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SYNTHESIS, STRUCTURE AND CYTOTOXIC ACTIVITY OF A 2-NITROPHENYLALANINE DERIVATIVE

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Summary. A new 2-nitrophenylalanine derivative has been synthesized. It was characterized by IR, ^{1}H NMR, ^{13}C NMR, mass-spectral data, elemental analysis, TLC, melting points determination and X-ray crystal structure analyses. The new compound was tested for cytotoxic activity in three tumor cell lines – HL-60, HL-60/Dox and SKW-3 using the MTT-dye reduction assay for cellular viability.

Key words: 2-nitrophenylalanine derivative, X-ray crystal structure analyses, cytotoxic activity, MTT-test

СИНТЕЗ, СТРУКТУРА И ЦИТОТОКСИЧНА АКТИВНОСТ НА ПРОИЗВОДНО НА 2-НИТРОФЕНИЛАЛАНИНА

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Резюме. Синтезирано е неописано в литературата производно ва 2-нитрофенилаланина. Съединението е охарактеризирано чрез ИЧ-, ^{1}Н-ЯМР-, ^{13}С-ЯМР-, масспектрални изследвания, данни от елементен анализ, контрол с тънкослойна хроматография, определяне т.т. и рентгеноструктурен анализ. Проведено е изследване за цитотоксична активност върху три туморни клетъчни линии – HL-60, HL-60/Dox и SKW-3, с помощта на MTT-теста.

Ключови думи: 2-нитрофенилаланин, рентгеноструктурен анализ, цитотоксична активност, MTT-тест

Introduction

A commonly used method for synthesis of imines is the condensation of an aldehyde or a ketone with a primary amine. Aldimines are readily prepared by simply mixing equimolar amounts of an aldehyde and an amine with provision for the removal of water. More recent studies have shown that it is often possible to prepare ketimines by a condensation at room-temperature. This procedure is very important in order to protect amino acid esters by high-yield preparation of the benzophenone Schiff base derivatives of amino acid esters. The procedure is based on transimination of the benzophenone imine with the amino acid ester salt [1]. This is a possible way to synthesize the nitro derivatives of phenylalanine and thyrosine.

Materials and methods

Chemistry

All starting materials were purchased from Merck, Sigma-Aldrich and Fluka. They were used without further purification. Melting points were measured in open capillary tubes on a Büchi 535 melting point apparatus. The IR spectra were recorded at Shimadzu FT-IR 8101 M spectrometer in nujol and frequencies were expressed in cm^{-1}. The ^{1}H NMR spectra were recorded in Brucker 250 MHz in DMSO-d_6 or acetone using TMS as an internal standard (chemical shifts are reported in ppm units, coupling constants (J) in Hz). Abbreviations are as follows: s – singlet, d – doublet, dd – double doublet, t – triplet, m – multiplet, br – broad.

Mass-spectral analysis was performed by electron ionization on masspectrometer Hewlett-Packard 5973 at 70 eV.

Synthesis of o-nitrobenzyl bromide (1)

3.0742 g (0.02 mol) of o-nitrobenzyl alcohol was slowly heated with 10.10 g (6.72 mL) of hydrobromic acid. The alcohol dissolved when the temperature reached 50°C. At 75°C the solution clouded and the bromide separated slowly. After 2 hours heating, the product was separated and washed with concentrated hydrochloric acid in order to remove any unchanged alcohol. The bromide obtained melted at about 38-39°C. After recrystallization from a mixture of alcohol and water the melting point was 45-47°C as earlier described [6].

*Synthesis of Methyl N-(diphenylmethylene)glycinate (**I**)*

10 g (9.3 ml, 0.0552 mol) of benzophenone imine, 6.93 g (0.0552 mol) methyl glycinate hydrochloride and 200 ml dichloromethane were stirred at room temperature for 24 h with the exclusion of moisture ($CaCl_2$ tube). The reaction mixture was filtered in order to remove ammonium chloride and evaporated to dryness on a rotary evaporator. The residue was taken up in 200 mL ether, filtered off, washed with 200 ml water, and dried over $MgSO_4$ [1]. After filtration the solvent was removed and the crude product was recrystallized (ether/hexane). Yield 11.37 g (81.3%), m.p 40-42°C. IR (KBr): 1745p 1615 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6), ppm: 3.7 (3H, s), 4.1 (2H, s), 7.1 – 7.8 (10H, m). Calcd for $C_{16}H_{15}NO_2$ (253.29): C 75.89%, H 5.93%, N 5.53%. Found: C 75.66%, H 6.07%, N 5.46%.

*Synthesis of Methyl-2-diphenylmethyleneimino-3-(o-nitrophenyl)propanoic acid (**II**)*

A solution of lithium diisopropylamide (5.44 ml, 1.8 M solution, 0.00979 mol, 1.2 eq.) in tetrahydrofurane (35 ml) was chilled to – 78°C. Methyl N-(diphenylmethylene)glycinate (2.4797 g, 0.00979 mol, 1.2 eq.) in 40 ml THF was added slowly. 1,3-dimethyltetrahydropyrimidin-2-one (DMPU) (1.18 mL, 0.00979 mol, 1.2 eq) was added dropwise. The mixture was stirred for 20 min and a solution of o-nitrobenzyl bromide (1.7687 g, 0.00816 mol, 1 eq.) in THF (40 ml) was added over a period of 40 min. The reaction mixture was maintained at – 78°C for 2 h, then warmed to room temperature over 3 h. The reaction was quenched by adding saturated aqueous NH_4Cl solution (50 ml) and ether. After phase separation the aqueous phase was extracted with ether, the combined organic layers were washed with brine and dried (Na_2SO_4) [2]. Filtration and evaporation to dryness provided the crude product which was purified by recrystallization with diisopropyl ether to give 2.75 g (86.5%). IR (KBr): 2940, 1729, 1623, 1596, 1576, 1519, 1489, 1443, 1345, 1313, 1277, 1220, 1177, 1144, 1066,857,789, 744, 693, 663 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6), ppm: 2.5 – 2.6 (s, 3H), 3.6 – 3.8 (d, 2H), 4.3 – 4.7 (t, 1H), 7.3 – 7.9 (m, 14 H). FAB MS: 389 $[M + H]^+$. Calcd for $C_{23}H_{20}N_2O_4$: C 71.13%, H 5.15%, N 7.22%. Found: C 70.98%, H 5.52%, N 7.18%.

X-Ray Crystal Structure Analysis

Data collection was carried out at –60°C using graphite-monochromated CuK_α radiation (λ = 1.5418 Å) on an ENRAF NONIUS four circle diffractometer. The unit cell was determined and refined using the CAD4-EXPRESS program. A semi-empirical absorption correction was performed using the PLATON/ABS PSI program [9]. The structure was solved with direct methods by SHELXS97 [7], and refined with SHELXL97 [8], by least-squares methods based on F^2. All non-hydrogen atoms were fully refined and all hydrogen atom positions were taken from the electron density map and refined isotropically. The plots of the molecular structure were made using the DIAMOND program (CRYSTAL IMPACT GbR, Bonn, Germany).

Complete data collection parameters and details of the structures solution and refinement are given in Tables 1 and 5. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Pharmacological study

MTT dye reduction assay

The MTT-dye reduction assay was carried out as described by Mossmann [5], with some modifications [4]. Briefly, 100 μL aliquots of cell suspension (1 x 10^5 exponentially dividing cells/ml) were seeded in 96-well microplates. Following a 24 h adaptation period at 37°C the cells were exposed to for 72 h. After the incubation MTT solution (10 mg/ml in PBS) was added (10 μl/well) and the plates were further incubated for 4 h at 37°C.

Thereafter the formazan crystals formed were dissolved through addition of 100 µl/well 5% formic acid in 2-propanol (Merck) and the absorption of the samples was measured with a microprocessor-controlled microplate reader (Labexim LMR-1) at 580 nm. 100 µl RPMI 1640 medium (Sigma), 10 µl MTT stock and 100 µl 5% formic acid in 2-propanol served as a blank solution. The results were expressed as survival fraction (% of untreated control).

Results and discussion

Crystal structure

Compound **I** crystallized from ether/hexane at a low temperature gave colorless crystals which belong to the triclinic system, space group P-1 with cell dimensions a = 7.7100 (18), b = 9.589 (3), c = 9.910 (2), α = 104.776 (19)°, β = 110.219 (18)° γ = 90.95 (2)°, V = 660.3 (3) Å^3, Z = 2. Crystallographic data for the investigated compound are listed in Table 1. The solid-state structure is shown in Fig. 2. DIAMOND drawing (50% probability level), selected bond lengths [Å] and angles: C1 – C8 1.4962 (18)°, C1 – C2 1.5008 (17)°, C1 – N 1.2796 (17)°, C15 – O2 1.1997 (16)°, C15 – O1 1.3440 (16)°. Examination of the bond lengths revealed that the atoms retained the character expected for an open-side chain compound. N – C14 bond length 1.4541 (17) Å was close to the expected value for a single bond adjacent to a carbonyl group. The length of the C1 – N double bond 1.2796 (17) Å was suitable for a C=N bond conjugated to two aromatic nuclei. The angle C2 – C1 – C8 118.14 (11)° showed that the bonding of the aromatic nucleus is disposed in one plane.

Colorless crystals (**II**) suitable for X-ray diffraction analysis were grown by slow evaporation of a diisopropyl ether solution. Crystallographic data for the investigated compound are listed in Table 5. The solid-state structure is shown in Fig. 3. A compound (**II**) crystallizes in the monoclinic system, space group P21 with cell dimensions a = 9.8281 (14), b = 9.5928 (5), c = 10.7475 (11), α = γ = 90°, β = 95.241 (13)°, V = 1009.02 (18) Å^3, Z = 2. DIAMOND drawing (50% probability level), selected bond lengths [pm] and angles: C15 – C14 – C21 117.4 (2), N13 – C14 – C15 117.9 (2), C14 – N13 – C8 120.2 (2). The angles studied showed that both aromatic rings of the protecting group were disposed at one plane. C1 – C7 – C8 112.7 (2), N13 – C8 – C7 108.8 (2) showed that the aromatic nucleus of the phenylalanine was disposed at a parallel plane to the plane of the diphenylmethyleneimino protecting group. An examination of the other bond lengths and angles revealed that the atoms retained the character expected for an open-side chain compound.

Table 1. *Crystal data, details for data collection and structural analysis of* ***Methyl N-(diphenylmethylene)glycinate (I)***

Empirical formula	**$C_{16}H_{15}NO_2$**
Formula weight	253.29
Temperature	173 (2) K
Wavelength/Radiation	0.71073 A / MoK\a
Crystal system/Space group	Triclinic / P-1
Unit cell dimensions	a = 7.7100 (18) Å α = 104.776 (19)° b = 9.589 (3) Å β = 110.219 (18)° c = 9.910 (2) Å γ = 90.95 (2)°
Volume	660.3 (3) x 10^6 Å^3
Z	2
Density (calculated)	1.274 Mg/m^3
Absorption coefficient	0.084 mm^{-1}
F(000)	268
Crystal size	0.1 x 0.1 x 0.5 mm
θ range for data collection [°]	3.39 to 29.27 deg.
Index ranges	$10 \le h \le 10$, $-13 \le k \le 13$, $-13 \le l \le 13$
Reflections collected/unique	11970/3541 [R(int) = 0.0525]
Completeness to 2 θ = 29.27	98.2%
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data/restraints/paramete	3541/0/174
Goodness-of-fit on F^2	1.033
Final R indices [I > 2σ (I)]	R1 = 0.0506, wR2 = 0.1155
R indices (all data)	R1 = 0.0720, wR2 = 0.1242
Extinction coefficient	0.025 (5)
Largest diff. peak and hole	0.348 and –0.197 e.A^{-3}

Pharmacology

The cell lines used in this study included the acute myeloid leukemia HL-60, its multidrug-resistant

sub-line HL-60/Dox, and the T-cell leukemia SKW-3. They were supplied from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The cells were maintained in a controlled environment (RPMI-1640 medium, supplemented with 10% heat-inactivated fetal calf serum and 2mM L-glutamine, at 37°C in a 'Heraeus' incubator with 5% CO_2 humidified atmosphere). In order to keep the cell cultures in log phase, cellular suspension aliquots were supplemented with fresh RPMI-1640 medium two or three times per week. HL-60/Dox were maintained in medium containing 0.2 μM doxorubicin in order to sustain their MDR phenotype. For 1 week prior to cytotoxicity determination however, they were kept in a drug-free medium in order to avoid synergistic interactions between the anthracycline and the tested compounds. The stock solutions of the tested compounds were prepared in DMSO and consequently diluted in RPMI-1640. At the final dilutions obtained, the concentration of the solvent never exceeded 0.5%.

***Table 2**. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for **Methyl N-(diphenyl-methylene)glycinate** U (eq) is defined as one third of the trace of the orthogonalized Uij tensor*

Atom	x	y	z	U (eq)
O 1	4517 (2)	1672 (1)	2815 (1)	32 (1)
O 2	6155 (2)	2508 (1)	5282 (1)	31 (1)
N	7268 (2)	5128 (1)	4918 (1)	24 (1)
C 1	8063 (2)	6194 (1)	4709 (1)	21 (1)
C 2	7799 (2)	6394 (1)	3200 (1)	22 (1)
C 3	6073 (2)	6671 (2)	2300 (2)	26 (1)
C 4	5834 (2)	6833 (2)	891 (2)	31 (1)
C 5	7296 (2)	6719 (2)	381 (2)	32 (1)
C 6	9006 (2)	6446 (2)	1269 (2)	31 (1)
C 7	9270 (2)	6292 (2)	2681 (2)	26 (1)
C 8	9326 (2)	7317 (1)	6067 (1)	21 (1)
C 9	10239 (2)	8530 (2)	5957 (2)	26 (1)
C 10	11376 (2)	9576 (2)	7235 (2)	30 (1)
C 11	11622 (2)	9426 (2)	8639 (2)	33 (1)
C 12	10720 (2)	8223 (2)	8760 (2)	32 (1)
C 13	9583 (2)	7180 (2)	7493 (2)	26 (1)
C 14	6021 (2)	4019 (2)	3626 (1)	27 (1)
C 15	5621 (2)	2678 (1)	4052 (1)	23 (1)
C 16	3955 (3)	327 (2)	3038 (2)	40 (1)

Table 3.** Selected bond lengths (Å) for **Methyl N-(diphenylmethylene)glycinate

Bond	Length	Bond	Length
O 1 – C 15	1.3440 (16)	N – C 14	1.4541 (17)
O 1 – C 16	1.4470 (18)	C 1 – C 8	1.4962 (18)
O 2 – C 15	1.1997 (16)	C 1 – C 2	1.5008 (17)
N – C 1	1.2796 (17)	C 14 – C 15	1.5094 (19)

Table 4.** Bond angles (°) for **Methyl N-(diphenylmethylene)glycinate

Bond	Angle	Bond	Angle
C 15 – O 1 – C 16	115.90 (12)	C 9 – C 8 – C 1	121.81 (11)
C 1 – N – C 14	119.08 (11)	C 13 – C 8 – C 1	119.77 (12)
N – C 1 – C 8	117.48 (11)	N – C 14 – C 15	111.28 (11)
N – C 1 – C 2	124.38 (11)	O 2 – C 15 – O 1	124.00 (13)
C 8 – C 1 – C 2	118.14 (11)	O 2 – C 15 – C 14	127.07 (12)
C 7 – C 2 – C 1	120.25 (12)	O 1 – C 15 – C 14	108.93 (11)
C 3 – C 2 – C 1	120.26 (13)		

Table 5. *Crystal data, details for data collection and structural analysis of Methyl-2-diphenylmethyleneimino-3-(2'-nitrophenylpropanoic acid ester (II)*

Empirical formula	**$C_{23}H_{20}N_2O_4$**
Formula weight	388.41
Temperature	213 (2) K
Wavelength/Radiation	0.71073 A / MoK\a
Crystal system/Space group	Triclinic/P21
Unit cell dimensions	a = 9.8381 (14) Å; α = 90°; b = 9.5928 (5) Å; β = 95.241 (13)°; c = 10.7475 (11) Å; γ = 90°
Volume	1009.02 (18) x 10^6 Å^3
Z	2
Density (calculated)	1.278 Mg/m^3
Absorption coefficient	0.088 mm^{-1}
F(000)	408
Crystal description	collourless plate
Crystal size	0.75 x 0.25 x 0.15 mm
No . of reflns. (lattice)	25
θ range (lattice)	6.90 to 15.23 deg.
θ range for data collection [°]	6.87 to 23.82 deg.
Index ranges	$11 \leq h \leq 11$, $-10 \leq k \leq 10$, $-12 \leq l \leq 12$
Reflections collected	6038
Independent reflections	3023 [R(int) = 0.0345]
Reflections observed	2521
Criterion for observation	2 sigma (I)
Absorption correction	Psi-Scan
Max. and min. transmission	0.9739 and 0.9398
Measurement method	Omega scan
No. of standard reflections	3
Interval count/time	300/3600
Decay	1.5%
Structure solution	SHELXS-97 (Sheldrick, 1990)
Structure refinement	SHELXS-97 (Sheldrick, 1997)
Molecular graphics	SCHAKAL, ZORTEP
Publication material	SHELXS-97 (Sheldrick, 1997)
Refinement method	Full-matrix least-squares on F^2
Weighting scheme	Calc w = 1/[\s^2(Fo^2) + (0.1043P)^2 + 0.0036P] Where P = (Fo^2 + Fc^2)/3
Solution primary	direct
Hydrogen treatment	mixet
Data/restraints/parameters	3023/1/310
Final R indices [I>2σ (I)]	R1 = 0.0511, wR2 = 0.1274
Goodness-of-fit on F^2	1.017
Final R indices (all) R1/wR2	0.0658/0.1369
Absolute structure parameter	0.0 (18)
Largest diff. peak and hole	0.232 and -0.134 e.A^{-3}

Table 6. *Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for* ***Methyl-2-diphenylmethyleneimino-3-(2'-nitrophenyl)propanoic acid ester*** *U (eq) is defined as one third of the trace of the orthogonalized Uij tensor*

Atom	x	y	z	U (eq)
C 1	1.0765 (3)	0.1997 (3)	0.7673 (3)	42 (1)
C 2	1.1189 (3)	0.2509 (3)	0.6559 (3)	49 (1)
C 3	1.1351 (3)	0.3933 (4)	0.6350 (3)	60 (1)
C 4	1.1071 (4)	0.4874 (4)	0.7247 (4)	61 (1)
C 5	1.0662 (3)	0.4418 (4)	0.8344 (3)	58 (1)
C 6	1.0523 (3)	0.2996 (3)	0.8570 (3)	47 (1)
C 7	1.0542 (3)	0.0487 (3)	0.7989 (3)	45 (1)
C 8	0.9056 (3)	0.0025 (3)	0.7690 (3)	42 (1)
C 9	0.8899 (3)	-0.1505 (3)	0.8014 (3)	45 (1)
O 10	0.9841 (2)	-0.2283 (2)	0.8260 (3)	66 (1)
O 11	0.7610 (2)	-0.1877 (2)	0.8016 (2)	62 (1)
C 12	0.7334 (4)	-0.3322 (4)	0.8277 (4)	74 (1)
N 13	0.8185 (2)	0.0857 (2)	0.8431 (2)	40 (1)
C 14	0.7232 (3)	0.1604 (3)	0.7892 (2)	36 (1)
C 15	0.6444 (3)	0.2518 (3)	0.8691 (3)	39 (1)
C 16	0.5202 (3)	0.3093 (4)	0.8237 (3)	54 (1)
C 17	0.4470 (4)	0.3911 (4)	0.9000 (4)	68 (1)
C 18	0.4960 (4)	0.4178 (4)	1.0194 (4)	66 (1)
C 19	0.6208 (4)	0.3632 (4)	1.0669 (4)	60 (1)
C 20	0.6942 (3)	0.2803 (3)	0.9915 (3)	48 (1)
C 21	0.6836 (2)	0.1625 (3)	0.6512 (2)	39 (1)
C 22	0.5989 (3)	0.0612 (4)	0.5957 (3)	55 (1)
C 23	0.5599 (4)	0.0657 (5)	0.4686 (3)	68 (1)
C 24	0.6082 (4)	0.1706 (5)	0.3973 (4)	72 (1)
C 25	0.6932 (4)	0.2690 (4)	0.4507 (3)	65 (1)
C 26	0.7307 (3)	0.2680 (3)	0.5770 (3)	52 (1)
N 27	1.1473 (3)	0.1587 (4)	0.5544 (3)	69 (1)
O 28	1.0886 (4)	0.0480 (4)	0.5423 (3)	90 (1)
O 29	1.2299 (6)	0.1934 (5)	0.4854 (4)	149 (2)

Table 7*. Selected bond lengths (Å) for* ***Methyl, 2-diphenylmethyleneimino-3-(2'-nitrophenyl)-propanoic acid ester***

Bond	Length	Bond	Length
C 1 – C 7	1.509 (4)	C 9 – O 11	1.317 (4)
C2 – N 27	1.452 (4)	O 11 – C 12	1.444 (5)
C 7 – C 8	1.532 (4)	N 13 – C 14	1.276 (3)
C 8 – N 13	1.459 (4)	C 14 – C 15	1.492 (4)
C 8 – C 9	1.520 (4)	N 27 – O 29	1.196 (5)
C 9 – O 10	1.200 (4)	N 27 – O 28	1.210 (5)
C 14 – C 21	1.499 (4)		

The cytotoxic effects of the novel compound were assessed against three human cell lines, namely the promyelocyte leukemia HL-60, its multi-drug resistant sub-line HL-60/Dox and the T-cell leukemia SKW-3, using the MTT-dye reduction assay for cellular viability. The concentration response curves from the study are depicted in fig. 1, whereas the corresponding IC50 values are summarized in Table 9.

Table 8. *Bond angles (°) for* ***Methyl-2-diphenylmethyleneimino-3-(2'-nitrophenyl)-propanoic acid ester***

Bond	Angle	Bond	Angle
C 2 – C 1 – C 6	115.8 (3)	C 15 – C 14 – C 21	117.4 (2)
C 2 – C 1 – C 7	126.4 (3)	C 16 – C 15 – C 14	121.2 (3)
C 6 – C 1 – C 7	117.8 (3)	C 20 – C 15 – C 14	120.3 (2)
C 1 – C 2 – N 27	121.6 (3)	C 22 – C 21 – C 26	118.9 (3)
C 3 – C 2 – N 27	116.3 (3)	C 22 – C 21 – C 14	120.7 (3)
C 1 – C 7 – C 8	112.7 (2)	C 26 – C 21 – C 14	120.4 (2)
N 13 – C 8 – C 9	109.0 (2)	O 29 – N 27 – O 28	121.5 (4)
N 13 – C 8 – C 7	108.8 (2)	O 29 – N 27 – C 2	118.9 (4)
O 11 – C 9 – C 8	112.3 (2)	O 28 – N 27 – C 2	119.6 (3)
C 9 – O 11 – C 12	117.2 (3)	N 13 – C 14 – C 15	117.9 (2)
C 14 – N 13 – C 8	120.2 (2)	N 13 – C 14 – C 21	124.7 (2)

It is evident from the results obtained that both I and II exerted cytotoxic effect in the tested cell lines, whereby II proved to be superior in terms of relative potency. Both compounds were found to be less active in the resistant HL-60/Dox cell line as compared to the sensitive prototype HL-60. In HL-60/Dox the IC_{50} value of II was approx. 2.8 fold higher than in the sensitive line, whereas I failed to induce 50% inhibition of cellular viability in the resistant cell line. The results were compared with the anticancer drug Melphalan.

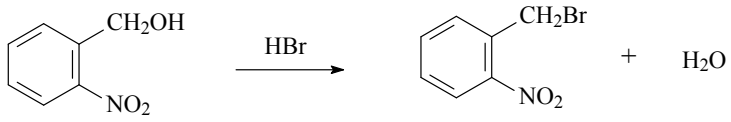
Table 9. *Cytotoxicity of tested compounds against human tumor cell lines as assessed by the MTT-dye reduction assay after 72 h continuous exposure*

Compounds	IC_{50} value (μM)		
	HL-60	HL-60/Dox	SKW-3
I	138.4	> 200.0	113.4
II	66.9	165.1	99.2

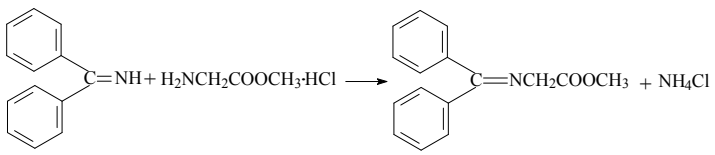
Statistics

The experimental data were fitted to sigmoidal dose-response curves and the corresponding IC_{50} values were calculated using non-linear regression analysis (GraphPad Prizm) as end-points to assess the relative cytotoxic/antiproliferative potency of the tested compounds. The data processing included the Student`s t-test with $p \leq 0.05$ taken as significance level, using Origin Plot and GraphPad Prizm software for PC.

1.

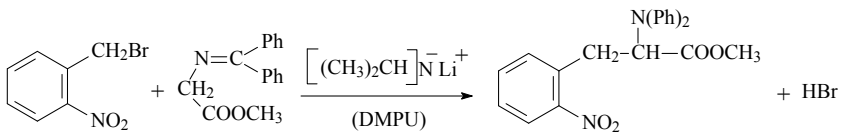


2.



(I)

3.



(II)

Fig. 1. *Synthetic scheme*

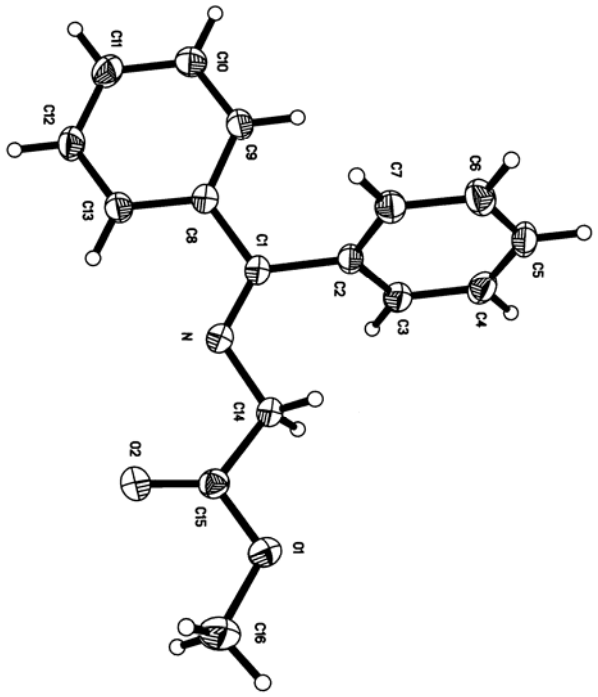


***Figure 2**. DIAMOND drawing (50% probability level) of Methyl N-(diphenylmethylene)-glycinate (**I**)*

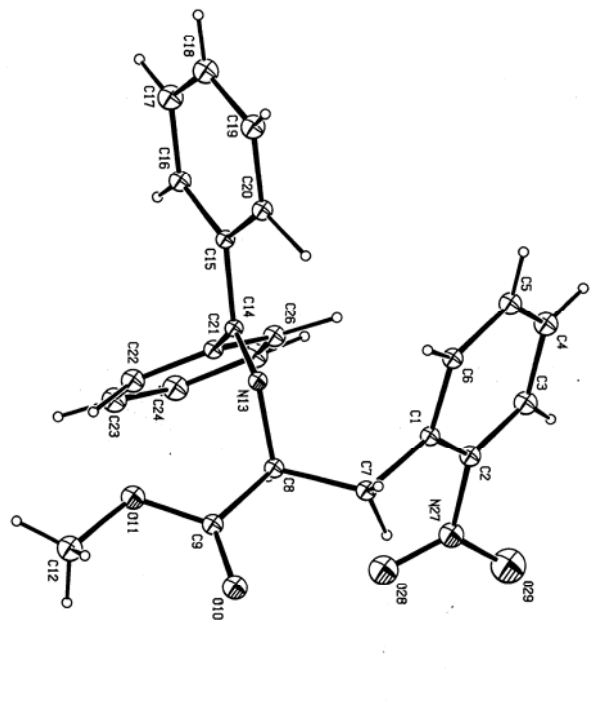


***Figure 3.** DIAMOND drawing (50% probability level) of Methyl-2-diphenylmethylene-imino-3-(2'-nitrophenyl)propanoic acid ester (**II**)*

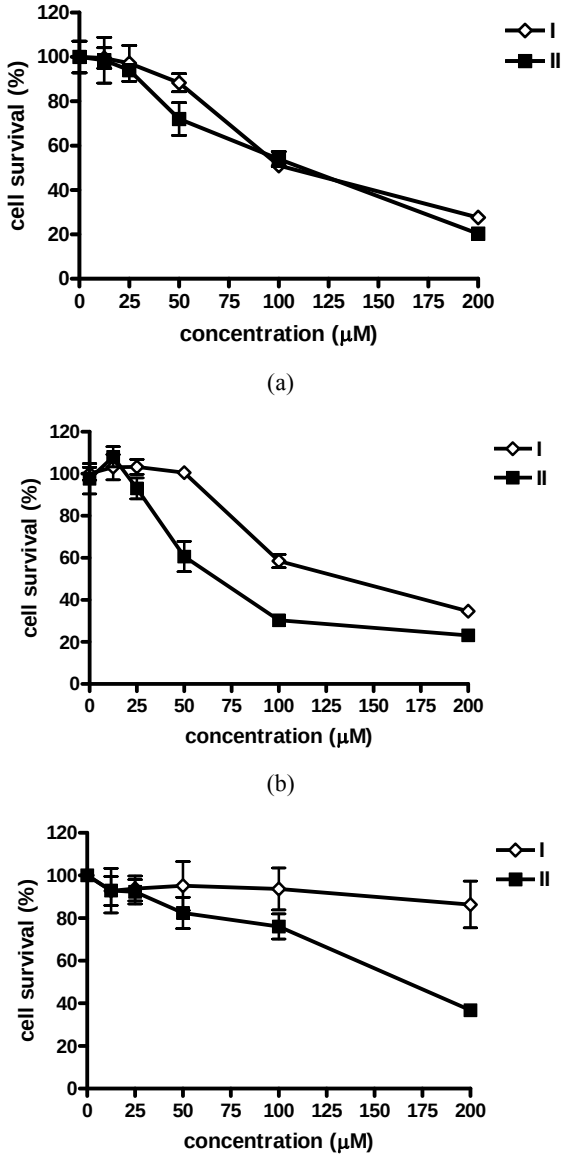


***Figure 4.** Cytotoxic effects of **I** and **II** as assessed by the MTT-dyer reduction assay after 72 h exposure on the human tumour cell lines SKW-3 (a), HL-60 (b) and HL-60/Dox (c)*

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