Asian Journal of Chemistry; Vol. 26, No. 23 (2014), 8118-8122

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17602

Synthesis, Crystal Structures and Xanthine Oxidase Inhibitory Activity of Benzohydrazone Compounds

WEI-MING ZHANG[1,*], GUI-HUA SHENG[2] and ZHONGLU YOU[3]

[1]Nanjing Institute for the Comprehensive Utilization of Wild Plant, Nanjing 210042, P.R. China
[2]School of Life Sciences, Shandong University of Technology, Zibo 255049, P.R. China
[3]Department of Chemistry and Chemical Engineering, Liaoning Normal University, Dalian 116029, P.R. China

*Corresponding author: E-mail: youzhonglu@126.com

Received: 14 March 2014; *Accepted*: 21 May 2014; *Published online*: 15 November 2014; AJC-16308

A series of three benzohydrazone compounds, 2*N*'-(5-hydroxy-2-nitrobenzylidene)-4-methylbenzohydrazide (**1**), *N*'-(5-chloro-2-hydroxybenzylidene)-3-methylbenzohydrazide (**2**) and *N*'-(2,4-dichlorobenzylidene)-3-methylbenzohydrazide (**3**), were prepared and structurally characterized by elemental analysis, IR spectra and single crystal X-ray determination. Xanthine oxidase inhibitory activities of the compounds were studied. Among the compounds, *N*'-(5-hydroxy-2-nitrobenzylidene)-4-methylbenzohydrazide (**1**) shows the most effective activity with IC_{50} value of 15.2 ± 2.3 μM. Docking simulation was performed to insert the compound into the crystal structure of xanthine oxidase at the active site to investigate the probable binding modes.

Keywords: Benzohydrazone, Xanthine oxidase, Inhibition, Crystal structure, Molecular docking study.

INTRODUCTION

Enzyme inhibitors can interact with enzymes and block their activity towards natural substrates. The importance of enzyme inhibitors as drugs is enormous since these molecules have been used for treating a number of pathophysiological conditions[1,2]. Xanthine oxidase (XO; EC 1.17.3.2), a molybdenum hydroxylase, catalyzes the hydroxylation of hypoxanthine and xanthine to yield uric acid and superoxide anions. These superoxide anions have been linked to post ischaemic tissue injury and edema as well as to vascular permeability[3]. Xanthine oxidase can oxidize synthetic purine drugs, such as anti-leukaemic 6-mercaptopurine, with the loss of their pharmacological properties[4]. Xanthine oxidase has also been linked to conditions such as hepatic and kidney damage, atherosclerosis, chronic heart failure, hypertension and sickle-cell disease due to the production of reactive oxygen species (ROS) alongside uric acid[5,6]. Then, control of the action of xanthine oxidase may help the therapy of some diseases. Nowadays, the treatment of gout makes use of allopurinol, a potent inhibitor of xanthine oxidase known for a long time[7]. The mode of action of allopurinol involves the direct coordination of its active metabolite, oxypurinol (alloxanthine), to the molybdenum centre in the active site of the enzyme[8]. However, given its side effects, toxicity and its inability to prevent the formation of free radicals by the enzyme[9], the research on new xanthine oxidase inhibitors is needed. Schiff base compounds have been of great interest in biological chemistry for a long time[10-12]. Leigh and co-workers have reported some Schiff bases as novel xanthine oxidase inhibitors[13]. Benzohydrazone compounds are a kind of Schiff bases, which show particular interesting biological activities. As an extension of the work on the exploration of effective xanthine oxidase inhibitors related to Schiff bases, in this paper, a series of benzohydrazone compounds, *N*'-(5-hydroxy-2-nitrobenzylidene)-4-methylbenzo-hydrazide (**1**), *N*'-(5-chloro-2-hydroxybenzylidene)-3-methyl-benzohydrazide (**2**) and *N*'-(2,4-dichlorobenzylidene)-3-methylbenzohydrazide (**3**), were synthesized and structurally characterized. The xanthine oxidase inhibitory activities of the compounds were investigated from both experimental and molecular docking study.

EXPERIMENTAL

Starting materials, reagents and solvents with AR grade were purchased from commercial suppliers and used without further purification. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. IR spectra were recorded on a Jasco FT/IR-4000 spectrometer as KBr pellets in the 4000-400 cm^{-1} region.

Synthesis of *N*'-(5-hydroxy-2-nitrobenzylidene)-4-methylbenzohydrazide (1): 5-Hydroxy-2-nitrobenzaldehyde (1 mmol, 0.167 g) and 4-methylbenzohydrazide (1 mmol, 0.150 g) were mixed in methanol and stirred at room temperature for 1 h. The methanol was evaporated to obtain yellow

crystalline product, which was washed with methanol and dried in air. Yield: 95 %; single crystals of compound **4** suitable for X-ray diffraction were obtained by recrystallization of the product in methanol. Anal. Calcd. for $C_{15}H_{13}N_3O_4$: C, 60.2; H, 4.4; N, 14; found: C, 60; H, 4.3; N, 14.2 %. IR data (KBr, ν_{max}, cm^{-1}): 3429 (m), 3320 (w), 1649 (s), 1557 (m), 1493 (m), 1316 (s), 1225 (m), 1127 (w), 1081 (w), 944 (w), 898 (w), 833 (w), 743 (w), 553 (w).

Synthesis of *N*'-(5-chloro-2-hydroxybenzylidene)-3-methylbenzohydrazide (2): 5-Chlorosalicylaldehyde (1 mmol, 0.156 g) and 3-methylbenzohydrazide (1 mmol, 0.150 g) were mixed in methanol and stirred at room temperature for 1 h. The methanol was evaporated to obtain colorless crystalline product, which was washed with methanol and dried in air. Yield: 87 %; single crystals of compound **5** suitable for X-ray diffraction were obtained by recrystallization of the product in methanol. Anal. Calcd. for $C_{15}H_{13}N_2O_2Cl$: C, 62.4; H, 4.5; N, 9.7; found: C, 62.4; H, 4.6; N, 9.5 %. IR data (KBr, ν_{max}, cm^{-1}): 3417 (m), 3208 (w), 1649 (s), 1557 (s), 1473 (m), 1356 (w), 1290 (s), 1219 (m), 1094 (w), 951 (w), 873 (w), 814 (m), 650 (w), 559 (w), 468 (w).

Synthesis of *N*'-(2,4-dichlorobenzylidene)-3-methylbenzohydrazide (3): 2,4-Dichlorobenzaldehyde (1 mmol, 0.175 g) and 3-methylbenzohydrazide (1 mmol, 0.150 g) were mixed in methanol and stirred at room temperature for 1 h. The methanol was evaporated to obtain colorless crystalline product, which was washed with methanol and dried in air. Yield: 95 %; single crystals of compound **6** suitable for X-ray diffraction were obtained by recrystallization of the product in methanol. Anal. Calcd. for $C_{15}H_{12}N_2OCl_2$: C, 58.7; H, 3.9; N, 9.1; Found: C, 58.5; H, 3.9; N, 9.2 %. IR data (KBr, ν_{max}, cm^{-1}): 3212 (w), 1647 (s), 1562 (s), 1461 (m), 1353 (w), 1288 (s), 1219 (m), 956 (w), 872 (w), 837 (m), 717 (w), 535 (w), 479 (w).

Measurement of the xanthine oxidase inhibitory activity: The xanthine oxidase activities with xanthine as the substrate were measured spectrophotometrically, based on the procedure reported by Kong *et al.*[14], with modification. The activity of xanthine oxidase is measured by uric acid formation monitored at 295 nm. The assay was performed in a final volume of 1 mL 50 mM K_2HPO_4 pH 7.8 in quartz cuvette. The reaction mixture contains 200 mL of 84.8 mg/mL xanthine in 50 mM K_2HPO_4, 50 mL of the various concentrations tested compounds. The reaction is started by addition of 66 mL 37.7 mU/mL xanthine oxidase. The reaction is monitored for 6 min at 295 nm and the product is expressed as mmol uric acid per minute. The reactions kinetic were linear during these 6 min of monitoring.

Docking simulations: Molecular docking study of the compounds into the 3D X-ray structure of xanthine oxidase (entry 1FIQ in the Protein Data Bank) was carried out by using the AutoDock version 4.2. First, Auto Grid component of the program precalculates a 3D grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of 60 × 70 × 60 $Å^3$ points in x, y and z direction with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitor within the macromolecules. The GALS search algorithm (genetic algorithm with local search) was chosen to search for the best conformers. The parameters were set using the software ADT (AutoDockTools package, version 1.5.4) on PC which is associated with AutoDock 4.2. Default settings were used with an initial population of 100 randomly placed individuals, a maximum number of 2.5 × 106 energy evaluations and a maximum number of 2.7 × 104 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Give overall consideration of the most favorable free energy of biding and the majority cluster, the results were selected as the most probable complex structures.

Data collection, structural determination and refinement: Diffraction intensities for the compounds were collected at 298(2) K using a Bruker D8 VENTURE PHOTON diffractometer with MoK_α radiation (λ = 0.71073 Å). The collected data were reduced using the SAINT program[15] and multi-scan absorption corrections were performed using the SADABS program[16]. The structures were solved by direct methods and refined against F^2 by full-matrix least-squares methods using the SHELXTL[17]. All of the non-hydrogen atoms were refined anisotropically. The amino and water H atoms were located in difference Fourier maps and refined isotropically, with N-H, O-H and H···H distances restrained to 0.90(1), 0.85(1) and 1.37(2) Å, respectively. All other H atoms were placed in idealized positions and constrained to ride on their parent atoms. The crystallographic data for the compounds are summarized in Table-1. Hydrogen bonding information is given in Table-2.

RESULTS AND DISCUSSION

Compounds **1-3** were readily synthesized by reaction of 1:1 molar ratio of aldehydes with benzohydrazides in methanol at room temperature, according to the literature method[18], with high yields (over 90 %) and purity. Single crystals suitable for X-ray diffraction were obtained by slow evaporation of the solutions containing the compounds in air. The compounds have been characterized by elemental analyses and IR spectra. Structures of the compounds were further confirmed by single crystal X-ray crystallography.

Structure description of the compounds: Figs. 1-3 give perspective views of compounds **1-3** with atomic labeling systems. X-ray crystallography reveals that the compounds are similar benzohydrazone derivatives. All the benzohydrazone molecules of the compounds adopt E configuration with respect to the methylidene units. The distances of the methylidene bonds, ranging from 1.26 to 1.29 Å, confirm them as typical double bonds. The shorter distances of the C-N bonds and the longer distances of the C=O bonds for the -C(O)-NH- units than usual, suggests the presence of conjugation effects in the molecules. The remaining bond lengths in the compounds are comparable to each other and are within normal values[18-20]. The dihedral angles between the two aromatic rings are 2.3(3)° for compound **1**, 14.2(4)° for compound **2** and 9.5(3)° for compound **3**. The crystal structures of the compounds are stabilized by intermolecular hydrogen bonds (Figs. 4-6).

TABLE-1
CRYSTALLOGRAPHIC AND
EXPERIMENTAL DATA FOR THE COMPOUNDS

Compound	1	2	3
m.f.	$C_{15}H_{13}N_3O_4$	$C_{15}H_{13}N_2O_2Cl$	$C_{15}H_{12}N_2OCl_2$
m.w.	299.3	288.7	307.2
T (K)	298(2)	298(2)	298(2)
Crystal shape/color	Block/yellow	Block/colorless	Bock/colorless
Crystal size (mm^3)	0.17×0.15×0.15	0.17×0.13×0.10	0.20×0.18×0.17
Crystal system	Triclinic	Monoclinic	Monoclinic
Space group	P-1	$P2_1/n$	$P2_1/n$
a (Å)	7.689(2)	7.129(4)	9.674(1)
b (Å)	9.068(2)	27.362(16)	4.799(1)
c (Å)	10.726(2)	7.742(4)	31.028(3)
α (°)	95.539(3)		
β (°)	108.625(3)	111.263(6)	92.594(2)
γ (°)	99.602(3)		
V ($Å^3$)	689.7(3)	1407.4(14)	1439.1(4)
Z	2	4	4
D_c (g cm^{-3})	1.441	1.363	1.418
μ (MoK_α) (mm^{-1})	0.107	0.274	0.447
F(000)	312	600	632
Reflections collected	5159	7216	6971
Unique reflections	2890	3020	3081
Observed reflections (I ≤ 2σ (I))	1922	1788	1781
Parameters	204	185	184
Restraints	1	1	1
Min. and max. transmission	0.982, 0.984	0.955, 0.973	0.916, 0.928
Goodness-of-fit on F^2	1.066	1.022	1.032
R_1, wR_2 [I ≤ 2σ (I)][a]	0.0501, 0.1336	0.0567, 0.1121	0.0565, 0.1132
R_1, wR_2 (all data)[a]	0.0789, 0.1634	0.1080, 0.1334	0.1052, 0.1312
Large diff. peak and hole ($eÅ^{-3}$)	0.226, –0.255	0.194, –0.201	0.198, –0.247

[a]$R_1 = F_o\text{-}F_c/F_o$, $wR_2 = [\Sigma w(F_o^2\text{-}Fc^2)/\Sigma w(F_o^2)^2]^{1/2}$

TABLE-2
HYDROGEN BOND DISTANCES (Å) AND
BOND ANGLES (°) FOR THE COMPOUNDS

D–H···A	d(D–H)	d(H···A)	d(D···A)	Angle (D-H···A)
1				
N2–H2···O3$^{\#1}$	0.90(1)	2.36(2)	3.247(2)	171(3)
O1–H1···O4$^{\#2}$	0.82	1.93	2.743(2)	168(3)
2				
O1–H1···N1	0.82	1.88	2.595(3)	146(3)
N2–H2···O2$^{\#3}$	0.90(1)	1.98(2)	2.876(3)	168(3)
3				
N2–H2···O1$^{\#4}$	0.90(1)	1.92(2)	2.776(3)	159(3)

Symmetry codes: #1) 1 – x, 1 – y, 1 – z; #2) – x, 2 – y, 1 – z; #3) –1/2 + x, 3/2 – y, –1/2 + z ; #4) x, –1 + y, z

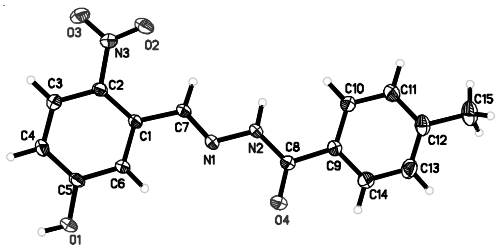

Fig. 1. A perspective view of the molecular structure of compound **1** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30 % probability level

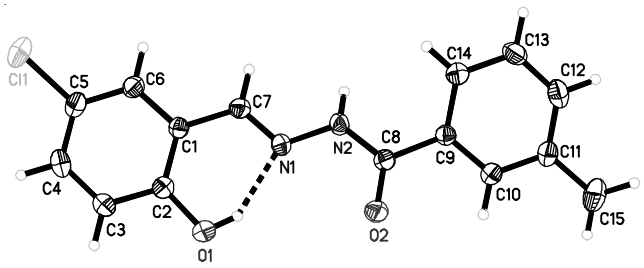

Fig. 2. A perspective view of the molecular structure of compound **2** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30 % probability level. Hydrogen bonds are shown as dashed lines

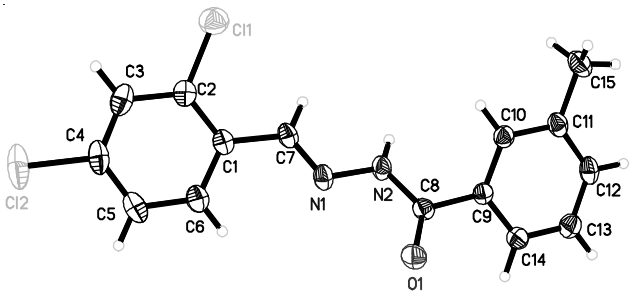

Fig. 3. A perspective view of the molecular structure of compound **3** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30 % probability level. Hydrogen bond is shown as a dashed line

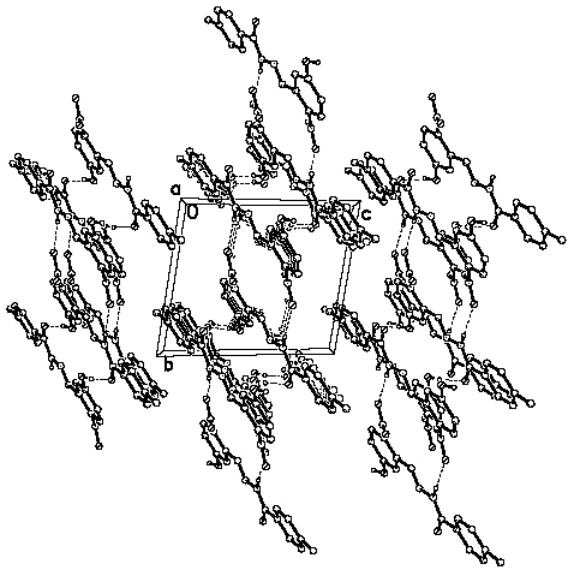

Fig. 4. Molecular packing diagram of compound **1**. Hydrogen bonds are shown as dashed lines

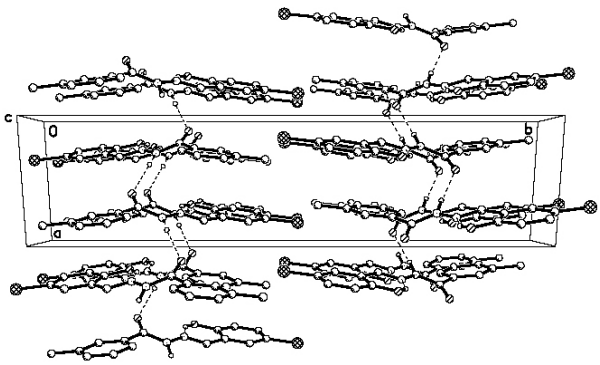

Fig. 5. Molecular packing diagram of compound **2**. Hydrogen bonds are shown as dashed lines

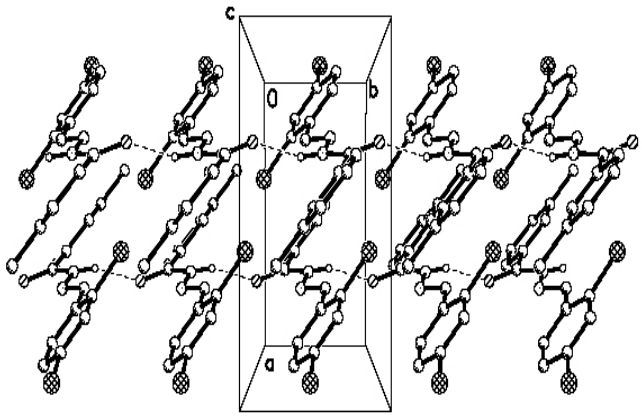

Fig. 6. Molecular packing diagram of compound **3**. Hydrogen bonds are shown as dashed lines

Pharmacology: The measurement of xanthine oxidase inhibitory activity was carried out for three parallel times. The percents of inhibition at the concentration of 100 μM and IC_{50} values for the compounds against xanthine oxidase are summarized in Table-3.

TABLE-3
INHIBITION OF XO BY THE TESTED MATERIALS

Tested materials	Percent of inhibition[b]	IC_{50}(μM)
1	67.2 ± 3.1	15.2 ± 2.3
2	35.9 ± 2.1	-
3	20.5 ± 2.0	-
Allopurinol	80.7 ± 4.3	8.7 ± 2.3

[b]The concentration of the tested material is 100 μM

Allopurinol was used as a reference with the per cent of inhibition of 80.7 ± 4.3 and with IC_{50} value of 8.7 ± 2.3 μM. Compound **1** shows the most effective activity with the percent of inhibition of 67.2 ± 3.1 and with IC_{50} value of 15.2 ± 2.3 μM. Although the number of tested compounds is limited, some structural features, important to the xanthine oxidase inhibitory effect, can be inferred. Compound **1**, bearing a nitrate group, has the most effective activity. These findings are coherent with the results reported in the literature that the existence of electron-withdrawing groups in the benzene rings can enhance the activities[21].

Molecular docking study: In order to give an explanation and understanding of potent inhibitory activity observed from the experiment, molecular docking study was performed to investigate the binding effects between the compound **1** and the active sites of xanthine oxidase (entry 1FIQ in the Protein Data Bank). Allopurinol was used to verify the model of docking and gave satisfactory results. Fig. 7 is the binding models for compound 1 in the enzyme active site of xanthine oxidase. The docking score is -5.73. As a comparison, the docking score for Allopurinol is -6.27.

From the docking results, it can be seen that the molecule of compound **1** is well filled in the active pocket of xanthine oxidase. The molecule of 1 is bind with the enzyme through three hydrogen bonds with SER876, GLU802 and THR1010. In addition, there exist hydrophobic interactions among the compound with the active sites of the enzyme. The results of

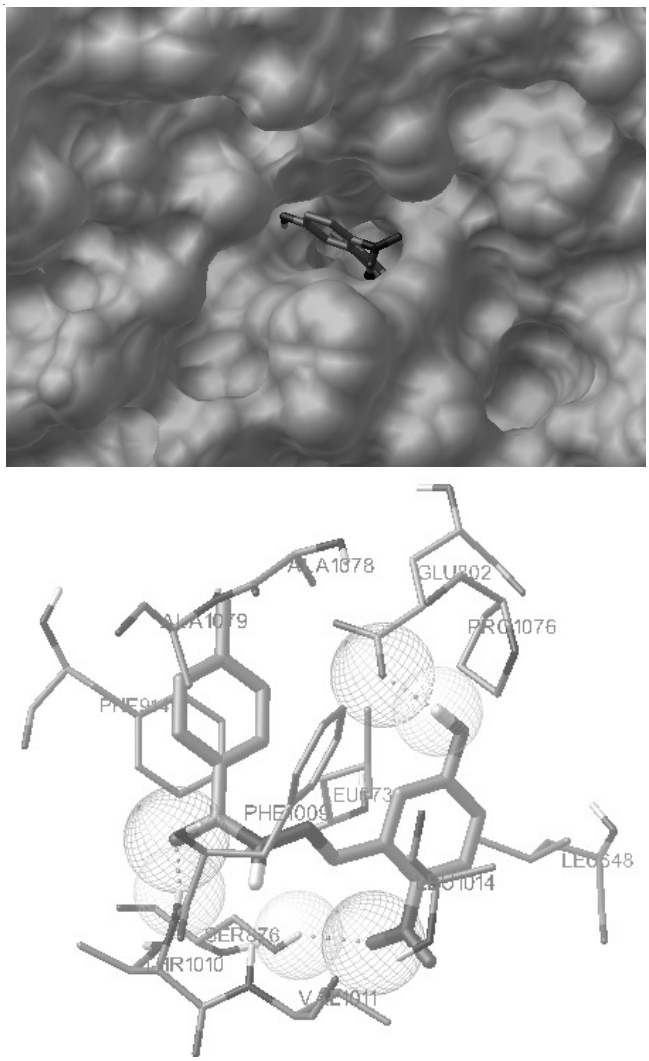

Fig. 7. 3D (above) and 2D (below) binding mode of compound **1** with the active site of XO. Hydrogen bonds are shown as dashed lines

the molecular docking study could explain the inhibitory activity of the compound on xanthine oxidase.

Supplementary information: CCDC-892366 for compound **1**, 892368 for compound **2** and 892369 for compound **3** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at http://www.ccdc.cam.ac.uk/const/retrieving.html or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

REFERENCES

1. D.W. Banner and P. Hadvary, *J. Biol. Chem.*, **266**, 20085 (1991).
2. E. Sandstrom, *Drugs*, **38**, 417 (1989).
3. P.H. Chan, J.W. Schmidley, R.A. Fishman and S.M. Longar, *Neurology*, **34**, 315 (1984).
4. H.S.D. Naeff, M.C.R. Franssen and H.C. van der Plas, *Recl. Trav. Pay-B*, **110**, 139 (1991).
5. P. Pacher, A. Nivorozhkin and C. Szabo, *Pharmacol. Rev.*, **58**, 87 (2006).
6. A.A. Fatokun, T.W. Stone and R.A. Smith, *Neurosci. Lett.*, **416**, 34 (2007).
7. R.K. Robins, G.R. Revankar, D.E. O'Brien, R.H. Springer, T.N.A. Albert, K. Senga, J.P. Miller and D.G. Streeter, *J. Heterocycl. Chem.*, **22**, 601 (1985).

8. K. Okamoto, B.T. Eger, T. Nishino, E.F. Pai and T. Nishino, *Nucleos. Nucleot. Nucl. Acids*, **27**, 888 (2008).
9. A. Haberland, H. Luther and I. Schimke, *Agents Actions*, **32**, 96 (1991).
10. H.-J. Zhang, X. Qin, K. Liu, D.-D. Zhu, X.-M. Wang and H.-L. Zhu, *Bioorg. Med. Chem*., **19**, 5708 (2011).
11. M.S. Alam, J.H. Choi and D.U. Lee, *Bioorg. Med. Chem*., **20**, 4103 (2012).
12. K. Cheng, Q.-Z. Zheng, Y. Qian, L. Shi, J. Zhao and H.-L. Zhu, *Bioorg. Med. Chem*., **17**, 7861 (2009).
13. M. Leigh, C.E. Castillo, D.J. Raines and A.K. Duhme-Klair, *Chem. Med. Chem*., **6**, 612 (2011).
14. L.D. Kong, Y. Cai, W.W. Huang, C.H.K. Cheng and R.X. Tan, *J. Ethnopharmacol*., **73**, 199 (2000).
15. Bruker, SMART and SAINT. Bruker AXS Inc., Madison, Wisconsin, USA (2002).
16. G.M. Sheldrick, SADABS, Program for Empirical Absorption Correction of Area Detector, University of Göttingen, Germany (1996).
17. G.M. Sheldrick, *Acta Crystallogr. A*, **64**, 112 (2008).
18. Y. Lu, D.-H. Shi, Z.-L. You, X.-S. Zhou and K. Li, *J. Coord. Chem*., **65**, 339 (2012).
19. F.H. Allen, O. Kennard, D.G. Watson, L. Brammer, A.G. Orpen and R. Taylor, *J. Chem. Soc., Perkin Trans*. **2**, S1 (1987).
20. M. Zhang, D.-M. Xian, H.-H. Li, J.-C. Zhang and Z.-L. You, *Aust. J. Chem*., **65**, 343 (2012).
21. S. Gupta, L.M. Rodrigues, A.P. Esteves, A.M.F. Oliveira-Campos, M.S.J. Nascimento, N. Nazareth, H. Cidade, M.P. Neves, E. Fernandes and M. Pinto, *Eur. J. Med. Chem*., **43**, 771 (2008).