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Note

Inhibition of Urease by Some Triazole, Urea, and Guanidine Derivatives

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Series of aminotriazole, guanidine and urea derivatives were prepared and tested as urease inhibitors. A greater part of the tested compounds, all containing the sequence ressembling the substrate, have shown an inhibitory effect on urease.

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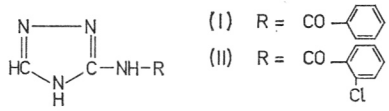
For many years urease seemed to be an enzyme with absolute specificity. The investigation of Fischbein[1], Shaw *et al.*[2], Kistiakowsky *et al.*[3] showed that urease can bind and transform some substrate analogs like thiourea, hydroxyurea, dihydroxyurea and methylurea. Today many urea-like compounds are known as inhibitors of urease activity[4].

We found that 3-benzamido-1,2,4-triazole (I) and 3-(o-chlorobenzamido)-1,2,4-triazole (II) are inhibitors of urease[5]:



This inhibition is unusual in so far as these compounds were known to inhibit only metalloproteic enxymes[6]. It was proved moreover that I and II are competitive inhitors of urease. The reactivation of the inhibited urease with thiol agents resulted in higher activity of the enzyme. The sulfhydryl content of the native and the inhibited enzyme was found to be the same. On the basis of all these observations it was assumed that —NH—C(NHR)=N— was the main sequence in the molecule of triazole compounds essential for the inhibition. To verify this assumption benzoylguanidine hydrochloride, *o*-chlorobenzoylguanidine hydrochloride, benzamidoguanidine hydrochloride and *o*-chlorobenzamidoguanidine hydrochloride were prepared and tested as inhibitors of urease[7]. All of them exerted an inhibitory effect. The results obtained stimulated the testing of various guanidine and urea derivatives as inhibitors of urease activity, to clarify the correlation between the chemical structure and the inhibitory action of these compounds.

EXPERIMENTAL

Enzyme source

100 mg urease (»Merck«) was dissolved in 100 ml of 0.02 M phosphate buffer (pH = 7.0, 0.02 units/mg protein). This solution was used for measurements of the inhibitory power of triazole, guanidine and urea derivatives.

Synthesis of some inhibitors

Inhibitors synthesized in our laboratory include five triazole and seven guanidine derivatives. Some of them were unknown compounds; their melting points and analytical data are listed in Table I. Phenylethylguanidine and 1-methyl-1-nitroso--3-nitroguanidine were obtained by courtesy from Prof. Prakash Chandra, Frankfurt. The other tested compounds were of commercial origin.

TABLE I

List of the compounds prepared

Compound	m. p. °C	Analysis calcd. %	Analysis found %	Ref
3-Amino-1,2,4-triazole	154			8
3-Benzamido-1,2,4-triazole	195			9
3-(o-Chlorobenzamido)-1,2,4-triazole	280	C 48.55 H 3.17 N 25.16	48.82 3.17 24.97	
3-(α-Bromobutyramido)-1,2,4-triazole	189	C 30.92 H 3.89	31.49 3.73	
3-(β-Bromobutyramido)-1,2,4-triazole	195	C 30.92 H 3.89	30.956: 4.41	
Nitroguanidine	232			10
Aminoguanidine bicarbonate	172			11
Benzoylguanidine hydrochloride	215			12
o-Chlorobenzoylguanidine hydrochloride	185	C 41.04: H 3.87	41.04: 3.85	
Benzamidoguanidine hydrochloride	223			13
o-Chlorobenzamidoguanidine hydrochloride	228	C 38.026: H 4.555	38.82: 4.033	
Dibenzoylguanidine	225			12

Enzyme assay

Urease was assayed by the Conway microdiffusion method[14] at 30 °C. The reaction mixture contained in a final volume of 3 ml: 1 ml of 3% urea solution in 0.02 M phosphate buffer (pH = 7), 1 ml of 10^{-2} M solution of tested compound and 1 ml of urease solution (0.02 units/ml), added in the order listed. In the control experiment 1 ml of redistilled water was added instead of the inhibitor. If the compound was insoluble in water stable suspension was prepared by adding 4 mg of Lyssapol per 100 ml of solution. In preliminary experiments Lyssapol was found to be without effect on urease activity in the concentration used. In kinetic measurements the final substrate concentrations were: 2.66×10^{-2} M, 5×10^{-2} M,

11.6×10^{-2} M and 16×10^{-2} M. In the reactivation experiment we compared the activity of triazole-inhibited enzyme in the absence and in the presence of the reactivator. The concentrations of the reactivator and of the inhibitor were equimolar.

Estimation of sulfhydryl groups

Sulfhydryl groups were determined according to Hamm and Hofmann[15] and by iodometric titration.

RESULTS AND DISCUSSION

By varying the substrate concentrations, a linear relationship was obtained in the Lineweaver-Burk double reciprocal plot indicating a competitive inhibition by 3-benzamido-1,2,4-triazole and 3-(*o*-chlorobenzamido)-1,2,4-triazole. Reactivation experiments were performed with glutathione and cysteine in the case of triazole-inhibited enzyme. Only with glutathione a positive result was obtained. If the activity of the inhibited enzyme is expressed as one arbitrary unit, then the ratio between the activities of native, the inhibited and the reactivated enzyme was 4 : 1 : 7. No significant difference was found

TABLE II

Inhibition of urease with triazolic, guanidinic and related compounds. The percentage of inhibition was calculated by the formula $H = 100\,(1 - A/A_0)$, *in which* A *and* A_0 *represent relative activities of enzyme solution with and without addition of an inhibitor. The final concentration of substrate was 0.16 M and that of the inhibitor* 3.3×10^{-3} *M.*

Compound	Percent inhibition[a]
1. 3-amino-1,2,4-triazole	no inhibition
2. 3-benzamido-1,2,4-triazole[b]	36.8
3. 3-(*o*-chlorobenzamido)-1,2,4-triazole[b]	45.1
4. 3-(α-bromobutyramido)-1,2,4-triazole[b]	no inhibition
5. 3-(β-bromobutyramido)-1,2,4-triazole[b]	no inhibition
6. Benzoylguanidine hydrochloride	16.5
7. *o*-chlorobenzoylguanidine hydrochloride	20.0
8. Benzamidoguanidine hydrochloride	29.0
9. *o*-chlorobenzamidoguanidine hydrochloride	20.0
10. *N*-ethylmaleinimide	95.0
11. Semicarbazide hydrochloride	7.5
12. Aminoguanidine bicarbonate[b]	8.1
13. Dibenzoylguanidine[b]	9.0
14. Arginine	10.8
15. Phenylethylguanidine[b]	12.4
16. Histidine	15.3
17. Thiosemicarbazide	small activ.
18. Creatine	small activ.
19. Guanidine hydrochloride	no inhibition
20. Cyanoguanidine	no inhibition
21. Thiourea	no inhibition
22. 1-methyl-1-nitroso-3-nitroguanidine[b]	no inhibition
23. Nitroguanidine	no inhibition
24. Adenine	no inhibition
25. Thymine	no inhibition
26. Uracil	no inhibition

[a] Percent of inhibition indicated in the table represents the mean of at least 6 measurements.
[b] Water-insoluble compound; Lissapol was applied.

between the sulfhydryl content of the triazole-inhibited and the noninhibited enzyme. The number of -SH groups determined by iodometric titration was 248 ± 10 per mole of urease, assuming that the oxidation proceeded to the disulfide stage.

As shown in Table II, urease was inhibited by most of the compounds. tested. As can be seen, most of the compounds containing the guanidino group showed an inhibitory effect. Only compounds having an electronegative group in the neighbourhood of the guanidine sequence were inactive. Under our experimental conditions thiourea was inactive, which is in accordance with the finding of Kistiakowsky and Shaw[3]. Uracil, adenine and thymine showed no effect on urease which is in accordance with the findings of Kobashi *et al.*[16] In all compounds tested in our experiments, a characteristic moiety showing a structural ressemblance to the substrate was present.

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SAŽETAK

Inhibicija ureaze nekim derivatima triazola, uree i gvanidina

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Pripravljeni su i testirani kao inhibitori ureaze neki derivati aminotriazola, gvanidina i uree. Većina testiranih spojeva, koji svi sadržavaju sekvenciju sličnu supstratu, pokazala je inhibitorsko djelovanje na ureazu.

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