Urinary Calcium Oxalate Crystal Growth Inhibitors

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ABSTRACT

Calcium stones occur because renal tubular fluid and urine are supersaturated with respect to calcium oxalate and phosphate. The process of stone formation includes crystal nucleation, growth, aggregation, and attachment to renal epithelia. Urine contains macromolecules that modify these processes and may protect against stone formation. Attention has focused especially on inhibitors of crystal growth, and several have been isolated from urine, including nephrocalcin, an acidic phosphorylated glycoprotein that contains several residues of γ-carboxyglutamic acid per molecule; osteopontin (uropontin), a phosphorylated glycoprotein also found in bone matrix; uronic acid-rich protein, which contains a covalently bound glycosaminoglycan residue; and several others. Abnormalities in structure and/or function have been detected in some of these proteins in stone formers' urine. However, the overall ability of urinary macromolecules to inhibit calcium oxalate crystal growth is often normal in stone formers. Recently, attention has been focused on the ability of these molecules to inhibit other stages in stone formation. Nephrocalcin can inhibit crystal nucleation, for example, and both nephrocalcin and Tamm-Horsfall protein inhibit crystal aggregation. Nephrocalcin and Tamm-Horsfall protein from stone formers are less active in preventing aggregation, and under some conditions, Tamm-Horsfall protein may promote the formation of crystal aggregates, especially in the presence of high concentrations of calcium. The structural abnormalities responsible for impaired inhibitory activity are not completely understood.

Key Words: Kidney calculi, crystal aggregation, crystal nucleation, nephrocalcin, Tamm-Horsfall protein

Nephrolithiasis is a common disorder, and about 75% of all kidney stones are composed mainly of calcium oxalate (1). The nucleation and growth of calcium oxalate or phosphate occur because renal tubular fluid and urine are often supersaturated with respect to these salts (1). Supersaturation is the result of the excretion of these poorly soluble minerals in a relatively small volume of water. Although the concentrations of calcium and oxalate in tubular fluid and urine often exceed the solubility limit, they less often exceed the higher concentration required for the spontaneous nucleation of crystals (called the formation product). However, because of increased excretions of calcium and/or oxalate, or the decreased excretion of citrate, the urine of stone formers is more likely to reach such levels, at least on some occasions. The nucleation of calcium crystals may also occur at lower levels of supersaturation in the presence of nucleating sites provided by other types of crystals (perhaps uric acid), cell membranes, or possibly, urinary proteins (2). Calcium oxalate crystals floating freely in tubular fluid are harmless, however, and are washed out in the urine. Only if a crystal attaches to an epithelial site and is retained in the kidney will continued growth lead eventually to the formation of a stone. The growth of newly formed crystals during tubular transit may not be sufficiently rapid to permit them to become big enough to lodge in the tubules; however, individual crystals may aggregate to form much larger crystal masses that could potentially do so (3). Other mechanisms of crystal attachment may involve binding to specific sites on renal epithelial cells (4).

CLINICAL OBSERVATIONS

Urinary Inhibitors of Calcium Oxalate Crystallization

Nucleation, growth, and aggregation of crystals are necessary steps in the formation of stones. However, urine contains substances that modify and inhibit these events. These substances include both small molecules, such as citrate and pyrophosphate, and macromolecules, such as glycoproteins, glycosaminoglycans, and proteoglycans (5). The presence of these inhibitors has been demonstrated by a number of investigators. For example, the formation product (for both calcium oxalate and brushite) of urine from normal subjects has been shown to exceed that of urine from stone formers or of simple salt solutions (6), which means that normal urine contains substances that can inhibit the nucleation of new crystals. Interest in these substances has been stimulated by the observation that, although normal urine is often supersaturated, it rarely contains crystals of calcium oxalate, and that, when found, these crystals are smaller and less aggregated than those found in the urine of recurrent calcium stone formers (7). The fact that this is not due solely to greater degrees of supersaturation in the urine of stone formers is demonstrated by the further observation that the crystals...
in normals remain fewer and smaller even after normal subjects are challenged with an oxalate load to induce levels of supersaturation comparable to those in stone formers (7). Dilute urine has also been shown to inhibit the growth and aggregation of calcium oxalate crystals in metastably supersaturated solutions in vitro (8). Substances that inhibit the nucleation, growth, and aggregation of calcium oxalate crystals could play a role in protecting normal subjects from calcium stone formation, and abnormalities of these molecules might lead to stone formation.

Investigators have sought to isolate the urinary components responsible for inhibitory activity for over 20 yr. Studies of urinary inhibitors are complicated by the fact that there is no universally accepted model system for measuring inhibition, nor is there agreement on what stage of crystal growth might be the critical point for inhibitory activity. Inhibitors might act at more than one stage of crystallization, and a molecule could potentially inhibit stone growth at one step while being inactive or even promoting it at another. It is also difficult to construct assays that mimic conditions in vitro, so all in vitro assays are to some degree artificial, and we should be duly cautious about generalizing to the in vitro situation. However, with these limitations in mind, some information has emerged about the urinary inhibitors of calcium oxalate crystallization.

Nephrocalcin

For calcium oxalate, the major inhibitors of crystal growth are anionic macromolecules (9). The first to be purified and partially characterized was nephrocalcin, a glycosylated and phosphorylated protein with a monomeric molecular weight of approximately 14,000, which contains two to three residues of γ-carboxyglutamic acid (Gla) per molecule (10). The presence of Gla implies that nephrocalcin is a vitamin K–dependent protein. It has been isolated from the urine and kidneys of a number of species (11) and from human kidney cell culture medium (12). The protein was initially purified by elution from anion exchange columns, testing fractions for crystal growth inhibitory activity (Figure 1). The active fractions were further purified by gel filtration chromatography. Nephrocalcin contains a large number of acidic amino acid residues; however, the protein's sequence is not yet known. The immunohistochemical data available thus far suggest that nephrocalcin is found in the proximal tubule and in the thick ascending limb of the loop of Henle (13). Little is known with respect to its physiologic regulation, although nephrocalcin excretion is markedly increased in the urine of pregnant women, who become hypercalciuric during gestation (14).

Nephrocalcin inhibits the growth of calcium oxalate seed crystals in metastably supersaturated solutions of calcium oxalate in vitro, and the addition of increasing amounts of nephrocalcin to the solution progressively slows crystal growth. This inhibition occurs at concentrations of nephrocalcin that are in the range of 10^-6 to 10^-8 M, similar to those reported in urine (15), but too low for the effect to be secondary to the chelation of calcium ions in the solution. Rather,
nephrocalcin’s ability to inhibit crystal growth is related to its ability to bind to crystal surface (16). The kinetic growth rate data are linear when plotted in the form of a Langmuir adsorption isotherm, implying that the inhibitor reversibly adsorbs to and blocks growth sites on the crystal surface (Figure 2B). This is confirmed by the incubation of calcium oxalate crystals in solutions of nephrocalcin and the measurement of the adherence of nephrocalcin to the crystal surface with antinephrocalcin antisera (Figure 2A). Adsorption measured in this manner also follows a Langmuir-type isotherm, and adsorption and inhibition increase in parallel (Figure 2C), suggesting that protein adsorbs to specific sites on the crystal and that the occupation of these sites blocks crystal growth. The slope of the Langmuir isotherm plot is the dissociation constant ($K_d$), a measure of the affinity of the protein for crystal surface; the dissociation constants calculated from growth inhibition or directly measured protein adsorption do not differ. When crystals of calcium oxalate are grown in the presence of nephrocalcin, growth along one crystal face is preferentially altered, which results in distorted and abnormal crystal morphology (17). This may be the general mechanism of action of all of the macromolecular inhibitors, all of which appear to exert their effects by binding to the crystal surface.

**Osteopontin**

Another inhibitor protein made by the kidney has recently been isolated (18). Serum-free media incubated with primary cultures of mouse kidney cortical cells contain an inhibitor of calcium oxalate crystal growth. Using a strategy much like the one used for nephrocalcin, the inhibitor was isolated by elution from an anion exchange column, testing fractions for inhibitory activity in a seeded crystal growth assay and active fractions further purified by gel filtration. The N-terminal sequence of the isolated protein is identical to that of osteopontin, a phosphorylated glycoprotein originally isolated from bone matrix, where it is made by osteoblasts and osteoclasts (19). A very similar protein has recently been isolated from human urine and named uropontin (20). The bone-derived and renal derived forms of this protein appear to be very similar or identical with respect to amino acid sequence, but there may be differences with respect to posttranslational modifications such as phosphorylation, glycosylation, and sulfation that are tissue specific. Like nephrocalcin, osteopontin slows the growth of calcium oxalate crystals in a supersaturated solution. By the use of the constant composition assay, in which the concentrations of calcium and oxalate are kept constant throughout the assay, more similar to the conditions found in urine, the effect of increasing concentrations of osteopontin on crystal growth was measured and the data were fitted to a Langmuir isotherm, with a dissociation constant of $3.7 \times 10^{-8}$ M (18).

The basal expression of osteopontin is most pronounced in bone matrix and kidney; expression in many other tissues can be induced or up-regulated by mitogenic stimuli (19). It is also widely associated with luminal epithelial cells, including those of the gastrointestinal tract, gallbladder, pancreas, lung, and reproductive tract (21). It is presumed to have a role in biomineralization, because of its presence in both normal and pathologically calcified tissue, such as

![Figure 2](https://example.com/figure2.png)

**Figure 2.** (A) Adsorption of nephrocalcin (NC) to calcium oxalate crystals measured with an antisera to NC to determine the amount of NC bound to the crystal surface ($y$ axis) versus the NC concentration in the incubation medium ($x$ axis). Values are means ± SE; regression is significant, $P < 0.001$. (B) Growth Inhibition of calcium oxalate crystals in the presence of the same concentrations of NC, plotted in the form of a Langmuir isotherm. $K_d$ and $K_c$ refer to crystal growth in the presence and absence of purified inhibitor, respectively. The dissociation constant for NC with respect to calcium oxalate crystal does not differ from that calculated in Panel A. (C) Relationship between reduction of crystal growth ($y$ axis) and adsorption of NC to crystals ($x$ axis). Reproduced from Reference 16 with permission.
stone matrix and atherosclerotic plaque (22, 23). Osteopontin contains a large percentage of acidic amino acid residues, as well as a number of posttranslational modifications, including a large number of potentially phosphorylated serine and threonine residues, and both N- and O-linked sugars (19). Sulfation of the protein occurs in mineralized tissues (24). The pattern of modification may mediate different protein functions (25) and may be influenced by hormonal stimuli (26). In addition, osteopontin contains an Arg-Gly-Asp (RGD) sequence, which mediates the binding of the protein to cell surface $\alpha_\beta_3$ integrin receptors, found on many cell types including osteoclasts, suggesting it plays a role in cell attachment and spreading (19). Which features of the protein are required for crystal growth inhibition is not known. The protein is upregulated by 1,25-\((\text{OH})_2\)-vitamin D, and a vitamin D response element has been found in the promoter region of the osteopontin gene (27); regulation in kidney or kidney cell lines has not been extensively studied.

We performed localization studies in rat kidney tissue using antisera raised in rabbits to the protein isolated from cell culture. The protein was seen primarily in cells of the thin descending limb of the loop of Henle and in the papillary surface epithelium (28). Immunogold label was localized to a population of dense vesicles distinct from lysosomes and endosomes. Osteopontin mRNA was localized to the same sites with in situ hybridization, with 2ar, a cDNA containing the coding region for mouse osteopontin (29). Other investigators, using antibodies to recombinant mouse or to human urinary osteopontin to stain mouse kidneys have found osteopontin expression primarily in a subset of thick ascending limbs and distal convoluted tubules (30). In both studies, staining was most intense along luminal surfaces.

Both nephrocalcin and osteopontin appear to be quite acidic proteins, with a large number of aspartic acid residues. It has been noted that a variety of aspartic acid–rich proteins have been found in other mineralized tissues, such as mollusk shells, and that these proteins can inhibit the growth of calcium salts when in solution and are able to nucleate calcium salts when they are surface immobilized (31, 32). Both nephrocalcin and osteopontin have been identified in the matrix of calcium stones (22, 23), and this has raised the possibility that these proteins might function, in some circumstances, as promoters of stone growth. The ability of these proteins to act potentially as both crystal growth inhibitors and as crystal nucleators means that their effects on the pathogenesis of stones may be complex. This may be true for other urinary macromolecules with inhibitory activity as well.

Other Macromolecular Inhibitors

A number of other macromolecules with inhibitory activity have also been found in urine. One such calcium oxalate crystal growth inhibitor has been termed uronic acid–rich protein (UAP) (34). The presence of uronic acid implies that this may be a proteoglycan, containing a covalently bound glycosaminoglycan side chain. Sequence data suggest that this protein is closely related to the light chain of inter-\(\alpha\)-trypsin inhibitor, also known as urinary trypsin inhibitor (35), a proteinase inhibitor found in the urine that contains a chondroitin sulfate moiety. The protein inhibits calcium oxalate crystal growth in a seeded crystal growth assay, but the dissociation constant of the protein with respect to crystal surface has not been reported, so that its crystal affinity cannot be compared with that of previously reported inhibitory proteins. Inter-\(\alpha\)-trypsin inhibitor has also been reported to be a crystal growth inhibitor by another group of investigators (36).

Recent studies report the immunolocalization in kidney tissue of another urinary protein, dubbed crystal matrix protein because it was isolated by virtue of its affinity for calcium oxalate crystals generated in human urine (37). This 31-kd protein reportedly is related to human prothrombin and inhibits calcium oxalate crystal aggregation, but detailed characterization and its effects on calcium oxalate crystal growth are as yet unreported.

Tamm-Horsfall protein (THP) is another glycoprotein found in urine that has long been felt to play a role in stone formation, although whether that role is as a promoter or an inhibitor has been controversial. THP does not appear to inhibit the growth of calcium oxalate seed crystals in supersaturated solutions (16); however, recent data suggest that THP is a potent inhibitor of crystal aggregation (38).

Glycosaminoglycans, which are polysaccharide chains composed of repeating disaccharides and which are generated by the degradation of higher molecular weight proteoglycans, are another group of macromolecules found in urine that have effects on calcium oxalate crystallization. They seem to be more potent inhibitors of crystal aggregation than of growth (39).

Abnormalities of Inhibitors in Stone Formers

For several of these molecules, abnormalities in structure and/or function have been identified in stone formers. Nephrocalcin isolated from the pooled urine of calcium stone formers has several molecular abnormalities (40). As shown in Figure 1, nephrocalcin normally elutes from anion exchange columns in four peaks, labeled A to D, perhaps reflecting heterogeneity in the posttranslational modification of the protein, such as the degree of phosphorylation (41). The nephrocalcin in Peaks C and D from stone formers’ urine, which comprises about 50% of the total nephrocalcin present, has a lower dissociation constant (that is, inhibits crystal growth more poorly) than comparable peaks from normal urine. Nephrocalcin from stone formers’ urine also lacks Gla. The
absence of Gla does not correlate with decreased growth inhibition, because Peaks A and B also lack Gla, but inhibit growth in in vitro assays as well as normal. However, although Peaks A, B, and C of normal nephrocalcin form stable films at an air-water interface, the stone former nephrocalcin (and Peak D from normal urine, which also lacks Gla) forms much less stable films, with lower collapse pressures. This may reflect a structural abnormality of the protein characterized by decreased amphipathicity or poor separation of the hydrophobic and hydrophilic areas. This might impair its ability to deter crystallization, in ways other than growth inhibition, as discussed below. Nephrocalcin isolated from the matrix of calcium stones shares this abnormality (33).

Another molecular abnormality has been identified in nephrocalcin isolated from the urine of patients with X-linked recessive nephrolithiasis (42). Nephrocalcin from both affected males and from carrier females reportedly is poorly phosphorylated, and some fractions have a decreased affinity for crystal surface. Abnormalities in amino acid composition and in the ability to form stable films at air-water interfaces were also detected. The underlying genetic abnormality is not known.

Uronic acid-rich protein (UAP) isolated from the urine of calcium stone formers was recently reported to inhibit calcium oxalate crystal growth less well than UAP isolated from normal urine (43). In a seeded crystal growth assay, the mean inhibitory activity of UAP (10 μg/mL) from five healthy subjects was 65%, compared with 35% inhibition by the same concentration of UAP from the urine of five stone formers. The difference was statistically significant. Whether this decreased activity is associated with specific structural abnormalities has not been reported.

Is Growth Inhibition the Major Function of Urinary Inhibitors?

The ability of urinary macromolecules to inhibit the growth of calcium oxalate crystals has often been used as a marker for inhibitory activity during isolation, because such assays can be performed rapidly on multiple samples. However, growth inhibition may not be their most important function, and defects in this ability may not be the inhibitor abnormalities that most often predispose to stone formation. Some recent data suggest that growth-inhibitory activity may not be deficient in the urine of many well-characterized calcium oxalate stone formers.

We studied the dialyzed urine from 19 hypercalciuric (10 male) and 21 normocalciuric (11 male) recurrent calcium stone formers, on no treatment, and compared them with urines from 20 control subjects (13 male) with no family history of nephrolithiasis (15). Nephrocalcin levels were measured by an ELISA with antiserum raised against human nephrocalcin. Crystal growth inhibition in the macromolecular fraction of the urine was measured by a seeded growth assay with polyaspartic acid as a standard. The excretion of nephrocalcin was lower in women than in men in all groups but was not lower in stone formers than in normals (Table 1). There was no difference in mean inhibitory activity, expressed as polyaspartic acid units per 24 h, in either group of stone formers compared with the controls or in men compared with women. There was no correlation between nephrocalcin levels and the ability of the urine to inhibit crystal growth.

Porile and associates have also recently presented data that support the impression that crystal growth inhibition is not the key attribute of macromolecular inhibitors (44). They studied 25 recurrent calcium oxalate stone formers (18 male), each with a history of at least 10 stones, who had minimal evidence of metabolic risk factors such as hypercalciuria, hyperoxaluria, hyperuricosuria, or hypocitraturia. Each patient was compared with an age-, sex-, and race-matched normal control. Each 24-h urine was dialyzed and lyophilized, and a uniform amount of protein (32 μg) was added to each seeded crystal growth assay. Crystal growth was tracked by the

<table>
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a Mean ± SD. PA, polyaspartic acid equivalents.

b P < 0.05 versus control male.
disappearance of oxalate from solution, which was measured by the change in absorbance of the metastably supersaturated solution monitored continuously at 214 nm. Proteins from control urine inhibited crystal growth by 59.6 ± 7.2%, whereas that from the patients' urine inhibited growth by 61.0 ± 10.8%. They concluded that, despite choosing patients with many stones unexplained by usual metabolic risk factors and comparison to carefully matched controls, they could find no evidence to suggest decreased growth inhibition by urinary macromolecules as a potential cause of these patients' stones.

Effects of Urinary Inhibitors on other Phases of Crystallization

Macromolecular crystal growth inhibitors may exert important inhibitory effects at other stages in crystal formation, however. The best studied example is nephrocalcin, which has been found to inhibit both crystal aggregation and nucleation. In studies of aggregation, by the use of a spectrophotometric assay in which calcium oxalate crystals were suspended in a solution that was just saturated with respect to calcium and oxalate, nephrocalcin was found to be a potent aggregation inhibitor at all concentrations tested, the lowest being 5 × 10⁻⁸ M (Figure 3) (38). At the same concentrations, nephrocalcin isolated from the urine of stone formers was significantly less effective at preventing crystal aggregation. This study also demonstrated that THP is a potent inhibitor of crystal aggregation at concentrations found in urine, although its activity is more dependent on the ionic strength and pH of the solution. This may be due to the self-aggregation of THP, which is known to be promoted by higher salt concentrations and lower pH (45). A later study by the same investigators showed that THP from the urine of recurrent stone formers had impaired ability to inhibit crystal aggregation, especially at higher concentrations of salt and of THP (46). This was interpreted to suggest that THP from stone formers was more prone to self-aggregation than normal THP, which impaired its ability to inhibit crystal aggregation. Raising calcium concentrations also promotes THP self-aggregation and, in the case of stone formers' THP, can turn it into a promoter of crystal aggregation (47). Viscosity measurements suggested that the greater the degree of self-aggregation, the poorer the inhibitory activity of the protein; this could be reversed by the addition of citrate (47). Data from one family suggest that the abnormality in THP may be inherited (46).

Nephrocalcin has also been shown to inhibit secondary nucleation of calcium oxalate crystals (48). When large, preformed crystals of calcium oxalate were incubated in a metastably supersaturated solution, the nucleation of many new crystals occurred on the surface of these crystals. When 5 × 10⁻⁷ M nephrocalcin or 20% dialyzed urine was added to the solution, little new crystal formation occurred. Nephrocalcin isolated from a stone former's urine inhibited less well than normal nephrocalcin, whereas THP did not inhibit nucleation.

Urinary macromolecules may also influence the adherence of crystals to the tubular epithelium. In an experimental system, a renal epithelial cell line (BSC-1) exposed to calcium oxalate crystals will bind and endocytose the crystals within a few minutes (49). Exposure of the cells, but not the crystals, to physiologic concentrations of THP for 15 min decreased crystal uptake by 37%. Heparin, fibronectin, and RGD peptide also decreased crystal uptake, whereas nephrocalcin did not. It is not known whether THP can inhibit crystal binding in the renal papilla, where most stones are found.

Clear differences in the inhibitory spectrum are emerging for the two best studied urinary inhibitors, nephrocalcin and THP. Although nephrocalcin inhibits crystal nucleation, growth, and aggregation (at least in vitro), another anionic urinary glycoprotein, THP, is a potent aggregation inhibitor but has little effect on crystal nucleation or growth and may inhibit crystal-cell interaction. In both cases, there appear to be structural abnormalities of these proteins in the urine of some stone formers that may interfere with their ability to inhibit one or more of these stages of crystallization.

What features of these proteins are required to permit the inhibitory activity is unknown at present. The protein inhibitors seem to be generally acidic, with many aspartic acid residues, and to have several posttranslational modifications, such as glycosylation (nephrocalcin, osteopontin, THP, UAP) and phosphorylation (nephrocalcin, osteopontin). Some limited evidence suggests that abnormalities of phosphoryla-

Figure 3. Percent inhibition of calcium oxalate crystal aggregation (y axis) by normal urine nephrocalcin (N NC), stone former urine NC (SF NC), and THP at three different protein concentrations (x axis) and two different ionic strengths: 0.10 (open bars) and 0.28 (hatched bars) (pH 7.2). Increased ionic strength reduced THP inhibition at 0.5 × 10⁻⁷ markedly, with more modest effects on other proteins. *Differences from same protein concentration and ionic strength 0.10, P < 0.05; **P < 0.005; ***P < 0.001. Reproduced from Reference 38 with permission.
tion may impair crystal growth inhibition (41,42). However, the proteins from stone formers, which inhibit poorly in in vitro systems, appear to have abnormalities of tertiary structure, such as decreased amphiphilicity in nephrocalcin (which correlates with a lack of Gla residues), and an enhanced tendency to self-aggregate in THP. These may interfere with proper alignment of the protein on the crystal surface, such that the protein cannot perform its function: modulating interactions of crystals with other crystals and with the surrounding solution.

It must be remembered that these studies reflect the activities of these inhibitors in vitro, in systems where conditions may not be like those in vivo. For example, each of these proteins is studied in isolation, without the presence of other, potentially interacting, macromolecules. Many proteins bind to the crystal surface, and the effect of the presence of other proteins on the inhibitory actions of nephrocalcin, THP, and the other inhibitors has not been studied. Of interest are the data on the effect of elevated ionic strength and raised solution calcium concentration on the activity of THP. The presence of high ionic strength (often found in the urine of stone formers who may ingest inadequate amounts of water) and of high calcium concentration (also a frequent finding in stone formers) can disable the inhibitory activity of THP and, in the case of an abnormal protein, even turn it into a promoter of larger crystal aggregates. This illustrates the importance of solution characteristics on the activity of these inhibitory proteins and the complexity it may introduce in understanding their roles.

These proteins appear to be made and secreted along the nephron, some in the proximal nephron (nephrocalcin), others distally (THP, crystal matrix protein), still others perhaps in several sites (osteopontin). It seems likely that they function, at least in part, to protect the kidney from the effects of crystalization induced by the generally supersaturated tubular fluid and urine and that at least some stone formers may be predisposed to crystal formation because of the structural abnormalities of these proteins, which are as yet incompletely understood. These abnormalities do not necessarily affect all protein functions equally; for example, nephrocalcin from stone formers’ urine and from stones may inhibit crystal growth normally, while inhibiting aggregation poorly. Future work will hopefully clarify the spectrum of activity and structural abnormalities of urinary inhibitors. Such work might allow the identification of those at risk and perhaps therapy directed at maximizing the efficacy of the inhibitors on the basis of knowledge about their activity in solution.

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REFERENCES