Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c5ob01005e

Exploring carbonic anhydrase inhibition with multimeric coumarins displayed on a fullerene scaffold†

Marta Abellán-Flos,[a] Muhammet Tanç,[b] Claudiu T. Supuran*[b] and Stéphane P. Vincent*[a]

Received 18th May 2015,
Accepted 4th June 2015

DOI: 10.1039/c5ob01005e

www.rsc.org/obc

Carbonic anhydrases (CAs) are ubiquitous Zn metallo-enzymes that catalyze the reversible hydration/dehydration of CO_2/HCO_3^-. CAs are involved in many key biological processes, therefore their inhibition has become an attractive research field. Distinct families of CA inhibitors (CAIs) have been reported, most of them interacting with the Zn(II) at the active site. Some compounds such as the coumarins are hydrolyzed before binding the entrance of the active site cavity, and thus behave as "suicide" inhibitors. This study reports the first synthesis of multimeric suicide inhibitors, designed to address the selectivity and the potency of CA multivalent inhibition. Twelve coumarin units have been grafted to a central fullerene scaffold thanks to a CuAAC reaction and the final dodecamers were assayed against 4 relevant CAs. The multimers were always stronger inhibitors than the monomeric species but no strong "multivalent effect" was found. However, our study showed that the multimeric presentation of the coumarin around the C_{60}, indeed affected the selectivity of the relative inhibition among the 4 CAs assayed.

Introduction

Multivalency has received much attention in biomedicine and bioorganic synthesis in the last few years because it plays a critical role in major biological phenomena.[1] Natural processes, especially at the cell interface, take advantage of the multimeric presentation of biological entities to enhance the efficiency of binding events. This is particularly true for carbohydrate–protein interactions, which are notoriously weak at the monomer level, but can be dramatically strengthened if the ligand is presented in a multivalent manner.[2] Inspired by these natural phenomena, scientists have dedicated tremendous effort to understanding the underlying mechanisms of multivalency but also to create artificial multivalent systems.[3] The most studied systems are probably lectin-carbohydrate dyads. Lectins, which bind carbohydrate epitopes without catalyzing reactions, often display more than one carbohydrate recognition domain or are structurally multimeric, thus favouring the interaction with multimeric presentation of their carbohydrate ligand. Therefore, a vast research in the lectin-carbohydrate field has been carried out to design potent inhibitors of biologically relevant lectins involved in pathological processes.[3g–i,4] A typical example is the inhibition of the adhesion of pathogenic bacteria to their host tissues in order to prevent bacterial infections.[5]

On the other hand, the multivalent inhibition of enzymes has not been substantially explored until 2009, when a clear multivalent effect was demonstrated and quantified in α-mannosidase inhibition by iminosugar clusters.[6] Shortly after, a fullerene bearing twelve copies of an iminosugar proved to be a very potent inhibitor of jack bean mannosidase.[7] In this study, a very strong binding enhancement was observed relative to the corresponding monomer. The term "multivalent effect", which was only employed with carbohydrate-binding proteins, could then be applied to the field of carbohydrate-processing enzymes. Importantly, an increase of the inhibition selectivity was also noticed thanks to the multivalent presentation of glycosidase inhibitors. One year later, our group discovered that glycofullerenes were also potent inhibitors of a bacterial glycosyltransferase.[8]

This research field being very recent, many key questions remain to be addressed and clarified. The first one is the question of the inhibition mechanism: why and how multivalent species do inhibit monomeric enzymes? The second important

[a]University of Namur (UNamur), Académie Louvain, Département de Chimie, Laboratoire de Chimie Bio-Organique, rue de Bruxelles 61, B-5000 Namur, Belgium. E-mail: stephane.vincent@unamur.be; Fax: +32 81 72 45 17
[b]Università degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy. E-mail: claudiu.supuran@unifi.it; Fax: +39055 457 3385; Tel: +39055 457 3005

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ob01005e

question is related to the type of enzymes that could possibly be inhibited in a multivalent manner: Presently, there is not enough data available to be able to predict whether a Multivalent Enzyme Inhibition (MEI) may occur or not. Strikingly, a very recent study demonstrated that polymeric iminosugars could improve the enzymatic activity of some glycosyl-hydrolases, and thus act as effectors, not inhibitors.[9]

To date, the glycosidase family is the enzyme class for which the largest amount of data on MEI is available.[6,7,9,10] First, it can be concluded that, among all glycosidases assayed, a minority could be inhibited with a significant multivalent effect. Second, MEI seems to be extremely sensitive to the structure of the multimeric inhibitor: the topology, the valency, the structure of the central scaffold and the length/rigidity of the tether to which each inhibitor is grafted to the scaffold are parameters that need to be fine-tuned in order to observe and optimize a multivalent inhibition.

Since the fullerene scaffold gave excellent multivalent inhibition of glycosidases, we decided to explore further this class of molecules to another biologically and therapeutically relevant class of enzymes. Therefore, we decided to study the multivalent binding of carbonic anhydrases since this family of enzymes, besides its essential biological functions, offers a large panel of macromolecular structures, and thus various binding features.

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous Zn based metallo-enzymes which catalyze the reversible hydration/dehydration of carbon dioxide/bicarbonate. All the human CAs (hCAs) belong to the α-class and differ mainly in their location within the cell and tissues. To date fifteen different CA isoforms and CA-related proteins (CARPs) have been reported.[11] CAs play an essential role in a great deal of physiological processes in animals such as respiration, pH regulation, biosynthesis, calcification and photosynthesis in plants. CA inhibitors (CAIs) are acknowledged as diuretics and antiglaucoma drugs. Besides, they have recently been demonstrated to be promising as anticonvulsant, antiobesity agents and in fighting pathological processes such as tumour formation, metastasis and the virulence of several pathogens.[12] The Zn(II) cation is crucial for the catalytic activity of these enzymes.[13] The active site of all the CA isoforms is highly conserved[14] and most CAIs act by binding either to the Zn(II) or to the water molecule coordinated to it. Namely, sulfonamides and their isosteres, metal complexing anions, phenols, thiophenols and polyamines. In addition, it was recently discovered that *ortho*-substituted benzoic acid derivatives can inhibit CA without entering the active site.[15] Moreover, coumarins[16] and lacosamide[17] inhibit the enzyme activity by occluding the entrance of the catalytic site. It has recently been proved that coumarins are suicide inhibitors which are hydrolyzed within the CA active site with formation of 2-hydroxycinnamic acids, displaying a lengthened two-arm conformation that blocks the entrance to the active site.[18] This new class of CAIs is very promising due to its mechanism of action. These inhibitors bind to the entrance of the active site cavity, which is the most differentiated region among the CA isoforms, offering the possibility of designing selective inhibitors, therefore with less side effects.[19] Moreover, coumarins can be readily synthesized and derived through distinct substitutions patterns without much difficulty. The interesting properties of coumarins prompted us to design a set of multivalent derivatives in order to evaluate their activity against several biologically relevant hCAs. The targeted molecules for this study are depicted in Fig. 1. The two fullerenes $\mathbf{C_{60}(A)_{12}}$ and $\mathbf{C_{60}(B)_{12}}$ bear twelve coumarins and differ in their structures only by the length of the spacer group linking the ligands and the C_{60} core. The four monomeric ligands A_1, A_2, B_1 and B_2 were designed to determine whether a multivalent effect occurs with the targeted enzymes by comparison with the dodecavalent fullerenes. In addition, having two kinds of monomeric coumarins with or without a triazole ring, allowed assessing how this heterocycle affects the inhibition activity.

The fullerene (C_{60}) was the core of choice due to its exclusive physical, electrochemical and chemical properties, which makes this carbon nanomaterial appealing for biotech and biomedical applications.[20] Several examples of clinical drugs which contain fullerene moieties can be already found and plenty of functionalized fullerenes are under intense research for a great variety of applications such as drug delivery, MRI contrast agents or gene delivery.[21] Previous research has been done in the field of coumarin-fullerene dyads,[22] though the fullerene derivatives synthesized for these studies displayed only one coumarin moiety. The objective of the latter studies was to investigate the photophysical properties and energy-transfers between the coumarin and the fullerene within the molecules.

Therefore, as far as we know, this study reports the first synthesis of multimeric suicide inhibitors, designed to address the selectivity and the potency of CA multivalent inhibition.

Synthesis

The synthesis of the monovalent coumarins (Scheme 1), started with the preparation of the linkers that would be later attached to 4-methylumbelliferone, a coumarin derivative already known as CA inhibitor.[19] An azidation was first carried out from 2-bromoethan-1-ol and 2-(2-chloroethoxy)ethan-1-ol to provide **1** and **3**, which were tosylated to give intermediates **2** and **4**, respectively. Williamson reactions of the latter molecules with 4-methylumbelliferone yielded azides **5** and **7**, which were then engaged in both the synthesis of the monovalent clicked control molecules and the final multivalent compounds. The monovalent controls **6** and **8** containing the 1,2,3-triazole moiety were carried out through the CuAAC click reaction by coupling the compounds **5** and **7** with 4-pentyn-1-ol. Therefore, molecules **6** and **8** are the exact monomeric units that will be present onto fullerenes $\mathbf{C_{60}(A)_{12}}$ and $\mathbf{C_{60}(B)_{12}}$.

The two targeted multimeric coumarins were prepared from known fullerene hexakis-adduct **10**[23] that displays twelve TMS-protected alkynes prone to cycloaddition reactions (Scheme 2). The azido-coumarins **5** and **7** were then grafted onto the fullerene scaffold through copper-catalyzed alkyne-

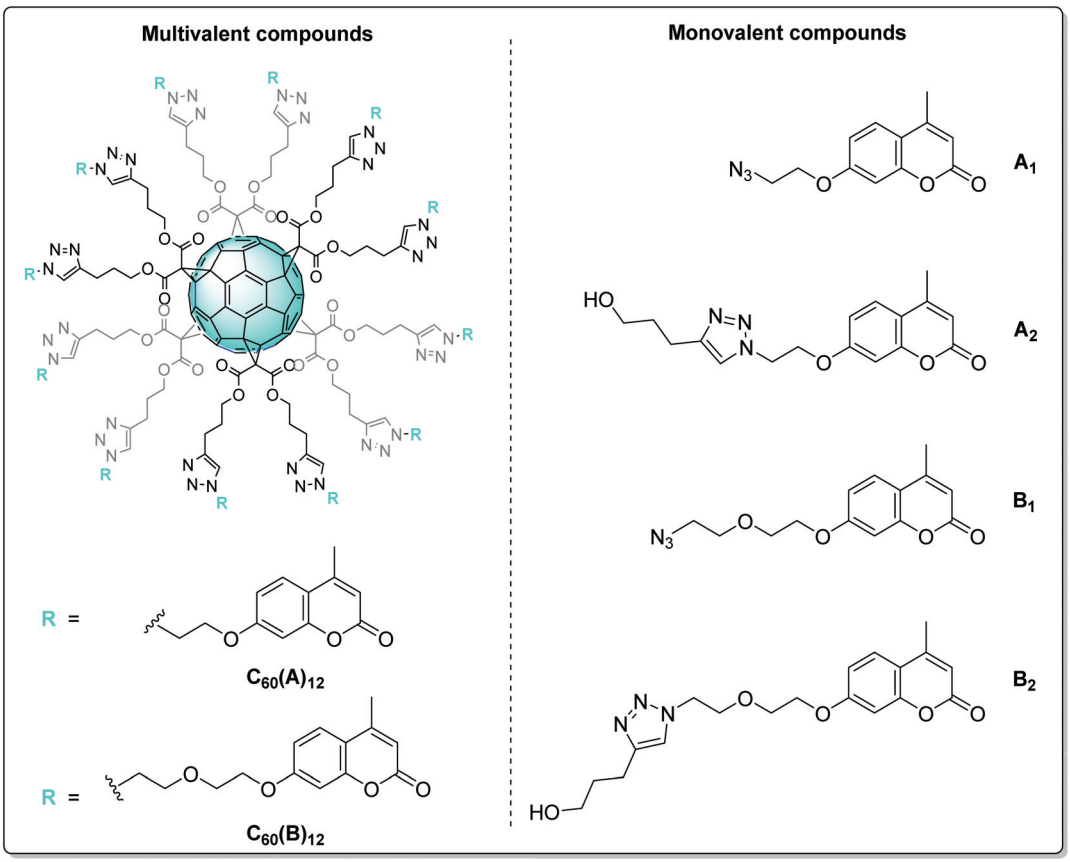
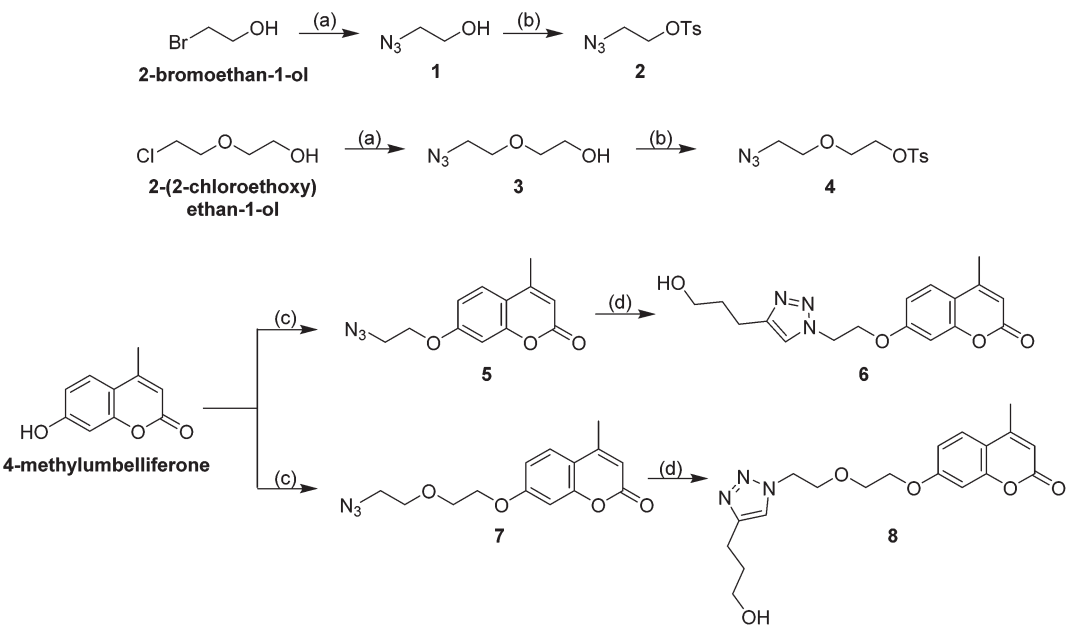
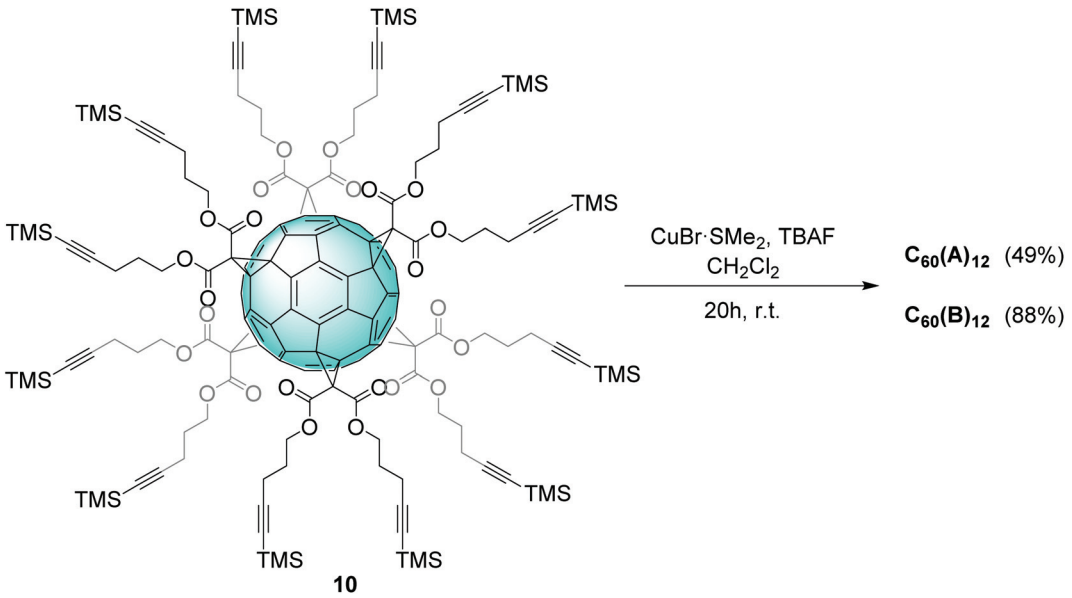


Fig. 1 Targeted multivalent coumarin derivatives and the corresponding monovalent controls.



Scheme 1 Synthesis of the monovalent coumarin derivatives. Reagent and conditions: (a) NaN_3, H_2O, 18 h, 80 °C (**1**: from 2-bromoethan-1-ol, 75%; **3**: from 2-(2-chloroethoxy)ethan-1-ol, 99%). (b) *p*-TsCl, Et_3N, CH_2Cl_2, 16 h, r.t. (**2**: from **1**, 90%; **4**: from **3**, 88%). (c) **2** or **4**, K_2CO_3, KI, DMF, 6 h, 60 °C (**5**: from **2**, 84%; **7**: from **4**, 96%). (d) **5** or **7**, 4-pentyn-1-ol, $CuSO_4$/NaAsc, 1,4-dioxane/CH_2Cl_2/H_2O, 5 h, 60 °C (**6**: from **5**, 86%; **8**: from **7**, 60%).



Scheme 2 Synthesis of the multivalent coumarin derivatives. Complete structures are shown in Fig. 1.

azide cycloaddition (CuAAC) based on the protocol developed by Nierengarten *et al.*[24]

Deprotection of the trimethylsilyl-protected alkynes was accomplished *in situ* by addition of TBAF 1 M in THF into the reaction mixture. To assure the complete functionalization of the fullerene core, 15 equivalents of the azido-coumarins were required. Dichloromethane was used as solvent due to the high solubility of all the compounds and $CuBr{\cdot}SMe_2$ was found to be the catalyst of choice compared to $CuSO_4$, which requires heterogeneous solvent systems.[24] After 20 h at room temperature, the reaction mixtures were diluted with dichloromethane and extracted with water. The purifications of the reaction mixtures were performed by silica gel chromatography followed by size exclusion chromatography on lipophilic Sephadex-LH20. The two final compounds were obtained in moderate to very good yields, bearing in mind that twelve reactions had taken place. Importantly, the possibility of purification of the final molecules by two successive chromatographies after the click reaction facilitates the total removal of Cu salts, as confirmed by ICP.

The structures of the final compounds were determined by ^1H and ^{13}C NMR, IR and mass spectrometry (see ESI†). The ^1H NMR clearly indicated the presence of the newly formed triazoles (around 7.5 ppm in $CDCl_3$ at 20 °C) and the disappearance of the TMS-alkynes and azides functionalities. Moreover, ^{13}C NMR spectra were instrumental for the demonstration of the final structures. The presence of only two sp^2 carbon atoms belonging to the fullerene core confirms the Th-symmetry of the core and the monodispersity of the final compounds. A comparison of the spectra of the precursors and the final molecule $\mathbf{C_{60}(B)_{12}}$ is shown in Fig. 2. Furthermore, mass spectrometry provided a definitive proof of structures (see ESI†). All the peaks correlate with the expected molecule and cannot arise from an incomplete functionalization of the fullerene scaffold.

CA Inhibition assays

The monovalent compounds **5–8** and the two fullerenes conjugated with coumarins $\mathbf{C_{60}(A)_{12}}$ and $\mathbf{C_{60}(B)_{12}}$ were assayed *in vitro* for inhibition against the four physiologically relevant CA isoforms, the cytosolic hCA I and II as well as the transmembrane, tumor-associated hCA IX and XII (Table 1). Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) was used as a standard in the assay.[25]

As many coumarin derivatives, the investigated compounds from the present paper also showed low inhibitory properties against the cytosolic isoforms hCA I and II, with most of them possessing inhibition constants >50 μM. The exceptions are the low molecular weight coumarin **7** and $\mathbf{C_{60}(B)_{12}}$ against hCA I, which were medium potency inhibitors with K_Is in the range of 7.0–8.1 μM. Against hCA II the simple coumarins **5–8** were ineffective inhibitors whereas the two fullerene conjugates $\mathbf{C_{60}(A)_{12}}$ and $\mathbf{C_{60}(B)_{12}}$ were medium efficacy inhibitors with K_Is in the range of 9.9–10.0 μM.

The tumor-associated hCA IX was better inhibited both by the coumarins **5–8** (K_Is in the range of 0.06–0.18 μM) as well as by the fullerene conjugates (K_Is of 0.04 μM for both derivatives). A similar behavior was observed for the inhibition of the second transmembrane isoform investigated here, hCA XII; against which coumarins **5–8** showed K_Is of 1.08–1.92 μM, whereas the two fullerene-coumarin conjugates of 0.16–0.19 μM. It is clear that the multimers are always more potent than their corresponding monomers, an effect likely due to the local ligand concentration around the fullerene core. It is also worth to note that a small positive triazole effect was observed for the extracellular isoforms hCA IX and hCA XII in every

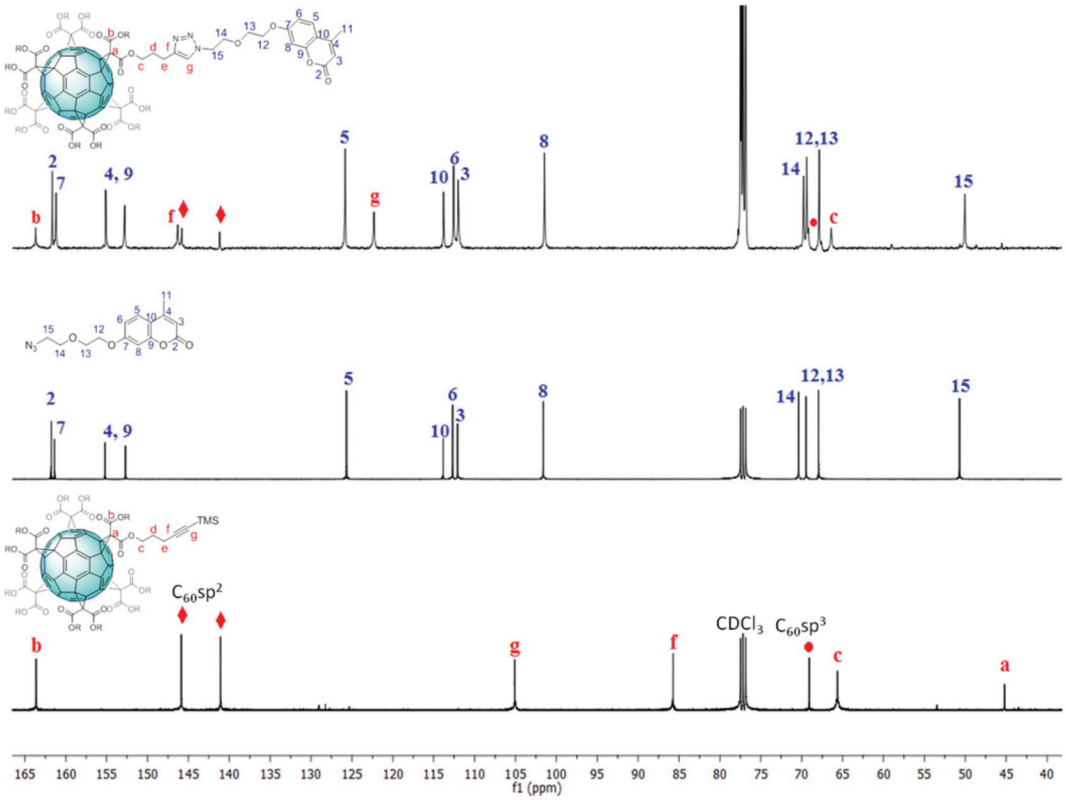


Fig. 2 Comparison of ^{13}C NMR spectra of molecules **10**, **7** and $\mathbf{C_{60}(B)_{12}}$ in $CDCl_3$ at 20 °C.

Table 1 Inhibition constants (K_Is) by a stopped flow CO_2 hydrase assay, with coumarins and fullerene-coumarin conjugates, and acetazolamide **AAZ** as standard drug[25]

	K_I (μM)			
Compound	hCA I	hCA II	hCA IX	hCA XII
5	>50	>50	0.14	1.92
6	>50	>50	0.06	1.44
7	8.1	>50	0.18	1.66
8	>50	>50	0.14	1.08
$\mathbf{C_{60}(A)_{12}}$	>50	9.9	0.04	0.16
$\mathbf{C_{60}(B)_{12}}$	7.0	10.0	0.04	0.19
AAZ[a]	0.25	0.012	0.025	0.006

[a] Acetazolamide (AAZ) was used as a standard inhibitor for all CAs investigated here. The data represent the mean of three different determinations. Errors were in the range of ±10% of the reported values (data not shown).

case. Indeed, the inhibitors **6** and **8** containing a 1,2,3-triazole moiety are slightly better than their corresponding azides **5** and **7**. However, in the particular case of the cytosolic hCA I, the monovalent azido-inhibitor **7**, showed a higher inhibitory power than the rest of monovalent molecules (K_Is of 8.1 μM, while for the others K_Is > 50 μM). Therefore, for this enzyme, there is a substantial effect of the linker length and the presence of triazole unit in the binding potency.

Discussion and conclusions

This work addressed the question of multivalent enzyme inhibition applied to a novel class of enzymes for this topic. The family of carbonic anhydrases was selected as enzymatic target because they play critical roles in numerous biological processes. For instance CAIs are already recognized as antiglaucoma and diuretic drugs while they are being investigated as anticancer and anti-virulence drugs, among other biomedical applications. In 2008, Supuran *et al.* synthesized nanoparticles functionalized by sulphonamides.[26] Although no multivalent effect was observed, the nanoparticles were potent inhibitors of CA and were shown not to penetrate into cells, contrarily to the corresponding monovalent species. This property could be extremely advantageous in case an extracellular enzymatic activity is targeted for a therapeutic purpose, which is the case for the tumour associated CA IX activity. In the present study, the synthesis of multimeric suicide CAIs is reported for the first time, and their activity against biologically relevant hCAs has been studied. Our main findings are (i) the modest inhibition power of coumarins against cytosolic hCAs are generally improved by displaying the inhibitors in a multivalent manner, (ii) the multimeric suicide inhibitors always showed a better activity against the extracellular CAs isoforms, (iii) a selective inhibition among cytosolic hCAs is possible by fine-tuning the length and structures of the linker bridging the C60

and the ligand without modifying the coumarin itself (iv) the effect of the triazole moiety can be either positive or negative depending on the hCA isoform. Although a clear multivalent effect could not be observed (if calculated on a "per-coumarin" basis), the multivalent compounds were always better inhibitors than the monovalent controls. Therefore, we believe that the multivalent inhibition of CAs is possible and that, as for the well-studied glycosidase family, the level of inhibition potency can be improved by optimizing the length and rigidity of the linker, the core scaffold itself and the substitution pattern on the coumarin derivative. We also believe that the field of multienzyme inhibition should indeed find biomedical applications especially if the enzymes are extracellular, as for the tumor-associated carbonic anhydrases, or if they are membrane-anchored and exposed to the external cellular medium such as the viral neuraminidases.[27]

Experimental

Synthetic procedures and analytical data: see ESI†

CA inhibition. A stopped-flow instrument (SX.18MV-R Applied Photophysics model) was used for assaying the CA-catalyzed CO_2 hydration activity.[25] Inhibitor and enzyme were preincubated for 15 min for allowing the complete formation of the enzyme-inhibitor adduct. IC_{50} values were obtained from dose response curves working at eight different concentrations of test compound (from 0.01 nM to 50 μM), by fitting the curves using PRISM (http://www.graphpad.com) and non-linear least squares methods, the obtained values representing the mean of at least three different determinations. The inhibition constants (K_I) were derived from the IC_{50} values by using the Cheng–Prusoff equation, as follows: $K_I = IC_{50}/(1 + [S]/K_m)$ where [S] represents the CO_2 concentration at which the measurement was carried out, and K_m the concentration of substrate at which the enzyme activity is at half maximal. All enzymes used were recombinant, produced in *E. coli* as reported earlier.[28] The concentrations of enzymes used in the assay varied between 8.4 nM and 12.8 nM.

Acknowledgements

We thank the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme "DyNano" FP7/2007-2013/under REA grant agreement no. 289033 for the financial support.

References

1 C. R. A. Varki and J. D. Esko, *et al.*, *Essentials of Glycobiology*, NY, 2nd edn, 2009.
2 C. Fasting, C. A. Schalley, M. Weber, O. Seitz, S. Hecht, B. Koksch, J. Dernedde, C. Graf, E. W. Knapp and R. Haag, *Angew. Chem., Int. Ed.*, 2012, **51**, 10472.
3 (*a*) R. J. Pieters, *Org. Biomol. Chem.*, 2009, **7**, 2013; (*b*) M. Lahmann, *Top. Curr. Chem.*, 2009, **288**, 17; (*c*) Y. M. Chabre and R. Roy, *Adv. Carbohydr. Chem. Biochem.*, 2010, **63**, 165; (*d*) M. Sanchez-Navarro, A. Munoz, B. M. Illescas, J. Rojo and N. Martin, *Chem. – Eur. J.*, 2011, **17**, 766; (*e*) J.-L. Reymond and T. Darbre, *Org. Biomol. Chem.*, 2012, **10**, 1483; (*f*) X. Zeng, C. A. Andrade, M. D. Oliveira and X. L. Sun, *Anal. Bioanal. Chem.*, 2012, **402**, 3161; (*g*) J. J.-B. A. Bernardi, A. Casnati, C. De Castro, T. Darbre, F. Fieschi, J. Finne, H. Funken, K.-E. Jaeger, M. Lahmann, T. K. Lindhorst, M. Marradi, P. Messner, A. Molinaro, P. V. Murphy, C. Nativi, S. Oscarson, S. Penadés, F. Peri, R. J. Pieters, O. Renaudet, J.-L. Reymond, B. Richichi, J. Rojo, F. Sansone, C. Schäffer, W. B. Turnbull, T. Velasco-Torrijos, S. Vidal, S. P. Vincent, T. Wennekes, H. Zuilhof and A. Imberty, *Chem. Soc. Rev.*, 2013, **42**, 4709; (*h*) A. K. Adak, H.-J. Lin and C.-C. Lin, *Org. Biomol. Chem.*, 2014, **12**, 5563; (*i*) S. Cecioni, A. Imberty and S. Vidal, *Chem. Rev.*, 2015, **115**, 525.
4 (*a*) D. Deniaud, K. Julienne and S. G. Gouin, *Org. Biomol. Chem.*, 2011, **9**, 966; (*b*) R. R. O. Renaudet, *Chem. Soc. Rev.*, 2013, **42**, 4515.
5 (*a*) A. Imberty, Y. M. Chabre and R. Roy, *Chem. – Eur. J.*, 2008, **14**, 7490; (*b*) M. Durka, K. Buffet, J. Iehl, M. Holler, J. F. Nierengarten, J. Taganna, J. Bouckaert and S. P. Vincent, *Chem. Commun.*, 2011, **47**, 1321; (*c*) M. Hartmann and T. K. Lindhorst, *Eur. J. Org. Chem.*, 2011, 3583; (*d*) M. Hartmann, H. Papavlassopoulos, V. Chandrasekaran, C. Grabosch, F. Beiroth, T. K. Lindhorst and C. Rohl, *FEBS Lett.*, 2012, **586**, 1459; (*e*) I. Nierengarten, K. Buffet, M. Holler, S. P. Vincent and J.-F. Nierengarten, *Tetrahedron Lett.*, 2013, **54**, 2398; (*f*) A. Kouki, R. J. Pieters, U. J. Nilsson, V. Loimaranta, J. Finne and S. Haataja, *Biology*, 2013, **2**, 918; (*g*) J. Luczkowiak, A. Munoz, M. Sanchez-Navarro, R. Ribeiro-Viana, A. Ginieis, B. M. Illescas, N. Martin, R. Delgado and J. Rojo, *Biomacromolecules*, 2013, **14**, 431; (*h*) A. M. Boukerb, A. Rousset, N. Galanos, J. B. Mear, M. Thepaut, T. Grandjean, E. Gillon, S. Cecioni, C. Abderrahmen, K. Faure, D. Redelberger, E. Kipnis, R. Dessein, S. Havet, B. Darblade, S. E. Matthews, S. de Bentzmann, B. Guery, B. Cournoyer, A. Imberty and S. Vidal, *J. Med. Chem.*, 2014, **57**, 10275.
6 J. Diot, M. I. Garcia-Moreno, S. G. Gouin, C. Ortiz Mellet, K. Haupt and J. Kovensky, *Org. Biomol. Chem.*, 2009, **7**, 357.
7 P. Compain, C. Decroocq, J. Iehl, M. Holler, D. Hazelard, T. Mena Barragan, C. Ortiz Mellet and J. F. Nierengarten, *Angew. Chem., Int. Ed.*, 2010, **49**, 5753.
8 M. Durka, K. Buffet, J. Iehl, M. Holler, J. F. Nierengarten and S. P. Vincent, *Chem. – Eur. J.*, 2012, **18**, 641.
9 Y. Brissonnet, S. Ladeveze, D. Teze, E. Fabre, D. Deniaud, F. Daligault, C. Tellier, S. Sestak, M. Remaud-Simeon, G. Potocki-Veronese and S. G. Gouin, *Bioconjugate Chem.*, 2015, **26**, 766.
10 (*a*) A. J. Cagnoni, O. Varela, S. G. Gouin, J. Kovensky and M. L. Uhrig, *J. Org. Chem.*, 2011, **76**, 3064; (*b*) C. Decroocq,

D. Rodríguez-Lucena, K. Ikeda, N. Asano and P. Compain, *ChemBioChem*, 2012, **13**, 661; (*c*) M. Almant, A. Mastouri, L. Gallego-Yerga, J. M. Garcia Fernandez, C. Ortiz Mellet, J. Kovensky, S. Morandat, K. El Kirat and S. G. Gouin, *Chem. – Eur. J.*, 2013, **19**, 729; (*d*) R. Risquez-Cuadro, J. M. Garcia Fernandez, J. F. Nierengarten and C. Ortiz Mellet, *Chem. – Eur. J.*, 2013, **19**, 16791; (*e*) Y. Brissonnet, C. Ortiz Mellet, S. Morandat, M. I. Garcia Moreno, D. Deniaud, S. E. Matthews, S. Vidal, S. Sestak, K. El Kirat and S. G. Gouin, *J. Am. Chem. Soc.*, 2013, **135**, 18427; (*f*) C. Decroocq, A. Joosten, R. Sergent, T. Mena Barragan, C. Ortiz Mellet and P. Compain, *ChemBioChem*, 2013, **14**, 2038; (*g*) C. Bonduelle, J. Huang, T. Mena-Barragan, C. Ortiz Mellet, C. Decroocq, E. Etame, A. Heise, P. Compain and S. Lecommandoux, *Chem. Commun.*, 2014, **50**, 3350; (*h*) P. Compain and A. Bodlenner, *ChemBioChem*, 2014, **15**, 1239.

11 S. Pastorekova, S. Parkkila, J. Pastorek and C. T. Supuran, *J. Enzyme Inhib. Med. Chem.*, 2004, **19**, 199.

12 V. Alterio, A. Di Fiore, K. D'Ambrosio, C. T. Supuran and G. De Simone, *Chem. Rev.*, 2012, **112**, 4421.

13 A. Maresca, C. Temperini, L. Pochet, B. Masereel, A. Scozzafava and C. T. Supuran, *J. Med. Chem.*, 2010, **53**, 335.

14 C. T. Supuran and A. Scozzafava, *Expert Opin. Ther. Pat.*, 2000, **10**, 575.

15 K. D'Ambrosio, S. Carradori, S. M. Monti, M. Buonanno, D. Secci, D. Vullo, C. T. Supuran and G. De Simone, *Chem. Commun.*, 2015, **51**, 302.

16 H. Vu, N. B. Pham and R. J. Quinn, *J. Biomol. Screening*, 2008, **13**, 265.

17 C. Temperini, A. Innocenti, A. Scozzafava, S. Parkkila and C. T. Supuran, *J. Med. Chem.*, 2010, **53**, 850.

18 A. Maresca, C. Temperini, H. Vu, N. B. Pham, S. A. Poulsen, A. Scozzafava, R. J. Quinn and C. T. Supuran, *J. Am. Chem. Soc.*, 2009, **131**, 3057.

19 A. Sharma, M. Tiwari and C. T. Supuran, *J. Enzyme Inhib. Med. Chem.*, 2014, **29**, 292.

20 N. Martin, *Chem. Commun.*, 2006, 2093.

21 J. L. C. R. Partha, *Int. J. Nanomedicine*, 2009, **4**, 261.

22 (*a*) S. Nascimento, M. J. Brites, C. Santos, B. Gigante, A. Fedorov and M. N. Berberan-Santos, *J. Fluoresc.*, 2006, **16**, 245; (*b*) M. J. Brites, C. Santos, S. Nascimento, B. Gigante, H. Luftmann, A. Fedorov and M. N. Berberan-Santos, *New J. Chem.*, 2006, **30**, 1036; (*c*) G. Pagona, S. P. Economopoulos, G. K. Tsikalas, H. E. Katerinopoulos and N. Tagmatarchis, *Chem. – Eur. J.*, 2010, **16**, 11969; (*d*) S. Nascimento, A. Fedorov, M. J. Brites and M. N. Berberan-Santos, *Dyes Pigm.*, 2015, **114**, 158.

23 J. Iehl and J. F. Nierengarten, *Chem. – Eur. J.*, 2009, **15**, 7306.

24 (*a*) J. Iehl, R. Pereira de Freitas, B. Delavaux-Nicot and J. F. Nierengarten, *Chem. Commun.*, 2008, 2450; (*b*) J. F. Nierengarten, J. Iehl, V. Oerthel, M. Holler, B. M. Illescas, A. Munoz, N. Martin, J. Rojo, M. Sanchez-Navarro, S. Cecioni, S. Vidal, K. Buffet, M. Durka and S. P. Vincent, *Chem. Commun.*, 2010, **46**, 3860.

25 R. G. Khalifah, *J. Biol. Chem.*, 1971, **246**, 2561.

26 M. Stiti, A. Cecchi, M. Rami, M. Abdaoui, V. Barragan-Montero, A. Scozzafava, Y. Guari, J. Y. Winum and C. T. Supuran, *J. Am. Chem. Soc.*, 2008, **130**, 16131.

27 S. J. F. Macdonald, R. Cameron, D. A. Demaine, R. J. Fenton, G. Foster, D. Gower, J. N. Hamblin, S. Hamilton, G. J. Hart, A. P. Hill, G. G. A. Inglis, B. Jin, H. T. Jones, D. B. McConnell, J. McKimm-Breschkin, G. Mills, V. Nguyen, I. J. Owens, N. Parry, S. E. Shanahan, D. Smith, K. G. Watson, W.-Y. Wu and S. P. Tucker, *J. Med. Chem.*, 2005, **48**, 2964.

28 (*a*) C. Temperini, A. Scozzafava, D. Vullo and C. T. Supuran, *Chem. – Eur. J.*, 2006, **12**, 7057; (*b*) C. Temperini, A. Scozzafava, D. Vullo and C. T. Supuran, *J. Med. Chem.*, 2006, **49**, 3019; (*c*) C. Temperini, A. Scozzafava and C. T. Supuran, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5152; (*d*) C. Temperini, A. Innocenti, A. Scozzafava, A. Mastrolorenzo and C. T. Supuran, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 628; (*e*) I. Nishimori, T. Minakuchi, S. Onishi, D. Vullo, A. Cecchi, A. Scozzafava and C. T. Supuran, *Bioorg. Med. Chem.*, 2007, **15**, 7229; (*f*) T. M. I. Nishimori, S. Onishi, D. Vullo, A. Cecchi, A. Scozzafava and C. T. Supuran, *J. Enzyme Inhib. Med. Chem.*, 2009, **24**, 70; (*g*) F. Carta, C. Temperini, A. Innocenti, A. Scozzafava, K. Kaila and C. T. Supuran, *J. Med. Chem.*, 2010, **53**, 5511; (*h*) D. V. R. Davis, C. T. Supuran and S. A. Poulsen, *BioMed. Res. Int.*, 2014, **374079**, 1.