

Antioxidant Activity of Synthetic Cytokinin Analogues: 6-Alkynyl- and 6-Alkenylpurines as Novel 15-Lipoxygenase Inhibitors

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Abstract—Synthetic cytokinin analogues as well as the well known CKs 6-benzylaminopurine (BAP), kinetin and *trans*-zeatin were examined for antioxidant activity. The compounds were tested as potential diphenylpicrylhydrazyl (DPPH) scavengers and as inhibitors of 15-lipoxygenase (15-LO). The natural plant hormones were essentially inactive in both assays, but several synthetic analogues have a profound inhibiting effect on 15-lipoxygenase from soybeans. The same compounds were only weak DPPH scavengers and they may therefore be regarded as so-called non antioxidant inhibitors of 15-LO. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

Lipoxygenase (LO) was first reported from soybeans more than 50 years ago, and has since then been found in a large number of higher plants.¹ In spite of the widespread occurrence of these enzymes in the plant kingdom, their physiological role is still disputed. At present, it is generally believed that lipoxygenases play a role in the response of plants to wounding,² possibly due to the toxicity of LO metabolites towards invading fungi and bacteria. Other activities of lipoxygenase metabolites have been suggested as well, such as plant hormonal activity,^{2,3} growth regulation and senescence induction,⁴ plant maturation⁵ and decreasing oxygen tension in seeds.⁶

Cytokinins (CK) are plant growth hormones which among other things, promote cell division and cell growth and are involved in retardation of senescence and protection against abiotic oxidative stress. The structures of the cytokinins kinetin, *N*⁶-benzylaminopurine (BAP) and *trans*-zeatin are shown in Figure 1.

Kinetin is reported to reduce lipoxygenase activity in detached pea leaves and induce chlorophyll retention, and some years ago it was proposed that CKs may act as radical scavengers and react directly with superoxide anion radical ($O_2^{\cdot-}$).⁷ The only support for this theory was, however, formation of amides when some aryl amines (not CKs) were reacted with superoxide. In fact, CKs does not react fast with the superoxide anion radical in aqueous solution.⁸ Antioxidant effects mediated by CKs even in mammalian cells are also reported. *trans*-Zeatin and derivatives inhibits lipoperoxidation in rat kidney homogenates. These results indicate that CKs may have a potential as preventors of lipid peroxidation in living animal cells.⁹ However, there are also studies that does not support cytokinin mediated lowering of lipoxygenase activity,¹⁰ and clearly the relationships between cytokinins and antioxidant effects are largely unexplored.

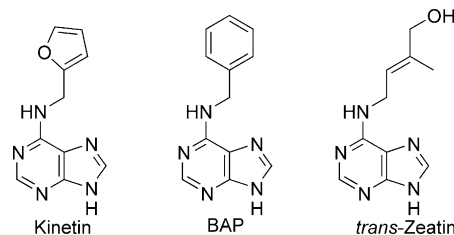


Figure 1. Structure of the cytokinins kinetin, BAP and *trans*-zeatin.

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We have prepared a number of cytokinin analogues where the $\text{-NHCH}_2\text{R}$ substituent found in the purine 6-position in naturally occurring CKs are replaced with a $\text{-C}\equiv\text{CR}$, -CH=CHR or $\text{-CH}_2\text{CH}_2\text{R}$ group. These compounds were believed to be less susceptible to degradation by cytokinin oxidase¹¹ and several of the compounds promoted cell growth within the same range as naturally occurring CKs.^{12,13} We have now examined the potential antioxidant abilities of the same CK analogues and we herein report their ability to scavenge diphenylpicrylhydrazyl (DPPH) and to inhibit 15-lipoxygenase.

Results and Discussion

Our synthetic cytokinin analogues as well as the well known CKs BAP, kinetin and *trans*-zeatin were examined as potential DPPH scavengers. All compounds examined can formally be represented as the general structures shown in Figure 2, all though the oxopurines ($\text{X}=\text{OH}$) exist mainly as lactam tautomers.

The results from the reaction with DPPH are shown in Table 1. The 6-aminopurines BAP, kinetin and *trans*-zeatin were not at all able to scavenge DPPH at 667 μM concn. None of the synthetic CK analogues was highly active DPPH scavengers, but several compounds were significantly more active than BAP, kinetin and *trans*-zeatin. Generally 2-oxopurines were somewhat more active than the corresponding purines without any substituent in the 2-position. No conclusion can be drawn at this point as to whether a 9-substituent is beneficial

for scavenging ability. Only alkynes **2** and **5** and *trans* alkenes **3** and **6** displayed any activity. The most active compounds were 6-(3-thienylethenyl)purine **3m** with 18% scavenging after 15 min and the furanes **3h** and **3i**. These were actually more active than the phenols **3d** and **3e** (entries 11 and 12). We found no correlation between DPPH scavenging and the plant growth stimulation we previously reported.^{12,13}

The CKs and analogues were also tested as potential inhibitors of 15-lipoxygenase (15-LO) from soybeans. Again the well known CKs BAP, and *trans*-zeatin were completely inactive and kinetin showed only a weak inhibitory activity. However, several synthetic CK analogues were profound enzyme inhibitors. All purines were tested for enzyme inhibition at 167 μM concn. IC_{50} values were determined for compounds showing more than 50% inhibition in the initial screening (Table 1). There were no correlations between the ability to mediate antioxidant activity and to stimulate plant growth. Furthermore, there were no direct correlations between enzyme inhibition and DPPH scavenging activity, indicating that the purines mediate their effect by binding to the enzyme and not by scavenging radicals formed from 15-LO oxidation of fatty acids. A $\text{-C}\equiv\text{CR}$ or *trans* -CH=CHR groups in the 6-position were required for high activity. Highest activity (IC_{50} values between 90 and 110 μM) were found for the alkynes and alkenes **2**, **3a**, **3b** and **3f**, which can be regarded as BAP analogues, and the *trans*-zeatin analogues **5a**, **5d**, **6a** and **6b**. Compounds **4** and **7** with a $\text{-CH}_2\text{CH}_2\text{R}$ group in the purine 6-position or a phenyl group bound directly to the purine ring (**1**) were only weakly active (5–25% inhibition at

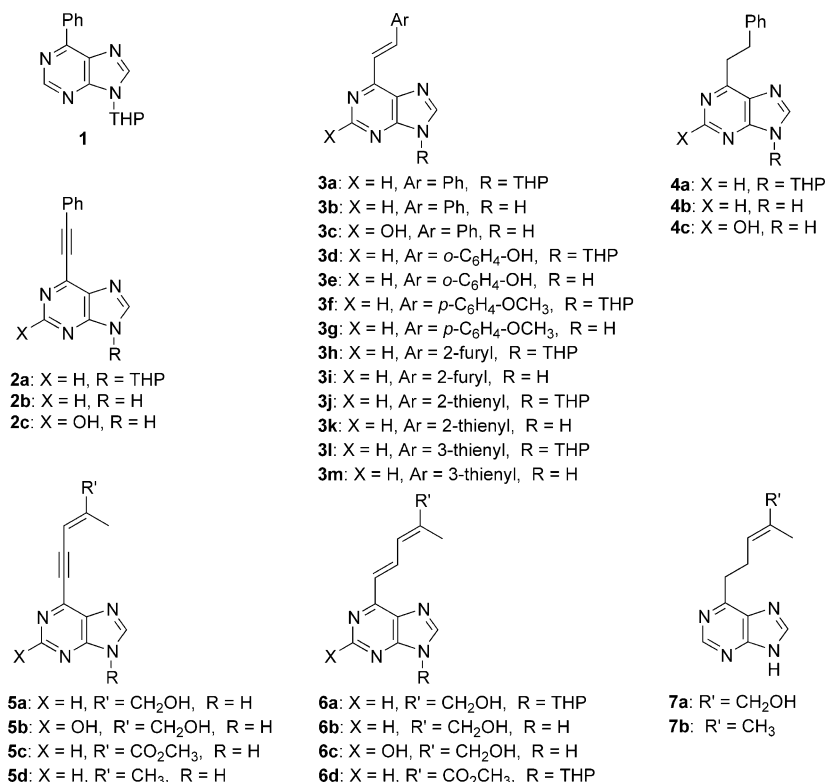


Figure 2. Structures of the cytokinin analogues 1–7.

167 μM concn). The substituents in the 2- and 9-position have no consistent effect on the enzyme inhibiting ability.

Most of the 15-LO inhibiting compounds reported herein have IC_{50} values between 90 and 120 μM . This compares well with previously reported 15-LO inhibitors of plant origin, such as quercetin (98 μM), sinenseitin (114 μM) and tetramethylscutellarein (110 μM),¹⁴ and also with synthetic inhibitors, for example dialkylphthalates (IC_{50} values between 93 and 108 μM).¹⁵

We can see no obvious structural relationship between the purines examined in this study and known LO inhibitors. To the best of our knowledge, this is the first report on lipoxygenase inhibitors with purine structure, except for the possible role of cytokinins as inhibitors of lipoxygenase activity. The only other relationship between purines and lipoxygenases we know of, is the fact that calcium dependent 5-lipoxygenase activity is stimulated by the addition of adenosine triphosphate (ATP)¹⁶ and that it has recently been reported that 15-LO from soybeans catalyze hydrolysis of ATP and the subsequent release of inorganic phosphate. However, the latter reaction was not affected by the addition of the lipoxygenase inhibitor phenidone¹⁷ and may not be

connected to the purine mediated enzyme inhibition described herein.

The results presented herein demonstrates that several 6-alkenyl and 6-alkynylpurines have a profound inhibiting effect on 15-lipoxygenase from soybeans. The same compounds were only weak DPPH scavengers and they may therefore be regarded as so-called non antioxidant inhibitors of 15-LO. We used 15-lipoxygenase from soybeans, but it has been shown that there is a good correlation for inhibitory activity for the soybean and mammalian 15-LO enzyme^{18,19} even though these two are not identical. 15-Lipoxygenase has been implicated in oxidation of low density lipoproteins (LDL). This process is believed to be important for the development of atherosclerosis^{20,21} and non antioxidant 15-LO inhibitors may have an anti-atherosclerotic effect in vivo.^{22–24} The current lack of lipoxygenase inhibitors limits further investigation of the biological role of these enzymes.²⁵ If continued research on the synthetic cytokinin analogues reported herein reveals that they also are inhibitors of human lipoxygenases, the purines may have a potential as drugs against atherosclerosis and maybe even against other major diseases as for instance cancer, Parkinson's disease, Alzheimer's disease, stroke, heart infarction and rheumatoid arthritis which are also linked to free radicals.^{26–28}

Table 1. Scavenging of DPPH and inhibition of 15-lipoxygenase

Compd	% DPPH scavenging after 5 min	Inhibition of 15-Lipoxygenase	
	667 μM concn ^a	% Inhibition at 167 μM ^a	IC_{50} ^a
BAP	-0.1 ± 0.4	-6.1 ± 7.6	n.d.
Kinetin	0.1 ± 0.2	9.2 ± 2.6	n.d.
<i>t</i> -Zeatin	-0.2 ± 0.1	-1.3 ± 10.4	n.d.
1	1.1 ± 0.3	17.1 ± 4.0	n.d.
2a	0.2 ± 0.3	76.2 ± 4.7	102 ± 11
2b	0.6 ± 0.1	85.4 ± 1.1	102 ± 3
2c	3.2 ± 0.1	83.8 ± 2.9	107 ± 6
3a	0.4 ± 0.0	84.9 ± 1.5	104 ± 6
3b	-0.2 ± 0.3	89.6 ± 3	96 ± 5
3c	1.0 ± 0.4^b	27.1 ± 6.5^d	n.d.
3d	5.9 ± 0.5	73.4 ± 2.2	119 ± 11
3e	3.5 ± 0.3	61.7 ± 3.5	138 ± 10
3f	0.2 ± 0.3	82.4 ± 1.2	95 ± 5
3g	0.8 ± 0.1	79.8 ± 1.2	> 166
3h	9.7 ± 0.7	54.1 ± 4.9	148 ± 22
3i	8.6 ± 0.4	45.0 ± 4.4	n.d.
3j	1.4 ± 0.1	39.8 ± 5.1	n.d.
3k	1.1 ± 0.1	45.9 ± 5.3	n.d.
3l	2.6 ± 0.9	59.8 ± 2.1	141 ± 8
3m	18.0 ± 0.9	63.8 ± 4.1	136 ± 11
4a	0.0 ± 0.3	24.5 ± 4.5	n.d.
4b	-0.1 ± 0.2	4.8 ± 8	n.d.
4c	1.2 ± 0.3^c	15.9 ± 7.7	n.d.
5a	1.6 ± 0.3	77.2 ± 4.9	108 ± 10
5b	3.6 ± 0.5	61.2 ± 4.3	130 ± 17
5c	4.2 ± 0.6	90.8 ± 1.5	116 ± 7
5d	1.9 ± 0.1	74.5 ± 1.5	105 ± 6
6a	4.0 ± 0.4	76.9 ± 1.6	106 ± 5
6b	2.9 ± 0.6	83.5 ± 2.5	92 ± 5
6c	7.0 ± 1.2	39.3 ± 5.1	n.d.
6d	0.8 ± 0.1	63.6 ± 4.8	131 ± 12
7a	1.1 ± 0.1	5.3 ± 5.5	n.d.
7b	0.5 ± 0.1	15.7 ± 6.4	n.d.

^aData are shown $\pm$ SD.

^b83.5 μM concn.

^c333 μM concn.

^d83.5 μM concn.

Experimental

6-Benzylaminopurine, kinetin and *trans*-zeatin were purchased from Fluka (Buchs, Switzerland) and diphenylpicrylhydrazyl (DPPH) and 15-lipoxygenase (Type 1) from Sigma (St. Louis, MO, USA). Silica gel for flash chromatography was available from Merck (Darmstadt, Germany) (Merck No. 9385) or Fluka (Fluka No. 60752). DMF, pyrrolidine, and diisopropylethylamine were distilled from CaH₂, and THF from Na/benzophenone. Tetramethylammonium fluoride trihydrate was dried by azeotropic distillation with abs ethanol. Compounds **2c**,¹³ **3a–b**,¹² **3c**,¹³ **3d–3m**,¹² **4c**,¹³ **5a**,¹² **5b**,¹³ **6a–b**,¹² **6c**,¹³ **6d**,¹² and **7a–b**¹³ were prepared as we have described before. 6-Iodopurine,²⁹ 6-iodo-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine³⁰ and 6-phenyl-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine **1**³¹ were available by literature methods. The ¹H NMR spectra were recorded at 500 MHz with a Bruker Avance DRX 500 instrument, at 300 MHz with a Bruker Avance DPX 300 instrument or at 200 MHz with a Bruker Avance DPX 200 instrument. The ¹³C NMR spectra were recorded at 125, 75 or 50 MHz using instruments mentioned above. Unless otherwise stated, the spectra are recorded at ambient temperature. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane. Mass spectra were recorded with a VG Prospec instrument at 70 eV ionizing voltage unless otherwise stated, and are presented as *m/z* (% rel. int.). Melting points are uncorrected. All measurements of DPPH scavenging and 15-lipoxygenase activity were carried out in a Shimadzu UV-160A spectrophotometer (Shimadzu, Kyoto, Japan).

6-(Phenylethynyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (2a). 6-Iodo-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (500 mg, 1.51 mmol), copper(I) iodide (29 mg, 0.15 mmol) and bis(triphenylphosphine)palladium(II) chloride (53 mg, 0.076 mmol) was dissolved in dry DMF (10 mL) under Ar atmosphere. Phenylethyne (3.35 mL, 3.03 mmol) and ethyldiisopropylamine (6.00 mL, 4.54 mmol) were added, and the mixture was heated at 60 °C for 17 h. After cooling to ambient temperature, a small amount of silica gel was added, and the volatiles were evaporated in vacuo. The crude product was purified by flash chromatography on silica gel eluting with pure hexane to pure ethyl acetate. Yield 386 mg (84%), mp 124–126 °C. ¹H NMR (200 MHz, CDCl₃) δ 8.92 (s, 1H, H-2), 8.32 (s, 1H, H-8), 7.75–7.68 (m, 2H, Ph), 7.41–7.33 (m, 3H, Ph), 5.81–5.75 (m, 1H, H-2 in THP), 4.20–4.13 (m, 1H, THP), 3.83–3.70 (m, 1H, THP), 2.17–2.01 (m, 3H, THP), 1.85–1.66 (m, 3H, THP). ¹³C NMR (50 MHz, CDCl₃) δ 152.6, 150.8, 143.0, 141.8, 134.3, 132.6, 129.9, 128.4, 121.4, 98.4, 84.1, 82.1, 68.8, 31.8, 24.8, 22.7, 19.0. MS (EI): 304 (*M* + , 42), 276 (16), 247 (7), 222 (12), 221 (79), 220 (100), 219 (9), 193 (10), 166 (10), 165 (7), 140 (8), 139 (17), 128 (6), 113 (8), 85 (31). HRMS: found: 304.1330, calcd for C₁₈H₂₆N₄O: 304.1324.

6-(Phenylethynyl)-1*H*-purine (2b). A mixture of 6-(phenylethynyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine **2a** (150 mg, 0.493 mmol), hydrochloric acid (10 mL, 1.0M)

and ethanol (20 mL, 96%) was stirred at ambient temperature for 1 h 45 min before neutralizing with solid NaHCO₃. A small amount of silica gel was added, and the volatiles were evaporated in vacuo. The crude product was purified by flash chromatography on silica gel eluting with 0–10% ethanol in ethyl acetate. Yield 85 mg (89%) off-white powder, mp 206–207 °C (lit.³² 198–199 °C). ¹H NMR (200 MHz, CD₃OD) δ 8.90 (s, 1H, H-2), 8.60 (s, 1H, H-8), 7.80–7.75 (m, 2H, Ph), 7.52–7.41 (m, 3H, Ph). MS (EI): 220 (*M* + , 100), 219 (5), 193 (6), 166 (7), 139 (12), 114 (4), 113 (5), 87 (1), 66 (3).

6-(2-Phenylethyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (4a). 6-Iodo-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (660 mg, 2.0 mmol), tris(dibenzylideneacetone)-dipalladium chloroform adduct (52 mg, 0.05 mmol) and triphenylphosphine (105 mg, 0.4 mmol) were dissolved in dry THF (5 mL) and stirred under N₂ atmosphere until the mixture turned yellow. Phenylethylzinc chloride (8 mL, 4 mmol, 0.5M in THF) was added and the mixture was stirred at 50 °C for 24 h and poured into NH₄Cl (satd, 50 mL). The resulting mixture was extracted with EtOAc (3×40 mL), and the combined extracts were dried (MgSO₄) and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel eluting with 0–70% EtOAc in hexane. Yield 411 mg (67%) off-white solid, mp 113–114 °C. ¹H NMR (200 MHz, CDCl₃) δ 8.85 (s, 1H, H-2), 8.20 (s, 1H, H-8), 7.26–7.05 (m, 5H, Ph), 5.71 (m, 1H, THP), 4.05 (m, 1H, THP), 3.68 (m, 1H, THP), 3.45 (m, 2H, CH₂), 3.18 (m, 2H, CH₂), 2.04–1.95 (m, 3H, THP), 1.67–1.56 (m, 3H, THP). ¹³C NMR (50 MHz, CDCl₃) δ 161.3, 152.0, 149.7, 141.3, 140.8, 132.3, 128.0, 127.98, 125.6, 81.5, 68.4, 34.5, 33.8, 31.3, 24.5, 22.4. MS (EI): 308 (*M* + , 6), 225 (15), 224 (100), 223 (60), 208 (9), 147 (30), 120 (5), 91 (33), 84 (6), 65 (13). HRMS: found: 308.1636, calcd for C₁₈H₂₀N₄O: 308.1637.

6-(2-Phenylethyl)-1*H*-purine (4b). A mixture of 6-(2-phenylethyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine **4a** (228 mg, 0.74 mmol), ethanol (15 mL, 96%) and hydrochloric acid (10 mL, 1.0M) was stirred at ambient temperature for 4 h before neutralizing with solid NaHCO₃. A small amount of silica gel was added, and the volatiles were evaporated in vacuo. The crude product was purified by flash chromatography on silica gel eluting with 0–20% ethanol in EtOAc. Yield 124 mg (74%) colorless powdery crystals, mp 138–139 °C (lit.³² 135–137 °C). ¹H NMR (500 MHz, CD₃OD) δ 8.81, (s, 1H, H-2), 8.44 (s, 1H, H-8), 7.19 (m, 4H, Ph), 7.11 (m, 1H, Ph), 3.42 (m, 2H, CH₂), 3.14 (m, 2H, CH₂). ¹³C NMR (125 MHz, CDCl₃, 50 °C) δ 160.6, 150.1, 153.2, 145.7, 142.2, 130.6, 129.4, 129.3, 127.1, 35.9, 35.2. MS (EI): 224 (*M* + , 100), 223 (70), 209 (4), 208 (9), 207 (3), 197 (2), 196 (3), 147 (36), 120 (5), 101 (7), 91 (42), 77 (5), 65 (16).

6-(5-Carbomethoxy-4-methyl-3-penten-1-yn-1-yl)-1*H*-purine (5c). To a solution of 2-methyl-pent-2-en-4-ynoic acid (435 mg) in methanol (30 mL) was added concd sulfuric acid (10 drops) and the resulting mixture was refluxed for 24 h, cooled to ambient temperature and poured into aqueous potassium carbonate (10%, 50 mL). The mixture was extracted with chloroform (4×50 mL).

After drying (Na_2SO_4) and evaporation, the product was purified by Kugelrohr distillation (17 mmHg, 150°C) to give methyl 2-methylpent-2-en-4-ynoate 178 mg (36%) as a pale yellow oil. ^1H NMR (200 MHz, CDCl_3) δ 6.57 (m, 1H, H-3), 3.75 (s, 3H, OCH_3), 3.51 (m, 1H, H-5), 2.06 (m, 3H, CH_3). 6-Iodopurine (295 mg, 1.20 mmol), bis(triphenylphosphine)palladium(II) dichloride (42 mg, 0.060 mmol) and CuI (23 mg, 0.12 mmol) were dissolved in dry DMF (6 mL) under nitrogen atmosphere. Ethyldiisopropylamine (620 μL , 3.60 mmol) was added via syringe, before methyl 2-methylpent-2-en-4-ynoate (178 mg, 1.43 mmol) was added and the mixture was stirred at 70°C for 18 h. The volatiles were removed in vacuo and the crude product was purified using flash chromatography on silica gel eluting with ethyl acetate. Yield 121 mg (42%) colorless powdery crystals, mp $177\text{--}181^\circ\text{C}$. ^1H NMR (500 MHz, CD_3OD) δ 8.90 (s, 1H, H-2), 8.59 (s, 1H, H-8), 6.95 (m, 1H, H-3'), 3.82 (s, 3H, CO_3CH_3), 2.26 (m, 3H, CH_3). ^{13}C NMR (125 MHz, CDCl_3 , 50°C) δ 168.2, 153.5, 147.7, 144.6, 133.7, 133.2, 130.0, 118.2, 94.8, 94.5, 53.5, 16.1. MS (EI): 242 (M^+ , 100), 211 (5), 184 (10), 183 (37), 182 (52), 156 (4), 129 (4), 128 (1), 102 (2), 76 (1). HRMS: found: 242.0830, calcd for $\text{C}_{12}\text{H}_{10}\text{N}_4\text{O}_2$: 242.0804.

6-(4-Methyl-3-penten-1-yn-1-yl)-1H-purine (5d). A mixture of tris(dibenzylideneacetone)dipalladium chloroform adduct (129 mg, 0.125 mmol), triphenylphosphine (262 mg, 1.00 mmol) and copper(I) iodide (95 mg, 0.50 mmol) in dry pyrrolidine (10 mL) was stirred at ambient temperature under N_2 -atm. 1-Bromo-2-methylpropene (675 mg, 5.0 mmol) and ethynyl(trimethyl)silane (1.40 mL, 10 mmol) were added and the resulting mixture was stirred at 40°C for 14 h. Satd aq NH_4Cl (40 mL) was added and the mixture was extracted with diethyl ether (4×20 mL). After drying (Na_2SO_4) and evaporation, the product was purified by Kugelrohr distillation (12 mmHg, $70\text{--}80^\circ\text{C}$) to give 4-methyl-1-trimethylsilyl-3-penten-1-yne 484 mg (64%) as a colorless liquid. ^1H NMR (CDCl_3 , 300 MHz): δ 5.27 (m, 1H, H-3), 1.89 (d, J 0.5 Hz, 3H, CH_3), 1.78 (d, J 1.2 Hz, 3H, CH_3), 0.15 (s, 9H, TMS); MS (EI) 152 (25, M^+), 137 (100). 4-Methyl-1-trimethylsilyl-3-penten-1-yne (1.34 g, 8.8 mmol) and ethyldiisopropylamine (0.51 mL, 3.0 mmol) were added to a mixture of 6-iodo-1H-purine (246 mg, 1.0 mmol), bis(triphenylphosphine)palladium(II) chloride (35 mg, 0.05 mmol), copper(I) iodide (19 mg, 0.1 mmol) and dry tetramethylammonium fluoride (10 mmol) in dry DMF (6 mL) and the resulting mixture was stirred for 24 h at 60°C . The mixture was evaporated and the product isolated after flash chromatography on SiO_2 eluting with EtOAc followed by $\text{EtOAc}\text{--EtOH}$ (95:5). Yield 69 mg (35%) yellow crystalline solid, mp $189\text{--}191^\circ\text{C}$. ^1H NMR (CD_3OD , 300 MHz) δ 8.81 (s, 1H, H-2), 8.52 (s, 1H, H-8), 5.69 (m, 1H, H-3'), 2.11 (s, 3H, CH_3), 1.95 (s, 3H, CH_3). ^{13}C NMR ($\text{DMSO}-d_6$, 125 MHz, 50°C) δ 154.4, 153.8, 151.7, 145.5, 138.9, 131.2, 103.9, 95.3, 86.9, 24.5, 21.2. HRMS: Found 198.0913, $\text{C}_{11}\text{H}_{10}\text{N}_4$ requires 198.0905.

DPPH scavenging

Scavenging activity towards the diphenylpicrylhydrazyl (DPPH) was measured as the decrease in absorbance at

517 nm of a methanolic DPPH solution ($A_{517} = 1.0$, 2.95 mL) over a 5-min period after addition of 50 μL of a DMSO solution of the test substance.³³ Appropriate corrections were made for dilution and for absorbance of the reaction product, reduced DPPH. Calculation of radical scavenging activity was carried out as previously described.³⁴

Inhibition of 15-lipoxygenase

Lipoxygenase activity was measured in borate buffer solutions (0.2 M, pH 9.00) as previously described^{14,33} by the increase in absorbance at 234 nm from 30 to 90 s after addition of the enzyme, using linoleic acid (134 μM) as substrate. The final enzyme concentration was 167 U/mL. Test substances were added as DMSO solutions (final DMSO concn 1.6%); DMSO alone was added in uninhibited control experiments. Six or more parallels of controls and three or more parallels for each test substance solution were measured. To ensure constant enzyme activity throughout the experiment, the enzyme solution was kept on ice, and controls were measured at regular intervals.

Calculation of enzyme activity was carried out as previously described¹⁴ and IC_{50} values were determined by linear interpolation between the measuring points closest to 50% activity. Values are expressed as means $\pm$ SD. Student's t -test was employed for determination of statistical significance.

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