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Cyclooctatetraene: A Bioactive Cubane Paradigm Complement

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Abstract

Cubane was recently validated as a phenyl ring (bio)isostere, but highly strained caged carbocyclic systems lack π character, which is often critical for mediating key biological interactions. This electronic property restriction associated with cubane has been addressed herein with cyclooctatetraene (COT), using known pharmaceutical and agrochemical compounds as templates. COT either out performed or matched cubane in multiple cases suggesting that versatile complementarity exists between the two systems for enhanced bioactive molecule discovery.

Graphical Abstract

COTton on: the potential of cyclooctatetraene (COT) as a bioactive motif was assessed using a portfolio of six pharmaceutical and agrochemical templates. The protean nature and π character of COT was found to compliment the attributes of the already established cubane (bio)isostere concept, and the facile conversion of the latter to the former inaugurate the pair as an effective tool for deployment in bioactive molecule discovery.

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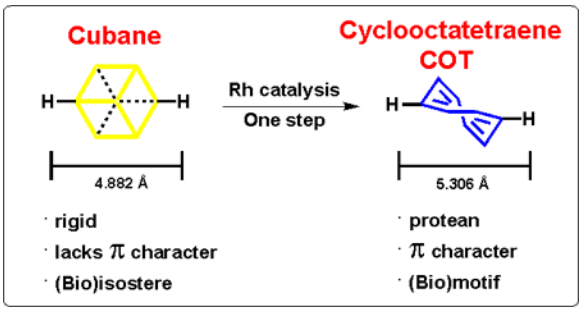
Conflict of interest

J.T., G. P. S., and C. M. W. have formal and informal commercial relationships with companies developing and supplying cubane intermediates.

Dedicated to Professors Armin de Meijere and Henning Hopf for their extraordinary contributions to hydrocarbon chemistry



Keywords

agrochemicals; pharmaceuticals; cubane; cyclooctatetraene; bioisostere

Eaton's 1992 conjecture,[1] that cubane (**1**) (Scheme 1) can act as a phenyl ring (bio)isostere,[2] was recently validated by our group using a variety of known pharmaceutical and agrochemical compounds.[3] Considering that cage hydrocarbon motifs (e.g. **1**), generally only provide steric replacement attributes,[4] and obviously lack **π** character, this aspect places substantial limits on bioactive compound discovery efforts using this approach i.e. due to a loss in potential **π** interactions[5] and/or even critical metabolic reactivity.[6] This situation is further compounded by the fact that contemporary bioactive molecule discovery is attempting to deviate from "flatland" chemical space[7] (i.e. highly planar aromatic compounds arising from ease of sp^2 coupling[8]) by adopting cage hydrocarbons for their physical attributes,[9] which introduce increased three dimensionality, increased lipophilicity and outright novelty.

In search of a readily accessible moiety that might deliver both steric and **π** components, 1,3,5,7-cyclooctatetraene (COT, **2**),[10] was identified as a motif that potentially fulfills the desired bimodal criteria. In addition, because of the dynamic equilibrium (shape shifting) that exists between ring inversion (e.g. **3**), **π**-bond shifts and valence tautomerisation (i.e. bicyclo[4.2.0]octatriene **4**),[11,12] COT offers a unique protean capability i.e. to potentially access an optimum conformation to engage biological targets in a "skeleton key" fashion (Scheme 1).

A number of synthetic protocols give access to the COT (**2**) ring system,[13] but a convenient method derives from the rhodium catalyzed valence isomerization of cubane (**1**),[1] which our group has extensively explored (Scheme 1).[14] This synthetic aspect is not only highly convenient, but allows simultaneous exploration of both cubane and COT frameworks for those working in any bioactive molecule discovery program.

Herein, is disclosed a broad examination of COT as a bioactive motif, using a wide range of known pharmaceutical and agrochemical templates (i.e. for blood coagulation, depression, pain, cancer, parasites, and agricultural pests), with direct comparison to the corresponding cubane system.

Warfarin (**6**)[15] was initially chosen to explore the COT concept. Although, warfarin is the most widely-used oral anticoagulant for the prevention and treatment of thrombosis in

humans globally, it is ranked among the top 10 drugs with serious adverse drug consequences.[16] In addition, controversy over treating individual genotypes of the target enzyme vitamin K epoxide reductase (VKOR),[17] and the fact that warfarin resistance is emerging,[18] make this a crucial pharmaceutical template for investigation.

Authentic warfarin along with cubyl-warfarin (**7**) and COT-warfarin (**8**) were prepared (Figure 1) (see SI, Schemes S1–S2). For the cubane (**7**) and COT (**8**) analogs both enantiomers were obtained separately (also for warfarin) by preparative enantioselective chromatography. In solution (^{1}H NMR, $CDCl_3$) the hemi-ketal form was partially observed for the cubane case, whereas the COT example existed solely in the hemi-ketal form (i.e. **8**) in $CDCl_3$ and a mixture of DMSO:D_2O (3:7). Initial VKOR inhibition evaluation was performed using FIXgla-PC/HEK293 reporter cells.[19] Results showed that the potency of inhibition of VKOR activity by (*S*)-warfarin was about 8-fold higher than (*R*)-warfarin, which was consistent with reported data.[20] The (*S*)-cubane analogue demonstrated *ca.* 3-fold lower activity than the (*R*)-enantiomer, but overall *ca.* 10-fold less activity than the warfarin enantiomers (. Pleasingly, however, the racemic COT derivative exhibited a *ca.* 2-fold increase in activity beyond the more active enantiomer [(*S*)–isomer] of warfarin (see SI, Figure S4). A subsequent evaluation showed that the (*S*)-enantiomer of COT-warfarin was 4-fold more potent than the (*R*)-enantiomer (see SI, Figure S5). Considering that the genetic variation in the VKOR gene is one of the largest factors contributing to the difficulty in the clinical use of warfarin,[16c] the performance of the racemic COT analogue (**8**) was explored against the 27 naturally occurring human VKOR mutants using VKOR/VKORC1L1 double gene knockout reporter cells[18a,c]. It was found that **8** and warfarin displayed a similar pattern of resistance to all naturally occurring VKOR mutants, suggesting they might bind to the same site in VKOR. Gratifyingly, however, the resistant variation of the COT surrogate to *all* the naturally occurring mutants was *ca.* 4-fold less than warfarin(Figure 1, see also SI, Figure S6).

Overall, these initial observations suggested that the π rich COT motif had potential to deliver improved activity, in a system where the cubane analogue failed to produce phenyl (bio)isosterism.

Other Pharmaceuticals and Pesticides.

Although the warfarin case provided promising evidence, additional examples were required to validate COT as a viable bioactive motif. Three pharmaceuticals [moclobemide (**9**), pravadoline (**10**) and SAHA (**11**)], an acaracide [benzyl benzoate (**12**)], and a pesticide [diflubenzuron (**13**)], were subjected to COT editing (Figure 2).

Synthesis of the COT analogues was simply achieved by first acquiring the cubane analog (**14**-**20**), or advanced precursor, followed by treatment with rhodium(I) norbornadiene chloride dimer {[Rh(nbd)Cl]$_2$},[14] which gave the corresponding COT derivatives (**21**-**27**) via valence isomerization (see SI, Schemes S3–S7).

The third-generation antidepressant moclobemide (**9**) was next pursued, as it displays a good selectivity profile towards monoamine oxidase A (MAO-A) i.e. important for patients with

dietary requirements.[21] An open field test (OFT) was used to assess the efficacy of **9**, **14** and **21** using adult male C57BL6/J mice[22] (see SI, Figures S27–S28). The locomotor profiles of cubane analog **14** were compared with those of **9**, where both were observed to decrease the total distance travelled by the same amount compared to the vehicle, indicating (in the OFT model of anxiolytic activity) that the cubane analogue **14** performed equally well as the clinically established antidepressant moclobemide. In comparison, the COT example (**21**) had a locomotor profile that was indistinguishable from the vehicle (water).

Pravadoline (**10**) was also evaluated (even though it did not proceed to clinical development beyond Phase 1 clinical trials[23]), because the pharmacological characteristics include an interesting dual mechanism of analgesia (i.e. both cyclooxygenase inhibitory and cannabinoid receptor agonist properties).[24] The cubane and COT analogues (**15** and **22**) were examined using the Freund's Complete Adjuvant (FCA) rat model of inflammatory pain in the hindpaw, and both were found to evoke similar pain relief to that of pravadoline itself (see SI, Figures S29–S30).

The histone deacetylase inhibitor SAHA (**11**, suberanilohydroxamic acid),[25] which is used clinically to treat cutaneous T-cell lymphoma (CTCL),[26] was chosen as the third pharmaceutical comparator. Evaluation of cubane derivative **16** and the COT analogue (**23**) against breast cancer (MCF7) and melanoma (MM96L) cell lines respectively, showed that **23** (IC_{50} 37±3 and 73±8 ng/ml respectively) was *ca.* 2-fold more active that **16** (IC_{50} 53±4 and 137±5 ng/ml respectively), although *ca.* 2-fold less active than SAHA (17±1 and 26±5 ng/ml respectively) (see SI, Figures S31–S34). That said, our earlier *in vivo* mouse studies showed that SAHA and the cubane derivative **16** had identical activity.[3]

The acaracide benzyl benzoate (**12**), which is used as a topical treatment of an infectious skin disease caused by scabies mites,[27] previously resulted in considerable losses in activity when interrogated using cubane.[3] All cubane analogs of benzyl benzoate (**17**-**19**) were converted in one step into the corresponding COT examples (**24**-**26**) (see SI, Figures S35–S37). Benzyl benzoate (**12**) caused 100% mite mortality within 5 min. At the same concentration, cubane surrogates caused substantially less mortality, even after an exposure time of 24 h. Although still not as effective as **12**, the COT analogs (**24**-**26**) displayed much higher mortality rates than the cubane analogues (**17**-**19**). Specifically, COT replacement of the benzoyl fragment resulted in the highest level of mortality (i.e. 75% at 8 h for COT **26**).

When exposed to late instar larvae of laboratory-cultured *T. castaneum* (rust-red flour beetle; a major pest of stored grain), the cubane analogue (**20**)[3] outperformed diflubenzuron (**13**).[28] However, when examined against field strain beetles it was found that COT replacement (i.e. **27**) did not outperform diflubenzuron, and was not as effective as cubane (see SI, Figures S38–S39).

Summarizing the six exemplars evaluated. COT-Warfarin (**8**) outperformed the corresponding cubane counterpart as did COT-SAHA (**23**) and the benzyl benzoate COT system **26**. However, both the COT and cubane surrogates (i.e. **22** and **15**) of pravadoline (**10**) showed essentially equal activity. Of the two remaining cases (i.e. moclobemide and

diflubenzuron) the cubane surrogates (i.e. **14** and **20**) outperformed the corresponding COT derivatives, but performed equally well against the benzene comparator.

Although the above results demonstrate a compelling case for COT as a bioactive complement to cubane, the remaining questions to address for pharmaceutical or agrochemical discovery concern the physical and metabolic properties of COT.

Lipophilicity.

Log *P*,[29] and associated lipophilicity measures,[9] are crucial assessment criteria for lead compounds and/or drug candidates. Although we previously used the clog *P* method,[3] for this study the traditional octanol water partition coefficient determination method was used for increased accuracy (see SI, Figure S1). In the warfarin (**6**, log *P* = 2.73; **7**, log *P* = 4.62; **8**, log *P* = 2.82), moclobemide (**9**, 1.85; **14**, 2.53; **21**, 1.95) and benzyl benzoate (**12**, 3.36; **19**, 4.22; **26**, 3.34) examples, the log *P* difference between COT and benzene counterparts was minimal (i.e. <0.10), and much less than that seen for the cubane replacements. For pravadoline (**10**, 3.19; **15**, 1.73; **22**, 2.52), SAHA (**11**, 0.99; **16**, 1.52; **23**, 1.38) and diflubenzuron (**13**, 3.33; **20**, 3.31; **27**, 4.36) COT substitution resulted in slightly increased log *P* values (i.e. 0.39–1.03), but in the case of **10** both COT and cubane systems had a lower log *P* than the corresponding benzene counterpart. Although no general trend regarding COT replacement can be claimed in relation to lowering or increasing log *P*, COT and cubane are certainly complementary in that one or the other appears to closely match the benzene counterpart, depending on the molecular and/or functional group context.

Metabolic Stability and Protein Plasma Binding.

For COT editing to be considered viable in bioactive discovery an appreciation of Phase I drug metabolism was required.[30] Therefore, a metabolic stability study was undertaken using both enantiomers of warfarin and the corresponding enantiomers of the COT system (i.e. **8**) in the presence of human liver microsomes (see SI, Figures S7–S10). The warfarin enantiomers showed little metabolism in a standard 60 min incubation, with (*S*)-warfarin having an observed half-life of 210±120 min, and (*R*)-warfarin showing no significant change. In the case of the two enantiomers of **8** these had similar metabolic stabilities [i.e. (*S*)-COT had a half-life of 110±38 mins, and the (*R*)-COT 130±20 mins]. Although the COT enantiomers appear slightly more metabolically labile, warfarin is known to have a long half-life (i.e. 36 h in the human body), whereas other clinically relevant warfarin analogues[31] are more rapidly metabolized, e.g., acenocoumarol (10 h)[32]. Further information on the metabolism of the (*S*)- and (*R*)-COT enantiomers was available through coupled liquid chromatography mass spectrometric analysis of the stored human liver microsomal incubates (see SI, Figures S14–S26). Analysis revealed substantial peaks exhibiting an M+16 (oxygen atom addition) in the case of (*S*)-COT warfarin, which is typical of Phase I cytochrome P450 metabolism and could correspond to a simple hydroxylated metabolite or an epoxide. Warfarin is known to be primarily metabolized by a variety of cytochrome P450 isoforms to monohydroxylated metabolites, although different isoforms process the different enantiomers.[31] Carbonyl reductases also reduce the side chain moiety to the corresponding alcohol in warfarin[31] and significant quantities of an M

+2 metabolite were also observed in the metabolism of (*R*)-COT warfarin, as were M+18 peaks (carbonyl reduction plus hydroxylation). However, any M+34 peak (epoxidation followed by epoxide hydration) was small, consistent with little epoxidation, or interception by glutathione (GSH) etc., as observed for (*S*)-COT warfarin. Certainly, epoxides of COT[33] are stable, and in the view that non-catalyzed ring opening of epoxides by thiols requires elevated temperatures,[34] these results suggest that the COT ring system is not a particular metabolic liability. It is possible that the variation in metabolic stability is due to differential binding of **8** to a cytochrome P450 (CYP2C9 is responsible for warfarin metabolism) promoting increased hydroxylation of the coumarin ring system, as is the case for acenocoumarol.[32] In addition, the enantiomers of warfarin and COT **8** were observed to bind human plasma proteins similarly (see SI, Tables S1–S8). The extent of binding was similar at 200 and 2000 ng/mL for most of the analytes. Lastly, the pharmacokinetics of substituted COT derivatives certainly warrant continued investigation, however, these results provide optimism in its ability to perform as a complementary motif.

Chemical Synthesis and Stability.

Although, a number of synthetic methods are available,[13] as mentioned above a convenient route to access the COT ring system is via rhodium catalyzed valence isomerization of cubane (**1**) (Scheme 1), which we recently demonstrated is broad in scope.[14] Furthermore, the COT system is also readily accessible via photochemical reaction of benzene with substituted acetylenes, as successfully utilized by us previously.[13] In terms of chemical stability from a bioactive COT development perspective, ^{1}H NMR analysis of COT-warfarin (**8**), COT-moclobemide (**21**), and COT diflubenzorun (**27**) samples, that had been stored at 8 °C for approximately two years, showed no signs of decomposition (see SI). Although, the COT ring system can be converted to semi-bullvalene on UV irradiation in acetone,[35] the photochemical aspects associated with COT synthesis[13] suggest concerns regarding light sensitivity are likely premature. Unlike cubane, COT can form cation−π interactions,[36] but very seldom reported (e.g. alkaline earth metals).[37]

Conformational aspects.

Cubane has a single rigid conformation, whereas COT is in dynamic equilibrium with multiple conformations and isomers (Scheme 1). This protean feature is especially relevant when COT is 1,4-disubstituted i.e. enabling comparison to both 1,3- and 1,4-disubstituted cubane. Inspection of the energy minimized geometries optimized using DFT (see SI, Figures S2–S3) suggests that the conformation of COT with substituents *anti* is a fair approximation for 1,4-disubstituted cubane, whilst *syn* is a better approximation for 1,3-disubstituted cubane (Figure 3). Gas-phase free energy calculations place the *syn*-isomer 0.34 kcal/mol lower in energy than the *anti*-isomer. This corresponds to a 2:1 ratio at equilibrium (from the Arrhenius equation), which is consistent with the ratio observed experimentally for 1,4-di-*t*-butyl COT (2:1 *syn*:*anti*).[38–40] The free energy difference is such that while *syn* is slightly preferred at room temperature, both isomers are readily accessible due to facile interconversion by bond-shifting, enabling sampling of both functional group arrangements e.g. in an enzyme active site. A further utility of COT in this

context lies in providing an easily accessible alternative to 1,3-disubstituted (or *meta*) cubanes, which are synthetically demanding.

Conclusion

In an era of multiple drug discovery platforms, the deployment of (bio)isostere replacement, and the sister concept of scaffold hopping, is prevalent. The benzene, or phenyl, (bio)isostere landscape has been dominated by saturated caged hydrocarbons, which lack the ability to provide π bond donation capabilities. The cyclooctatetraene (COT) motif was examined as a potential π containing complement to cubane (bio)isosterism using the known pharmaceutical and agrichemical templates, warfarin, moclobemide, pravadoline, SAHA, benzyl benzoate and diflubenzuron. The dynamic equilibrium and conformational isomer attributes possessed by COT provide both electronic and steric properties unmatched by classical saturated hydrocarbon systems if a π component is required by the biological partner for optimum binding. This was most evident in the case of the popular anti-coagulant warfarin, and with the synthetic cannabinoid pravadoline responding well to COT editing. In the case of moclobemide it was clear that cubane outperformed COT, further reinforcing complementarity between the two systems i.e. if cubane fails then investigate COT or *vice versa*. The demonstration herein that the COT (bio)motif acts in concert with cubane, opens additional opportunities to explore chemical space, rejuvenate pioneer drugs, fight resistance and revitalize failed clinical and agrochemical candidates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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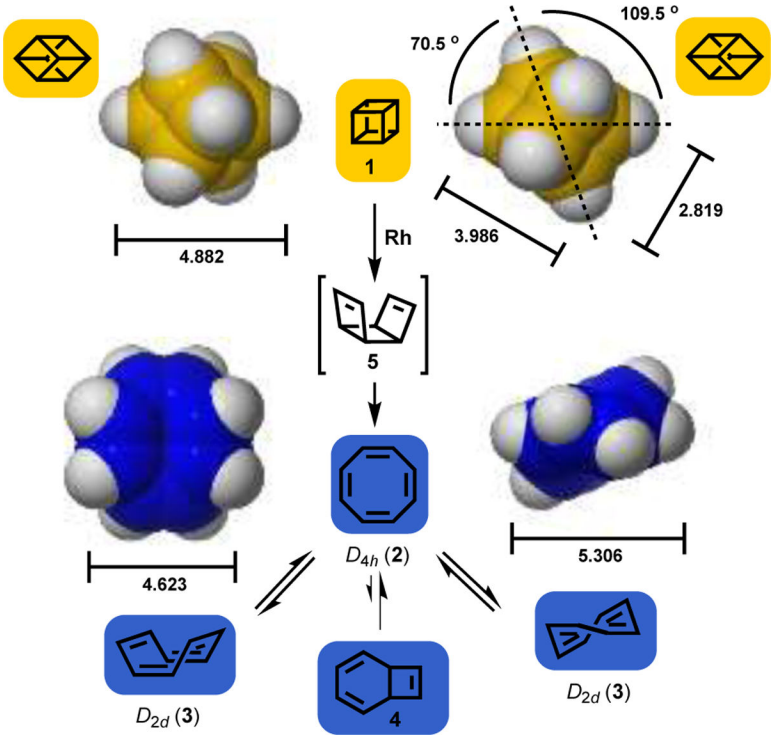
[39]. We thank the reviewer for drawing our attention to references [38].

[40]. Variations in ratios are observed depending on the functional group and substitution pattern, see [14].



Scheme 1.

Space filling model comparisons of cubane (**1**), and the ring inversion isomers (D_{2d} **3**) of cyclooctatetraene (**2**, COT shown as one of two **π**-bond shift isomers); bond distances given in angstroms [M06–2X/6–311+G(d,p)], and angles in degrees. These values are consistent with the available X-ray crystallographic data (see SI). Valence isomer of COT (i.e. **4**), and rhodium catalyzed valence tautomerization of cubane to COT, via the intermediate tricyclo[4.2.0.$0^{2,5}$]octa-3,7-diene (**5**) is also shown

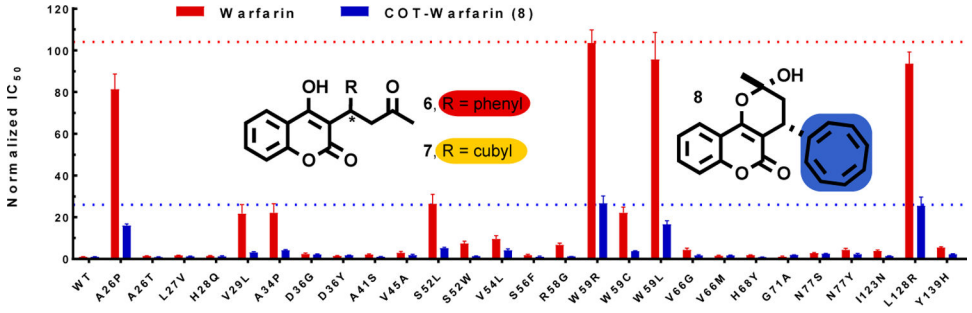


Figure 1:

Comparison of the resistance of warfarin (6) and COT-warfarin (8) against the 27 naturally occurring VKOR mutants. The potency of warfarin and COT-warfarin (8) on VKOR activity inhibition expressed as IC50 (concentrations required for 50% inhibition of VKOR activity) and normalized (resistance for wild-type VKOR is normalized as 1).

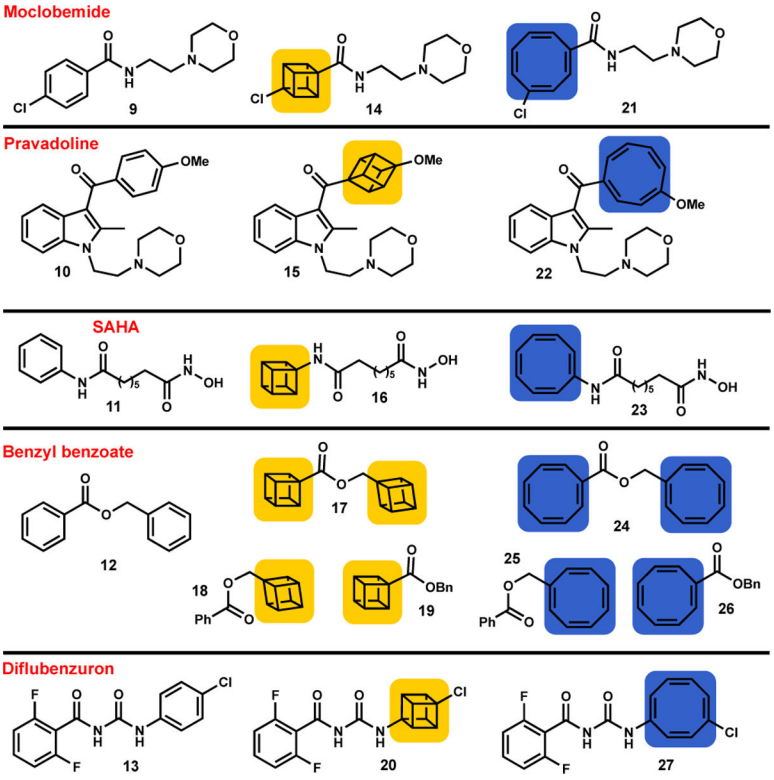


Figure 2: Additional pharmaceuticals and pesticides investigated.

Moclobemide, pravadoline, benzyl benzoate, diflubenzuron and SAHA and the corresponding cubane (yellow) and COT (blue) analogues. For synthetic procedures and characterization data see the SI.

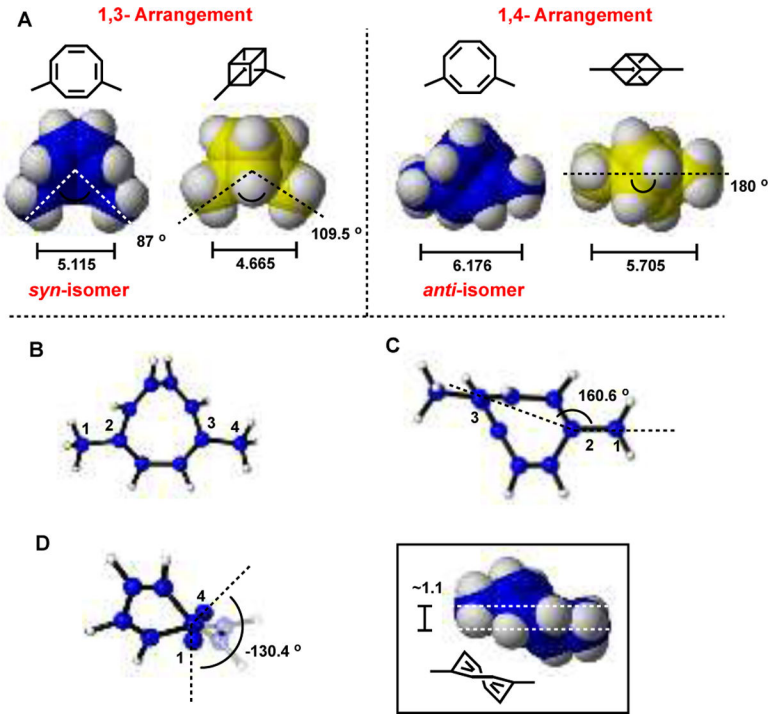


Figure 3:

A) Energy minimized geometries [M06–2X/6–311+G(d,p)] with selected distances and angles comparing 1,4-disubstituted COT with that of 1,3- and 1,4- disubstituted cubane. Bond distances are in angstroms, angles are in degrees; B) Face centered view of 1,4-COT; C) Angle between C1–C2–C3; D) Dihedral angle of C1 and C4 along the C2–C3 axis. Methyl hydrogen atoms have been omitted for clarity.