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O-Methylated Catechins from Tea Leaves Inhibit Multiple Protein Kinases in Mast Cells

Mari Maeda-Yamamoto,* Naoki Inagaki,† Jiro Kitaura,‡ Takao Chikumoto,‡ Hiroharu Kawahara,§ Yuko Kawakami,¶ Mitsuaki Sano,† Tosho Miyase,† Hirofumi Tachibana,¶ Hiroichi Nagai,‡ and Toshiaki Kawakami*‡

Tea contains a variety of bioactive compounds. In this study, we show that two O-methylated catechins, (-)-epigallocatechin-3-O-(3-O-methyl) gallate and (-)-epigallocatechin-3-O-(4-O-methyl) gallate, inhibit in vivo mast cell-dependent allergic reactions more potently than their nonmethylated form, (-)-epigallocatechin-3-O-gallate. Consistent with this, these O-methylated catechins inhibit IgE/Ag-induced activation of mouse mast cells: histamine release, leukotriene release, and cytokine production and secretion were all inhibited. As a molecular basis for the catechin-mediated inhibition of mast cell activation, Lyn, Syk, and Bruton’s tyrosine kinase, the protein tyrosine kinases, known to be critical for early activation events, are shown to be inhibited by the O-methylated catechins. In vitro kinase assays using purified proteins show that the O-methylated catechins can directly inhibit the above protein tyrosine kinases. These catechins inhibit IgE/Ag-induced calcium response as well as the activation of downstream serine/threonine kinases such as Akt and c-Jun N-terminal kinase. These observations for the first time have revealed the molecular mechanisms of antiallergic effects of tea-derived catechins. The Journal of Immunology, 2004, 172: 4486–4492.

Most cells play a critical role in the effector phase of IgE-dependent immediate hypersensitivity and allergic diseases (1). Cross-linking of high affinity IgE receptors (FcεRI) with IgE and allergen initiates the activation process, leading to the release of preformed and de novo synthesized vasoactive amines, proteases, leukotrienes, cytokines, and chemokines. These chemical and polypeptide agents elicit various allergy-associated pathophysiological changes locally and systemically: for instance, amines such as histamine and serotonin enhance vascular permeability, and cytokines such as TNF-α recruit inflammatory cells to the site of allergen exposure.

Over the past two decades, we have seen a tremendous advancement in our understanding of what happens inside mast cells following FcεRI cross-linking (2). FcεRI is a heterotetrameric receptor composed of an IgE-binding α subunit, a four-transmembrane β subunit, and disulfide-bonded two γ subunits (3). γ subunits are critical for both membrane expression of the receptor and signal transduction, while β subunits amplify the γ subunit-generated signals and stabilize the receptor. β subunits are associated constitutively with a predominant Src family protein tyrosine kinase (PTK)3 (3), Lyn (4). Upon FcεRI cross-linking, Lyn becomes activated and phosphorylates tyrosine residues in the immunoreceptor tyrosine-based activation motifs in β and γ subunits (5, 6). Tyrosine-phosphorylated immunoreceptor tyrosine-based activation motifs of β and γ subunits recruit Lyn and Syk (another PTK with two tandem Src homology 2 domains), respectively, to the plasma membrane and activate these PTKs (4, 7–9). Active Lyn and Syk phosphorylate numerous signaling proteins. Another PTK, Bruton’s tyrosine kinase (Btk) (10), is phosphorylated at its critical activation-loophop-binding by Lyn and activated (11). Active Syk and Btk in turn phosphorylate and activate phospholipase C (PLC)-γ in a concerted manner (12, 13). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol 1,4,5-trisphosphate. Inositol 1,4,5-trisphosphate mobilizes Ca2+ from intracellular storage sites. Diacylglycerol and Ca2+ activate some isoforms of protein kinase C (PKC). Downstream of these early activation events, at least four major mitogen-activated protein kinase (MAPK) subfamilies of protein serine/threonine kinases (PS/TKs), i.e., extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinase (JNK) 1/2, p38, and big mitogen-activated kinase 1/ERK5 (14–19), and a critical survival kinase, Akt/protein kinase B (Akt) (20), are also activated. Many of these kinases are shown to be involved in production and secretion of cytokines.

Tea (Camellia sinensis L.) has been consumed all over the world, particularly in large quantities in Japan and China, where it has also been used for medicinal purposes for tens of centuries. It has been reported that tea has various bioregulatory activities, such as anticarcinogenic (21, 22) and antimetastatic activities (23–26), antioxidative activity (27–29), antihypertensive activity (30), anti-cholesterolemic activity (31, 32), antiallergic activity (33), antibacterial activity (34), and intestinal flora amelioration activated protein kinase; PCA, passive cutaneous anaphylaxis; PKC, protein kinase C; PLC, phospholipase C; PS/TK, protein serine/threonine kinase; PVDF, polyvinylidene difluoride.
activity (35). Major principles for these activities were shown to be catechins, a group of polyphenolic compounds. In previous studies, we have reported that O-methylated forms of (-)-epigallocatechin-3-O-gallate (EGCG), i.e., (-)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3Me) and (-)-epigallocatechin-3-O-(4-O-methyl) gallate (EGCG4Me), which were contained in some cultivars of tea such as 'Benifuuki' or 'Benifujii,' had antiallergic action in in vivo type I and type IV allergies (36, 37). In this study, we present evidence that two O-methylated catechins, EGCG3Me and EGCG4Me, have an antiallergic activity and inhibit several protein kinases as a molecular basis for such an activity.

Materials and Methods

Antibodies

Sources of commercial Abs are as follows: anti-phosphotyrosine mAb 4G10 from Upstate Biotechnology (Chattanooga, VA); anti-Lyn (38), anti-Syk (C-20), anti-Akt (C-20), and anti-JNK (C17) from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phospho-ERK1/2, anti-phospho-JNK, and anti-phospho-p38 from Cell Signaling Technology (Beverly, MA); anti-mouse IgG F(ab')2 from BioSource International (Camarillo, CA); anti-Btk from BD PharMingen (San Diego, CA); anti-ERK1/2 from Zymed Laboratories (South San Francisco, CA); and DNP-specific IgE from Sigma-Aldrich (St. Louis, MO). Anti-Flt3Rβ subunit mAb IRK was kindly provided by J. Rivera (National Institutes of Health).

Chemicals

OVA and DNP conjugates of human serum albumin (DNP-HSA) were purchased from Sigma-Aldrich; IFA and diphenhydramine hydrochloride were from Wako Pure Chemical (Osaka, Japan).

Purification of EGCG3Me and EGCG4Me

Dried tea leaves (cultivars 'Benihomare' and 'Benifuki' or 'Ton-chin oolong tea) were extracted with boiling water for 30 min. The extract was passed through a porous polymer gel (Mitsubishi Diaion HP-20; Mitsubishi Oil Chemicals, Tokyo, Japan) column (9 × 40 cm). After the column had been washed with water, the absorption fraction was eluted with water/methanol (1:1) to give a pale brown residue (31 g). The residue was chromatographed on a silica gel column (9 × 25 cm) and eluted with chloroform/methanol/water (40:50:10) to yield six fractions. The peak fraction (2.657 cm) that contained EGCG3Me and 101 mg of EGCG4Me were recovered by brief centrifugation following another 30-min incubation with Pansorbin (Calbiochem, San Diego, CA) for rabbit polyclonal Abs or anti-immune Ig-conjugated agarose (Sigma-Aldrich) for mouse mAbs. Immune complexes were washed in buffer four times. Cleared cell lysates or immunoprecipitates were separated by SDS-PAGE and blotted electrophoretically to polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore, Billerica, MA). Membranes were blocked and incubated with a primary Ab and then with a HRP-conjugated secondary Ab. Visualization of immunoreactive proteins was performed with ECL reagents (PerkinElmer Life Sciences, Wellesley, MA). Relative amounts of immunoreactivity were quantified by National Institutes of Health Image Analysis software (v.1.62).

Immune complex kinase assays

Immunoprecipitates were washed four times in lysis buffer and once with kinase buffer (50 mM Tris, pH 7.4, 0.1% Nonidet P-40, 10 mM MnCl2, 10 mM MgCl2) without ATP. Washed immune complexes were incubated in kinase buffer with or without exogenous substrate (Btk and Lyn without exogenous substrate; Syk with 2 μg of GST-HS1 containing the sequence from position 352 to position 486 of the human HS1 protein (40)) in the presence of [γ-32P]ATP (ICN Biomedicals, Irvine, CA). Reaction products were analyzed by SDS-PAGE, followed by electroblotting onto PVDF membranes and autoradiography. Phosphorylated protein bands were quantified by densitometry.

In vitro kinase assays using purified or recombinant enzymes

Lyn and Btk were expressed in Sf9 insect cells and partially purified, as described (41). Recombinant human Syk was purchased from Upstate Biotechnology. Kinase assays were performed, as described above, for immune complex assays, except for the presence of various concentrations of EGCGs throughout the assay period. Kinase activities of PKCe, PKCβII, and PKCβIII (all recombinant human isoforms from Panvera, Madison, WI) in the absence or presence of EGCG3Me and EGCG4Me were measured using the PKC pseudosubstrate peptide PKD (19–27) in a PKC assay kit (Invitrogen). Assays on ERK2 (recombinant mouse; New England Biolabs, Beverly, MA) and JNK1 (recombinant mouse; Upstate) in the absence or presence of O-methylated EGCGs were performed using myelin basic protein and GST-c-Jun (1–79), respectively, as substrate.

Kinetic analysis of EGCG3Me inhibition of Lyn kinase was performed at pH 7.2 in kinase buffer, using 1 μg of purified Lyn per assay in the presence of various concentrations of [γ-32P]ATP with or without 0.4 μg/ml EGCG3Me. Reaction products were analyzed by SDS-PAGE, followed by electroblotting onto PVDF membranes and autoradiography. Phosphorylated protein bands were quantified by densitometry. Kinetic data were analyzed by Lineweaver-Burk plotting.

Transcription assays

BMMC were transiently transfected by electroporation with a reporter construct, IL-2/lac (42). Twenty-four hours later, the cells were sensitized with IgE and stimulated by Ag in the absence or presence of O-methylated EGCGs for another 7 h. Luciferase activity was measured, as described previously (42).

 FIGURE 1. Structural formula of EGCG and O-methylated EGCGs isolated from tea leaves.

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Measurement of Ca^{2+} influx

Ca^{2+} influx was analyzed by spectrofluorometry, as previously described (43). Briefly, BMNCs were incubated at 37°C for 30 min with 5 μM fura 2-AM (Dojindo, Osaka, Japan) in Tyrode/HEPES solution after overnight sensitization with anti-DNP IgE. After washing with HBSS, aliquots of the cell suspension were placed in a cuvette and incubated with O-methylated EGCGs at 37°C for 10 min, and then 100 ng/ml DNP-HSA was added. Fluorescence was recorded using a fluorometer (CAF-100; Spectroscopic, Tokyo, Japan) by alternating excitation at 340 and 380 nm and reading the emission at 510 nm. O-methylated EGCGs did not exhibit autofluorescence nor interfere with fura 2 fluorescence.

Passive cutaneous anaphylaxis (PCA) reactions

As a model of in vivo type I allergic reactions, we performed mast cell-dependent PCA experiments, as previously described (44). Briefly, male ddY mice (Japan SLC, Hamamatsu, Japan) were intradermally injected with anti-DNP IgE at both ears. Catechin samples (or distilled water as control) were administered i.v. or orally to the mice 60 min before Ag administration. Intravenous administration of a 0.25-ml mixed solution of Ag (0.1 mg/ml DNP-BSA) and Evans blue dye (0.5%) was i.v. injected 24 h after IgE sensitization. The mice were killed by cervical dislocation 30 min later, and the ears were removed. Evans blue dye leaked into ear tissues was extracted and quantified by spectrophotometry. To confirm the role of histamine released from activated mast cells in inducing PCA reactions, diphenhydramine, an inhibitor of the histamine H_{1} receptor, at 20 mg/kg, was i.p. injected 30 min before Ag challenge. As shown in Fig. 2, this treatment consistently inhibited PCA reactions by 70–85%.

![Figure 2](http://www.jimmunol.org/)

FIGURE 2. Effects of EGCG and O-methylated EGCGs on vascular permeability increase caused by PCA reactions. Mice were sensitized intradermally at the ear with anti-DNP IgE 24 h before Ag challenge. Catechin samples (or distilled water as control) were administered i.v. or orally to the mice 60 min before Ag administration. Intravenous administration of a 0.25-ml mixed solution of Ag (0.1 mg/ml DNP-BSA) and Evans blue dye (0.5%) was i.v. injected 24 h after IgE sensitization. The mice were killed by cervical dislocation 30 min later, and the ears were removed. Evans blue dye leaked into ear tissues was extracted and quantified by spectrophotometry. To confirm the role of histamine released from activated mast cells in inducing PCA reactions, diphenhydramine, an inhibitor of the histamine H_{1} receptor, at 20 mg/kg, was i.p. injected 30 min before Ag challenge. As shown in Fig. 2, this treatment consistently inhibited PCA reactions by 70–85%.

Statistics

Statistical analysis was performed using Student’s t test or Dunnett’s multiple comparison test. Values with p < 0.05 were considered statistically significant.

Results

Effects of tea catechins on PCA reactions

EGCG is known to inhibit immediate hypersensitivity reactions (38, 45). We first compared in vivo effects on mast cell-dependent PCA reactions between different tea catechins, EGCG and its O-methylated derivatives (Fig. 2). Mice were sensitized for 24 h by intradermal injection of anti-DNP IgE at the ear, and they were i.v. injected with DNP-BSA solution containing Evans blue dye. Thirty minutes later, the mice were sacrificed, and the dye leaked into the ear was extracted and quantified. Intraperitoneal administration of EGCG 60 min before Ag/dye injection substantially inhibited allergic reactions (10, 27, and 43% inhibition by a single dose of EGCG at 5, 10, and 20 mg/kg, respectively). Both 3-O- and 4-O-methylated derivatives, EGCG3Me and EGCG4Me, were 2 times more potent in inhibiting PCA reactions than EGCG. Oral administration of EGCG and O-methylated derivatives 60 min before Ag/dye injection exhibited similar inhibition of PCA reactions, with slightly weaker inhibition compared with i.p. administration. Because our experiments were designed to monitor early phase reactions, these results suggest that these catechins, particularly the O-methylated EGCGs, inhibit immediate hypersensitivity reactions by suppressing activation of mast cells. Alternatively, the catechins might antagonize effects of chemical mediators or affect vascular permeability nonspecifically.

O-methylated EGCGs inhibit mast cell cell activation

Because mast cells are the major effector cells in the early phase of immediate hypersensitivity, we explored the effect of the O-methylated EGCGs on mast cell activation. To this end, BMNCs sensitized with IgE were pretreated with these catechins for 30 min before Ag stimulation. As readouts of mast cell activation, we measured histamine release (as a surrogate marker of degranulation), leukotriene release, and cytokine secretion. As shown in Fig. 3A, both EGCG3Me and EGCG4Me inhibited histamine release in a dose-dependent manner. Inhibition by EGCG3Me and EGCG4Me at 100 μg/ml reached 70%. Leukotriene release and IL-2 production/secretion were also inhibited by these catechins (Fig. 3, B and C). It is noteworthy that more than control levels of leukotriene release and IL-2 secretion were observed at lower doses (5 or 25 μg/ml) of the O-methylated EGCGs.

O-methylated EGCGs inhibit PTK activity in mast cells

To gain insight into the mechanism of mast cell inhibition by O-methylated EGCGs, we investigated their effects on early signaling events. IgE-sensitized BMNCs were preincubated with the catechins for 30 min and stimulated with Ag for 3 min in the continued presence of the catechins. Effects on one of the earliest signaling events, tyrosine phosphorylation of the FcεRI β subunit, were examined by immunoprecipitation with anti-FcεRI β Ab, followed by immunoblotting with anti-phosphotyrosine Ab. As shown in Fig. 4A, up to 50 μg/ml of either EGCG derivative did not substantially affect FcεRI β phosphorylation. Interestingly, however, tyrosine phosphorylation of other cellular proteins, as revealed by immunoblotting of total cell lysates (Fig. 4B), was more strongly inhibited: tyrosine phosphorylation of several proteins (100-, 85-, 75-, 56-, 53-, 40-, 38-, and 30-kDa proteins) was obviously inhibited by both catechins in a dose-dependent manner.

We next performed in vitro kinase assays on Lyn, Syk, and Btk immunoprecipitated from O-methylated EGCG-treated, IgE/
most completely by EGCG3
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stantial Syk activity remained at 100
of cellular histamine content was released upon stimulation in the absence
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release are representative of four independent experiments, and those of
lyst event, and cytokine secretion (17, 20, 47, 48), we previously
their effects on the activation of MAPKs (ERKs, JNK, and p38)
through c-Jun phosphorylation (49). BMMC pretreated with
response (Fig. 6).
we performed kinase assays on purified Lyn, Syk, and Btk proteins in the
presence of various concentrations of O-methylated EGCGs (Fig. 5A). We
found that the catalytic activity of all these PTKs is inhibited by both O-methylated EGCGs in a dose-dependent manner. These
PTKs were much more sensitive to O-methylated EGCGs in these
A
B
C
FIGURE 4. Effects of O-methylated EGCGs on early mast cell activation events. A, IgE-sensitized BMMC were left unstimulated (−) or stimulated with Ag for 3 min (+) in the presence of the indicated concentrations of O-methylated EGCGs. FceRI β was immunoprecipitated with anti-FceRI β mAb from cell lysates and immunoblotted with anti-phosphotyrosine 4G10 mAb. B, Immunoblotting of total cell lysates with 4G10 mAb was performed with mast cells left unstimulated (−) or stimulated with Ag for 3 min (+) in the presence of the indicated concentrations of O-methylated EGCGs. C, Cells were left unstimulated (−) or stimulated with Ag for 3 min (+). In vitro kinase assays were done on immune complexes precipitated with anti-Syk using GST-HS1 as an exogenous substrate. Lyn and Btk immunoprecipitates were subjected to kinase assays without substrate. Phosphorylated GST-HS1 and autophosphorylated Lyn or Btk bands were detected by SDS-PAGE and blotting, followed by autoradiography. The amounts of the respective PTKs immu
nonoprecipitated were measured by reprobing the same blots. Approximate IC50 values estimated from these kinase assays are also presented.

Ag-stimulated cells (Fig. 4C). The results demonstrated that both O-methylated EGCGs inhibit catalytic activity of these critical PTKs: EGCG3"Me inhibited Lyn more preferably (IC50 of 30 μg/ml) than Syk (IC50 of 100 μg/ml). Lyn activity was inhibited almost completely by EGCG3"Me at 100 μg/ml, but substantial lev
eels of Lyn activity remained at 100 μg/ml EGCG4"Me. In contrast, Syk activity was totally abrogated by 100 μg/ml EGCG4"Me, but substantial Syk activity remained at 100 μg/ml EGCG3"Me. Btk was also inhibited by EGCG4"Me with an IC50 of 50 μg/ml (Fig. 4C). However, Btk activity was not affected by EGCG3"Me. Treatment with 50 or 100 μg/ml O-methylated EGCGs did not change kinetics of activation of these PTKs or tyrosine phosphorylation (data not shown).

We next investigated whether inhibition of PTKs by O-methylated EGCGs is a direct action or not. To this end, we performed kinase assays on purified Lyn, Syk, and Btk in the presence of various concentrations of O-methylated EGCGs (Fig. 5A). We found that the catalytic activity of all these PTKs is inhibited by both O-methylated EGCGs in a dose-dependent manner. These PTKs were much more sensitive to O-methylated EGCGs in these

Effects of O-methylated EGCGs on late signaling events
Subsequent to activation of the PTKs, various signaling pathways are activated upon FceRI stimulation. Increased intracellular Ca2+ concentration levels following PLC-γ activation by Syk and Btk are critical for FceRI-induced degranulation (46). As expected from PTK inhibition (Figs. 4 and 5) and inhibition of degranulation, leukotriene release, and cytokine production (Fig. 3) by O-
methylated EGCGs, both EGCG3"Me and EGCG4"Me inhibited IgE/Ag-induced Ca2+ response (Fig. 6).

In light of inhibition of IL-2 production/secretion by O-methylated EGCGs at a high concentration of 100 μg/ml, we examined their effects on the activation of MAPKs (ERKs, JNK, and p38) and Akt. Among these P/TKs that were shown to be involved in cytokine production and secretion (17, 20, 47, 48), we previously showed that Akt regulates production of IL-2 and TNF-α (20) and JNK is believed to control the transcription of the IL-2 gene through c-Jun phosphorylation (49). BMMC pretreated with O-
methylated EGCGs were stimulated with Ag, and cell lysates were analyzed by immunoblotting with Abs that react with the activated forms of these kinases. As shown in Fig. 7A, EGCG3"Me inhibited

FIGURE 3. Effects of O-methylated EGCGs on biologic outcomes of mast cell activation. IgE-sensitized BMMC were pretreated with EGCGs and stimulated with Ag for 30 min (histamine release), 45 min (leukotrienes release), or 24 h (cytokine secretion). A, Inhibition of histamine release was expressed as a percentage of reduction compared to control values (histamine release induced by Ag without inhibitor). At least 30–70% of cellular histamine content was released upon stimulation in the absence of inhibitor. * Significantly different from the EGCG-treated group (p < 0.05). B, Release of leukotrienes. C, IL-2 secretion. Results of histamine release are representative of four independent experiments, and those of leukotriene release and cytokine secretion of at least three independent experiments.

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methylated EGCGs were stimulated with Ag, and cell lysates were analyzed by immunoblotting with Abs that react with the activated forms of these kinases. As shown in Fig. 7A, EGCG3"Me inhibited
Akt and JNK1, but not ERK1, ERK2, or p38. Effects of EGCG4\textsubscript{Me} were more complex: it inhibited Akt at 25 mg/ml; it inhibited JNK activity less potently than EGCG3\textsubscript{Me}, but p38 activity was rather enhanced by EGCG4\textsubscript{Me}. Potentially related to the increased IL-2 production/secretion in cells treated with low concentrations of O-methylated EGCGs (Fig. 3C), increased phosphorylations of some of these kinases were observed in cells treated with low concentrations of O-methylated EGCGs: for instance, ERK1, ERK2, and p38 in EGCG3\textsubscript{Me}-treated cells and p38 in EGCG4\textsubscript{Me}-treated cells (Fig. 7A).

Downstream of these serine/threonine kinases, transcription factors are activated. Such activation of transcription factors was monitored by transfecting BMMC with IL-2\textsubscript{luc}, followed by pretreatment of the cells with O-methylated EGCGs and stimulation with IgE and Ag (Fig. 7C). Again, consistent with the inhibition of IL-2 production/secretion with O-methylated EGCGs at high concentrations, IL-2\textsubscript{luc} activity was inhibited by both O-methylated EGCGs at 100 µg/ml. Lower concentrations of O-methylated EGCGs rather enhanced the IL-2\textsubscript{luc} activity.

**Direct effects of high concentrations of O-methylated EGCGs on PS/TK**

Given the broad specificity of PTK inhibition by O-methylated EGCGs, we explored whether these catechins might directly affect the catalytic activity of serine/threonine kinases as well. For this purpose, effects of O-methylated EGCGs were measured in in vitro kinase assays using pure preparations of ERK2, JNK1, and PKC\textsubscript{II} (Fig. 7B). All of these kinases were inhibited by either EGCG in a dose-dependent manner. However, their IC\textsubscript{50} were at least 10- to 50-fold lower than those against the PTKs. Therefore, it is likely that the inhibition of several PS/TKs observed in mast cells treated with the O-methylated catechins is due to inhibition of upstream PTKs. Effects of O-methylated EGCGs on mast cell signal transduction appear more complex than simply inhibiting PTKs: although Lyn kinase activity was inhibited by these agents, FceRI β phosphorylation that is mediated by Lyn was not substantially affected by them. They rather increased the activity of PS/TKs, particularly p38, at low concentrations. These results suggest that O-methylated EGCGs influence other signaling molecules, e.g., a protein phosphatase(s). These catechins are unique in their broad specificity among other known purposes.
mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents. Other PTK inhibitors that were shown to inhibit mast cell activation exhibit more stringent selectivity: for mast cell-inhibitory agents.

**References**


