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Synthesis of modified homo-*N*-nucleosides from the reactions of mesityl nitrile oxide with 9-allylpurines and their influence on lipid peroxidation and thrombin inhibition

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ABSTRACT

9-(3-Mesityl-4,5-dihydroisoxazol-5-yl) homo-*N*-nucleosides were prepared from the 1,3-dipolar cycloaddition reactions of mesityl nitrile oxide with 9-allyl derivatives of 6-chloropurine, 6-piperidinylpurine, 6-morpholinylpurine, 6-pyrrolidinylpurine, and 6-*N*,*N*-dibenzoyladenine. The new compounds were tested in vitro for their ability: (i) to interact with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, (ii) to inhibit lipid peroxidation, (iii) to scavenge the superoxide anion, (iv) to inhibit the activity of soybean lipoxygenase, and (v) to inhibit in vitro thrombin. Most of them found to be potent thrombin inhibitors and to inhibit in vitro lipid peroxidation. The majority of the compounds showed significant lipoxygenase inhibitory activity.

Oxidation is an important process which produces free radicals in food, chemicals, and in living systems. Persistently high levels of reactive oxygen species (ROS) are believed to produce pathological conditions. The rate of ROS production is increased in many diseases.[1] ROS, like superoxide radical anion, hydrogen peroxide and hydroxyl radical, are produced during the inflammation process by phagocytic leukocytes at the inflamed site and they are involved in the biosynthesis of prostaglandins and in the cycloxygenase-(CO) and lipoxygenase-(LO) mediated conversion of arachidonic acid into proinflammatory intermediates.[2,3] LO products are regulators of platelet $[Ca^{2+}]_i$ mobilization and aggregation in response to some agonists. LO inhibitors may work in part by modifying platelet cyclic AMP metabolism. The most of the LO inhibitors are antioxidants or free radical scavengers,[4] since lipoxygenation occurs via a carbon-centered radical. Antioxidants are defined as substances that even at low concentration significantly delay or prevent oxidation of easy oxidizable substrates and there is an increased interest of using antioxidants for medical purposes in recent years. Evidence points toward extensive cross-talk between coagulation and inflammation, whereby inflammation not only activate the coagulation pathway, but coagulation also considerably affects inflammation. It has been proved that inflammation plays an important role in post-thrombolytic complications whereas it is induced by thrombolytic therapy in patients with acute myocardial infarction.[5] It is now widely accepted that activation of the coagulation cascade, with initiation of thrombin and fibrin deposition is a consequence of inflammation. Once thought to be completely different processes, the boundaries between inflammation and coagulation are now nearly indistinguishable.[6]

Thrombine, a serine protease of the trypsin family, is a proteolytic enzyme[7] that can elicit many inflammatory responses in microvascular endothelium. Its multiple role in thrombosis makes thrombin an important target for the therapeutic agents designed for thrombus prevention.[8] LO inhibitors reduce platelet aggregation induced by thrombin and U46619 and modify release of Ca^{2+} from intracellular[9] stores. The development of selective, orally active, low molecular weight synthetic thrombin inhibitors have been an intense research focus in the search for new anticoagulants.[10]

Nucleosides represent a class of compounds that possess very interesting biological activities,[11,12] especially antiviral and anticancer. The adenosine (**I**) generated at inflamed site is receiving increasing interest as an endogenous anti-inflammatory agent and presents potential pharmacological uses as anti-inflammatory agent.[13,14] Initial studies of the effects of adenosine on human neutrophiles[14] indicated that adenosine inhibits stimulated $O_2^{\cdot-}$ or H_2O_2 generation. The modified derivatives of adenine (with carbocyclic ring in the place of the sugar moiety) aristeromycin (**II**) and neplanocin A (**III**) are natural products with antibiotic and antioncogenic activities,[15] whereas the synthetic derivative abacavir (**IV**)

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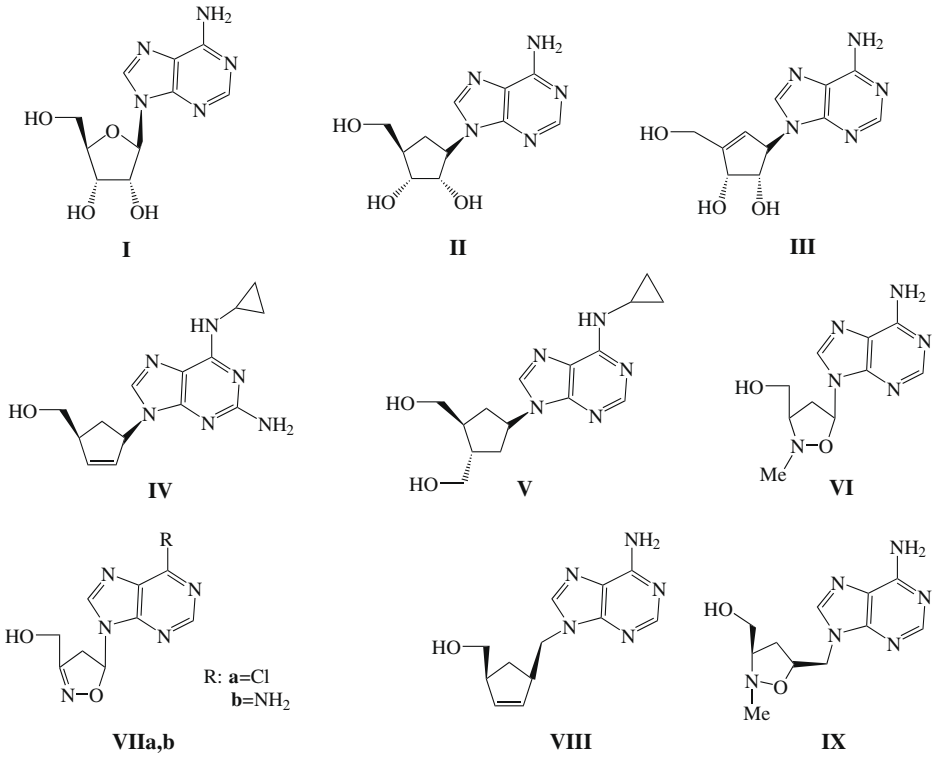
is an HIV inhibitor,[16] while the derivative **V** has been tested for its antitumorial activity.[16] Modified nucleosides with heterocyclic rings,[17] such as isoxazolidine[18–20] **VI** and isoxazoline derivatives[18–21] **VIIa, b** have been studied for anti-HIV and anticancer activities. The homo-*N*-nucleosides with a CH_2-group between adenine and carbocyclic or heterocyclic ring possess higher conformational[22,23] flexibility to combine with the bases of DNA/RNA by a lowering of the electrostatic repulsion.[23] The derivative **VIII** was prepared[24] in the search for agents against HIV and hepatitis B viruses in comparison to carbovir. The isoxazolinyl-derivative **IX**[23] is a special glucosidase inhibitor.

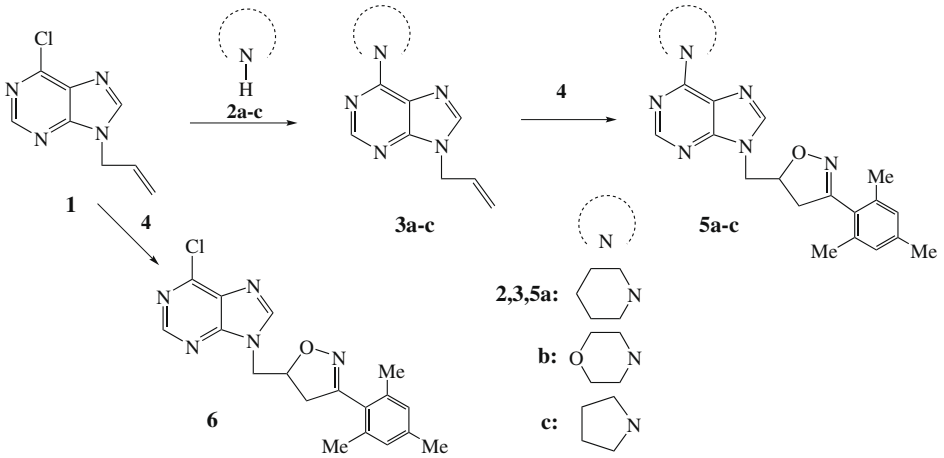
is therefore evident that the treatment of coronary artery diseases could benefit from the use of drugs that combine anti-inflammatory, antioxidant, and antithrombotic activity. Several methods are used to estimate the efficiency of synthetic/natural antioxidants, like the 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH)/linoleic acid assay,[26] and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.[27]

The adenine derivatives **3a–c** were prepared[28] by amination of compound **1** with piperidine (**2a**), morpholine (**2b**), or pyrrolidine[29] (**2c**) under microwave (MW) irradiation at 100 °C in H_2O. We synthesized also the piperidinyl and morpholinyl derivatives[29]

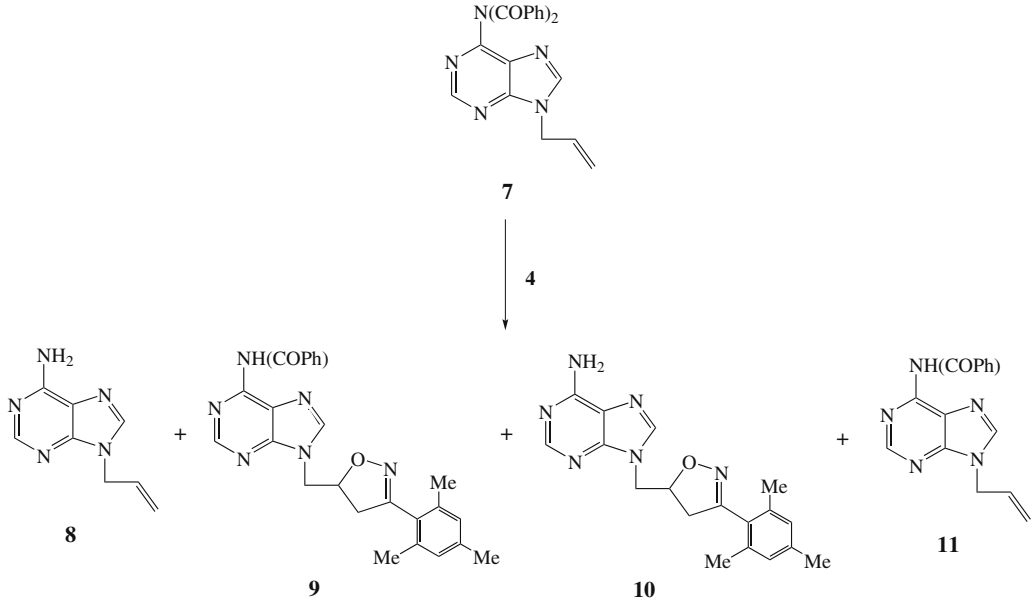


In this work we present the reactions of mesityl nitrile oxide (**4**) with 9-allyl-6-chloropurine (**1**),[25] the adenine derivatives **3a–c** and **7** (Schemes 1 and 2) and we evaluate the resulting isoxazolines 5a–**c**, **6**, **9**, and **10** as lipid peroxidation and thrombin inhibitors. It

3a and **3b** by refluxing in water in better yields (95% and 91%, respectively) than before.[28] The reaction[29] of the 6-piperidinylpurine derivative **3a** with the nitrile oxide **4** in refluxing toluene resulted to the isoxazolinyl derivative **5a** (52%) after separation by



Scheme 1. Reagents and conditions: (i) H_2O, reflux, 24 h (for **3a**, **3b**); H_2O, MW, 100 °C, 7 min (for **3c**); (ii) toluene, reflux, 2 d (for **5a**), 6 d (for **5b**, **5c**); (iii) Toluene, reflux, 5 d.



Scheme 2. Reagent and conditions: (i) toluene, reflux, 7 d.

column chromatography, while 12% of the starting compound remained unchanged. The analogous reactions of the 6-morpholinylpurine **3b** or 6-pyrrolidinylpurine **3c**, gave[29] the isoxazolinyl nucleosides **5b** (62%) or **5c** (61%) along with unreacted compounds **3b** (23%) or **3c** (25%). From the reflux of a mixture of 9-allyl-6-chloropurine (**1**) with the nitrile oxide **4** in toluene we isolated the isoxazolinyl derivative **6** (72%) (22% of compound **1** recovered).

The dibenzoyl protected adenine derivative **7**, prepared[30] by benzoylation of 9-allyladenine (**8**), was heated under reflux with mesityl nitrile oxide (**4**) to give,[29] after separation and column chromatography, the mono-benzoyl isoxazolinyl nucleoside **9** (37%) followed by the 9-allyl-6-*N*-benzoyladenine (**11**)[31] (22%), the 9-allyladenine (**8**) (35%) and the isoxazolinyl adenine derivative **10** (6%). The products obtained above are produced through hydrolization of the 1,3-cycloaddition reaction product and of the starting material **7** under the reaction conditions.

Compounds with antioxidant properties are expected to offer protection in inflammation and thrombosis and to lead to effective drugs. Reduction of the DPPH stable free radical, by the examined compounds, was studied[32,33] at 0.05 mM and 0.1 mM after 20 and 60 min (Table 1). The DPPH assay is the best-known, most frequently employed, and most accurate method. The new compounds (Table 1) did not present any reducing activity in both concentrations and after 20/60 min, in comparison to the well known antioxidant agent nordihydriguaiaretic acid (NDGA).

In our studies AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to the conjugated diene hydroperoxide.[26,34] Compounds **5a, 5b, 5c, 6**, and **9** showed excellent inhibition of lipid peroxidation (Table 1) compared to 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), used as a standard (73%), whereas compound **10** was found to provide very low inhibition of lipid peroxidation. No differences are observed between compounds **5b** (morpholinyl) and **9** (–NHCOPh).

Our results pointed out that the homo-*N*-nucleosides derivatives highly inhibit lipid peroxidation. These compounds may prove useful for treating a variety of inflammatory and coronary artery diseases and may lead to the development of new drugs. Compound **10** with a free NH_2 group did not present any biological activity under our experimental conditions. Thus, the presence of a substituent to the amino group seems to be crucial for the inhibition of lipid peroxidation. The role of lipophilicity, as well as, the role of the 6-substituent's stereochemistry is not well defined.

Enzymatic superoxide anion radicals were generated by a hypoxanthine and xanthine oxidase (XOD) reaction system.[34–36] The majority of the compounds did not present/ or presented low scavenging activity at 0.1 mM compared to caffeic acid used as a standard (10%) (Table 1).

Compounds were further evaluated for inhibition of soybean lipoxygenase (LO) by the UV absorbance based enzyme assay.[32] Compounds **5b** and **5c** present equipotent inhibition (IC_{50} = 100 μM). No differentiation was observed between the pyrrolidi-

Table 1
Interaction% with 1,1-diphenyl-2-picrylhydrazyl (DPPH%) at 0.05 mM and at 0.1 mM; inhibition of lipid peroxidation at 100 μM (LP%); superoxide radical scavenging activity (O_2^- %) at 100 μM; in vitro inhibition of soybean lipoxygenase (LO)% at 100 μM; in vitro inhibition of thrombin (Thr%)[34]

No.	DPPH% 20/60 min 0.05 mM	DPPH% 20/60 min 0.1 mM	LP% at 100 μM	O_2^- % at 100 μM	LO% inhb. at 100 μM	Thr% inhb. at 100 μM
5a	nd/nd	1/1	60	12	42	67
5b	nd/nd	4/4	81	1	IC_{50} = 100 μM	28
5c	11/11	nd/nd	52	nd	IC_{50} = 100 μM	69
6	2/2	2/2	52	13	66	41
9	nd/nd	8/10	83	19	84	100
10	nd/nd	nd/nd	5	nd	nd	nd
NDGA	68/72	81/83				
CA				10		
NAPAP						100
Trolox			73			

NDGA: nordihydroguaiaretic acid; CA: caffeic acid; NAPAP: N^{α}-(2-naphthyl-sulfonyl-glycyl)-D,L-*p*-amidinophenylalanyl-piperidine); nd: not determined results under the reported experimental conditions; each experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

nyl-(**5c**) and morpholinyl-(**5b**) substituents. No inhibition was found for compound **10** under the reported experimental conditions. Compound **9** presents higher % inhibition values, followed by **6** and **5a**. For all the compounds the LO% inhibition values are in agreement with these on lipid peroxidation.

We evaluated the ability of the compounds to inhibit thrombin.[37,38] Compound **9** is the most potent inhibitor, followed by **5c** and **5a** which are almost equipotent. The presence of a –COPh group as substituent at position 6- is significant for the inhibition of thrombin (100% inhibition). (Table 1) The presence of a piperidinyl or of a pyrrolidinyl ring leads to similar biological responses (67% and 69%).

Our studies confirm that the presence of substituent at position 6- is an important structural feature for the antioxidant/antiinflammatory and anti-thrombin activity. This provides an impetus for designing new dual acting agents using the N-substituted 6-amino purine scaffold as the starting point.

It is evident that, from all the tested compounds, **9** exhibits satisfactory combined antioxidant-antiinflammatory activity and thrombin inhibitory ability (100% inhibition at 100 μM), whereas compound **5c** presents high LO inhibitory activity in combination to significant anti-thrombin activity. Therefore the design of this type of dual acting molecules should be further explored.

References and notes

1. Jacob, R. A.; Burri, B. J. *Am. J. Clin. Nutr.* **1996**, *63*, 985.
2. Garrido, G.; Gonzalez, D.; Delporte, C.; Backhouse, N.; Quintero, G.; Nunez-Selles, A. J.; Morales, M. A. *Phytother. Res.* **2001**, *15*, 18.
3. Weber, V.; Coudert, P.; Rubat, C.; Duroux, E.; Vallee-Goyet, D.; Gardette, D.; Bria, M.; Albuisson, E.; Leal, F.; Gramain, J.-C.; Couquelet, J.; Madesclaire, M. *Bioorg. Med. Chem.* **2002**, *10*, 1647.
4. Muller, K. *Arch. Pharm.* **1994**, *327*, 3.
5. Merlini, P. A.; Cugno, M.; Rossi, M. L.; Agricola, P.; Repetto, A.; Fetiveau, R.; Diotallevi, P.; Canosi, U.; Mannucci, P. M.; Ardissino, D. *Am. J. Cardiol.* **2004**, *93*, 822.
6. Viles-Gonzalez, J. F.; Badimon, J. J. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 25.
7. Becker, R. C.; Spencer, F. A. *J. Thromb. Thrombolysis* **1998**, *5*, 73.
8. Maraganore, J. M. *Thromb. Haemostasis.* **1993**, *70*, 208.
9. Nyby, M. D.; Sasaki, M.; Ideguchi, Y.; Wynne, H. E.; Hori, M. T.; Berger, M. E.; Golub, M. S.; Brickman, A. S.; Tuck, M. L. *J. Pharm. Exp. Therap.* **1996**, *278*, 503.
10. Vacca, J. P. *Annu. Rep. Med. Chem.* **1998**, *33*, 81.
11. De Clercq, E. *J. Med. Chem.* **1995**, *38*, 2491.
12. De Clercq, E. *Curr. Med. Chem.* **2001**, *8*, 1543.
13. Serhan, C. N.; Ward, P. A. In *Molecular and Cellular Basis of Inflammation*; Totowa, N. J., Ed.; Humana Press series, Current Inflammation Research; Cronstein, B. N., 1999; p 259. Chapter 12.
14. Cronstein, B. N.; Kramer, S. B.; Weissmann, G.; Hirschhorn, R. *J. Exp. Med.* **1983**, *158*, 1160.
15. Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229.
16. Blanco, J. M.; Caamano, O.; Fernandez, F.; Rodriguez-Borges, J. E.; Balzarini, J.; De Clercq, E. *Chem. Pharm. Bull.* **2003**, *51*, 1060.
17. Merino, P. *Curr. Med. Chem. Anti-Infective Agents* **2002**, *1*, 389.
18. *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*; Padwa, A., Pearson, W. H., Eds.; J. Wiley & Sons: N. Jersey, 2003; p 3361.
19. Chiacchio, U.; Padwa, A.; Romeo, G. *Curr. Org. Chem.* **2009**, *13*, 422.
20. Pan, S.; Amankulor, N. M.; Zhao, K. *Tetrahedron* **1998**, *54*, 6587.
21. Xiang, Y.; Chen, J.; Schinazi, R. F.; Zhao, K. *Bioorg. Med. Chem Lett.* **1996**, *6*, 1051.
22. Herdewijn, P. *Biochim. Biophys. Acta* **1999**, *1489*, 167.
23. Chiacchio, U.; Genovese, F.; Iannazzo, D.; Librando, V.; Merino, P.; Rescifina, A.; Romeo, R.; Procopio, A.; Romeo, G. *Tetrahedron* **2004**, *60*, 441.
24. Balo, C.; Blanco, J. M.; Fernandez, F.; Lens, E.; Lopez, C. *Tetrahedron* **1998**, *54*, 2833.
25. Gundersen, L.-L. *Acta Chem. Scand.* **1996**, *50*, 58.
26. Liegois, C.; Lermusieau, G.; Colin, S. *J. Agric. Food Chem.* **2000**, *48*, 1129.
27. Szabo, M. R.; Iditeoiu, C.; Chambre, D.; Lupea, A. X. *Chem. Pap.* **2007**, *61*, 214.
28. Qu, G.-R.; Zhao, L.; Wang, D.-C.; Wu, J.; Guo, H.-M. *Green Chem.* **2008**, *10*, 287.
29. *Selected data*: (a) *General procedure. Amination of 9-allyl-6-chloropurine under reflux*. A mixture of compound **1** (195 mg, 1 mmol) and piperidine (**2a**) (170 mg, 0.198 ml, 2 mmol) in H_2O (3 ml) was refluxed for 24 h. After cooling the mixture was extracted with DCM (2 × 10 ml) and the organic layer was washed with H_2O (2 × 10 ml), dried with $MgSO_4$ and evaporated to give *9-allyl-6-piperidin-1-yl-9H-purine* (**3a**) (230 mg, 95% yield), mp 53–55 °C (54–56 °C).[28]
(b) *9-Allyl-6-morpholin-4-yl-9H-purine* (**3b**) (91% yield), mp 122–124 °C (122–124 °C).[28]
(c) *General procedure. Amination of 9-allyl-6-chloropurine under MW irradiation*. A mixture of purine **1** (195 mg, 1 mmol) and pyrrolidine (**2c**) (139 mg, 0.16 ml, 2 mmol) in H_2O (3 ml) was irradiated at 100 °C in a Biotage (initiator 2.0) scientific MW oven for 7 min. After cooling and evaporation the residue was crystallized from Et_2O to give *9-allyl-6-pyrrolidin-1-yl-9H-purine* (**3c**) (90% yield), mp 105–106 °C, ^{1}H NMR ($CDCl_3$) δ 1.92–2.13 (m, 4H), 3.78 (br s, 2H), 4.18 (br s, 2H), 4.80 (d, 2H, J = 5.5 Hz), 5.17 (d, 1H, J = 16.4 Hz), 5.29 (d, 1H, J = 10.9 Hz), 5.97–6.11 (m, 1H), 7.72 (s, 1H), 8.37 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.1, 45.5, 47.5, 118.4, 120.1, 132.1, 138.5, 150.0, 152.9, 153.1.
(d) *General procedure. 1,3-Dipolar cycloaddition reaction with mesityl nitrile oxide* (**4**). A mixture of the allylpurine **3a** (122 mg, 0.5 mmol) and mesityl nitrile oxide (**4**) (81 mg, 0.5 mmol) in dry toluene (15 ml) was heated under reflux for 2 days. After cooling and evaporation of the solvent the residue was chromatographed on a column (Silica Gel No. 60, ethyl acetate) to give from the faster moving band *9-[(3-mesityl-4,5-dihydroisoxazol-5-yl)methyl]-6-piperidin-1-yl-9H-purine* (**5a**) (52% yield), mp 117–118 °C (DCM–hexane), IR (KBr): 3023, 2930, 2851, 1586 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.64–1.80 (m, 6H), 1.99 (s, 6H), 2.25 (s, 3H), 2.99 (dd, 1H, J_1 = 7.7 Hz, J_2 = 18.0 Hz), 3.29 (dd, 1H, J_1 = 10.9 Hz, J_2 = 18.0 Hz), 4.25 (br s, 4H), 4.50 (d, 2H, J = 4.5 Hz), 5.08–5.18 (m, 1H), 6.83 (s, 2H), 8.00 (s, 1H), 8.29 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.3, 21.0, 24.8, 26.2, 41.3, 45.7, 46.4, 78.2, 119.4, 125.4, 128.4, 136.3, 139.0, 151.0, 152.6, 153.9, 157.5; MS (ESI): 405 [M+H]$^+$, 427 [M+Na]$^+$; Anal. Calcd for $C_{23}H_{28}N_6O$: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.46; H, 6.81; N, 20.65.
(e) *9-[(3-Mesityl-4,5-dihydroisoxazol-5-yl)methyl]-6-morpholin-4-yl-9H-purine* (**5b**) (62% yield, after refluxing for 6 days), mp 179–180 °C (DCM–ethanol), IR (KBr): 3015, 2947, 2859, 1584 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.26 (s, 3H), 2.98 (dd, 1H, J_1 = 7.7 Hz, J_2 = 18.0 Hz), 3.31 (dd, 1H, J_1 = 10.9 Hz, J_2 = 18.0 Hz), 3.84 (t, 4H, J = 4.8 Hz), 4.32 (br s, 4H), 4.51 (d, 2H, J = 4.5 Hz), 5.08–5.18 (m, 1H), 6.84 (s, 2H), 8.02 (s, 1H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.3, 21.0, 41.4, 45.6, 45.8, 67.1, 78.1, 119.6, 125.3, 128.5, 136.3, 139.0, 139.7, 151.2, 152.4, 153.9, 157.5; MS (ESI): 407 [M+H]$^+$, 429 [M+Na]$^+$; Anal. Calcd for $C_{22}H_{26}N_6O_2$: C, 65.01; H, 6.45; N, 20.68. Found: C, 65.16; H, 6.70; N, 20.86.
(f) *9-[(3-Mesityl-4,5-dihydroisoxazol-5-yl)methyl]-6-pyrrolidin-1-yl-9H-purine* (**5c**) (61% yield, after refluxing for 6 days), mp 131–132 °C (DCM–hexane), IR (KBr): 3050, 2973, 2872, 1593 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.92–2.12 (m, 4H), 2.02 (s, 6H), 2.26 (s, 3H), 2.98 (dd, 1H, J_1 = 7.7 Hz, J_2 = 18.0 Hz), 3.31 (dd, 1H, J_1 = 10.9 Hz, J_2 = 18.0 Hz), 3.84 (br s, 2H), 4.19 (br s, 2H), 4.50 (d, 2H, J = 3.9 Hz), 5.09–5.19 (m, 1H), 6.84 (s, 2H), 8.01 (s, 1H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.4, 21.0, 24.1, 41.4, 43.2, 45.8, 78.3, 119.8, 125.4, 128.5, 136.3, 139.0, 139.8, 150.4, 152.9, 153.2, 157.5; MS (ESI): 391 [M+H]$^+$, 413 [M+Na]$^+$; Anal. Calcd for $C_{22}H_{26}N_6O$: C, 67.67; H, 6.71; N, 21.52. Found: C, 67.46; H, 6.79; N, 21.27.
(g) *6-Chloro-9-[(3-mesityl-4,5-dihydroisoxazol-5-yl)methyl]-9H-purine* (**6**) (72% yield, after refluxing for 5 days), mp 135–136 °C (DCM-ethanol), IR (KBr): 3040, 2949, 2860, 1596 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 2.26 (s, 3H), 2.95 (dd, 1H, J_1 = 7.7 Hz, J_2 = 18.0 Hz), 3.39 (dd, 1H, J_1 = 10.9 Hz, J_2 = 18.0 Hz), 4.57 (dd, 1H, J_1 = 5.8 Hz, J_2 = 14.8 Hz), 4.65 (dd, 1H, J_1 = 3.5 Hz, J_2 = 14.8 Hz), 5.13–5.25 (m, 1H), 6.85 (s, 2H), 8.45 (s, 1H), 8.76 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.4, 21.1, 41.7, 46.6, 77.7, 125.0, 128.5, 128.6, 130.1, 136.2, 139.3, 146.4, 151.4, 152.1, 157.6; MS (ESI): 378/380 [M+Na]$^+$. Anal. Calcd for $C_{18}H_{18}ClN_5O$: C, 60.76; H, 5.10; N, 19.68. Found: C, 60.53; H, 5.09; N, 19.39.
(h) *N-{9-[(3-Mesityl-4,5-dihydroisoxazol-5-yl)methyl]-9H-purin-6-yl}benzamide* (**9**) (37% yield, after refluxing for 7 days, eluting first from the column), mp 90–92 °C (DCM), IR (KBr): 3291, 3098, 1694, 1615, 1579 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 6H), 2.25 (s, 3H), 3.00 (dd, 1H, J_1 = 7.4 Hz, J_2 = 18.0 Hz), 3.38 (dd, 1H, J_1 = 10.8 Hz, J_2 = 18.0 Hz), 4.58 (dd, 1H, J_1 = 5.4 Hz, J_2 = 14.3 Hz), 4.67 (dd, 1H, J_1 = 3.4 Hz, J_2 = 14.3 Hz), 5.12–5.24 (m, 1H), 6.84 (s, 2H), 7.47 (t, 2H, J = 7.9 Hz), 7.61 (t, 1H, J = 7.9 Hz), 8.11 (d, 2H, J = 7.9 Hz), 8.40 (s, 1H), 8.82 (s, 1H), 9.94 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.4, 21.0, 41.7, 46.4, 77.9, 123.7, 128.3, 128.4, 128.6, 128.7, 129.5, 130.1, 132.7, 133.6, 143.6, 144.1, 147.2, 152.6, 155.4, 171.0; MS (EI): 440 (5%) [M]$^+$, 412 (3%), 395 (5%), 253 (30%), 220 (53%), 149 (80%), 105 (100%). Anal. Calcd for $C_{25}H_{24}N_6O_2$: C, 68.15; H, 5.49; N, 19.09. Found: C, 68.42; H, 5.67; N, 19.30.
(i) *9-[(3-Mesityl-4,5-dihydroisoxazol-5-yl)methyl]-9H-purin-6-amine* (**10**) (6% yield, after refluxing for 7 days, eluting at the end of the column), mp 256–258 °C (dec.) (DCM), IR (KBr): 3415, 3342, 3050, 1601, 1573 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 6H), 2.25 (s, 3H), 3.00 (dd, 1H, J_1 = 7.6 Hz, J_2 = 18.0 Hz), 3.32 (dd, 1H, J_1 = 11.2 Hz, J_2 = 18.0 Hz), 4.52 (d, 2H, J = 3.9 Hz), 5.10–5.20 (m, 1H), 5.64 (br s, 2H, exchanged by D_2O), 6.83 (s, 2H), 8.10 (s, 1H), 8.34 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.4, 21.1, 41.0, 45.1, 77.9, 122.4, 126.4, 128.3, 130.6, 136.9, 138.0, 149.8, 151.2, 153.5, 157.3; MS (ESI): 359 [M+Na]$^+$. Anal. Calcd for $C_{18}H_{20}N_6O$: C, 64.25; H, 6.00; N, 24.99. Found: C, 64.46; H, 5.74; N, 25.30.
30. Thibon, J.; Latxague, L.; Deleris, G. *J. Org. Chem.* **1997**, *62*, 4635.
31. Miyaki, M.; Shimizu, B. *Chem. Pharm. Bull.* **1970**, *18*, 1446.
32. Kontogiorgis, C.; Litinas, K. E.; Makri, A.; Nicolaides, D. N.; Vronteli, A.; Hadjipavlou-Litina, D. J.; Pontiki, E.; Siohou, A. *J. Enz. Inh. Med. Chem.* **2008**, *23*, 43.
33. DPPH inhibition (%) = $[(A_{blank} - A_{sample})/A_{sample}] \times 100$
34. Symeonidis, T.; Chamilos, M.; Hadjipavlou-Litina, D. J.; Kallitsakis, M.; Litinas, K. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1139.
35. Saint-Cricq de Gaulejac, N.; Vivas, N.; de Freitas, V.; Bourgeois, G. *J. Sci. Food Agric.* **1999**, *79*, 1081.
36. At pH 7.4 superoxide anion reduces the tetrazolium blue into formasan blue (λ_{max} = 560 nm). The production of superoxide was estimated by the nitroblue tetrazolium method.
37. Michaelidou, A.; Hadjipavlou-Litina, D. J.; Matsini, I.; Tsitsogianni, E. *Med. Chem.* **2007**, *3*, 439.
38. As a substrate tosyl-Gly-Pro-Arg-pNA was used at 1 mM final concentration. Compounds were dissolved at a final concentration of 0.1 mM in a Tris-buffer (0.05 M Tris, 0.154 M NaCl, ethanol 5%, pH 8.0). Three minutes after the addition of bovine thrombin (2.5 unit/mg), the reaction was ended by adding 1 ml acetic acid 50%. The absorption of the released p-nitroaniline was measured at 405 nm.