Research Square

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Structural investigation of aaptourinamine by a time-saving module-assembly based calculation

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Research Article

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Abstract

Unambiguously confirming natural products with high C/H ratio or proton-deficient and multi heteroatoms is challenging. A time-saving module-assembly calculation method named ‘Dooerafa’ including of constructing the meta-structures by a grafting method based on the crucial and the limited two dimensional nuclear magnetic resonance (2D NMR) correlations, ‘ring-contraction strategy’ based on mechanic force field and quantum chemical theory, and self-assemble calculations in Python programing for shaping up the structural candidates along with DFT-GIAO calculation was studied. And this method, verified by a known alkaloid spiroreticulatine with structure determined by X-ray diffraction, was performed for the structural elucidation of aaptourinamine which was isolated from marine sponge *Aaptos suberitoides*, showing us a brand new scaffold of imidazo[4,5,1-ij]pyrrolo[3,2-f]quinolin-7(8H)-one which has a biosynthetically relationship with the bioactive and structurally unique aaptamine alkaloid.

Introduction

For Natural products have highly diverse chemical structures [1, 2], and some have complicated skeleton, which is a challenge to the organic chemistry [3]. From a structure-elucidation point of view, it is still a fussy work for those complicated structures which are originated from certain natural organisms and especially have high C/H ratio or proton-deficient and multi heteroatoms. Because there are many unanticipated structural misassignment such as sclerophytin A [4, 5], isoschizogamine [6, 7], and calyculaglycoside A [8, 9], despite our advantages nowadays including nuclear magnetic resonance (NMR), electron circular dichroism (ECD), mass spectrometry (MS), and X-ray crystallography [10], as well as the biosynthesis-assisted structural elucidation [11]. A natural structure, a meta-fashion related issue [12–14]. Overall, how many eyes are needed to watch them?

As an important alternative and hopeful tool, computer-assisted structural elucidation (CASE) plays more and more irreplaceable role [15]. There are four CASE programs including ACD Labs Structure Elucidator, Bruker CMC-se, Mestrelab MNova Structure Elucidator and Logic for Structure Determination (LSD) which were mainly based on empirical estimates and comparison of database. Whereas it is an unreliable means for those compounds with less two dimensional (2D) NMR cross peaks, besides, the HMBC correlation is hard to be predicted.

With the increasing speed of computers, it is now feasible to carry out accurate chemical shift calculations for even complex natural products using Density Functional Theory (DFT) programs. This has been proved to be a very useful tool for natural product structure elucidation [16, 17]. Moreover, Python with legibility and extensibility has become a popular computer programming language in the world contrasting to other programming languages such as Java and C++, which has been applied to DP4-AI method for confirming the molecular structure [18].

Hence, a time-saving module-assembly based calculation method called ‘Dooerafa’ (doors refer to further) which was constructed by a grafting method based on the crucial and limited 2D NMR

correlations, self-assemble in Python programing for shaping up the structural candidates, and DFT-GIAO calculation was exploited to elucidate the natural structures.

Materials And Methods

General experimental procedures

NMR spectra were measured on a Bruker AVANCE NEO 400 (1H, 400 MHz; 13C, 101 MHz), an Aglient DD2-500 (1H, 500 MHz; 13C, 126 MHz), or a JEOL JNM-ECP 600 (1H, 600 MHz; 13C, 151 MHz) spectrometer. The 2.48 and 40.1 ppm resonances of DMSO were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. HRESIMS spectra were measured on Micromass Q-Tof Ultima GLOBAL GAA076LC mass spectrometers. Semi-preparative HPLC was performed using a Waters 1525 pump equipped with a 2998 photodiode array detector and a YMC C18 column (YMC, 10 × 250 mm, 5 μmol/L). Preparative HPLC was performed using a Shimadzu LC-20AR pump equipped with an LC-20A array detector and a SilGreen C18 column (SilGreen,20 × 250 mm, 5 μm). And Sephadex LH-20 (Amersham Pharmacia Biotech AB) was used for column chromatography. Silica gel [(200—300 mesh, 300—400 mesh and silica gel H), Qingdao, China] were used for column chromatography, and pre-coated Silica gel plates (GF254, Qingdao, China) were used for TLC, and spots visualized by heating SiO2 plates sprayed with 10% H2SO4 in EtOH.

Animal material

The marine sponge *Aaptos suberitoides* was collected from Yongle Islands of Xisha Islands of the South China Sea in May 2012 and was identified by Leen van Ofwegen, National Museum of Natural History, Netherlands. The voucher specimen (No. XS-2012-30), which was frozen at − 20°C, was deposited at the School of Medicine and Pharmacy, Ocean University of China, P. R. China.

Extraction and isolation

The frozen sponge *Aaptos suberitoides* (5.4 kg, wet weight) was shredded and extracted with MeOH (five times, each time for 3 days). Then the combined solution was concentrated under vacuum and desalinated to yield 325.0 g of residue. The residue was subjected to vacuum liquid chromatography (VLC) on a silica gel column by gradient mixtures of petroleum ether/acetone (from 50/1 to 0/1, v/v) and CH_2Cl_2/MeOH (from 10/1 to 0/1, v/v) to yield nine major fractions (Fr.1 − 9). Fr.6 (12 g) showed cytotoxic activity against A549 and H1299 tumor cell lines with inhibition ratio of more than 92% at the concentration of 50 μg/mL. And this fraction was divided into eight subfractions (Fr.6 − 1 − 6–8) by silica gel CC eluting with petroleum CH_2Cl_2/MeOH (50/1 − 0:1, v/v). Fr.6 − 5 (0.4 g) was separated by Sephadex LH-20 (MeOH) to give six fractions (Fr.6-5-1 − 6-5-6). Fr.6-5-5 was purified by semipreparative HPLC (YMC C8, 10×250 mm, 5 μm; MeOH/H_2O 40:60, v/v; 1.5 mL/min; detection UV: 230 nm) to give compound aaptourinamine (1.5 mg).

Aaptourinamine

yellow oil; ^{1}H NMR and ^{13}C NMR data, Table S2 (see Additional file 1); HRESIMS m/z 210.0661 [M + H]+ (calcd for $C_{12}H_8ON_3$, 210.0662) and 232.0481 [M + Na]+ (calcd for $C_{12}H_7ON_3Na$, 232.0481).

Procedure for the 'Dooerafa'

In 'Dooerafa', the meta-groups were deduced on the basis of experimental data, and the meta-structures were assembled by grafting method to make the connection points less than 14 for saving time. Then a self-assemble procedure written by Python is expected to assemble the meta-groups based on meta-structures to obtain all the structure candidates which were converted from the calculated group data and were drawn by manual in Chemoffice 14. InChI key of each structure was obtained in Excel embedded with Chemoffice 14. The duplicates were removed according to InChI code. The molecular energy calculation in mechanic force field is performed by Python controlling Chem 3D and Microsoft Excel software. Conformational search were performed on Maestro 11.9 of Schrödinger software package [19]. DFT-GIAO calculation of ^{1}H and ^{13}C NMR data were performed on Gaussian 16 at PCM/mPW1PW91/6–31 + G** level. 'Dooerafa' calculations were performed in Supercomputing Center in Pilot National Laboratory for Marine Science and Technology (Qingdao).

Quantum chemical calculations

The quantum chemical calculations were carried out by Gaussian 16 [20] software using the density functional theory (DFT). The input geometries were built by the Chemdraw Pro 14.1 software with an MM2 force field. The lowest energy conformers within 10 kcal/mol were subjected to further DFT calculations at the B3LYP-D3(BJ)/6-31g(d) level in the gas phase, and all minima displayed no imaginary frequencies by vibrational frequency analysis at the same level. Thermal corrections to Gibbs energies were obtained from frequency calculations at 298 K. The population of each conformer and Gibbs energies ware calculated by Boltzmann distribution based on Gibbs free energy with the Shermo [21]. Then, conformers with distributions higher than 1% were chosen for the GIAO calculations of NMR shielding constants with spin-spin interactions were accomplished using the DFT method at the PCM/mPW1PW91/6–31 + G**//B3LYP/6-31G* level in DMSO, using the PCM model as the optimal method for error analysis. The calculated shielding constants of these conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy. The shielding constants (including C and H) obtained were directly statistically analyzed with experimental chemical shifts.

Results And Discussion

A case study of spiroreticulatine

For the efficiency and accuracy of 'Dooerafa', spiroreticulatine, a previously reported new alkaloid which was isolated from the marine sponge *Fascaplysinopsis reticulata* and unambiguously determined by X-ray diffraction [22], was used as a verification module.

Spiroreticulatine displayed good ^{1}H and ^{13}C NMR experimental data while HMBC cross peaks can only establish fragments of the structure. And there were at least two structures with good relation with 2D NMR experiments (Fig. 1). The problem of structure conformation can be easily distinguished by DFT-GIAO calculations of ^{13}C NMR data for now. But the real problem could be which kind and how many of the structural candidates the compound implied. So the 'Dooerafa' for spiroreticulatine was performed.

Because ^{2}J and ^{3}J correlations are ambiguous, which led the structure assignment difficult and more uncertain, only the segments which were determined unambiguously by the very certain ^{1}H-^{1}H COSY correlations and the HMBC correlations of methyl groups were assigned as the original issues (meta-groups, Figs. 1 and 2A). In that case, there are six meta-groups A-F for spiroreticulatine (Fig. 2A). However, there are 16 connection points (consistent with Additional file 1 Section 2) for shaping up a whole molecule which took about two weeks long for all the structure assembling in the preliminary 'Dooerafa'.

For the efficiency, 11 meta-structures (eleven assembling types for meta-groups A, C-F on B) which were lowered down to 14 connection points were constructed based on the grafting method and were used for the assembling calculation. And that took less than 16 hours (Fig. 2B). Then 854 group data by Python programming code based on the deduced connection points (see Additional file 3) were obtained and were converted to chemical structures.

Subsequently, five kinds of energies related to stability of the structures including stretch, bend, stretch-bend, torsion and total energy were calculated automatically by Python (see Additional file 3) in Chem 3D software using MM2 molecular mechanic force field [23, 24], because there must be a chemical reasonability for the natural products under a certain structural assembling strategy. That is, the natural products with more stable chemical features tend to be the real and especially chemically comfortable structures. As a result, 50 structures showing lower energy before the inflection point in the most important trend of the total energy were selected to calculate ^{1}H and ^{13}C NMR data using DFT-GIAO method (Fig. 3).

Furthermore, a related analysis of the fitting between experimental and calculated data using coefficient of determination (R^2) as well as mean absolute error (MAE) and truncated absolute difference (TAD) gave us the correct structure of spiroreticulatine which is the 5th one in all the 854 structure candidates. Other structures displayed good but not best match of ^{13}C NMR data and were contradicted with the HMBC correlations. Thus, the verification of spiroreticulatine indicated that 'Dooerafa' is suitable for determining the structure of natural products.

Then, the 'Dooerafa' was applied to confirm the structure of an unknown alkaloid named aaptourinamine which was lack of the 2D NMR correlations as well. Firstly, the structure of aaptourinamine was preliminarily analysed by NMR data.

Structural investigation of aaptourinamine

Aaptourinamine was isolated from marine sponge *Aaptos suberitoides*. It has a molecular of $C_{12}H_7ON_3$ as deduced from HRESIMS data m/z 210.0661 $[M + H]^+$ (calcd for $C_{12}H_8ON_3$, 210.0662) and 232.0481 $[M + Na]^+$ (calcd for $C_{12}H_7ON_3Na$, 232.0481) supported by the ^1H and ^{13}C NMR spectral data (see Additional file 1 Section 6). It was a potentially new compound with good purity and distinct ^1H and ^{13}C NMR experimental data having no match with the ^{13}C NMR database in MICRONMR [25]. However, it had high C/H ratio and was lack of efficient HMBC cross peaks to be unambiguously determined, neither is it possible for efficient ^{15}N NMR due to the insufficient sample amount [26].

With the aid of HMQC and ^1H-^1H COSY experiments, aaptourinamine displayed one NH proton at δ_H12.31 (1H, s), and six olefinic protons at δ_H8.71 (2H, one doublet J = 6.85 Hz and one overlapped singlet), 8.03 (1H, d, J = 6.85 Hz), 7.29 (1H, t, J = 6.82 Hz), 7.25 (1H, t, J = 2.57 Hz), 6.95 (1H, dd, J = 2.35, 2.05 Hz) in ^1H NMR. The profile of an aromatic compound was shown in ^{13}C NMR (DEPT) displaying eleven olefinic carbons (five olefinic CH) with chemical shifts around δ_C105-134, as well as one carbonyl group at δ_C168.8. The ^1H-^1H COSY and HMBC correlations indicated a dihydropyrrole moiety in which the crucial cross peaks of δ_H7.25/δ_C122.4 and δ_H8.03/δ_C122.4 were distinguished from a cross peak group of all the olefinic protons with the adjacent δ_C122.4/122.5. Besides, there must be an imine group except for five pairs of double bonds and the carbonyl group according to HRESIMS data. Thus, there are three potential core structure candidates A-C except for D with an unreasonable location of the carbonyl group (Fig. 4A), because chemical shift of the carbonyl carbon in D indicating macrocyclic structure candidates will be greatly deshielded compared with δ_C168.8 [27].

To save calculation time, the grafting method (see Additional file 1 Section 2) based on the core structure candidates was applied to obtain the meta-structures with 14 connection points (see Additional file 1 Section 2) as in the case of candidate A (Fig. 4B). Thus, there are totally 44 meta-structures as shown in Figs. 4 and 5, which was constructed on the basis of that an imine group and a carbonyl group were certain and were connected with the core structures A-C or outside the core structures to make sure of less than 14 connection points totally for each meta-structure.

Subsequently, 2507 structures were drawn manually by the group data obtained through the program code in Python. These structures showed very huge diversity with varied kinds of assembling. Then, the energies related molecular stability were calculated by Python controlling the Microsoft Excel and Chem 3D. The molecular energy in mechanic force field displayed an interesting trend for the structural change. And there is an inflection point at which the structures tend to be unstable with a sudden changing especially of the torsion, stretch-bend and total energies (Fig. 6, see Additional file 2), which indicated the structures are chemically unfavourable in the case that the main structural moieties of meta-groups have been confirmed based on the crucial experimental NMR data. So there must be a very knowledgeable reason to shape up a complex and chemically unfair structures existing in nature [28].

Then 150 structures before the inflection point showing lower energy were calculated for ^1H and ^{13}C NMR using DFT-GIAO method (Fig. 6, see Additional file 1 Section 4) and analysed the linear regression

parameters which were used to reflect the fitting between experimental and calculated data. Finally, the correct structure of aaptourinamine (A-205, the 9th one in the 2507 candidate structures, see Additional file 1 Section 5) with best match of calculated NMR data was shown as imidazo[4,5,1-ij]pyrrolo[3,2-f]quinolin-7(8H)-one which is structurally related to aaptamine alkaloid as predicted in the biosynthesis pathway in Scheme 1.

Therefore, aaptourinamine is shown as a new scaffold of aaptamine family which has totally different type featured by formation of a pyrrolo[3,2-f]quinolone core which is firstly found in nature, indicating an intriguing profile of the biosynthesis and bioactivity. The chemical shift of the carbonyl carbon C-8 at δ_C168.8 (Table S2, see Additional file 1) in aaptourinamine is greatly upshielded comparing to the normal ketone group probably due to the large and favourable conjugated system as in the case of exiguamine A [27b]. And the nitrogen atoms must play an important role for the unique conjugated aromatic system of 18 π-electrons over 16 centers [29].

To summarize, a calculation study of aaptourinamine by a time-saving module-assembly based calculation was performed in ‘Dooerafa’ which was validated through spiroreticulatine with structure determined by X-ray diffraction, providing the credible and accurate result. In this method, first, meta-groups are very important which were assigned from the believable NMR and HRESIMS experimental data. Then the grafting method is practical and efficient to obtain the meta-structures to save calculation time. Subsequently, the self-created program code in Python which shows all group data converting to the structure candidates is the first to report for determining the structure. And running the code only takes less than 16 hours, indicating the efficiency of ‘Dooerafa’.

On the other hand, DFT-GIAO calculation of NMR data has been proved to be a reliable method to predict the right structure. Especially ^{13}C NMR data which is almost not changing in different solvent in the practical experiments is absolutely suitable for the DFT calculation [30]. Nevertheless, ^{1}H NMR data is impressible to the solvent and is not a good choice to determine structure as in the cases of spiroreticulatine and aaptourinamine in the present study. Thus, a logical use of mechanic force field and quantum chemical theory of ‘ring-contraction strategy’ is the key point for the study.

Hence, the molecular energies trend in mechanic force field which showed clear inflection points is suitable to select the stable structures with great efficiency (Fig. 7), in which the correct structure of aaptourinamine occupied 9th in the top 150 structures. And the molecular energy with DFT calculation generally displayed a similar trend but showed a closer relation with the correct structure. Nevertheless, the 150 structures with lower energies are biogenically irregular but chemically favourable. Finally the right skeleton of the natural product was figured out according to the NMR calculation showing the best coefficient of determination (R^2). Thus, the structures which were ticked from the numerous candidates and showed the best match with the experimental data must be the right and unique natural occurring structure.

Conclusion

In conclusion, ‘Dooerafa’ provides a new method to elucidate the structures of natural products with high C/H ratio and multi heteroatoms. Meanwhile, aaptourinamine with an unknown scaffold of imidazo[4,5,1-ij]pyrrolo[3,2-f]quinolin-7(8H)-one, indicating an intriguing profile of the biosynthesis and bioactivity, was found. Besides, a meta (called ‘Yuan’ in Chinese) study time for the characteristic real issues also gives us a new opportunity. Since we have much evidence probably due to our having knowledge, the structure of a certain natural compound must be known in solution. But how many eyes shall we have. A different point of view is needed for the novel seeing of nature.

Abbreviations

NMR: nuclear magnetic resonance, ECD: electron circular dichroism, MS: mass spectrometry, CASE: computer-assisted structural elucidation, LSD: Logic for Structure Determination, 2D: two-dimensional, DFT: Density Functional Theory, VLC: vacuum liquid chromatography, R^2: coefficient of determination, MAE: mean absolute error, TAD: truncated absolute difference.

Declarations

Acknowledgments

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Author’s Contributions

P. L. conceived and designed the experiments. X. S., Z. W., T. J., C. W. performed the experiments and prepared the Supplementary materials. X. S. performed calculations. P. L., X. S. wrote the paper. All authors analyzed and discussed the results, and commented on the paper.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Scheme

Scheme 1 is available in supplementary section.

Figures

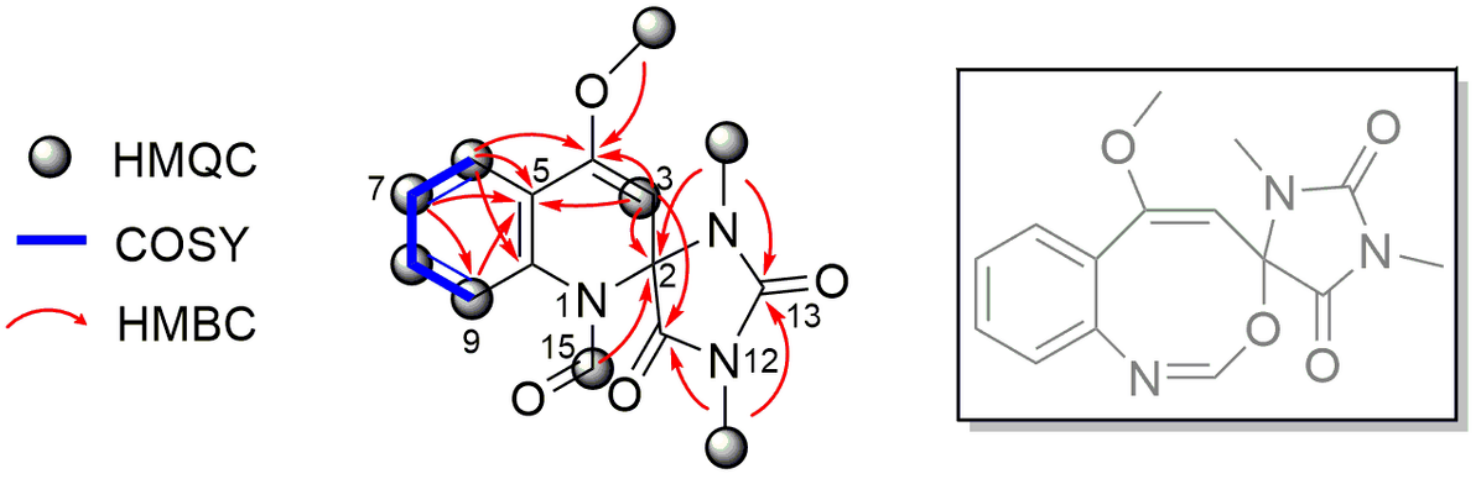

Figure 1

Key 2D NMR correlations of spiroreticulatine and the related possible structure candidate.

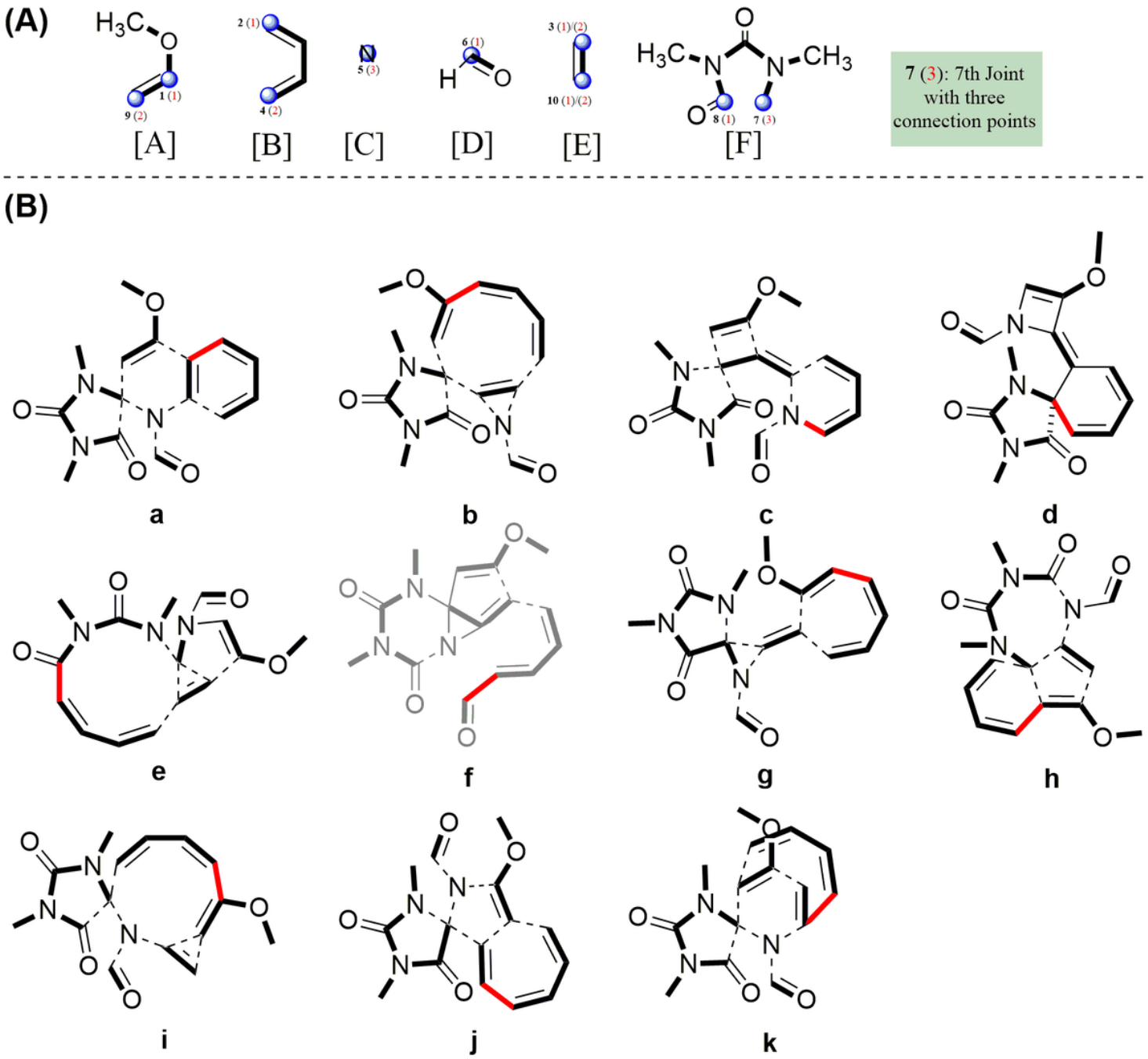

Figure 2

(A) Meta-groups of spiroreticulatine. **(B)** Meta-structures of spiroreticulatine (the greys are left out for the unreasonable ^{13}C NMR chemical shift of aldehyde group, dashed bond can be freely assembled).

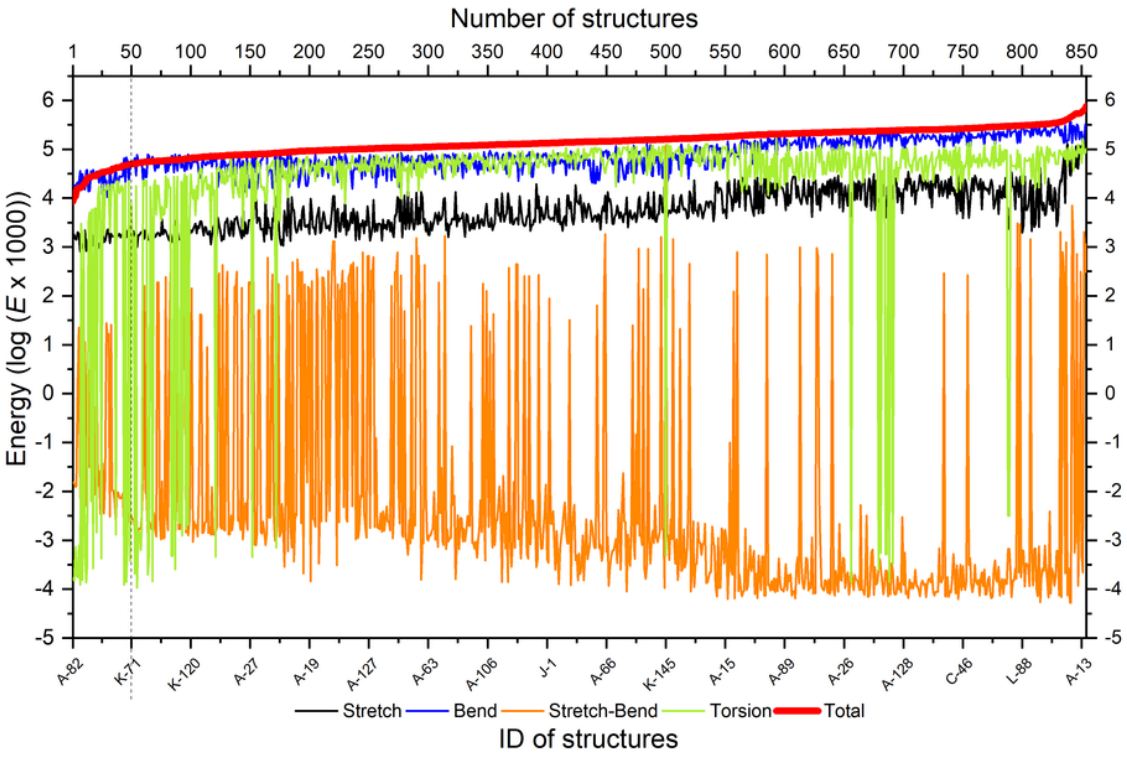

Figure 3

Energy trend of the structures for spiroreticulatine (the values were converted with $\log_{10}$(E×1000)).

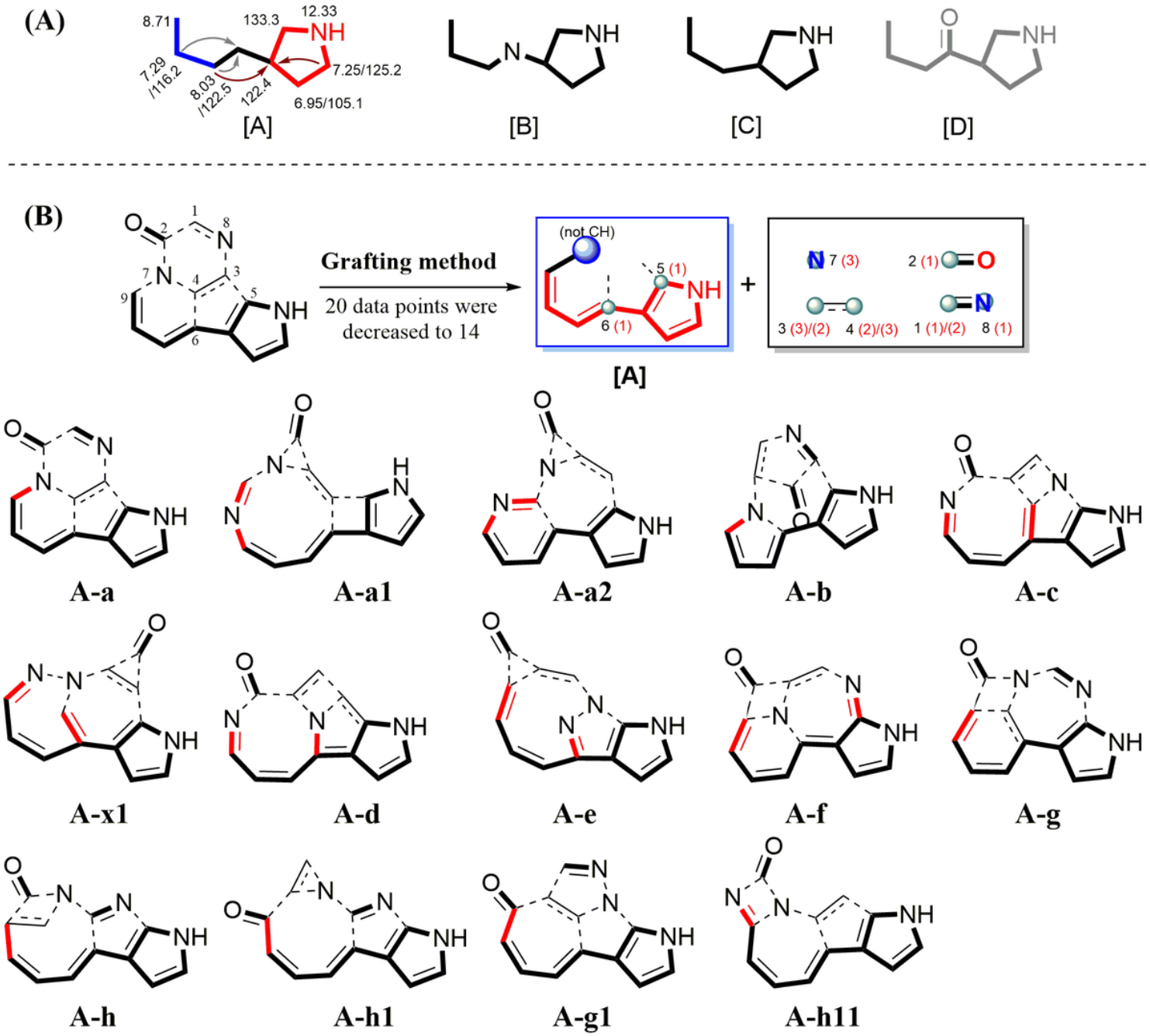

Figure 4

(A) The core structure candidates according to the key HMBC, ^{1}H-^{1}H COSY, and HMQC experimental correlations (the expected correlations in grey were not reasonable). **(B)** Meta-groups and meta-structures based on candidate A (the red bond was grafted on the basis of core structure candidate A, dashed bond can be freely assembled).

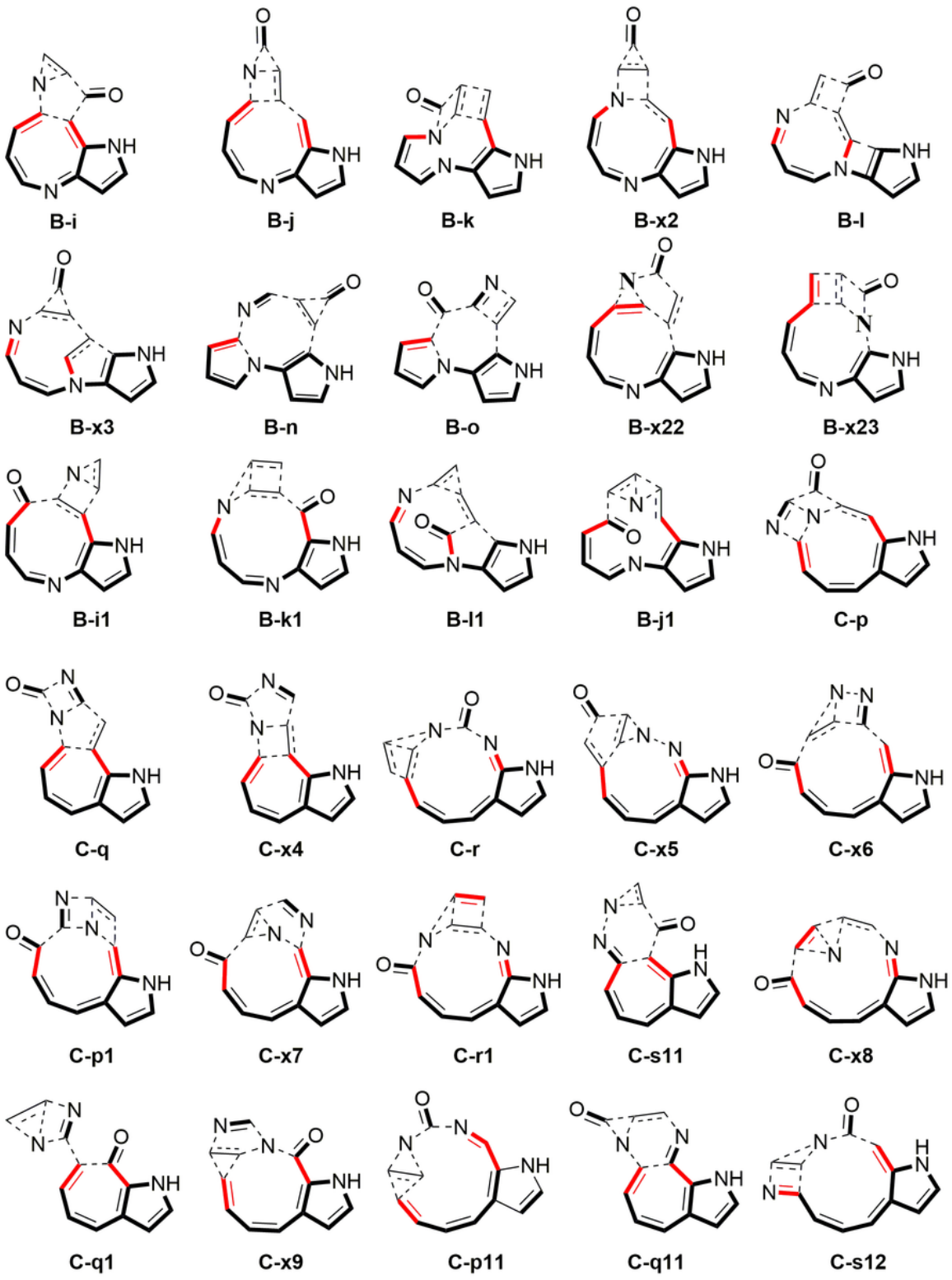

Figure 5

Meta-structures based on candidates B and C (the red bond was grafted on the basis of core structure candidates, and dashed bond can be freely assembled).

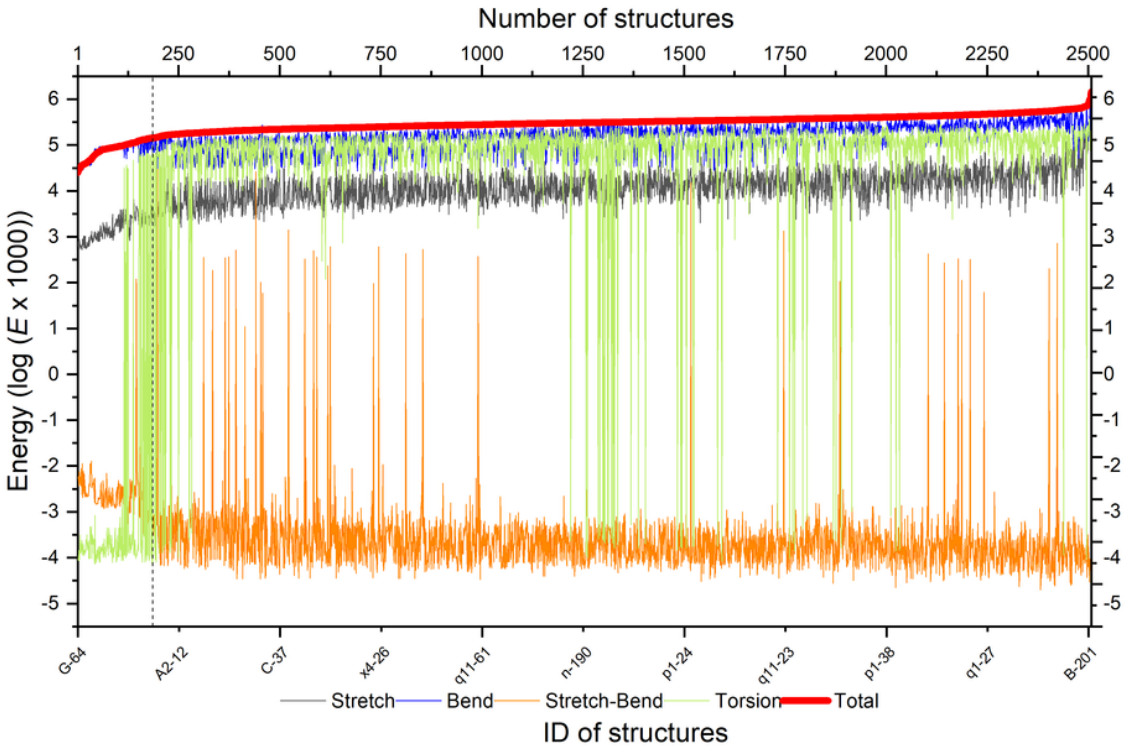

Figure 6

Energy trend of the structures related to aaptourinamine (the values were converted with $\log_{10}(E\times1000)$).

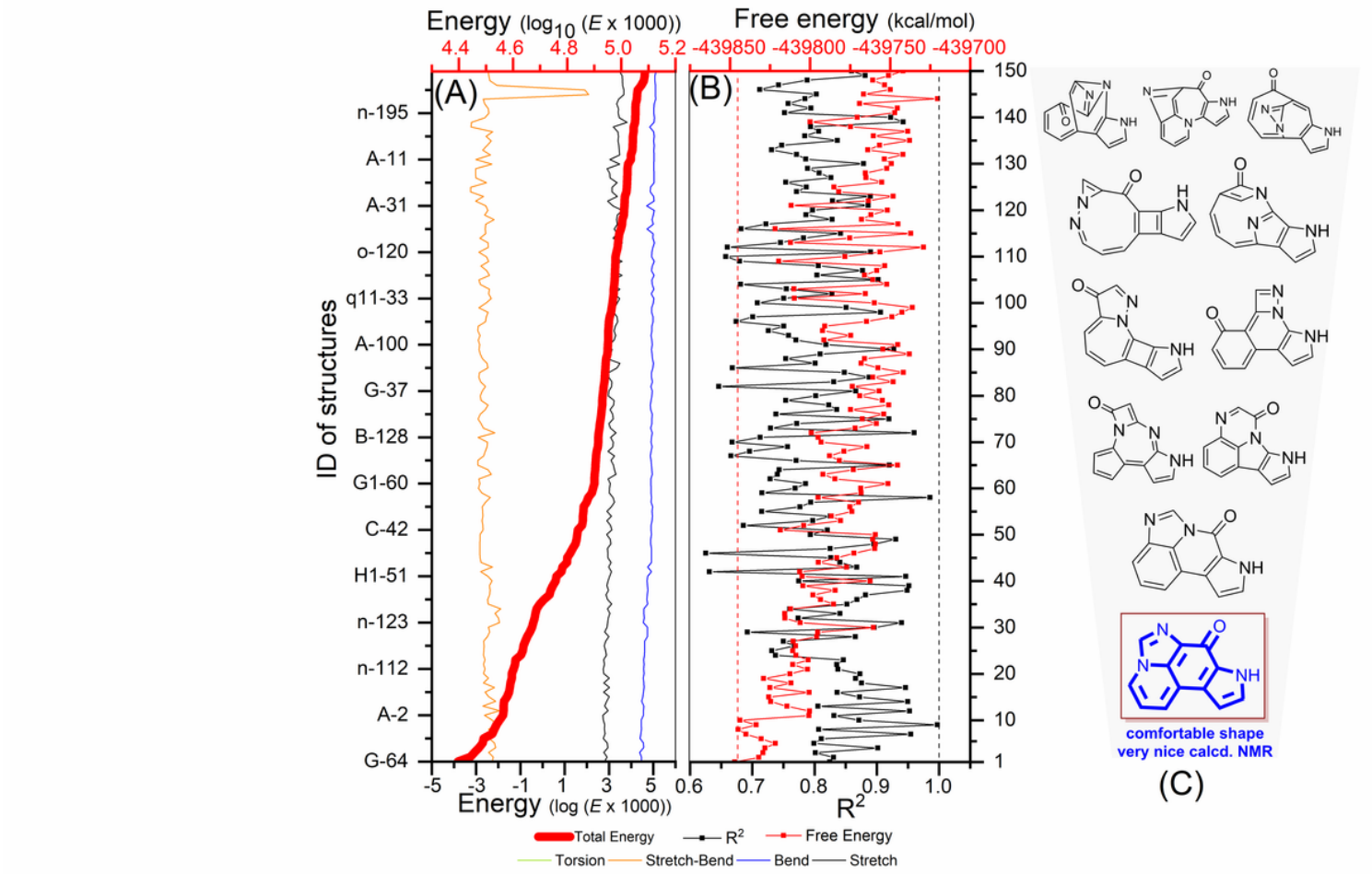

Figure 7

(A) Molecule energy trend of the 150 structures before the inflection point related to aaptourinamine in MM2 molecular mechanic force field (the values were converted with $\log_{10}(E\times1000)$). **(B)** Molecule energy trend of the 150 structures in DFT calculated Gibbs free energy, and the related coefficient of determination (R^2) comparing the experimental and calculated ^{13}C NMR data. **(C)** 'ring-contraction strategy' calculation for the correct aaptourinamine.

Supplementary Files

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