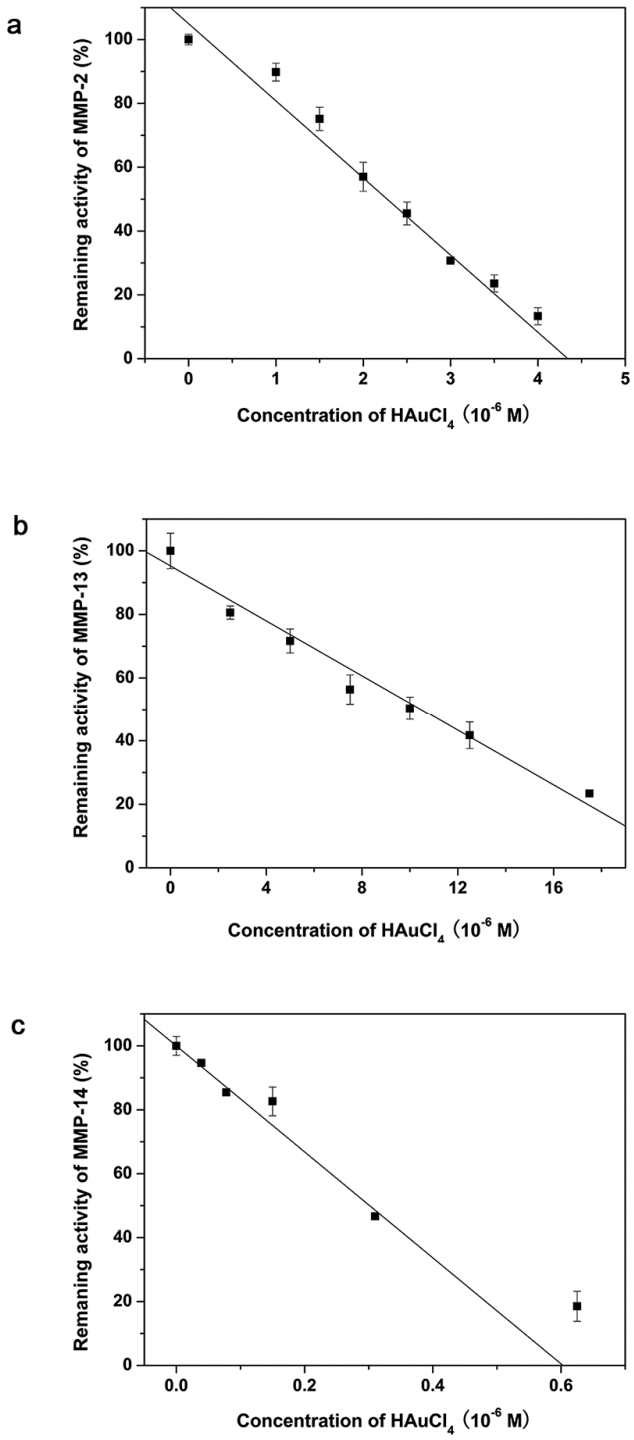


Fig. 1

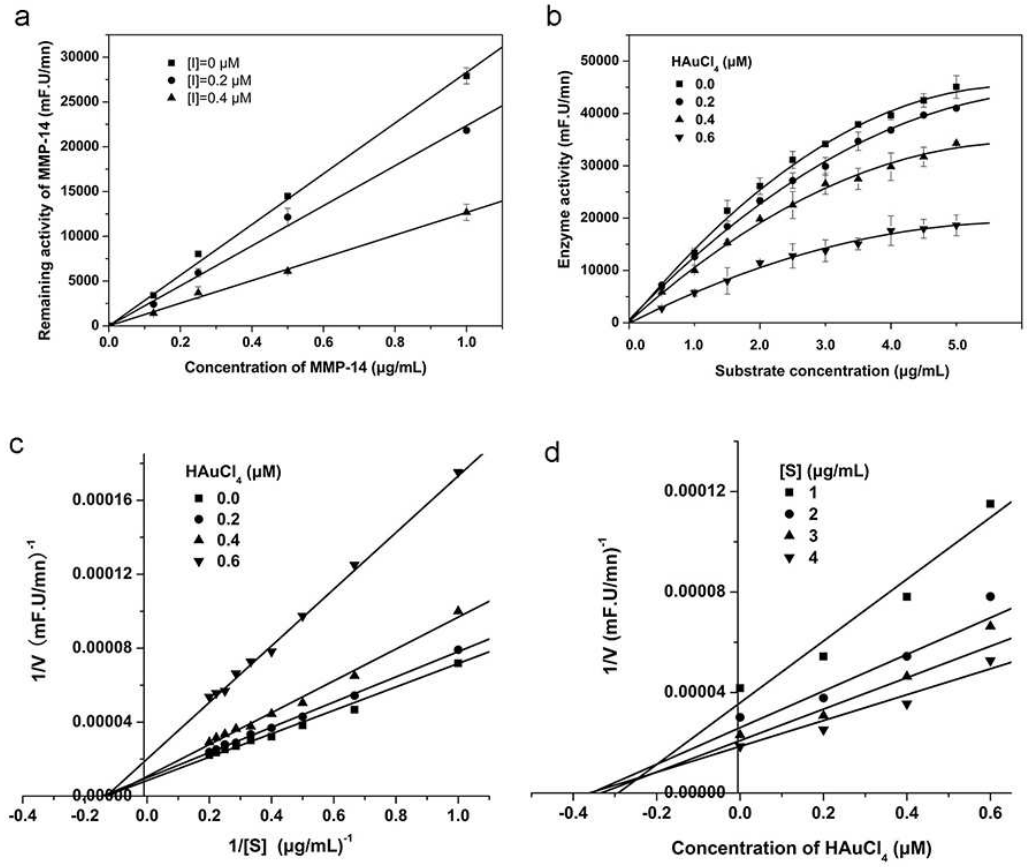


Fig. 2

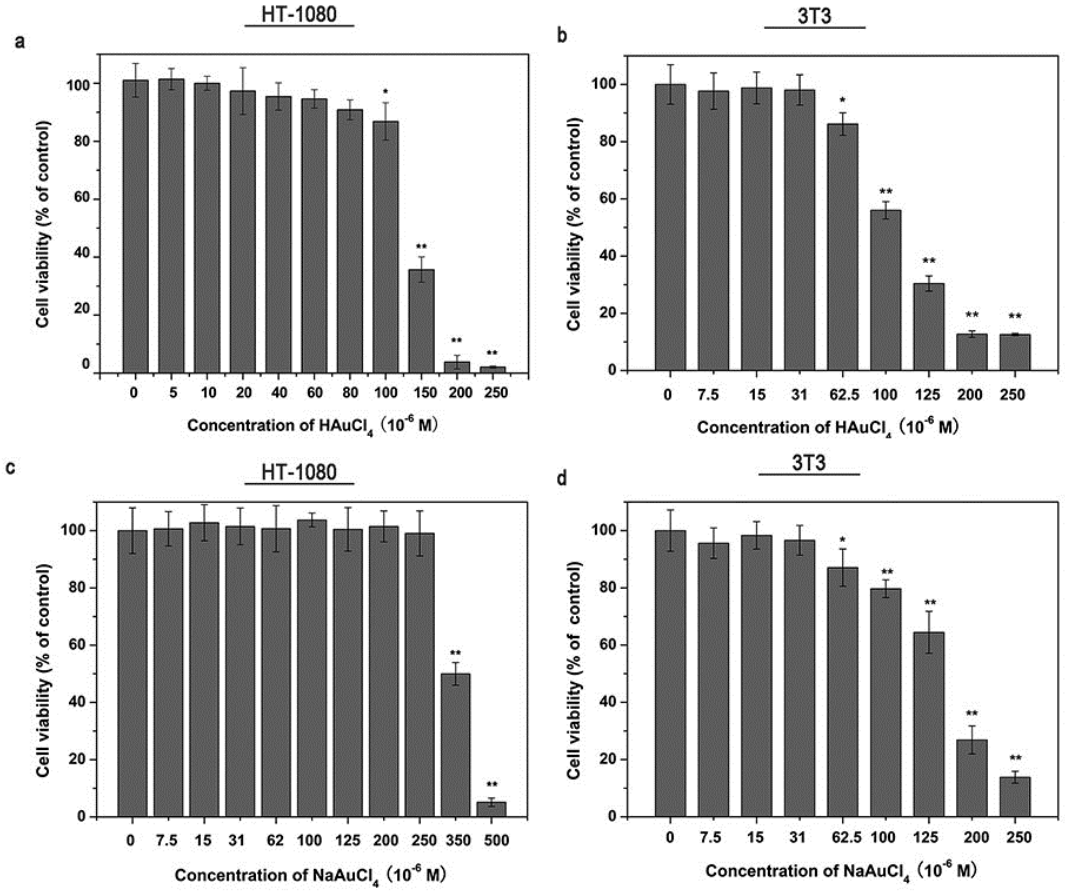


Fig. 3

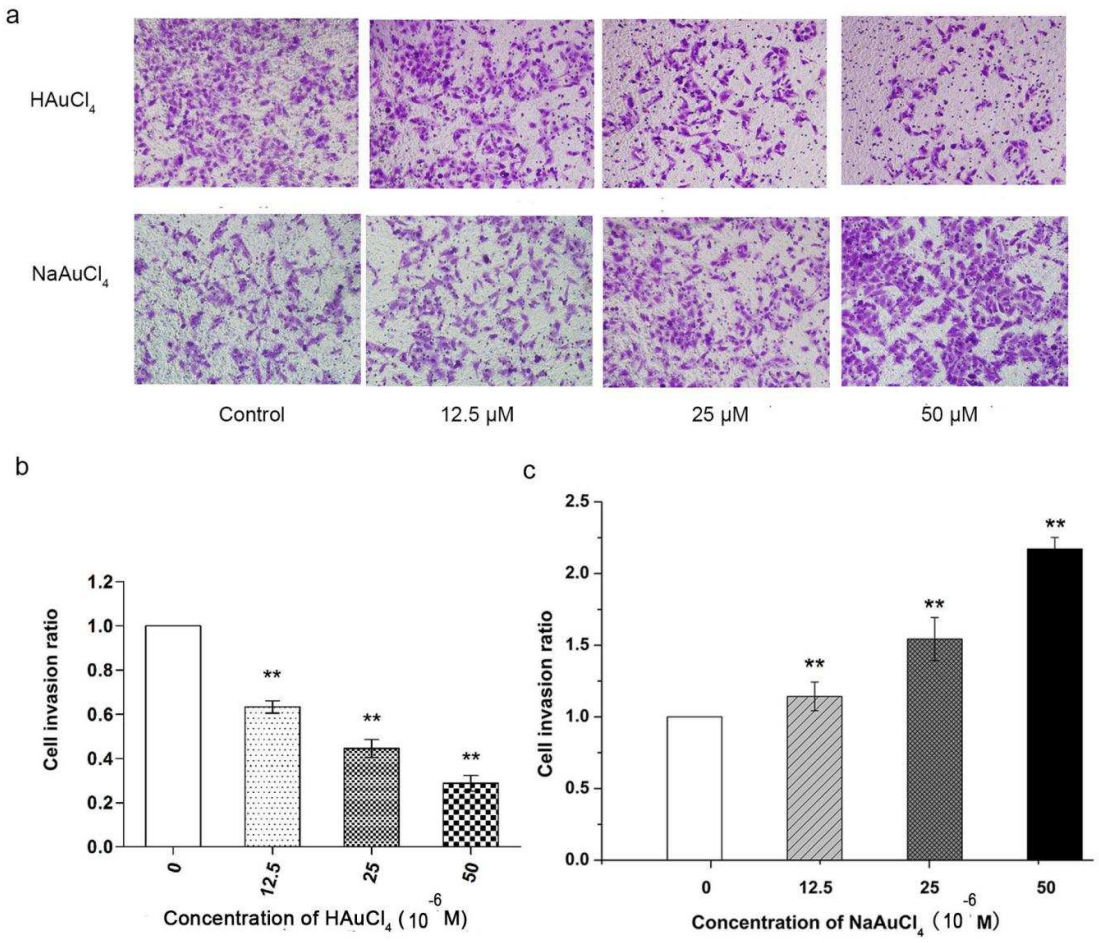
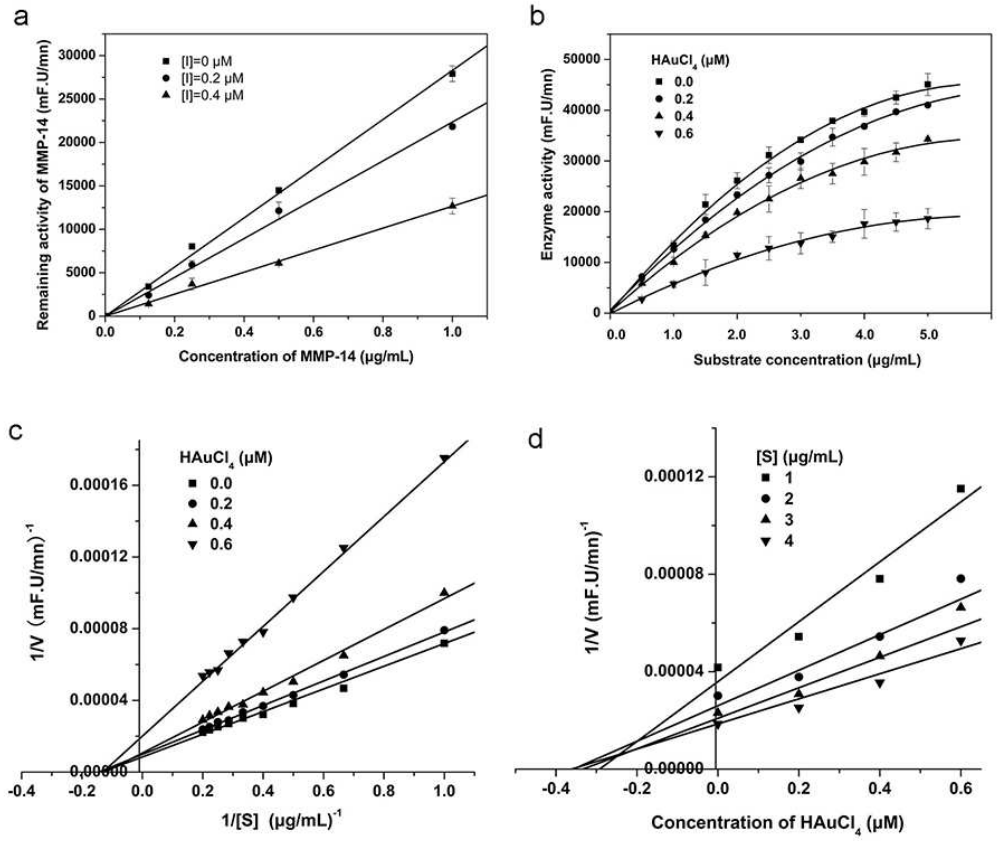


Fig. 4



Enzyme kinetics and **matrigel invasion assay** indicated that the specific inhibition of $HAuCl_4$ on MMP-14 involves a non-competitive reversible inhibitory mechanism and $HAuCl_4$ inhibits HT-1080 cell invasion in a dose-dependent manner.