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*Dedicated to Professor Victor-Emanuel Sahini on the occasion of his 85th anniversary*

# THEORETICAL STUDY OF DHEA. A POSSIBLE NEW DRUG FROM A COMPLEX BETWEEN DHEA AND 5-HTP

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Molecular parameters (interatomic distances and angles, total atomic charge, dipole moments) of DHEA (Dehydroepiandrosterone), 5-HTP (5-hydroxytryptophan) and of their possible complex including its heat of formation, have been computed in an *ab-initio* comparative study involving HF and DFT calculations. The 6-31G* basis set and the B3LYP functional were employed. The aim of this study is to emphasize the possible existence of a complex between DHEA and 5-HTP that may have the properties of a new drug. A Natural Bond Orbital analysis (NBO) description offers supplementary details for the structure of the molecular units and their interaction.

## INTRODUCTION

In the last few years literature references emphasize the fact that DHEA is the active form of a steroidal hormone, with very desirable physiological and beneficial health properties in animals and humans.[1-7]

For instance as DHEA levels diminish with age, depression is far more likely to take hold.[8]

On the other side, when serotonin deficiency exists, depression is also far more likely to occur.

The nutrient amino acid L-tryptophan is the precursor of 5-HTP, which is itself the precursor of the neurotransmitter serotonin. In one study, when normal, healthy humans were deprived of L-tryptophan, a rapid lowering of mood occurred, resulting in greater frive to distraction, similar to that of depressed individuals.[9] In another study, depression occurred when supplies of serotonin were depleted.[10] Also, when normal, healthy humans were given 5-HTP, mood-elevation characteristics developed similar to those that occur when antidepressants are taken.[11-13] Indeed, 5-HTP has become very popular with the public for just this reason: people feel better when they use it.

Many recent studies on biological systems were carried out to identify the active molecules involved in vivo and to understand their interactions and functions. The advances made in various areas of chemistry with the help of the supramolecular paradigm emphasize the importance of a theoretical analysis of intermolecular interactions in relevant couples of weakly bound biologically active molecules.

The supramolecular approach advocated by Lehn provides a universal model to study such interactions.[14]

The present paper targets such a goal by characterizing DHEA, generated in the suprarenal glands and in the brain, and the 5-HTP, as well as their association in a complex. The electronic struc-

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ture of DHEA and a possible complex DHEA with serotonine, has been described by the authors.[15-16]

## METHODS

Using the crystallographic data reported in the literature as a starting point,[17] the geometry of DHEA was fully optimized. The initial input for 5-HTP was obtained from a molecular mechanics calculation ($MM^+$ force field).[18–20] The molecular geometries were optimized without any constraints. The *ab initio* calculations were carried out using the Gaussian 03 program.[21] The geometries of DHEA, 5-HTP and of their complex were optimized at the 3-21G* level, starting from an INDO guess. A stationary point was found. At this point, a refinement was carried out by a single point at the 6-31G* level (Raffenetti integral calculation was used) and at the B3LYP/6-31G* level.[22]

In the last part of this paper, an analysis of the molecular wave function performed in terms of localized electron-pair bonding units using the NBO program is given.[23-24] This analysis is deemed very important to understand the various interactions involving each component of the complex under study.

The structure and the numbering of selected atoms in the DHEA–5-HTP complex are shown in Fig. 1.

## RESULTS AND DISCUSSION

### Structure and bonding in the molecular units and their intermolecular complex

The formation of the DHEA–5-HTP complex is analyzed in terms of geometry, charge and energy parameters. Finding the absolute minimum for a complex is a nontrivial question, given the subtle balance of the intra- and intermolecular factors. The different nature of the overall molecular constitution of the two biomolecules, DHEA, which has a rigid σ skeleton, and 5-HTP, which possesses essentially a planar $\pi$-conjugated core, practically precludes a significant association of the $\pi-\pi$ stacking type. The isolated C=C and C=O bonds in DHEA obviously do not offer enough support for such a cohesion. The strongest association involves hydrogen bonds. There are several possible patterns for hydrogen bonding (O–H...O, O–H...N, involving the various heteroatom combinations). The supramolecular association presented here is the optimal one found after a thorough analysis due to the supplementary stabilization resulting from the alignment of the dipoles on the molecular constituents.

In HF calculation the bonding associations are :O(75).....H(47) with a regular length of 2.60Å and O(75).....H(43) with a regular length of 3.75 Å.The geometry of associations are characterized by H44....O75-H77 and H77-O75....H43 angle values ($94.68^0$ and $101.96^0$ respectively). In B3LYP calculation the bonding associations are: O(75)....H(47) with a regular length of 2.60Å and O(75)....H(43) with a regular length of 3.74 Å. The geometry of associations is characterized by H44....O75-H77 and H77-O75....H43 angle values ($94.68^0$ and $63.44^0$ respectively).

A methodological conclusion is that the HF and DFT behave similarly in both the optimized geometry.

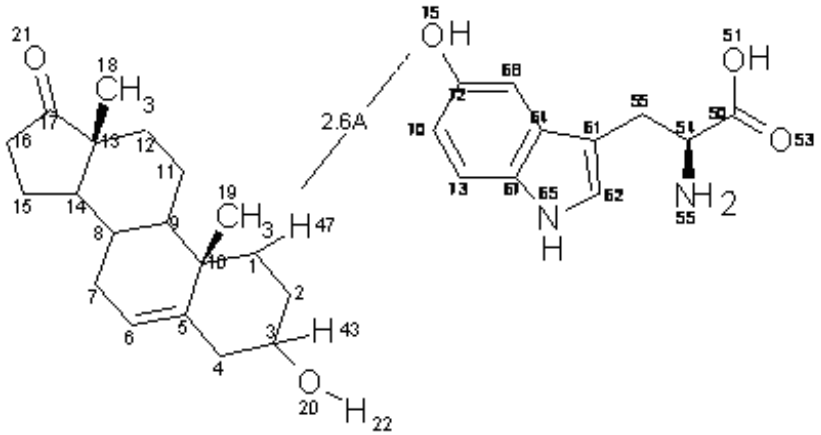


Fig. 1 – DHEA- 5HTP complex structure.

Generally, the DFT results can be credited with a higher confidence in the quantitative respects because of their treatment of correlation effects. On the other hand, it is acknowledged that the regular DFT functionals face intrinsic problems in the long-range regime.[18] Therefore, the comparative use of HF and DFT methods in computing weakly bonded systems is a technical necessity.

Selected reactivity parameters for DHEA, 5-HTP and for the complex obtained at the HF and DFT levels are shown in Table 1. It results that a higher stability for the complex is predicted from the HF data.

The dipole moment in the complex suggests a symbiotic action with the hydrogen bonding, due to some polarization of the electron density and to conformational changes in the side-chain of 5-HTP.

A detailed description of these interactions is obtained from a comparative analysis of the atomic charges in the isolated molecules and in their association complex (Table 2).

Table 2 shows that atom O75 from 5-HTP acquires the largest negative charge in B3LYP calculations. A larger positive increase of the charge is noted for the bridge hydrogen, $H_{44}$, in HF calculations. It is also interesting to note that the inductive effect produces a positive charge increase on atom $H_{27}$ from the outer O–H bond of the intermolecular association region.

*Table 1*

Reactivity parameters calculated at the HF/6-31G* and B3LYP/6-31G* levels

| Method | Reactivity parameter | DHEA | 5-HTP | DHEA– 5-HTP | $E_{complex}-(\Sigma E_{complex})$ | BSSE |
|---|---|---|---|---|---|---|
| HF/ 6-31G* single point | Total energy (au) | −885.519 | -757.0154 | -1642. 536 | -0.0016 (-1.00 Kcal/mol) | |
| | HOMO (au) | −0.33916 | -0.28449 | -0.28837 | | |
| | LUMO (au) | 0.15761 | 0.12666 | 0.12037 | | |
| | μ(D) | 2.87 | 3.69 | 5.95 | | |
| B3LYP/ 6-31G* single point | Total energy (au) | −891.087 | -761.3439 | -1652.880 | -0.4491 | -0.0011 (-0.69 Kcal/mol) |
| | HOMO (au) | -0.23353 | -0.20137 | -0.20053 | | |
| | LUMO (au) | -0.02304 | -0.01512 | -0.01532 | | |
| | μ(D) | 2.71 | 3.49 | 4.96 | | |

*Table 2*

Total atomic charge on selected atoms in the molecular components and in the association complex, from HF and DFT Mulliken population analysis

**HF analysis**

| | **Fragment** | **Complex** | **Variation** | | **Fragment** | **Complex** | **Variation** |
|---|---|---|---|---|---|---|---|
| ATOM | DHEA | DHEA–5-HTP | ΔQ | ATOM | 5-HTP | DHEA–5-HTP | ΔQ |
| $O_{20}$ | -0.7698 | -0.7712 | -0.0014 | **$O_{75}$** | **-0.7840** | **-0.7893** | **-0.0053** |
| $O_{21}$ | -0.5533 | -0.5813 | -0.0280 | $H_{77}$ | 0.4545 | 0.4574 | 0.0029 |
| $H_{44}$ | 0.1537 | 0.1844 | 0.0307 | $H_{74}$ | 0.2174 | 0.2206 | 0.0032 |
| $H_{46}$ | 0.1840 | 0.1660 | -0.0180 | $H_{60}$ | 0.2092 | 0.1591 | 0.0501 |
| **$H_{47}$** | **0.1729** | **0.2074** | **0.0345** | | | | |

**B3LYP analysis**

| | **Fragment** | **Complex** | **Variation** | | **Fragment** | **Complex** | **Variation** |
|---|---|---|---|---|---|---|---|
| ATOM | DHEA | DHEA–5-HTP | ΔQ | ATOM | 5-HTP | DHEA–5-HTP | ΔQ |
| $O_{20}$ | -0.6052 | -0.6269 | -0.0217 | **$O_{75}$** | **-0.2161** | **-0.6683** | **-0.4522** |
| $O_{21}$ | -0.4208 | -0.4652 | -0.0444 | $H_{77}$ | 0.1966 | 0.4091 | 0.2125 |
| $H_{44}$ | 0.1207 | 0.1478 | 0.0271 | $H_{74}$ | 0.0697 | 0.1385 | 0.0668 |
| $H_{46}$ | 0.1438 | 0.1332 | -0.0106 | $H_{60}$ | 0.0913 | 0.1256 | 0.0343 |
| **$H_{47}$** | **0.1298** | **0.1658** | **0.0360** | | | | |

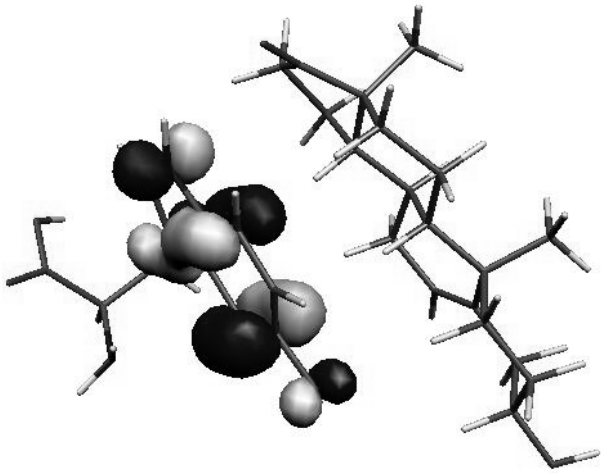

Fig. 2 – The HOMO of the complex in the HF/6-31G* (SinglePoint) calculations.

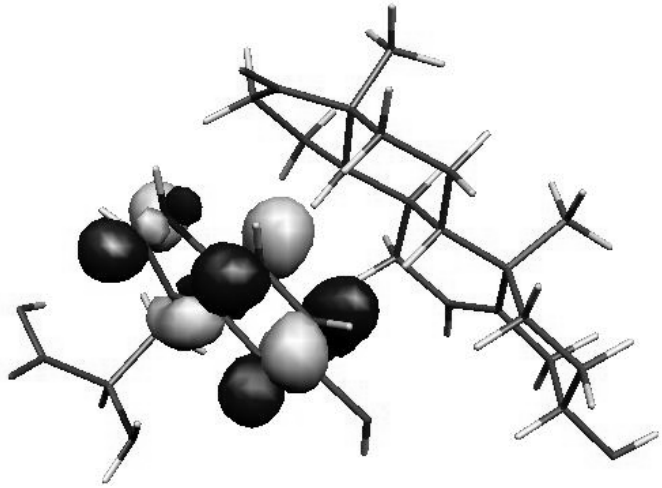

Fig. 3 – The LUMO of the complex in the HF/6-31G* (SinglePoint) calculations.

The effect of the association with DHEA is then an activation of 5-HTP induced by this electronic distribution change. The resulting activation mechanism of 5-HTP would then be due to a potentially significant structural rearrangement as shown by the data in Table 2.

Comparing the methods, one observes that the DFT based population analysis gives smaller absolute charge values on each atom. This can be regarded as a better account of the density using DFT, as the HF approach seemingly overestimates the absolute values of the charge separation in covalent polar bonds. However, both HF and DFT account similarly for the density flow resulting from the molecular association. For such weak donor–acceptor complexes, it is also clear that the outcome of the detailed results depends on the choice of the density function.

The dipole moment in the complex is smaller than the sum of the individual components, suggesting it is not affected by the hydrogen bonding; that is due to some polarization of the electron density and to conformational changes in the side chain of 5-HTP and of DHEA.

At the HF/6-31G* level, the frontier orbitals in the complex are localized on 5-HTP.

Conversely in the B3LYP/6-31G* model, one notes that the frontier orbitals in the complex derive from the HOMO of 5-HTP and the LUMO of DHEA.

The HOMO and LUMO orbitals (at the HF/6-31G* level) in 5-HTP are mainly localized on the whole molecule (Figs. 2-3).

The HOMO–LUMO gap in the DFT calculations is smaller than in the HF case. This is a consequence of systematic positive energy shifts in the occupied MOs and negative energy shifts in the virtual ones, due to the nature of the HF and KS functions.

In 5-HTP, the HOMO and LUMO orbitals are seen as pure π orbitals. Because the orbital shape in a π-type system is merely determined by topological reasons, the frontier orbital shapes are similar in the HF and DFT calculations. The frontier orbitals from 5-HTP lose their almost pure $\pi$-nature, and acquire a hybrid character in the complex. This reveals a subtle influence of the electronegativity factors involved in the donor–acceptor interactions that is accounted for in the frame of the DFT approach. It is seen (Figs. 4-5) that in complex, unlike HF results, the HOMO from B3LYP calculations is localized on 5-HTP whereas the LUMO is localized on DHEA.

## NBO analysis of the complex by B3LYP calculations

The natural bond orbital (NBO) method[23,24] offers supplementary structural information. The simplest analysis consists in checking the composition of the natural hybrid orbitals (NHO), which may reveal details about differential hybridization, that is sometimes rather important deviations from the usual $sp^2$ (*s*:*p*=33:67%) or $sp^3$ (*s*:*p*=25:75%) compositions.

In B3LYP calculations for the double bond between $C_5$ and $C_6$, one observes that the corresponding hybrid orbitals, with $C_5$ s (38.96%) and p 1.57 (61.00%), $C_6$ s(39.39%) and p 1.54 (60.57%) correspond to a rather higher s

percentage than the usual 33%. The larger s content can be associated with the strengthening of the bond, noting then the nontrivial consequence that the presence of the double bond in the skeleton slightly weakens the single C–C bonds surrounding it, while giving in turn a supplementary stabilization of the σ-component of the C=C bond. The NBO shows that the p bond is established, as expected with pure p-Aos ($C_5$ s(0.01%) and p 1.00 (99.9%) in BD (2) $C_5$–$C_6$), (Some results are presented in Table 3).

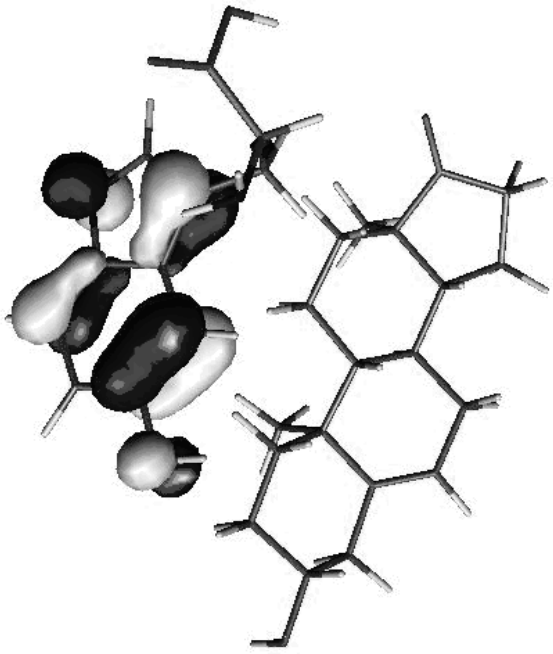

Fig. 4 – The HOMO of the complex in the B3LYP/6-31G* (SinglePoint ) calculations.

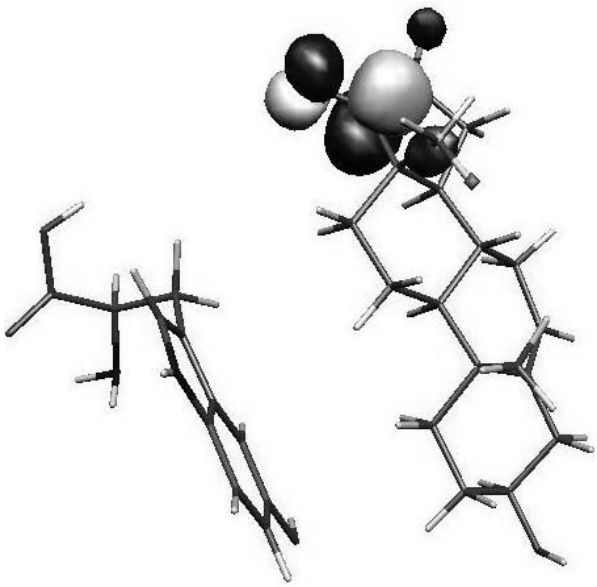

Fig. 5 – The LUMO of the complex in the B3LYP/6-31G* (SinglePoint) calculations.

*Table 3*

NATURAL BOND ORBITAL ANALYSIS obtained by B3LYP/6-31G*

(Occupancy) Bond orbital/ Coefficients/ Hybrids

4. (1.98083) BD ( 1) C 1 - H 47
( 61.50%) 0.7842* C 1 s( 24.23%)p 3.50( 76.00%)d 0.00( 0.07%)
( 38.50%) 0.6205* H 47 s(100.00%)

14. (1.97943) BD ( 1) C 5 - C 6
( 50.81%) 0.7128* C 5 s( 38.96%)p 1.57( 61.00%)d 0.00( 0.03%)
( 49.19%) 0.7014* C 6 s( 39.39%)p 1.54( 60.57%)d 0.00( 0.04%)

15. (1.94514) BD ( 2) C 5 - C 6
( 49.73%) 0.7052* C 5 s( 0.01%)p 1.00( 99.92%)d 0.00( 0.07%)
( 50.27%) 0.7090* C 6 s( 0.00%)p 1.00( 99.93%)d 0.00( 0.07%)

NATURAL LOCALIZED MOLECULAR ORBITAL (NLMO) ANALYSIS:

Maximum off-diagonal element of DM in NLMO basis: 0.10814E-09
Hybridization/Polarization Analysis of NLMOs in NAO Basis:
NLMO/Occupancy/Percent from Parent NBO/ Atomic Hybrid Contributions

136. (2.00000) 98.9439% LP ( 1) O 75
0.038% C15 s( 33.35%)p 2.00( 66.65%)
0.092% H30 s(100.00%)
0.010% H39 s(100.00%)
0.048% C64 s( 58.28%)p 0.72( 41.72%)

*Table 3 (continued)*

| | |
|---|---|
| | 0.257% C68 s( 56.23%)p 0.78( 43.77%) |
| | 0.425% C72 s( 7.87%)p11.71( 92.13%) |
| | 0.099% C73 s( 72.50%)p 0.38( 27.50%) |
| | 0.031% H74 s(100.00%) |
| | 98.944% O75 s( 43.80%)p 1.18( 54.20%) |
| | 0.016% H77 s(100.00%) |
| 86. (2.00000) | 99.6983% BD ( 1) C 72 O 75 |
| | 0.047% C64 s( 41.69%)p 1.40( 58.31%) |
| | 0.063% C68 s( 8.51%)p10.75( 91.49%) |
| | 0.020% H69 s(100.00%) |
| | 0.032% C70 s( 36.79%)p 1.72( 63.21%) |
| | 32.847% C72 s( 22.85%)p 3.38( 77.15%) |
| | 0.063% C73 s( 1.45%)p67.84( 98.55%) |
| | 0.022% H74 s(100.00%) |
| | 66.856% O75 s( 34.437%)p 1.90( 65.50%) |
| | 0.014% H77 s(100.00%) |
| 88. (2.00000) | 99.5150% BD ( 1) O 75 H 77 |
| | 0.011% C 7 s( 52.43%)p 0.91( 47.57%) |
| | 0.022% H30 s(100.00%) |
| | 0.015% H39 s(100.00%) |
| | 0.053% C68 s( 51.43%)p 0.94( 48.57%) |
| | 0.014% C70 s( 49.22%)p 1.03( 50.78%) |
| | 0.184% C72 s( 16.92%)p 4.91( 83.08%) |
| | 0.156% C73 s( 40.83%)p 1.45( 59.17%) |
| | 74.406% O75 s( 21.83%)p 3.58( 78.08%) |
| | 25.113% H77 s(100.00%) |

Particularly interesting are the hybrid orbitals associated with the intermolecular hydrogen bond formed by the sequence of atoms $C_{(1)}$–$H_{(47)}$...$O_{(75)}$. The hybrid orbitals of the $C_{(1)}$ atom are close to the regular $sp^3$ (s:p=24:76%) composition.

The NBO analysis shows that the hybrid composition is not so standard, and is in fact better characterized by an $sp^2$ differential hybridization. The lone pair devoted to the $H_{(47)}$...$O_{(75)}$ hydrogen bond has the nonstandard composition s (43.41%) p 1.30 (56.53%) which practically suggests an sp hybrid character. The hybrids along the $C_{(72)}$–$O_{(75)}$ and $O_{(75)}$–$H_{(77)}$ bonds have the following compositions s (34.43%) p 1.90 (65.50%) and s (21.83 %) p 3.58 (78.08 %) respectively.

The first one has an $sp^2$ hybrid character and the last one has an $sp^3$ hybrid character. The heterogeneous nature of the bond is measured by the 67.13% participation of the oxygen hybrid orbitals in the C–O bond. Similarly in the O–H bond described, the oxygen hybrid orbital percentage is 74.69%.

The NBO analysis automatically identifies two molecular units corresponding to the steroid and 5-HTP molecules. The perturbation donor–acceptor analysis of the NBO method offers information about intermolecular interactions. A look at the corresponding data shows that the most important intermolecular donor– acceptor contact occurs between an antibonding NBO function (NBO no. 137. LP ( 2) $O_{75}$ /511. BD*( 1) $C_1$ - $H_{47}$ of the C–H group and the lone pair of $O_{(75)}$, corresponding to an energy of 1.2 Kcal/mol. The empty antibonding NBO of the C–H group displays a 62.66 % character for atomic orbital s of $H_{(47)}$, which corresponds to an unshielded proton pulling electron density from the occupied lone pair hybrid orbitals (NBO no. 137. LP ( 2) $O_{75}$).

These conclusions are valuable also for NBO analysis in HF/6-31g. Though the only difference between the B3LYP and HF/6-31g in this study is the following: in the NLBO calculations in HF/6-31g there are a contribution to NLBO136 LP ( 1) $O_{75}$ and to NLBO137 LP( 2)$O_{75}$ from $H_{47}$ that emphasizes the presences of the H bond in the complex.

## CONCLUSIONS

From these comparative calculations, it appears that the possibility of forming an association

complex between DHEA and 5-HTP is emphasized by the HF/6-31g and B3LYP calculations.

The NBO analysis reveals several nonstandard hybrid compositions and the associated donor–acceptor perturbative schemes support the idea of a moderate strength hydrogen bond, cumulated with electrostatic effects, leading to a firmly bound molecular complex.

The present theoretical study on the electronic changes brought about by complexation leads to the hypothesis that a change in the biological action of 5-HTP and/or DHEA could result from their interaction. This hypothesis should now be reinforced by the experimental observation of an interaction between those two molecules.

The possibility for a functional connection between 5-HTP and DHEA opens up new vistas. The role of DHEA as a neurohormone associated to 5-HTP, a precursor in the synthesis of serotonine, may lead to the design of a new drug.

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