Antiproliferative Plant and Synthetic Polyphenolics Are Specific Inhibitors of Vertebrate Inositol-1,4,5-trisphosphate 3-Kinases and Inositol Polyphosphate Multikinase*

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Inositol-1,4,5-trisphosphate 3-kinases (IP3K) A, B, and C as well as inositol polyphosphate multikinase (IPMK) catalyze the first step in the formation of the higher phosphorylated inositols InsP5 and InsP6 by metabolizing Ins(1,4,5)P3 to Ins(1,3,4,5)P4. In order to clarify the special role of these InsP5-phosphorylating enzymes and of subsequent anabolic inositol phosphate reactions, a search was conducted for potent enzyme inhibitors starting with a fully active IP3K-A catalytic domain. Seven polyphenolic compounds could be identified as potent inhibitors with IC50 \(200\) nM (IC50 given): ellagic acid (36 nM), gossypol (58 nM), (-)-epicatechin-3-gallate (94 nM), (-)-epigallocatechin-3-gallate (EGCG, 120 nM), aurintricarboxylic acid (ATA, 150 nM), hypericin (170 nM), and quercetin (180 nM). All inhibitors displayed a mixed-type inhibition with respect to ATP and a non-competitive inhibition with respect to Ins(1,4,5)P3. Examination of these inhibitors toward IP3K-A, -B, and -C and IPMK from mammals revealed that ATA potently inhibits all kinases while the other inhibitors do not markedly affect IPMK but differentially inhibit IP3K isoforms. We identified chlorogenic acid as a specific IPMK inhibitor whereas the flavonoids myricetin, 3',4',7,8-tetrahydroxyflavone and EGCG inhibit preferentially IP3K-A and IP3K-C. Mutagenesis studies revealed that both the calmodulin binding and the InsP3 binding domain in IP3K are involved in inhibitor binding. Their absence in IPMK and the presence of a unique insertion in IPMK were found to be important for selectivity differences from IP3K. The fact that all identified IP3K and IPMK inhibitors have been reported as antiproliferative agents and that IP3Ks or IPMK often are the best binding targets deserves further investigation concerning their antitumor potential.

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D-my-o-Inositol-1,4,5-trisphosphate 3-kinase (IP3K), an enzyme ubiquitously found in all mammalian tissues (1), plays a central role in the anabolic routes of inositol phosphates in vertebrate cells because it phosphorylates the second messenger Ins(1,4,5)P3 as the starting substrate of these reactions. The IP3K family consists of three isoenzymes referred to as IP3K-A (2–4), IP3K-B (5), and IP3K-C (6) differing in their degree of activation by Ca2⁺-CaM, affinity for Ins(1,4,5)P3 (reviewed in Ref. 7), tissue and intracellular distribution. IP3K-B is expressed in nearly all human tissues and is localized at actin filaments (8, 9) and the endoplasmic reticulum (9, 10) whereas IP3K-A was identified mainly in brain and testis (9, 11) and shows an exclusive F-actin localization (12). IP3K-C is also widely expressed in mammalian cells and in rat it was shown to shuttle actively between nucleus and cytoplasm (13). An additional Ins(1,4,5)P3 kinase enzyme, inositol polyphosphate multikinase (IPMK), was identified in yeast, rat, man, and plants (14–18). IPMK exhibits nuclear localization and evidently plays a role in nuclear InsP metabolism (16). In vertebrates, the Ins(1,3,4,5,6)P5 product of these enzymes is the starting substrate to be metabolized by different InsP kinases and phosphatasas leading to Ins(1,3,4,5,6,7)P6, InsP7, and the pyrophosphorylated isoforms of InsP6 and InsP7 (19, 20). InsP6 is normally the most abundant InsP in eukaryotic cells and appears to be important in several processes; in yeast it is essential for intact vesicle structures (21), mRNA export (22, 23) and is involved in chromatin remodeling (24). For mammalian cells a stimulation of DNA repair (25), endocytosis (26), and exocytosis (27) in pancreatic β-cells, Ca2⁺ channel activity (28, 29), an involvement in synaptic vesicle trafficking (30, 31), and other effects were reported for InsP6 (reviewed in Refs. 19 and 32).

To ease studies of the importance of higher InsPs for cell proliferation or other cellular functions in vivo, one possible approach is to block their biosynthesis by pharmacological inhibition of IP3K isoforms and IPMK. Other approaches are knock-out or RNAi experiments shutting down enzymes generating Ins(1,3,4,5)P4 or higher InsPs. An individual knock-out of IP3K-A in mice resulted in a very weak CNS phenotype in the former case (34). IP3K-B knockout revealed an essential role of the enzyme or its products for T-cell immunity (35). That deletion of the broadly expressed IP3K-B did not generate further phenotypes may be caused by rescue phenomena based on the fact that a total of four enzymes are able to convert Ins(1,4,5)P3 to Ins(1,3,4,5,6)P5. These considerations led us to start a search for pharmacological IP3K and IPMK inhibitors. We first examined herbal and synthetic compounds belonging to the flavonoids, anthraquinones, coumarins, triphenylmeth-
anes, tyrphostins, perylenequinones, and staurosporine derivatives for their ability to inhibit a catalytic domain of avian IP3K-A. This enzyme form, when expressed in bacteria, exhibits $V_{\text{max}}$ and $K_m$ values indiscernible from those of enzyme purified from native tissue (36) and recent crystal structures of the catalytic domain of IP3K-A have revealed intact folding (37, 38). The most potent inhibitors found by this prescreen were analyzed in detail for their mechanism of enzyme inhibition of avian IP3K-A and subsequently tested for their inhibitory effect on all three recombinant mammalian IP3K isoforms and human IPMK. Selectivity profiles derived from inhibitors identified here indeed provide promising lead structures for both broad spectrum and isoform specific inhibitors. The intriguing finding that all inhibitors identified in our search have reported antiproliferative effects suggests a correlation between cell growth and IP3K and/or IPMK action.

**EXPERIMENTAL PROCEDURES**

**Materials**—The enzymes SpeI and NdeI were purchased from NEB (Leusden, Netherlands), NheI, XhoI, and Eco31I from MBI (St. Leon-Roth, Germany), Pfu polymerase from Stratagene (La Jolla, CA). The plasmid pZErO 2.1 was obtained from Invitrogen, pGEM T-easy from Promega (Madison, WI), expression vector pET 17b from Novagen (Madison, WI), and expression vector pASK IBA 3 from IBA GmbH (Göttingen, Germany). *Escherichia coli* strain BL21 (DE3) was obtained from Novagen. Primers were purchased from MWG (Ebersberg, Germany). Quercetin and ellagic acid were obtained from Fluka (Buchs, Switzerland).

| Mutant          | Sequence of primer$^a$      | Sense |
|-----------------|-----------------------------|-------|
| GgIP3K-A<sub>K268E</sub> | 5'-GAA GAG CTC ACT GAA GCC CGT GAG AAA CC-3' | Forward |
| GgIP3K-A<sub>K272D</sub> | 5'-GCC CGT GAG GAT CCA AAG CTG CGT AAA G-3' | Forward |
| HsIPMK<sup>$^c$</sup> | 5'-GGTTCATCTCAGGCAACCATACACTGTTGCGAAGATGCT-3' | Forward |

$^a$ Changed bases are underlined.
$^b$ Fragment comprising the CaM binding and catalytic domains.
$^c$ Deletion of amino acids 266–371.

**Table I**

Oligonucleotides used for site-directed mutagenesis

| Mutant          | Sequence of primer$^a$      | Sense |
|-----------------|-----------------------------|-------|
| GgIP3K-A<sub>K268E</sub> | 5'-GAA GAG CTC ACT GAA GCC CGT GAG AAA CC-3' | Forward |
| GgIP3K-A<sub>K272D</sub> | 5'-GCC CGT GAG GAT CCA AAG CTG CGT AAA G-3' | Forward |
| HsIPMK<sup>$^c$</sup> | 5'-GGTTCATCTCAGGCAACCATACACTGTTGCGAAGATGCT-3' | Forward |

$^a$ Changed bases are underlined.
$^b$ Fragment comprising the CaM binding and catalytic domains.
$^c$ Deletion of amino acids 266–371.

**Table II**

Structure-activity relationship for flavonoids tested in GgIP3K-A

| Type     | Compound               | $IC_{50}$ [μM] |
|----------|------------------------|----------------|
| Flavanoids | Quercetin              | 0.18           |
| Flavones | Amentoniflavone        | 0.29           |
| Flavonoids | Myricetin              | 0.54           |
| Flavan-3-ole | Myricetin            | 0.50           |
|                     | Luteolin              | 3.0            |
|                     | Chrysin               | 7.0            |
|                     | Biscaritin            | 8.3            |
|                     | Apigenin              | 18.1           |
|                     | 3',4',7,8-(OH)<sub>2</sub>-flav. | 70.0          |
|                     | 4',6'-OH<sub>2</sub>-flav. | 0.09           |
| Flavones | (+)-Naringenin         | 2,3-Dihydroapigenin | 18.0        |
| Flavonoids | (-)-Taurilolin        | 2,3-Dihydroquercetin | 58.0        |
| Flavan-3-ole | (-)-Epicatechin        | 10.0           |
|                     | (+)-Catechin          | >200           |
|                     | ECG                   | 0.09           |
|                     | EGCG                  | 0.12           |
| Isoflavones | Genistein             | 11.6           |
|                     | Daidzein             | 131            |
| Anthocyanins | Peonidin              | 8.6            |
| Chalcones | Phloretin             | 19.0           |
| other Flavonoids | Stigmasterol         | 31.4           |

**Materials**—The enzymes SpeI and NdeI were purchased from NEB (Leusden, Netherlands), NheI, XhoI, and Eco31I from MBI (St. Leon-Roth, Germany). Pfu polymerase from Stratagene (La Jolla, CA). The plasmid pZErO 2.1 was obtained from Invitrogen, pGEM T-easy from Promega (Madison, WI), expression vector pET 17b from Novagen (Madison, WI), and expression vector pASK IBA 3 from IBA GmbH (Göttingen, Germany). *Escherichia coli* strain BL21 (DE3) was obtained from Novagen. Primers were purchased from MWG (Ebersberg, Germany). Quercetin and ellagic acid were obtained from Fluka (Buchs, Switzerland).
Polyphenolic Inhibitors of Inositol Phosphate Kinases

IC_{50} values for other structural groups of inhibitors of GgIP3K-A

| Class           | Inhibitor          | IC_{50} \( \mu M \) | IC_{50} \( \mu M \) |
|-----------------|--------------------|----------------------|----------------------|
| Phenylbutyrate  | Arbutin            | 0.06                 | 0.06                 |
| Polyphenol      | Kaempferol         | 0.12                 | 0.12                 |
| Polyphenol      | Genistein          | 0.32                 | 0.32                 |
| Polyphenol      | Quercetin          | 0.71                 | 0.71                 |
| Polyphenol      | Chlorogenic acid   | >100                 | >100                 |

Germany, amentoflavone from Roth (Karlsruhe, Germany), hypericin from Calbiochem (Bad Soden, Germany), phenylmethylsulfonyl fluoride, gossypol, ECG, and EGCG from Sigma, and ATA from Aldrich (Steinheim, Germany). All other tested inhibitor compounds were also purchased from the suppliers mentioned above at highest purity available (>95%). Me_{6}SO was of “Uvasol” quality and obtained from Merck (Darmstadt, Germany). L-Tartate dehydrogenase from pig muscle, phosphokinase from rabbit muscle, bovine erythrocytes (36). Subsequently, a recombinant fragment comprising the catalytic domain and the CaM binding domain (GgIP3K-ACaM/cat) was further purified by CaM affinity chromatography (36).

Cloning, Expression, and Purification of RpIP3K-A Isoform—The full-length cDNA coding for rat IP3K-C, RpIP3K-C, was cloned from a rat cDNA library (13). Two different recombinant fragments of rat IP3K-C, one comprising the catalytic domain and the calmodulin binding domain (RpIP3K-C_{CaM}), the other comprising only the catalytic domain (RpIP3K-C_{cat}), were expressed in E. coli BL21(DE3)RIL cells and purified by phosphocellulose (13). HsIP3K-BCaM/cat was further purified by CaM affinity chromatography (36).

Cloning, Expression, and Purification of HsIPMK—An expression vector was created as described in Ref. 16. Recombinant protein fragments of rat IP3K-C, one comprising the catalytic domain and the calmodulin binding domain (RpIP3K-C_{CaM}), the other comprising only the catalytic domain (RpIP3K-C_{cat}), were expressed in E. coli BL21(DE3)pLys[pREP4] and purified on DEAE-Sepharcl and phosphocellulose (16).

Mutation of GgIP3K-A and HsIPMK—The HsIPMK deletion mutant, HsIPMK (amino acids 266–371), and the point mutants GgIP3K-A_{N235E} and GgIP3K-A_{K239E} were created by using PCR-based site-directed QuikChange mutagensis (39). GgIP3K-A_{CaM}, pET17b (see above) cDNA were used as templates and specific oligonucleotides were designed (see Table I). Expression in E. coli and purification was described as wild-type GgIP3K-A_{CaM} and HsIPMK (16, 36).

IP3K and IPMK Enzyme Inhibition Assay—The inhibitors were dissolved in Me_{6}SO at a concentration of 3–10 mM under light exclusion and stored at −20 °C. Prior to use, aliquots were diluted to concentrations of 0.05–1 mM in Me_{6}SO. The enzyme activities of IP3K isozymes and IPMK were measured by an optical assay coupling ADP formation to NADH consumption via pyruvate kinase and lactate dehydrogenase reactions and using a wavelength of 339 nm. IP3K assays were routinely performed without Ca^{2+}-CaM. The final assay mixture was: 0.2 mM NADH, 1 mM phosphoenolpyruvate, 10 mM triethanolamine-HCl, 20 mM KCl, 500 mM NTP, 5 units/mlL-lactate dehydrogenase, 2.5 units/ml pyruvate kinase, pH 7.5. The final volume of the assay was 800 μl. GgIP3K-A, HsIP3K-A, HsIP3K-B, RpIP3K-C, or IPMK were added to the mixture (preincubated at 30 °C for 10 min) to a final concentration of between 3 and 20 mM. The mixture was further incubated at 30 °C for 10 min. After determining the low basal rate of ATP consumption without Insa_{5} (due to coupling enzyme constant activity), ATP was added stepwise in this period in 1–5-μl volumes until maximum inhibition or a concentration of 100 μM was reached. The final concentration of Me_{6}SO was kept below 3% (v/v). This concentration did not affect IP3K or IPMK activity. To preclude time dependent irreversible effects and possible photodecomposition of inhibitors, multiple assays with varying initial concentrations of inhibitors were also performed.

Assay for the Influence of Inhibitors on the K_{m} for ATP—Measurements were carried out with GgIP3K-A as described above with the concentration of Insa_{1,4,5}P_{5} kept at 25 μM (saturation); the concentration of inhibitor was varied as well as the initial concentration of ATP, the range of which was from 25 to 800 μM.

Assay for the Influence of Inhibitors on the K_{m} for ATP—Measurements were carried out with GgIP3K-A as described above with the concentration of ATP kept constant at 500 μM (close to saturation); that of Insa_{1,4,5}P_{5} was varied between 10 μM and <0.1 μM by means of single transient recordings (40). In the range of apparent half-maximal substrate concentration (about 1 μM), 9 μM of the product, Insa_{1,4,5}P_{5}, was present, acting as a competitive type of product inhibitor. Although the derived K_{m} values thus were higher than the true K_{m} (−0.1 μM), the assay was adequate to rapidly analyze influence of inhibitors on the K_{m} for Insa_{1,4,5}P_{5}.

Test of Influence of Inhibitors on Coupling Enzymes—The standard reaction mixture was incubated at 30 °C for 10 min in the presence and absence of inhibitor but without adding IP3K. Subsequently, ATP was added to a final concentration of 10 μM and the observed rapid consumption of NADH was recorded. In no case was any of the coupling assays detectably inhibited by the tested inhibitors or by their solvent.

Reversal of Enzyme Inhibition by Addition of Triton X-100 and Ca^{2+}—CaM—The standard reaction mixture was preincubated at 30 °C for 10 min, and enzyme inhibition was brought about with inhibitor concentration. After 5-fold above (3–10 μM) or 5 or 20 min, and CaCl_{2} (final concentration 50 μM) and CaM (final concentration 4 μM), or Triton X-100 (final concentration 0.2%) were added from concentrated stock solutions of 1 mM, 100 mM, and 10% (w/v), respectively, and the reaction was followed for another 5 min.

Test of Enzyme Aggregation—Isoform and IPMK inhibition assays with ATA were performed according to the standard conditions but IC_{50} values were derived for low (3 mM GgIP3K-A or 9 mM IPMK) and high...
(9 nM GgIP3K-A or 27 nM IPMK) enzyme concentrations.

Assay for an Activating or Inactivating Influence of Photometer Light on Photosensitive Inhibitors—For each compound, four reaction mixtures were incubated at 30 °C for 15 min under irradiation with photometer light (λ = 339 nm), two in the presence and two in the absence of a concentration of inhibitor about 3-fold above its IC50. In each pair of mixtures, one only contained Ins(1,4,5)P3 while the other only contained ATP. After pre-illumination, the assay was started either with 500 μM ATP or 25 μM Ins(1,4,5)P3, respectively. In the pair of reaction mixtures still lacking inhibitor the latter was supplemented together with the second substrate employed to start the reaction.

Test of Covalent Inhibitor Binding by MALDI-Re-TOF MS—GgIP3K-A (final concentration 15 μM) was mixed with inhibitors (ATA or THF; final concentration 30 μM). These mixtures were incubated (buffer: 10 mM triethanolamine-HCl pH 7.5, 1 mM dithiothreitol, 0.1% Triton X-100, 250 mM NaCl) for 20 min and diluted 1:50 with H2O. After another 20 min of incubation, 1 μl of this sample was applied onto the MALDI target and allowed to air-dry. Once dry, 1 μl of a saturated solution of sinapinic acid (matrix) in acetonitrile/water (1:1 with 0.1% (v/v) trifluoroacetic acid) was pipetted directly onto the sample surface. The solvent was allowed to evaporate and MALDI-Re-TOF MS analysis was performed using theBruker relex IV system. Mass spectra (arbitrary intensity versus m/z data, z = 1) were numerically smoothed, baseline subtracted, and submitted to a peak analysis employing the program Peak Fit 4.11 (SPSS Inc). For baseline subtraction, the best D2 function at a tolerance level of 4.5% was employed, smoothing was by the Fast Fourier Transform method with a smoothing factor of 12.5%, and fitting of peaks (maxima, widths, and areas) was by the residuals method employing spectroscopy type peak functions of the Voigt type with area-based optimization at an amplitude threshold of 4.5%.

Test on Topoisomerase II Inhibition—Inhibition of Topo II was assayed by the Topoisomerase II Drug Screening Kit obtained by Topogen. Activity was visualized by the change of linking number in superhelical plasmid DNA. Assays (20 μl) containing 0.25 μg of plasmid DNA and 0.05 to 50 μM inhibitor in 20 μl of 120 mM KCl, 10 mM, MgCl2, 0.5 mM ATP, 0.5 mM dithiothreitol, and 50 mM Tris-HCl, pH 8.0 (final concentrations) preincubated at 37 °C in the dark were started by adding 4 units of Topo II. Me2SO, the solvent of inhibitor stocks, was ≤ 1% (v/v) in the assay, which had no effect on Topo II activity. After 30 min at 37 °C in the dark, the reaction was stopped and subsequent agarose gel electrophoresis of extracted DNA was done according to the manufacturer's suggestion.

RESULTS

Screening for Inhibitors—On the basis of published inhibitors of PI3K, phosphodiesterases, and protein kinases (41–44) a series of substances (listed in Table II) belonging to the flavonoid group were first tested for inhibition of avian IP3K-A. The most potent inhibitors among the flavonols and flavones were quercetin (IC50, 180 nM), 3’,4’,7,8-tetrahydroxyflavone (THF; IC50, 290 nM), and myricetin (IC50, 540 nM). Starting from the molecular structures of these compounds, further flavonols were tested which differed from the latter with respect to the position and number of hydroxyl groups at the chromone ring system and at the B ring (Table II). Catechin and epicatechin, belonging to the flavan-3-ol group, inhibited IP3K to a low extent (IC50 > 100 μM). In contrast, gallic acid substituted catechin derivatives like ECG and EGCG (Table II) showed a strong inhibitory effect on IP3K (IC50 94 and 120 nM, respectively). The isoflavones daidzein and genistein (Table II), potent tyrosine kinase inhibitors (45,46), were only very weak...
inhibitors of GgIP3K-A (IC₅₀, 131 and 116 μM, respectively). Because of their known inhibitory effect on protein kinases (47), pseudohypericin (Table III) and hypericin (Table IV) were also tested. Hypericin reduced the enzyme activity of IP3K very effectively already in absence of illumination (IC₅₀, 170 nM). Irradiation of enzyme plus hypericin just with lab light (15 min) reduced the apparent IC₅₀ to 75 nM. Photometer light (λ = 339 nm) did not enhance the inhibitory potential of this inhibitor. Pseudohypericin and calphostin C, both structural perylenequinone analogues of hypericin (47–50), showed a markedly higher IC₅₀ of 2.4 μM and 1 μM, respectively, than hypericin (Table III, assays done in the dark). Anthraquinones, reported inhibitors of Topo II, were also analyzed (Table III); quinolizidin displayed the most potent inhibition (IC₅₀, 2.5 μM). Adria-nycin (Table III), having been reported by indirect evidence as a potential inhibitor of IP3K (51), did not strongly affect the enzyme activity of IP3K neither in the glycoside form (IC₅₀, >200 μM) nor in the aglycone form (IC₅₀, 62 μM). Based on the apparent structure-function relationship derived for flavonoids, the triterpene gossypol, the phenolic bis-lactone ellagic acid, and the triphenylmethane analogue ATA (Table IV), were analyzed. All three are highly potent inhibitors, the former two being the most potent inhibitors of IP3K identified so far (gossypol, 58 nM; ellagic acid, 36 nM; ATA, 150 nM). Since most of the identified inhibitors structurally exhibit a certain bandedness of ring structures (see Tables II-IV) we also tested biflavonoids. In fact, the weak inhibitor apigenin (IC₅₀, 8.3 μM), when dimerized to the related biflavonoid amentoflavone (52), exhibited a 10-fold increased potency (IC₅₀, 0.5 μM, see Table II). The protein kinase inhibitor rottlerin (53), distantly resembling complex flavonoids, also inhibited IP3K with IC₅₀ of 1.21 μM (Table III). For the most potent inhibitors (Table IV), representative dose response relationships observed at saturating Ins(1,4,5)P₃ and close to maximal ATP concentration are plotted in Fig. 1, A and B. A 100% inhibition was found for hypericin, gossypol, ECG, EGCG, and ATA. In contrast, ellagic acid, quercetin, myricetin, and THF inhibited the enzyme only by about 80% at a concentration of 100 μM.

Inhibition of Mammalian IP3K Isoforms and Human IPMK—The most potent inhibitors of avian IP3K-A were now tested for their effects on mammalian HsIP3K-A, HsIP3K-B, RnIP3K-C, and IPMK. All inhibitors identified in the extensive screening with GgIP3K-A also reduced the enzyme activity of mammalian IP3K isoforms, but the inhibitor selectivity of mammalian IP3K isoforms A and C differed strongly from that of isoform B (Table V and Fig. 1C). The flavonoids myricetin, THF, and EGCG had a markedly stronger effect on isoforms A and C than on isoform B. Ellagic acid was the only inhibitor showing preferred selectivity for isoform B. Many of the potent IP3K inhibitors (EGCG, ECG, quercetin, THF, myricetin, hypericin) did not affect IPMK activity at up to 100 μM. Obviously, only acidic polyphenolic compounds containing carboxylic acid or other acidic side groups are potent inhibitors of this InSP kinase. The most potent inhibitors were (IC₅₀ values given) ATA (44 nM) > rose bengal (620 nM) > chlorogenic acid (1.15 μM) > ellagic acid (1.37 μM). Gossypol lacking an acidic group showed also a weak IPMK inhibition (IC₅₀, 3.4 μM). Chlorogenic acid was the only substance exclusively inhibiting IPMK but not IP3K.

Inhibition of Topoisomerase II by IP3K/IPMK Inhibitors—Since for some of the identified IP3K/IPMK inhibitors a strong inhibition of Topo II has been reported in the literature (see discussion below), but reported assay conditions were strongly differing, we submitted all identified potent inhibitors to a standardized assay of Topo II inhibition. These data (Table V) revealed that for all inhibitors except for EGCG and ECG the IC₅₀ for Topo II inhibition was distinctly above the IC₅₀ for IP3K inhibition.

Partial Irreversibility of Inhibition by IP3K Inhibitors—Trition X-100 was identified as a substance antagonizing the effect of all inhibitors identified with GgIP3K-A. When 0.2% (w/v) Triton X-100 was added to assays containing the respective inhibitors at ≥IC₅₀ the previous inhibition of GgIP3K-A was immediately reversed by an extent between 49 and 100%, depending on the inhibitor and the time when Triton X-100 was added.
Triton ranging from 0.012% (aggregation. Therefore, we investigated the dependence of IC50 Triton X-100 could be an indication of such inhibitory enzyme The observed partial or full reversal of IP3K inhibition by inhibitors by Triton X-100 (about 1:1 stoichiometry, bition could mainly be attributed to a direct complexing of Triton X-100 (0–0.2% w/v) revealed that the reversal of inhi- nation was almost complete (75–100%) for all tested inhibitors except for amentoflavone, whose inhibition was fully addition of (data not shown). When the detergent was added imme- diately or 5 min after inhibitor addition, the reversal of inhibition was almost complete (75–100%) for all tested inhibitors (quercetin, myricetin, amentoflavone, EGC, EGCG, ellagic acid, gossypol, hypericin in the dark, ATA). When it was added (data not shown). Difference spectro- cwork analyses (data not shown) performed with solutions of inhibitors (20 or 50 μM) mixed with an increasing concentration Triton X-100 (0–0.2% w/v) revealed that the reversal of inhibition could mainly be attributed to a direct complexing of inhibitors by Triton X-100 (about 1:1 stoichiometry, Kapp for Triton ranging from 0.012% (~184 μM) to 0.034% (~521 μM)) and a decrease of the free inhibitor concentration by this scav- enging reaction.

IP3K and IPMK Inhibitors Do Not Induce Enzyme Aggregation— For a number of proteins of pharmacological interest it has been shown that certain inhibitors mainly cause inactivating irreversible protein aggregation instead of a specific inhibition of ligand binding. Such an action is accompanied by decreasing IC50 values with increasing protein concentration. The observed partial or full reversal of IP3K inhibition by Triton X-100 could be an indication of such inhibitory enzyme aggregation. Therefore, we investigated the dependence of IC50 values of GgIP3K-A and IPMK for the common inhibitor ATA on enzyme concentration. As shown in Fig. 2A, neither of these two enzymes revealed a decrease of its IC50 with increasing protein concentration. Instead, a slight increase of IC50 with increasing enzyme concentration was found as predicted for unchanged binding affinity. Inactivating enzyme aggregation caused by inhibitors of GgIP3K-A and IPMK thus could be ruled out.

Effect of Ca2+-CaM on IP3K and IPMK Inhibition—Ca2+-CaM (4 μM), when added to GgIP3K-A after 5 min or 20 min of inhibition also partially reversed the previous inhibition (by 10–28%, the same inhibitors were employed as in the Triton X-100 reactivation experiments, data not shown). This Ca2+-CaM-induced reactivation never reached the extent observed with Triton X-100 (see above). Again the duration of previous inhibition negatively influenced the degree of reactivation, with the exception of amentoflavone, whose inhibition was fully reversible. Reactivation by Ca2+-CaM never brought the enzyme activity to the level measured for uninhibited Ca2+-CaM-activated IP3K-A (about 150% of the activity of IP3K-A alone, see Ref. 7). Other IP3K isoforms exhibited similar partial re- activation by Ca2+-CaM (data not shown). These data allowed no discrimination between a direct scavenging of inhibitor by Ca2+-CaM and a “normal type” of Ca2+-CaM activation of inhibited enzyme.

Table V

Comparison of potent inhibitors with respect to their effect on GgIP3K-A, HsIP3K-A, HsIP3K-B, RnIP3K-C, HsIPMK topoisomerase II, and HsIPMKΔ

Mean and S.E. of triplicate determinations of IC50 are given with the exception of Topo II, where IC50 was only determined once. All assays were performed as described under “Experimental Procedures.”

| Inhibitor | HsIP3K-A | GgIP3K-A | RnIP3K-C |
|-----------|-----------|-----------|-----------|
|           | IC50 (nM) | Max (%)   | IC50 (nM) | Max (%)   | IC50 (nM) | Max (%)   |
| Gossypol  | 115 ± 14  | 100       | 58 ± 3    | 100       | 175 ± 7   | 100       |
| ATA       | 138 ± 8   | 100       | 150 ± 9   | 100       | 62 ± 3    | 100       |
| THF       | 180 ± 5   | 74 ± 3    | 290 ± 10  | 97 ± 1    | 190 ± 6   | 75 ± 2    |
| EGCG      | 150 ± 9   | 100       | 120 ± 5   | 100       | 210 ± 15  | 100       |
| Quercetin | 300 ± 4   | 81 ± 3    | 180 ± 10  | 80 ± 3    | 390 ± 20  | 71 ± 4    |
| ECG       | 400 ± 15  | 100       | 94 ± 5    | 100       | 385 ± 15  | 100       |
| Ellagic acid | 550 ± 11 | 62 ± 3    | 36 ± 4    | 75 ± 3    | 690 ± 15  | 85 ± 2    |
| Hypericin | 180 ± 9   | 100       | 170 ± 11  | 100       | 170 ± 12  | 100       |
| Myricetin | 150 ± 19  | 65 ± 4    | 540 ± 13  | 85 ± 2    | 290 ± 11  | 62 ± 4    |
| Chlorogenic acid | >100,000 | >100,000 | >100,000 |
| Rose bengal | 2,190 ± 20 | 100       | 3,120 ± 13 | 100       | 1,890 ± 65 | 100       |

| Inhibitor | HsIP3K-B | HsIPMK | Topo II | HsIPMKΔ |
|-----------|-----------|---------|---------|---------|
|           | IC50 (nM) | Max (%) | IC50 (nM) | Max (%) | IC50 (nM) | IC50 (nM) |
| Gossypol  | 340 ± 15  | 100     | 3,400 ± 50 | 100     | 5,000     | 700 ± 40  |
| ATA       | 110 ± 8   | 100     | 44 ± 4    | 100     | 500       | 65 ± 4    |
| THF       | 3,400 ± 350 | 79 ± 3 | >100,000 | 100     | 6,000     | 520 ± 120 |
| EGCG      | 2,780 ± 25 | 100     | >100,000 | 100     | 400       | 390 ± 55  |
| Quercetin | 1,250 ± 25 | 97 ± 1  | >100,000 | 100     | 100,000   | ND        |
| ECG       | 560 ± 40  | 100     | >100,000 | 100     | 150       | ND        |
| Ellagic acid | 220 ± 10 | 92 ± 8  | 1,270 ± 40 | 100     | 50,000    | ND        |
| Hypericin | 210 ± 10  | 100     | >100,000 | 100     | 4,500     | ND        |
| Myricetin | 4,200 ± 80 | 61 ± 3  | >100,000 | 100     | 40,000    | ND        |
| Chlorogenic acid | >100,000 | >100,000 | >100,000 | >100,000 | ND | 1,160 ± 50 |
| Rose bengal | 520 ± 20  | 100     | 620 ± 20  | 100     | ND | 570 ± 20  |

* Maximal degree of inhibition in %.
* Mutant with a segmental deletion as described under “Experimental Procedures” and Table I. Maximal inhibition was 100% for gossypol, ATA, and rose bengal, and 85, 90, and 65% for THF, EGCG, and chlorogenic acid, respectively.
* ND, not determined.
inhibition most likely is due to covalent binding of inhibitors or a tight binding, which is not reversed under MS conditions. Insufficient purity of IPMK precluded corresponding experiments with IPMK.

**Kinetic Analysis of the Reversible Part of Inhibition of IP3K**—The reversible type of interaction of inhibitors with the enzyme was analyzed in detail in GgIP3K-A. In order to perform these analyses at close to steady state conditions uninfluenced by the slow irreversible inhibition (above), only short time enzyme assays (up to 5 min) were employed (see "Experimental Procedures" for details). All potent inhibitors increased the \( K_{m} \) for ATP (uninhibited 75 \( \mu \)M, inhibited see Table VI). However, none of the inhibitors behaved purely competitive with ATP, but all showed linear mixed-type inhibition with respect to this substrate (54). For inhibition by ellagic acid and hypericin, double reciprocal plots are depicted in Fig. 3. The \( K_{m} \) for \( \text{Ins}(1,4,5)\text{P}_{3} \) determined in absence of \( \text{Ins}(1,3,4,5)\text{P}_{4} \) is about 0.1 \( \mu \)M (7). To simplify \( K_{m} \) determinations for \( \text{Ins}(1,4,5)\text{P}_{3} \) in presence of inhibitors, single transient assays (40) were performed in these transients, product inhibition by \( \text{Ins}(1,3,4,5)\text{P}_{4} \) accumulating during the reaction led to higher apparent \( K_{m} \) values for \( \text{Ins}(1,4,5)\text{P}_{3} \) (about 1 \( \mu \)M, see Ref. 7). But these apparent \( K_{m} \) values still reveal a competition of inhibitor with \( (1,4,5)\text{P}_{3} \) binding. As compiled in Table VI, none of the most potent inhibitors showed any influence on the \( K_{m} \) value for \( \text{Ins}(1,4,5)\text{P}_{3} \). In Fig. 3 the \( 1/v \) versus \( 1/[\text{InsP}_{3}] \) data for hypericin and ellagic acid are also plotted. The derived linear non-competitive inhibition with respect to \( \text{InsP}_{3} \) proofs that none of the inhibitors apparently interferes directly with the binding of \( \text{Ins}(1,4,5)\text{P}_{3} \) to the catalytic site.

**Search for Inhibitor Binding Sites**—Because the addition of a molar excess of \( \text{Ca}^{2+} \)-CaM could partially antagonize the effect of inhibitors on our recombinant IP3K isoforms, all containing a functional CaM binding domain and exhibiting normal activation by \( \text{Ca}^{2+} \)-CaM (see Refs. 7, 13, 36), we investigated whether the CaM binding domain of IP3Ks is involved in inhibitor binding. For these analyses, we compared the inhibitory effect of the potent IP3K-A and IP3K-C inhibitor THF on RnIP3K-C_{CaMoot} (an enzyme comprising the \( \text{Ca}^{2+} \)-CaM binding domain and the catalytic domain) with RnIP3K-C_{cat} (an enzyme comprising only the catalytic domain). The latter enzyme revealed a lower affinity for \( \text{InsP}_{3} \) in comparison to the former one and a significantly higher specific activity, the \( K_{m} \) for ATP was almost unchanged (see Table VII and Ref. 13). Under conditions where both enzyme forms were saturated with \( \text{Ins}(1,4,5)\text{P}_{3} \), RnIP3K-C_{CaMoot} in fact showed a 3-fold lower IC\textsubscript{50} value for THF than RnIP3K-C_{cat}. The maximum degree of inhibition was unchanged. This result together with the finding that \( \text{Ca}^{2+} \)-CaM partly reverses IP3K inhibition indicates a facilitating but not essential involvement of the CaM binding domain in inhibitor binding. That \( \text{Ca}^{2+} \)-CaM apparently competes with this interaction and not directly binds and thus scavenges inhibitors can also be deduced from the ineffectiveness of \( \text{Ca}^{2+} \)-CaM in de-inhibiting IPMK, which lacks a CaM binding domain (Ref. 16, see Fig. 4A). Because the deletion of the CaM binding domain in IP3K only weakened but not abolished inhibitor binding, there must be further subdomains in the IP3K catalytic domain involved in inhibitor binding. In a search for amino acids involved in inhibitor binding we mutated several amino acids within the catalytic domain of GgIP3K-A_{CaMoot} and analyzed their inhibition by THF. Two of the obtained point mutants revealed a drastically reduced inhibition by THF (Table VII). Substitution of Lys\textsuperscript{566} with Glu led to a 45-fold increase in the IC\textsubscript{50} and substitution of Lys\textsuperscript{522} with Asp even led to a 260-fold increase. On the other hand, kinetic parameters of the enzyme with respect to both substrates were nearly unchanged (Table VII). Thus, by substituting either one of these two lysines in a basic segment involved in InsP\textsubscript{3} and InsP\textsubscript{4} binding (55) and located in the IP-binding lobe (37, 38) we have created inhibitor-resistant IP3K-A with full enzymatic activity and normal substrate affinity. As shown in the sequence alignment of IP3K isoforms and IPMK in Fig. 4B, the corresponding segment is missing in HsIPMK but present in all isoforms of IP3K. Both the absence of the CaM binding domain (above) and the missing "InsP\textsubscript{3} binding core domain" (55) may thus contribute to the observed differences in inhibitor selectivity of IPMK as compared with IP3K (see Fig. 1C).

In contrast to IP3K, IPMK harbors a unique basic segment of 105 amino acids, which is inserted between the SSLL motif and the DFG motif (56). In the three-dimensional structure of IP3K-A (37, 38) this insert is extending a surface loop between \( \beta \)-strands \( \beta_{4c} \) and \( \beta_{5c} \) in the C-lobe, and functionally it was
shown to be responsible for nuclear localization of IPMK (Ref. 16, see Fig. 4A). As the enzyme activity of IPMK is preferentially inhibited by acidic polyphenolic compounds, this segment might be involved in inhibitor binding. Therefore we deleted the whole segment. A fully active enzyme, termed IPMK/H9004, with almost unchanged kinetic parameters (only $K_m$ for ATP and $V_{max}$ were about doubled, see Table VII) resulted. Its $IC_{50}$ values for most of the potent IPMK inhibitors, namely ATA, rose bengal, and chlorogenic acid, showed only slight differences as compared with wild-type enzyme. But surprisingly, gossypol, the weak wild-type IPMK-inhibitor ($IC_{50}$ 3.4 $\mu$m) but strong IP3K inhibitor ($IC_{50}$ 0.06–0.34 $\mu$m) revealed a 5-fold lower $IC_{50}$ in IPMK$\Delta$ of 0.7 $\mu$m (Table V). Still more intriguingly, THF and EGCG, inhibitors which did not significantly inhibit wild-type IPMK up to 100 $\mu$m, became almost as potent inhibitors in IPMK$\Delta$ ($IC_{50}$, 0.52 $\mu$m and 0.39 $\mu$m, respectively) as they are for IP3K-A and IP3K-C ($IC_{50}$, 0.18–0.29 $\mu$m and 0.12–0.21 $\mu$m, respectively, Table V).

**DISCUSSION**

**Structural Features of Potent Inhibitors**—With one exception, namely the common inhibitor ATA, we found that certain polyphenolic compounds inhibit IP3K and IPMK in a specific, almost mutually exclusive manner. IP3K isoforms are inhibited most effectively by polyphenolic substances containing multiple (≥2) aromatic ring systems with more than three

| Parameters of inhibition | Non-competitive with respect to Ins(1,4,5)P$_3$ | Mixed-type with respect to ATP |
|--------------------------|---------------------------------------------|---------------------------------|
| Inhibitor                | $IC_{50}^a$ $^b$ | $K_i^c$ | Slope $d$ | $a^e$ | $1/v$- Interceptor $^c$ |
| Ellagic acid             | 36 ± 4$^f$ | 60 ± 7 | Linear | 43 ± 4 | Linear | 3.2 | Linear |
| Gossypol                 | 55 ± 3   | 77 ± 8 | Linear | 61 ± 6 | Linear | 2.8 | Linear |
| ECG                      | 94 ± 5   | 43 ± 4 | Linear | 20 ± 3 | Linear | 4.0 | Linear |
| EGCG                     | 120 ± 5  | 59 ± 7 | Linear | 59 ± 7 | Linear | 3.1 | Linear |
| ATA                      | 150 ± 9  | 100 ± 13 | Linear | 96 ± 11 | Linear | 1.7 | Linear |
| Hypericin                | 170 ± 11 | 156 ± 17 | Linear | 74 ± 8 | Linear | 2.0 | Linear |
| Quercetin                | 180 ± 10 | 197 ± 19 | Linear | 312 ± 22 | Linear | 1.6 | Linear |

$^a$ Determined at $V_{max}$ for Ins(1,4,5)P$_3$.

$^b$ Determined from re-plots of the slopes and the $1/v$-axis intercepts (obtained from Lineweaver-Burk plots at each concentration of inhibitor) versus concentration of inhibitor. (See Segal et al. (54), non-competitive inhibition, pp. 125–139).

$^c$ Determined from re-plots of the slopes (obtained from Lineweaver-Burk plots at each concentration of inhibitor) versus concentration of inhibitor. (See Ref. 54, mixed type inhibition, pp. 170–191).

$^d$ Parameter $\alpha$ is the $K_i$ or $K_s$ changing factor, when inhibitor binds to the enzyme-substrate complex or substrate binds to the enzyme-inhibitor complex, respectively. (See Ref. 54, mixed type inhibition, pp. 170–191). With $K_i$ determined from the slope versus inhibitor re-plot, the factor $\alpha K_i$ was determined from the re-plot of the $1/v$-axis intercepts (obtained from Lineweaver-Burk plots at each concentration of inhibitor) versus concentration of inhibitor.

$^e$ Means and S.E. of linear regression parameters are given.

![Enzyme kinetic analysis of the reversible type of inhibition of GgIP3K-A.](image)
Phenolic hydroxyls and one or more carbonyl groups, the phenol rings thereby forming a “bi-handed” structure, optimally represented by several flavonoids. IPMK is best inhibited by acidic triphenylmethanes or similar acidic polyphenolic substances. For avian IP3K-A a comparison of structure and inhibitory effects of flavonoids indicates that the degree of inhibition is likely, presumably via a Schiff base formation with the enamine NH2 groups of lysines. Inhibitor binding core domain downstream of this motif are aligned. Amino acids in GgIP3K-A (bold) shown by mutagenesis to be involved in inhibitor binding are boxed.

Mechanism of Interaction of Inhibitors with IP3K—MS data (Fig. 2B) as well as the slowly developing irreversible inhibition indicate that after a rapid pre-equilibrium there is a subsequent slow covalent or quasi irreversible “tight binding” of the most potent inhibitors. Since all inhibitors exhibiting such slow irreversible inhibition contain carbonyl or carboxylic groups, the enzyme is likely, presumably via e-NH2 groups of lysines. Inhibitor binding core domain downstream of this motif are aligned. Amino acids in GgIP3K-A are strongly distorted with angles of up to 60° between the planes of the ring moieties leading to distorted bihanded structures. Propeller-like symmetry in such bihanded molecular structures that represent other two regions divergent between IP3K and IPMK, namely the Ip6 binding core domain unique for all IP3Ks and a polybasic and hydrophobic insert unique in IPMK (16).

**Fig. 4. Differences in the amino acid sequence of IP3K and IPMK and sites involved in inhibitor binding in GgIP3K-A.** A, schematic alignment of the homologous sequences of HsIPMK and GgIP3K-A. Segments strongly conserved between these two enzymes are in dark gray and designated with segment names. The CaM binding domain present in all IP3K isoforms but absent in IPMK is marked in black. Bright gray bars represent the other two regions divergent between IP3K and IPMK, namely the Ip6 binding core domain unique for all IP3Ks and a polybasic and hydrophobic insert unique in IPMK (16). B, identification of sites of GgIP3K-A involved in inhibitor binding. Partial sequences from the catalytic domains of mammalian IP3K isoforms and HsIPMK comprising the PDGK motif (underlined) being essential for the catalytic activity of all PDGK-type InsP kinases (56) and the Ip6 binding core domain downstream of this motif (55) are aligned. Amino acids in GgIP3K-A (bold) shown by mutagenesis to be involved in inhibitor binding are boxed.
type inhibition with respect to ATP and a non-competitive one with respect to Ins(1,4,5)P3. Ligands with such complex type of inhibition of protein or substrate kinases were discussed to be advantageous in searches for highly selective inhibitors whereas pure binding competitors of ATP, a substrate broadly used by many cellular enzymes, are less suited to selectively act against only one out of these many enzymes (58).

Inhibitory Selectivity Profiles for Different IP3K Isoforms and IPMK—The inhibitory action of most strong IP3K inhibitors, from that of IP3K isoforms. Only acidic polyphenolic compounds potently inhibit this enzyme. Except for gossypol, all other potent IP3K inhibitors lacking an acidic function do not significantly influence IPMK activity. A correlation exists between negative charge of the phenolic substances and IPMK inhibition. ATA, which contains three carboxylic groups, is the best inhibitor; polyphenols containing fewer acidic groups (ellagic acid, rose Bengali, and chlorogenic acid, see Table IV and Fig. 5) are weaker inhibitors, followed by gossypol completely lacking an acidic group. ATA, the best inhibitor of IPMK is also an excellent inhibitor of all IP3K isoforms and thus is the best candidate for a common inhibitor generally preventing Ins(1,4,5)P3 phosphorylation in cells. Chlorogenic acid, showing no effect on IP3K activity likely because of the lack of the second aromatic ring structure may be useful as a lead to more specific IPMK inhibitors (see Fig. 5).

Structural Basis of Inhibitor Selectivity—The markedly differing inhibitor selectivity of IP3K and IPMK reflects different inhibitor binding sites within these two homologous enzymes (see the structural sketch in Fig. 4A). One important position in the IP3K catalytic domain for inhibitor binding seems to be located within the InsP3 binding segment, since substitution of one of two closely neighboring lysine residues downstream of the PDKG motif (Ref. 56, see Fig. 4) caused a 45–260-fold decrease of the inhibitory effect. No corresponding site is present in IPMK (see Fig. 4B). The location of corresponding residues in the three-dimensional structure of IP3K-A (37, 38) and IPMK (see Table IV and Fig. 5) apparently protects IPMK from the inhibitory action of most strong IP3K inhibitors, i.e. is kind of an inhibitor resistance domain, while its deletion renders IPMK more IP3K-like in its inhibitor selectivity. Its steric loca-

### Table VIII

| Inhibitor | Cellular effects | Refs. |
|----------|-----------------|-------|
| Quercetin | Suppression of tumor cell growth | 75–77 |
| ATA | Prevention of apoptosis | 79 |
| Ellagic acid | Suppression of tumor cell growth | 90–98 |
| Gossypol | Suppression of tumor cell growth | 99–104 |
| Hypericin | Suppression of tumor cell growth | 98–100 |
| Myricetin | Suppression of tumor cell growth | 105–108 |
| ATA | Prevention of apoptosis | 109–111 |
| Myricetin | Suppression of tumor cell growth | 112–115 |
| Rose bengal | Suppression of tumor cell growth | 116–118 |
| Myricetin | Suppression of tumor cell growth | 119–125 |
| ATA | Suppression of tumor cell growth | 126–128 |
| Myricetin | Suppression of tumor cell growth | 79,129,130 |
| ATA | Suppression of tumor cell growth | 131,132 |
| Chlorogenic acid | Suppression of tumor cell growth | 133 |

### Table IX

| Inhibitor | Cellular effects | Refs. |
|----------|-----------------|-------|
| Quercetin | Suppression of tumor cell growth | 75–77 |
| ATA | Prevention of apoptosis | 79 |
| Ellagic acid | Suppression of tumor cell growth | 90–98 |
| Gossypol | Suppression of tumor cell growth | 99–104 |
| Hypericin | Suppression of tumor cell growth | 98–100 |
| Myricetin | Suppression of tumor cell growth | 105–108 |
| ATA | Prevention of apoptosis | 109–111 |
| Myricetin | Suppression of tumor cell growth | 112–115 |
| Rose bengal | Suppression of tumor cell growth | 116–118 |
| Myricetin | Suppression of tumor cell growth | 119–125 |
| ATA | Suppression of tumor cell growth | 126–128 |
| Myricetin | Suppression of tumor cell growth | 79,129,130 |
| ATA | Suppression of tumor cell growth | 131,132 |
| Chlorogenic acid | Suppression of tumor cell growth | 133 |

*Fig. 5. Structure of rose bengal and chlorogenic acid.*
tation near the nucleotide binding domain (insert 2 region) in the IP3K-A three-dimensional structure (37) is compatible with this protective role. All three described structural differences between IP3K and IPMK together thus may contribute to the markedly different inhibitor selectivity of these two enzyme families.

Alternative Targets of IP3K and IPMK—Since many of the identified IP3K and IPMK inhibitors influence other cellular enzymes or metabolic systems, a detailed examination of reported IC_{50} or K_{i} values for these effects obtained in vitro was performed. In most cases, the IC_{50} for IP3K and IPMK inhibition is perceptibly lower than for other enzymes (Table VIII). Only two other enzyme families, DNA polymerase and Top II are inhibited by some of our identified inhibitors with comparable efficiency. Quercetin, EGCG, and ECG inhibit DNA polymerases \( \alpha \) and \( \beta \) as potently as IP3K-A and -C. ATA and, according to our analyses, ECG and EGCG are comparably potent Top II inhibitors (see Tables V and VIII). EGCG has a further potent inhibitory effect on prolyl-endopeptidase (66).

**Cellular Effects of Inhibitors of IP3K and IPMK**—An actual literature search (see references in Table IX) revealed that most discovered potent IP3K and IPMK inhibitors have been reported to exert antiproliferative effects on cultured cells (designated “in vitro”) or in animal experiments/tumor treatment studies (designated “in vivo”). An ongoing screen for further inhibitors of IP3K and IPMK up to now only revealed compounds also inhibiting cell growth in cell lines of non-transformed and transformed phenotype. THF is one of these novel compounds. IP3K and/or IPMK should therefore be put onto the list of potential targets of natural and synthetic antiproliferative drugs and future studies have to show whether IP3K or IPMK are key players of proliferative signal transduction. It will also be of high interest to know how plant cells manage both inhibitory polyphenol biosynthesis and the action of their IPMKs (18).

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**Polyphenolic Inhibitors of Inositol Phosphate Kinases**
