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Research paper

Spectroscopic and DFT studies on 6-Aminophenanthridine and its derivatives provide insights in their activity towards ribosomal RNA

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ABSTRACT

6-Aminophenanthridine (6AP), a plant alkaloid possessing antiprion activity, inhibits ribosomal RNA dependent protein folding activity of the ribosome (referred as PFAR). We have compared 6AP and its three derivatives 6AP8Cl, 6AP8CF_3 and 6APi for their activity in inhibition of PFAR. Since PFAR inhibition requires 6AP and its derivatives to bind to the ribosomal RNA (rRNA), we have measured the binding affinity of these molecules to domain V of 23S rRNA using fluorescence spectroscopy. Our results show that similar to the antiprion activity, both the inhibition of PFAR and the affinity towards rRNA follow the order 6AP8CF_3 > 6AP8Cl > 6AP, while 6APi is totally inactive. To have a molecular insight for the difference in activity despite similarities in structure, we have calculated the nucleus independent chemical shift using first principles density functional theory. The result suggests that the deviation of planarity in 6APi and steric hindrance from its bulky side chain are the probable reasons which prevent it from interacting with rRNA. Finally, we suggest a probable mode of action of 6AP, 6AP8CF_3 and 6AP8Cl towards rRNA.

1. Introduction

Phenanthridine derivatives are a class of plant alkaloids, which display a wide variety of biological activities [1]. Recently, some phenanthridine derivatives have been shown to be active against prions in yeast PSI+ system and mammalian cell-based assays [2]. Prions are the misfolded form of the prion protein and are the causative agents of fatal neurodegenerative disorders [3,4]. It has been shown that substitution on the phenanthridine core of 6AP significantly alters the antiprion activity of the compounds. The introduction of the chloride and trifluoromethyl groups (Fig. 1), respectively, at the 8 position of the phenanthridine ring of 6AP retained or even increased the antiprion activity of these compounds [2]. Thus, 6-Amino 8-chloro phenanthridine (6AP8Cl) and 6-Amino 8-trifluoromethyl phenanthridine (6AP8CF_3) are also active antiprion compounds, the activity being in the order 6AP8CF_3 > 6AP8Cl > 6AP. However, the substitution of the 6 amino group of 6AP molecules with a 2-(butan-1-ol) group (Fig. 1) renders the molecule inactive in antiprion activity [5]. This inactive form is referred to as 6APi. Previous studies suggest that 6AP demonstrates the antiprion activity by a trans mechanism, where it binds to ribosomal RNA (rRNA) and not to the prion protein [5]. However, the exact mechanism by which 6AP prevents prion propagation is still unclear.

Independent studies have demonstrated that 6AP selectively inhibits the protein folding activity of the ribosome [5,6]. This activity of the ribosome, referred commonly as PFAR, is a function of the large subunit of the ribosome and dependent on 23S ribosomal RNA (rRNA) in bacteria and 25S/28S rRNA in eukaryotes [7–11]. The domain V of 23S rRNA has been identified as the activity center for PFAR in bacterial ribosome [12,13]. This RNA domain (referred hereafter as domain V rRNA) is highly conserved in all species and also responsible for peptide bond formation. The *in vitro* transcribed domain V rRNA retains protein folding activity [11,12,14,15], although so far there are no reports of peptide bond formation with just this RNA fragment. It has been recently demonstrated that 6AP inhibits folding activity of domain V rRNA [14]. Interestingly, 6APi, inactive in antiprion activity, is also inactive in inhibiting PFAR [5,14]. These observations suggest that PFAR is likely to be involved

Abbreviations: 6AP, 6-Aminophenanthridine; 6AP8Cl, 6-Amino 8-chloro phenanthridine; 6AP8CF_3, 6-Amino 8-trifluoromethyl phenanthridine; PFAR, protein folding activity of ribosome; rRNA, ribosomal RNA; DFT, density functional theory.

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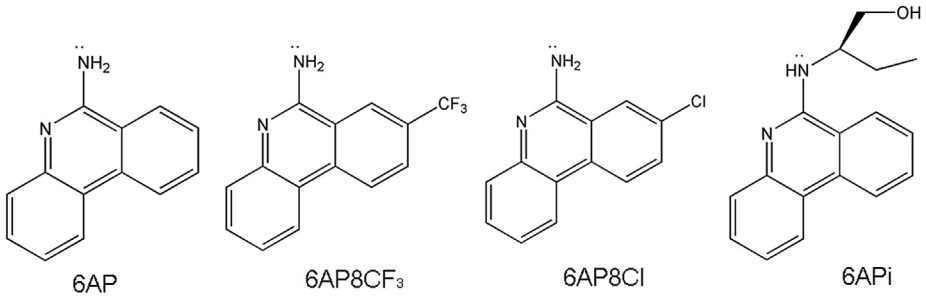

Fig. 1. Schematic representation of 6AP derivatives.

in prion propagation. Thus, 6AP and its derivatives constitute important tools to understand the mechanism of PFAR and its role in prion propagation.

Despite these findings, till date, there is no direct evidence demonstrating the interaction of 6AP or its derivatives with RNA or more specifically with rRNA. Thus, the mode of interaction of 6AP with rRNA is not known and also, why 6APi lacks the biological activity in spite of its similarity to 6AP remains unclear. Moreover, the activity of $6AP8CF_3$ and 6AP8Cl in PFAR inhibition has not been tested so far, which is important to see if the same order of activity exists among the 6AP derivatives in inhibiting PFAR as well. Here, we have tested the 6AP derivatives in PFAR assay. Furthermore, we have employed the fluorescence property of these molecules (Vovusha et al. *in preparation*) to study their interaction with domain V rRNA. Complementary to the experiments using spectroscopy, quantum chemical calculations have emerged as vital tools to study the properties of organic molecules [16,17]. In order to understand the molecular basis for the difference in the mode of action of 6AP and its derivatives towards rRNA, we have analyzed aromaticity of these compounds using density functional theory (DFT) calculations. Our results pinpoint, on one hand, why 6APi does not show any activity towards rRNA, and explain, on the other hand, why $6AP8CF_3$ shows relatively higher activity among 6AP molecules. Our quantitative comparison of the 6AP derivatives for rRNA affinity and the DFT based molecular analysis provided, for the first time, the basis for the difference of their rRNA dependent activity. Thus, this study significantly advances our understanding of the rRNA based biological activity of 6AP and derivatives.

2. Materials and methods

2.1. Preparation of components

6AP and its derivatives were synthesized according to the procedure described previously [18]. The model protein for PFAR assay, human carbonic anhydrase (HCA) was purified as described earlier [6,14]. Ribosomes were purified from *Escherichia coli* (*E. coli*) MRE600 following sucrose gradient ultracentrifugation [19].

2.2. Preparation of domain V rRNA

The domain V rRNA was synthesized from plasmid pGEM4Z, which contained DNA sequences for domain V rRNA from *E. coli*. Linear templates were prepared by restriction digestion with enzyme *EcoRI*. 1.5 μg of the linearized DNA template was mixed with transcription buffer (800 mM Hepes-NaOH (pH 7.5), 120 mM $MgCl_2$, 120 mM dithiothreitol and 8 mM spermidine). Next, 7 mM rNTP mix, 60 U Fermentas RiboLock RNase inhibitor and 2 μM T7 RNA polymerase were added to the mixture to initiate the transcription and incubated overnight at 37 °C. After overnight transcription, DNA templates were digested with RNase-free DNase I. The domain V rRNA was precipitated with 3 M sodium acetate (pH 5.2) and ethanol after extraction with phenol and chloroform (1:1). Then it was made free from nucleotides using nucleospin RNA cleanup kit (Macherey–Nagel GmbH & Co. KG). The purity of the domain V rRNA was checked by running in a 4% denaturing urea-polyacrylamide gel and the concentrations were measured in NanoDrop™ 1000 spectrophotometer (Thermo Scientific, V.3.6). The molar concentration was estimated using a molecular weight of 205,958 Da, using the exact sequence of 661 nt long domain V rRNA [14].

2.3. PFAR assay

The PFAR assays were carried out as described in earlier reports [6,14]. HCA was denatured with 6 M guanidinium-HCl, overnight at room temperature. Refolding was started by diluting denatured HCA 100 times (final concentration 300 nM) in the refolding buffer (20 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 100 mM NaCl and 0.05 mM $ZnSO_4$) containing 300 nM of 70S ribosome, without or with 6AP and derivatives in gradually increasing concentration. Each refolding reaction was carried out for 30 min at room temperature and assayed for HCA activity, which involved the measurement of the enzyme catalyzed break down of *p*-nitrophenol acetate through monitoring the change in absorption at 340 nm. The resulting activity was plotted as percentage of native HCA activity (stored undiluted in ice) representing the extent of refolding.

2.4. Fluorescence studies

The fluorescence spectra of 6AP, 6AP8Cl, $6AP8CF_3$ and 6APi were recorded in Hitachi F-7000 fluorescence spectrophotometer using a quartz cuvette of path length 1 cm. To study the interaction of 6AP derivatives with rRNA, domain V rRNA was progressively added to 8 μM solutions of 6AP derivatives (in 20 mM phosphate buffer, pH 7.0). The samples were excited at 330 nm. For measurement of K_d values for RNA binding, the emission at 378 nm was recorded and the change in fluorescence (F_0–F, where F_0 represents the fluorescence in the absence of domain V rRNA and F represents the fluorescence in the presence of increasing amounts of domain V rRNA), was plotted as a function of RNA concentration. The K_d values were estimated from the X-intercept of the double reciprocal plots. All experiments were done in triplicates.

To determine the nature of fluorescence quenching, the parameter F_0/F was plotted against the concentration of domain V rRNA in accordance to the Stern Volmer equation,

$$F_0/F = 1 + K_{sv}[Q],$$

where K_{sv} denotes the Stern Volmer constant and Q the concentration of the quencher (here domain V rRNA). The experiments were carried out at three different temperatures in triplicates.

2.5. Computational methods

The geometries of 6AP, 6APi, 6AP8Cl and 6AP8CF_3 were optimized using density functional theory (DFT) with Becke 3-parameter-Lee-Yang-Parr (B3LYP) hybrid functional and 6-311G (d,p) basis set. Nucleus independent chemical shift (NICS (1)) values have been calculated by the procedure suggested by Schleyer et al. [20] using Gaussian 09 package [21].

3. Results

3.1. Inhibition of the protein folding activity of the ribosome (PFAR)

The effect of 6AP and its derivatives on PFAR has been tested in PFAR assay using HCA as a model protein and *E. coli* 70S ribosome as the refolding modulator. As shown in Fig. 2, in the absence of the 6AP or its derivatives, 60% refolding was achieved. Consistent with earlier reports [6], ribosome assisted refolding decreased gradually with addition of 6AP in increasing concentration, while 6APi had no inhibitory effect (Fig. 2). Interestingly, both 6AP8Cl and 6AP8CF_3 also showed inhibition as 6AP, only with higher efficiency. While the total inhibitory concentration for 6AP was 300 μM, the same level of inhibition could be achieved with 250 μM 6AP8Cl and 150 μM of 6AP8CF_3 (Fig. 2). Thus, 6AP8CF_3 is the most effective inhibitor of PFAR among the 6AP derivatives and the activity against PFAR followed the order 6AP8CF_3 > 6AP8Cl > 6AP, while 6APi was totally inactive.

3.2. Interaction with domain V rRNA

The fluorescence intensity of 6AP decreased gradually upon addition of domain V rRNA in increasing concentrations (Fig. 3A). A similar or greater decrease in fluorescence by adding domain V rRNA was also observed for 6AP8Cl and 6AP8CF_3 (data not shown), while 6APi did not show any detectable change in the spectrum (Fig. 3A inset). The quenching of fluorescence of 6AP, 6AP8Cl and 6AP8CF_3 was indicative of interaction between the compounds and domain V rRNA. On the other hand, the absence of quenching in 6APi indicated that it did not interact with this rRNA. Additionally, we also observed that 6APi did not interfere in the binding of 6AP to domain V rRNA (data not shown).

In order to investigate the nature of quenching, the binding of 6AP to domain V rRNA was studied at different temperatures. The quenching in fluorescence was plotted as a Stern Volmer plot in which the parameter $(F_0/F) - 1$ (where F_0 indicates the 6AP fluorescence in the absence of domain V rRNA and F represents the fluorescence in the presence of domain V rRNA) was plotted against the concentration of domain V rRNA (Fig. 3B). The data agrees to a linear fit in accordance with the Stern Volmer equation. The Stern Volmer constants (K_{SV}) at different temperatures were estimated from the slopes of the corresponding linear fits. The K_{sv} values were estimated to be 0.0182, 0.0136 and 0.0119 at 295 K, 305 K and 315 K respectively. It was observed that the quenching decreases at higher temperatures (inset of Fig. 3B). This inverse temperature dependence of the quenching indicates that the quenching is an example of static quenching which involves the complex formation of 6AP and domain V rRNA in the ground state [22,23]. Interestingly, there was no significant shift of the emission maximum of 6AP on the addition of domain V rRNA. However, this is not an unusual observation as there are many reports in the literature, where binding of macromolecules to fluorophores does not involve a spectral shift [24–28]. The absence of spectral shift only indicates that there is no significant change in the polarity of the environment of 6AP upon binding to RNA.

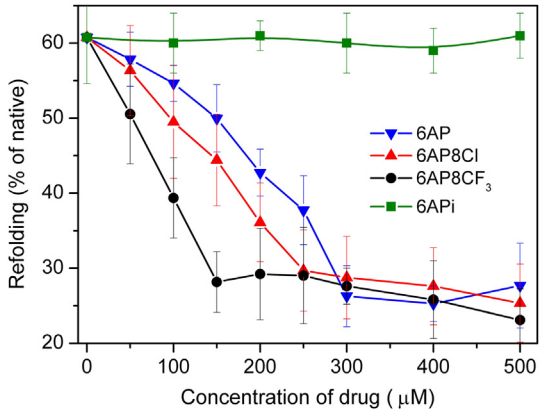

Fig. 2. Effect of 6AP derivatives on PFAR. The plots represent 70S ribosome assisted refolding of HCA (see Materials and Methods) with increasing concentration of 6AP derivatives.

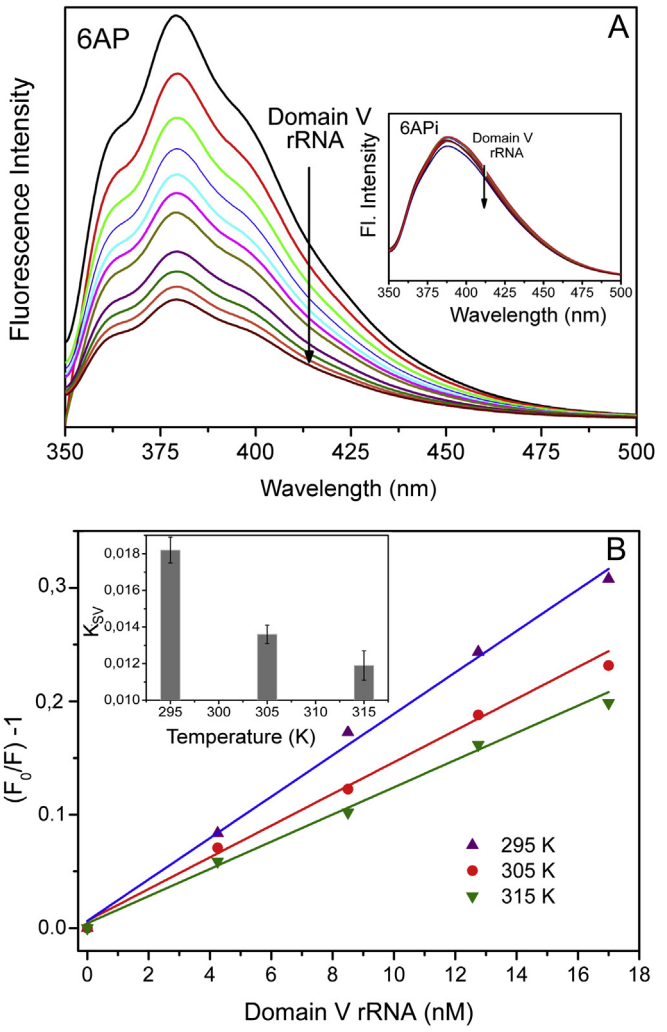

Fig. 3. (A) The fluorescence spectra of 6AP and 6APi (inset) (8 μM) with domain V rRNA in increasing concentration (indicated by the arrow); black 0 nM, red 25 nM, green 45 nM, blue 90 nM, cyan 115 nM, magenta 140 nM, dark yellow 170 nM, purple 225 nM, olive 280 nM, orange 335 nM and brown 390 nM. (B) The Stern–Volmer plots representing fluorescence quenching of 6AP (378 nm) with domain V rRNA (quencher) at three different temperatures. The solid lines represent linear fit to the data (average of three independent measurements). The Stern–Volmer constants (K_{SV}) were estimated from the slopes, which are plotted as bars in the inset against different temperatures.

The decrease in fluorescence of 6AP derivatives upon addition of domain V rRNA has been utilized to construct the saturation binding curves of the drugs with domain V rRNA (Fig. 4A–C). In the figure, the change in fluorescence intensity has been plotted as a function of the concentration of domain V rRNA. The results have been fitted with a hyperbolic function. The curves are converted to double reciprocal plots to get a linear fit, from which an estimate of the dissociation constant (K_d) could be obtained (Fig. 4D). The K_d values for 6AP8CF$_3$, 6AP8Cl and 6AP are 10 ± 0.25 nM, 100 ± 4.7 nM and 140 ± 7.2 nM respectively, which indicates that 6AP8CF$_3$ is the strongest binder of domain V rRNA, followed by 6AP8Cl and 6AP. It is highly interesting to note that the order of affinity towards domain V rRNA parallels the order of antiprion activity [2] and PFAR (Fig. 2).

3.3. Aromaticity of 6AP, 6APi, 6AP8Cl and 6AP8CF$_3$

Fig. 5 presents side view of the three dimensional model structures of 6AP and its derivatives. It can be clearly seen that 6AP has a planar structure. The introduction of the chlorine (Cl) and trifluoromethyl (CF$_3$) groups at the 8 position of 6AP in 6AP8Cl and 6AP8CF$_3$ does not significantly affect the planarity of the phenanthridine ring. However, the substitution on the amine nitrogen of 6AP with 2-(butan -1-ol) group makes 6APi visibly non planar.

Since 6AP and its derivatives contain the phenanthridine ring, it is possible to correlate the planarity of the 6AP and its derivatives through a quantitative estimate of their aromaticity. There are several theoretical methods to estimate the aromaticity of compounds using DFT. Schleyer and his coworkers have established that a negative value of NICS (1) provides appropriate information about the aromaticity of various hydrocarbons [20]. We have calculated the NICS (1) values of 6AP and its derivatives (Fig. 5). It is evident from the tabulated values that the aromaticity and thus the planarity of 6AP, 6AP8Cl and 6AP8CF$_3$ are comparable (−10.6 ± 0.1) and significantly higher than that of 6APi (−8.44). Hence, compared to other 6AP molecules 6APi is relatively nonplanar.

4. Discussion

The antiprion activity of 6AP was first identified in yeast PSI+ prion system and further it was verified in URE3 prion system and also in murine cell based antiprion assay [2]. Later several derivatives of 6AP were synthesized and tested for antiprion activity. Different derivatives of 6AP showed varied degree of antiprion activity. It was reported that 6AP, 6AP8Cl and 6AP8CF$_3$ showed antiprion activity in the order 6AP8CF$_3$ > 6AP8Cl > 6AP, while 6APi was totally inactive [5]. It was suggested that the antiprion activity of 6AP is implemented by inhibiting PFAR through its interaction

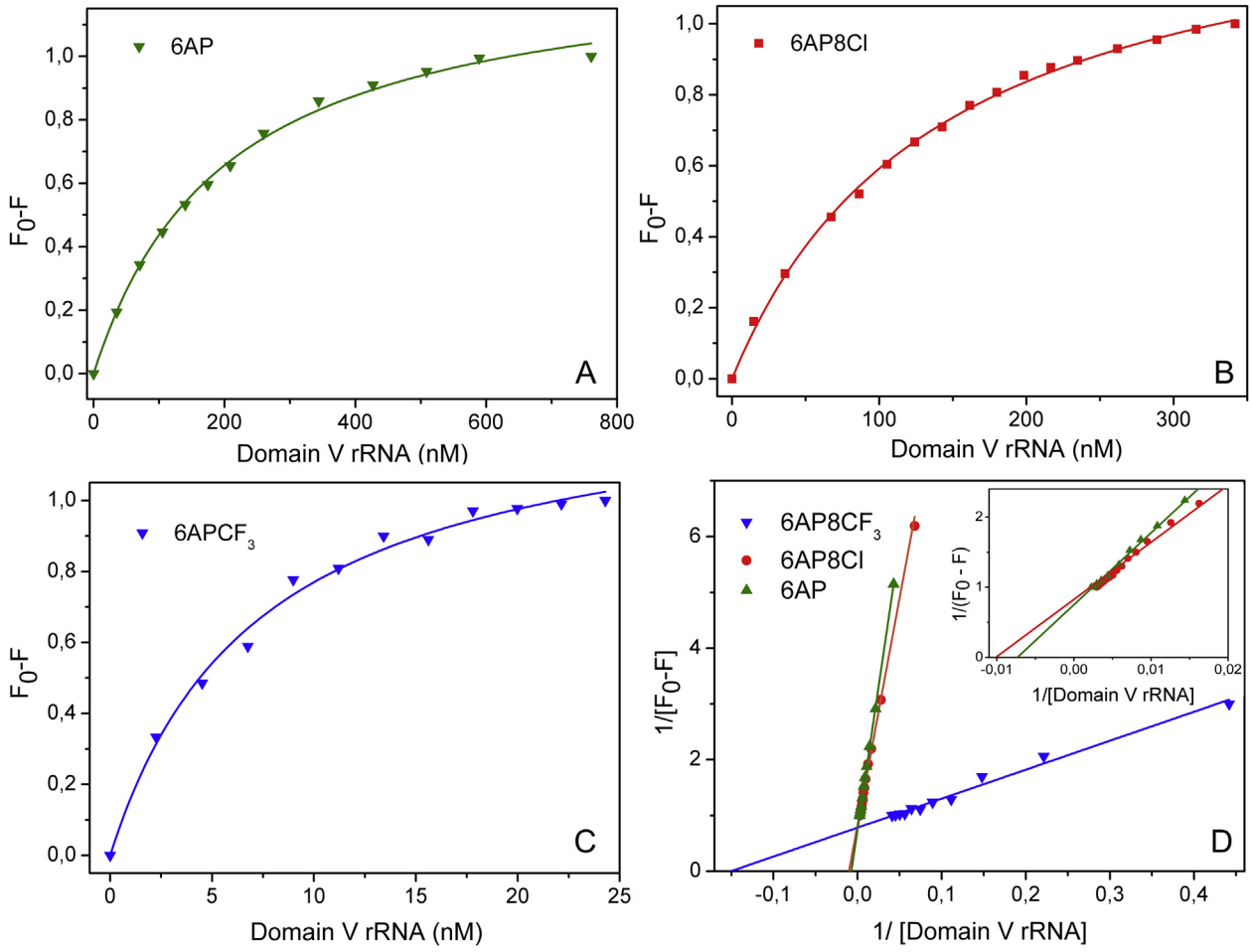

Fig. 4. Saturation binding curves of 6AP (A) 6AP8Cl (B) and 6AP8CF$_3$ (C) (8 μM) as a function of domain V rRNA at 20 °C. The solid lines represent hyperbolic fit to the experimental data. (F_0–F) indicates the change in fluorescence emission of 6AP at 378 nm upon addition of domain V rRNA (quencher); F_0 is 6AP fluorescence without quencher. Replotting the binding curves as double reciprocals yields straight line fits (D), the X-intercepts indicate K_d values for domain V rRNA of the respective 6AP molecules. The inset in (D) shows zoomed double reciprocal plot for 6AP8Cl and 6AP for better visualization.

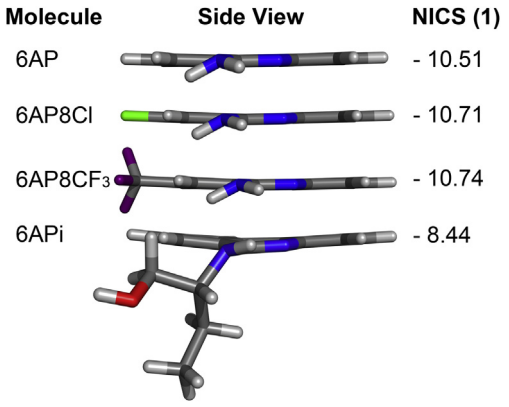

Fig. 5. The side view of 6AP and its derivatives enabling visual comparison of planarity of these molecules. The corresponding NICS (1) values are listed in the right column.

with domain V rRNA [5,6,14]. Should this proposition be true, one would expect the same order of activity in 6AP derivatives in PFAR inhibition and domain V rRNA interaction. Our results of PFAR assay show that while 6APi is inactive in PFAR inhibition, 6AP and derivatives indeed show the same order of activity 6AP8CF_3 > 6AP8Cl > 6AP (Fig. 2), which corresponds well with their antiprion activity reported earlier [2].

It is well established that PFAR is a function of domain V rRNA in bacterial ribosome [6,14]. This RNA domain is an integral part of the catalytic RNA in the large subunit of the ribosome, highly conserved and not masked by any ribosomal protein in the free 50S subunit. It has been shown recently that 6AP inhibits PFAR by binding to the domain V rRNA [14]. 6AP binding competitively prevents the protein substrates from accessing the folding center of the ribosome. In the same study, the 6AP interaction sites on domain V rRNA has been mapped using UV-crosslinking followed by a primer extension assay. Interestingly, 6APi was not only inactive in PFAR inhibition, but also it could not be photo-crosslinked to this rRNA domain [14]. These observations, although suggested that 6AP but not 6APi binds to domain V rRNA, a direct demonstration of the binding was lacking. Moreover, these studies did not provide any quantitative assessment of 6AP − domain V rRNA interaction. The understanding that 6AP derivatives possess intrinsic fluorescence due to the phenanthridine moiety led us design experiments to follow the domain V rRNA interaction quantitatively in a direct manner.

Our results of fluorescence quenching of 6AP, 6AP8CF_3 and 6AP8Cl on addition of domain V rRNA are the first direct demonstrations of rRNA interaction with these molecules. Despite similarity in chemical structure and fluorescence properties, the absence of quenching in case of 6APi indicated that it did not interact with this rRNA domain. This is consistent with previous reports showing that 6APi is inactive in all rRNA dependent biological activities and that it couldn't be photo-crosslinked with domain V rRNA [5,6,14]. The progressive fluorescence quenching for 6AP, 6AP8CF_3 and 6AP8Cl allowed us to estimate their binding affinity to domain V rRNA. Our results show that the K_d values estimated for these molecules also follow the same order 6AP8CF_3 > 6AP8Cl > 6AP as their biological activities. 6AP8CF_3 being the strongest binder of domain V rRNA shows highest degree of inhibition of PFAR and strongest antiprion activity. Thus, these results strengthen the notion that a strong correlation exists between PFAR and prion propagation. It is generally accepted that prion propagation involves nonfibrillar intermediates formed by disintegration of the long amyloid prion fibrils to the smaller units [29,30] We suspect that probably PFAR is engaged in this process; 6AP and its active derivatives (6AP8CF_3 and 6AP8Cl) inhibit PFAR by competing with the protein substrate [14] thereby preventing prion propagation.

The experimental demonstrations, irrespective of direct (e.g. fluorescence quenching) or indirect measurements (e.g. PFAR inhibition), are limited in explaining the molecular basis for the differences in the activities of 6AP derivatives. This led us to analyze the molecular structure of these molecules using quantum chemical calculations (Vovusha et al., *in preparation*). All phenanthridine molecules are predominantly planar, which enables them to interact with nucleic acid bases. The presence of an aromatic phenathridine ring in 6AP and its derivatives allowed us to compare their aromaticity and relate to their planarity. Our results show that the NICS (1) values of 6AP, 6AP8Cl and 6AP8CF_3 (-10.6 ± 0.1, Fig. 5) are closely comparable with that of the parent phenanthridine molecule (-11.74). Thus, the substitution of the phenanthridine nucleus with a Cl or CF_3 group did not affect the planarity of 6AP derivatives. In contrast, there is a significant difference in aromaticity of inactive 6APi. The NICS (1) value (-8.44) suggests that 6APi is sufficiently non-planar, so as to compromise its aromaticity (Fig. 5).

Since 6AP (also 6AP8Cl and 6AP8CF_3) is planar, an electron delocalization involving the amine nitrogen exists in these compounds (Fig. 6), which is absent in non-planar 6APi. We perceive that this electron delocalization is particularly important for their interaction with domain V rRNA. The deviation in planarity in 6APi does not allow the electron delocalization to occur. In addition, the steric hindrance offered by the bulky side chain is probably another reason, which hinders its association with nucleobases, thus rendering it inactive. In contrast, the existing electronic delocalization is highly stabilized in 6AP8CF_3 due to the presence of a highly electron withdrawing CF_3 (which is a $-I$ (inductive), $-R$ (resonance) group), that makes it most active in interaction with domain V rRNA. Also, the Cl group in 6AP8Cl, although not as strong as the CF_3 group in attracting electrons (attracts electron only through $-I$ effect), stabilizes electron delocalization and thus, 6AP8Cl shows an intermediate activity between 6AP and 6AP8CF_3.

It can be perceived that 6AP, 6AP8Cl and 6AP8CF_3 possess RNA/DNA binding property in general due to the phenanthridine moiety, although there are no reports or quantitative assessments available so far. It is to be emphasized that the biological functions of these molecules involve rRNA and domain V rRNA in particular. Thus, a comparative analysis of the action of 6AP molecules towards domain V rRNA is highly relevant for understanding the basis of their biological activities. It is possible that a special recognition motif exists in the structure or sequence of domain V rRNA which facilitates binding of the active 6AP molecules. A detailed comparison of 6AP action towards different nucleic acid substrates will

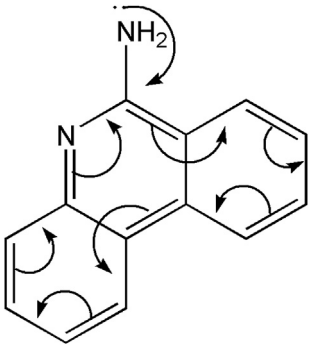

Fig. 6. The proposed scheme for electron delocalization in 6AP.

illuminate the mechanism of its substrate recognition and specificity, which is a definitive objective for future research.

5. Conclusions

In summary, the biological activities of 6AP and its derivatives as antiprion drugs, inhibitors of PFAR and rRNA binders, follow the order 6AP8CF$_3$ > 6AP8Cl > 6AP, while 6APi is inactive. The inactivity of 6APi is due to the lack of planarity in the molecule and steric hindrance from the bulky side chain. Alternatively, the higher activity of 6AP8CF$_3$ is due to the presence of the electron attracting group (−CF$_3$), which probably stimulates electron delocalization in the phenanthridine moiety required for RNA interaction. Thus, this study has provided molecular insight into the RNA based biological activities of 6AP and its derivatives.

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References

[1] J. Urbanová, P. Lubal, I. Slaninová, E. Táborská, P. Táborský, Fluorescence properties of selected benzo[c]phenantridine alkaloids and studies of their interaction with CT DNA, Anal. Bioanal. Chem. 394 (2009) 997–1002.
[2] S. Bach, N. Talarek, T. Andrieu, J.-M. Vierfond, Y. Mettey, H. Galons, D. Dormont, L. Meijer, C. Cullin, M. Blondel, Isolation of drugs active against mammalian prions using a yeast-based screening assay, Nat. Biotechnol. 21 (2003) 1075–1081.
[3] A. Aguzzi, M. Polymenidou, Mammalian prion biology: one century of evolving concepts, Cell 116 (2004) 313–327.
[4] S.B. Prusiner, Prions, Proc. Natl. Acad. Sci. 95 (1998) 13363–13383.
[5] D. Tribouillard-Tanvier, S. Dos Reis, F. Gug, C. Voisset, V. Béringue, R. Sabate, E. Kikovska, N. Talarek, S. Bach, C. Huang, N. Desban, S.J. Saupe, S. Supattapone, J.-V. Thuret, S. Chédin, D. Vilette, H. Galons, S. Sanyal, M. Blondel, Protein folding activity of ribosomal RNA is a selective target of two unrelated antiprion drugs, PLoS One 3 (2008) e2174.
[6] S.D. Reis, Y. Pang, N. Vishnu, C. Voisset, H. Galons, M. Blondel, S. Sanyal, Mode of action of the antiprion drugs 6AP and GA on ribosome assisted protein folding, Biochimie 93 (2011) 1047–1054.
[7] S. Chattopadhyay, B. Das, A.K. Bera, D. Dasgupta, C. Dasgupta, Refolding of denatured lactate dehydrogenase by *Escherichia coli* ribosomes, Biochem. J. 300 (1994) 717–721.
[8] C. Voisset, S.J. Saupe, M. Blondel, The various facets of the protein-folding activity of the ribosome, Biotechnol. J. 6 (2011) 668–673.
[9] D. Das, A. Das, D. Samanta, J. Ghosh, S. Dasgupta, A. Bhattacharya, A. Basu, S. Sanyal, C. Dasgupta, Role of the ribosome in protein folding, Biotechnol. J. 3 (2008) 999–1009.
[10] B. Das, S. Chattopadhyay, A.K. Bera, C. Dasgupta, In vitro protein folding by ribosomes from *Escherichia coli*, wheat germ and rat liver, Eur. J. Biochem. 235 (1996) 613–621.
[11] S.C. Sanyal, S. Pal, S. Chaudhuri, C. Dasgupta, 23S rRNA assisted folding of cytoplasmic malate dehydrogenase is distinctly different from its self-folding, Nucleic Acids Res. 30 (2002) 2390–2397.
[12] S. Chattopadhyay, B. Das, C. Dasgupta, Reactivation of denatured proteins by 23S ribosomal RNA: role of domain V, Proc. Natl. Acad. Sci. 93 (1996) 8284–8287.
[13] D. Pal, S. Chattopadhyay, S. Chandra, D. Sarkar, A. Chakraborty, C. Dasgupta, Reactivation of denatured proteins by domain V of bacterial 23S rRNA, Nucleic Acids Res. 25 (1997) 5047–5051.
[14] Y. Pang, S. Kurella, C. Voisset, D. Samanta, D. Banerjee, A. Schabe, C. Dasgupta, H. Galons, M. Blondel, S. Sanyal, The antiprion compound 6-Aminophenanthridine inhibits protein folding activity of the ribosome by direct competition, J. Biol. Chem. 288 (2013) 19081–19089.
[15] S. Pal, S. Chandra, S. Chowdhury, D. Sarkar, A.N. Ghosh, C. Dasgupta, Complementary role of two fragments of domain V of 23 S ribosomal RNA in protein folding, J. Biol. Chem. 274 (1999) 32771–32777.
[16] A.-G. Zhang, Y.-Z. Zhang, Z.-M. Duan, K.-Z. Wang, H.-B. Wei, Z.Q. Bian, C.-H. Huang, Dual molecular light switches for pH and DNA based on a novel Ru(II) complex. A non-intercalating Ru(II) complex for DNA molecular light switch, Inorg. Chem. 50 (2011) 6425–6436.
[17] Q. Zhao, J.L. Freeman, J. Wang, Y. Zhang, T.P. Hamilton, C.M. Lawson, G.M. Gray, Syntheses, X-ray crystal structures, and optical, fluorescence, and nonlinear optical characterizations of diphenylphosphino-substituted bithiophenes, Inorg. Chem. 51 (2012) 2016–2030.
[18] F. Gug, N. Oumata, D. Tribouillard-Tanvier, C. Voisset, N. Desban, S. Bach, M. Blondel, H. Galons, Synthesis of conjugates of 6-Aminophenanthridine and guanabenz, two structurally unrelated prion inhibitors, for the determination of their cellular targets by affinity chromatography, Bioconjugate Chem. 21 (2010) 279–288.
[19] M. Johansson, E. Bouakaz, M. Lovmar, M. Ehrenberg, The kinetics of ribosomal peptidyl transfer revisited, Mol. Cell 30 (2008) 589–598.
[20] Z. Chen, C.S. Wannere, C. Corminboeuf, R. Puchta, P. Schleyer, Nucleus-independent chemical shifts (NICS) as an aromaticity criterion, Chem. Rev. 105 (2005) 3842–3888.
[21] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Gaussian, Inc., Wallingford CT, 2009.
[22] J.R. Lackowicz, Principles of Fluorescence Spectroscopy, Springer, New York, 2006.
[23] B. Pan, Y. Liu, D. Xiao, F. Wu, M. Wu, D. Zhang, B. Xing, Quantitative identification of dynamic and static quenching of ofloxacin by dissolved organic matter using temperature-dependent kinetic approach, Environ. Pollut. 161 (2012) 192–198.
[24] S. Arttamangkul, V. Alvarez-Maubecin, G. Thomas, J.T. Williams, D.K. Grandy, Binding and internalization of fluorescent opioid peptide conjugates in living cells, Mol. Pharmacol. 58 (2000) 1570–1580.
[25] R.M. De Lorimier, J.J. Smith, M.A. Dwyer, L.L. Looger, K.M. Sali, C.D. Paavola, S.S. Rizk, S. Sadigov, D.W. Conrad, L. Loew, H.W. Hellinga, Construction of a fluorescent biosensor family, Protein Sci. 11 (2002) 2655–2675.
[26] Y. Elbaz, N. Tayer, E. Steinfels, S. Steiner-Mordoch, S. Schuldiner, Substrate-Induced tryptophan fluorescence changes in EmrE, the smallest ion-coupled multidrug transporter, Biochemistry 44 (2005) 7369–7377.
[27] T. Mondol, S. Batabyal, S.K. Pal, Ultrafast electron transfer in the recognition of different DNA sequences by a DNA-binding protein with different dynamical conformations, J. Biomol. Struct. Dyn. 30 (2012) 362–370.
[28] P. Rajdev, T. Mondol, A. Makhal, S.K. Pal, Simultaneous binding of anti-tuberculosis and anti-thrombosis drugs to a human transporter protein: a FRET study, J. Photochem. Photobiol. B Biol. 103 (2011) 153–158.
[29] S.R. Collins, A. Douglass, R.D. Vale, J.S. Weissman, Mechanism of prion propagation: amyloid growth occurs by monomer addition, PLoS Biol. 2 (2004) e321.
[30] A. Aguzzi, T. O'Connor, Protein aggregation diseases: pathogenicity and therapeutic perspectives, Nat. Rev. Drug Discovery 9 (2010) 237–248.