

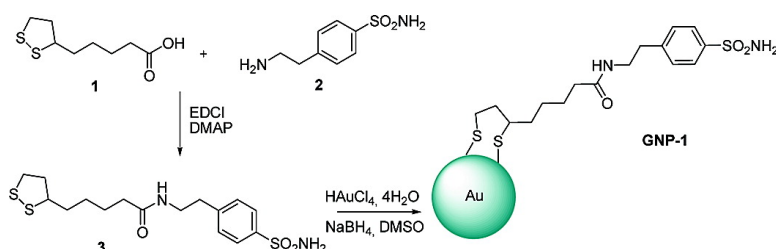
Communication

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Carbonic Anhydrase Inhibitor Coated Gold Nanoparticles Selectively Inhibit the Tumor-Associated Isoform IX over the Cytosolic Isozymes I and II

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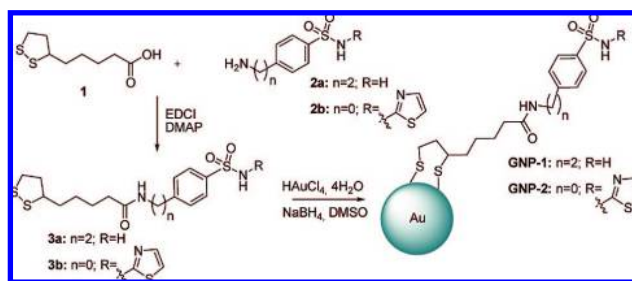
The carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metallo-enzymes with five independently evolved ($\alpha, \beta, \gamma, \delta, \zeta$) classes reported up to date.^{1–3} These enzymes catalyze the reversible hydration of carbon dioxide to bicarbonate and protons by means of a metal-hydroxide ($\text{Lig}_3\text{M}^{2+}(\text{OH})^-$) mechanism.²

In addition to the established role of the carbonic anhydrase inhibitors (CAIs) as diuretics and antiglaucoma drugs, it has recently emerged that CAIs could have potential as novel antiobesity, anticancer, and anti-infective drugs.^{1,2} A critical problem in the design of CAIs is related to the high number of isoforms in mammals (15), their diffuse localization in many tissues/organs, and the lack of isozyme selectivity of the presently available inhibitors.^{1–4}

Recently, nanoparticles received great attention for their biomedical applications both for the site-specific delivery of drugs⁷ or for imaging purposes.^{7,8} Several recent such examples include HIV inhibition with a CCR5 antagonist attached to multivalent gold nanoparticles (Au NPs), tumor targeting with NPs loaded with hydroxycamptothecin, paclitaxel, or fumagillin, and magnetic resonance imaging (MRI) techniques of tumors based on integrins targeting with Fe_3O_4 NPs, computer tomography (CT) or MRI imaging with gadolinium chelate Au NPs, etc.^{7,8}

Human CA IX (hCA IX) is an extracellular, transmembrane isoform which was recently shown to constitute a novel and interesting target for the anticancer therapy due to its overexpression in many cancer tissues and not in their normal counterparts.^{1b,9} Its expression is strongly induced by hypoxia present in many tumor tissues and correlated with a bad response to classical chemo- and radiotherapies.^{1b,9} CA IX was shown to acidify the extratumoral medium leading both to the acquisition of metastatic phenotypes and to chemoresistance with many anticancer drugs, these processes being reverted by inhibition of the enzyme catalytic activity with sulfonamide inhibitors.^{1b,9} The development of selective CA IX inhibitors might provide useful tools for highlighting the exact role of CA IX in hypoxic cancers, to control the pH imbalance of tumor cells and lead to novel diagnostic or therapeutic applications for the management of such tumors.^{1b,9} Although many sulfonamide/sulfamate/sulfamide potent CA IX inhibitors were reported, few of them show an acceptable level of selectivity for inhibiting the transmembrane-tumor associated target isoform IX over the cytosolic, ubiquitous isozymes hCA I and II.^{1b,9} Considering the extracellular localization of the target CA isoform and the fact that nanomaterials generally show membrane impermeability,¹⁰ we

Scheme 1. Synthesis of Au NPs Coated with Sulfonamide CAI, of Type **GNP-1** and **GNP-2**



report here the synthesis of CAI coated Au NPs which show excellent CA IX inhibitory properties and selectivity for the inhibition of the tumor-associated isoform over hCA I and II.

The key intermediate **3a** was synthesized by coupling lipoic acid **1** with 4-aminoethylbenzene sulfonamide **2a** in the presence of EDCI/DMAP, as outlined in Scheme 1. The CAI coated Au NPs (**GNP-1**) were then prepared in a single step by reduction of chloroaurate with NaBH₄ in the presence of the lipoic acid tailed sulfonamide **3a**.^{11,12} The same strategy has been used to prepare a sulfathiazole–lipoic acid Au NPs conjugate (**GNP-2**) which has been used as a control, since substituted sulfonamides do not act as CAIs.¹ The NPs were characterized by transmission electron microscopy (TEM) (Supporting Information, Figure 1) being observed that they are roughly spherical in shape. These particles are monodispersed with an average particle size of 3.3 nm which corresponds to ~720–724 Au atoms. Energy dispersive X-ray analysis (EDX) and elemental analysis allowed us to estimate the number of sulfonamide ligands attached to the NP as being 144 for **GNP-1** and 135 for **GNP-2**. The average surface area occupied by one ligand unit **3a** is ~0.24 nm². **GNP-1** has the empirical formula [Au₇₂₀(C₁₆H₂₄O₃N₂S₃)₁₄₄], and **GNP-2** [Au₇₂₄(C₁₇H₂₁O₃N₃S₄)₁₃₅], which were confirmed by elemental analysis data. The powdered materials **GNP-1/2** showed an intense plasmon band absorbance at 540 nm characteristic of Au(0) NPs.¹² The powder X-ray diffraction (XRD) patterns of the Au NPs **GNP-1/2** in the range 2θ (20°–140°) (Supporting Information Figure 2) presented seven of the main diffractions characteristic of the Au cubic phase at 38.6°, 44.9°, 64.9°, 78.1°, 82.2°, 111.2°, and 135.5°. The peak broadness may be explained by the small size of the crystalline domains. The peak of the d₁₁₁ reflection has been deconvoluted to the Lorentzian curve for determining the full width at half-maximum (FMWH) value. The crystalline domain has been calculated from the Debye–Scherrer formula using the FMWH value of the corresponding index peaks giving an average

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Table 1. CA Inhibition Data Against Isoforms CA I, II and IX With the Standard Sulfonamide in Clinical Use Acetazolamide **AZA**, the New Sulfonamides **3a,b**, and **GNP-1/2**^a

compound	K_i (nM)		
	hCA I	hCA II	hCA IX
AZA	250 ± 12	12 ± 1	25 ± 1
3a	214 ± 9	230 ± 10	41 ± 2
GNP-1	581 ± 18 (128)	451 ± 21 (116)	32 ± 2 (2.4)
3b	>50 000	>50 000	>50 000
GNP-2	28 550	30 400	31 050
Au@	32 000	31 600	29 560

^a Data in parentheses show the inhibition constants when enzyme and inhibitor were incubated for 2 h.¹⁴

value of ~4.0 nm which is concomitant with the value 3.3 ± 1.4 nm obtained from TEM images.¹²

Inhibition data of **3a,b**, **GNP-1/2**, the standard, clinically used CAI acetazolamide **AZA** (5-acetamido-1,3,4-thiadiazole-2-sulfonamide), and Au NPs (Au@) as control, against isoforms hCA I and II (cytosolic isoforms) and the transmembrane, tumor-associated isozyme hCA IX (a construct incorporating the catalytic domain and proteoglycan regions of the enzyme),¹³ are shown in Table 1.¹⁴ A stopped-flow method has been used for assaying the CA-catalyzed CO₂ hydration activity with Phenol red as indicator, working at the absorbance maximum 557 nm, following the initial rates of the CA-catalyzed CO₂ hydration reaction for 10–100 s. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.01 μM) were prepared in distilled-deionized water with 5% DMSO, and dilutions up to 0.01 nM were done thereafter with distilled-deionized water. The NPs were soluble in this solvent mixture. Inhibitor (concentration range 0.01 μM–0.01 nM) and enzyme solutions ([E] = 10 nM) were preincubated together for 15 min–2 h at room temperature prior to assay, to allow for the formation of the E–I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3, and represent the mean from at least three different determinations. In the standard conditions used to determine the inhibition constants, i.e., incubation time 15 min, the sulfonamides **AZA**, **3a** and **GNP-1** were modest hCA I inhibitors (K_i 's of 214–581 nM), **AZA** was an effective CA II and IX inhibitor (K_i 's of 12–25 nM) whereas the new sulfonamides **3a** and **GNP-1** were moderate–weak CA II inhibitors (K_i 's of 230–451 nM) and effective CA IX inhibitors (K_i 's of 32–41 nM). However, when inhibitors and enzymes were incubated for 2 h (or longer) **GNP-1** (but not the other sulfonamides) showed an enhanced inhibitory activity against all three isozymes, with inhibition constants of 128 nM against hCA I, 116 nM against hCA II, and 2.4 nM against hCA IX, respectively (Table 1). In control experiments, the sulfathiazole liponic acid conjugate **3b**, its Au NPs derivative **GNP-2**, and uncoated Au NPs were assayed under the same conditions. It may be observed that **3b**, **GNP-2**, and Au@ show very weak, micromolar inhibition or no inhibition at all against all CA isoforms. Thus, the inhibition observed with **GNP-1** is due to the interactions of its sulfonamide moiety with the enzyme active site. Inhibitors **3a** and **GNP-1** also showed good selectivity for inhibiting CA IX over CA I and II (Table 1), probably due to the fact that some key residues for the binding of inhibitors^{1,2,4} (such as Phe131 and Gly132) are different in the cytosolic and transmembrane isozymes, as rationalized earlier by us.⁹

We investigated the penetrability of these CAIs through membranes using red blood cells (RBCs) as an experimental model for *in vivo* inhibition of transmembrane versus cytosolic isozymes.¹⁵ The experi-

ments were performed by incubation of RBCs with millimolar concentrations of sulfonamide inhibitors (**AZA**, **3a**, and **GNP-1**). Incubation with **AZA** and sulfonamide **3a** led to saturation with the inhibitor of the two isozymes present in erythrocytes (CA I and II), after 30–60 min (Supporting Information, Table 2).^{15,16} This is due to the high diffusibility through membranes of these inhibitors. On the contrary, **GNP-1** was only detected in negligible amounts within the RBCs (even after 2–24 h incubation time), proving that the CAI coated Au NPs are unable to penetrate through biological membranes. These experiments show that the CAI coated Au NPs are totally membrane-impermeant, which is a highly desirable feature for a compound that should inhibit selectively only CA IX which possesses and extracellular active site. Thus, **GNP-1** constitutes an interesting candidate to be investigated for both imaging and treatment purposes of tumors overexpressing CA IX.

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Supporting Information Available: The synthesis, characterization, and *in vitro*/*in vivo* enzymatic studies are described in detail. This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

References

- (1) (a) Krishnamurthy, V. M.; Kaufman, G. K.; Urbach, A. R.; Gitlin, I.; Gudiksen, K. L.; Weibel, D. B.; Whitesides, G. M. *Chem. Rev.* **2008**, *108*, 946–1051. (b) Supuran, C. T. *Nat. Rev. Drug Discovery* **2008**, *7*, 168–181.
- (2) (a) Supuran, C. T.; Scozzafava, A.; Conway, J., Eds. *Carbonic anhydrase - Its inhibitors and activators*; CRC Press: Boca Raton, FL, 2004; pp 1–376 and references cited therein. (b) Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146–189.
- (3) (a) Tripp, B. C.; Smith, K. S.; Ferry, J. G. *J. Biol. Chem.* **2001**, *276*, 48615–8. (b) Klengel, T.; Liang, W. J.; Chaloupka, J.; Ruoff, C.; Schropel, K.; Naglik, J. R.; Eckert, S. E.; Morgensen, E. G.; Haynes, K.; Tuite, M. F.; Levin, L. R.; Buck, J.; Mühlischlegel, F. A. *Curr. Biol.* **2005**, *15*, 2021–6.
- (4) Aaron, J. A.; Chambers, J. M.; Jude, K. M.; Di Costanzo, L.; Dmochowski, I. J.; Christianson, D. W. *J. Am. Chem. Soc.* **2008**, *130*, 6942–3.
- (5) (a) Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Ilies, M. A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4884–892. (b) Supuran, C. T.; Clare, B. W. *Eur. J. Med. Chem.* **1999**, *34*, 41–50.
- (6) Nishimori, I.; Onishi, S.; Takeuchi, H.; Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 622–30.
- (7) (a) Bowman, M. C.; Ballard, T. E.; Ackerson, C. J.; Feldheim, D. L.; Margolis, D. M.; Melander, C. *J. Am. Chem. Soc.* **2008**, *130*, 6896–7. (b) Xie, J.; Chen, K.; Lee, H. Y.; Xu, C.; Hsu, A. R.; Peng, S.; Chen, X.; Sun, S. *J. Am. Chem. Soc.* **2008**, *130*, 7542–3. (c) Wang, A.; Li, S. *BMC Biotechnol.* **2008**, *8*, 46–53.
- (8) Ansell, S. M.; Johnstone, S. A.; Tardi, P. G.; Lo, L.; Xie, S.; Shu, Y.; Harasym, T. O.; Harasym, N. L.; Williams, L.; Bermudes, D.; Liboiron, B. D.; Saad, W.; Prud'homme, R. K.; Mayer, L. D. *J. Med. Chem.* **2008**, *51*, 3288–96.
- (9) (a) Winum, J. Y.; Rami, M.; Scozzafava, A.; Montero, J. L.; Supuran, C. T. *Med. Res. Rev.* **2008**, *28*, 445–63. (b) Thiry, A.; Dogné, J. M.; Masereel, B.; Supuran, C. T. *Trends Pharmacol. Sci.* **2006**, *27*, 566–73. (c) Svastova, E.; Hulikova, A.; Rafajova, M.; Zatovicova, M.; Gibadulinova, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C. T.; Pastorek, J.; Pastorekova, S. *FEBS Lett.* **2004**, *577*, 439–45. (d) Alterio, V.; Vitale, R. M.; Monti, S. M.; Pedone, C.; Scozzafava, A.; Cecchi, A.; De Simone, G.; Supuran, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 8329–35.
- (10) Verma, A.; Uzun, O.; Hu, Y.; Hu, Y.; Han, H. S.; Watson, N.; Chen, S.; Irvine, D. J.; Stellacci, F. *Nat. Mater.* **2008**, *7*, 588–95.
- (11) Brust, M.; Fink, J.; Bethell, D.; Schiffrin, D. J.; Kiely, C. *J. Chem. Soc., Chem. Commun.* **1995**, 1655–1656.
- (12) Daniel, M. C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293–346.
- (13) Hilvo, M.; Baranauskienė, L.; Salzano, A. M.; Scaloni, A.; Matulis, D.; Innocenti, A.; Scozzafava, A.; Monti, S. M.; De Simone, G.; Lindfors, K.; et al. *J. Biol. Chem.* **2008**, *283*, 27799–809.
- (14) Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561–2573.
- (15) Wistrand, P. J.; Lindqvist, A. In *Carbonic Anhydrase—From Biochemistry and Genetics to Physiology and Clinical Medicine*; Botrè, F.; Gros, G.; Storey, B. T., Eds.; VCH: Weinheim, 1991; pp 352–378.
- (16) Scozzafava, A.; Briganti, F.; Ilies, M. A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 292–300.

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