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SYNTHESIS OF 8,9,10,11-TETRAHYDROINDOLO[2,1-*c*]BENZO[1,2,4]-TRIAZINE. A NEW RING SYSTEM

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Abstract – Indolo[2,1-*c*]benzo[1,2,4]triazine, a new ring system, was synthesized by diazotization of 2-amino-1 aryltetrahydroindoles followed by an intramolecular coupling of the diazonium group with the aryl moiety. All the new compounds were screened at the NCI for antiproliferative property but none of them showed significant activity.

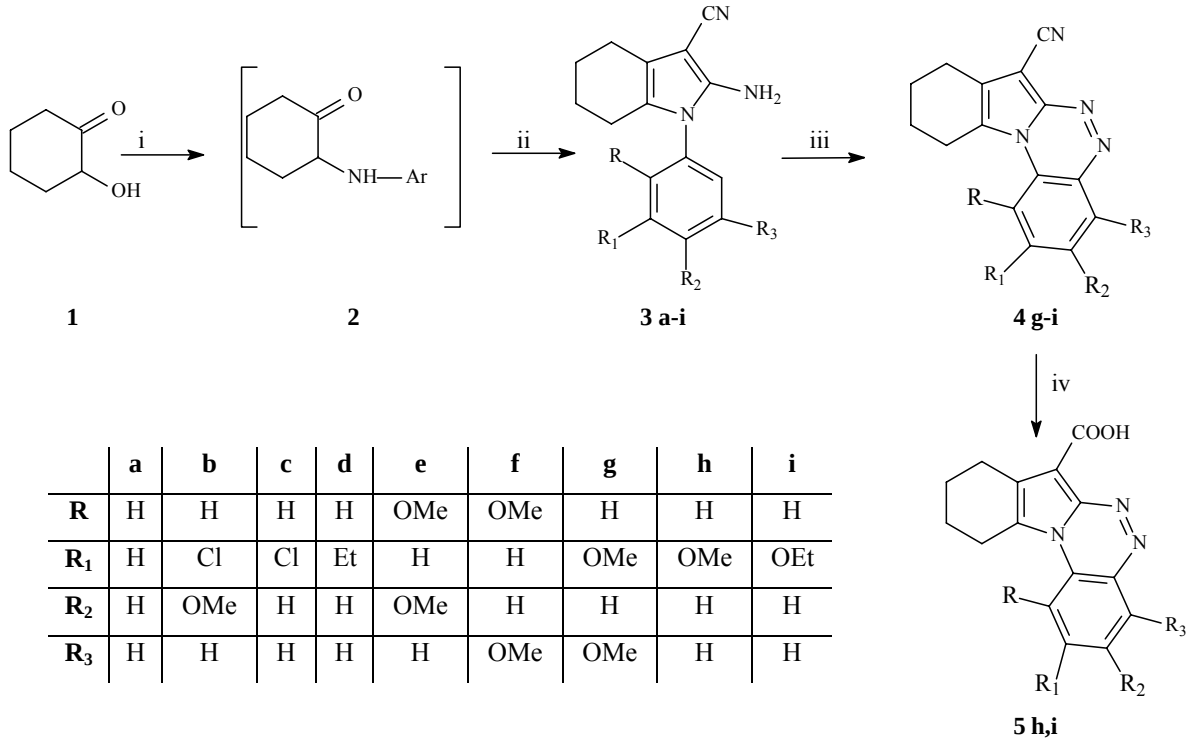
Many derivatives of the 1,2,4-triazine nucleus are well known compounds endowed with a wide range of biological activities. In fact some components of this class of heterocycles have found use in agrochemistry (Sencor® and Goltix®), and many 1,2,4-triazines are used in therapy, their activity ranging from antitumor (Lamotrigine®) to antibacterial and tubercolostatic (Panfuran®), antibiotic (Rocephin®).[1] Moreover 1,2,4-triazine can also be regarded as azapyrimidines. A number of 6-azapyrimidine nucleotides and nucleosides are known in literature and their biological properties have been intensively studied. Most significant among these are 6-azauracil, 6-azauridine, 6-azathymine, 6-azacytosine, and 6-azacytidine. Such compounds were tested for many biological activities and showed good antitumor and antiviral properties.[1] Also annelated 1,2,4-triazines have shown antineoplastic activities. Infact benzo-condensation originates compounds that modulate the biological activity, many of them showing IC_{50} of 8-500 μM in the inhibition of growth of Hep-2, HeLa, peritoneal carcinoma and pleurocarcinoma.[2] Moreover pyrazolo[5,1-*c*][1,2,4]triazine derivatives exhibited antineoplastic activity against the mouse sarcoma 180 and methylcolanthrene-induced rat sarcoma.[3]

These findings prompted us to synthesize condensed 1,2,4-triazines with azole nuclei. Infact, in connection with our studies on polycondensed nitrogen heterocycles of biological interest, we successfully set the new versatile synthesis of the pyrrolo[2,1-*c*][1,2,4]triazine ring system, obtained in moderate to good yelds (50-90%), by reaction of 2-diazopyrroles with the sodium salts of β-diketones, β-carbonitriles, and β-keto esters. All these pyrrolotriazines, screened by NCI, demonstrated inhibitory

effects in the growth of a wide range of cancer cell lines at 10^{-5} – 10^{-6} M level.[4]

Also derivatives of the indolo[2,1-*c*][1,2,4]triazine ring system, obtained by an analogous synthetic pathway, showed *in vitro* antiproliferative activity (GI_{50} 10^{-5} – 10^{-6}M).

Therefore, continuing our studies on pyrrole- and indole-fused heterocycles we now report the synthesis of the new ring system tetrahydroindolo[2,1-*c*]benzo[1,2,4]triazine.



	a	b	c	d	e	f	g	h	i
R	H	H	H	H	OMe	OMe	H	H	H
R_1	H	Cl	Cl	Et	H	H	OMe	OMe	OEt
R_2	H	OMe	H	H	OMe	H	H	H	H
R_3	H	H	H	H	H	OMe	OMe	H	H

Scheme 1 **i** $ArNH_2$, *p*-Toluensulfonic acid, cyclohexane; **ii** malononitrile, piperidine, cyclohexane; **iii** Acetic acid/sodium nitrite; **iv** 37% HCl

The synthetic approach to such ring system was achieved by reacting 2-hydroxycyclohexanone **(1)** with substituted anilines to give the non-isolated intermediates α-arylamino ketones **(2)** which, by condensation with malononitrile, afforded the 2-amino-1-aryl-tetrahydroindoles **(3)** in good yields (60-85%) and with a broad range of substituents. The amines **(3a-i)** were diazotized at 0°C with stoichiometric amount of sodium nitrite in aqueous acetic acid. The diazonium group, as soon as formed, whould have cyclized to the tetracyclic system by an intramolecular coupling with the position 6 or 2 (if not substituted) of the phenyl bounded to the nitrogen of the tetrahydroindole nucleus. However the indolobenzotriazines were obtained in reasonable yields (50-60%) only in the case of amines **(3g-i)** in which the electron donating groups in the phenyl ring were suitably placed to activate the position 6 to undergo the electrophilic attack by the diazonium group. In the case of amines **(3a-f)** it was impossible to isolate the indolobenzotriazines even when reaction mixture was heated (60°C or reflux), or when hydrochloric acid (6N or 12N) was employed. This is attributable to the fact that the 6 position was not nucleophilic enough either for the absence of an electron donating group or the presence of a methoxy group in a non conjugated position with the reaction centre. The case of amine **(3f)**, bearing a methoxy group in the position 5' is not easy to

understand. However, calculation of the atomic charge[5] using the software PIMMS (V.143), supplied by Oxford Molecular-Accelrys on the unsubstituted ortho position at the phenyl ring for the amines of type **(3)** showed that in compound **(3f)** the charge at the position 6 was -0.07 whereas for derivatives **(g-i)** the charge resulted high enough (from -0.09 to -0.1) to justify the reactivity.

The cyano group in the position 3 of the indolobenzotriazines **(4g-i)** was hydrolyzed with concentrated hydrochloric acid and in the case of **(4h** and **i)** the corresponding carboxy acids **(5h** and **i)** respectively, were isolated whereas in the case of **4g** the reaction mixture was complex and the expected acid was not isolated.

The indolobenzotriazines **(4g-i** and **5h** and **i)** were submitted to the NCI and screened against a panel of 60 human tumor cell lines, but none of them showed significant antiproliferative activity.

EXPERIMENTAL

All melting points were taken on a Buchi-Tottoli capillary apparatus and are uncorrected; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.3 MHz respectively in DMSO-d_6 , unless otherwise specified, using a Bruker AC-E series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with a BIOTAGE FLASH40i chromatography module (prepacked polyethylene cartridge system).

General Procedure for the Synthesis of 2-Amino-1-aryl-4,5,6,7-tetrahydroindole-3-carbonitrile (3a-i).

A mixture of 11.3 g (0.1 mol) of 2-hydroxycyclohexanone **(1)**, the appropriate amine (0.1 mol), and a catalytic amount of *p*-toluensulfonic acid in cyclohexane (80 mL) was heated under reflux for 1 h by using a Dean-Stark trap. After the addition of piperidine (2 mL), 6.6 g (0.1 mol) of malononitrile dissolved in hot cyclohexane (10 mL) was added in such a manner so as to mantain gentle boiling. After cooling, the precipitate was collected by filtration and recrystallized from ethanol.

2-Amino-1-phenyl-4,5,6,7-tetrahydroindole-3-carbonitrile (3a). Yield: 85%. The analytical as well as mp, IR and NMR data were identical to those reported in literature.[6]

2-Amino-1-(3′-chloro-4′-methoxyphenyl)-4,5,6,7-tetrahydroindole-3-carbonitrile (3b). Yield: 60%; mp: 162-164°C; IR: 3317 and 3426 (NH_2), 2197 (CN) cm^{-1}; ^{1}H NMR δ: 1.95 (4H, br s, CH_2 x 2), 2.36 (2H, s, CH_2), 2.62 (2H, s, CH_2), 4.20 (3H, s, CH_3), 5.81 (2H, s, NH_2), 7.54 (1H, s, H-2'), 7.55-7.69 (2H, m, H-5' and H-6'); ^{13}C NMR δ: 21.2 (t), 21.3 (t), 22.5 (t x 2), 56.3 (q), 69.4 (s), 113.1 (d), 115.0 (s), 117.9 (s), 121.0 (s), 121.3 (s), 127.9 (d), 128.0 (s), 129.2 (d), 146.9 (s), 154.4 (s). Anal. Calcd for $C_{16}H_{16}N_3OCl$: C, 63.68; H, 5.34; N, 13.93. Found: C, 63.80; H, 5.41; N, 14.10.

2-Amino-1-(3′-chlorophenyl)-4,5,6,7-tetrahydroindole-3-carbonitrile (3c). Yield: 60%; mp: 139-141°C; IR: 3329 and 3442 (NH_2), 2200 (CN) cm^{-1}; ^1H NMR δ: 1.65 (4H, br s, CH_2 x 2), 2.09 (2H, s, CH_2), 2.33 (2H, s, CH_2), 5.60 (2H, s, NH_2), 7.25-7.53 (4H, m, Ar-H); ^{13}C NMR δ: 21.2 (t), 21.5 (t), 22.5 (t), 22.6 (t), 70.1 (s), 115.6 (s), 117.7 (s), 120.8 (s), 126.4 (d), 127.6 (d), 128.2 (d), 131.1 (d), 133.7 (s), 136.5 (s), 146.7 (s). Anal. Calcd for $C_{15}H_{14}N_3Cl$: C, 66.30; H, 5.19; N, 15.46. Found: C, 66.44; H, 5.20; N, 15.34.

2-Amino-1-(3′-ethylphenyl)-4,5,6,7-tetrahydroindole-3-carbonitrile (3d). Yield: 65%; mp: 141-143°C; IR: 3342 and 3450 (NH_2), 2196 (CN) cm^{-1}; ^1H NMR δ: 1.21 (3H, t, J = 7.8 Hz, CH_3), 1.66 (4H, br s, CH_2 x 2), 2.10 (2H, s, CH_2), 2.36 (2H, s, CH_2), 2.61 (2H, q, J = 7.8 Hz, CH_2), 5.41 (2H, s, NH_2), 7.09 (1H, d, J = 7.3 Hz, H-4'), 7.13 (1H, s, H-2'), 7.27 (1H, d, J = 7.3 Hz, H-6'), 7.42 (1H, t, J = 7.3 Hz, H-5'); ^{13}C NMR δ: 15.3 (q), 21.3 (t), 21.7 (t), 22.6 (t), 22.7 (t), 27.9 (t), 69.9 (s), 115.3 (s), 117.9 (s), 120.9 (s), 124.6 (d), 126.7 (d), 126.7 (d), 129.5 (d), 133.1 (s), 145.5 (s), 146.5 (s). Anal. Calcd for $C_{17}H_{19}N_3$: C, 76.95; H, 7.22; N, 15.84. Found: C, 77.20; H, 7.25; N, 16.00.

2-Amino-1-(2′,4′-dimethoxyphenyl)-4,5,6,7-tetrahydroindole-3-carbonitrile (3e). Yield: 60%; mp: 178-180°C; IR: 3345 and 3424 (NH_2), 2195 (CN) cm^{-1}; ^1H NMR δ: 1.63 (4H, br s, CH_2 x 2), 1.90-2.00 (2H, m, CH_2), 2.33 (2H, s, CH_2), 3.75 (3H, s, CH_3), 3.81 (3H, s, CH_3), 5.22 (2H, s, NH_2), 6.59 (1H, dd, J = 8.4 and 1.9 Hz, H-5'), 6.72 (1H, d, J = 1.9 Hz, H-3'), 7.10 (1H, d, J = 8.4 Hz, H-6'); ^{13}C NMR δ: 21.0 (t), 21.2 (t), 22.6 (t), 22.8 (t), 55.5 (q), 55.7 (q), 68.9 (s), 99.7 (d), 105.2 (d), 114.3 (s), 116.0 (s), 118.3 (s), 121.6 (s), 130.4 (d), 147.3 (s), 156.4 (s), 160.8 (s). Anal. Calcd for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.40; H, 6.40; N, 14.31.

2-Amino-1-(2′,5′-dimethoxyphenyl)-4,5,6,7-tetrahydroindole-3-carbonitrile (3f). Yield: 60%; mp: 114-115°C; IR: 3322 and 3408 (NH_2), 2192 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.73 (4H, br s, CH_2 x 2), 2.01-2.29 (2H, m, CH_2), 2.48 (2H, s, CH_2), 3.77 (6H, s, CH_3 x 2), 4.01 (2H, s, NH_2), 6.72 (1H, d, J = 2.3 Hz, H-6'), 6.91-7.03 (2H, m, H-3' and H-4'); ^{13}C NMR ($CDCl_3$) δ: 21.4 (t), 21.4 (t), 22.7 (t), 22.8 (t), 55.6 (q), 56.2 (q), 72.4 (s), 113.4 (d), 114.9 (d), 115.0 (d), 116.1 (s), 117.6 (s), 122.7 (s), 123.8 (s), 145.2 (s), 149.0 (s), 153.5 (s). Anal. Calcd for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.90; H, 6.53; N, 14.30.

2-Amino-1-(3′-5′-dimethoxyphenyl)-4,5,6,7-tetrahydroindole-3-carbonitrile (3g). Yield: 65%; mp: 174-176°C ; IR: 3330 and 3412 (NH_2), 2196 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.73-1.77 (4H, br s, CH_2 x 2), 2.23 (2H, s, CH_2), 2.50 (2H, s, CH_2), 3.81 (6H, s, CH_3 x 2), 4.07 (2H, s, NH_2), 6.39 (2H, d, J = 2.5 Hz, H-2' and H-6'), 6.51 (1H, t, J = 2.5 Hz, H-4') ; ^{13}C NMR ($CDCl_3$) δ: 21.4 (t), 21.9 (t), 22.8 (t), 22.9 (t), 55.5 (q x 2), 72.0 (s), 100.5 (d), 105.5 (d x 2), 116.4 (s), 117.5 (s), 122.1 (s), 136.5 (s), 114.6 (s), 161.4 (s x 2). Anal. Calcd for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.65; H, 6.51; N, 14.32.

2-Amino-1-(3′-methoxyphenyl)-4,5,6,7-tetrahydroindole-3-carbonitrile (3h). Yield: 71%; mp: 153-155°C; IR: 3333 and 3415 (NH_2), 2195 (CN) cm^{-1}; ^1H NMR δ: 1.66 (4H, s, CH_2 x 2), 2.12 (2H, s, CH_2), 2.35 (2H, s, CH_2), 3.80 (3H, s, CH_3), 5.47 (2H, s, NH_2), 6.85 (1H, t, *J* = 1.9 Hz, H-2'), 6.90 (1H, dt, *J* = 8.4 and 1.9 Hz, H-4'), 7.00 (1H, dd, *J* = 8.4 and 1.9 Hz, H-6'), 7.40 (1H, td, *J* = 8.4 and 1.9 Hz, H-5'); ^{13}C NMR δ: 21.3 (t), 21.6 8 (t), 22.6 (t), 22.7 (t), 55.4 (q), 113.0 (d), 114.2 (d), 115.3 (s), 118.0 (s), 119.5 (d), 121.0 (s), 122.4 (s), 130.3 (d), 136.2 (s), 146.6 (s), 160.0 (s). Anal. Calcd for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72. Found: C, 71.90; H, 6.40; N, 15.70.

2-Amino-1-(3′-ethoxyphenyl)-4,5,6,7-tetrahydroindole-3-carbonitrile (3i). Yield: 61%; mp: 145-147°C; IR: 3355 and 3440 (NH_2), 2188 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.43 (3H, t, *J* = 7.3 Hz, CH_3), 1.74 (4H, br s, CH_2 x 2), 2.20 (2H, s, CH_2), 2.49 (2H, s, CH_2), 3.97 (2H, s, NH_2), 4.04 (2H, q, *J* = 7.3 Hz, CH_2), 6.76 (1H, t, *J* = 1.9 Hz , H-2'), 6.81 (1H, dt, *J* = 7.8 and 1.9 Hz, H-4'), 6.94 (1H, td, *J* = 7.8 and 1.9 Hz, H-6'), 7.34 (1H, t, *J* = 7.8 Hz, H-5'); ^{13}C NMR ($CDCl_3$) δ: 14.7 (q), 21.5 (t), 21.9 (t), 22.9 (t), 23.0 (t), 63.9 (t), 72.3 (s), 113.5 (d), 114.9 (d), 116.6 (s), 117.6 (s), 119.2 (d), 122.3 (s), 130.5 (d), 136.1 (s), 144.7 (s), 160.0 (s) Anal. Calcd for $C_{17}H_{19}N_3O$: C, 72.57; H, 6.81; N, 14.93. Found: C, 72.58; H, 6.73; N, 15.11.

General Procedure for the Synthesis of Substituted 7-Cyano-8,9,10,11-tetrahydroindolo[2,1-*c*]-benzo[1,2,4]triazines (4g-i)

To a solution of the amino derivatives **(3a-i)** (5 mmol) in glacial acetic acid (10 mL), sodium nitrite (379.4 mg, 5.5 mmol) dissolved in the minimum amount of water, was added dropwise at 0-5°C with stirring. The mixture was stirred for additional 2 h at rt and then neutralized with a 10% sodium carbonate solution. The solid precipitated was filtered and air dried. In the case of the amines **(3a-f)** the reaction mixture showed to be very complex with no prevalent product. In the case of the amines **(3g-i)** a major product was formed, therefore the reaction mixture was purified by column chromathography (eluent: dichloromethane: ethyl acetate 9:1).

7-Cyano-2,4-dimethoxy-8,9,10,11-tetrahydroindolo[2,1-*c*]benzo[1,2,4]triazine (4g). Yield: 50%; mp: >270°C; IR: 2221 (CN) cm^{-1}; ^1H NMR δ : 1.88-2.10 (4H, m, CH_2 x 2), 2.97 (2H, t, *J* = 5.6 Hz, CH_2)**,** 3.36 (2H, t, *J* = 5.6 Hz, CH_2), 4.00 (3H, s, CH_3), 4.13 (3H, s, CH_3), 6.61 (1H, d, *J* = 1.5 Hz, H-3), 7.16 (1H, d, *J* = 1.5 Hz, H-1). Anal. Calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.32; N, 18.17. Found: C, 66.32; H, 5.22; N, 17.98.

7-Cyano-2-methoxy-8,9,10,11-tetrahydroindolo[2,1-*c*]benzo[1,2,4]triazine (4h). Yield: 50%; mp: >270°C; IR: 2218 (CN) cm^{-1}; ^1H NMR δ: 1.25 (3H, s, CH_3), 1.84-1.98 (4H, m, CH_2 x 2), 2.87 (2H, t, *J* = 5.8 Hz, CH_2), 3.37 (2H, t, *J* = 5.8 Hz, CH_2), 7.42 (1H, dd, *J* = 9.0 and 2.6 Hz, H-3), 7.68 (1H, d, *J* = 2.6 Hz, H-1), 8.44 (1H, d, *J* = 9.0 Hz, H-4). Anal. Calcd for $C_{16}H_{14}N_4O$: C, 69.05; H, 5.07; N, 20.13. Found: C, 69.30; H, 4.99; N, 20.28.

7-Cyano-2-ethoxy-8,9,10,11tetrahydroindolo[2,1-*c*]benzo[1,2,4]triazine (4i). Yield: 50%; mp: >270°C; IR (nujol): 2221 (CN) cm^{-1}; ^1H NMR δ: 1.45 (3H, t, *J* = 6.9 Hz, CH_3), 1.87-2.00 (4H, m, CH_2 x 2), 2.88 (2H, t, *J* = 5.7 Hz, CH_2), 3.41 (2H, t, *J* = 5.7 Hz, CH_2), 4.34 (2H, q, *J* = 6.9 Hz, CH_2), 7.41 (1H, dd, *J* = 8.8 and 2.5 Hz, H-3), 7.63 (1H, d, *J* = 2.5 Hz, H-1), 8.43 (1H, d, *J* = 8.8 Hz, H-4). Anal. Calcd for $C_{17}H_{16}N_4O$: C, 69.85; H, 5.52; N, 19.16. Found: C, 70.00; H, 5.50; N, 19.12.

Hydrolysis of the substituted 7-cyano-8,9,10,11-tetrahyndolo[2,1-*c*]benzo[1,2,4]triazines (4g-i):
A solution of the tetrahydroindolobenzotriazines **(4g-i)** (0.5 mmol) in hydrochloric acid (37%, 15 mL), was heated under reflux for 10 h. In the case of **4g** an oily and very complex mixture was obtained, from which it was not possible to isolate any product. For the triazines **(4h** or **i)** a solid separated from the reaction mixture which was filtered off, rinsed with cold water and recrystallized from ethanol.

2-Methoxy-8,9,10,11-tetrahydroindolo[2,1-*c*]benzo[1,2,4]triazine-7-carboxylic acid (5h): Yield: 56%; mp: 263-265°C from; IR: 3430 (OH), 1721 (CO) cm^{-1}; ^1H NMR δ: 1.78-1.96 (4H, m, CH_2 x 2), 3.00 (2H, t, *J* = 6.6. Hz, CH_2), 3.45 (2H, t, *J* = 6.6 Hz, CH_2), 4.02 (3H, s, CH_3), 7.34 (1H, dd, *J* = 9.5 and 2.1 Hz, H-3), 7.69 (1H, d, *J* = 2.1 Hz, H-1), 8.36 (1H, d, *J* = 9.5 Hz, H-4), 12.30 (1H, br s, OH). Anal. Calcd for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13. Found: C, 64.80; H, 5.19; N, 14.30.

2-Ethoxy-8,9,10,11tetrahydroindolo[2,1-*c*]benzo[1,2,4]triazine-7-carboxylic acid (5i). Yield: 60%; mp: 264-266°C; IR: 3428 (OH), 1730 (CO) cm^{-1}; ^1H NMR δ: 1.43 (3H, t, *J* = 6.6 Hz, CH_3), 1.80-1.93 (4H, m, CH_2 x 2), 3.01 (2H, t, *J* = 6.6 Hz, CH_2), 3.43 (2H, t, *J* = 6.6 Hz, CH_2), 4.31 (2H, q, *J* = 6.6 Hz, CH_2), 7.33 (1H, dd, *J* = 8.8 and 2.2 Hz, H-3), 7.66 (1H, d, *J* = 2.2 Hz, H-1), 8.37 (1H, d, *J* = 8.8 Hz, H-4), 12.26 (1H, br s, OH). Anal. Calcd for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 14.50. Found: C, 65.31; H, 5.54; N, 14.70.

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REFERENCES

1. H. Neunhoeffer, "Comprehensive Heterocyclic Chemistry: 1,2,4-Triazines and Their Benzoderivatives", Vol. 3, ed. by A. R. Katritzky, Pergamon Press, Oxford, 1984, pp. 385-456; H. Neunhoeffer and P. F. Wiley, "Chemistry of Heterocyclic Compounds" Vol. 33, 1978, pp. 189-574.
2. U. Stottmeister, M. Schoenfelder, H. Wilde, D. Sicker, and J. Andersch (Ufz Umweltforschungszentrum Leipzig-Halle G.m.b.H., Germany) patent WO2000-EP13028 20001220, 2001 (*Chem. Abstr.*, 2001, **135**, 472687).
3. M. W. Partridge and M. F. G. Stevens, *J. Chem. Soc., C*, 1966, 1127.

4. P. Diana, P. Barraja, A. Lauria, A. Montalbano, A. M. Almerico, G. Dattolo, and G. Cirrincione, *Eur. J. Med. Chem*., 2002, **37,** 267.
5. R. J. Abraham and P. E. Smith, *J. Comput.-Aided Mol. Design*, 1989, **3**, 175.
6. K. Eger, W. Lanzner, and K. Rothenhausler, *Liebigs Ann. Chem.,* 1993, 465.