ORIGINAL PAPER

Synthesis and biological activity of (Z) and (E) isomers of 3-(3,4-diaryl-1,2,4-triazole-5-yl)prop-2-enoic acid

Bożena Modzelewska-Banachiewicz · Barbara Michalec · Teresa Kamińska · Liliana Mazur · Anna E. Kozioł · Jacek Banachiewicz · Marzena Ucherek · Martyna Kandefer-Szerszeń

Received: 30 July 2008/Accepted: 23 August 2008/Published online: 14 October 2008 © Springer-Verlag 2008

Abstract (*Z*)-3-(3,4-diaryl-1,2,4-triazole-5-yl)prop-2-enoic acid derivatives were obtained in the course of the reaction of N^3 -substituted amidrazones with maleic anhydride, and isomerized into the (*E*) isomers by heating under reflux in acetic acid solution. The molecular structure of the compounds obtained was confirmed by IR and ¹H NMR spectroscopy, and by X-ray crystallography for (2*E*)-3-(4, 5-diphenyl-4*H*-1,2,4-triazol-3-yl)prop-2-enoic acid. The antiviral and immunomodulating activity of several of the compounds was examined.

B. Modzelewska-Banachiewicz · J. Banachiewicz · M. Ucherek
Department of Organic Chemistry,
Faculty of Pharmacy,
Nicolaus Copernicus University,
Jagiellonska 13, 85-067 Bydgoszcz, Poland
e-mail: maru@cm.umk.pl

B. Michalec (⊠) Department of Molecular Biology, Maria Curie-Sklodowska University, Akademicka 19, 20-033 Lublin, Poland e-mail: basiam@hektor.umcs.lublin.pl

T. Kamińska · M. Kandefer-Szerszeń Department of Immunology and Virology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland

L. Mazur · A. E. Kozioł Department of Crystallography, Maria Curie-Sklodowska University, M. Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland **Keywords** (*Z*) and (*E*) isomers \cdot 3,4-Diaryl-1,2,4-triazoles \cdot Propenoic acid derivatives \cdot Antiviral activity \cdot Immunomodulation

Introduction

1,2,4-Triazole derivatives are constituents of many pharmaceuticals with diverse therapeutic action—antiviral, antifungal, sedative, and anxiolytic [1]. Moreover, the derivatives of carboxylic acids (e.g. acetic, propionic, and benzoic) which contain aromatic systems in their structure (phenyl, naphthyl, indolyl, 2-pyridyl) are used as non-steroidal anti-inflammatory drugs (NSAIDs) and analgesic drugs (e.g. ibuprofen, naproxen, piroxicam, diclofenac, aspirin) [1]. Recently, the antiviral [2, 3] and anti-inflammatory [4–13] activity of 1,2,4-triazole derivatives has been studied.

In the course of the reaction of N^3 -substituted amidrazones 1-7 [14] with maleic anhydride, (Z)-8-14 of 3-(3,4-diaryl-1,2,4-triazole-5-yl)prop-2-enoic acid have been obtained [15]. The presence of a double C=C bond in compounds 8–14 enabled the formation of (Z) and (E)isomers differing in their energetic stability, which could affect their biological activity also [16, 17]. In this paper we present a method of transformation of the (Z)-8-14 parent isomers into the (E)-15–21 diastereoisomers. The reactions are presented in Scheme 1. Due to the presence of the 1,2,4-triazole system, which has also been found in the structure of numerous drugs with antiviral activity (e.g. ribavirin), we decided to conduct antiviral and virucidal tests. The prepared compounds were also bioassayed for their potential to stimulate or modulate mitogen phytohaemaglutinin (PHA) with lipopolysaccharide (LPS)-induced interferon gamma (IFN-y) and tumour



Scheme 1

necrosis factor (TNF) production in human blood leukocytes.

Results and discussion

The synthesis of (*Z*)-3-(3,4-diaryl-1,2,4-triazole-5-yl)prop-2-enoic acid derivatives was performed according to the procedure described in a previous report [15]. Condensation of N^3 -substituted amidrazones **1–7** with maleic acid anhydride was carried out at ambient temperature. Substrates were dissolved in anhydrous diethyl ether prior to mixing them together (Scheme 1).

Based on results from spectral analysis (IR, ¹H NMR) and X-ray crystallography, it was revealed that the reaction led directly to the formation of (*Z*)-3-(3,4-diaryl-1,2,4-triazole-5-yl)prop-2-enoic acids (**8–14**). In the IR spectra of cyclic compounds, characteristic absorption bands of the carbonyl group were present in the range 1,700– 1,708 cm⁻¹. In the ¹H NMR spectra, signals of vinyl-type hydrogens are observed at 6.2–6.5 ppm. The coupling constants for (*Z*) diastereoisomers are 6–12 Hz. The carboxylic group hydrogens were observed as broad singlets in the range 13.5–13.8 ppm. Only for compound **12**, containing two pyridyl rings, the carbonyl group hydrogen signal was moved downfield. Signals of methyl group hydrogens of compounds **8** and **10** were observed as singlets in the range 2.3–2.4 ppm.

Isomerization to (E) diastereoisomers 15–21 occurred when the (Z)-isomers were heated under reflux in the glacial acetic acid. The spatial configuration at the double C=C bond was confirmed, by spectroscopic means, to be (Z) for compounds 8–14 and (E) for compounds 15–21. In the IR spectra of both series of isomers, the characteristic absorption band of a carbonyl group was present in the 1,698–1,710 cm⁻¹ range. In the ¹H NMR spectra, signals of vinyl-type hydrogens were observed at 6.5-6.9 ppm. The coupling constants for (Z)-8-14 and (E)-5-21 measured in the spectra were in good accordance with the literature J = 12-18 Hz for the (E) configuration [18]. The carboxvlic group hydrogens were observed as broad singlets in the 12.9-13.8 ppm range. Only for compound 15, containing pyridinyl and methylphenyl substituents, the COOH hydrogen signal was moved downfield to 15.0 ppm. Signals of methyl group hydrogens were observed as singlets in the range 2.3–2.4 ppm for compounds 15 and 17.

The molecular structure of the isomerization products of (Z)-3-(3,4-diaryl-1,2,4-triazole-5-yl)prop-2-enoic acid derivatives **8–14** into (E)-**15–21** was confirmed by the X-ray analysis of crystalline **20**. The numbering scheme and general view of two symmetrically independent molecules of **20** are shown in Fig. 1.

Equivalent bond distances and angles for both molecules are equal within experimental error. The triazole N1=C5 and N2=C3 bonds have double-bond character, with respective bond distances of 1.312(8) Å (molecule A), 1.311(9) Å (molecule B) and 1.302(8) Å (molecule A), 1.316(9) Å (molecule B), whereas the N4–C3 and N4–C3 bonds have an intermediate character, indicating delocalization of the electron cloud. The triazole and two phenyl rings are each essentially planar but are not coplanar. The symmetrically independent molecules are connected by alternate O1a–H…N1 and O1–H…N1a short hydrogen bonds to form extended ribbons along the crystallographic *a* axis. These ribbons are joined by numerous C–H…O/N and C–H… π interactions.

Antiviral activity

Anti-herpes simplex virus type 1 (HSV-1) activity of the 1,2,4-triazole derivatives was examined in a cytopathic effect (CPE) reduction assay and as a result the virus titre was calculated. All compounds examined inhibited viral replication during 24 h, however, when CPE was estimated after 48 h, only partial inhibition of viral replication was observed. No significant differences in the antiviral activity of (*Z*)-**11** and (*Z*)-**13** in comparison with (*E*)-**18** and (*E*)-**20** were detected. The compounds **18** and **20** most effectively

Fig. 1 Molecular structure and atom numbering scheme for two symmetrically independent molecules of 20. Displacement ellipsoids are shown at the 50% probability level, hydrogen atoms are shown as *spheres* of arbitrary size. Hydrogen bonding is indicated by *dashed lines*



reduced the viral CPE. Most potent antiviral activity was exhibited by compound **20** with 50% of virus inhibition. The compounds **11**, **13**, and **18** were less active with virus inhibition above 30% (Table 1).

Virucidal activity

The concentration of compounds used in this assay was $100 \ \mu g \ cm^{-3}$. After incubation of the viral suspension with the compound for 1 h at 37°C, the most potent virucidal effect on HSV-1 was exerted by compound **14**. Compounds **10** and **11** did not inactivate HSV-1 in direct contact whereas the rest of the derivatives did reduce the titre of HSV-1, but less effectively than compound **14** (Table 2).

The immunomodulatory activity of the examined derivatives

The prepared compounds were bio-assayed for their potency to stimulate or modulate mitogen (PHA with LPS)-induced IFN- γ and TNF production in human blood leukocytes. The results indicated that compounds **8**, **9**, **11**, and **14** induced low but detectable amounts of TNF (Table 3).

Moreover, compounds **9** and **12** slightly enhanced mitogen-stimulated TNF production in human blood leukocytes (Table 4).

None of the compounds induced IFN production (data not shown) and compounds **8**, **13**, and **14** inhibited mitogen-stimulated level of IFN (Table 5).

The production of inflammatory mediators by monocytes and macrophages is a crucial step in the early response to infections and tissue damage. TNF and IFN- γ are proinflammatory cytokines produced mainly by activated monocytes, macrophages, and Th1 lymphocytes and play a key role in immunological response, which causes rapid neutralization of factors dangerous to the organism.

Table 1 The antiviral activity of compounds **11** and **13**, and their (*E*) isomers, **18** and **20**, respectively (6 μ g cm⁻³) in comparison with ACV

HSV titre \pm SD (log ₁₀ <i>CCID</i> ₅₀ cm ⁻³)					
Incubation	24 h	48 h	Inhibition (%)		
Virus control	2.7 ± 0.4	4.0 ± 0.5			
ACV	0	0			
11	0	2.7 ± 0.4	33.2		
13	0	2.5 ± 0.4	37.5		
18	0	2.5 ± 0.3	37.5		
20	0	2.0 ± 0.5	50		

 Table 2
 The virucidal activity of the compounds

Compound	Virus titre \pm SD (log ₁₀ <i>CCID</i> ₅₀ cm ⁻³)			
	48 h incubation	72 h incubation	96 h incubation	120 h incubation
Control of virus	3.3 ± 0.4	3.5 ± 0.4	3.7 ± 0.4	4.0 ± 0.5
8	2.5 ± 0.3	2.5 ± 0.3	2.7 ± 0.4	3.3 ± 0.4
9	2.7 ± 0.4	3.0 ± 0.5	3.2 ± 0.5	3.2 ± 0.5
10	3.3 ± 0.4	3.8 ± 0.5	3.5 ± 0.4	3.5 ± 0.4
11	3.3 ± 0.4	3.5 ± 0.4	3.5 ± 0.4	3.5 ± 0.4
12	2.5 ± 0.3	2.7 ± 0.4	2.8 ± 0.5	3.2 ± 0.5
13	2.5 ± 0.3	2.5 ± 0.3	2.7 ± 0.4	3.5 ± 0.4
14	0	0	2.0 ± 0.5	3.0 ± 0.5

On the other hand, overproduction of these cytokines may lead to pathology [19] observed for example in autoimmune diseases (rheumatoid arthritis, lupus erythematosus, insulin-dependent diabetes) or in sepsis, with fatal consequences [20]. Thus, modulators of TNF and IFN- γ production have been sought for many years and it seems that some of the compounds examined might be considered as a candidates for such an agent. Further experiments in this field are needed.

 Table 3 Examined compounds as inducers of TNF production

Compound	TNF (pg cm ⁻³) \pm SD
Control (without induction)	0
8	84.6 ± 107.7
9	30.9 ± 27.8
10	<5
11	30.4 ± 52.5
12	<5
13	<5
14	53.3 ± 76.5

Table 4 Examined compounds as modulators of TNF production

Compound	TNF (pg cm ^{-3}) \pm SD	Control (%)
Control (mitogen induced)	638 ± 295	100
8	648 ± 283	102
9	752 ± 211	118
10	649 ± 352	102
11	611 ± 258	96
12	757 ± 327	119
13	650 ± 243	102
14	685 ± 423	107

Compounds 8–14, 18, 20 (10 $\mu g~cm^{-3})$ with inducers: LPS (5 $\mu g~cm^{-3})$ + PHA (10 $\mu g~cm^{-3})$

Table 5 Examined compounds as modulators of IFN-y production

Compound	IFN- γ (pg cm ⁻³) \pm SD	Control (%)
Control (mitogen induced)	$1,614 \pm 426$	100
8	$1,259 \pm 597$	78
9	$1,329 \pm 376$	82
10	$1,529 \pm 648$	95
11	$1,442 \pm 714$	89
12	$1,450 \pm 766$	90
13	$1,186 \pm 243$	73
14	$1,\!226\pm202$	76

Control of inducers: LPS (5 μ g cm⁻³) + PHA (10 μ g cm⁻³); compounds **8–14**, **18**, **20** (10 μ g cm⁻³) with inducers: LPS (5 μ g cm⁻³) + PHA (10 μ g cm⁻³)

Experimental

Chemistry

Melting points were measured on a Boetius apparatus. ¹H NMR spectra were recorded on a Tesla BS 567A (300 MHz) apparatus in DMSO- d_6 with TMS as an external standard. Chemical shifts are given in ppm (δ -scale), coupling constants in Hz. IR spectra (v, cm⁻¹) were recorded in KBr using a Specord IR-75 spectrometer.

Elemental analysis (C. H. N) was conducted in the Department of Organic Chemistry, Medical University of Lublin (Poland). Their results were in good agreement with calculated values. All reactions were monitored by thinlayer chromatography (TLC). Chromatography was performed on $10 \text{ cm} \times 10 \text{ cm}$ TLC plates precoated with silica gel RP-18 F₂₅₄ and silica gel Si 60 F₂₅₄ (Merck, Darmstadt, Germany). The mobile phase for the normalphase system was acetone-toluene-acetic acid 8:2:1; for the reversed-phase system the mixture methanol-waterformic acid 5:5:0.5) was applied. Compounds were dissolved in methanol (5 mg cm⁻³) and 10-µL samples of these solutions were spotted on the plates. After development in horizontal Teflon DS chambers (Chromdes, Lublin, Poland) and drying, the spots were visualized under UV light at $\lambda = 254$ nm. Chemicals were purchased from Merck or Lancaster and used without further purification.

Synthesis of (E)-3-(3,4-diaryl-1,2,4-triazole-5-yl)prop-2-enoic acids derivatives **15–21**: general procedure

A mixture of 0.01 mol (*Z*)-3-(3,4-diaryl-1,2,4-triazole-5-yl)prop-2-enoic acid **8–14** and 5 cm³ glacial acetic acid was heated under reflux for 2.5 h (**8–11, 13, 14**) or 0.5 h (**12**). Acetic acid was removed under reduced pressure. The residue was washed with cold methanol and purified by crystallization from methanol.

(2E)-3-[4-(4-methylphenyl)-5-pyridin-2-yl-4H-1,2,

4-triazol-3-yl]prop-2-enoic acid (**15**, $C_{17}H_{14}N_4O_2$) Yield 2.45 g (80%); mp 266–268°C; IR (KBr): $\bar{\nu} = 3,450$ (OH), 2,971, 1,450 (CH_{al}.), 1,700 (C=O), 1,191 (C–O) cm⁻¹; ¹H NMR (*DMSO*-d₆): $\delta = 2.5$ (s, CH₃), 6.6, 6.7 (d, J = 15.9 Hz, 2HC2=), 7.0–7.5 (m, 8*Ar*-H), 15.00 (s, COOH).

(2E)-3-(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-

yl)*prop-2-enoic acid* (**16**, $C_{16}H_{12}N_4O_2$)

Yield 2.05 g (70%); mp 240–242°C; IR (KBr): $\bar{\nu} = 3,433$ (OH), 1,705 (C=O), 1,199 (C–O) cm⁻¹; ¹H NMR (*DMSO*-d₆): $\delta = 6.7, 6.8$ (d, J = 15.9, 2HC2=); 7.2–8.5 (m, 9*Ar*-H); 13.8 (s, COOH).

(2E)-3-[4-(4-methylphenyl)-5-pyridin-4-yl-4H-1,2,4triazol-3-yl]prop-2-enoic acid (**17**, C₁₇H₁₄N₄O₂)

Yield 2.08 g (68%); mp 243–245°C; IR (KBr): $\bar{\nu} = 3,430$ (OH), 2,970, 1,450 (CH_{al.}), 1,705 (C=O), 1,190 (C–O) cm⁻¹; ¹H NMR (*DMSO*-d₆): $\delta = 2.5$ (s, CH₃), 6.7, 6.9 (d, J = 15.9 Hz, 2HC2=), 7.1–8.2 (m, 8*Ar*-H), 12.9 (s, COOH).

(2E)-3-(4-phenyl-5-pyridin-2-yl-4H-1,2,4-triazol-3-

yl)prop-2-enoic acid (18, C₁₆H₁₂N₄O₂)

Yield 2.45 g (72%); m.p. 268–270°C; IR (KBr): $\bar{\nu} = 3,468$ (OH), 1,698 (C=O), 1,190 (C–O) cm⁻¹. ¹H NMR (*DMSO*-d₆): $\delta = 6.6$, 6.9 (d, J = 15.8 Hz, 2HC2=), 7.3–8.3 (m, 9*Ar*-H), 12.9 (s, COOH).

(2*E*)-3-(4,5-dipyridin-2-yl-4*H*-1,2,4-triazol-3-yl) prop-2-enoic acid (**19**, C₁₅H₁₁N₅O₂) Yield 1.9 g (65%); mp 168–170; °C; IR (KBr): $\bar{\nu} = 3,420$ (OH), 1,708 (C=O), 1,180 (C–O) cm⁻¹; ¹H NMR (*DMSO*d₆): $\delta = 6.4$, 6.6 (d, J = 15.8 Hz, 2HC2=), 6.9–7.9 (m, 8*Ar*-H), 13.2 (s, COOH).

(2E)-3-(4,5-diphenyl-4H-1,2,4-triazol-3-yl)

prop-2-enoic acid (**20**, C₁₇H₁₃N₃O₂)

Yield 2.1 g (72%), mp 262–264°C. IR (KBr): $\bar{\nu} = 3,434$ (OH); 1,702 (C=O); 1,191 (C–O) cm⁻¹; ¹H NMR (*DMSO*-d₆): $\delta = 6.6, 6.9$ (d, J = 15.9 2H C2=), 7.3–7.6 (m, 10*Ar*-H), 12.90 (s, COOH).

Crystal data for **20**: monoclinic space group P2₁/*n*, unitcell parameters a = 10.498(2) Å, b = 18.977(4) Å, c = 14.997(3) Å, $\beta = 100.17(2)^{\circ}$, V = 2,940.8(10) Å³; Z = 8, $d_{calc} = 1.316$ g cm⁻³, $\mu = 0.725$ mm⁻¹.

Diffraction data for **20** were measured at 293(2) K on a KM4 diffractometer using variable scan speed ($\omega - 2\theta$ scan mode) and graphite-monochromatized CuK_{α} radiation ($\lambda = 1.54,178$ Å). A single crystal of dimensions 0.68 × 0.35 × 0.06 mm³ was used. Reflections were collected up to $\theta_{max} = 80.21^{\circ}$; 6,480 reflections were measured. Crystal structure was solved by direct methods using the SHELXS97 program [21] and refined by the fullmatrix least squares on F^2 using the SHELXL97 program [22].

Non-hydrogen atoms of the phenyl substituents were refined with isotropic displacement parameters. The remaining non-hydrogen atoms were refined anisotropically. Hydrogen atoms of the carboxyl groups were located in a difference map and their positional parameters were not refined. All the other hydrogen atoms were positioned geometrically and were given isotropic factors of 1.2 U_{eq} of the bonded C/O atoms; the "riding" model was used in the refinement. Final discrepancy factors are $R_1 = 0.1068$, $wR_2 = 0.2049$, and S = 0.93, for 1,299 reflections with $I > 2\sigma(I)$.

Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 271179. Copies of the data may be obtained on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

$\begin{array}{l} (2E) \hbox{-} 3-[4-(4-nitrophenyl) \hbox{-} 5-pyridin \hbox{-} 2-yl \hbox{-} 4H-1, 2, 4-triazol-3-yl] prop-2-enoic acid (\mathbf{21}, \mathbf{C}_{16}\mathbf{H}_{11}\mathbf{N}_5\mathbf{O}_4) \end{array}$

Yield 2.36 g (70%); mp 266–268°C; IR (KBr): $\bar{\nu} = 3,419$ (OH), 1,710 (C=O), 1,182 (C–O) cm⁻¹; ¹H NMR (*DMSO*-d₆):

 $\delta = 6.5, 6.9 \text{ (d, } J = 15.6, 2 \text{H} \text{ (C2=)}, 7.3\text{--}8.4 \text{ (m, 8Ar-H)}, 13.1 \text{ (s, COOH)}.$

Pharmacology

Compounds

Compounds 8–21 were dissolved in DMSO at a concentration of 1,000 μ g cm⁻³ as a stock solution. Just before experiments compounds were diluted with 2% minimal essential medium (MEM).

The cells and viruses

HSF were obtained by a routine method of trypsynization of an adult human skin fragment and cultured in MEM (Sigma Chemical, St Louis, MO, USA) supplemented with 10% FCS (Life Technologies, Karlsruhe, Germany), 100 U cm⁻³ penicillin, and 100 µg cm⁻³ streptomycin. HeLa-human cells of cervical carcinoma were cultured in MEM supplemented with 5% FCS (Gibco, BRL), 100 U cm⁻³ penicillin, and 100 µg cm⁻³ streptomycin. HSV-1 McIntyre strain was obtained from the National Institute of Health (Warsaw, Poland). Virus stock was prepared from infected HeLa cells. The titre of the virus in HeLa cells was $10^{4.71 \pm 0.3} CCID_{50}$ cm⁻³.

The blood donors

The heparinized blood samples were obtained from volunteers aged 21–23 (two men and eight women). None of the volunteers reported any history of acute or chronic medical problems.

Cytopathic effect reduction assay

The compounds were examined for their antiviral activity in the CPE reduction assay. Human skin fibroblasts (HSF) were seeded into 96-well plates at a density of 1×10^5 cells cm⁻³ in MEM supplemented with 10% foetal calf serum (FCS) and grown at 37°C for 24 h. The cells were covered with HSV-1 suspension at a multiplicity of infection (MOI) of 0.01 and with non-toxic concentrations of triazole derivatives (6 µg cm⁻³) diluted in MEM supplemented with 2% FCS. As a control ACV (6 µg cm⁻³) was used. The cells were then incubated for 24 h and 48 h at 37°C in 5% CO₂ atmosphere. After incubation, the virus CPE was read under the light microscope and virus titre—log₁₀ Cell Culture Infecting Dose (*CCID*₅₀) cm⁻³—was calculated.

The virucidal activity of the compounds

The virucidal activity of the compounds was estimated by incubation of undiluted stock virus samples with equal volumes of the compounds used at a concentration of 100 μ g cm⁻³. After 1 h of incubation at 37°C, the titre of the virus (log₁₀ *CCID*₅₀ cm⁻³) was estimated in HSF cells.

The influence of the compounds on cytokine production The ability of compounds to induce IFN- γ and TNF production was also tested. Heparinized blood obtained from healthy donors was diluted in MEM (supplemented with antibiotics) to obtain 1×10^6 leukocytes cm⁻³. The examined derivatives were added to diluted blood at 10 μ g cm⁻³ concentration, samples of blood from donors were incubated for 24 h at 37°C in 5% CO₂ atmosphere. After centrifugation, the harvested supernatants were analysed for cytokines by enzyme-linked immunosorbent assay (ELISA) techniques with commercially available kits and according to the manufacturer's instructions. The IFN- γ kit was obtained from Endogen (Woburn, MA, USA) and the TNF kit was purchased from Bender MedSystem (Vienna, Austria). The lower limits of detection for the individual assays were IFN- γ 2 pg cm⁻³ and TNF 5 pg cm $^{-3}$. To estimate the ability of the compounds to modulate IFN- γ and TNF production, a mixture of mitogens (LPS at a concentration of 5 μ g cm⁻³ and PHA at a concentration of 10 μ g cm⁻³) was used. The mixture of mitogens and the tested compounds was added to diluted blood samples and incubated for 24 h at 37°C in 5% CO₂ atmosphere. The harvested supernatants were analysed for IFN- γ and TNF as described above.

References

- O'Neil MJ, Heckelman PE, Koch CB, Roman KJ, Kenny CM, D'Arecca MR (eds) (2006) The Merck Index, 14th edn, entries 4881, 6417, 7506, 3081, 851. Merck, Whitehouse Station, NJ, USA
- Abdel-Hafez AA, Elsherief HA, Kurokawa M, Shiraki K, Kawahata T, Otake T, Nakamura N, Hattori M (2002) Arzneimittelforschung 52:833

- Al-Soud YA, Al-Dwori MN, Al-Masoudi NA (2004) Farmaco 59:775
- 4. De La Rosa M, Kim HW, Gunic E, Jenket C, Boyle U, Koh YH, Korboukh I, Allan M, Zhang W, Chen H, Xu W, Nilar S, Yao N, Hamatake R, Lang SA, Hong Z, Zhang Z, Giradet JL (2006) Bioorg Med Chem Lett 16:4444
- Zhou L, Amer A, Korn M, Burda R, Balzarini J, De Clercq E, Kern ER, Torrence PF (2005) Antivir Chem Chemother 16:375
- Chun MW, Kim JH, Kim MJ, Kim BR, Jeong LS (2005) Nucleos Nucleot Nucleic Acids 24:979
- 7. Tozkoparan B, Aktay G, Yesilada E (2002) Farmaco 57:145
- Labanauskas L, Udrenaite E, Gaidelis P, Brukstus A (2004) Farmaco 59:255
- Labanauskas L, Kalcas V, Undrenaite E, Gaidelis P, Brukstus A, Dauksas V (2001) Pharmazie 56:617
- Novidopour L, Shafaroodi H, Abdi K, Amini M, Garemani MH, Dehpour AR, Shafiee A (2006) Bioorg Med Chem 14:2507
- Turan-Zitouni G, Kaplancikli ZA, Ozdemir A, Chevallet P, Kandilci HB, Gümüsel B (2007) Arch Pharm (Weinheim) 11:586
- 12. Amir M, Kumar H, Javed SA (2007) Arch Pharm (Weinheim) 11:577
- 13. Amir M, Kumar H, Javed SA (2007) Bioorg Med Chem Lett 16:4504
- Modzelewska-Banachiewicz B, Pyra E (1995/1996) Annales UMCS, Sec. AA, 50/51:111
- Modzelewska-Banachiewicz B, Banachiewicz J, Chodkowska A, Jagiello-Wojtowicz E, Mazur L (2004) J Eur Med Chem 39:873
- Cysewski P, Modzelewska-Banachiewicz B (2004) Internet J Chem 7:2
- 17. Dugave C, Demange L (2003) Chem Rev 103:2475
- Zieliński W, Rajcy A (2000) Metody spektroskopowe i ich zastosowanie do identyfikacji związków organicznych WNT, Warszawa, p 571
- 19. Miles EA, Allen E, Calder PC (2002) Cytokine 20:215
- 20. Fiers W (1991) FEBS Lett 285:199
- 21. Sheldrick GM (1997) SHELXS97. Program for a Crystal Structure Solution. University of Göttingen, Göttingen
- 22. Sheldrick GM (1997) SHELXL97. Program for the Refinement of a Crystal Structure from Diffraction Data. University of Göttingen, Göttingen