Tetracycline speciation during molecular imprinting in xerogels results in class-selective binding

Elmer-Rico E. Mojica, Jochen Autschbach, Frank V. Bright* and Diana S. Aga*

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The creation of tetracycline (TC) responsive molecularly imprinted xerogels (MIXs) was investigated using electronic absorbance, liquid chromatography-ion-trap mass spectrometry (LC-ITMS), and first-principles theory. Experimental results show that the template molecule converts to its epimer, 4-epitetracycline (ETC), during the imprinting process. Additionally, end capping of the MIX surface silanols transforms TC into anhydrotetracycline (ATC) and 4-epianhydrotetracycline (EATC). Hence, despite aiming to imprint for a single analyte (TC), one simultaneously imprints for up to four analogs (TC, ETC, EATC and ATC) within a MIX. Binding studies using LC-MS showed the binding of the prepared xerogels with the four analogs. In some formulations, preferential uptake of ETC, EATC and ATC relative to the template molecule (TC) was observed. Computations of the interaction energies between silane monomers and the four analogs reveal that ETC, EATC and ATC have higher interaction energies and are more likely to be imprinted in comparison to TC.

Introduction

Molecular imprinting, the concept of which has been proposed by Pauling back in the 1930s,¹ has become a useful tool to develop materials with selective binding properties.² The typical protocol for preparing molecularly imprinted polymers (MIPs) involves the polymerization of functionalized monomers around a target molecule or structural analog and subsequent template removal to create the molecular recognition site that binds the target molecule (analyte). Molecularly imprinted materials have been used in separation sciences,³ sensor design,⁴ drug design⁵ and catalysis.⁶ These materials have also been used as selective enrichment and pretreatment sorbents in complex sample analysis.⁷⁻⁹

Most MIPs are based on organic acrylate or acrylic type polymers¹⁰ which exhibit high selectivity and affinity towards the target compound, however, these platforms generally exhibit poor analyte accessibility and mass transport.¹¹ Molecularly imprinted materials have also been developed using sol–gel processing techniques,¹²⁻¹⁴ which provide a facile means to create porous materials (xerogels) under ambient conditions.¹³ Molecularly imprinted xerogels (MIXs) can exhibit good selectivity and superior mass transport in comparison to MIPs.¹⁴⁻¹⁵ In addition, the MIXs ease of preparation and gelation at ambient conditions (particularly important when preserving weak interactions) are other advantages over MIPs.¹⁶ Although target analyte rebinding to MIPs and MIXs has been verified in numerous reports, the imprinting process per se is scarcely discussed. It is commonly believed that imprinting results in the formation of shape-complementary microcavities with defined spatial arrangement of functional groups.¹⁷⁻¹⁸

In this paper, we investigate the binding site speciation of tetracycline (TC) (Fig. 1) within a simple MIX platform by electronic absorbance, liquid chromatography with ion-trap mass spectrometry (LC-ITMS), and first-principles theory. It is well-known that TC is not a stable molecule and it undergoes transformation over time.¹⁹⁻²¹ The change in pH readily transforms TC to its anhydro form,²² anhydrotetracycline (ATC) at pH < 2, and to its epimer forms,²³ 4-epitetracycline (ETC) and 4-epianhydrotetracycline (EATC), at pH 2–6 (see Fig. 1 for their chemical structures). The objective of this study is to determine whether or not the TC molecule that is initially used as the template is able to maintain its chemical structure during the imprinting process. We hypothesize that the changes in the chemical composition and pH during the xerogel formation

Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, NY, 14260-3000, USA. E-mail: dianaaga@buffalo.edu; chefvb@buffalo.edu

Fig. 1 Chemical structures of tetracycline and its analogs (with their respective molecular ions, m/z) used in this research.
process could cause TC to be transformed, resulting in critical changes in the final imprinted cavities.

The TCs are widely used class of antibiotics in human and animal therapy, and also are added as growth promoters in animal feeds. Due to the high use rate of TCs in animal agriculture, this class of antibiotics has been detected in soil and water as environmental contaminants. We have previously developed a TC-responsive MIX albeit using a more complex xerogel platform.

Experimental

Reagents

Water was purified by using a Nanopure Diamond™ water purifier (Barnstead, Dubuque, IA). The following reagents were used: tetracycline (TC), oxytetracycline (OTC) and hydrochloric acid (ACS Grade) (Fisher Scientific); chlortetracycline (CTC) hydrochloride and 4-epianhydrotetracycline (EATC) hydrochloride (Sigma-Aldrich); anhydrotetracycline (ATC) hydrochloride and 4-epitetracycline (ETC) hydrochloride (Acros Organics); methanol (HPLC grade), acetonitrile (HPLC grade) and tetrahydrofuran (Burdick & Jackson); allyltrimethoxysilane (AtEOS); tetramethoxysilane (TMOS), tetraethoxysilane (TEOS) and trimethylchlorosilane (TMCS) (Gelest); and ethanol (200 proof ACS/USP grade) (Pharmeco).

TC-imprinted xerogel preparation

A TC stock solution (28.31 mg mL⁻¹) was prepared in EtOH. The TEOS sol was formed by mixing (in order) 4.53 mL TEOS, 6.21 mL of TC stock solution, and 1.20 mL of 0.1 M HCl. The TMOS sol was formed by mixing (in order) 3.00 mL TMOS, 6.21 mL of TC stock solution, and 1.20 mL 0.1 M HCl. In addition, to examine the behavior of other TC analogs, OTC and CTC were used as templates in preparing imprinted materials. However, the low solubility of CTC in the reaction mixture posed problems in the xerogel preparation and its use was discontinued. Also, OTC was not pursued because preliminary calculations showed weak interactions as discussed below. For AtEOS, the sol was formed by mixing (in order) 2.30 mL AtEOS, 2.27 mL TEOS, 6.21 mL of TC stock solution and 1.20 mL 0.1 M HCl. The corresponding blanks (non-imprinted xerogels, NIXs) were prepared by replacing TC in ethanol with pure ethanol. The amount of reagents used was based on optimization done in preparing the xerogels at the preliminary stage.

Sols were sonicated under ambient conditions for 5–10 min to form a visually homogenous sol. The sol was then sealed and mixed for 30 s with a touch mixer (Fisher Scientific Model 231). Sols were then stored at 45 °C for 3 days to form xerogel monoliths.

MIXs and NIXs were crushed by using a mortar and pestle, and further powderized by using a ball mill (Fritsch Mini-Mill Pulverisette 23). Each MIX and NIX was divided into half. One portion was end capped while the other was used as it is. For end capping, a 0.5 g portion of MIX or NIX was mixed with 3 mL of TMCS for 24 h at room temperature. Excess reagents were removed by washing with tetrahydrofuran (4 × 5 mL) and acetonitrile (2 × 5 mL). End capping is done to remove the surface silanols that formed and can result in non-specific interactions with the target analyte.

Pressurized liquid extraction

Xerogel powders were subjected to pressurized liquid extraction (PLE) (Dionex model ASE 200) for template removal, monomer extraction, and MIX clean-up. Methanol served as the PLE extraction solvent (60 cycles, 70 °C, 105 bars, static time = 10 min).

Absorbance measurements

Electronic absorbance spectra were measured by using a HP 8452A diode array spectrophotometer. Quartz cuvettes (1 cm²) were used for all experiments. Appropriate blanks were used.

Liquid chromatography with ion-trap mass spectrometry (LC-ITMS)

The LCQ LC-ITMS system (Thermo Finnigan) was used to elucidate the chemical structures of the template molecules within the MIX. Separation was achieved by using a BetaBasic C18 column (2.1 × 100 mm, 3 mm particles) (Thermo Fisher Scientific) under gradient elution (t = 0–3 min, 5% acetonitrile and 95% water with 0.3% formic acid; t = 3–15 min, acetonitrile increased linearly from 5% to 95%; t > 15 min, 95% acetonitrile and 5% water with 0.3% formic acid). The flow rate was 200 mL min⁻¹, the column temperature was 30 °C, and the full loop injection volume was 20 mL. The LC-ITMS system was equipped with electrospray ionization and was operated in positive ionization mode. The capillary temperature was 200 °C, the capillary voltage was 10 V, and the spray voltage was 4.5 kV for all applications. Nitrogen was used as the sheath gas at a flow rate of 20 mL min⁻¹, and helium gas was used to induce dissociation of selected ions using 48% normalized collision energy. Initial MS scan was performed and two molecular ions (445 and 427 m/z) were observed, corresponding to the molecular ions (M + H)⁺ of TC and ATC, respectively. These molecular ions were isolated and fragmented (MS²) to monitor 445 → 427 and 445 → 410 transitions for TC, and the 427 → 410 transition for the anhydro forms of TC.

Binding site speciation determination

To evaluate the impact of TC instability under our imprinting conditions, we measured the time-dependent electronic absorbance spectra and LC-ITMS profiles for TC dissolved in solution sans silanes in the dark at 45 °C (with and without acid). Because the acid used as catalyst in the sol–gel process may transform TC to ETC and ATC, it is important to test the effect of acid on the stability of the TC solutions. PLE washings from MIXs (end capped and uncapped) were also measured by electronic absorbance and LC-MS.

Binding studies

The binding capability of the prepared xerogels was tested by incubating 1 mL solution containing the four TC analogs (5 μg L⁻¹) in methanol with 50 mg of xerogels (MIXs/NIXs) for
10 minutes. A 100 μL aliquot was obtained and dried with nitrogen gas. The sample was then reconstituted with 100 μL of deionized distilled water. A 1 mL solution containing the four TC analogs in deionized distilled water was also used of which 100 μL aliquot was obtained and used to analyze TCs content. The amount of TC that binds with xerogels was known by injecting the solution in LC-MS determining the unbound TC remaining in the solution. Losses of TC in terms of degradation from the effect of temperature and storage were taken in consideration ensuring that these are accounted for.

The binding of each TC analog to the MIX was characterized by its imprinting factor (IF), which is defined as:

\[
IF = \frac{\text{amount of TC bound per mg MIX}}{\text{amount of TC bound per mg NIX}}
\]  

(1)

The higher the IF value, the higher is the relative amount of the TC analog binding to the MIX compared to the NIX, indicating a higher specific binding.

Analysis of TC concentrations was performed on the Agilent 6410 triple quadrupole MS equipped with the 1100 HPLC system (Palo Alto, CA). A 10 μL aliquot was used for sample injection. Separation was achieved on a Phenomenex Inc. (Torrance, CA) Kinetex C18 column (100 × 2.1 mm, 2.6 μm particle size) at a flow rate of 200 μL min⁻¹. A gradient mobile phase of 0.3% formic acid (A) and acetonitrile (B) was used. The gradient profile consisted of a mobile phase of 95% A held for the first 30 s, and then gradually increased to 55% B in the next 4 min, held for 3.5 min isocratically and then ramped back to 95% within 1 min. The total run time was 15 min.

Ionization was achieved through positive electrospray ionisation (+ESI) at a spray voltage of 4 kV situated at a 90° angle to the entrance. The highest sensitivity was achieved when using a drying gas temperature of 350 °C, a nebulizer pressure (N₂) of 22 psi, and drying gas (N₂) of 11 L min⁻¹. The TC analogs were monitored using two product ions (445 → 410 and 427 → 410) in multiple reaction monitoring (MRM). The fragmentor and collision voltages were tuned for each TC analog to achieve the optimum signal intensity. All data were collected and analyzed by Agilent Technologies MassHunter™ Software Version B (Palo Alto, CA).

### Statistical analysis

All results are the average of at least three separate experiments under a given set of conditions. All error bars represent ± one standard deviation. Microsoft Excel Analysis Toolpak was used to carry out all statistical calculations. Statistical data treatment used analysis of variance (ANOVA) to determine the significance of the different TC analogs and end capping on the TC reuptake.

### Computational methods

Each possible form of the TC template (TC, ETC, EATC, ATC and OTC) and each silane monomer (TEO, TMOS and AtEOS) (Fig. 2) were subjected to conformational searches at the quantum mechanical level of theory using the Spartan software package.²⁶ The PM3 semi-empirical Hamiltonian was initially used for structural optimizations. The three lowest energy conformers were then further optimized at the Hartree–Fock (HF) level of theory by using three Gaussian-type basis sets [3-21G, SV(P) and SVP]. Additional computations were also performed using the B3LYP/3-21G density functional theory (DFT) method to check that inclusion of electron correlation (as approximated by this DFT method) does not alter the structures substantially. The systems with the lowest energies represent $E_{\text{template}}$ or $E_{\text{monomer}}$, respectively, for each basis set.

For the monomer–template complexes, initial conformational searches were carried out by using the molecular mechanics force field (MMFF94²⁷) in Spartan. The geometries of the 10 most stable conformers were optimized by using the PM3 semi-empirical Hamiltonian. The geometries with the three lowest energies were further optimized by using HF and DFT as described above. The structure with the lowest energy was used to obtain the uncorrected interaction energy, $\text{IE}_{\text{un}}$:

\[
\text{IE}_{\text{un}} = E_{\text{template–monomer}} - E_{\text{template}} - E_{\text{monomer}}
\]  

(2)

Basis set superposition error (BSSE) corrections to the interaction energies were also computed. This was done at the HF level of theory by using the SV(P) and SVP basis sets, utilizing the counterpoise approach, to give the BSSE-corrected interaction energy $\text{IE}_{\text{cor}}$.²⁸ The computational methods were chosen with efficiency in mind since we plan to extend the protocol to larger systems in subsequent works and sample a larger conformational space for the IE calculations.

### Results and discussion

#### TC stability under imprinting conditions

The TC electronic absorbance spectrum arises from two separate chromophores namely the A chromophore that is made up of the β-tricarbonyl system of ring A and the BCD chromophore which comprises the π-electronic system located on rings B, C and D⁹ (Fig. 1). The peak around 360 nm (resulting in the yellow solution color) is associated with ring BCD chromophore. The absorbance at 360 nm gradually disappeared over time in TC imprinting solutions, either with acid (Fig. 3A) or without acid (Fig. 3B), and was replaced by bands between 300–350 nm and 200–260 nm, over four days of observation. The solutions under acidic conditions mimic MIX preparations.

Ratiometric analysis of the absorbances at 220, 260, 300 and 360 nm was performed to determine how TC is affected during the imprinting process. Fig. 4 summarizes the time-dependent changes in the TC absorbance ratios for solutions (line graph)
and MIX washings (bar graph). All the absorbance ratios (\(A_{360}/A_{300}\), \(A_{360}/A_{260}\) and \(A_{360}/A_{220}\)) initially decreased on day 1 and then remained constant over the next 3 days of observation. The observed changes in ratios can be attributed to the decrease in the absorbance at 360 nm. It was also observed that the ratiometric values for the TC solutions are close to those of the PLE washings of the TC-imprinted xerogels. The PLE washings from both end capped xerogels (TEOS and TMOS) have lower ratiometric values than their uncapped counterparts.

**Fig. 5** Total ion chromatogram of (A) TC solution exposed to imprinting condition over a period of 3 days, (B) extracted ion chromatogram (\(m/z = 427\) and 445, for ATC and TC, respectively) of PLE washings from xerogels that were prepared with TMOS or TEOS, uncapped and end capped, and (C) total ion chromatogram of freshly prepared OTC solution and washings from the end capped OTC:TEOS.

**LC-ITMS**

Fig. 5A presents typical total ion chromatograms for a freshly prepared TC solution, and solutions after 1, 2, and 3 days of exposure to imprinting conditions. The major peak at 9.5 min corresponds to TC (\(m/z = 445\)), while the minor peaks at 9.3 min...
correspond to ETC ($m/z = 445$). There is an increase in the peak area of ETC (109% and 150% increase after a day and two days of exposure) until a decrease on the third day. The increase in the ratio and peak height suggests that more ETC was present relative to TC during the imprinting process. This is possible since the acidic condition within xerogel allows the epimerization of TC to ETC. However, by the third day of the imprinting process, degradation might have taken place as the peak heights of the ETC and TC are only 30% and 19% relative to their original peak heights. These two peaks decrease with time as other peaks appear at longer retention time.

The extracted ion chromatograms of the washings from both end capped MIX (TEOS and TMOS) (Fig. S5B) revealed the appearance of two major peaks which correspond to ATC ($m/z = 427$, retention time = 10.60 min) and its epimer EATC ($m/z = 427$, retention time = 10.40 min), and corresponding decrease in TC and ETC peaks. This change was not obvious in the uncapped MIX washings, suggesting that the conditions during the end capping process promote TC transformations to ATC. The trimethylchlorosilane used as an end capping reagent usually releases chloride ion at the same time that the hydrogen from the hydroxyl group is released during the end capping process. For the AtEOS MIX, the same trend can be shown wherein the TC and ATC are higher in uncapped while EATC and ATC are higher in the end capped xerogels. Similarly, Fig. 5C revealed the same changes in the OTC:TEOS formulation where the major peak of OTC ($m/z = 461$) in the washings from non-end capped MIX was replaced by a different peak which has $m/z = 443$, eluting at a different retention time in the end capped MIX washings. Similar to TC, the formation of the anhydro form of OTC occurred under acidic conditions. It has been reported previously that the hydroxyl group of TC at the C-6 position rapidly dehydrates at acidic pH or higher temperature.

Taken together, the results from the absorption and LC-ITMS experiments provide additional evidence of the instability of TC reported previously. The acidic conditions within the xerogel may contribute to the transformation of TC, which undergoes epimerization reaction at pH 2-6 to form ETC and EATC. Furthermore, ATC is reported to be formed at pH < 2 (ref. 22) (Fig. 1). The observed results indicate that the binding sites of the imprinted xerogels will not be exclusively selective towards TC, but will more likely recognize the TC transformation products, ATC, EATC and ETC. A previous study that used acrylic MIP and TC as template molecule also reported that TC itself is not imprinted, and instead was converted to other forms during the imprinting process. Using HPLC-UV, ATC (56%), EATC (28%), and an unknown component (~15%) was reported to be responsible for imprinting in one formulation, while in another formulation TC accounts for only 7% compared to ETC (67%) and ATC (21%).

**Binding studies**

The selectivity of the imprinted xerogel was tested by performing binding studies using a mixture of the four TC analogs. Fig. 6 showed the imprinting factor (IF) for each TC analog in the different selected xerogels using methanol as the rebinding solvent. End capping of the xerogels significantly improved the IF of most TC analogs in all xerogels (except the EATC in TEOS xerogel, TC and ETC in TMOS xerogel and EATC and ATC in AtEOS xerogel). There is no significant difference among the IFs of the TC analogs within xerogel formulation (uncapped and end capped) with the exception of TMOS formulation (end capped) wherein the IFs of EATC and ATC are significantly higher than those of TC and ETC.

The results indicate that the resulting xerogel materials are generally class-selective to TC group. The ATC and EATC tend to have higher IFs than the other compounds tested, and can be explained by the conversion of the template molecule to ATC and ETC during the imprinting process, which occurs under acidic conditions.

**Computational**

The use of four TC compounds (TC, ATC, EATC and ETC) as template for calculations has provided the interaction energies that are shown in Tables 1 and 2. The complex containing ETC as template gave the highest interaction energy with the monomer (TEOS and TMOS) in the HF calculations of Table 2. In the PM3, HF (3-21g) and B3LYP (3-21g) calculations, EATC with TMOS had higher interaction energy than the ETC counterpart.

**Table 1 Calculated interaction energies, IE_{min} (in kJ mol⁻¹) using different computational methods and ranking (in parentheses)**

<table>
<thead>
<tr>
<th>Complex</th>
<th>PM3</th>
<th>HF (3-21g)</th>
<th>B3LYP (3-21g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATC–TMOS</td>
<td>−37.3 (3)</td>
<td>−100.6 (3)</td>
<td>−151.1 (2)</td>
</tr>
<tr>
<td>EATC–TMOS</td>
<td>−44.6 (1)</td>
<td>−130.5 (1)</td>
<td>−180.6 (1)</td>
</tr>
<tr>
<td>ETC–TMOS</td>
<td>−69.5 (2)</td>
<td>−127.9 (2)</td>
<td>−131.9 (3)</td>
</tr>
<tr>
<td>TC–TMOS</td>
<td>−12.5 (4)</td>
<td>−69.6 (4)</td>
<td>−125.5 (4)</td>
</tr>
<tr>
<td>ATC–TEOS</td>
<td>−48.8 (2)</td>
<td>−46.7 (3)</td>
<td>−95.6 (3)</td>
</tr>
<tr>
<td>EATC–TEOS</td>
<td>−39.4 (3)</td>
<td>−123.7 (2)</td>
<td>−174.7 (1)</td>
</tr>
<tr>
<td>ETC–TEOS</td>
<td>−69.9 (1)</td>
<td>−126.2 (1)</td>
<td>−125.5 (2)</td>
</tr>
<tr>
<td>TC–TEOS</td>
<td>−31.6 (4)</td>
<td>−24.2 (4)</td>
<td>−51.0 (4)</td>
</tr>
<tr>
<td>OTC–TEOS</td>
<td>−38.8 (5)</td>
<td>−12.8 (5)</td>
<td>−38.8 (5)</td>
</tr>
<tr>
<td>ATC–AIEOS</td>
<td>−25.4 (3)</td>
<td>−128.3 (1)</td>
<td>−106.8 (1)</td>
</tr>
<tr>
<td>EATC–AIEOS</td>
<td>−45.1 (1)</td>
<td>−111.8 (3)</td>
<td>−84.6 (3)</td>
</tr>
<tr>
<td>ETC–AIEOS</td>
<td>−36.2 (2)</td>
<td>−119.1 (2)</td>
<td>−69.7 (4)</td>
</tr>
<tr>
<td>TC–AIEOS</td>
<td>−19.6 (4)</td>
<td>−72.2 (4)</td>
<td>−99.8 (2)</td>
</tr>
</tbody>
</table>

*— = no structure with a negative IE was found.*
The EATC-TEOS has the highest interaction energy with B3LYP (3-21g). For the interaction of TC and its analogs with AtEOS, either ATC or EATC has the highest interaction energy in the different theoretical models. The computations also revealed that TC has the lowest interaction energy among the four TCs in all models except in the B3LYP level where TC has the second highest interaction with AtEOS. Calculations were also performed using OTC with TEOS, but the calculated interaction energies were even lower than that of TC. In some calculations, no structures with negative interaction energies could be found, thus providing a rationale on why excess OTC reagent did not imprint in the xerogel.

In the geometry optimizations with the B3LYP density functional theory, another tautomer was predicted to be lower in energy (Tautomer 2) (Fig. 7). This change in structure was observed for all the four TCs. This tautomer was already reported in literature as one of the stable tautomer forms using AMI/SCRF calculations. Both tautomer structures were used in calculation using Hartree–Fock with the SV(P) and SVP basis sets. The results showed that the dimers with Tautomer 2 had larger magnitudes of the interaction energies. The trend with regard to the magnitude of the interaction energy for the template is mostly ETC > EATC > ATC > TC for both tautomers (Fig. 8), with the exception in TEOS (Tautomer 1) where TC is slightly higher than ATC in SV(P) basis set. In terms of the BSSE-corrected energies, the trend still remains the same wherein ETC > EATC > ATC > TC. For the AtEOS, EATC has the highest BSSE-corrected energies instead of ETC. Under the assumption that larger magnitudes of the interaction energies are positively correlated with the quality of the imprint, the computational results support the notion that other TC products are more preferably imprinted than the TC itself.

**Conclusions**

The imprinting process of TC in xerogel was monitored by absorbance and LC-ITMS. The absorbance profile of the TC solutions under conditions that mimic the xerogel formation process (without the silanes and monomers) showed the transformation of TC over time, suggesting that the template that imprints on the xerogel is not necessarily the starting template molecule. Analysis of TC from the washings of xerogels using LC-ITMS revealed the formation of ETC during the imprinting process, and the formation of EATC and ATC during the end capping process. The combination of absorption spectroscopy and mass spectrometry provided evidence indicating that TC is transformed during the imprinting processes such that the template is actually a combination of ETC, EATC, TC, and ATC. Therefore, it is possible that the transformation products of TC are preferentially imprinted, rather than the TC itself, as

### Table 2

<table>
<thead>
<tr>
<th>Complex</th>
<th>Tautomer 1</th>
<th></th>
<th>Tautomer 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SV(P)</td>
<td>SVP</td>
<td>SV(P)</td>
<td>SVP</td>
</tr>
<tr>
<td>Uncorrected IE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATC–TMOS</td>
<td>−62.0(3)</td>
<td>−61.4(3)</td>
<td>−65.3(3)</td>
<td>−64.2(3)</td>
</tr>
<tr>
<td>EATC–TMOS</td>
<td>−73.2(2)</td>
<td>−72.6(2)</td>
<td>−74.9(2)</td>
<td>−73.9(2)</td>
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<tr>
<td>ETC–TMOS</td>
<td>−90.7(1)</td>
<td>−91.9(1)</td>
<td>−92.5(1)</td>
<td>−93.3(1)</td>
</tr>
<tr>
<td>TC–TMOS</td>
<td>−26.1(4)</td>
<td>−26.8(4)</td>
<td>−35.1(4)</td>
<td>−34.7(4)</td>
</tr>
<tr>
<td>ATC–TEOS</td>
<td>−14.2(4)</td>
<td>−15.2(3)</td>
<td>−19.5(3)</td>
<td>−19.8(3)</td>
</tr>
<tr>
<td>EATC–TEOS</td>
<td>−71.3(2)</td>
<td>−69.2(2)</td>
<td>−72.4(2)</td>
<td>−71.1(2)</td>
</tr>
<tr>
<td>ETC–TEOS</td>
<td>−89.9(1)</td>
<td>−90.1(1)</td>
<td>−90.2(1)</td>
<td>−89.9(1)</td>
</tr>
<tr>
<td>TC–TEOS</td>
<td>−16.6(3)</td>
<td>−14.5(4)</td>
<td>−17.6(4)</td>
<td>−16.5(4)</td>
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<tr>
<td>ATC–AtEOS</td>
<td>−18.8(1)</td>
<td>−18.9(2)</td>
<td>−18.8(2)</td>
<td>−18.4(1)</td>
</tr>
<tr>
<td>EATC–AtEOS</td>
<td>−18.4(2)</td>
<td>−18.9(1)</td>
<td>−18.8(1)</td>
<td>−18.4(2)</td>
</tr>
<tr>
<td>ETC–AtEOS</td>
<td>−17.0(3)</td>
<td>−17.3(3)</td>
<td>−17.6(3)</td>
<td>−17.6(3)</td>
</tr>
<tr>
<td>TC–AtEOS</td>
<td>−11.3(4)</td>
<td>−10.8(4)</td>
<td>−11.3(4)</td>
<td>−10.5(4)</td>
</tr>
<tr>
<td>Corrected IE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATC–TMOS</td>
<td>−46.8(3)</td>
<td>−47.7(3)</td>
<td>−46.4(3)</td>
<td>−46.3(3)</td>
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<tr>
<td>EATC–TMOS</td>
<td>−58.8(2)</td>
<td>−60.3(2)</td>
<td>−60.5(2)</td>
<td>−61.4(2)</td>
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<tr>
<td>ETC–TMOS</td>
<td>−77.9(1)</td>
<td>−81.2(1)</td>
<td>−79.6(1)</td>
<td>−82.3(1)</td>
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**Fig. 7** Two tautomers of tetracyclines considered in the computations.

**Fig. 8** The structures of the different TC analogs showing an optimized orientation for the interaction with silane (TEOS). Note that the two analogs (ETC and EATC) with the highest interaction energies with TEOS exhibited an orientation that promotes hydrogen-bonding interaction relative to the other two analogs (ATC and TC). (Structure images were generated using UCSF Chimera. 36)
supported by computational calculations that showed higher interaction energies between the monomers and the ETC, EATC, and ATC. This research underscores the importance of considering the stability of template molecules, and characterizing the extracted template after the imprinting process when designing selective cavities in molecular imprinting procedures.

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