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Preparation of 5-diaminomethylenebarbiturates by barbituric acid addition to carbodiimides

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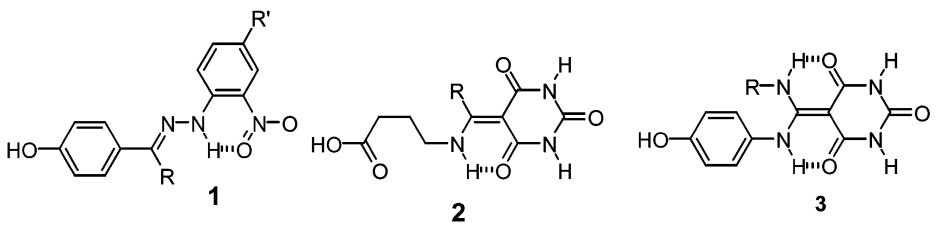
Abstract—Through NMR spectroscopic monitoring of barbituric acid addition to carbodiimide, a general synthetic procedure for the preparation of 5-diaminomethylenebarbiturates (DABA) was developed. This procedure is very simple and applicable to the preparation of large quantities of DABA derivatives. Through an X-ray structural study of one of the DABA derivatives, it was established that these compounds have an ylide-type structure with strong charge separation within the molecule.

1. Introduction

It was previously determined that some simple nitrophenylhydrazones of aromatic ketones (compound **1**, Scheme 1) possess strong anticancer activity.[1] In addition, it was also determined that these compounds possess immuno-modulating[2] and strong molecular binding capabilities.[3] Therefore, one might speculate that the anticancer activity of **1** could be the result of the immuno-modulating capabilities of these compounds.[4] There are also some literature reports on both the anticancer[5] and immuno-modulating[6] capabilities of barbituric acid derivatives, which has directed our research toward the development of new barbituric acid derivatives as anticancer drugs that act as immuno-modulating agents. This research has led to the design of a new barbituric acid derivative **2** with both anticancer and immuno-modulating activity.[7]

By close comparison of the structural properties of the biologically active compounds that have been prepared and tested in our laboratories, we have extended our search for anticancer compounds to a new family of barbituric acid derivatives that have the structural properties of compound **3** (Scheme 1). If thorough biological evaluation of the structural isomers of **3** is to be successful, then reliable synthetic procedures for the preparation of these isomers must be developed. These procedures should ensure the synthesis of large quantities of material of high purity, and a common synthetic procedure should be applicable for the preparation of a large number of structural variations of compound **3**.

Examining the structural characteristics of our target molecule (**3**), it is obvious that DABA contains two major moieties, urea and barbituric acid (Scheme 2). These readily available starting reagents[8] can be condensed by virtue of one water molecule elimination. Although condensation reactions between carbonyl compounds and barbituric acid are well documented, the reaction is mostly successful with aromatic aldehydes and with some activated ketones.[9] To be able to perform urea–barbituric acid condensation, urea should be activated through its transformation into a more reactive intermediate, such as carbodiimide (Scheme 2). Using this rationale, DABA derivatives can be prepared by barbituric acid addition to carbodiimides. To the best of our knowledge there is no available literature procedure for the



Scheme 1. Structures of known anticancer compounds **1** and **2** and our general synthetic target **3**.

Keywords: barbituric acid; 5-diaminomethylenebarbiturates; immuno-modulating agents.

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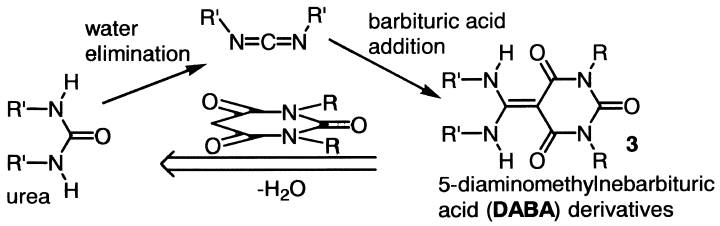


Scheme 2. Transformation of barbituric acid and urea derivatives into DABA derivatives.

preparation of DABA derivatives from urea (carbodiimides)[10] and barbituric acid.

However, there are some literature data that suggest that this synthetic approach might be successful. It was demonstrated that compounds with active methylene groups such as diethyl malonate can add to carbodiimides.[11] This condensation reaction was carried out at a high temperature with diphenyl ether as the reaction solvent. In many chemical reactions, barbituric acid is considered to be a compound with an active methylene group. If barbituric acid and 1,3-dicyclohexylcarbodiimide (DCC) are mixed together in THF in the presence of pyridine, immediately the color of the reaction mixture changes to fluorescent purple. In fact, DCC in combination with pyridine has been used for the analytical detection of barbituric acid,[12] but the products for this reaction have not been isolated.[13]

2. Results and discussion

In our initial experiments, we demonstrated that in THF barbituric acid adds to DCC, but the reaction requires a long time (several weeks at room temperature) to be completed.[14] Additionally, besides the major product of this reaction, one can also obtain the 1,3-dicyclohexylurea byproduct of water addition to DCC. If the reaction is performed in refluxing THF, products of decomposition were formed. Therefore, we have carefully explored reaction conditions for the barbituric acid addition to carbodiimides through the NMR spectroscopic monitoring.

Experiments are performed in aprotic solvents such as tetrahydrofuran, dioxane, benzene, toluene, dimethylformamide, and dimethyl sulfoxide. The solubility of barbituric acid in some of these solvents is very low. The most reliable experimental data that can also be applied to large scale (100 g) preparations of target compound **3** are obtained in DMSO as a reaction solvent. Although reaction conditions for every reported compound in this paper were optimized through NMR spectroscopic monitoring, only results for barbituric acid and 1,3-dimethylbarbituric acid addition to 1,3-di(4-methylphenyl)carbodiimide will be discussed. The addition of barbituric acid is a very slow process and with water present in the reaction media, water addition to carbodiimide becomes the dominant reaction. That was demonstrated with NMR spectroscopic monitoring of barbituric acid addition to di(4-methylphenyl)carbodiimide at room temperature (Fig. 1). Using a tenfold barbituric acid excess ensured that large quantities of water were added into the reaction media. All starting material was practically consumed after 24 h at room temperature and 1,3-di(4-methylphenyl)urea was formed (Fig. 1). We believe that this urea derivative immediately forms a molecular complex with the surrounding barbituric acid, which is evident by the appearance of the NH singlet of the barbituric acid moiety in the complex at 10.15 ppm. Same NMR pattern is obtained for DMSO solution of barbituric acid and 1,3-di(4-methylphenyl)urea. There is only a small amount of the desirable product **4**. With elevated temperatures the ratio of the product, with regards to water addition to the carbodiimide, increases (Fig. 1). It seems that the best reaction temperature is 150°C, at which the reaction is completed after 15 min and more than 95% of the target product **4** is formed.

Similar results were observed when other barbituric acid derivatives were added to di(4-methylphenyl)carbodiimide. That is demonstrated by the methyl portion of the NMR spectrum in the 1,3-dimethylbarbituric acid addition to 1,3-di(4-methylphenyl)carbodiimide (Fig. 2). The water concentration is substantially lower than in the previous experiment (the bulk of the water in the reaction media is associated with barbituric acid), and the desirable product **5** is present in a much higher ratio. The addition of the barbituric acid is very slow at room temperature and 9 days

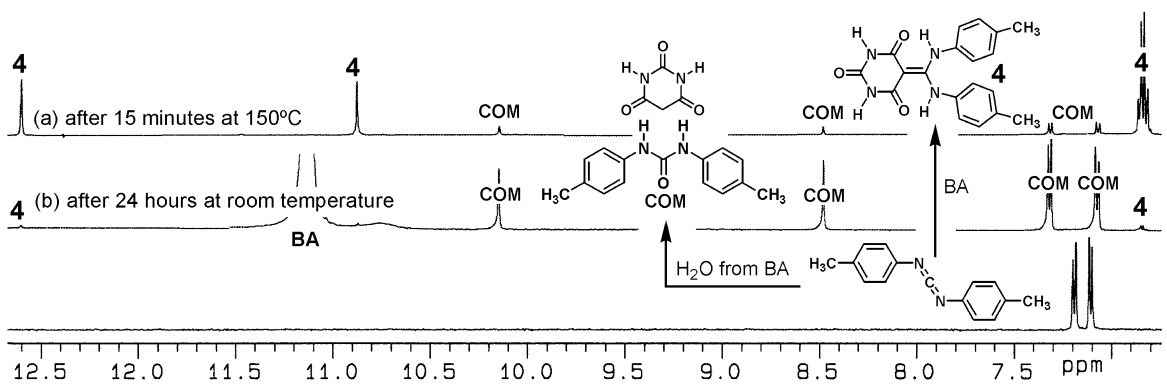


Figure 1. The ^{1}H NMR spectroscopic monitoring of barbituric acid (10 mM) addition to 1,3-di(4-methylphenyl)carbodiimide (1 mM) in DMSO-d_6 (1 mL).

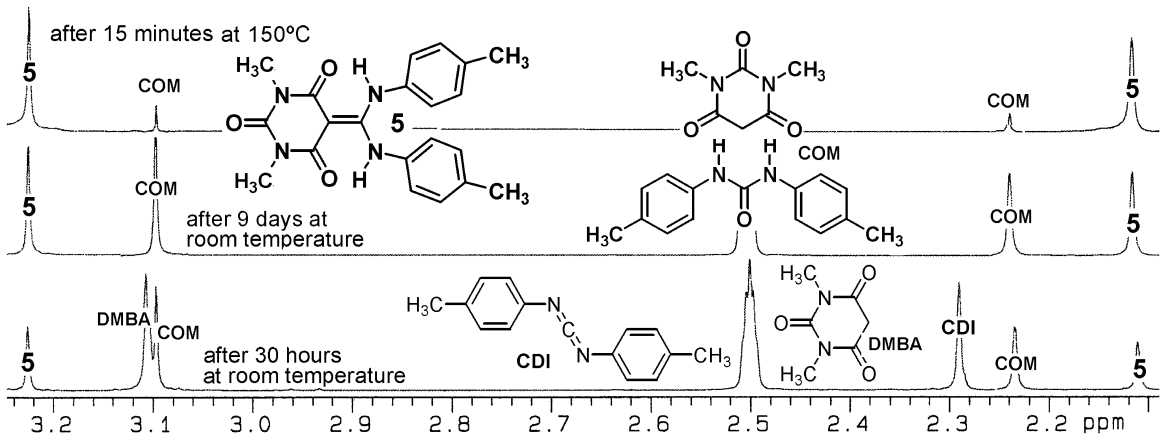
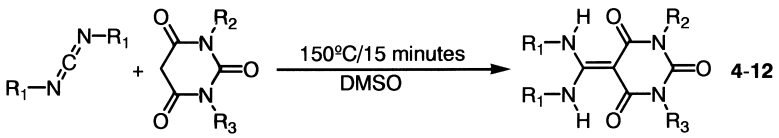


Figure 2. The methyl region of the ^{1}H NMR spectra for 1,3-dimethylbarbituric acid (1 mM) addition to 1,3-di(4-methylphenyl)carbodiimide (1 mM) in DMSO-d_6 (1 mL).

are required for both reactants to be consumed (Fig. 2); 1 mmol of each reactant in 1 mL of DMSO yielded an almost equimolar ratio of product **5** and the molecular complex (Fig. 2). If the reaction was performed at 150°C for 15 min then the major product of the reaction (95% conversion) is condensation product **5**.

From these experiments, it is obvious that our target compounds can be prepared at elevated temperatures (150°C) in a very short reaction period (15 min). Satisfactory yields of the condensation products can also be obtained if the reaction is performed in other inert solvents, such as tetrahydrofuran at room temperature. Tetrahydrofuran solution of barbituric acid should be dried over molecular sieves for a few days to eliminate water that usually accompanies barbituric acid. Into such dried solution corresponding carbodiimide should be added and the reaction mixture should be kept in a closed container at room temperature for 14 days. Solvent was evaporated and the remaining residue was crystallized from methanol, resulting in the pure target compounds **4**–**12** in 85–95% yield. Due to low solubility of barbituric acid derivatives in tetrahydrofuran this procedure is practical for the preparation of small quantities of 5-diaminomethylenebarbiturates. For large-scale preparation DMSO is much more suitable as reaction solvent. Isolated yields and applied methods for the preparation of 5-diaminomethylenebarbiturates **4**–**12** are listed in Table 1.

Table 1. Isolated yields of 5-diaminomethylenebarbiturates **4**–**12**



	Compound	Method	R_1	R_2	R_3	Yield (%)
1	**4**	A	4-$CH_3C_6H_4$	H	H	89
2	**5**	B	4-$CH_3C_6H_4$	CH_3	CH_3	90
3	**6**	A	4-$CH_3C_6H_4$	C_6H_5	H	89
4	**7**	C	$(CH_3)_2CH$	H	H	83
5	**7**	D	$(CH_3)_2CH$	H	H	86
6	**8**	C	$(CH_3)_2CH$	CH_3	CH_3	87
7	**8**	D	$(CH_3)_2CH$	CH_3	CH_3	85
8	**9**	C	$(CH_3)_2CH$	C_6H_5	H	92
9	**9**	D	$(CH_3)_2CH$	C_6H_5	H	87
10	**10**	E	C_6H_{11}	H	H	89
11	**11**	B	C_6H_{11}	CH_3	CH_3	95
12	**12**	B	C_6H_{11}	C_6H_5	H	93

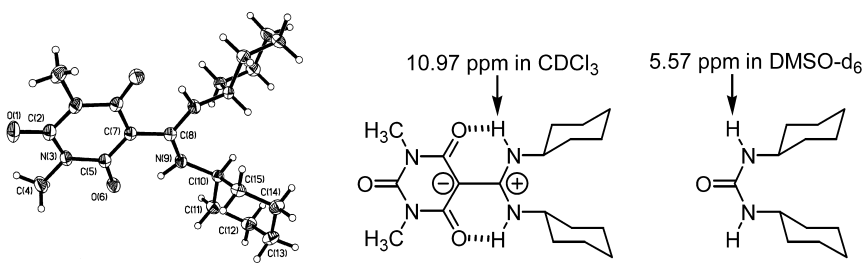


Figure 3. ORTEP drawing of X-ray crystallographic structure of **11** and its ylide structure.

To confirm the structural properties of the prepared target barbituric acid derivatives, compound **11** was selected for X-ray structural studies. Our spectroscopic data for 5-diaminomethylidenebarbiturates are in full agreement with the X-ray determined structure of **11** (Fig. 3). It is interesting to mention that the C(7)–C(8) bond between urea and the barbituric acid moiety has only partial double bond character. The compound can be better described as an ylide with a positive charge located on the urea carbon and a negative charge on the barbituric acid moiety. This charge separation is perfectly demonstrated with strong ═O···HN hydrogen bonding and a very high chemical shift for the urea NH hydrogen (Fig. 3).

3. Conclusion

In conclusion it can be stated that through careful NMR spectroscopic monitoring it was possible to develop a general synthetic procedure for the preparation of large quantities of 5-diaminomethylenebarbiturates from substituted carbodiimides (urea) and barbituric acid. Preparation of a wide variety of structural isomers of these compounds is now possible. It was also demonstrated that strong electrostatic interactions between these two moieties (urea and barbituric acid) exist and that these interactions are responsible for strong charge separation throughout the molecule that has ylide-like structural properties.

4. Experimental

4.1. General

All reagents and starting materials were purchased from Aldrich Chemical Company and were used without further purification. Solvents were purchased from Fisher Chemical Company and were used without previous distillation or drying. All NMR spectra were recorded on a 500 MHz Unity Varian NMR instrument with DMSO-d_6 (2.50 ppm, for hydrogen and 39.51 ppm for carbon) and $CDCl_3$ (7.27 ppm for hydrogen and 77.23 ppm for carbon) as an internal reference. Electro-spray mass spectrometric analysis was performed on a Micromass Quattro 2 Triple Quadropole Mass spectrometer. X-Ray structure determination was performed on a Bruker SMART 1KCCD automated diffractometer. Crystals of compound **11** were obtained by slow crystallization from a diluted methanol solution of **11**. Melting points were determined on an Electrothermal 9100 melting point apparatus and are not corrected.

4.1.1. Typical procedure A. Preparation of 5-[di(4-methylphenylamino)methylene]pyrimidine-2,4,6-trione (4)

Barbituric acid (128 mg; 1 mmol), di(4-methylphenyl)carbodiimide (222 mg; 1 mmol) and dimethyl sulfoxide (1 mL) were heated at 150°C for 15 min. Initially, the reaction mixture was a suspension that first turned to a yellow solution and then to a dark brown solution. The ^{1}H NMR test of the reaction mixtures suggests that all starting carbodiimide was consumed and transferred into the desired product **4** (~95%) and 1,3-di(4-methylphenyl)urea (~5%). The reaction mixture was cooled to room temperature, diluted with methanol (~100 mL), and left at room temperature overnight for the solvent to slowly evaporate to a volume of ~30 mL. The white precipitate was separated by filtration, washed with cold methanol (3×5 mL) and dried at 110°C for 30 min to give 310 mg (89%) pure product. Mp 278.7°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.61 (2H, s, NHAr), 10.88 (2H, s, NH), 6.84 (4H, d, J=8.0 Hz, m-H), 6.81 (4H, d, J=8.0 Hz, o-H), and 2.10 (6H, s, CH_3). ^{13}C NMR (DMSO-d_6, 500 MHz) δ 167.2, 157.9 (carbonyl carbons), 149.1, 80.3 (C═C carbonyls), 134.4, 133.7, 128.9, 122.9 (aromatic carbons, and 20.3 ppm (methyl carbon). MS-ES$^+$ (CH_3OH) m/z 405 (M+CH_3-OH+Na^+, 100%)$^+$, 723 (2M+Na^+, 90%)$^+$. Anal. calcd for $C_{19}H_{18}N_4O_3$ (350.14): C, 65.13; H, 5.18; N, 15.99. Found: C, 65.11; H, 5.20; N, 15.95.

4.1.2. Typical procedure B. Preparation of 5-[di(4-methylphenylamino)methylene]-1,3-dimethylpyrimidine-2,4,6-trione (5)

A mixture of 1,3-dimethylbarbituric acid (156 mg; 1 mmol), di(4-methylphenyl)carbodiimide (222 mg; 1 mmol) and dimethyl sulfoxide (1 mL) was heated at 150°C for 15 min. The reaction solution was left at room temperature for 2 h, and a white crystalline product precipitated. This suspension was diluted with methanol (15 mL), and solid product was separated by filtration, washed with methanol (3×5 mL) and dried at 110°C for 30 min resulting in 340 mg (90%). Mp 211.8°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.85 (2H, s, NH), 6.75 (4H, d, J=9.0 Hz, m-H), 6.69 (4H, d, J=9.0 Hz, o-H), 3.36 (6H, s, NCH_3), and 2.12 ppm (6H, s, CH_3). ^{13}C NMR ($CDCl_3$, 500 MHz) δ 165.7, 158.8 (carbonyl carbons), 151.0, 81.8 (C═C carbons), 135.2, 134.0, 128.1, 123.5 (aromatic carbons), 27.9 (NCH_3 carbon), and 20.8 ppm (CH_3 carbon). MS-ES$^+$ (CH_3COOH) m/z: 379 (M+1, 55%), 767 (2M+1, 25%). Anal. calcd for $C_{21}H_{22}N_4O_3$ (378.42): C, 66.65; H, 5.86; N, 14.81. Found: C, 66.46; H, 5.90; N, 14.70.

4.1.3. Preparation of 5-[di(4-methylphenylamino)methylene]-1-phenylpyrimidine-2,4,6-trione (6). Compound **6** was prepared by following procedure outlined in typical procedure A. The isolated yield is 89%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.85 (2H, broad s, ArNH), 10.90 (1H, s, NH), 7.40 (2H, t, J=8.0 Hz), 7.35 (1H, t, J=8.0 Hz), 7.18 (2H, d, J=8.0 Hz), 6.77 (4H, d, J=9.0 Hz), 6.65 (4H, d, J=9.0 Hz), 2.15 ppm (6H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ 166.5, 166.3, 165.9, 165.7, 160.1, 151.0, 125.8, 135.2, 134.0, 129.8, 128.6, 128.1, 127.9, 123.5, 80.3, and 20.6 ppm. MS-ES$^+$ (CH_3OH) m/z: 449 (M+Na^+, 10%), 481 (M+CH_3OH+Na^+ 27%), 875 (2M+Na^+, 100). Anal. calcd for $C_{25}H_{22}N_4O_3$ (426.17): C, 70.41; H, 5.20; N, 13.14. Found: C, 70.29; H, 5.31; N, 13.09.

4.1.4. 5-(Diisopropylaminomethylene)pyrimidine-2,4,6-trione (7). This compound was prepared by applying either typical procedure C or D, obtaining the target product in 83 and 86% yields, respectively. ^{1}H NMR (DMSO-d_6, 400 MHz) δ, 10.78 (2H, s, NHCHMe$_2$), 10.43 (2H, s, NH), 3.824 (2H, m, J=6.0 Hz, CH), 1.21 (12H, d, J=6.0 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 166.8, 160.5, 151.7, 79.3, 45.4, and 23.2 ppm. ES-MS$^+$

(CH_3CO_2H), 255 ($M+H^+$, 100%), 337 ($M+CH_3CO_2H+Na^+$, 25%), 509 ($2M+H^+$, 30%). Anal. calcd for $C_{11}H_{18}N_4O_3$ (254.14): C, 51.96; H, 7.13; N, 22.03. Found: C, 51.88; H, 7.23; N, 21.95.

4.1.5. Typical procedure C. Preparation of 5-[di(1-methylethylamino)methylene]-1,3-dimethylpyrimidine-2,4,6-trione (8)

A tetrahydrofuran (50 mL) solution of 1,3-dimethylbarbituric acid (156 mg; 1 mmol) and diisopropylcarbodiimide (138 mg; 1.1 mmol) was stirred in a closed round bottle flask for 10 days. Solvent was evaporated, the oily residue was dissolved in absolute ethanol–benzene (1:3) solution (50 mL), and the solvent was evaporated. This procedure was repeated three more times. The semi-solid residue was mixed with methanol (100 mL) and the resulting mixture was refluxed for 1 h, filtered and left in an open beaker (150 mL) at room temperature for several days. Methanol slowly evaporated at room temperature almost to dryness, and formed large glassy-like needles of the product were separated by filtration, washed with cold methanol (3×5 mL) and dried at 110°C for 2 h to give 245 mg (87%) of pure product. Mp 169.3°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.75 (2H, s, NH), 3.86 (2H, m, CH), 3.12 (6H, s, NCH_3), and 1.22 ppm (12H, d, J=6.0 Hz, $C(CH_3)_2$). ^{13}C NMR (DMSO-d_6, 500 MHz) δ 164.1, 160.3 (carbonyl carbons), 150.4, 81.0 (C=C carbons), 45.5, 23.0 (isopropyl carbons), and 27.3 ppm (NCH_3 carbon). MS-ES$^+$ (CH_3COOH) m/z: 283 ($M+H^+$, 100%), 365 ($M+CH_3COOH+Na^+$, 18%), 565 ($2M+H^+$, 22%), 869 ($3M+Na^+$, 25%). Anal. calcd for $C_{13}H_{22}N_4O_3$ (382.17): C, 55.30; H, 7.85; N, 19.84. Found: C, 55.28; H, 7.90; N, 19.72.

4.2. Typical procedure D

Compound **8** was also prepared by typical procedure D. The reaction was performed with the same quantities of the reactants as in typical procedure C, except dimethyl sulfoxide (1 mL) was used as solvent. The reaction mixture was heated in a closed container at 150°C for 15 min and the solvent was evaporated at 10^{-3} mm pressure to an oily residue. This residue was crystallized from methanol in the same manner as described in method C. The product was prepared in 85% yield.

4.2.1. 5-(Diisopropylaminomethylene)-1-phenylpyrimidine-2,4,6-trione (9). Compound **9** was prepared by following either typical procedures C or D to obtain the target product in 92 and 87% yields, respectively. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.75 (2H, s, NH), 2.03 (1H, s, NH), 7.45 (2H, t, J=7.5 Hz), 7.34 (1H, t, J=7.5 Hz), 7.19 (2H, d, J=7.5 Hz), 3.86 (2H, m, CH), and 1.22 ppm (12H, d, J=6.0 Hz, $C(CH_3)_2$). ^{13}C NMR (DMSO-d_6, 500 MHz) δ 164.2, 163.7, 160.3, 150.2, 135.7 129.2 128.3 127.5, 81.0, 45.3, and 22.7 ppm. MS-ES$^+$ (CH_3COOH) m/z: 331 ($M+H^+$, 30%). Anal. calcd for $C_{17}H_{22}N_4O_3$ (330.17): C, 61.80; H, 6.71; N, 16.96. Found: C, 61.72; H, 6.81; N, 16.83.

4.2.2. Typical procedure E. Preparation of 5-[di(cyclohexylamino)methylene]pyrimidine-2,4,6-trione (10)

A dimethyl sulfoxide (150 mL) suspension of barbituric acid (12.8 g; 0.1 mol) and dicyclohexylcarbodiimide (20.6 g; 0.1 mol) was heated at 140°C for 20 min. The clear, deep brown reaction mixture was left at room temperature for 4 h, and glass like plates of 1,3-dicyclohexylurea (1.3 g) were separated by filtration. The dimethyl sulfoxide filtrate was diluted with water (1 L). The resulting suspension was stirred at room temperature for 30 min, and a white solid was separated by filtration, washed with water (3×50 mL) and mixed with methanol (150 mL). The resulting methanol suspension was stirred at room temperature for 30 min; a solid residue was separated by filtration, washed with methanol (3×30 mL) and dried at 110°C for 1 h. In this way pure product in 89% (29.7 g) yield was obtained. If necessary, the product can be purified by crystallization from a large volume of methanol. Mp 295.1°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.91 (2H, d, J=5.5 Hz, NH), 10.46 (2H, s, barbituric acid NH), 3.49 (2H, m, CH), 1.87 (4H, m), 1.66 (4H, m), 1.50 (2H, m), and 1.34 ppm (10H, m). ^{13}C NMR (DMSO-d_6, 500 MHz) δ 166.8, 160.2 (carbonyl carbons), 149.1, 79.3 (CC double bond carbons) 51.8, 32.6, 24.9, 23.2 ppm (cyclohexane moiety carbons). MS-ES$^+$ (CH_3OH) m/z: 389 ($M+CH_3OH+Na^+$, 60%), 581 ($M+DCU+Na^+$, 40%), 805 ($M+2DCU+Na^+$, 60%), 915 ($2M+DMU+Na^+$, 35%). Anal. calcd for $C_{17}H_{26}N_4O_3$ (334.20): C, 61.06; H, 7.84; N, 16.75. Found: C, 61.05; H, 7.89; N, 16.69.

4.2.3. Preparation of 5-[di(cyclohexylamino)methylene)-1,3-dimethylpyrimidine-2,4,6-trione (11). Compound **11** was prepared by following the procedure outlined in typical procedure B. The isolated yield is 85%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.97 (2H, d, J=7.5 Hz, NH), 3.29 (2H, m), 3.14 (6H, s, CH_3), 1.82 (4H, m), 1.65 (4H, m), 1.46 (2H, m), 1.30 (4H, m), and 1.20 ppm (6H, m). ^{13}C NMR ($CDCl_3$, 500 MHz) δ 165.3, 160.9 (two carbonyl carbons), 150.8, 81.1 (two CC double bond carbons) 53.2, 33.4, 27.4, 25.2, and 24.2 ppm (four cyclohexane carbons). MS-ES$^+$ (CD_3OD) m/z: 363 (M+1+, 7%), 417 ($M+CH_3OH+Na^+$, 45%), 609 (M+dicyclohexylurea+Na^+, 55%), 747 ($2M+Na^+$, 75%). Anal. calcd for $C_{19}H_{30}N_4O_3$ (362.23): C, 62.96; H, 8.34; N, 15.46. Found: C, 62.89; H, 8.31; N, 15.32.

4.2.4. Preparation of 5-[di(cyclohexylamino)methylene)-1-phenylpyrimidine-2,4,6-trione (12). Compound **12** was prepared by following the procedure outlined in typical procedure B. The isolated yield is 95%. ^{1}H NMR (DMSO-d_6, 500) δ, 10.87 (3H, broad singlet), 7.43 (2H, t, J=7.5 Hz), 7.37 (1H, t, J=7.5 Hz), 7.21 (2H, d, J=7.5 Hz), 3.51 (2H, m), 1.89 (4H, m), 1.67 (4H, m), 1.52 (2H, m), 1.35 ppm (10H, m). ^{13}C NMR (DMSO-d_6, 500 MHz) δ 165.9, 165.7, 160.1 (three different carbonyl carbons), 149.2, 135.8, 129.4, 128.6, 127.7, 79.8 (aromatic and CC double bond carbons), 52.1, 32.7, 24.9, and 23.4 ppm (cyclohexane moiety carbons). MS-ES$^+$ (CH_3OH) m/z: 433 ($M+Na^+$, 20%), 465 ($M+CH_3OH+Na^+$, 30%), 843 ($2M+Na^+$, 100%). Anal. calcd for $C_{23}H_{30}N_4O_3$ (410.23): C, 67.29; H, 7.37; N, 13.65. Found: C, 67.12; H, 7.36; N, 13.49.

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