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Short communication

Synthesis and antiproliferative activity of 3-substituted 1H-indole [3,2-*d*]-1,2,3-triazin-4 (3H)-ones

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Abstract – Some 3-substituted indole-triazin-4-ones were synthesized. Their antiproliferative activity against chronic myeloid leukaemia and non-Hodgkin lymphoma human cells was compared in vitro with that of daunorubicin. One compound appeared to be very effective against human CML. © Elsevier, Paris

3-substituted-indole-triazin-4-ones / synthesis / antiproliferative activity / daunorubicin

1. Introduction

1,2,3-Triazine-4-ones fused to a heterocyclic moiety have a high reactivity both chemically and biologically. Their 'masked' diazonium character infers the potential for versatile interaction with important macromolecules [1]. The cytostatic activity of many diazoazoles is known [2, 3, 4] but the instability of these structures led to the study of fused 1,2,3-triazines **1**. In view of the well-known antitumour properties of several indole derivatives [5, 6, 7], we report, as a part of our research program in this field, the synthesis and the antitumour activity of some 3-substituted indole-triazin-4-ones, with the aim to evaluate the importance of the heterocycle bearing the NNN linkage. The different substituents at the 3-position of the triazinone could influence their diazonium character, increasing the DNA alkylation capability [8].

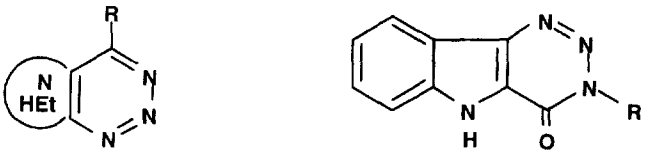

Figure 1.

2. Chemistry

The synthesis of the indoletriazinones **2** (**a–h**) was carried out according to *figure 1*. The starting compound was 3-nitroindole-2-carboxylic acid **3** [9]. Condensation of **3** with the appropiate amines was effected with 1,1'-carbonyldiimidazole (CDI) in dry DMF to afford the nitroamides **4** (**a–h**). Reduction of the nitro group with zinc and hydrochloric acid [10], followed by diazotization of the crude amines **5** (**a–h**), resulted in cyclization to the desidered indole 1,2,3,-triazin-4-ones **2** (**a–h**).

3. Pharmacological results

From the results reported in *table I*, four derivatives (**2a**, **2b**, **2d**, **2e**) appeared to be quite interesting antitumour agents. Compound **2a** was the most effective: its antiproliferative activity against human CML was very intensive even if less than that of daunorubicin (*figure 2*). The cytotoxicity of **2a** was strongest

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Table I. Comparison between the activity[a] of Daunorubicin and compounds **2a–h** against human CML[b] and non-Hodgkin lymphoma.

Compounds	μM	CML (% inhibition)	Non-Hodgkin lymphoma (% inhibition)
2a	15.5	77.3 ± 2.80	70.8 ± 0.26
	7.7	72.1 ± 1.59	57.0 ± 0.55
	3.8	69.7 ± 0.96	43.6 ± 0.23
	1.9	69.7 ± 0.57	28.2 ± 0.20
2b	15.5	54.8 ± 0.90	56.1 ± 1.16
	7.7	40.1 ± 1.10	49.4 ± 0.31
	3.8	9.1 ± 0.26	43.6 ± 0.40
	1.9	0	35.9 ± 0.61
2c	15.5	0	0
2d	15.5	65.6 ± 2.27	46.4 ± 1.21
	7.7	46.4 ± 0.81	34.2 ± 0.90
	3.8	12.2 ± 0.53	20.6 ± 0.58
	1.9	0	20.6 ± 0.83
2e	15.5	69.9 ± 0.87	48.8 ± 0.20
	7.7	48.4 ± 0.878	24.1 ± 0.15
	3.8	13.6 ± 1.71	18 ± 0.12
	1.9	9.1 ± 0.55	18 ± 0.50
2f, 2g, 2h	15.5	0	0
Daunorubicin	15.5	96.03 ± 0.80	96.7 ± 0
	7.7	89.1 ± 1.59	89.17 ± 0.84
	3.8	87.04 ± 1.29	83.2 ± 0.20
	1.9	83.37 ± 0.79	72.45 ± 0.78

[a]Data are expressed as mean ± S.E.; [b]CML = Chronic Mieloid Leukaemia.

against human CML cells and less against non-Hodgkin lymphoma cells. The lower activity with regard to daunorubicin and the greater selectivity of action against CML might be used in polychemoterapy protocols in which **2a** could be associated with other valid antitumorals. The results indicate that the presence of bulky hydrophobic substituents at the 3-position of indole-triazinone decreases activity.

4. Experimental protocols

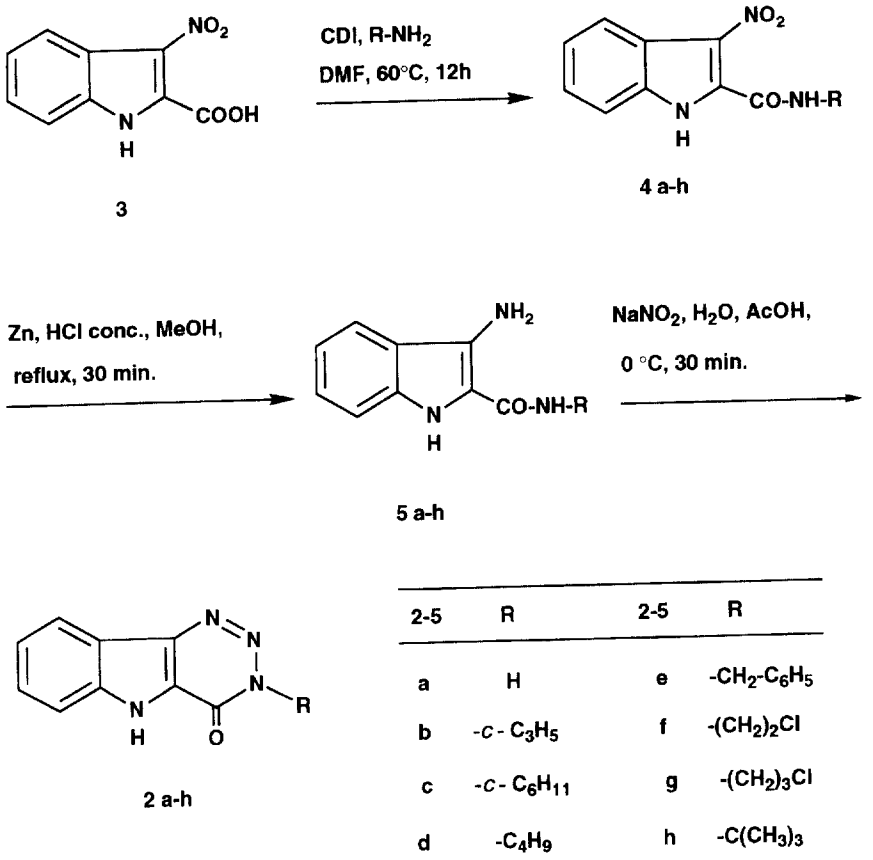
4.1. Chemistry

Organic chemicals were from Aldrich and Fluka. Biochemicals were from Sigma. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) and visualized with iodine or aqueous potassium permanganate. ^{1}H-NMR and ^{13}C-NMR were determined in DMSO-d_6 solutions with a Varian VXR 300 spectrometer, peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard. Melting points were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatographies were performed with Merck 60–200 mesh silica gel. All products gave ^{1}H-NMR spectra in agreement with the assigned structures. Organic solutions were dried over anhydrous magnesium sulfate. Elemental analyses were performed by the microanalytical laboratory of the Chemistry Department, University of Ferrara, and agreed within 0.4% with the theoretical values.

*4.1.1. N-substituted 3-nitroindole-2-carboxamides **4a–h***

Solid 1,1'-carbonyldiimidazole (CDI) (1.1 mmol) was added to a solution of **3** (1 mmol) in dry DMF (10 mL) and the mixture was stirred for 15 min at 55 °C. The appropriate amine (1 mmol) was added and the mixture was stirred at 60 °C for 12 h, then evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (eluting with toluene–acetone, 7:3) and crystallized from ethyl acetate–petroleum ether.

4a: (80%); mp 250 °C; ^{1}H-NMR (DMSO-d_6): δ 7.39–7.59 (m, 2H), 8.10–8.15 (m, 1H), 8.30 (bs, 2H), 13.20 (bs, 1H).



2-5	R	2-5	R
a	H	e	$-CH_2-C_6H_5$
b	$-c-C_3H_5$	f	$-(CH_2)_2Cl$
c	$-c-C_6H_{11}$	g	$-(CH_2)_3Cl$
d	$-C_4H_9$	h	$-C(CH_3)_3$

Figure 2.

4b: (75%); mp 244 °C; ^{1}H-NMR (DMSO-d_6): δ 0.56–0.59 (m, 2H), 0.76–0.79 (m, 2H), 2.8–2.9 (m, 1H), 7.38–7.41 (m, 2H), 7.52–7.55 (m, 1H), 8.08–8.11 (m, 1H), 9.06 (bs, 1H), 12.6 (bs, 1H).

4c: (82%); mp 265 °C; ^{1}H-NMR (DMSO-d_6): δ 1.29–1.92 (m, 10 H), 3.9 (m, 1H), 7.39–7.42 (m, 2H), 7.50–7.53 (m, 1H), 8.11–8.13 (m, 1H), 8.95 (bs, 1H), 12.6 (bs, 1H).

4d: (77%); mp 212 °C; ^{1}H-NMR (DMSO-d_6): δ 0.88–0.92 (m, 3H), 1.40–1.45 (m, 2H), 1.54–1.59 (m, 2H), 3.32–3.38 (m, 2H), 7.41–7.44 (m, 2H), 7.56–7.57 (m, 1H), 8.12–8.15 (m, 1H), 9.06 (bs, 1H), 12.6 (bs, 1H).

4e: (83%); mp 268 °C; ^{1}H-NMR (DMSO-d_6): δ 4.60–4.62 (m, 2H), 7.33–7.61 (m, 8H), 8.15–8.18 (m, 1H), 9.6 (bs, 1H), 12.6 (bs, 1H).

4f: (74%); mp 215 °C; ^{1}H-NMR (DMSO-d_6): δ 3.68–3.76 (m, 2H), 3.78–3.80 (m, 2H), 7.41–7.44 (m, 2H), 7.56–7.59 (m, 1H), 8.12–8.14 (m, 1H), 9.4 (bs, 1H), 13.3 (bs, 1H).

4g: (65%); mp 232 °C; ^{1}H-NMR (DMSO-d_6): δ 2.0–2.06 (m, 2H), 3.36–3.49 (m, 2H), 3.76–3.81 (m, 2H), 7.40–7.44 (m, 2H), 7.56–7.57 (m, 1H), 8.12–8.14 (m, 1H), 9.2 (bs, 1H), 12.5 (bs, 1H).

4h: (68%); mp 218 °C; ^{1}H-NMR (DMSO-d_6): δ 1.41 (s, 9H), 7.38–7.40 (m, 2H), 7.45–7.50 (m, 1H), 8.05–8.1 (m, 1H), 8.4 (bs, 1H), 12.6 (bs, 1H).

4.1.2. N-substituted 3-aminoindole-2-carboxamides ***5a–h***

A solution of nitroamide **4a–h** (3 mmol) in MeOH (15 mL) was treated with zinc dust (2 g) and concentrated hydrochloric acid (3 mL) was added slowly. The mixture was stirred under reflux for 30 min; then the excess of zinc dust was removed. The filtrate was evaporated, diluted with water, made alkaline with 2 M aqueous ammonia and extracted with ethyl acetate (3 x 50 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness. Owing to their instability, the amines **5a–h** were used directly in the next step.

4.1.3. 3-Substituted 1H-indole-[3,2-d]-1,2,3-triazin-4(3H)-ones ***2a–h***

The crude aminocarboxamide **5a–h** (8.2 mmol) was dissolved in a mixture of H_2O (6 mL) and AcOH (12 mL) at 0 °C. Sodium nitrite (0.62 g, 9 mmol) in H_2O (6 mL) was added slowly. The mixture was kept at 10 °C for 30 min, neutralized with sodium carbonate and extracted with CH_2Cl_2 (3 x 30 mL). The organic phase was dried (Na_2SO_4) and evaporated to

dryness. The residue was purified by column chromatography on silica gel eluting with ethyl acetate–petroleum ether (1:1) and crystallized from ethyl acetate–petroleum ether.

2a: (74%); mp 270 °C; ^{1}H-NMR (DMSO-d_6): δ 7.05–7.12 (m, 1H), 7.15–7.37 (m, 2H), 8.12–8.15 (m, 1H), 12.5 (bs, 1H), 14.5 (bs, 1H); ^{13}C-NMR (DMSO-d_6): δ 114.15, 120.25, 121.13, 123.43, 123.66, 129.43, 136.52, 139.23, 152.82.

2b: (71%); mp 225 °C; ^{1}H-NMR (DMSO-d_6): δ 1.21–1.26 (m, 4H), 4.08 (m, 1H), 7.47 (m, 1H), 7.69 (m, 2H), 8.24–8.28 (m, 1H), 13.1 (bs, 1H); ^{13}C-NMR (DMSO-d_6): δ 6.68, 31.28, 113.35, 119.71, 120.28, 122.24, 122.35, 128.20, 134.84, 138.83, 152.14.

2c: (68%); mp 195 °C; ^{1}H-NMR (DMSO-d_6): δ 1.40–2.01 (m, 10 H), 5.06 (m, 1H), 7.44–7.47 (m, 1H), 7.62–7.65 (m, 2H), 8.22–8.27 (m, 1H), 12.55 (bs, 1H); ^{13}C-NMR (DMSO-d_6): δ 25.35, 25.73, 32.32, 56.04, 113.63, 119.97, 120.69, 122.56, 122.73, 128.65, 135.21, 139.20, 151.10.

2d: (73%); mp 185 °C; ^{1}H-NMR (DMSO-d_6): δ 0.91–0.96 (m, 3H), 1.33–1.41 (m, 2H), 1.81–1.86 (m, 2H), 4.47–4.57 (m, 2H), 7.41–7.46 (m, 1H), 7.62–7.68 (m, 2H), 8.23–8.25 (m, 1H), 12.5 (bs, 1H); ^{13}C-NMR (DMSO-d_6): δ 14.30; 20.06, 31.51, 49.53, 114.24, 119.51, 120.18, 121.22, 123.52, 129.44, 136.23, 139.51, 151.96.

2e: (86%); mp 120 °C; ^{1}H-NMR (DMSO-d_6): δ 5.61 (s, 2H), 7.39 (m, 5H), 8.0 (m, 1H), 8.1 (m, 1H), 8.2 (m, 2H), 12.5 (bs, 1H); ^{13}C-NMR (DMSO-d_6): δ 52.75, 119.73, 124.99, 128.13, 128.22, 128.46, 128.99, 133.45, 135.90, 136.73, 144.07, 155.06.

2f: (77%); mp 181 °C; ^{1}H-NMR (DMSO-d_6): δ 4.12–4.16 (t, 2H, J = 0.66 Hz), 4.82–4.86 (t, 2H, J = 0.66 Hz), 7.44–7.49 (m, 1H), 7.64–7.71 (m, 2H), 8.25–8.27 (m, 1H), 12.6 (bs, 1H); ^{13}C-NMR (DMSO-d_6): δ 42.06, 50.20, 113.53, 119.76, 120.45, 122.51, 122.71, 128.55, 135.25, 138.91, 151.39.

2g: (69%); mp 200 °C; ^{1}H-NMR (DMSO-d_6): δ 2.28–2.35 (m, 2H), 3.73–3.79 (t, 2H, J = 1.59 Hz), 4.58–4.65 (t, 2H, J = 1.70 Hz), 7.40–7.47 (m, 1H), 7.60–7.69 (m, 2H), 8.23–8.25 (m, 1H), 12.5 (bs, 1H); ^{13}C-NMR (DMSO-d_6): δ 42.63, 46.62, 49.71, 113.56, 119.78, 120.36, 122.51, 122.74, 128.36, 135.36, 138.84, 151.27.

2h: (66%); mp 178–180 °C; ^{1}H-NMR (DMSO-d_6): δ 1.79 (s, 9H), 7.4 (m, 1H), 7.62 (m, 2H), 8.18 (m, 1H), 12.93 (bs, 1H); ^{13}C-NMR (DMSO-d_6): δ 28.33, 64.54, 113.32, 119.47, 120.33, 122.24, 123.65, 128.19, 134.77, 138.92, 151.96.

4.2. Biology

In the various experiments conducted in vitro two different types of human tumor cell lines were used: JJhan cells from a chronic myeloid leukemia and SupT1 cells from a non-Hodgkin lymphoma. All the cells were maintained by serial passage in this laboratory [11].

The cell cultures were performed in RPMI medium supplemented with 10% heat-inactivated (56 °C, 30 min) foetal calf serum, penicillin (50 iu/mL) and streptomycin (50 μ/mL). The initial inoculation for the various tests was about 5 x 10^4 cells. All the cells were grown in suspension in cup-trays of 24 wells, incubated at 37 °C in a 95% humid atmosphere of 5% CO_2 and 95% air and kept in contact with the various substances being tested for 48 h. At the end of the incubation period, a count of both alive and dead cells was performed using a solution of 4% Trypan Blue and Bürker's globule counter. All tests were carried out in triplicate and the results compared with those obtained using equimolecular doses of daunorubicin (Pharmacia, Milan).

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References

[1] Stevens M.F.G., in: Ellis G.P., West G.B. (Eds.), Progress in Medicinal Chemistry, North Holland, Amsterdam, 1976, pp. 205–269.
[2] Sava G., Giraldi T., Lassiani L., Nisi C., Cancer Treatment Rep. 63 (1979) 93–98.
[3] Shealy Y.F.J., Pharm. Sci. 59 (1970) 1533–1558.
[4] Saunders P.P., Schultz G.A., Biochem. Pharmacol. 19 (1970) 911–919.
[5] Angerer E., Prekajac J., Strohmeier J., J. Med. Chem. 27 (1984) 1439–1447.
[6] Angerer E., Prekajac J., J. Med. Chem. 26 (1983) 113–116.
[7] Angerer E., Prekajac J., Strohmeier J., J. Med. Chem. 30 (1987) 131–136.
[8] Threadgill M.D., in: William D.E.V. (Ed.), Chemistry of Antitumour Agents, Blackie & Sons, New York, 1990, p. 187.
[9] Angelico F., Velardi G., Gazzetta 34 (1904) 57–66.
[10] Garuti L., Roberti M., Rossi M., Il Farmaco 51 (1996) 757–760.
[11] Ishioka C., Kanamuru R., Sato T., Dei T., Konishi Y., Asamura M., Wakui A., Cancer Res. 48 (1988) 2813–2818.