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Cytotoxicity of Novel Hydrazide-hydrazone Derivatives towards Tumor and Normal Cell Lines

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THE REACTION of cyanoacetyl hydrazone with ninhydrin gave the hydrazide – hydrazone derivative 3. The reactivity of the later product towards different chemical reagents was studied. The cytotoxicity of the newly synthesized products was measured towards the three cancer cell lines, namely breast adenocarcinoma (MCF-7), non-small cell lung cancer(NCI-H460) and CNS cancer (SF-268).

Keywords: Ninhydrin, Hydrazide-hydrazone, Pyridine and Pyridazone.

The hydrazone group has been known for its antimicrobial activity and a number of hydrazide–hydrazones were claimed to possess interesting antibacterial, antifungal[1-3], anticonvulsant[4-6], anti-inflammatory[7,8] antimalarial[9] and anti-tubercular activities[10-15].

Results and Discussion

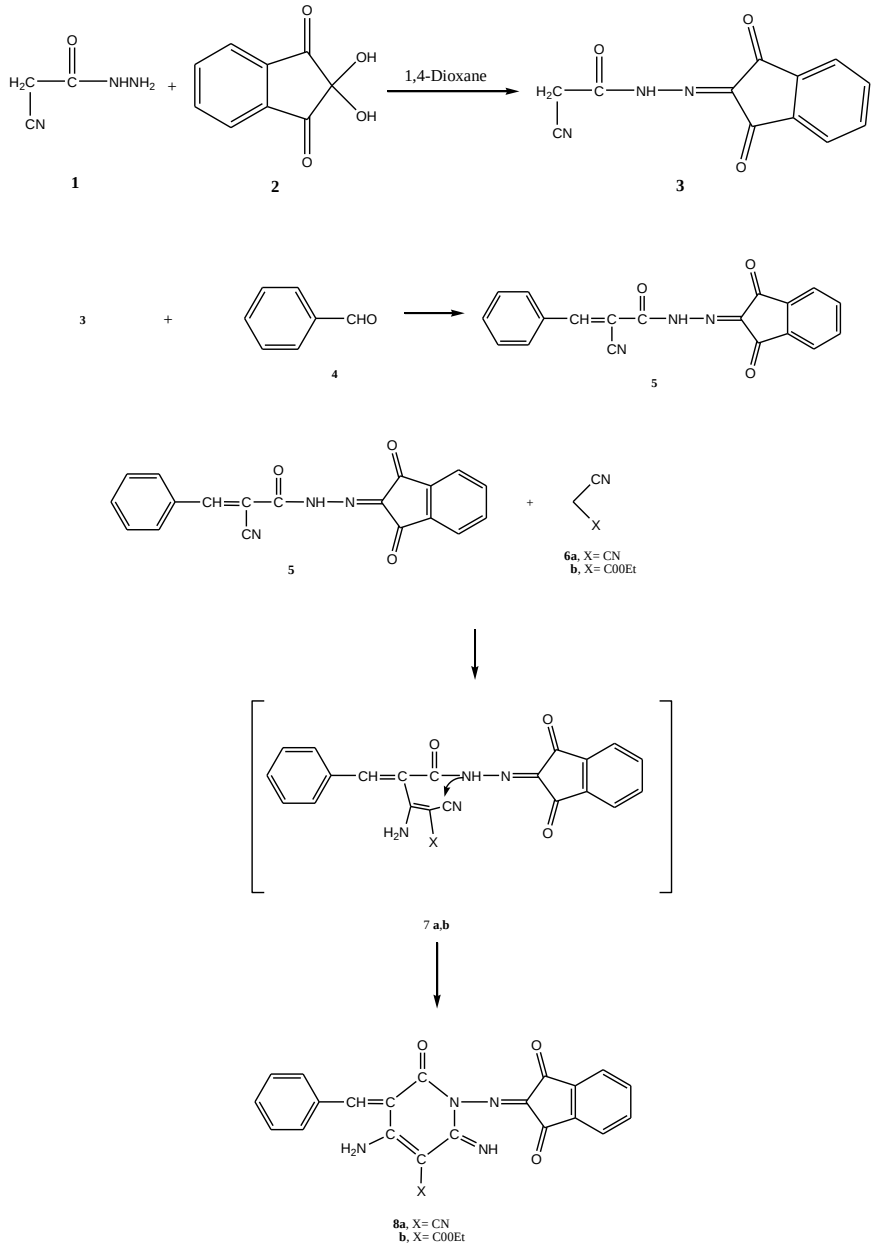
Chemistry

We herein report the synthesis of a series of biologically-active hydrazide-hydrazones whose precursor, the hydrazide-hydrazone derivative 3[16], was obtained via the reaction of cyanoacetic acid hydrazide (1) with ninhydrin (2) .

The structure of the latter product was based on analytical and spectral data. Thus, the H^1NMR spectrum of the reaction product showed a singlet at δ 3.82 corresponding to the active methylene group, a singlet at δ 8.80 for the NH group, and a multiplet at δ 7.30-7.39 resulting from the four C_6H_4 protons.

Further structural confirmation of compound 3 was achieved through studying its reactivity towards several chemical reagents. Thus, the reaction of compound 3 with benzaldehyde (4) gave the benzal derivative 5, whose analytical and spectral data are in agreement with the proposed structure. It is noteworthy that many arylidine derivatives were previously obtained via the reaction of aromatic aldehydes with cyanomethylene reagents[17- 19].

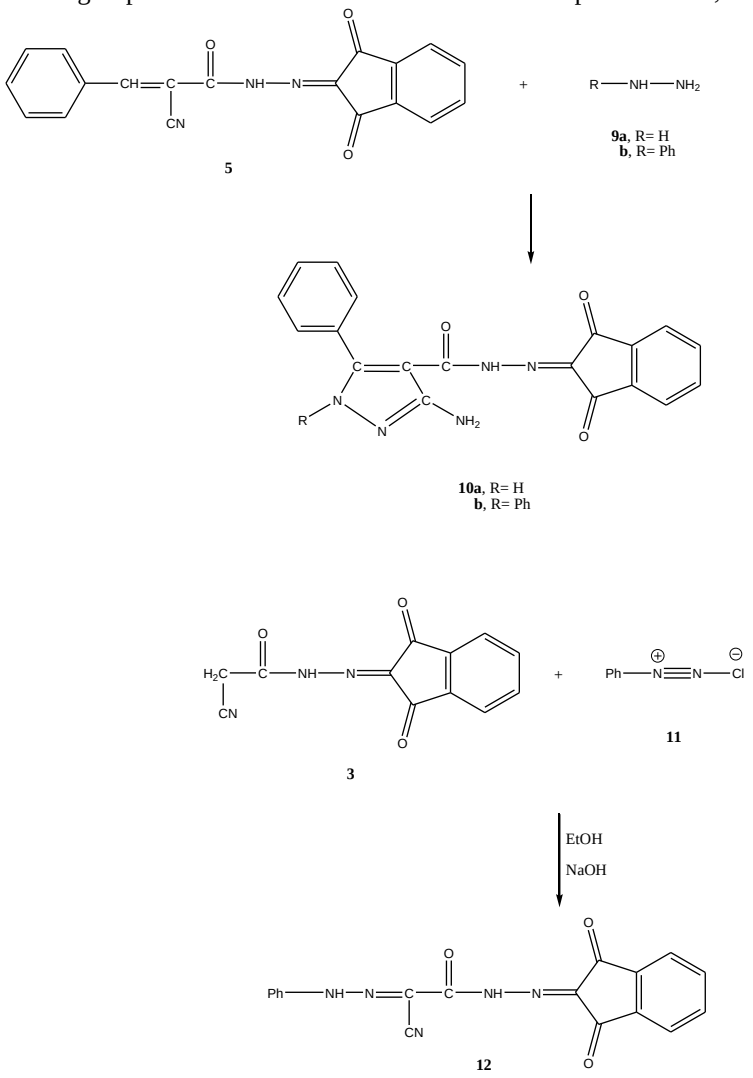
Compound 5 has reacted with either malononitrile (6a) or ethyl cyanoacetate (6b) to afford pyridine derivatives 8a,b, respectively (Scheme 1). Structure elucidation for the latter products was based on analytical and experimental data (see Experimental section).



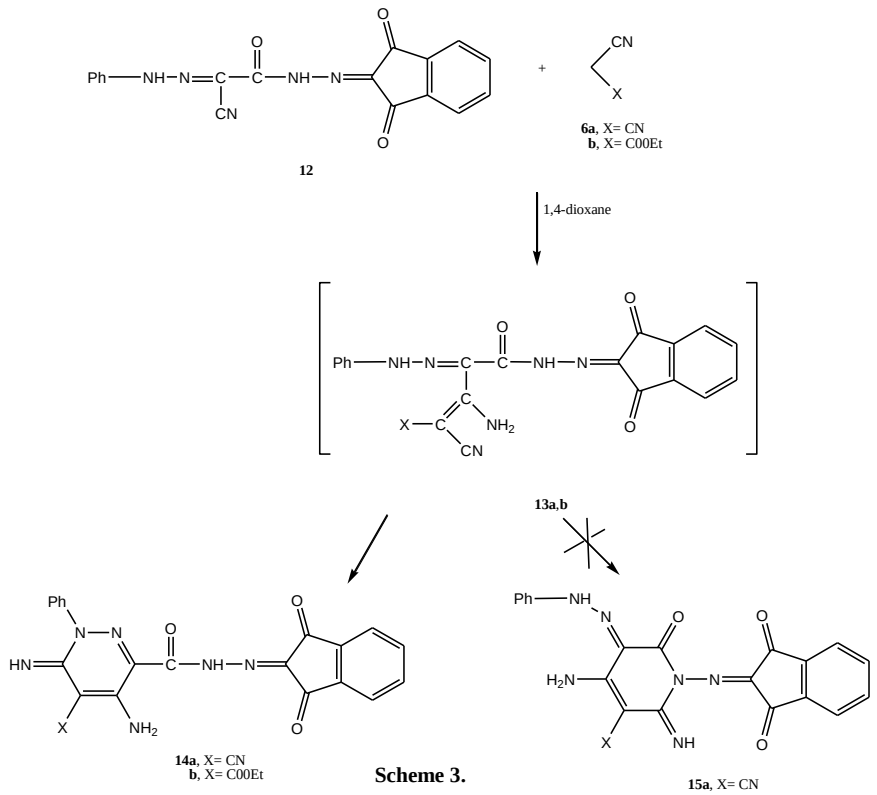
Scheme 1 .

On the other hand, the reaction of compound 5 with either hydrazine hydrate (9a) or phenyl hydrazine (9b) gave the pyrazole derivatives 10a,b, respectively.

The reaction of compound 3 with benzenediazonium chloride (11) in ethanol/sodium acetate at 0-5 ^{o}C gave the hydrazone derivative 12 (Scheme 2). Compound 12 reacted with either malononitrile (6a) or ethyl cyanoacetate (6b) and afforded products with molecular formulae $C_{21}H_{13}N_7O_3$ and $C_{23}H_{18}N_6O_5$, respectively. Two possible isomeric structures were proposed for the mentioned structures; either 14a,b or 15a,b (Scheme 3). Structures 14a,b were considered for the reaction products based on the H^1NMR data which asserted the presence of the -CO-NH- group of the hydrazone moiety through the chemical shift at δ 8.72-8.83, together with the absence of the shift characteristic of the phenyl hydrazo NH group which would have been eminent in the spectra of 15a,b.



Scheme 2.



Scheme 3.

Biological evaluation

TABLE 1. Effect of the obtained compounds on the growth of three human tumor cell lines.

Compound	IC_{50} (μmol L^{-1})		
	MCF-7	**NCI-H460**	**SF-268**
3	30.4 ± 2.8	20.1 ± 4.6	36.3 ± 4.5
5	0.01 ± 0.008	0.01 ± 0.006	0.08 ± 0.08
8a	77.8 ± 10.0	64.2 ± 8.4	70.2 ± 12.6
8b	34.2 ± 12.6	33.7 ± 6.6	44.2 ± 8.2
10a	32.0 ± 2.5	24.0 ± 4.6	26.5 ± 2.8
10b	10.0 ± 0.8	8.3 ± 2.8	16.5 ± 4.0
12	44.4 ± 6.8	26.1 ± 2.6	34.3 ± 2.5
14a	0.01 ± 0.008	0.01 ± 0.006	0.08 ± 0.08
14b	70.8 ± 10.0	66.2 ± 8.4	74.2 ± 12.6
Doxorubicin	0.04±0.008	0.09±0.008	0.09±0.007

Results are given in concentrations that were able to cause 50 % of cell growth inhibition (GI_{50}) after a continuous exposure of 48 hr and show means ± SEM of three-independent experiments performed in duplicate.

Structure activity relationship

Ten compounds were tested towards the three cancer cell lines namely breast adenocarcin oma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268). It is obvious from Table 1 that compound 5 which is 2-cyano-N'-(1,3-dioxo-1H-inden-2(3H)-ylidene)-3-phenylacrylohydrazide and 14a which is 4-amino-5-cyano-N'-(1,3-dioxo-1H-inden-2(3H)-ylidene)-6-imino-1-phenyl-1,6-dihydropyridazine-3-carbohydrazide showed the maximum inhibition effect towards the three cancer cell lines and such inhibition is much higher than the reference doxorubicin. Among the tested compounds, 10b which is 3-amino-N'-(1,3-dioxo-1H-inden-2(3H)-ylidene)-1,5-diphenyl-1H-pyrazole-4-carbohydrazide showed moderate activity. However, compounds 3, 8a, 8b, 10a, 12 and 14b showed the least activity towards the three cancer cell lines.

Experimental

Chemistry

All melting points were determined in open capillaries and are uncorrected. IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^{1}H-NMR spectra were measured on a Varian EM390-200 MHz instrument in CD_3SOCD_3 as solvent using TMS as internal standard, and chemical shifts are expressed as δ in units of parts per million (ppm). Analytical data were obtained from the Micro analytical Data Unit at Cairo University.

2-Cyano-N'-(1,3-dioxo-1H-inden-2(3H)-ylidene)acetohydrazide (3)

To a solution of cyanoacetic acid hydrazide (1) (6.00 g, 0.061 mol) in 80 ml of 1,4-dioxane, ninhydrin (2) (10.79 g, 0.061 mol) was added and the reaction mixture subjected to heat under reflux for 3 hr, after which it was poured on a mixture of ice and water and a few concentrated HCl drops were added to enhance precipitation. The formed precipitate was then collected by suction filtration.

Orange crystals from ethanol, yield 84.94 %, 12.50 g, m.p. 225-228 ^{o}C. IR (KBr): υ/cm^{-1}= 3470-3321 (NH), 2892 (CH_2), 3059 (CH aromatic), 2258 (CN), 1693-1680 (3CO), 1660 (C=N), 1644 (C=C). H^{1}NMR (DMSO) δ = 3.82 (s, 2H, CH_2), 7.30-7.39 (m, 4H, C_6H_4), 8.80 (s, 1H, NH). Calc. for $C_{12}H_7N_3O_3$ (241.20): C, 59.75; H, 2.93; N, 17.42 %. Found: C, 59.88; H, 3.01; N, 17.20 %.

2-Cyano-N'-(1,3-dioxo-1H-inden-2(3H)-ylidene)-3-phenylacrylohydrazide (5)

Compound 3 (2.00 g, 8.292×10^{-3} mol) has been dissolved in 50 ml of 1,4-dioxane and benzaldehyde (0.88 g, 8.292×10^{-3} mol) and 0.5 ml of piperidine stirred in. The reactants were then heated under reflux for 2 hr and then poured on ice and a few drops of concentrated HCl were added to enhance precipitation. The formed precipitate was then filtered out.

Pale yellow crystals from ethanol, yield 73.23 %, 2.00 g, m.p. 256-259 ^{o}C. IR (KBr): υ/cm^{-1}= 3482-3340 (NH), 3055 (CH aromatic), 2256 (CN), 1690-1683

(3CO), 1656 (C=N), 1640 (C=C). H^1NMR (DMSO) δ = 6.58 (s, 1H, CH), 7.26-7.38 (m, 9H, C_6H_5, C_6H_4), 8.80 (s, 1H, NH). Calc. for $C_{19}H_{11}N_3O_3$ (329.31): C, 69.30; H, 3.37; N, 12.76 %. Found: C, 69.51; H, 3.44; N, 12.81 %.

4-Amino-5-benzylidene-1-(1,3-dioxo-1H-inden-2(3H)-ylideneamino)-2-imino-6-oxo-1,2,5,6-tetrahydropyridine-3-carbonitrile (8a)

Compound 5 (0.30 g, 9.110×10^{-4} mol) was dissolved in 50 ml of 1,4-dioxane and malononitrile (0.06 g, 9.110×10^{-4} mol) was added with 0.5 ml of triethylamine as a catalyst. The reaction mixture was then heated under reflux for 3 hr and poured on ice, a few concentrated HCl drops were added and stirring was carried out for a few minutes. The obtained precipitate was then collected by suction filtration.

Pale brown black crystals from ethanol, yield 69.45 %, 0.25 g, m.p. 288-291 ^oC. IR (KBr): υ/cm^{-1}= 3494-3324 (NH_2, NH), 3059 (CH aromatic), 2220 (CN), 1692-1684 (3CO), 1669 (C=N), 1636 (C=C). H^1NMR (DMSO) δ = 4.88 (s, 2H, NH_2), 6.59 (s, 1H, CH), 7.28-7.37 (m, 9H, C_6H_5, C_6H_4), 7.89 (s, 1H, NH). Calc. for $C_{22}H_{13}N_5O_3$ (395.37): C, 66.83; H, 3.31; N, 17.71 %. Found: C, 66.98; H, 3.49; N, 17.86 %.

Ethyl 4-amino-5-benzylidene-1-(1,3-dioxo-1H-inden-2(3H)-ylideneamino)-2-imino-6-oxo-1,2,5,6-tetrahydropyridine-3-carboxylate (8b)

Compound 5 (0.40 g, 1.215×10^{-3} mol) was dissolved in 50 ml of 1,4-dioxane and ethyl acetoacetate (0.14 g, 1.215×10^{-3} mol) was added with 0.5 ml of triethylamine as a catalyst. The reaction mixture was then heated under reflux for 2.5 hr and poured onto ice and water, a few HCl (concentrated) drops were added and stirring was carried out for a few minutes. The obtained precipitate was then collected by suction filtration.

Orange crystals from ethanol yield 64.82 %, 0.35 g, m.p. 233-236 ^oC. IR (KBr): υ/cm^{-1}= 3473-3326 (NH), 3052 (CH aromatic), 2987, 2879 (CH_3, CH_2), 1679-1680 (4CO), 1650 (C=N), 1637 (C=C). H^1NMR (DMSO) δ = 1.14 (t, 3H, J = 7.01 Hz, CH_3), 4.22 (q, 2H, J = 7.02 Hz, CH_2), 4.80 (s, 2H, NH_2), 6.61 (s, 1H, CH), 7.28-7.40 (m, 9H, C_6H_5, C_6H_4), 7.80 (s, 1H, NH). Calc. for $C_{24}H_{18}N_4O_5$ (442.42): C, 65.15; H, 4.10; N, 12.66 %. Found: C, 65.27; H. 4.23; N, 12.73 %.

5-Amino- N'-(1,3-dioxo -1 H-inden -2 (3H) -ylidene) -3- phenyl -1H –pyrazole -4-carbohydrazide (10a)

To a solution of compound 5 (0.30 g, 9.110×10^{-4} mol) in 50 ml of 1,4-dioxane, hydrazine hydrate (0.05 g, 9.110×10^{-4} mol) was stirred in and the reaction mixture was heated under reflux for 2.5 hr, after which it was poured on ice and a few drops of concentrated HCl were added. The formed precipitate was collected by suction filtration.

Brown crystals from ethanol, yield 54.55 %, 0.18 g, m.p. 295-298^oC. IR (KBr): υ/cm^{-1}= 3453-3320 (NH_2, 2NH), 3061 (CH aromatic), 1690-1680 (3CO), 1663

(C=N), 1639 (C=C). H^1NMR (DMSO) δ = 4.60 (s, 2H, NH_2), 7.31-7.48 (m, 9H, C_6H_5, C_6H_4), 7.77, 8.81 (2s, 2H, 2NH). Calc. for $C_{19}H_{13}N_5O_3$ (359.34): C, 63.51; H, 3.65; N, 19.49 %. Found: C, 63.60; H, 3.70; N, 19.68 %.

3-Amino-N'-(1,3-dioxo-1H-inden-2(3H)-ylidene)-1,5-diphenyl-1H-pyrazole-4-carbohydrazide (10b)

To a solution of compound 5 (0.45 g, 1.367×10^{-3} mol) in 60 ml of 1,4-dioxane, phenyl hydrazine (0.15 g, 1.367×10^{-3} mol) was stirred in and the reaction mixture was heated under reflux for 2 hr, after which it was poured on ice and a few drops of concentrated HCl were added. The formed precipitate was collected by suction filtration.

Pale yellow crystals from ethanol yield 30 %, 0.18 g, m.p. 233-236 °C. IR (KBr): υ/cm^{-1}= 3483-3336 (NH), 3059 (CH aromatic), 1693-1683 (3CO), 1653 (C=N), 1640 (C=C). H^1NMR (DMSO) δ = 4.59 (s, 2H, NH_2), 7.29-7.46 (m, 14H, $2C_6H_5$, C_6H_4), 7.79 (s, 1H, NH). Calc. for $C_{25}H_{17}N_5O_3$ (435.43): C, 68.96; H, 3.94; N, 16.08 %. Found: C, 69.11; H, 4.04; N, 16.33 %.

2- (2- (1,3- Dioxo - 1H- inden - 2(3H) - ylidene) hydrazinyl) - 2- oxo- N' phenylacetohydrazonoyl cyanide (12)

To a solution of compound 3 (1.11 g, 4.60×10^{-3} mol) in 30 ml of ethanol containing 0.5 g of sodium hydroxide pellets, benzenediazonium chloride (0.65 g, 4.60×10^{-3} mol) [prepared by adding an aqueous sodium nitrite solution (0.32 g, 4.60×10^{-3} mol in 25 ml of water) to a cold solution of aniline (0.43 g, 4.60×10^{-3} mol) in 50 ml of conc. HCl at 0-5 °C, with continuous stirring] was added with stirring. The reaction mixture was left for the precipitate to coagulate, and the formed precipitate was collected by suction filtration.

Light brown crystals from ethanol, yield 45.31 %, 0.720 g, m.p. >300°C. IR (KBr): υ/cm^{-1}= 3477-3326 (2NH), 3063 (CH aromatic), 2256 (CN), 1692-1683 (3CO), 1650 (C=N), 1638 (C=C). H^1NMR (DMSO) δ = 7.26-7.41 (m, 9H, C_6H_5, C_6H_4), 7.77, 8.78 (2s, 2H, 2NH). Calc. for $C_{18}H_{11}N_5O_3$ (345.31): C, 62.61; H, 3.21; N, 20.28 %. Found: C, 62.93; H, 3.04; N, 19.98 %.

4-Amino-5-cyano-N'-(1,3-dioxo-1H-inden-2(3H)-ylidene)-6-imino-1-phenyl-1,6-dihydropyridazine-3-carbohydrazide (14a)

Compound 12 (0.25 g, 7.24×10^{-4} mol) was dissolved in 50 ml of 1,4-dioxane containing 0.5 ml of triethylamine. To this solution, malononitrile (0.05 g, 7.24×10^{-4} mol) has been added and the reaction mixture was then heated under reflux for 3 hr, after which it was poured on ice and a few drops of concentrated HCl were added. The formed precipitate was then collected by suction filtration.

Brown crystals from ethanol, yield 40 %, 0.12 g, m.p. >300 °C. IR (KBr): υ/cm^{-1}= 3467-3328 (2NH), 3055 (CH aromatic), 2222 (CN), 1689-1681 (3CO), 1651 (C=N), 1643 (C=C). H^1NMR (DMSO) δ = 4.62 (s, 2H, NH_2), 7.31-7.43 (m, 9H,

C_6H_5, C_6H_4), 7.81, 8.83 (2s, 2H, 2NH). Calc. for $C_{21}H_{13}N_7O_3$ (411.37): C, 61.31; H, 3.19; N, 23.83 %. Found: C, 61.57; H. 3.31; N, 23.92 %.

Ethyl 5-amino-6-(2-(1,3-dioxo-1H-inden-2(3H)-ylidene)hydrazinecarbonyl)-3-imino-2-phenyl-2,3-dihydropyridazine-4-carboxylate(14b)

Compound 12 (0.18 g, 5.21×10^{-4} mol) was dissolved in 40 ml of 1,4-dioxane containing 0.5 ml triethylamine. To this solution, ethyl cyanoacetate (0.06 g, 5.21×10^{-4} mol) has been added and the reaction mixture was then heated under reflux for 2.5 hr, after which it was poured on ice and a few drops of concentrated HCl were added. The formed precipitate was then collected by suction filtration.

Dark brown crystals from ethanol, yield 58.33 %, 0.14 g, m.p. 283-286 ^{o}C. IR (KBr): υ/cm^{-1}= 3479-3341 (2NH), 3053 (CH aromatic), 1690-1684 (4CO), 1654 (C=N), 1636 (C=C). H^1NMR (DMSO) δ = 1.16 (t, 3H, J= 6.34 Hz, CH_3), 4.24 (q, 2H, J= 6.34 Hz, CH_2), 4.67 (s, 2H, NH_2), 7.29-7.39 (m, 9H, C_6H_5, C_6H_4), 7.83, 8.72 (2s, 2H, 2NH). Calc. for $C_{23}H_{18}N_6O_5$ (458.43): C, 60.26; H, 3.96; N, 18.33 %. Found: C, 60.52; H, 4.02; N, 18.49 %.

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السمية لمشتقات الهيدرايزيد – هيدرازون تجاه خطوط الخلايا السرطانية والطبيعية

ميسون يوسف زكى
الهيئة القومية للرقابة والبحوث الدوائية – القاهرة – مصر.

اعطى تفاعل سيانواستيل هيدرازون مع النينهيدرين مشتقات الهيدرازيد – هيدرازون (3) . تم دراسة تفاعلات هذا المركب الاخير تجاه الكواشف الكيميائية المختلفة . تم قياس وتسجيل السمية للمركبات المخلقة حديثاً تجاه خطوط الخلايا السرطانية الثلاث : الادينوكارسينوما الثديية والخلايا الغير – صغيرة لسرطان الرئة وسرطان الجهاز العصبى المركزى.