Structure–Activity Studies on γ-Aminobutyric Acid-Like Agents

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Abstract □ Molecular orbital calculations were conducted on the γ-aminobutyric acid-like agents hydrazinopropionic acid, taurine, homotaurine, and imidazoleacetic acid. The results predict that all but taurine prefer conformations in which the previously proposed γ-aminobutyric acid pharmacophore is contained. The evidence supports the hypothesis that the γ-aminobutyric acid pharmacophore is composed of an onium group separated from an oxygen atom by 5.8 ± 0.2 Å.

Keyphrases □ γ-Aminobutyric acid, related agents—structure–activity studies, molecular orbital calculations, relation to previously proposed pharmacophore □ Molecular orbital calculations—γ-aminobutyric acid-like agents (hydrazinopropionic acid, taurine, homotaurine, and imidazoleacetic acid), relation to proposed pharmacophore, structure–activity relationships □ Structure–activity relationships—γ-aminobutyric acid-like agents, evidence supporting possible pharmacophore, molecular orbital calculations

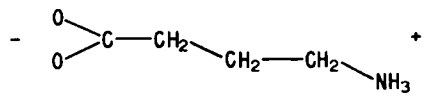
Accumulated evidence strongly supports the role of γ-aminobutyric acid (I) as an inhibitory transmitter at certain interneuronal synapses in the mammalian central nervous system (CNS) (1–5). γ-Aminobutyric acid appears to be associated with the excitability level of the brain as evidenced by relationships with epileptiform seizures (6), sodium barbital withdrawal convulsions (7), electroshock convulsions (8), and convulsions following high oxygen exposure (9). In view of its apparent role in maintaining a level of brain activity and its possible role in pathological conditions when deficient, structural characteristics influencing its biological effect are of considerable importance.

THEORETICAL

Some γ-aminobutyric acid-like agonists have been identified, including β-hydroxy-γ-aminobutyric acid (10) and muscimol (II) (11). More recently, the alkaloid bicuculline (III) was studied and concluded to be a competitive inhibitor of the central inhibitory effect of γ-aminobutyric acid (12), although some controversy has emerged on the specific nature of this antagonism (13–15).

In contrast to the central inhibitory transmission postulated for γ-aminobutyric acid, the amino acid glycine has been postulated as a distinctly different inhibitory transmitter functioning principally on motoneurons in the spinal cord (16–18). The effect of glycine was reported to be prevented by strychnine (18). The postsynaptic inhibition of strychnine has been questioned (19, 20) and defended (21).

The singularity of inhibitory effects produced by γ-aminobutyric acid and glycine is supported by the observations that strychnine, an inhibitor of glycine effects, does not depress the inhibitory effects of γ-aminobutyric acid (18). Likewise, bicuculline, an inhibitor of γ-aminobutyric acid effects, does not depress glycine-induced inhibition (12, 22). Taurine (2-aminoethanesulfonic acid) (23) and imidazoleacetic acid (14) have been suggested as inhibitory transmitters in addition to γ-aminobutyric acid and glycine. Further neurochemical evidence is required to support these possibilities.

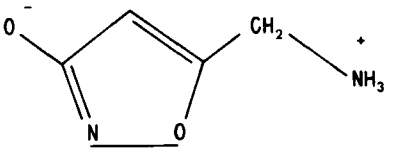


I

Some structural considerations have been studied among the γ-aminobutyric acid inhibitors in an effort to relate agonists and to deduce something about a γ-aminobutyric acid pharmacophore. In 1970, a molecular orbital study of the preferred conformation of γ-aminobutyric acid and γ-aminobutyric acid agonist muscimol (24) was reported. The calculations revealed a preference for an all-*trans* γ-aminobutyric acid zwitterion molecule, with free rotation predicted for the carboxylate. When assuming that the onium group and some part of the carboxylate are receptor active, it was predicted that the distance separating the nitrogen and one oxygen atom was 5–6 Å. The calculations on the muscimol betaine molecule predicted a conformation in which the O to N distance was 5.8 Å. From the calculated conformations, it was concluded that interaction of the two molecules with a common receptor was structurally allowed on the basis of similar charged regions separated by similar distances. It was also predicted that the γ-aminobutyric acid pharmacophore contained these two charged centers at least 5 Å, but more likely approximately 6 Å, apart.

Curtis *et al.* (12) commented on the similarity in distances separating these same structural features of γ-aminobutyric acid and muscimol on the basis of scale model examination. At the same time, they speculated that the γ-aminobutyric acid inhibitor bicuculline could conceivably present similarly charged structural features to a γ-aminobutyric acid receptor, thereby functioning as a competitive antagonist.

Another conclusion was reached by Van Gelder (25) who compared possible molecular arrangements of glutamic acid and γ-aminobutyric acid. He proposed that the steric dimension of the γ-hydrogens in γ-aminobutyric acid were important at a receptor and that cell wall transport was dependent on the diameter of the hydrated ions. He did presume a folded conformation for γ-aminobutyric acid. A preliminary report on the crystal structure of γ-aminobutyric acid zwitterion led to a provisional conclusion of some folding in the molecule, making the charged centers 4.5 Å or less apart (26). An X-ray study of γ-aminobutyric acid hydrochloride conformation revealed a *trans* or fully extended structure (27).



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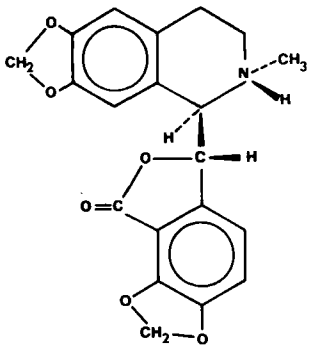

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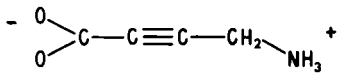

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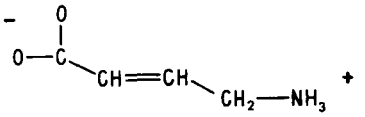

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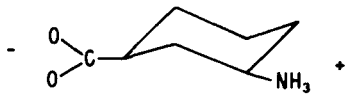


VI

More recent studies from this laboratory on β-hydroxy-γ-aminobutyric acid predicted the molecule to prefer both *trans-* (fully extended) and *gauche-* (Cα–Cβ bond) conformations (28). The *trans*-conformation is identical to the prediction of the γ-aminobutyric acid structure and is in agreement with the crystal structure (29), while the *gauche*-conformation is more consistent with the crystal study of Steward *et al.* (26) of γ-aminobutyric acid.

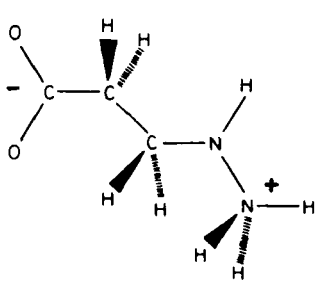
In the same study (28), the conformation of bicuculline was reported. It was found that the onium group distance from the carbonyl oxygen atom, in the preferred conformation, corresponds closely (5.6 Å) with the hypothesis of the γ-aminobutyric acid pharmacophore. Another study on bicuculline at the same time using molecular orbital calculations and NMR analysis appears to be in agreement (30).

An important consideration is the pharmacological mechanism whereby a compound may be exerting a γ-aminobutyric acid-like action. Actions mimicking the direct effect of γ-aminobutyric acid and requiring structural features comparable to γ-aminobutyric acid are: *(a)* a direct agonist action at the postsynaptic γ-aminobutyric acid receptor, *(b)* a competitive inhibition of γ-aminobutyric acid transaminase activity, and *(c)* a competitive inhibition of cellular uptake of γ-aminobutyric acid following postsynaptic action. γ-Aminobutyric acid transamination and cellular uptake constitute mechanisms whereby postsynaptic action is terminated. Inhibition of either process results in the preservation of γ-aminobutyric acid, presumably leading to the opportunity for continued interaction events at the postsynaptic receptor.

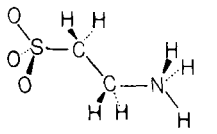
Some recent studies examined structural requirements promoting inhibition of γ-aminobutyric acid transaminase *in vitro* (31) and γ-aminobutyric acid uptake in cerebral cells *in vitro* (32, 33). The competitive inhibition of γ-aminobutyric acid transaminase was accomplished with 4-aminotetrolic acid (IV), a rigid molecule with N to O distances of 5.2 and 5.8 Å, quite similar to γ-aminobutyric acid in its fully extended conformation. A similar observation has been made that the rigid molecule *trans*-4-aminocrotonic acid (V) is an excellent substrate for this enzyme (34). Again the O to N distances are comparable to those found for the extended γ-aminobutyric acid conformation.

These studies led to the conclusion that the extended conformation of γ-aminobutyric acid is important in the interaction with γ-aminobutyric acid transaminase (31). It follows that substrates and competitive inhibitors should have this pattern of receptor-functional moieties.

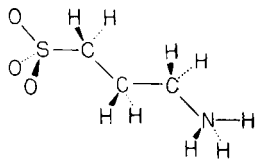
Studies of competitive inhibitors of γ-aminobutyric acid uptake into cerebral gray matter cells *in vitro* have established that compounds with the potential for mimicking the extended γ-aminobutyric acid conformation are active (32). *trans*-4-Aminocrotonic acid was a competitive inhibitor of γ-aminobutyric acid uptake while the *cis*-isomer, with a much closer N to O distance, was inactive. Only the *cis*-3-aminocyclohexanedicarboxylic acid isomer (VI) was active—not the *trans*-isomer. Only in the diequatorial conformer of VI is the N to O distance identical to that found in the extended γ-aminobutyric acid.



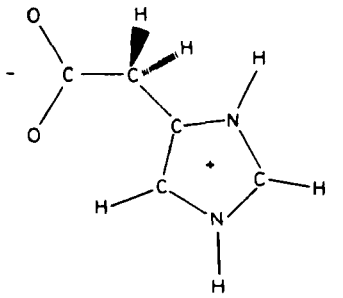
VII



VIII



IX



X

This study consolidated previous results and tested the pharmacophore hypothesis by predicting the conformations of several agents acting at receptors responsive to γ-aminobutyric acid.

Hydrazinopropionic Acid—Hydrazinopropionic acid (VII) has been found to be an inhibitor of γ-aminobutyric acid transaminase and should, therefore, possess features comparable to γ-aminobutyric acid to be active at this enzyme (35). Van Gelder (35) commented on this possibility from an examination of scale molecular models. Accordingly, molecular orbital calculations have been performed on hydrazinopropionic acid to assess its preferred conformation and, therefore, its potential for mimicking γ-aminobutyric acid at the enzyme.

Taurine and Homotaurine—Taurine (VIII) and homotaurine (IX) have been observed to behave like γ-aminobutyric acid in their CNS effects (36). Taurine is a very prominent amino acid in the CNS and has been proposed as a possible neurotransmitter (23). Homotaurine has been reported to produce CNS effects more like γ-aminobutyric acid, while taurine appears to resemble the inhibitory effects of glycine. To determine whether either molecule possesses structural features resembling the hypothesized γ-aminobutyric acid pharmacophore, the conformational preferences of both molecules have been calculated using molecular orbital theory.

Imidazoleacetic Acid—Imidazoleacetic acid (X) has been found to produce an effect in the CNS mimicking γ-aminobutyric acid (37). Tests with inhibitors led to the conclusion that imidazoleacetic acid is acting on γ-aminobutyric acid-like receptors. To test the possibility that imidazoleacetic acid possesses a γ-aminobutyric acid pharmacophore, the conformational preferences of the protonated and neutral molecule in both tautomeric forms have been calculated using molecular orbital theory.

EXPERIMENTAL

Calculations of the total energies were made using extended Huckel theory (38) with parameters previously discussed (39) and

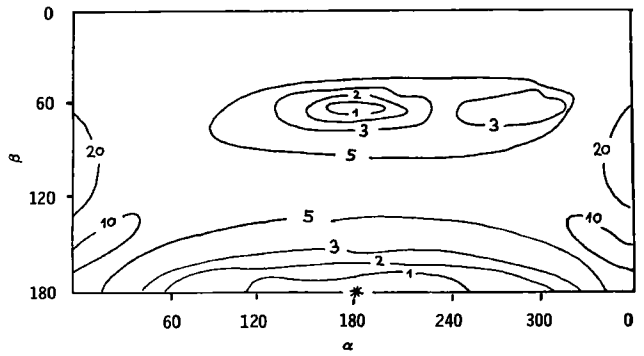


Figure 1—*Calculated energy contour map for hydrazinopropionic acid (VII). The 180° angle for bond α is a* trans*-conformation around bond CH_2—NH. The 180° angle for bond β is a* trans*-conformation around bond CH_2—CH_2. The carboxylate group is freely rotating.*

employed (24, 28). The minimum calculated energy was assumed to be associated with the preferred conformation.

Calculations on taurine and homotaurine were made using bond distances of 1.80 Å for C—S and 1.44 Å for S—O. The Coulomb integrals were used for S(3*s*) = −30.00 ev and S(3*p*) = −14.20 ev. The Slater exponent employed for sulfur was 1.817.

Imidazoleacetic acid at physiological pH certainly exists as the anion, and this form was used in the calculations. An additional problem is created by the high probability that the imidazole ring can tautomerize. The position of N—H is thus uncertain. In addition, the pKa for the ring may be very close to physiological pH. The pKa for imidazole is 6.7. It is probably more basic with the inductive effect of the methylene group. As a consequence, three forms must be considered as coexisting, the two tautomeric forms and the ring-protonated form. All three forms were considered in the calculations of preferred conformation.

RESULTS

Hydrazinopropionic Acid—Calculations of the minimum energy conformation of the hydrazinopropionic acid zwitterion revealed a preference for the fully extended molecule. The energy contours for the variation of the CH_2—CH_2 (β) and C—N (α) bonds are shown in Fig. 1. The carboxylate was found to rotate freely in the fully extended conformation while the N—N bond preferred a hydrogen eclipsing conformation. All possible hydrogen bonding conformations were calculated and found to have no preference. The β bond showed a modest flexibility, ±60°, from the fully extended conformation. The distances separating the onium nitrogen from the two oxygen atoms in the preferred conformation with the carboxylate group in the plane of the molecule were 5.1 and 6.1 Å.

Taurine—The calculations on the taurine zwitterion revealed a preference for a fully extended molecule. The contour diagram is shown in Fig. 2 for the rotations of the C—S and C—C bonds. The onium nitrogen to oxygen distances in the preferred conformation were 4.7, 4.7, and 5.3 Å.

Homotaurine—Calculations on the homotaurine zwitterion revealed a preference for a fully extended molecule. The contour diagram for the two C—C bonds is shown in Fig. 3. The calculated onium nitrogen to oxygen distances were 5.6, 5.6, and 6.6 Å.

Imidazoleacetic Acid—N_1—*H Tautomer*—The preferred conformation of this tautomer anion is shown in the contour diagram of Fig. 4. The plane of CO_2—CH_2—C_1 (ring) was found to be perpendicular to the ring plane. Some flexibility was revealed in this relationship. The carboxylate group was virtually free to rotate.

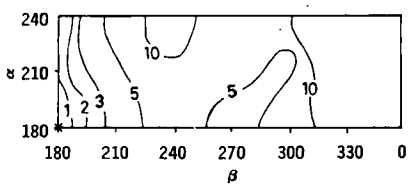


Figure 2—*Calculated energy contour map for taurine (VIII). The 180° angle for bond α is a staggered arrangement of oxygens and adjacent carbon atom substituents. The 180° angle for bond β is a* trans*-conformation around the CH_2—CH_2 bond. See Structure VIII.*

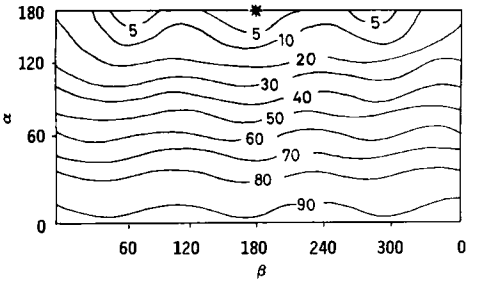


Figure 3—*Calculated energy contour map for homotaurine (IX). The 180° angle for bond α is a* trans*-conformation around the SO_3CH_2—CH_2CH_2 bond. The 180° angle for bond β is a* trans*-conformation around the CH_2CH_2—CH_2NH_3 bond. The SO_3 group is staggered to the adjacent methylene group.*

If the protonated nitrogen and the oxygens are identified as being significant structural features, interatom distances can be predicted in the preferred conformations, ranging from 3.8 to 4.2 Å.

N_3—*H Tautomer*—Calculations on the N_3—H tautomer anion revealed a conformational preference almost identical to that predicted for the N_1—H tautomer. The energy *versus* angle contour diagram is shown in Fig. 5. The range of N(H) to oxygen distances calculated was 5.5–5.9 Å.

The absolute energy values found for the N_3—H tautomer were consistently lower than those found for the N_1—H tautomer for comparable conformations. The energy differences (tautomer N_3—H minus tautomer N_1—H) were averaged at appropriate positions in the contour diagrams where there is a predicted energy <0.5 kcal above the global minimum. The average value of the increments is about 0.45 kcal. This corresponds to a predicted ratio of 2.1 in favor of the N_3—H tautomer.

Calculations on the conformational preference of the zwitterion

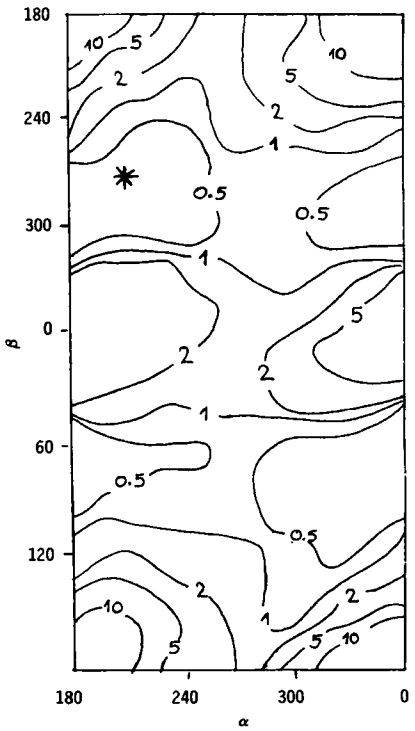


Figure 4—*Calculated energy contour map for imidazoleacetic acid anion (X) as the N_1(H) tautomer. The 0/180° angle for bond α is the conformation in which a C—O bond eclipses the ring—CH_2 bond. The 0° angle for bond β is the planer structure depicted by X.*

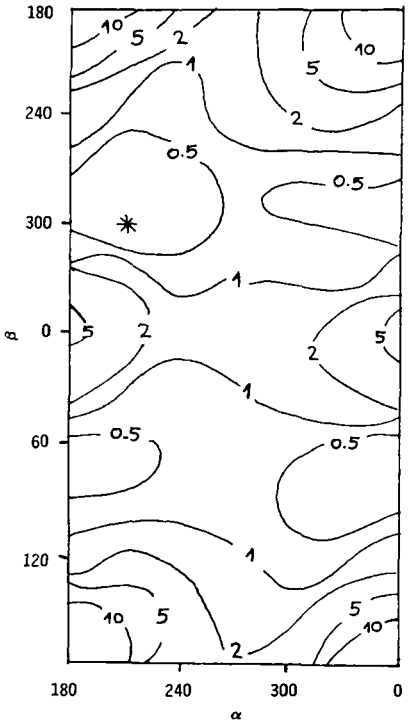


Figure 5—*Calculated energy contour map for imidazoleacetic acid anion as the $N_3(H)$ tautomer. The angles are the same as defined in Fig. 4.*

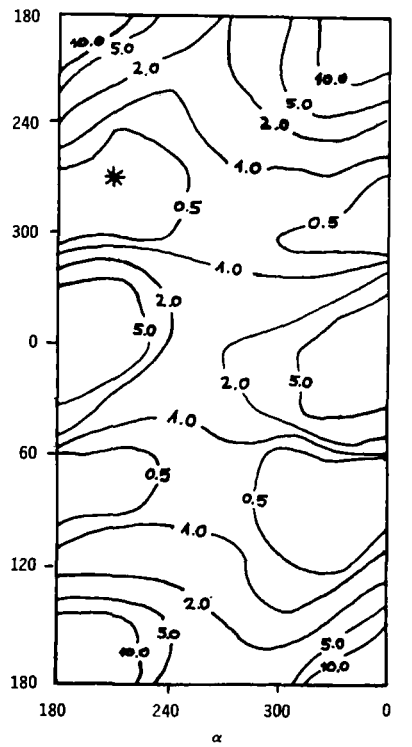


Figure 6—*Calculated energy contour map for imidazoleacetic acid zwitterion. The angles are the same as defined in Fig. 4.*

form of imidazoleacetic acid are shown in Fig. 6; the results are very similar to the two tautomeric anions. The distance range separating the N_3 and the most distal oxygen atom was identical, 5.5–5.9 Å, to that predicted for the N_3—H tautomer.

DISCUSSION

Calculations of the preferred conformation of hydrazinopropionic acid reveal a structure comparable to that of γ-aminobutyric acid. The oxygen to terminal nitrogen distance mirrors that of γ-aminobutyric acid. The competitive inhibition of γ-aminobutyric acid transaminase may be accounted for on the basis of this structural similarity. The comparison supports the hypothesis of the γ-aminobutyric acid pharmacophore (24). It further suggests that the same basic structural pattern may obtain in the enzyme reactions of the molecule as well as the postsynaptic receptor response.

Calculations on taurine predict a fully extended molecule, in contrast to the X-ray analysis revealing a *gauche* preference (40). The polar nature of the molecule would lead one to suspect that binding events in the crystal may be quite different from those found in solution. Nevertheless, an N to O distance in taurine is not predicted to be particularly close to that described by the hypothesized γ-aminobutyric acid pharmacophore. In taurine, this dimension is predicted to be 4.7, 4.7, and 5.3 Å, invoking each oxygen atom as being a candidate for receptor binding. Only the 5.3 Å value comes close to the range predicted for γ-aminobutyric acid. It is still short of the 5.6 or 5.8 Å that was predicted for comparable atoms in bicuculline (28) and muscimol (24).

If taurine is a neurotransmitter, as was discussed by Davison and Kaczmarek (23), the present structural studies argue against the possibility that it is acting at a γ-aminobutyric acid receptor. These results are in harmony with the findings of Curtis *et al.* (36) who observed that taurine is not γ-aminobutyric acid like based on the effect of competitive inhibitors.

Calculations on homotaurine predict a fully extended molecule to be preferred. This is in agreement with X-ray analysis (41), although it is not felt to be highly relevant to solution structure. The N to O distances in the predicted preferred conformation are 5.6, 5.6, and 6.6 Å. The 5.6-Å separation is closely related to the predicted γ-aminobutyric acid dimension. As a result, it is predicted that the γ-aminobutyric acid action observed (36) can be explained by the presence of the appropriate pharmacophore in homotaurine.

The calculations on the conformational preferences of the imidazoleacetic acid anions reveal a modest preference for the N_3—H tautomer. From a comparison of relative energies at highly preferred conformations, it can be concluded that this tautomer is in approximately a 2:1 ratio with the N_1—H tautomer. Recent experimental evidence on the tautomer ratio in histamine supports this prediction (42). The pKa of the acid is close to neutrality, so it can be concluded that approximately one-half of the molecules are zwitterionic. At physiological pH, over 80% of the population will be molecules with an N_3—H.

If it is presumed that the molecule is γ-aminobutyric acid like in its activity by virtue of possessing a pharmacophore, then the salient structural features of imidazoleacetic acid are the carboxylate oxygen atoms and an N—H group. This latter structural feature may be the receptor equivalent of an N—H moiety of γ-aminobutyric acid.

The distance interposing between at least one carboxyl oxygen atom and a protonated nitrogen atom should be comparable to that of γ-aminobutyric acid if this molecule is functioning at a γ-aminobutyric acid receptor in an agonistic or competitively antagonistic manner. The calculations reveal that this distance is indeed comparable in the preferred conformation of the imidazoleacetic acid N_3—H tautomer and zwitterion. Thus, it can be concluded that the molecule possesses the predicted pharmacophore in the preferred conformations. From a structural point of view, it is predicted that the molecule is capable of interacting with a γ-aminobutyric acid receptor.

In conclusion, the present predictions, coupled with previous studies, support the hypothesis that the γ-aminobutyric acid pharmacophore for the inhibitory receptor and very likely the transaminase and reuptake enzyme actions is the N—H feature separated from an oxygen atom by 5.8 ± 0.2 Å.

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