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Incorporating Thiobarbituric Acid with 1,2,4-Triazin-6-one Containing Nitroarginine Moiety

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Abstract

Nitrilimines (**2a,b**), react with nitroarginine methyl ester (**3**) at room temperature, through cyclocondensation reaction, to give 1-aryl-3-substituted-1,2,4-triazin-6-ones (**4a,b**). Condensation of these compounds with thiosemicarbazide give the thiosemicarbazone derivatives (**5a,b**) which upon heterocyclization with malonic acid give the thiobarbituric acid derivatives (**6a,b**). The structures of these compounds were deduced from: IR, mass, ^{1}H and ^{13}C NMR spectra.

Keywords Nitrilimine , nitroarginine methyl ester, 1,2,4-triazin-6-ones, thiosemicarbazone, thiobarbituric acid derivatives.

دمج حامض الثيوباربيتيوريك مع 4,2,1-ترايازين-6-أون المحتوي على حامض النيتروأرجنين

ملخص

يتفاعل النيترايل إمين مع النيتروأرجنين ميثيل إستر على درجة حرارة الغرفة من خلال التكاثف الحلقي ليعطي 1-أريل-4,2,1-ترايازين-6-أون (**4a,b**). تكاثف هذا المركب مع الثيوسيميكاربازايد أعطي مشتقات الثيوسيميكاربازون (**5a,b**)، والتي تحلقت من خلال التفاعل مع حامض المالونيك منتجة حلقة سداسية غير متجانسة من حامض الثيوباربيتيوريك (**6a,b**) ملتحمة مع هذا المركب. وقد تم تحديد بنى المركبات المحضرة من خلال دراسة أطيافها المختلفة مثل طيف الأشعة تحت الحمراء و طيف الكتلة وأطياف الرنين النووي المغناطيسي البروتوني والكربوني.

كلمات مفتاحية: النيترايل إمي، النيتروأرجنين ميثيل إستر ، 4,2,1-ترايازين-6-أون، الثيوسيميكاربازون، مشتقات حامض الثيوباربيتيوريك.

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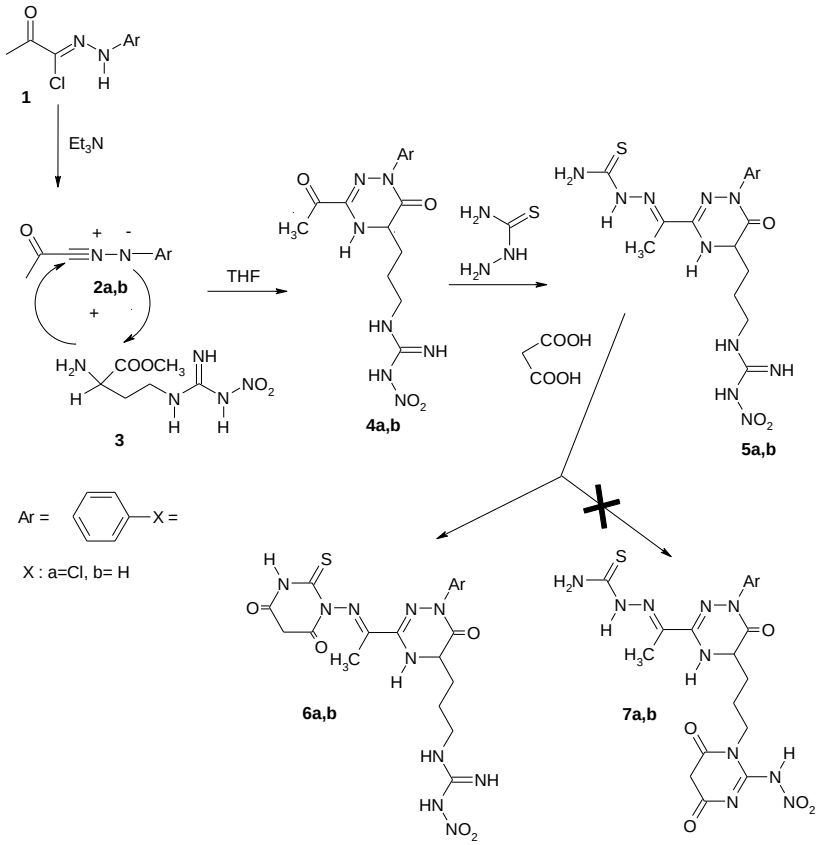
1. Introduction

1,2,4-Triazines play a vital role in many biological processes and synthetic drugs. Furthermore, many heterocyclic systems bearing 1,2,4-triazines exhibit remarkable pharmacological effects [1-5]. In search for new anti-HIV and anticancer agents, some additional heterocyclic moieties were incorporated in the 1,2,4-triazine nucleus *via* the interaction between functionalized 1,2,4-triazines with various nucleophilic and electrophilic reagents in different media [6].

A lot of information about the important methods of synthesis, structure, physical properties and chemical reactivity of these compounds are described in the literature [7-9].

A facile preparation of 1,2,4-triazin-6-ones from the reaction of nitrilimines with α-amino esters was reported [10], as well as the reaction of nitrilimines with α-aminoacetonitrile [11]. Nitrilimines react with α-hydrazinoester to give 1,2,4-triazin-6-ones [12], also the synthesis of 1,2,4-triazin-6-one containing nitroarginine have been reported [13]. Barbituric acid or thiobarbituric acid are well known for their biological activity [14-16]. The incorporation of compounds (**4**) (scheme 1) and thiobarbituric acid into a single molecule may enhance the activity of the target molecule. The new triazinone derivatives will be bioassayed, and the results will be communicated separately.



Scheme 1 *Synthesis of compounds 6*

2. Results And Discussion

Nitrilimines **(2),** generated in situ from the respective hydrazonyl halides **(1)** in THF upon the addition of triethylamine, are found to react with nitroarginine methyl ester **(3)** in methanol as a solvent at room temperature, through cyclocondensation reaction, to give 1,2,4-triazin-6-ones (**4**)**.** Compounds **(4)** are achieved via a nucleophilic attack of the amino group of the nitroarginine **(3)** at nitrilimines followed by intracyclization. Synthesis of some new heterobicyclic systems bearing a 1,2,4-triazinone moiety was performed starting from 1,2,4-triazinones (**4**). Condensation of these compounds with thiosemicarbazide in boiling ethanol and few drops of acetic acid produced the semicarbazone derivative (**5**). Refluxing of **5** with malonic acid caused competitive heterocyclization on the thiosemicarbazide moiety and yielded the thiobarbituric acid derivative (**6**).

Structures of compounds (**5**) and compounds (**6**) have been deduced from IR, mass, ^{1}H-NMR and ^{13}C-NMR spectra.

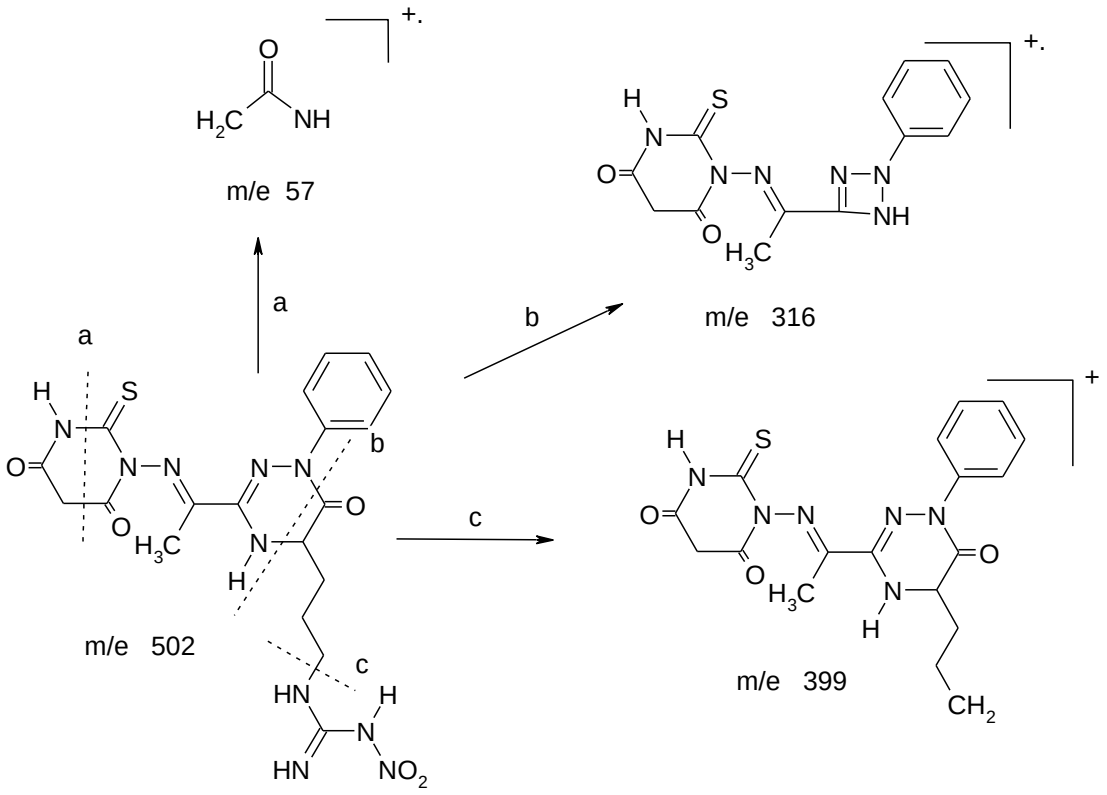
The IR spectra of compounds (**5**) in KBr revealed strong bands for all types of (NH) stretching in the region 3450-3280 cm^{-1}. One absorption band is also observed about 1660 cm^{-1} (C=O triazinone). Three stretching bands of C=N appeared in the region 1640-1610 cm^{-1}. The IR spectra of compounds (**6**) in KBr revealed two new strong bands around 1670 cm^{-1} for (C=O) indicating amides produced from reaction of compounds (**5**) with malonic acid.

The structure of compound (**6b**) for example was also deduced from mass spectrum fragmentation pattern of this compound which shows the molecular ion peak M^{+} 502, the base peak m/e 57 $[CH_2CONH]^{+}$ path a. An important fragmentation is observed (m/e 316) $M^{+\cdot}$–186 $[COCH(CH_2)_3NHC{=}NHNHNO_2]^{+\cdot}$ (path b). Another important fragmentation is observed (m/e 399) $M^{+\cdot}$ – 103 $[NHC{=}NHNHNO_2]^{+\cdot}$ (path c) (Scheme 2).

The ^{1}H-NMR spectra of compounds (**5**) show a singlet at 10.0 ppm for NH proton of thiosemicarbazone and a singlet at 10.6 ppm for NH_2 protons. The ^{1}H-NMR spectra of compounds (**6**) show a singlet at 7.3 ppm for NH proton of triazinone and three singlets for 3NH of nitroguanidine are observed between 8.5-7.6 ppm. The NH and CH_2 protons of pyrimidine appear as singlets at 10.4 and 3.15 ppm respectively.

^{13}C-NMR spectra of compounds (**6**) show two new signals for CH_2 and C=O of pyrimidine at 45 and 170 ppm, respectively. Compounds (**7**) contain primary NH_2 and secondary NH groups, However, compounds (**6**) contain only secondary NH groups. The ^{1}H-NMR shows clearly the triazine ring NH which appears as a singlet at 7.3 ppm, three NHs for nitroarginine as three singlets between 8.5-7.9 ppm and the NH of thiobarbituric acid appear as singlet around 10.4 ppm. The singlet signal of NH of semicrbazone at 9.89 in compound (**5**) disappeared in compound (**6**) and the two H's of NH_2 converted to one hydrogen for NH means that the cyclization occurred on the thiosemicarbazone moiety. The mass spectrum fragmentation pattern of compounds (**6**) support the the cycliztion of malonic acid on the thiosemicarbazone moiety, through the fragmentation pattern b (Scheme 2).

These results agree with our expectation of obtaining compound (**6**) and not (**7**) due to the deactivating group NO_2 on the guanidine moiety which diminishes its nucleophilicity and consequently prevents the cyclization at that position.



Scheme 2 *Mass fragmentation pattern of compound 6b*

3. Experimental

Melting points (uncorrected) were determined on Steuart melting point apparatus. IR spectra (in cm^{-1}) records as KBr discs on Perkin-Elmer 237 infrared spectrometer. Mass spectra were obtained using GCMS-QP10000 EX Schimadzu spectrometre at 70 eV. ^{1}H and ^{13}C-NMR were recorded on a Bruker AM 300 MHz NMR spectrometer using DMSO-d_6 as a solvent at 21°C and TMS as an internal reference. Chemical shifts are expressed in δ (ppm) downfield from TMS and coupling constants are in Hertz (Hz).

The appropriate hydrazonoyl chlorides employed in this work were prepared following standard procedure [17]. Nitroarginine methyl ester hydrochloride (**3**) employed in this work was obtained by reaction of nitroarginine with thionyl chloride in methanol following standard procedure [13]. 1,2,4-triazin-6-ones (**4a,b**) were obtained by following the standard procedure [13].

Synthesis of (5a, b) :

To a stirred solution of the particular 1,2,4-triazin-6-one (**4**) (10 mmol) in absolute ethanol (60 mL) acidified with drops of glacial acetic acid was added the thiosemicarbazide and the stirring reaction mixture was refluxed for 2-3 hours. The solvent was then evaporated in vacuo and the residue was collected and recrystallized from ethanol to yield the thiosemicarbazone derivatives (**5a,b**).

The following compounds were synthesized using this method:

1-(4-Chlorophenyl)-3-(thiosemicarbazonoacetyl)-4,5-dihydro-5-[3 nitroguanidineprop-1-yl] –(*1H*)- 1,2,4-triazin-6-one (5a)

Yield = 75%, M. P. = 175-176 °C, IR (KBr): cm^{-1} 3475-3290 (all NH and NH_2 bands), 1660 (C=O lactam), 1638 - 1590 (3 C=N). ^{1}H-NMR (DMSO-d_6): δ / ppm 1.56 (m, 2H, CH_2), 1.85 (m, 2H, CH_2), 2.10 (s, 3H, CH_3C=N), 3.13 (m, 2H, CH_2NH), 4.06 (m, 1H, H-5), 7.5 (s, 1H, NH), 7.5-7.4 (2d, 4H aromatic protons, J = 7 Hz), 8.5-7.8 (s, 3H, 3NH nitroguanidine), 9.89 (s, 1H, NH thiosemicarbazone), 10.4 (s, 2 H, NH_2). ^{13}C NMR (DMSO-d_6): δ / ppm 11.8 (CH_3C=N), 24.3, 30.6 $(CH_2)_2$, 40.8 (CH_2NH), 53.1 (C-5), 126.3, 128.8, 130.5, 140.3, 143.2 (aromatic carbons), 145.05 (C-3), 152 (CH_3*C*=N), 160 (*C*=N nitroguanidine), 162.7 (C=O lactam), 179.6 (C=S). MS: m/z ($C_{16}H_{21}ClN_{10}O_3S$) = 468/470 ($M^{+\cdot}$), 267/269 [$CH_3C(NNHCSNH_2)C$=NN-p-ClC_6H_4]$^{+\cdot}$,153/155 [p-ClC_6H_4NCO]$^{+\cdot}$, 125/127 [p-ClC_6H_4N]$^{+\cdot}$, 111/113 [p-ClC_6H_4]$^{+\cdot}$, 103 [$NO_2NHCNHNH$]$^{+\cdot}$, 90 [$NH_2CSNHNH$]$^{+\cdot}$, 75 [NH_2CSNH]$^{+\cdot}$, 60 [NH_2CS]$^{+\cdot}$.

1-Phenyl-3-(thiosemicarbazonoacetyl)-4,5-dihydro-5-[3 nitroguanidineprop-1-yl]-(*1H*)-1,2,4-triazin-6-one (5b)

Yield = 77%, M. P. = 179-180 °C, IR (KBr): cm^{-1} 3475-3280 (all NH bands), 1662 (C=O lactam), 1640 - 1590 (3 C=N). ^{1}H-NMR (DMSO-d6): δ / ppm 1.86 (m, 2H, CH_2), 2.00 (m, 2H, CH_2), 2.10 (s, 3H, CH_3C=N), 3.20 (m, 2H, CH_2NH), 4.05 (m, 1H, H-5), 6.85 (s, 1H, NH) 7.40-7.20 (m, 5H aromatic protons), 8.5-7.9 (s, 3H, 3NH nitroguanidine), 10.1 (s, 1H, NH thiosemicarbazone), 10.5 (s, 2 H, NH_2). ^{13}C NMR (DMSO-d_6): δ / ppm 14.0 (CH_3C=N), 24.3, 30.6, $(CH_2)_2$, 40.8 (CH_2NH), 53.3 (C-5), 114.2, 123.2, 129.9, 138.4 (aromatic carbons), 144.0 (C-3), 151.2 (CH_3*C*=N), 159.7 (*C*=N nitroguanidine), 162.7 (C=O lactam), 179.4 (C=S). MS: m/z ($C_{16}H_{22}N_{10}O_3S$) = 434 ($M^{+\cdot}$), 233 [$CH_3C(NNHCSNH_2)C$=NNC_6H_5]$^{+\cdot}$, 103 [$NO_2NHCNHNH$]$^{+\cdot}$, 91 [C_6H_5N]$^{+\cdot}$, 90 [$NH_2CSNHNH$]$^{+\cdot}$, 77 [C_6H_5]$^{+\cdot}$,75 [NH_2CSNH]$^{+\cdot}$, 60 [NH_2CS]$^{+\cdot}$.

Synthesis of (6a,b) :

To a stirred solution of the thiosemicarbazone derivatives (2 mmol) in methanol (30 mL) was added a solution of malonic acid (2.5 mmol) in methanol (30 mL). The resulting reaction mixture was heated at refluxed 4 hours and the solvent was then removed in vacuo. The residue was collected and recrystallized from ethanol to yield **(6a,b)**.

The following compounds were synthesized using this method:

1-(4-aryl)-3-(2′-thioxo-dihydropyrimidine-4′,6′-dione)ethydiden-4,5-dihydro-5-[3-nitroguanidineprop-1-yl]-(*1H*)-1,2,4-triazin-6-one (6a)

Yield = 76%, M. P. = 200-201 °C, IR (KBr): cm^{-1} 3480-3250 (3NH nitroguanidine, 1NH triazinone and 1NH pyrimidine), 1665, 1640 (two C=O), 1635 - 1580 (3 C=N).

^{1}H-NMR (DMSO-d_6): δ / ppm 1.20 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 2.00 (s, 3H, CH_3C=N), 3.00 (m, 2H, CH_2NH), 3.15 (s, 2H, CH_2, pyrimidine), 4.06 (m, 1H, H-5), 7.3 (s, 1H, NH triazinone), 7.58 - 7.40 (2d, 4H aromatic protons, J = 7 Hz), 8.5-7.9 (s, 3H, 3NH nitroguanidine), 10.40 (s, 1NH, pyrimidine). ^{13}C NMR (DMSO-d_6): δ / ppm 11.7 (CH_3C=N), 46.0 (CH_2 pyrimidine), 18.1, 30.1, $(CH_2)_2$, 40.2 (CH_2NH), 53.0 (C-5), 126.3, 128.7, 130.5, 140.0 (aromatic carbons), 145.3 (C-3), 150.0 (CH_3C=N), 159 (C=N nitroguanidine), 163.0 (C=O triazine), 170.0 (C=O pyrimidine), 179.6 (C=S). MS: m/z ($C_{19}H_{21}ClN_{10}O_5S$) = 536/538 ($M^{+\cdot}$), (433/435) [$C_{18}H_{18}ClN_6O_3S$]$^{+\cdot}$ $M^{+\cdot}$–103, (350/352) [$C_{13}H_{11}ClN_6O_2S$]$^{+\cdot}$ $M^{+\cdot}$–186, 103 [$NO_2NHCNHNH$]$^{+\cdot}$, 91 [C_6H_5N]$^{+\cdot}$, 77 [C_6H_5]$^{+\cdot}$, 57 [CH_2CONH] $^{+\cdot}$.

1-Phenyl-3-(2′-thioxo-dihydropyrimidine-4′,6′-dione)ethydiden-4,5-dihydro-5-[3-nitroguanidineprop-1-yl]-(*1H*)-1,2,4-triazin-6-one (6b)

Yield = 84%, M. P. = 202-204 °C, IR (KBr): cm^{-1} 3480-3250 (3NH nitroguanidine, 1NH triazinone and 1NH pyrimidine), 1665 (C=O amide), 1655 (C=O lactam), 1640 - 1590 (3 C=N). ^{1}H-NMR (DMSO-d_6): δ / ppm 1.55 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 2.00 (s, 3H,

$CH_3C=N$), 3.16 (m, 2H, CH_2NH), 3.20 (s, 2H, CH_2, pyrimidine), 4.10 (m, 1H, H-5), 7.2 (s, 1H, NH triazinone), 7.52 – 6.98 (m, 5H aromatic protons), 8.5-7.8 (s, 3H, 3NH nitroguanidine), 10.50 (s, 1NH, pyrimidine). ^{13}C NMR (DMSO-d_6): δ / ppm 14.1 ($CH_3C=N$), 24.0, 30.0, $(CH_2)_2$, 40.0 (CH_2NH), 45.0 (CH_2 pyrimidine), 53.0 (C-5), 126.2, 128.9, 130.5, 140.3 (aromatic carbons), 143.60 (C-3), 151.00 (CH_3**C=N**), 159.00 (C=N nitroguanidine), 162.81 (C=O triazine), 170.00 (C=O pyrimidine), 179.40 (C=S). MS: m/z ($C_{19}H_{22}N_{10}O_5S$) = 502 ($M^{+\cdot}$), (399) $[C_{18}H_{19}N_6O_3S]^{+\cdot}$ $M^{+\cdot}$–103, (316) $[C_{13}H_{12}N_6O_2S]^{+\cdot}$ $M^{+\cdot}$–186, 103 $[NO_2NHCNHNH]^{+\cdot}$, 91 $[C_6H_5N]^{+\cdot}$, 77 $[C_6H_5]^{+\cdot}$, 57 $[CH_2CONH]^{+\cdot}$.

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