**1. Introduction**

Carbonic anhydrase (EC 4.2.1.1, CA) is a metalloenzymes family that catalyzes the rapid conversion of CO$_2$ to HCO$_3^-$ and H$^+$. CA isoforms are found in a variety of tissues where they participate in several important biological processes such as acid-base balance, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis and balance, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis and bone resorption. Furthermore, the phenyl moiety of this inhibitor was found to lay in the hydrophobic part of the hCA II active site, through a network of two hydrogen bonds with the Zn(II) ion from the enzyme active site in tetrahedral or trigonal bipyramidal geometries of the metal ion (Fig. 1A and B). This study has been known since ancient times, and it was used as an anti-inflammatory drug.

Indeed, phenol binds to CA in a diverse manner compared to the classical inhibitors of the sulfonamides/sulfamates/sulfamides, which coordinate to the Zn$^{2+}$ ion from the enzyme active site by substituting the fourth, non-protein ligand, a water molecule or hydroxide ion. Recently, Christianson’s group then reported the X-ray crystal structure for the adduct of hCA II with phenol, showing indeed this inhibitor to bind to hCA II by anchoring its OH moiety to the zinc-bound H$_2$O/hydroxide ion of the enzyme through a hydrogen bond as well as to the NH amide of Thr 199, an amino acid conserved in all $\alpha$-CAs and critically important for the catalytic cycle of these enzymes. Furthermore, the phenyl moiety of this inhibitor was found to lay in the hydrophobic part of the hCA II active site, where presumably CO$_2$, the physiologic substrate of the CAs, binds in the precatalytic complex, explaining thus the behaviour of phenol as a unique CO$_2$ competitive inhibitor.

The CAs belong to four main classes: (i) sulfonamides (and their isosteres, such as sulfamates, sulfamides and similar derivatives) and metal complexing anions, which coordinate to the Zn(II) ion from the enzyme active site in tetrahedral or trigonal bipyramidal geometries of the metal ion (Fig. 1A and B), (ii) phenols (such as the simple phenol C$_6$H$_5$OH), (iii) polyamines, (such as spermine, spermidine and congeners, which bind rather similar but not identical to phenols, that is, by anchoring to the water molecule/hydroxide...
linear optics, photovoltaics, semiconductors, photodynamic sensors, electrochromic and electroluminescent displays, non-such as liquid crystals, electronic devices, gas and chemical activators, occluding the entrance to the active site (Fig. 1E).

bind (in hydrolyzed form) in the same active site region as the widespreadly have been used in dynamic therapy applications. Such dyes and their derivatives have been attracting increasing interest. Pcs dyes have found techniques.

trans hydrolyzed coumarin, /C23 represent distances (in Å), as determined by X-ray crystallographic techniques. Hydrogen bonds are represented as dashed lines. All these binding modes have been proven by means of X-ray crystallography on enzyme-inhibitor adducts.

2. Experimental section

2.1. Materials and methods

All reagents and solvents were of reagent grade quality and were obtained from commercial suppliers. All solvents were dried and purified as described by Perrin and Armarego. Sulphonlamide, Sepharose 4B, protein assay reagents, 4-nitrophenylacetate were obtained from Sigma-Aldrich Co. All other chemicals were analytical grade and obtained from Merck.

The IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrophotometer, using KBr pellets. 1H and 13C-NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometers in CDCl3 and chemical shifts were reported (δ) relative to Me2Si as internal standard. MALDI-MS of complexes were obtained in dihydroxybenzoic acid as the MALDI matrix, using a nitrogen laser accumulating 50 laser shots, with a Bruker Microflex LT MALDI-TOF mass spectrometer. Optical spectra in the UV-Vis region were recorded with a Perkin Elmer Lambda 25 spectrophotometer.

2.2. Synthesis

2.2.1. Bis[(2E)-3-[4-(dimethylamino)phenyl]-1-(4-phenoxy)prop-2-en-1-one]phthalocyaninato silicon(IV) (ZT-Si). A mixture of SiPcCl2 (1) (100 mg, 0.16 mmol) and (2E)-3-[4-(dimethylamino)phenyl]-1-(4-hydroxyphenyl)prop-2-en-1-one (2) (85 mg, 0.32 mmol) in toluene (10 mL) was stirred and then sodium hydride (7.7 mg, 0.32 mmol) was added to this mixture. After heating at reflux temperature under nitrogen atmosphere for 24 h, toluene was evaporated to dry under reduced pressure. The product was purified by column chromatography [silica gel/CHCl3 : CH3OH (100 : 6)]. FT-IR (KBr pellet) ν (cm⁻¹): 3021 (Ar–H), 2984–2848 (Aliph. C–H), 1645, 1579, 1550, 1503, 1430, 1334, 1289, 1263, 1210, 1160, 1120, 1079, 1038, 912, 881, 759, 729, 680. 1H-NMR (400 MHz, DMSO-d₆), (δ ppm): 9.74–9.72 (m, 8H, Pe–H₄), 8.58–8.55 (m, 12H, Ar–H), 7.85–7.79 (m, 12H, Pe–H₄), 7.59–7.57 (m, 12H, Pe–H₄), 7.38–7.32 (m, 12H, Pe–H₄), 6.85–6.77 (m, 12H, Pe–H₄), 4.80–4.75 (m, 12H, Pe–H₄), 4.25–4.20 (m, 12H, Pe–H₄).
3. Results and discussion

3.1. Synthesis and characterization

The synthesis of axially disubstituted silicon phthalocyanines ZT-Si, ZM-1-Si, ZM-5-Si and their quaternized derivatives ZT-Siq, ZM-1-Siq, ZM-5-Siq were given in Fig. 2 and 3, respectively. (2E)-3-[4-(Dimethylamino)phenyl]-1-(4-hydroxyphenyl)prop-2-en-1-one 2a, silicon(v) phthalocyanine dichloride 1, silicon(v) phthalocyanine ZM-1-Si, ZM-1-Siq and silicon(v) phthalocyanine ZM-5-Si, ZM-5-Siq were synthesized according to previously published methods. Reaction of silicon(v) phthalocyanine dichloride 1 with (2E)-3-[4-(dimethylamino) phenyl]-1-(4-hydroxyphenyl)prop-2-en-1-one 2 in the present of NaH in toluene led to the target axially disubstituted silicon(v) phthalocyanine ZT-Si yielded 35%. Quaternized silicon(v) phthalocyanine ZT-Siq was achieved by the reaction of silicon(v) phthalocyanine ZT-Si with excess methyl iodide which is a quaternization agent in CHCl₃ at room temperature. The newly synthesized silicon(v) phthalocyanine ZT-Si and its quaternized derivative ZT-Siq were characterized by various spectroscopic methods including FT-IR, ¹H NMR, ¹³C NMR, UV-Vis, mass. All the results were consistent with the predicted structures for all newly phthalocyanines as shown in the Experimental section.

The formation of silicon(v) phthalocyanine ZT-Si was clearly confirmed by the disappearance of the OH band at 3318 cm⁻¹ for compound 2 in the IR spectrum of phthalocyanine ZT-Si. The ¹H NMR spectrum of axially disubstituted silicon(v) phthalocyanine ZT-Si showed peaks belonging to Hₐ and Hᵦ protons at between 9.74–9.72 and 8.58–8.35 ppm, respectively. In the ¹³C-NMR spectra of silicon(v) phthalocyanine ZT-Si, the observation of new signals at δ = 8.31, 6.86, 6.75 ppm belonging to aromatic protons on the substituents proved the synthesis of this phthalocyanine ZT-Si. On the other hand, the appearance of new signal at δ = 2.99 ppm belonging to aliphatic protons (CH₃-N) also confirmed the formation of target compound. The ¹³C-NMR spectra showed signals for relative carbon atoms for silicon(v) phthalocyanine ZT-Si. The mass spectra of silicon(v) phthalocyanine ZT-Si also confirmed the proposed structures of this phthalocyanine. The molecular ion peak was observed at m/z: 1103 [M + H⁺] (Fig. 4). No major change in the IR spectra was also observed after quaternization (for ZT-Siq) of silicon(v) phthalocyanine ZT-Si. The fragment peaks was observed to the mass spectra of quaternized cationic silicon(v) phthalocyanine ZT-Siq at m/z: 1103 as [M – 2I]⁺. This result support the proposed formula for silicon(v) phthalocyanine ZT-Siq.

The ground state electronic absorption spectra of the novel non-ionic silicon(v) phthalocyanine ZT-Si showed characteristic absorptions in the Q band region at 683 in DMF. The methyl group on the nitrogen atom of the substituents did not any affect on the absorption wavelengths of the studied phthalocyanine. The B band absorption of silicon(v) phthalocyanine ZT-Si was observed at 405 and 354 nm (Fig. 5). The ground state electronic spectra of the quarternized silicon phthalocyanine ZT-Siq showed characteristic absorption in the...
Q band region at 684 nm in DMF (Fig. 5). The quaternization of
the non-ionic phthalocyanines did not affect to the absorption
wavelength of the studied phthalocyanines. The B bands were
observed at 414, 355 and 324 nm which are similar wavelength
with non-ionic phthalocyanine ZT-Si in DMF.

3.2. Biological evaluation of the synthesized and reference
compounds for CA inhibitory activity
The purification of the two CA isozymes used here was performed
with a simple one step method by a affinity chromatography.\textsuperscript{14}
Inhibitory effects of silicon phthalocyanines ZM-1-Si, ZM-5-Si, ZT-Si

Fig. 2  The synthesis of the silicon(IV) phthalocyanine ZT-Si and its quaternized derivative ZT-SiQ. (i) Toluene, NaH, reflux. (ii) CHCl\textsubscript{3}, CH\textsubscript{3}I, room
temperature.
and their quaternized derivatives ZM-1-SiQ, ZM-5-SiQ, ZT-SiQ on enzyme activities were tested for the first time under in vitro conditions; IC50 values are given in Table 1.

We report here the first study on the inhibitory effects of ZM-1-Si, ZM-1-SiQ, ZM-5-Si, ZM-5-SiQ, ZT-Si and ZT-SiQ on the esterase activity of hCA I and II. Data of Table 1 show the following regarding inhibition of hCA I and II with these compounds, by an esterase assay, with 4-nitrophenylacetate (4-NPA) as substrate:

(i) Against the slow cytosolic isozyme hCA I were moderately inhibited by compound ZM-1-Si. A second group of derivatives, including ZM-5-Si, ZM-1-SiQ and ZM-5-SiQ showed better inhibitory activity as compared to the previously mentioned phthalocyanine, with IC50 values in the range of 0.0243–0.0840 μM. Molecules ZT-SiQ and ZT-Si were among the best inhibitors in this series of phthalocyanines. Data of Table 1 also show that
Similarly to acetazolamide (AZA), some of the investigated phthalocyanines bind in the same regions of the active site cavity as the substrate. However the binding site of 4-NPA itself is unknown, it is presumed to be in the same region as that of CO$_3^-$, the physiological substrate of this enzyme.$^{28}$

(ii) A rather similar activity of these compounds has been observed also for the inhibition of the rapid cytosolic isozyme, hCA II (Table 1). Thus, a first group of derivatives, ZM-1-Si showed modest hCA II inhibitory activity with IC$_{50}$ in the range of 0.1212 μM (Table 1), whereas the remaining five phthalocyanines, that is, the same compounds acting as efficient hCA II inhibitors, showed IC$_{50}$ in the range of 0.0172–0.0762 μM. The best hCA II inhibitor in this series of derivatives were ZT-Si and ZT-SiQ, which with a IC$_{50}$ of 0.0172–0.026 μM. Considering the data of Table 1, structure–activity relationship was thus quite similar in these small groups of N,N-dimethylaniline derivatives (phthalocyanines), for both the inhibition of hCA I and II, although differences of affinity between the two isozymes are evident. The N,N-dimethylaniline substituent on phenyl ring could easily be predicted to be involved in making hydrogen bonds with the active site as observed in classical CAI sulfonamide inhibitors (Fig. 1F). Again most of these compounds acted as competitive inhibitors with 4-NPA as substrate (Table 1).

The new compounds ZT-Si and ZT-SiQ showed promising inhibitory profiles compared to the standard drug AZA and they all had comparable IC$_{50}$ values against hCA I and hCA II.

In a recent study it was reported that different phenolic compounds,$^{37}$ a simple compound lacking the sulfonamide, sulfamate, or related functional groups that are typically found in all known CA inhibitors, acts as a CAI inhibitor, and could represent the starting point for a new class of inhibitors that may have advantages for patients with sulfonamide allergies.$^{38}$ However, it is critically important to explore further classes of potent CAIs in order to detect compounds with a different inhibition profile as compared to the sulfonamides and their bioisosteres and to find novel applications for the inhibitors of these widespread enzymes.

| Table 1  | Silicon phthalocyanines and their IC$_{50}$ values$^a$ |
|----------|-----------------------------------------------------|
| Test compounds | IC$_{50}$ (μM) | hCA I | hCA II |
| ZM-1-Si | 0.1653 | 0.1212 |
| ZM-1-SiQ | 0.0710 | 0.0544 |
| ZM-5-Si | 0.0840 | 0.0762 |
| ZM-5-SiQ | 0.0243 | 0.0363 |
| ZT-Si | 0.0178 | 0.0172 |
| ZT-SiQ | 0.0223 | 0.0260 |
| AZA (Acetazolamide)$^{25}$ | 0.9857 | 0.4894 |

$^a$ Errors in the range of 2–5% of the shown data, from three different assays.

4. Conclusion

In the presented work, novel silicon(IV) phthalocyanine axially substituted with [(2E)-3-[4-(dimethylamino)phenyl]-1-(4-phenoxy)prop-2-en-1-one] groups ZT-Si and its quaternized derivative ZT-SiQ were synthesized for the first time. A novel class of efficient CAIs, interacting with the CA isozymes I and II (cytosolic) in a different manner compared to sulfonamides, sulfamates and other classes of inhibitors, is reported in this paper. Kinetic measurements allowed us to identify N,N-dimethylaniline substituted phthalocyanines as well as ZT-SiQ as submicromolar–low micromolar inhibitors of the two CA isozymes. This new class of inhibitors binds differently of all other CAIs known to date, being found between the phenol-binding site within the enzyme cavity. They exploit different interactions with amino acid residues and water molecules from the CA active site compared to other classes of inhibitors, offering the possibility to design compounds with a better inhibition profile compared to the clinically used sulfonamides/sulfamates. As a result, this study is the first example of carbonic anhydrase enzyme inhibition of phthalocyanines. These results showed that silicon phthalocyanines have potential as carbonic anhydrase inhibitors.

Conflicts of interest

There are no conflicts to declare.

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