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Original research paper

Use of 2-aminoprop-1-ene-1,1,3-tricarbonitrile for the synthesis of tetrahydronaphthalene, hexahydroisoquinoline and hexahydrocinnoline derivatives with potential antitumor activities

RAFAT M. MOHAREB[1,2]*
HOSAM E. MOUSTAFA[3]

[1] *October University for Modern Sciences and Arts, El Wahaat Road October City, A.R. Egypt*

[2] *Chemistry Department, Faculty of Science, Cairo University Giza, A.R. Egypt*

[3] *Applied Medical Sciences Department Community College in Quaryat, Al Jouf University Al Jouf, Saudi Arabia*

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The aim of the work was to synthesize heterocyclic compounds from 2-aminoprop-1-ene-1,1,3-tricarbonitrile and to study their antitumor activities. The title reagent reacted with cyclohexanone to give the ethylidene derivative **2**. The reactivity of the latter product towards different chemical reagents was studied to give tetrahydronaphthalene, hexahydroisoquinoline and hexahydrocinnoline derivatives. The newly synthesized products were screened as antitumor agents on the *in vitro* growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268). It was found that some of these compounds showed inhibitory effects on the three cell lines, indicating their potential use in the development of oncology products.

Keywords: tetrahydronaphthalene, hexahydroisoquinoline, hexahydrocinnoline, antitumor activity

2-Aminoprop-1-ene-1,1,3-tricarbonitrile has attracted a great deal of interest due to its wide applications in the field of pharmaceuticals (1). The title reagent had great applicability in heterocyclic synthesis since it was used for the synthesis of pyridines, pyrimidines, pyridazines, thiophenes, thiazoles and their analogs (2, 3). The importance of such compounds is due to their diverse pharmaceutical activities including for neurological disorders (4), as receptor antagonists (5), antidiabetics (6), tubulin inhibitors (7), kinase inhibitors (8) and anticancer agents (9). In continuation of our interest in the development of new and simple methods for the synthesis of polyfunctionally substituted heterocycles with anticipated biological activity (10), we report here the uses of the title reagent for the synthesis of new tetrahydronaphthalene, hexahydroquinoline and hexahydrocinnoline derivatives together with their antitumor evaluation.

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* Correspondence; e-mail: raafat_mohareb@yahoo.com

EXPERIMENTAL

All melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and were uncorrected. Elemental analyses were performed on a Yanaco CHNS Corder elemental analyzer (Japan). IR spectra (υ, cm^{-1}) were recorded in KBr pellets on a PA-9721 IR spectrophotometer (Shimadzu, Japan). ^{1}H NMR and ^{13}C NMR spectra were obtained on a Jeol 300 MHz (Japan) spectrometer in DMSO-d_6 as solvent, using TMS as internal reference, and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

Synthetic pathways are presented in Schemes 1–3 and physicochemical and spectral data of the synthesized compounds are given in Tables I and II. 2-Amino-3-cyclohexylideneprop-1-ene-1,1,3-tricarbonitrile (**1**) was synthesized according to the literature procedure (11). Compounds **4a,b** and **6a,b** were formed through non-isolable intermediates **3a,b** and **5a,b**, respectively.

Syntheses

*2-Amino-3-(2-benzylidenecyclohexylidene)prop-1-ene-1,1,3-tricarbonitrile (**2**).* – To a solution of compound **1** (0.212 g, 1 mmol) in ethanol (40 mL), piperidine (0.5 mL) and benzaldehyde (0.106 g, 1 mmol) were added. The reaction mixture was heated under reflux for 3 h, then poured onto an ice/water mixture containing a few drops of hydrochloric acid. The formed solid product was collected by filtration.

*3-Amino-4-(1-amino-2,2-dicyanovinyl)-1-phenyl-5,6,7,8-tetrahydro-1-phenylnaphthalene-2-carbonitrile (**4a**) and ethyl 3-amino-4-(1-amino-2,2-dicyanovinyl)- 5,6,7,8-tetrahydro-1-phenylnaphthalene-2-carboxylate (**4b**). General procedure. – Method A*. To a solution of compound **2** (0.3 g, 1 mmol), either malononitrile (0.066 g, 1 mmol) or ethyl cyanoacetate (0.113 g, 1 mmol) was added in ethanol (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 3 h, then poured onto an ice/water mixture containing a few drops of hydrochloric acid. The solid product formed in each case was collected by filtration. *Method B.* To a solution of compound **1** (0.212 g, 1 mmol) in ethanol (40 mL) containing a catalytic amount of triethylamine (0.50 mL), either α-cyanocinnamonitrile (0.150 g, 1 mmol) or ethyl α-cyanocinnamate (0.201 g, 1 mmol) was added. The reaction mixture in each case was heated under reflux for 3 h, then neutralized by pouring onto an ice/ water mixture containing a few drops of hydrochloric acid. The solid product formed in each case was collected by filtration.

*2-(Amino(2,3-diamino-1-phenyl-1,2,5,6,7,8-hexahydroisoquinolin-4-yl)methylene)malononitrile (**6a**) and 2-(amino(3-amino-1-phenyl-2-(phenylamino)-1,2,5,6,7,8-hexahydroisoquinolin-4-yl)methylene)-malononitrile (**6b**).* – To a solution of compound **2** (0.30 g, 1 mmol) in ethanol (40 mL), either hydrazine hydrate (0.050 g, 1 mmol) or phenylhydrazine (0.108 g, 1 mmol) was added. The reaction mixture in each case was heated under reflux for 3 h, then poured onto an ice/water mixture containing a few drops of hydrochloric acid. The formed solid product in each case was collected by filtration.

*2-Amino-3-(2-benzylidene-6-bromocyclohexylidene)prop-1-ene-1,1,3-tricarbonitrile (**7**).* – A solution of compound **2** (0.30 g, 1 mmol) in glacial acetic acid (30 mL) was warmed to

60 °C, then bromine solution (0.16 g, 1 mmol) in acetic acid (10 mL) was added dropwise under continuous stirring. The reaction mixture was stirred for 1.5 h, then poured into an ice/water mixture and the formed solid product was collected by filtration.

4-(2-Benzylidenecyclohexylidene)(cyano)methyl)-1,2,3,6-tetrahydro-6-imino-1-phenyl-2-thioxopyrimidine-5-carbonitrile (8). – To a solution of compound **2** (0.30 g, 1 mmol) in ethanol (40 mL) containing triethylamine (0.50 mL), phenylisothiocyanate (0.135 g, 1 mmol) was added. The reaction mixture was heated under reflux for 3 h, then poured into an ice/water mixture containing a few drops of hydrochloric acid. The formed product was collected by filtration.

*3-Amino-2-cyano-4-(2-(*p*-tolyl-hydrazono)-cyclohexylidene)prop-1-ene-1,1,3-tricarbonitrile (9)*. – To a cold solution (0–5 °C) of compound **1** (0.212 g, 1 mmol) in pyridine (15 mL), an equimolar amount of diazotized-4-methyl-benzenediazonium chloride was gradually added under stirring. The solid product separated during stirring in an ice bath was collected by filtration and washed thoroughly with water.

Table I. Physical properties of the newly synthesized compounds

Compd. No.	M.p. (°C) (solvent of cryst.)	Yield (%)	Mol. formula (M_r)	Analysis calcd./found (%)			
				C	H	N	S
2	143–145	67	$C_{19}H_{16}N_4$	75.98	5.37	18.65	
	(dioxane)		(300.14)	75.86	5.29	18.73	
4a	221–224	79	$C_{21}H_{17}N_5$	74.32	5.05	20.63	
	(EtOH)		(339.39)	74.14	5.12	20.77	
4b	98–102	76	$C_{23}H_{22}N_4O_2$	71.48	5.74	14.50	
	(EtOH)		(386.45)	71.29	6.00	14.76	
6a	192–196	79	$C_{19}H_{20}N_6$	68.65	6.06	25.28	
	(EtOH)		(332.40)	68.58	6.24	25.54	
6b	137–140	67	$C_{25}H_{24}N_6$	73.51	5.92	20.57	
	(EtOH)		(408.50)	73.50	5.67	20.61	
7	210–212	77	$C_{19}H_{15}BrN_4$	50.17	3.99	14.77	
	(AcOH)		(379.25)	49.96	4.08	14.64	
8	109–112	73	$C_{26}H_{21}N_5S$	71.70	4.86	16.08	7.36
	(EtOH)		(435.54)	71.74	4.86	15.85	7.18
9	140–144	77	$C_{19}H_{18}N_6$	69.07	5.49	25.44	
	(EtOH)		(330.39)	69.14	5.26	25.61	
10	165–169	75	$C_{19}H_{18}N_6$	69.07	5.49	25.44	
	(EtOH)		(330.39)	69.13	5.64	25.67	
11	120–122	80	$C_{19}H_{17}N_5O$	68.87	5.17	21.13	
	(dioxane)		(331.37)	68.62	5.08	21.25	
12a	143–146	75	$C_{12}H_{16}N_6$	59.00	6.60	34.40	
	(dioxane)		(244.14)	58.81	6.54	34.24	
12b	165–169	71	$C_{18}H_{20}N_6$	67.48	6.29	26.23	
	(dioxane)		(320.17)	67.35	6.30	26.48	

Table II. Spectral data of the newly synthesized compounds

Compd. No.	IR (υ, cm^{-1})	^{1}H NMR (DMSO-d_6) (δ, ppm)	^{13}C NMR (DMSO-d_6) (δ, ppm)	MS (M$^+$)
2	3420, 3345 (NH$_2$) 3059 (CH aromatic) 2229–2204 (3CN) 1635 (C=C)	2.22–2.24 (m, 4H, 2CH$_2$), 2.30–2.34 (m, 4H, 2CH$_2$), 3.65 (s, 2H, NH$_2$), 6.98 (s, 1H, CH=C), 2CH$_2$)	26.8, 27.3, 29.0, 30.3 (cyclohexyl C), 116.0, 116.9, 117.3 (3CN), 87.2, 90.5, 104.2, 106.2, 132.1, 140.3 (3C=C), 124.1, 125.3, 128.8, 129.5 (benzene C)	300
4a	3420–3346 (2NH$_2$) 3058 (CH aromatic) 2228–2202 (3CN) 1630 (C=C)	2.22–2.24 (m, 4H, 2CH$_2$), 2.32–2.36 (m, 4H, 2CH$_2$), 3.89– 4.64 (2s, 4H, 2NH$_2$), 7.29–7.49 (m, 5H, C$_6$H$_5$)	24.2, 26.8, 28.3, 39.2 (cyclohexyl C), 115.8, 116.4, 115.1 (3CN), 54.8, 88.2 (C=C), 123.0, 124.8, 126.8, 129.0, 130.3, 133.7, 135.9, 144.3 (two benzene C)	339
4b	3460–3325 (2NH$_2$) 3060 (CH aromatic) 2882–2787 (CH$_3$, CH$_2$) 2230–2218 (2CN) 1693 (C=O)	1.36 (t, 3H, *J* = 7.22 Hz, CH$_3$), 2.20–2.27 (m, 4H, 2CH$_2$), 2.31–2.37 (m, 4H, 2CH$_2$), 4.22(q, 2H, *J* = 7.22 Hz, CH$_2$), 4.42 4.45 (2s, 4H, 2NH$_2$), 7.30–7.35 (m, 5H, C$_6$H$_5$)		386
6a	3473–3325 (3NH$_2$) 3058 (CH aromatic) 2890–2780 (CH$_3$, CH$_2$) 2229–2222 (2CN) 1690 (C=O)	2.34–2.46 (m, 4H, 2CH$_2$), 2.46–2.50 (m, 4H, 2CH$_2$), 4.25–4.62, 4.80 (2s, 4H, 2NH$_2$), 5.70 (s, 1H, pyridine, H-6), 7.26–7.36 (m, 5H, C$_6$H$_5$)	23.9, 25.2, 28.6, 38.9 (cyclohexyl C), 116.8, 117.3 (2CN), 54.9, 85.3 (C=C), 122.8, 126.7, 129.0, 130.6, 132.9, 135.3, 142.9 (benzene C, pyridine C)	332
6b	3377–3370 (2NH$_2$) 3058 (CH aromatic) 2875 (CH$_2$) 2225, 2220 (2CN), 1621 (C=C)	2.36–2.49 (m, 4H, 2CH$_2$), 2.50–252 (m, 4H, 2CH$_2$), 4.62, 4.78 (2s, 4H, 2NH$_2$), 5.68 (s, 1H, pyridine, H-6), 7.30–7.33 (m, 10H, 2C$_6$H$_5$), 7.51 (s, 1H, NH)		408
7	3443–3333 (NH$_2$) 3052 (CH aromatic) 2783 (CH$_2$), 2229–2215 (3CN), 1646 (C=C)	2.21–2.26 (m, 4H, 2CH$_2$), 2.33 (m, 2H, CH$_2$), 3.63 (m, 1H, cyclohexan H-6), 4.31 (s, 2H, NH$_2$), 6.22 (s, 1H, CH=C), 7.30–7.38 (m, 5H, C$_6$H$_5$)	23.6, 25.3, 37.9, 44.2 (cyclohexyl C), 55.3, 84.2, 95.2, 101.1, 11.7, 113.0 (3 C=C), 116.1, 116.8, 117.2 (3 CN), 120.8, 121.1, 122.3, 124.7, 126.0, 128.1, 130.2, 138.0 (two benzene C)	378
8	3420–3222 (2NH), 3053 (CH aromatic), 2873 (CH$_2$), 2225, 2220 (2CN) 1655 (C=N) 1630 (C=C)	2.48–2.49 (m, 4H, 2CH$_2$), 2.50–2.51 (m, 4H, 2CH$_2$), 6.23 (s, 1H, CH=C), 7.43–7.45 (m, 10H, 2C$_6$H$_5$), 8.23, 8.45 (2s, 2H, 2NH)	24.2, 25.8, 35.8, 43.8 (cyclohexyl C), 55.2, 80.6, 94.0, 111.2 (2C=C), 115.9, 116.3 (2CN), 122.8, 124.1, 126.3, 129.0, 131.8, 132.1, 132.9, 144.0 (two benzene C). 172 (C=N), 180.3 (C=S)	435

9	3465–3238 (NH_2, NH) 3050 (CH aromatic) 2880 (CH_2), 2228, 2222, 2220 (3CN) 1659 (C=N) 1635(C=C)	2.41–2.46 (m, 4H, 2CH_2), 2.52–2.54 (m, 4H, 2CH_2), 4.25 (s, 2H, NH_2), 7.32–7.40 (m, 5H, C_6H_5), 8.09 (s, 1H, NH)	22.8 (CH_3), 24.6, 26.2, 35.3, 43.5 (cyclohexyl C), 55.0, 78.3, 90.2, 110.3 (2 C=C), 115.8, 116.2, 116.9 (3CN), 122.3, 123.8, 125.7, 128.0 (benzene C), 176.3 (C=N)	330
10	3450–3322 (NH_2, NH) 3055 (CH aromatic) 2980, 2788 (CH_3,CH_2) 2227–2222 (2CN) 1655 (C=N) 1640(C=C	2.23–2.26 (m, 4H, 2CH_2), 2.32–2.36 (m, 4H, 2CH_2), 3.03 (s, 3H, CH_3), 5.44 (s, 2H, NH_2), 7.22–7.38 (m, 4H, C_6H_4), 8.25 (s, 1H, NH)		330
11	3463, 3343 (NH_2) 3050 (CH aromatic) 2982, 2884 (CH_3, CH_2) 2229–2220 (2CN) 1688 (CO), 1643 (C=N)	2.24–2.26 (m, 4H, 2CH_2), 2.35–2.38 (m, 4H, 2C_2), 3.14 (s, 3H, CH_3), 4.68 (s, 2H, NH_2), 7.30–7.39 (m, 4H, C_6H_4)		331
12a	3448–3315 (3NH_2) 3056 (CH aromatic) 2789 (CH_2), 2225 (CN), 1666 (C=N), 1639 (C=C)	2.21–2.26 (m, 4H, 2CH_2), 2.30–2.38 (m, 6H, 3CH_2), 4.21, 5.20, 5.24 (3s, 6H, 3NH_2)		244
12b	3470–3321 (2NH_2, NH) 3056 (CH aromatic) 2875 (CH_2) 1670 (exocyclic C=N) 1638 (C=C)	2.21–2.34 (m, 10H, 5CH_2), 4.76, 5.66 (2s, 4H, 2NH_2), 7.32–7.39 (m, 5H, C_6H_5), 8.32 (s, 1H, NH)	24.8, 25.9, 36.0, 43.3 (cyclohexyl C), 55.0, 78.3, 90.2, 110.3 (2C=C), 116.9 (CN), 120.2, 122.5, 123.4, 126.2, 128.0 (benzene C), 169.2, 172.8 (2C=N)	320

*2-(Amino(2,3,5,6,7,8-hexahydro-3-imino-2-*p*-tolylcinnoline-4-yl)methylene)malononitrile* (***10***). – A suspension of compound **9** (0.33 g, 1 mmol) in sodium ethoxide solution (obtained by dissolving sodium metal (0.046 g, 0.2 mmol) in absolute ethanol (20 mL) was heated in boiling water for 2 h and then left to cool. The solid product formed upon pouring onto ice/water was collected by filtration.

*2-(Amino(3-oxo-2-(*p*-tolylamino)-2,3,5,6,7,8-hexahydrocinnolin-4-yl)methylene)malononitrile (**11**).* – A solution of compound **10** (0.330 g, 1 mmol) in ethanol (30 mL) containing one pellet of sodium hydroxide was heated under reflux for 3 h, then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

*3-Amino-3-(3,5-diamino-4*H*-pyrazol-4-ylidene)-2-cyclohexylidene-propanonitrile (**12a**) and 3-amino-3-(3-amino-5-imino-1-phenyl-1*H*-pyrazol-4-(5*H*)-ylidene)-2-cyclohexylidenepropanonitrile (**12b**).* – To a solution of compound **1** (0.212 g, 1 mmol) in ethanol (40 mL), either hydrazine hydrate (0.05 g, 1 mmol) or phenylhydrazine (0.108 g, 1 mmol) was added. The reaction mixture in each case was heated under reflux for 3 h and then poured onto an ice/water mixture containing a few drops of hydrochloric acid. The solid product formed in each case was collected by filtration.

Antitumor activity tests

Fetal bovine serum (FBS) and L-glutamine were from Gibco Invitrogen Co. (UK). RPMI-1640 medium was from Cambrex (USA), dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (USA).

Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, UK) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). Their growth as a monolayer was routinely maintained in RPMI-1640 medium supplemented with 5 % heat inactivated FBS, 2 mmol L^{-1} glutamine and antibiotics (penicillin 100 U mL^{-1}, streptomycin 100 μg mL^{-1}), at 37 °C in a humidified atmosphere containing 5 % CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells mL^{-1} for MCF-7 and SF-268 and 0.75×10^4 cells mL^{-1} for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all experiments by exposing untreated control cells to the maximum concentration (0.5 %) of DMSO used in each assay.

The effects of compound **2-12b** on the *in vitro* growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the '*In vitro* Anticancer Drug Discovery Screen' which uses the protein-binding dye sulforhodamine B to assess cell growth (12). Briefly, cells growing exponentially in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μmol L^{-1}. Following this exposure period, adherent cells were fixed, washed and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Powerwave XS, Bio-Tek Instruments Inc., USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50 % (GI_{50}), corresponding to the concentration of the compounds that inhibited 50 % of net cell growth, was calculated as described elsewhere (13). Doxorubicin was used as a positive control and was tested in the same manner.

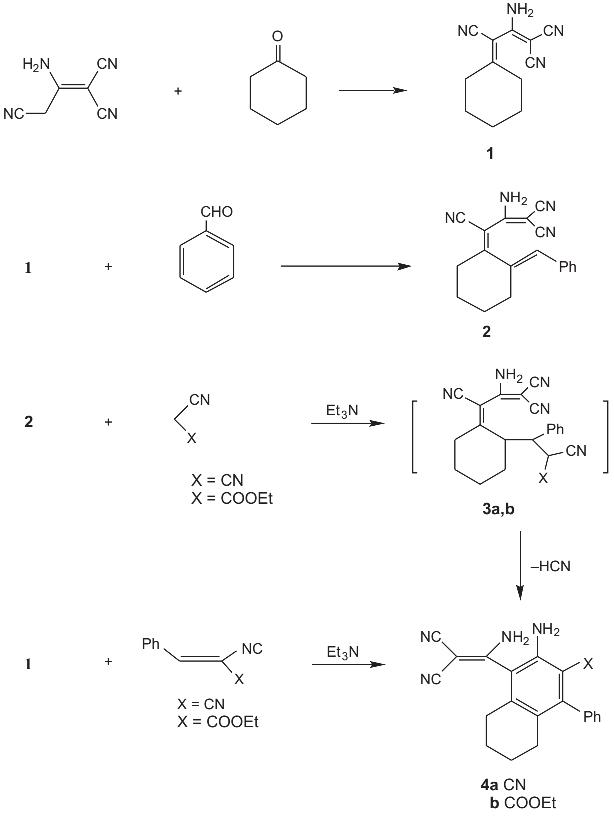
RESULTS AND DISCUSSION

Chemistry

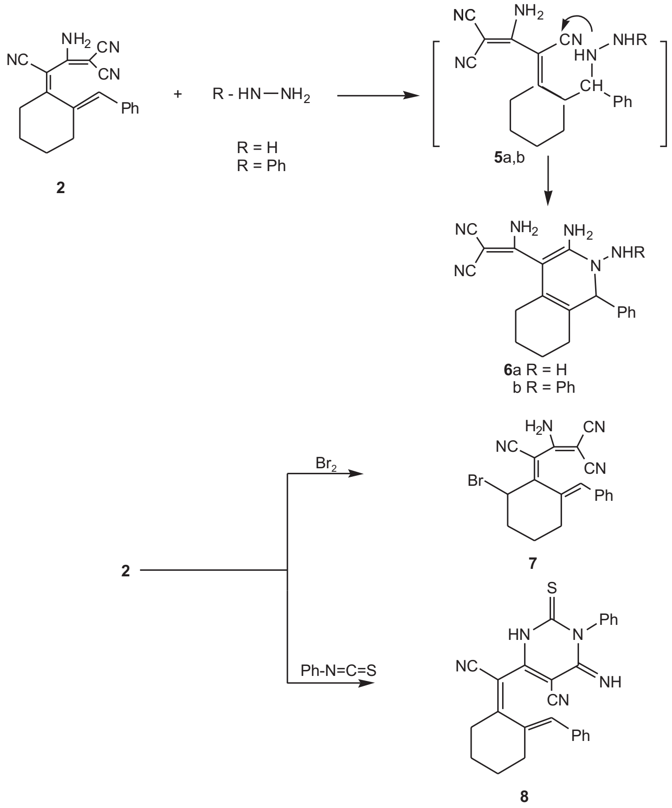
The reaction of compound **1** with benzaldehyde gave the benzal derivative **2**, the structure of which was established on the basis of analytical and spectral data. The ^1H NMR spectrum showed the presence of a multiplet at δ 2.22–2.24 and 2.30–2.34 ppm coresponding to four CH_2 groups, a singlet at δ 3.65 ppm (D_2O exchangeable corresponding to the NH_2 group) and a singlet at δ 6.98 ppm for the CH=C proton. Moreover, the ^{13}C NMR spectrum showed the presence of signals at δ 26.8, 27.3, 29.0, 30.3 (cyclohexyl C), 116.0, 116.9, 117.3 (3 CN), 87.2, 90.5, 104.2, 106.2, 132.1, 140.3 (3 C=C), 124.1, 125.3, 128.8, 129.5 ppm (benzene C). The reactivity of the latter product with cyanomethylene reagents was studied. Thus, the reaction of compound **2** with either malononitrile or ethyl cyanoacetate gave the tetrahydronaphthalene derivatives **4a,b**. The latter products were formed through intermediate formation of compounds **3a,b** (Scheme 1). Compounds **4a** and **4b** were obtained through another reaction route as well. Thus, the reaction of compound **1** with either α-cyanocinnamonitrile or ethyl α-cyanocinnamate gave the same tetrahydronaphthalene derivatives **4a** and **4b** (m.p. and mixed m.p.), respectively (Scheme 1). The analytical and spectral data (Tables I and II) of the latter products are consistent with the proposed structures (see experimental section).

On the other hand, the reaction of compound **2** with either hydrazine hydrate or phenylhydrazine gave the hyrazide derivatives **6a,b** through intermediate formation of **5a,b**.

The reaction of compound **2** with bromine in glacial acetic acid gave the monobromo derivative **7**. The reaction of compound **2** with phenylisothiocynate gave the pyrimidine derivative **8**. The analytical and spectral data of **7** and **8** (Tables I and II) are in agreement with the proposed structures (Scheme 2). The reaction of compound **1** with 4-methylbenzenediazonium chloride gave the hydrazone derivative **9**. The structure of compound **9** was also based on analytical and spectral data. Thus the ^{1}H NMR spectrum showed two multiplets at δ 2.22–2.28 and 2.33–2.37 ppm for four methylene groups, one



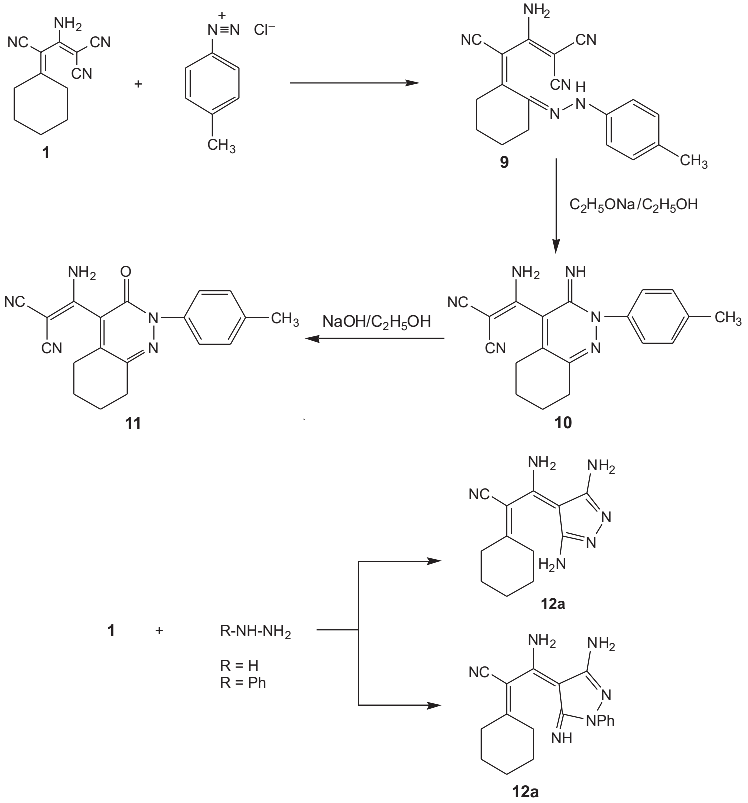
Scheme 1



Scheme 2

singlet at δ 3.21 ppm corresponding to CH_3 group, and two singlets at δ 4.55 and 8.88 ppm (D_2O exchangeable) corresponding to NH_2 and NH groups. Moreover, ^{13}C NMR showed signals at 22.8 (CH_3), 24.6, 26.2, 35.3, 43.5 (cyclohexyl C), 55.0, 78.3, 90.2, 110.3 (2 C=C), 115.8, 116.2, 116.9 (3 CN), 122.3, 123.8, 125.7, 128.0 (benzene C), 176.3 ppm (C=N). Compound **9** underwent ready cyclization in sodium ethoxide solution to give the pyridazine derivative **10** (Scheme 3). The 6-imino group in compound **10** underwent ready hydrolysis when heated in ethanolic sodium hydroxide to give the 6-oxopyridazine derivative **11** (Scheme 3). Such hydrolysis of an imino group into an oxo group had been reported before (14, 15). The reaction of compound **2** with either hydrazine hydrate or phenylhydrazine gave the pyrazole derivatives **12a** and **12b**, respectively (Scheme 3).

It is interesting to note that all compounds synthesized in this work can exist in either *Z* or *E* structures. However, according to the concept of push-pull alkenes reviewed by Sandstrom (16), conjugated systems with unsaturated heterocyclic rings with conjugated alkyl chains, exemplify typical push-pull compounds, which can exist in *Z*/*E* equilibrium depending on the temperature and the nature of solvent. One can say that all the newly synthesized compounds in this work are typical push-pull compounds existing in *Z*/*E* equilibrium.



Scheme 3

Antitumor activity

The effect of compounds **1**, **2**, **4a**, **4b**, **6a**, **6b**, **7**, **8**, **9**, **10**, **11, 12a** and **12b** was evaluated on the *in vitro* growth of three human tumor cell lines, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268), after continuous exposure for 48 h. The results are summarized in Table III.

All compounds were able to inhibit the growth of the three human tumor cell lines in a dose-dependent manner (data not shown). The 2-(amino(3-phenylamino-1-phenyl--2-(phenylamino)-1,2,5,6,7,8-hexahydroisoquinolin-4-yl)methylene)malononitrile (**6b**) and the 3-amino-3-(3,5-diamino-4*H*-pyrazol-4-ylidene)-2-cyclohexylidene-propanenitrile (**12a**) exhibited the highest inhibitory effect on the three tumor cell lines, but still much lower than that of the control doxorubicin. On the other hand, compounds **4a**, **7** and **12b** showed a moderate growth inhibitory effect. Comparing the activities of **6a** and **6b**, it was assumed that the N-phenyl group in **6b** coused weaker growth inhibitory effect although the results in the NCI-H460 cell line were comparable. Moreover, it is convenient to compare the activity of 3-amino-3-(3,5-diamino-4*H*-pyrazol-4-ylidene)-2-cyclohexylidenepropanenitrile (**12a**) with that of 3-amino-3-(3-amino-5-imino-1-phenyl-1*H*-pyrazol--4-(5*H*)-ylidene)-2-cyclohexylidenepropanenitrile (**12b**): the former compound (with pyrazole moiety) showed higher inhibitory effect than the latter (with N-pyrazole moiety).

*Table III. Effects of compounds **1-12b** on the growth of three human tumor cell lines*

Compd.	GI_{50} (μmol L^{-1})		
	MCF-7	NCI-H460	SF-268
1	30.1 ± 0.6	17.3 ± 1.4	22.3 ± 1.5
2	20.6 ± 0.4	24.3 ± 0.8	32 ± 0.8
4a	70.6 ± 16.9	38.9 ± 10.8	50.8 ± 8.6
4b	40.6 ± 12.2	32.6 ± 8.6	60.4 ± 14.8
6a	35.4 ± 10.2	24.1 ± 0.8	18.9 ± 6.8
6b	11.8 ± 0.6	14.5 ± 0.8	16.7 ± 1.6
7	72.7 ± 17.5	40.2 ± 12.8	50.0 ± 9.0
8	50.1 ± 0.7	23.2 ± 4.8	18.4 ± 1.8
9	22.0 ± 0.2	30.6 ± 1.4	38.4 ± 0.6
10	38.0 ± 1.8	44.0 ± 0.8	20.5 ± 1.1
11	20.0 ± 0.6	22.0 ± 0.4	31.5 ± 8.0
12a	11.9 ± 0.6	14.1 ± 0.6	20.3 ± 0.5
12b	70.9 ± 0.9	43.6 ± 1.8	56.8 ± 0.8
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

Results are given as concentrations that were able to cause 50 % cell growth inhibition (GI_{50}) after continuous exposure of 48 h; Mean ± SEM of three-independent experiments performed in duplicate.

CONLUSIONS

In this work, we described a new series of tetrahydronaphthalene, hexahydrophthalazine and hexahydrocinnoline derivatives obtained starting from the 2-amino-3-cyclohexylidene-1-propen-1,1,3-tricarbonitrile. Some of synthesized products showed potential anti-tumor activity. This finding encourages us to explore new molecules by introducing potent moieties, such as heterocyclic and fused ring systems described in this work, into other enaminonitriles. Our prediction is that these compounds with new ring systems may show better antitumor activity.

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SAŽETAK

Uporaba 2-aminoprop-1-en-1,1,3-trikarbonitrila u sintezi derivata tetrahidronaftalena, heksahidroizokinolina i heksahidrocinolina s potencijalnim antitumorskim djelovanjem

RAFAT M. MOHAREB i HOSAM E. MOUSTAFA

Cilj rada bio je sintetizirati heterocikličke spojeve polazeći od 2-aminoprop-1-en-1,1,3-trikarbonitrila. Taj spoj je prvo u reakciji s cikloheksanonom dao derivat etilidena **2**, iz kojeg su zatim priređeni derivati tetrahidronaftalena, heksahidrokinolina i heksahidrocinolina. Novosintetizirani spojevi ispitani su na antitumorsko djelovanje *in vitro* na tri humane tumorske stanične linije: adenokarcinoma grudi (MCF-7), tumora pluća (NCI-H460) i tumora CNS-a (SF-268). Neki od ispitanih spojeva pokazali su snažan inhibitorni učinak na ispitivane stanične linije, što ukazuje na mogućnost njihove primjene u razvoju onkoloških lijekova.

Ključne riječi: tetrahidronaftalen, heksahidrokinolin, heksahidrocinolin, antitumorsko djelovanje

October University for Modern Sciences and Arts, October City, A.R. Egypt

Chemistry Department, Faculty of Science, Cairo University, Giza, A.R. Egypt

Applied Medical Sciences Department, Community College in Quaryat, Al Jouf University, Al Jouf Saudia Arabia