Slow-binding inhibitors of prolyl oligopeptidase with different functional groups at the P1 site

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POP (prolyl oligopeptidase) specifically hydrolyses a number of small proline-containing peptides at the carboxy end of the proline residue and POP inhibitors have been shown to have cognition-enhancing properties. It has been noted that certain functional groups at the P1 site of the inhibitor, which correspond to the substrate residue on the N-terminal side of the bond to be cleaved, increase the inhibitory potency. However, detailed mechanistic and kinetic analysis of the inhibition has not been studied. In the present study, we examined the effect of different functional groups at the P1 site of the parent inhibitor isophthalic acid bis-(L-prolylpyrrolidine) amide on the binding kinetics to POP. Addition of CHO, CN or COCH₂OH groups to the P1 site increased the inhibitory potency by two orders of magnitude ($K_i = 11.8\text{--}0.1\text{ nM}$) and caused a clear slow-binding inhibition. The inhibitor containing a CHO group had the lowest association

rate constant, $k_{on} = (2.43 \pm 0.12) \times 10^5\text{ M}^{-1} \cdot \text{s}^{-1}$, whereas the inhibitor with a CN group exhibited the fastest binding, $k_{on} = (12.0 \pm 0.08) \times 10^5\text{ M}^{-1} \cdot \text{s}^{-1}$. In addition, the dissociation rate was found to be crucially dependent on the type of the functional group. Compounds with COCH₂OH and CHO groups had much longer half-lives of dissociation (over 5 h) compared with the compound with the CN group (25 min), although the K_i values of the compounds were relatively similar. A possibility to optimize the duration of inhibition by changing the functional group at the P1 site is important when planning therapeutically useful POP inhibitors.

Key words: enzyme kinetics, prolyl oligopeptidase, slow- and tight-binding inhibition, Z-prolylprolinal.

INTRODUCTION

POP (prolyl oligopeptidase; EC 3.4.21.26) is a large enzyme (80 kDa), which belongs to a POP family of serine proteases, unrelated to trypsin, subtilisin and carboxypeptidase Y [1]. The POP family includes POP, dipeptidyl peptidase IV (EC 3.4.14.5), oligopeptidase B (EC 3.4.21.83) and acylaminoacyl peptidase (EC 3.4.19.1), and this enzyme family is of ancient origin [2]. POP preferentially hydrolyses small peptides at the carboxy side of a proline residue. Although POP is predominantly a cytosolic enzyme, a membrane-bound form has also been characterized [3].

Several POP substrates, such as substance P, vasopressin, neurotensin and thyroliberin, are claimed to be involved in learning and memory [4]. In addition, low levels of substance P are commonly found in the brains of patients suffering from Alzheimer's disease, and administration of substance P has been reported to block β -amyloid-induced neurotoxicity [5]. It was recently reported that POP gene transcription was decreased when rats were exposed to an enriched environment and the transcription was increased manyfold in hypothalamus and cortex from aged rats [6,7]. Therefore it has been postulated that centrally acting POP inhibitors might be beneficial in patients with cognitive disturbances. Indeed, POP inhibitors have been shown to reverse scopalamine-induced amnesia in rats and to improve cognition in old rats and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys [8–10]. In addition, POP inhibitors have been reported to prevent β -amyloid deposition in a neuroblastoma cell line and in senescence-accelerated mouse [11,12].

The overall principles of catalysis and inhibition of the serine protease POP are reasonably well documented, and several very potent POP inhibitors have been developed [13]. However, the relationship between the structure of the inhibitor and the rate of the reaction is still scarcely known. This would be useful information, since it could provide the means to regulate the duration of enzyme inhibition when using POP inhibitors as a therapeutic agent.

Previously, one POP inhibitor, Z-prolylprolinal (see Figure 1), has been reported to act in a slow-binding manner [14]. This compound contains a CHO group at the P1 site and has been shown to form a hemiacetal adduct with the active-site serine residue, mimicking the tetrahedral transition state of the enzyme-catalysed reaction [15]. In addition to CHO group, also CN and COCH₂OH groups at the P1 site have been reported to increase the affinity of isophthalic acid bis-(L-prolylpyrrolidine) amide, probably by interacting with the active-site serine residue [16]; however, no details are available of their binding kinetics and the importance of different functional groups. Slow-binding inhibition is a phenomenon in which inhibition occurs relatively slowly and not at diffusion-controlled rates. Analysis of slow-binding inhibition provides insight into the binding mode of the inhibitors and also allows the calculation of enzyme–inhibitor association and dissociation constants [17].

In the present study, we performed a detailed kinetic analysis of isophthalic acid bis-(L-prolylpyrrolidine) amide containing different 2(S) substituents at the pyrrolidine ring at the P1 site (see Figure 1). Indeed, all of the three functional groups were shown to lead to a slow-binding inhibition mechanism and this

Abbreviations used: DTT, dithiothreitol; POP, prolyl oligopeptidase; Z-Gly-Pro-AMC, N-benzyloxycarbonylglycylprolyl-7-amino-4-methylcoumarin.

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Figure 1 Z-prolyl-prolinal and the inhibitors used in the study

allowed us to determine the association and dissociation rates of the inhibition. Measurements indicated that both of these kinetic parameters are crucially dependent on the characteristics of the functional group, which can be used to optimize the duration of action of POP inhibitors.

EXPERIMENTAL

Reagents

Z-Gly-Pro-AMC (*N*-benzyloxycarbonyl-glycyl-prolyl-7-amino-4-methylcoumarin) was obtained from Bachem AG (Bubendorf, Switzerland). Na₂HPO₄ and KH₂PO₄ were from Merck (Darmstadt, Germany), methanol from Labscan (Dublin, Ireland) and DMSO from Riedel-de Haën (Seelze, Germany).

Compounds

The four inhibitors that were used in the experimental study are presented in Figure 1. The studied inhibitors were synthesized at the University of Kuopio [16]. Stock solutions of 0.1 M were made in DMSO; further dilutions were made in 0.1 M sodium/potassium phosphate buffer (pH 7.0).

Expression and purification of porcine POP

Expression and purification of porcine POP were performed as reported previously, and the active enzyme concentration was determined using the tight-binding POP inhibitor JTP-4819 [18]. Purified porcine POP was used as the enzyme in all measurements.

Determination of association and dissociation rate constants

Slow-binding inhibition measurements were performed at 23 °C in 0.1 M sodium/potassium phosphate buffer (pH 7.0), containing 0.1 mM DTT (dithiothreitol) at an enzyme concentration of 0.14 nM. From the two commercially available POP substrates, Z-Gly-Pro-AMC was used, since it does not exhibit substrate inhibition [18], which could lead to erroneous estimation of the association and dissociation constants. Z-Gly-Pro-AMC was used at 10 µM, which is 0.5 *K_m*. Owing to its poor solubility, Z-Gly-Pro-AMC was dissolved and diluted in methanol. The final methanol concentration in the reaction mixture was 1%, which inhibited POP by < 10%. The reactions were initiated by the addition of the enzyme, and the progress curves of AMC liberation were monitored every 1 min for 60 min using a Bio-Tek FL500 fluorescence plate reader at excitation and emission wavelengths of 360 and 460 nm respectively. Five or six inhibitor concentrations were

used for each inhibitor. Blank values (substrate without enzyme) were subtracted from data and the progress curves were fitted to the rate equation of slow-binding inhibition [17]

$$[P] = v_s t + \frac{v_0 - v_s}{k_{\text{obs}}} (1 - e^{-k_{\text{obs}} t}) \quad (1)$$

using GraphPad Prism version 3.02 software (GraphPad Software, San Diego, CA, U.S.A.). In the above equation, [P] is the product concentration, *v*₀ and *v*_s are the initial and final steady-state reaction rates, *t* the time and *k*_{obs} the apparent first-order rate constant for determining the final steady-state velocity.

The apparent association and dissociation constants were then calculated as described in the Results section.

Recovery of enzyme activity

The dissociation rate constants were also determined directly by measuring the recovery of enzyme activity after dilution of the enzyme–inhibitor complex. In this assay, 5 nM of the inhibitor was incubated with 5 nM of the enzyme for 1 h at 23 °C. After incubation, the mixture was diluted 200-fold into the assay buffer containing 100 µM Z-Gly-Pro-AMC and the reaction was monitored every 1 min for 100 min. The data were fitted to eqn (1), in which *k*_{obs} corresponds to the dissociation rate constant.

Determination of *K_i* values

The *K_i* value for the inhibitor with R=H was calculated using eqn (2) and the results from the progress-curve experiments:

$$\frac{v_s}{v_0} = \frac{1}{\frac{[I]}{K_i^{\text{app}}} + 1} \quad (2)$$

K_i (app) is an apparent inhibition constant that was converted to a real *K_i* value according to:

$$K_i = \frac{K_i^{\text{app}}}{1 + \frac{[S]}{K_m}} \quad (3)$$

where [S] is the substrate concentration (10 µM) and *K_m* the Henry–Michaelis–Menten constant for Z-Gly-Pro-AMC (20 µM). The *K_m* value was determined as reported previously [18]. Since the compound with R=H did not show any time-dependent inhibition, the *v*₀ value was calculated from the control reaction in the absence of an inhibitor.

The *K_i* values for compounds with R=CHO, R=COCH₂OH and R=CN could not be determined by the same method as for the compound with R=H, because they were shown to be tight-binding inhibitors of POP (their *K_i* values are of the same order of magnitude as the enzyme concentration in the reaction mixture). Instead, their slow dissociation from the enzyme allowed direct determination of the real *K_i* value. The enzyme (0.57 nM) was incubated for 2 h in the presence of various concentrations of inhibitors at 23 °C. The reaction was started with Z-Gly-Pro-AMC (final concentration 10 µM) and the reaction was monitored every 1 min for 10–15 min. Over that time scale, the product formation was linear, indicating that the inhibitor did not dissociate markedly from the enzyme. The *K_i* values were calculated using the Morrison equation, which takes the tight-binding inhibition into account [19]:

$$\frac{v_i}{v_0} = 1 - \frac{(E + I + K_i) - \sqrt{(E + I + K_i)^2 - 4E \cdot I}}{2E} \quad (4)$$

where v_0 and v_i are reaction velocities in the absence and presence of the inhibitor (I) respectively, K_i is the inhibition constant of the inhibitor and E the active enzyme concentration in the reaction medium (0.57 nM). Since the inhibitor did not dissociate from the enzyme during the measurement, competition of binding between substrate and inhibitor did not occur and, hence, the calculated K_i value is the real dissociation constant of the inhibitor [20].

Inhibitor stability

The possible degradation of inhibitors by POP was tested with the parent inhibitor (compound with R=H). The compound (5 μ M) was incubated with 10 nM enzyme for 0 or 24 h in 20 mM sodium/potassium phosphate buffer (pH 7.0) at 23 °C. The incubations were stopped by filtration with Amicon Ultra-4 centrifugal filter device with 30 kDa-cut-off membrane. Samples (50 μ l) were injected into a reversed-phase HPLC system consisting of Shimadzu LC-10AD pump, SPD-10A UV-detector, SIL-9A autoinjector and Supelco Supelcosil LC-18-DB column (25 cm $\times$ 4.6 mm; inner diameter, 5 μ m). The mobile phase was 25 % acetonitrile, 75 % 20 mM sodium/potassium phosphate buffer (pH 7.0) and the detection wavelength and flow rate were 215 nm and 0.5 ml/min respectively. The HPLC method exhibited excellent linearity and repeatability.

RESULTS

Slow-binding inhibition

As shown in Figures 2(A) and 2(B), POP inhibition by the compound with R=CHO showed a clear time- and concentration-dependent approach to steady state, whereas the steady-state rate of substrate hydrolysis was reached instantaneously. Similar slow-binding inhibition was also observed for compounds with R=CN and R=COCH₂OH. In contrast, the time course of inhibition by the compound with R=H was linear, indicating rapid binding.

In principle, there are two ways in which slow-binding inhibition can occur: the direct binding of inhibitor (Scheme 1a) or the isomerization of an initial enzyme-inhibitor complex (Scheme 1b) [17]. In the direct-binding model, the binding of the inhibitor to the enzyme active site is either a slow process (low-association constant, k_{on}) or both k_{on} and k_{off} are low. In the latter case, the low association and dissociation rates would lead to inhibition of slow binding, even though k_{on} was of a similar magnitude to that seen with diffusion-controlled interaction. In the isomerization model described in Scheme 1(b), the inhibitor first binds rapidly to the enzyme (complex EI formation), which then undergoes a slow conformational change to form the inactive complex EI*.

The association and dissociation constants of a slow-binding inhibitor can be determined by plotting the apparent first-order rate constant k_{obs} against inhibitor concentration. Depending on the inhibition mechanism, the plot is a straight line (for the direct-binding model; Scheme 1a) or a hyperbola (for the isomerization model; Scheme 1b). Figure 2(C) shows that the plot of k_{obs} versus [I] is linear for all of the studied compounds, which indicates that the binding of the inhibitors to the enzyme active site is a single-step process. Therefore the data could be fitted to:

$$k_{obs} = k_{off} + k'_{on}[I] \quad (5)$$

where k_{off} and k'_{on} are the dissociation and association rate constants respectively. k'_{on} was determined from the slope of the plot and then corrected for substrate competition using:

$$k_{on} = k'_{on}(1 + S/K_m) \quad (6)$$

Figure 2 Slow-binding inhibition of POP

(A) Progress curves for the hydrolysis of Z-Gly-Pro-AMC in the presence of various concentrations of compound with R=CHO, monitored in 0.1 M sodium/potassium phosphate buffer (pH 7.0), containing 0.1 mM DTT at 23 °C. (B) Region of the initial velocity of the progress curve. (C) k_{obs} as a function of inhibitor concentration for the compounds with R=CHO, R=CN and R=COCH₂OH. Results are expressed as means $\pm$ S.E.M. for 3–5 independent experiments.

The k_{off} values of the inhibitors were so low that the intercepts of the y-axis in eqn (5) did not provide a very accurate estimate of the dissociation rate constant. Instead, the k_{off} values were calculated with the help of K_i value according to:

$$k_{off} = k_{on}K_i \quad (7)$$

K_i values were calculated using the Morrison equation (eqn 4). A representative plot for the compound with R=CHO is shown in Figure 3.

The kinetic parameters were determined similarly for the compounds with R=CN and R=CH₂OH groups. For the compound with R=H, only the K_i value was determined, since the

Scheme 1 Kinetic schemes for slow-binding inhibition

(a) Binding of the inhibitor to the enzyme active site is a single-step process, whereas in the two-step scheme (b), the inhibitor first binds rapidly to the enzyme (formation of complex EI), which then undergoes a slow conformational change to form the inactive complex EI*.

Figure 3 Measurement of K_i for the compound with R=CHO

The enzyme (0.57 nM) was incubated for 2 h in the presence of various concentrations of the compound with R=CHO, after which the reaction was started with Z-Gly-Pro-AMC. v_i/v_0 was fitted with the Morrison equation (eqn 4). Results are expressed as means $\pm$ S.E.M. for three independent experiments.

Table 1 Kinetic parameters for POP inhibitors

The inhibition kinetic assays were conducted with Z-Gly-Pro-AMC as a substrate in 0.1 M sodium/potassium phosphate buffer (pH 7.0), containing 0.1 mM DTT at 23 °C. Results are expressed means $\pm$ S.E.M. for 3–5 measurements; ND, not detected.

Compound	K_i (nM)	$K_{\text{on}} \times 10^5$ ($\text{M}^{-1} \cdot \text{s}^{-1}$)	$k_{\text{off}} \times 10^{-5}$ (s^{-1})	EI half-life
R=H	11.8 ± 0.5	ND	ND	ND
R=CN	0.39 ± 0.03	(12.0 ± 0.08)	(46.7 ± 0.46)	25 ± 3 min
R=CHO	0.15 ± 0.01	(2.43 ± 0.12)	(3.56 ± 0.34)	5.4 ± 0.5 h
R=COCH ₂ OH	0.079 ± 0.010	(4.66 ± 0.23)	(3.68 ± 0.50)	5.2 ± 0.7 h

inhibitor did not exhibit slow-binding inhibition kinetics. The results of the kinetic measurements are presented in Table 1.

It can be seen that the presence of CHO, CN and COCH₂OH groups greatly increases the inhibitory potency of the compound, as previously noted in IC₅₀ determinations [16]. The K_i values of these compounds are in the sub-nanomolar range, in contrast with the K_i value of 11.8 nM for the compound having R=H. The inhibitor containing the COCH₂OH group is the most potent inhibitor of POP, having a K_i value of 0.079 ± 0.010 nM. All these compounds also exhibited slow-binding inhibition kinetics. The lowest association rate constant was measured for the compound with R=CHO; the difference in association constants between the inhibitors with R=CHO and R=CN was 5-fold. The compounds with R=COCH₂OH and R=CHO had equal, very low dissociation rate constants, which correspond to half-lives of more than 5 h for dissociation. In contrast, for the compound

Figure 4 Association (black bars, left y-axis) and dissociation (white bars, right y-axis) half-lives of slow-binding POP inhibitors at a concentration of 10 nM

Results are means $\pm$ S.E.M. for 3–5 independent measurements.

Figure 5 Recovery of enzyme activity after the dilution of the enzyme-inhibitor complex

Inhibitor and enzyme (5 nM) were incubated for 1 h at 23 °C, after which the complex was diluted 200-fold into the assay buffer containing 100 μ M Z-Gly-Pro-AMC. The liberation of AMC was monitored every 1 min for 100 min.

with R=CN, the half-life was only 25 min for dissociation. Thus, even though the difference in K_i values was only 2-fold between the inhibitors with R=CN and R=CHO, the half-life for dissociation was 13 times longer for the compound with R=CHO. To visualize the considerable differences in the kinetic parameters between the compounds, the association and dissociation half-lives are presented in Figure 4. The association half-life was calculated for the inhibitor concentration of 10 nM. The three compounds can be categorized as follows: (1) R=CN, fast association, fast dissociation; (2) R=CHO, slow association, slow dissociation; and (3) R=COCH₂OH, intermediate association rate, slow dissociation.

It is important to mention that the inhibitors are not cleaved by POP, as incubation with 5 μ M compound with R=H during 24 h did not decrease the amount of the inhibitor as measured by HPLC (results not shown). The degradation of compounds with R=CN, R=CHO and R=COCH₂OH is also unlikely, since they contain only the same peptide bonds similar to the tested compound.

The k_{off} values were also determined by enzyme dilution experiments (Figure 5) for the compounds with R=CN, R=CHO and R=COCH₂OH. The recovery of activity was so fast for the inhibitor with R=H that the dissociation rate could not be measured. In these assays, the inhibitor concentrations used were $> 10K_i$ before dilution and $< 35K_i$ (app) after dilution. The 95% confidence intervals for the half-lives of the dissociations were 29–36 min for the compound with R=CN, 3.9–10.0 h for the compound with R=CHO and 2.9–5.3 h for the compound with R=COCH₂OH. These experimental values agree with the

calculated dissociation rates, further supporting the direct-binding model of the inhibition (Scheme 1a).

The kinetic parameters of the compound with R=CHO can be compared with those of the previously reported slow-binding inhibitor, Z-prolylprolinol, because they share the same functional formyl group [14]. The association and dissociation constants were found to be almost equal (2.4×10^5 versus $1.6 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}$ and $3.6 \times 10^{-5} \text{ s}^{-1}$ versus $4 \times 10^{-5} \text{ s}^{-1}$) for these inhibitors, although their P3 and P4 sites are quite different. Hence, it seems that the functional CHO group is the principal factor determining the inhibition kinetics of those compounds.

DISCUSSION

We have shown that the addition of a CHO, CN or COCH₂ group to the P1 site of isophthalic acid bis-(L-prolylpyrrolidine) amide decreases the IC₅₀ value strongly [16]. However, as the IC₅₀ value is an overall result of the association and dissociation rates of the inhibition and it is also affected by the enzyme and substrate concentrations used in the measurement, it does not provide any insight into the reaction mechanism or kinetic behaviour. In the present study, we have analysed the inhibition reaction with these potent inhibitors in detail and found that the inhibitors achieve their high potency in different ways.

The kinetic measurements indicated that the addition of a CHO, CN or COCH₂OH group to the P1 site of the inhibitor leads to a slow-binding inhibition of POP. Inhibition with each of the studied inhibitor was a single-step process, as can be deduced from the linear relationship in the plot of k_{obs} versus [I]. This inhibition mechanism is described in Scheme 1(a). However, distinguishing between the direct binding and the isomerization mechanisms is not straightforward. If the k_2/k_1 ratio in the isomerization model is much greater than the k_4/k_3 and the measurements are made using an inhibitor concentration lower than k_2/k_1 , the plot of k_{obs} versus [I] would also be linear for an inhibitor that obeys this mechanism (Scheme 1b) [19]. Irrespective of that complication, we suggest that the slow-binding inhibitors studied here follow the direct-binding model for two reasons. First, the progress curves of the compound with a CHO group (Figures 2A and 2B) show that the initial velocity, v_0 , does not decrease even at the highest inhibitor concentration (20 nM), when compared with the uninhibited reaction velocity. This concentration is higher than the K_i value of the compound with R=H, which was seen to bind rapidly. Secondly, if the slow-binding compounds were obeying the two-step mechanism, the core of the inhibitor should first bind rapidly to the active site (corresponding to the binding of the compound with R=H) and form a complex, as was observed with the compound having R=H. In the second step, the enzyme–inhibitor complex would undergo a slow conformational change and form a tighter complex EI* due to the CHO group. However, this first complex would have a dissociation constant of 11.8 nM (K_i for compound R=H) and, thus, the highest measured inhibitor concentration of 20 nM should cause a marked decrease in the initial velocity of the reaction. Since that was not the case (see Figures 2A and 2B), we conclude that the inhibition of POP by these slow-binding inhibitors is really a single-step process. The single-step reaction is also supported by the fact that the calculated and directly measured dissociation constants were equal.

Although addition of each of the groups, CHO, CN and COCH₂OH, led to highly potent POP inhibitors and revealed single-step slow-binding inhibition mechanism, there were considerable differences in the inhibition rates between the compounds. The inhibitor with R=CN was found to have the highest association constant, followed by the compounds with R=

COCH₂OH and R=CHO. It has been shown that the Ser-554 at the enzyme active site attacks the CHO carbon atom of the inhibitor, resulting in a covalent hemiacetal adduct [15]. Similarly, it is probable that the CN and COCH₂OH groups interact with the same Ser-554 residue and form an imino ether and a hemiketal adduct respectively, and these covalent adducts increase the affinity of the inhibitors [16]. The rate of the hemiacetal formation is considerably slower than the formation of the imino ether and the hemiketal adducts, demonstrated by the observation that the association rate of the inhibitor with R=CHO is 2- and 5-fold lower than the association rates of the inhibitors with R=COCH₂OH and R=CN respectively. It seems that the formation of these adducts is a relatively slow process and mainly responsible for the slow-binding inhibition.

In addition to the differences in the association rates, differences in the dissociation rates between these slow-binding inhibitors were also found. Namely, the inhibitor with R=CN had a half-life of 25 min for dissociation, in contrast with the inhibitors with R=CHO and R=COCH₂OH, which dissociated much more slowly from the enzyme, having the half-life of over 5 h. The differences in the dissociation rates often affect the K_i values of the inhibitors since $K_i = k_{\text{off}}/k_{\text{on}}$. However, the K_i values of the inhibitors studied here do not predict the dissociation rates. For example, the difference in K_i values is only 2-fold between the inhibitors with R=CN and R=CHO, even though the half-life for dissociation is 13 times higher for the compound with R=CHO. This is because the association rate of the compound with R=CN is much higher than that of the compound with R=CHO. The findings reported in the present study are of relevance, as POP inhibitors are under consideration for therapeutic use in the treatment of cognitive deficits associated with aging and neurodegenerative diseases. The slow dissociation would be predicted to result in a long duration of action and, consequently, inhibition of POP could persist even after the drug has been cleared from the plasma, as was recently shown with the anti-Alzheimer drug rivastigmine [21]. Here, we have demonstrated that the duration of action can be adjusted by changing the functional group at the P1 site of the inhibitor. This information can be used to develop potent short- or long-acting POP inhibitors.

In summary, in the present study, we have demonstrated that (i) the addition of COCH₂OH, CN or CHO group at the P1 site of isophthalic acid bis-(L-prolylpyrrolidine) amide leads to slow-binding inhibition of POP and the inhibition appears to be a single-step process; and (ii) although the K_i values of the inhibitors are relatively similar, both the association and dissociation rates are crucially dependent on the type of the functional group: fast association, fast dissociation with R=CN; slow association, slow dissociation with R=CHO; intermediate association rate, slow dissociation with R=COCH₂OH. These findings will be valuable when developing POP inhibitors for therapeutic use.

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