A New Method for the Preparation of Intermediates for 2,6-Substituted Anthrapyridazones

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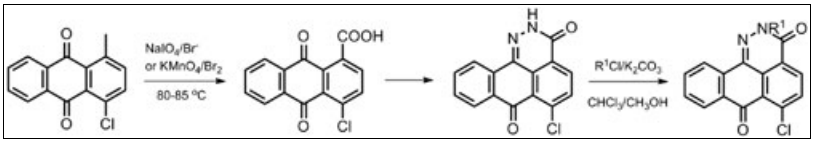

A new method for the preparation of 2-substituted 6-chloro-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cynnoline-3,7-diones has been developed. The compounds have been obtained in an original three-step procedure comprising the oxidation of 1-methyl-9,10-anthraquinones with periodate or permanganate/brominating reagent systems, cyclization to 6-chloro-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cynnoline-3,7-dione, and selective alkylation thereof. The selected processes were applied in the efficient scale-up of specific 2,6-substituted 2,7-dihydro-3*H*-dibenz[*de*,*h*]cinnolin-3,7-dione derivatives, currently being investigated pre-clinically as anticancer agents.

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INTRODUCTION

Natural and synthetic 9,10-anthraquinone derivatives are widely used in the chemical industry and in medicine. The 9,10-anthraquinone moiety occurs as a natural pigment in plants, fungi, lichens, and insects [1–5]. One of the anthraquinone classes contains compounds with a carbonyl substituent, such as those isolated from plants: rhein, diacerein, and emodic acid [6]. These natural or synthetic anthraquinones find their application as building blocks in the synthesis of the compounds with a biological activity (Fig. 1) [7–10]. Anthraquinones are known for their laxative [11], antifungal [12], antimalaric [13], and anticancer properties (Fig. 2) [14]. Mitoxantrone, a 9,10-anthraquinone derivative, has long been registered as an anticancer therapeutic [15–17]. However, like other clinical anticancer drugs, it undergoes the phenomenon of phenotypic, multidrug cross-resistance of tumor cells (multidrug resistance (MDR)), related to the removal of drugs from cells via specific transport proteins. The observed overexpression of transporter proteins in tumors diminishes the therapeutic effect of drugs and therefore the effectiveness of therapy. Thus, the occurrence of the multidrug resistance effect has necessitated studies on new active compounds against the resistant cells, including anthraquinone derivatives. To date, a group of compounds with an additional heterocyclic ring connected to the anthraquinone core has revealed some activity toward resistant cancer cells. Among them, the most interesting profile is displayed by tetracyclic compounds with an additional pyridazone ring fused to the anthraquinone moiety, that is, anthrapyridazone derivatives. It has been described that an improved solubility and the increase in the cytotoxic activity may be obtained by introducing [(alkylamino)alkyl]amino side chains into the positions *2* and *6* of the anthrapyridazone ring. Recently, another group of asymmetrically 2,6-disubstituted 2,7-dihydro-3*H*-dibenz[*de*,*h*]cinnoline-3,7-diones has been disclosed. These analogs exhibit a high cytostatic activity against MDR tumor cells with the overexpression of different exporting pumps, MDR-1, BCRP, and MRP, and against a broad spectrum of resistant cell lines derived from different tissues and patient organs. Although the new MDR compound class was successfully synthetized in a small laboratory scale, we observed several problems during the preparation of their two intermediates in a multigram scale: anthraquinone acid (**2**) and 2-substituted anthrapyridazones (**6**, Scheme 1) [18,19].

The established methods for the preparation of 4-substituted 9,10-anthraquinone-1-carboxylic acids (**2**) are based on the oxidation of the methyl group in a particular anthraquinone derivative (**1**). Both scientific and patent literature sources describe several methods of the methyl group oxidation in 1-methyl-9,10-anthraquinones. The most common one is the oxidation with the diluted nitric acid under high pressure in a sealed tube at the temperature of 195–220°C [20,21]. As an alternative, patent literature discloses a method for the oxidation of the starting 1-chloro-4-methyl-9,10-antrhtaquinone (**1a**): in the solution of the acetic acid in the presence of air and daylight [22]; in nitrobenzene, by passing chlorine gas through the reaction mixture at the temperature of

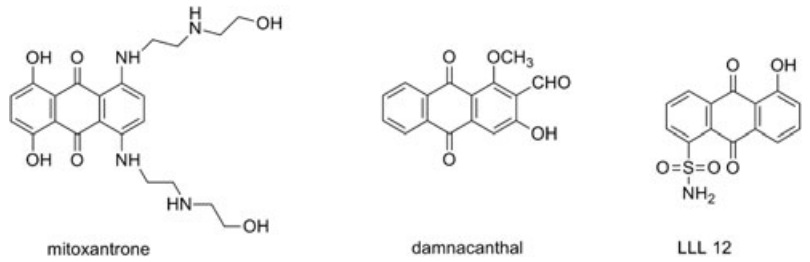


Figure 1. Chemical structures of some derivatives with the anthraquinone core exhibiting an anticancer and/or antifungal activity.

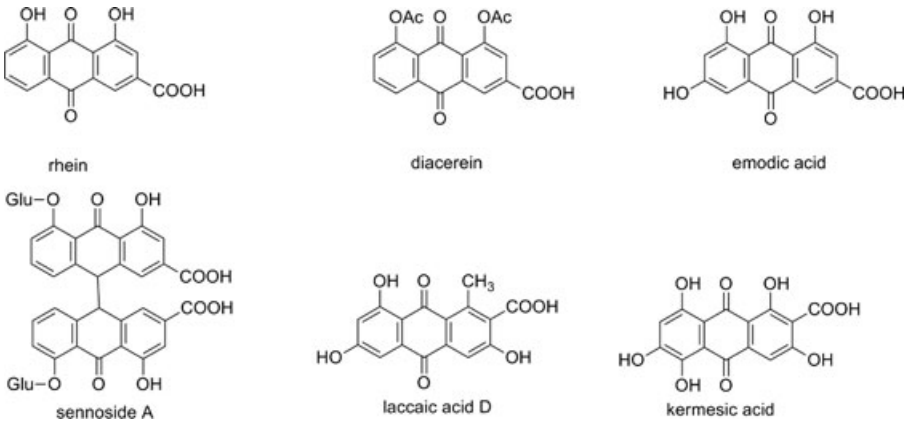
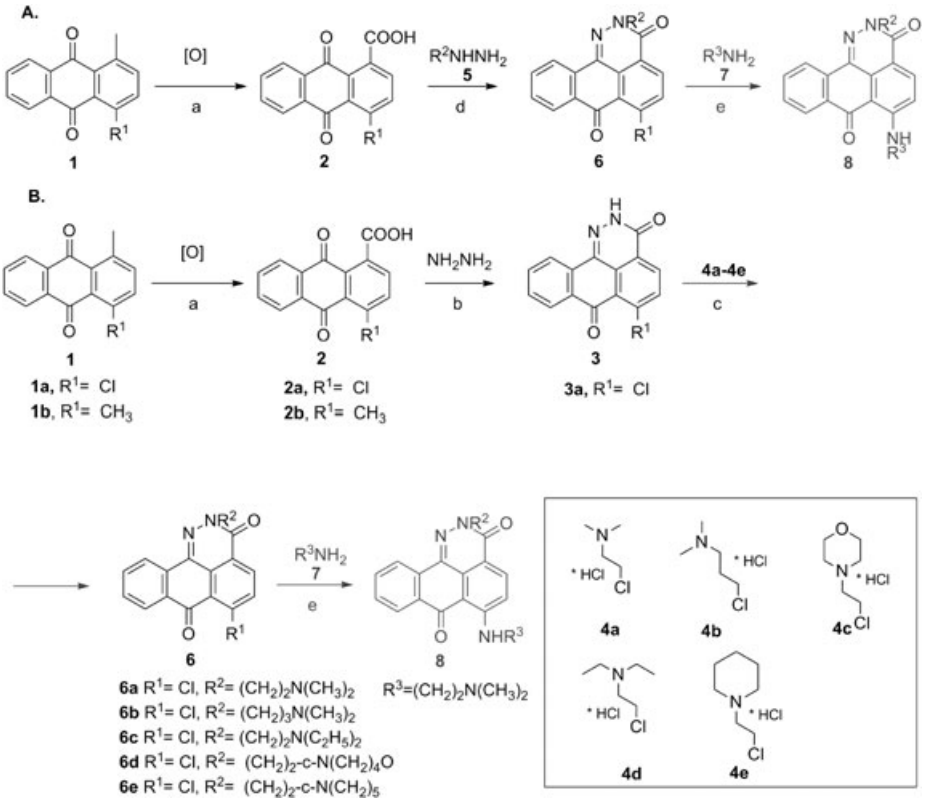


Figure 2. Chemical structures of some anthraquinones isolated from plants (rhein, diacerein, emodic acid, and sennoside A) [6] and insect pigments (laccaic acid D and kermesic acid) [4].

Scheme 1. Synthetic routes for obtaining **8** (reference route **A**: (a) 16% HNO_3 aq/200°C, (d) PCl_5/benzene; present route **B**: (a) $NaIO_4$/LiBr/80°C, (b) SO_2Cl_2/toluene/80% hydrazine monohydrate, and (c) $K_2CO_3/CHCl_3/CH_3OH$).



160–170°C [23]. Another known methods involve oxidations with the fuming sulfuric acid or oleum [24,25]. All the aforementioned procedures require aggressive reagents and harsh reaction conditions including high pressure and temperature.

Another major problem in the synthesis of anthrapyridazones **8** is obtaining their monosubstituted intermediates **6**. According to the known literature methodology [18,19], 2,7-dihydro-3*H*-dibenz[*de*,*h*]cinnoline-3,7-diones with the (alkylamino)alkyl substituent in position *2* of the tetracyclic ring can be obtained through the condensation of 4-chloroanthraquinone-1-carboxylic acid (**2**), previously converted to the respective chloride, and (alkylamino)alkyl hydrazine derivative (**5**) (Scheme 1, reaction d). Thereby, a group of 2-substituted 2,7-dihydro-3*H*-dibenz[*de*,*h*]cinnoline-3,7-diones with (dimethylamino)ethyl, (dietylamino)ethyl, (morfoline)ethyl, and (piperidineamino)ethyl spacers was isolated in the yield of only 20–60% after the purification by column chromatography. The aforementioned strategy seems to be problematic in the upscaling process because of the necessity to obtain harmful (alkylamino)alkyl hydrazine precursors (**5**). The synthesis of **5** includes several hours of heating of the corresponding (alkylamino)alkyl chloride (**4a–e**) with 60–80% hydrazine solution in water [26]. The resulting product is difficult to characterize, a high boiling mixture of different compounds, such as disubstituted (alkylamino)alkyl hydrazines. The purification requires distillation under reduced pressure of 15–20 mbar and eventually a reliable analytical method to characterize the expected product. It results in low yields of the hydrazine derivatives **5a–e** (20–30%).

Taking into consideration the aforementioned limitations, we have decided to search for new, effective, and reproducible synthetic methods that by allowing us to prepare intermediates **2** and **6** would lead us to obtain 2,7-dihydro-3*H*-dibenz[*de*,*h*]cinnolin-3,7-dione derivatives (**8**) for preclinical tests.

Herein, we describe new improvement methods for the synthesis of **2** and **6** intermediates: building blocks for compounds with a cytostatic activity against multidrug resistance cell lines.

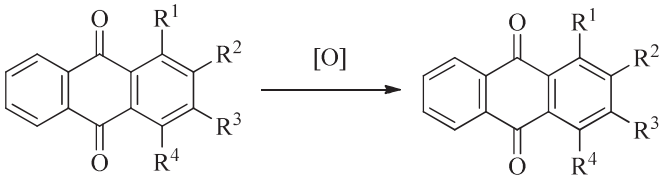
RESULTS AND DISCUSSION

During our studies on the 2,7-dihydro-3*H*-dibenz[*de*,*h*]cinnolin-3,7-diones (**8**) synthesis, it was experimentally verified that only the method using the diluted nitric acid guarantees an adequate purity of anthraquinone-1-carboxylic acids. However, it seemed to be problematic in the upscaling process, because it forced us to use an autoclave resistant to the corrosive nitric acid and nitrogen oxides.

While searching for a milder, easier to handle, and more economical oxidation of 1-methyl-9,10-anthraquinones, numerous methods for the oxidation of the methyl group in aromatic compounds were found [27–30]. We examined literature procedures with the commonly used oxidants, that is, potassium permanganate, sodium dichromate, CrO_3, and HNO_3 in the atmospheric pressure with no positive result. It had been reported before that the direct oxidation of alkylarenes mediated by the sodium periodate/lithium bromide combination produces benzyl acetates through benzyl bromides in the acetic acid or benzoic acids in the diluted inorganic acid [31,32]. Based on these results, we examined a variety of reaction conditions with or without the bromine source (Table 1) and the sodium periodate or potassium permanganate in order to find an effective oxidizing system for 1-chloro-4-methyl-9,10-anthraquinone (**1a**) and 1,4-dimethyl-9,10-anthraquinone (**1b**). Contrary to literature data, the oxidation was observed not only in the presence of the lithium bromide. A comparable efficiency was achieved when using potassium or sodium bromides as the halogen source. A low transformation level to 4-chloroanthraquinone-1-carboxylic acid (**2a**), that is, 3–7%, took place in the absence of the bromine source or while using sodium chloride or cuprous bromide as the accompanying salts. The consumption of the starting 1-methyl-9,10-anthraquinone derivatives in the presence of the oxidation reagents was examined by HPLC. The HPLC samples were obtained after the final workup of the reaction mixtures. An almost insoluble acid was isolated by a simple filtration. The filtrate was extracted with chloroform and ethyl acetate in order to recover the starting material after evaporating the solvent excess. The organic residues were combined and homogenized before the HPLC analysis. Attempts to use other solvent systems (chloroform/diluted sulfuric acid, tetrahedrofuran/diluted sulfuric acid, methylene chloride/process water) in order to increase the yield and shorten the reaction time all failed. The extended reaction time proved to be an essential factor for the effectiveness of the oxidation (Table 1). The purity of 4-chloroanthraquinone-1-carboxylic acid (**2a**) exceeding 83% was reached in 144 h, while the quenching after 48 h resulted in only 16% of the transformation. An increase in the reaction temperature to 110°C neither shortened the reaction time nor improved the yield significantly. The electrophilic ring bromination was neither observed. Interestingly, the oxidation product was determined in the bromine-treated reaction mixture with the yield of 22.4% (Table 1). One of the highest transformations was observed while using the potassium permanganate/bromine system; however, that condition was very difficult to apply in a larger scale because of the problematic separation of hardly soluble 4-chloroanthraquinone-1-carboxylic from the insoluble manganese dioxide inorganic impurity.

Table 1

Oxidative bromination[a] of **1a** with $NaIO_4$ or $KMnO_4$ and a bromine source.



1a R^1=CH_3, R^2=R^3=H,R^4=Cl
1b R^1=R^4=CH_3, R^2=R^3=H
1c R^1=R^3=R^4=H, R^2=CH_3
1d R^1=R^4=H, R^2=CH_3, R^3=Cl
1e R^1=R^4=H, R^2=CH_3, R^3=Br

2a R^1=COOH, R^2=R^3=H,R^4=Cl
2b R^1=COOH, R^2=R^3=H, R^4=CH_3

						HPLC purity[c]	
Entry	Bromine source	Oxidant	Solvent	Reaction time (h)	Temp (°C)	**1**	**2**
1a	—	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	90.55	5.48
1a	LiBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	48	85	80.54	15.60
1a	LiBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	41.36	56.67
1a	LiBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	144	85	14.14	83.80
1a	LiBr	$NaIO_4$	Chlorobenzene/H_2O	96	85	76.44	21.01
1a	LiBr	$NaIO_4$	Chloroform/H_2O	96	60	91.13	5.09
1a	KBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	30.55	68.06
1a	NaBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	34.93	63.02
1a	CuBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	88.33	7.24
1a	NaCl	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	91.74	3.35
1a	LiBr	$KMnO_4$	chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	81.83	12.18
1a	Br_2[b]	$KMnO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	23.26	75.03
1a	Br_2[b]	—	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	75.74	22.40
1b	LiBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	38.50	61.19
1c [33]	LiBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	—[d]	—
1c [33]	KBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	—[d]	—
1d [20]	LiBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	—[d]	—
1d [20]	KBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	—[d]	—
1e [34]	LiBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	—[d]	—
1e [34]	KBr	$NaIO_4$	Chlorobenzene/0.5 *M* H_2SO_4 1:1	96	85	—[d]	—

[a]Conditions: substrate (3.9 mmol), oxidant (11.7 mmol), metal bromide (11.7 mmol), solvent (20 mL), tetrabutylammonium bromide (10 mol%).
[b]For bromine (5.9 mmol).
[c]Determination of the HPLC purity for the substrate and product combined after the reaction workup.
[d]Lack of product **2** on the TLC plates.

In addition, effective reaction conditions for obtaining 4-substituted 9,10-anthraquinone-1-carboxylic acids (**2a–b**) were examined for their ability to oxidize 2- and 3-methyl-9,10-anthraquinone derivatives [20,33,34] (**1c–e**) to respective acids (**2c–e**). Unfortunately, when the sodium periodate/bromide salt systems were used (Table 1), the formation of the expected products was not observed on the TLC plates.

In order to develop an efficient method for the synthesis of intermediates **6,** we first verified the literature data. As it was reported, any attempts to repeat the known procedure of direct formation of the tetracyclic ring from acid **2** [18,19] and (alkylamino)alkyl hydrazine substrates (**5**) [26] showed that the main problems included: low yields of the non-commercial substrates **5** synthesis, their purification by distillation under reduced pressure, and difficult analytical evaluation. In addition, following the distillation process the decomposition of compounds **5a** and **b** was observed on the ^{1}H NMR spectrum. Because of these technological complications, we preferred to find original conditions for obtaining **6** in a two-step procedure, that is, the reaction of **2** with hydrazine, then the alkylation of **3** with commercially certified 2-(dimethylamino)ethyl chloride hydrochloride (**4a**). Compound **3** was obtained with 4-chloroanthraquinone-1-carboxylic acid **2** and 80% hydrazine hydrate in the

presence of thionyl chloride with a good 74% yield. In the second step, the alkylation of **3** required us to try a variety of electrophilic reagents as well as different solvents and temperature conditions in order to find the most efficient method. First of all, literature procedures for the alkylation of pyridazinones [35–41] were tested. However, the attempts to directly transfer the published reaction conditions to the alkylation of **3** by 2-(dimethylamino)ethyl chloride hydrochloride (**4a**) failed. Initial experiments were performed in DMF or toluene in the presence of NaH, KOH, or NaOH (Table 2; entries 1, 2, and 3) because of the fair solubility of substrate **3** in that solvent/base systems. Unfortunately, we detected fast decomposition of starting material **3** for those strong bases. After changing the base for potassium carbonate, different results for toluene and DMF were observed. A reaction in nonpolar toluene and at ambient temperature did not lead to the alkylation of substrate **3**. However, an increase in the reaction temperature resulted in the decomposition of the starting material. A better effect was observed for the reaction carried out in DMF at ambient temperature (Table 2; entry 5), although **6a** was detected with a 55.8% purity. Taking into consideration the results collected from the DMF reactions, a conversion in other polar solvents, that is, acetone, acetonitrile, DMSO, or NMP, was evaluated. Unfortunately, a decrease in the **6a** formation was observed during these attempts. The purity varied from about 8% to 33% in crude reaction mixtures, and numerous unknown impurities were detected, except for the substrate and the product. While the reactions were carried out in alcohols MeOH and EtOH (Table 2; entries 16, 17), only traces of the product were observed. During our systematic modifications of reaction conditions, followed by the examination of the substrate conversion and the purity of the alkylation processes, it was revealed that chloroform may be the most promising medium for this type of reaction (Table 2; entry 18). The reaction carried out in the presence of 6 equiv of potassium carbonate at 40°C for 24 h provided 80% of **6a** in a crude reaction mixture. Extending the reaction time to 48 h caused a partial decomposition of product **6a**. As a consequence, a decrease in purity to 70% (Table 2; entry 19) was observed. Further investigation showed that the alkylation procedure can be improved by using a mixture of chloroform with some amount of polar MeOH as a solvent. The best result was observed at 20% of MeOH in relation to the chloroform volume in the presence of 6 equiv of potassium carbonate and 5 equiv of 2-(dimethylamino)ethyl chloride hydrochloride (Table 2; entry 20). This optimized procedure was verified through the alkylation of **3** with alkylaminoalkyl chlorides (**4b**, **4c**, **4d**, and **4e**). In all experiments, an appropriate product (**6b**, **6c**, **6d**, and **6e**) was obtained with a good yield ranging from 80 to 100% (see Experimental section). The structures of all compounds were confirmed by extended ^{1}H and ^{13}C NMR experiments as well as HR-MS (Experimental section). The purity of all products was verified by the

Table 2

Optimization of **3** alkylation condition in a model compound **6a**.

					HPLC	
Entry	Base	Solvent	Reaction time (h)	Temp. (°C)	**3**	**6a**
1.	NaH (60%, 1.5 eq.)	DMF	24	rt	Decomp.	—
2.	KOH, TBAB	toluene	5	rt	66.6	5.8
3.	NaOH, TBAB	toluene	5	50	Decomp.	—
4.	K_2CO_3 (1.5 eq.)	DMF	24	rt	50.3	5.6
5.	K_2CO_3 (3 eq.)	DMF	24	rt	13.5	55.8
6.	K_2CO_3 (2 eq.)	DMF	24	40	3.7	40.4
7.	K_2CO_3 (2 eq.)	acetone	48	rt	32.8	35.5
8.	K_2CO_3 (2.3 eq.)	acetonitrile	48	rt	26.7	28.7
9.	K_2CO_3/KJ (2 eq.)	DMF	48	rt	4.2	31.3
10.	K_2CO_3/H_2O (2 eq.)	DMF	48	rt	9.5	12.4
11.	K_2CO_3 (2 eq.)	DMSO	4	rt	10.3	30.7
12.	K_2CO_3 (2 eq.)	DMSO	24	rt	1.0	33.7
13.	K_2CO_3 (2 eq.)	NMP	6	rt	54.4	8.1
15.	K_2CO_3 (4 eq.)	DMF	24	50	11.6	47.9
16.	K_2CO_3 (6 eq.)	MeOH	24	40	—	—[a]
17.	K_2CO_3 (6 eq.)	EtOH	24	40	—	—[a]
18.	K_2CO_3 (6 eq.)	$CHCl_3$	24	40	**1.17**	**80.0**
19.	K_2CO_3 (6 eq.)	$CHCl_3$	48	40	0.08	70.15
20.	K_2CO_3 (6 eq.)	$CHCl_3$/MeOH	24	40	—	**90.0**

[a]Monitoring by the TLC method.

C-18 RPHPLC method using acetonitrile–water as the mobile phase.

In conclusion, our results show that the new current procedure for the preparation of 2-substituted 6-chloro-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cynnoline-3,7-diones (**6**) offers an attractive alternative to the reported methods for the preparation thereof. These compounds are used as intermediates in the synthesis of potential anticancer agents **8** [19]. The three-step process comprises efficient methods of 1-methyl-9,10-anthraquinones (**1a–b**) oxidation to the 4-substituted 9,10-anthraquinone-1-carboxylic acids (**2a–b**) and selective alkylation of **3** with commercially available (alkylamino)alkyl chlorides hydrochloride (**4a–e**). Compound **3** may be easily obtained from 4-chloroanthraquinone-1-carboxylic acid **2a** in a sequence of reactions with thionyl chloride and 80% hydrazine hydrate. However, the application of the proposed oxidation method to the strategy of the 2,7-dihydro-3*H*-dibenz[*de*, *h*]cinnolin-3,7-dione derivatives (**8**) synthesis prolongs the reaction time, and the ease of manipulation (lower temperature, easier-to-handle oxidation reagents, atmospheric pressure, andstandard laboratory equipment) seems to be advantageous in a bigger scale. Although we did not fully optimize the conditions of the processes, the proposed experimental procedure allowed us to reproducibly obtain hundreds of grams of **2a** with pharmaceutical purity. Therefore, it is our belief that further optimization might shorten the reaction time and be attractive to chemists.

EXPERIMENTAL

All reagents and solvents were purchased from common commercial suppliers and were used without further purification. The NMR spectra were recorded on a Varian VNMRS 600, Varian VNMRS 500, and Varian Gemini 200 spectrometers (at 298 K) in CDCl3 and DMSO-d_6, using TMS ($\delta = 0.0$ ppm) as an internal standard. Standard experimental conditions and standard Varian programs were used. The melting points were measured on a Büchi melting point apparatus and were uncorrected. The chromatographic analysis was performed using Dionex UltiMate 3000 UHPLC systems. The chromatographic separation for **2a–b** was achieved on a Kinetex C18, 150×4.6-mm, 2.6-μm column using a gradient mixture of solvent A (0.1% orthophosphoric acid in water) and solvent B (acetonitrile). The flow rate of 1.0 mL/min was used. The UV detection was carried out at 254 nm. The samples were prepared at the concentration of about 0.25 mg/mL and were dissolved in methanol. The injection volume was 10 μL. The column was placed in a thermostated column heater at 30°C. The chromatographic separation for **3**, **6a–b** was achieved on a XBridge C18, 150×4.6-mm, 3.5-μm column using a gradient mixture of solvent A (0.1% trifluoroacetic acid in water) and solvent B (0.1% trifluoroacetic acid in acetonitrile). The flow rate of 1.0 mL/min was used. The UV detection was carried out at 248 nm. The samples were prepared at the concentration of about 0.20 mg/mL and were dissolved in acetonitrile–methanol (4:1). The injection volume was 10 μL. The column was placed in a thermostated column heater at 30°C.

4-Chloro-9,10-dihydro-9,10-dioxo-1-anthracenecarboxylic acid (2a)

Preparation with the $NaIO_4$/LiBr system. The diluted sulfuric acid (10 mL, 2.5% (v/v)), sodium metaperiodate (2.5 g, 11.7 mmol), lithium bromide (1.02 g; 11.7 mmol), and tetrabutylammonium bromide (0.1 g, 0.3 mmol) were added to the solution of 1-chloro-4-methylanthraquinone [22] (1.00 g, 3.9 mmol) in chlorobenzene (10 mL). The reaction was stirred and heated for 144 h at 80–85°C. After cooling to the ambient temperature, the crude product was filtered, washed with saturated sodium thiosulfate solution, then with deionized water. The yellow crystals were air dried and purified by crystallization from methanol/water. Yield 0.82 g (73%). HPLC purity 99.96%. 126.26. M.p. = 231.5 (228–229°C) [20].

^{1}H NMR (DMSO-d_6; 600 MHz) δ 13.25 (m, 1H, COO*H*), 8.16 (dd, $J = 7.5$ Hz, $J = 1.5$ Hz, 1H, Ar–*H*), 8.11 (dd, $J = 7.5$ Hz, $J = 1.5$ Hz, 1H, Ar–*H*), 7,99 (d, $J = 8.2$ Hz, 1H Ar–*H*), 7.94 (ddd, $J = 7.5$ Hz, $J = 1.5$ Hz, 1H, Ar–*H*), 7.91 (ddd, $J = 7.5$ Hz, $J = 1.5$ Hz, 1H, Ar–*H*), 7.78 (dd, $J = 8.2$ Hz, 1H, Ar–*H*); ^{13}C NMR (DMSO-d_6; 600 MHz) δ 181.39, 180.90, 169.53, 137.39, 135.46, 134.84, 134.27, 133.94, 133.85, 132.48, 132.29, 131.99, 129.67, 126.87.

Preparation with the Br_2/$KMnO_4$ system. The diluted sulfuric acid (10 mL, 2.5% (v/v)), potassium permanganate (0.92 g; 5.8 mmol), and bromine (0.93 g; 5.8 mmol) were added to the solution of 1-chloro-4-methylanthraquinone (1.00 g, 3.9 mmol) in chlorobenzene (10 mL). The reaction was stirred and heated for 96 h at 80–85°C. After cooling to the ambient temperature, the crude product was filtered and washed with deionized water. The precipitate was suspended in acetone (200 mL) and stirred for 30 min in the ambient temperature. The inorganic salt was filtered off, and the filtrate was evaporated *in vacuo* to give a crude product. After crystallization from methanol/water, 0.84 g of 4-chloro-9,10-dihydro-9,10-dioxo-1-anthracenecarboxylic was obtained with the yield 75%. HPLC purity 99.91%.

4-Methyl-9,10-dihydro-9,10-dioxo-1-anthracenecarboxylic acid (2b). Sulfuric acid (10 mL, 2.5% (v/v)), sodium metaperiodate (2.72 g, 12.7 mmol), lithium bromide (1.10 g; 12.7 mmol), and tetrabutylammonium bromide (0.1 g, 0.3 mmol) were added to the solution of 1,4-dimethylanthraquinone [28] (1.00 g, 4.2 mmol) in chlorobenzene (10 mL). The reaction was stirred and heated for 144 h at 80–85°C. After cooling to the ambient temperature, the crude product was filtered,

washed with the saturated sodium thiosulfate solution, then with deionized water. The yellow crystals were air dried (96.07% of the HPLC purity) and purified by crystallization from methanol/water. Yield 0.69 g (61%). HPLC purity 99.25%. M.p. = 231–232 (230–231°C) [42].

^{1}H NMR (DMSO-d_6; 600 MHz) δ 13.52 (m, 1H, COO*H*), 8.13 (dd, J = 7.5 Hz, J = 1.5 Hz, 1H, Ar–*H*), 8.09 (dd, J = 7.5 Hz, J = 1.5 Hz, 1H, Ar–*H*), 7.90 (ddd, J = 7.5 Hz, J = 1.5 Hz, 1H, Ar–*H*), 7.88 (ddd, J = 7.5 Hz, J = 1.5 Hz, 1H, Ar–*H*), 7.74 (d, J = 8,0 Hz, 1H Ar–*H*), 7.66 (dd, J = 8.0 Hz, 1H, Ar–*H*), 2.77 (s, 3H, C*H*$_3$); ^{13}C NMR (DMSO-d_6; 600 MHz) δ 184.07, 182.47, 170.40, 142.05, 137.89, 134.98, 134.64, 134.12, 133.89, 132.21, 131.43, 131.17, 131.00, 126.78, 126.19, 22.96.

6-Chloro-2,7-dihydro-3H-dibenzo[*de,h*]cynnoline-3,7-dione (3). To the suspension of 4-chloroanthraquinone-1-carboxylic acid (**5**) (6 g, 21 m*M*) in 150 mL of toluene, $SOCl_2$ (3.06 mL, 42 m*M*) was added, and the mixture was stirred at reflux for 1 h. Next, the solution was cooled to 40–50°C, and the hydrazine hydrate (3.06 mL, 63 m*M*, 80%) was added. The reaction mixture was stirred at reflux for 1.5 h. Then AcOEt (75 mL) was added to the cooled mixture, and the suspension was stirred for 30 min. The precipitate was filtered and washed with AcOEt, water, and MeOH. The crude product was refluxed with MeOH (70 mL) for 30 min, next the product was filtered, washed with MeOH, and dried (4.39 g, HPLC purity 94.61%, 74% yield). HR-MS (ESI) Calcd for $C_{15}H_7N_2O_2$ $[M+Na]^+$: 305.0079. Found: 305.0094; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 13.52 (s, 1H), 8.51 (d, 1H, J = 8.4 Hz), 8.40 (d, 1H, J = 7.7 Hz), 8.28 (m, 1H), 8.10 (d, 1H, J = 8.5 Hz), 7.90–7.81 (m, 1H), 7.80–7.65 (m, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 180.17, 158.74, 140.44, 135.73, 134.27, 134.07, 132.54, 132.11, 130.76, 130.14, 128.63, 127.11, 125.76, 124.08, 122.70.

2-[2-(Dimethyloamino)ethyl]-6-chloro-2,7-dihydro-3H-dibenzo[*de,h*]cynnoline-3,7-dione (6a). To the suspension of **3** (0.5 g, 1.77 m*M*) in chloroform (30 mL), K_2CO_3 (1.5 g, 10.85 m*M*) and 2-chloro-*N*,*N*-dimethylethylamine hydrochloride (1.4 g, 8.85 m*M*) were added, then the mixture was stirred at 40°C for 24 h (HPLC monitoring). The inorganic solid was filtered and washed with chloroform (40 mL). The organic layer was evaporated to dryness, and product **6a** was obtained as an orange solid (0.56 g, HPLC purity 90.5%, 86% yield). HR-MS (ESI) Calcd for $C_{19}H_{16}ClN_3O_2$ $[M+H]^+$: 354.1009. Found: 354.1000; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.56 (d, 1H, J = 8.4 Hz), 8.50 (dd, 1H, J = 1.1 Hz, J = 8.0 Hz), 8.39 (dd, 1H, J = 1.1 Hz, J = 7.7 Hz), 7.95 (d, 1H, J = 8.4 Hz), 7.80 (ddd, 1H, J = 1.5 Hz, J = 7.3 Hz), 7.65 (ddd, 1H, J = 1.4 Hz, J = 7.6 Hz), 4.51 (t, 2H, J = 6.6 Hz, J = 13.2 Hz), 2.95 (t, 2H, J = 6.5 Hz, J = 13.3 Hz), 2.36 (s, 6H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 180.65, 158.37, 142.22, 135.78, 134.41, 133.73, 132.56, 132.46, 131.39, 130.16, 128.34, 127.80, 125.56, 124.36, 123.11, 57.38, 49.51, 45.56.

2-[2-(Dimethyloamino)propyl]-6-chloro-2,7-dihydro-3*H*-dibenzo[*de,*h]cynnoline-3,7-dione (6b). To the suspension of **3** (0.5 g, 1.77 m*M*) in chloroform (30 mL), K_2CO_3 (1.5 g, 10.85 m*M*) and 3-dimethylamino-1-propylchloride hydrochloride (1.27 g, 8.85 m*M*) were added, then the mixture was stirred at 40°C for 48 h (HPLC monitoring). The inorganic solid was filtered and washed with chloroform (40 mL). The organic residue was evaporated to dryness, and product **6b** was obtained as an orange solid (0.423 g, HPLC purity 88.02%, 68% yield). HR-MS (ESI) Calcd for $C_{20}H_{18}ClN_3O_2$ $[M+H]^+$: 368.1166. Found: 368.1159; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.63 (d, 1H, J = 8.7 Hz), 8.49 (dd, 1H, J = 1.1 Hz, J = 8.0 Hz), 8.40 (dd, 1H, J = 1.1 Hz, J = 7.7 Hz), 7.97 (d, 1H, J = 8.7 Hz), 7.75 (ddd, 1H, J = 1.4 Hz, J = 7.3 Hz), 7.65 (ddd, 1H, J = 1.4 Hz, J = 7.3 Hz), 4.44 (t, 2H, J = 7.3 Hz, J = 14.4 Hz), 2.52 (t, 2H, J = 6.9 Hz, J = 14.6 Hz), 2.27 (s, 6H), 2.22–2.10 (m, 2H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 180.66, 158.26, 142.22, 135.82, 134.35, 133.75, 132.44, 132.40, 131.36, 130.17, 128.27, 127.79, 125.53, 123.13, 56.74, 50.18, 45.40, 26.73.

2-(2-Morfolinethyl)-6-chloro-2,7-dihydro-3*H*-dibenzo[*de, h*]cynnoline-3,7-dione (6c). To the suspension of **3** (0.1 g, 0.35 m*M*) in chloroform (10 mL) and MeOH (1 mL), K_2CO_3 (0.29 g, 2.12 m*M*) and 4-(2-chloroethyl)morpholine hydrochloride (0.33 g, 1.77 m*M*) were added, then the mixture was stirred at 50°C for 48 h (HPLC monitoring). The inorganic solid was filtered and washed with chloroform (20 mL). The organic residue was evaporated to dryness. The product was purified by column chromatography (eluent: chloroform) to afford compound **6c** as an orange solid (0.15 g, HPLC purity 89.2%, 100% yield). HR-MS (ESI) Calcd for $C_{21}H_{18}ClN_3O_3$ $[M+H]^+$: 396.1115. Found: 396.1102; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.56 (d, 1H, J = 8.54 Hz), 8.39 (d, 1H, J = 7.95 Hz), 8.33 (dd, 1H, J = 0.6 Hz, J = 7.95 Hz), 7.92 (d, 1H, J = 8.55 Hz), 7.75–7.71 (m, 1H), 7.64–7.60 (m, 1H), 4.53 (t, 2H, J = 6.5 Hz, J = 13.3 Hz), 3.68–3.67 (m, 4H), 2.96 (t, 2H, J = 6.5 Hz, J = 13.3 Hz), 2.63 (brs, 4H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 180.56, 158.31, 142.27, 135.86, 134.38, 133.74, 132.44, 132.41, 131.37, 130.22, 128.28, 127.83, 125.47, 124.37, 122.97, 66.79, 56.52, 53.72, 53.55, 48.35, 29.67.

2-[2-(Diethylamino)ethyl]-6-chloro-2,7-dihydro-3*H*-dibenzo[*de,h*]cynnoline-3,7-dione (6d). To the suspension of **3** (0.1 g, 0.35 m*M*) in chloroform (5 mL) and MeOH (1 mL), K_2CO_3 (0.29 g, 2.12 m*M*) and 2-chloro-*N*,*N*-diethylethylamine hydrochloride (0.30 g, 1.77 m*M*) were added, then the mixture was stirred at 50°C for 48 h (HPLC monitoring). The inorganic solid was filtered and washed with chloroform (20 mL). The organic residue was evaporated to dryness. The product was purified by column chromatography in the solvent system, successively: chloroform, chloroform/methanol 80:1, to afford compound **6d** as an orange solid (0.110 g, HPLC purity 95%, 83% yield). HR-MS (ESI) Calcd for

$C_{21}H_{20}ClN_3O_2$ $[M+H]^+$: 382.1322. Found: 382.1310; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.58 (d, 1H, J = 8.54 Hz), 8.40 (dd, 1H, J = 0.79 Hz, J = 8.14 Hz), 8.33 (dd, 1H, J = 0.8 Hz, J = 7.95 Hz), 7.92 (d, 1H, J = 8.54 Hz), 7.75–7.72 (m, 1H), 7.64–7.61 (m, 1H), 4.51 (t, 2H, J = 6.9 Hz, J = 14.1 Hz), 3.03 (t, 2H, J = 6.9 Hz, J = 14.1 Hz), 2.70–2.66 (m, 4H), 1.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 180.67, 158.31, 142.22, 135.81, 134.32, 133.76, 132.52, 132.43, 131.38, 130.16, 128.28, 127.81, 125.51, 124.36, 123.08, 50.61, 49.54, 47.33, 11.94.

2-[2-(Piperidinamino)ethyl]-6-chloro-2,7-dihydro-3*H*-dibenzo[*de,h*]cynnoline-3,7-dione (6e). To the suspension of **3** (0.1 g, 0.35 m*M*) in chloroform (5 mL) and MeOH (1 mL), K_2CO_3 (0.29 g, 2.12 m*M*) and 1-(2-chloroethyl)piperidine hydrochloride (0.33 g, 1.77 m*M*) were added, then the mixture was stirred at 40°C for 48 h (HPLC monitoring). The inorganic solid was filtered and washed with chloroform (20 mL). The organic residue was evaporated to dryness. The product was purified by column chromatography (eluent:chloroform) to afford compound **6e** as an orange solid (0.170 g, HPLC purity 91.1%, 100% yield). HR-MS (ESI) Calcd for $C_{22}H_{20}ClN_3O_2$ $[M+H]^+$: 394.1322. Found: 394.1310; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.57 (d, 1H, J = 8.54 Hz), 8.45–8.42 (m, 1H), 8.35–8.32 (m, 1H), 7.92 (d, 1H, J = 8.54 Hz), 7.75–7.71 (m, 1H), 7.63–7.60 (m, 1H), 4.52 (t, 2H, J = 6.9 Hz, J = 14.1 Hz), 2.93 (t, 2H, J = 6.9 Hz, J = 14.0 Hz), 2.58 (brs, 4H), 1.60–1.58 (m, 4H), 1.45–1.43 (m, 2H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 180.64, 158.26, 142.21, 135.78, 134.32, 133.75, 132.53, 132.42, 131.36, 130.16, 128.29, 127.78, 125.52, 124.35, 123.11, 56.74, 54.72, 54.55, 48.96, 25.78, 24.16.

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REFERENCES AND NOTES

[1] Wolkenstein, K.; Schoefberger, W.; Müller, N.; Oji, T. J Nat Prod 2009, 72, 2036.

[2] Huang, H.; Wang, F.; Luo, M.; Chen, Y.; Song, Y.; Zhang, W.; Zhang, S.; Ju, J. J Nat Prod 2012, 75, 1346.

[3] Muangsin, N.; Wisetsakdakorn, W.; Chaichit, N.; Sihanonth, P.; Petsom, A.; Sangvanich, P. Dyes Pigm 2008, 77, 653.

[4] Shamim, G.; Ranjan, S. K.; Pandey, D. M.; Ramani, R. Eur J Entomol 2014, 111, 149.

[5] Anthraquinone The Complete Book on Natural Dyes & PigmentsNIIR Board of Consultants & Engineers Ed.; Asia Pacific Business Press Inc.: Delhi, India, 2005.

[6] Dave, H.; Ledvani, L. Indian J Nat Prod Resour 2012, 3, 291.

[7] Bhasin, D. k.; Etter, J. P.; Chettiar, S. N.; Mok, M.; Li, P. K. Bioorg Med Chem Lett 2013, 23, 6864.

[8] Bu, X.; Chen, J.; Deady, L. W.; Smith, C. L.; Baguley, B. C.; Greenhalgh, D.; Yang, S.; Denny, W. A. Bioorg Med Chem 2005, 13, 3657.

[9] Bu, X.; Deady, L. W.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. J Med Chem 2001, 44, 2004.

[10] Garcia-Vilas, J. A.; Quesada, A. R.; Medina, M. A. Sci Rep 2015, 5, 8021.

[11] Sakulpanich, A.; Gritsanapan, W. Southeast Asian J Trop Med Public Health 2009, 40, 835.

[12] Singh, D. N.; Verma, N.; Raghuwanshi, S.; Shukla, P. K.; Kulshreshtha, D. K. Bioorg Med Chem 2006, 16, 4512.

[13] Batista, R.; de Jesus Silva, A. Jr.; de Oliveira, A. B. Molecules 2009, 14, 3037.

[14] Huang, Q.; Lu, G.; Shen, H. M.; Chung, M. C.; Ong, C. N. Med Res Rev 2007, 27, 609.

[15] Wiseman, L. R.; Spencer, C. M. Drugs Aging 1997, 10, 473.

[16] Dunn, C. J.; Goa, K. L. Drugs Aging 1996, 9, 122.

[17] Faulds, D.; Balfour, J. A.; Chrisp, P.; Langtry, H. D. Drugs 1991, 41, 400.

[18] Stefanska, B.; Arciemiuk, M.; Bontemps-Gracz, M. M.; Dzieduszycka, M.; Kupiec, A.; Martelli, S.; Borowski, E. Bioorg Med Chem 2003, 11, 561.

[19] BS-154 SP. Z O.O. Gdansk, Poland, US 9,096,536 B2, 2015.

[20] Heller, G.; Schulke, K. Ber dd chem Ges 1908, 41, 3627.

[21] Castle, R. N.; Kudo, H.; Lee, M. L. Collect Czech Chem Commun 1991, 56, 2269.

[22] Alfred Eckert D. R., German Patent 383030, 1921; Great Britain Patent 182487, 1921; Chem Abstr 1922, 16, 4214.

[23] B. Anilin & S. Fabrik, German Patent 259365, 1912; Chem Abstr 1914, 8, 1190.

[24] Ullman, F.; Minajeff, W. Liebigs Ann 1912, 388, 217.

[25] Fischer, O.; Sapper, A.; et al. J Prakt Chem 1911, 83, 201.

[26] Elslager, E. F.; Weinstein, E. A.; Worth, D. F. J Med Chem 1964, 7, 493.

[27] Lee, D. G. Oxidation of Organic Compounds by Permanganate Ion and Hexavalent Chromium; Open Court, Pub. Co.: La Salle, IL, 1980.

[28] Fatiadi, A. J. Synthesis 1987, 106, 85.

[29] Abell, R. D. J. Chem Soc 1951, 1379.

[30] Bastock, T. W.; Clark, J. H.; Martin, K.; Trenbirth, B. W. Green Chem 2002, 4, 615.

[31] Shaikh, T. M.; Sudalai, A. Tetrahedron Lett 2005, 46, 5587.

[32] Shaikh, T. M. A.; Emmanuvel, L.; Sudalai, A. JOC 2006, 71, 5043.

[33] Naeimi, H.; Namdariyes, R. Dyes Pigm 2009, 81, 259.

[34] Du Pont. US, US 2,519,037, 1950.

[35] Brzezinski, J. Z.; Bzowski, H. B.; Epsztajn, J. Tetrahedron 1996, 52, 3261.

[36] Kaila, N.; Huang, A.; Moretto, A.; Follows, B.; Janz, K.; Lowe, M.; Thomason, J.; Mansour, T. S.; Hubeau, C.; Page, K.; Morgan, P.; Fish, S.; Xu, X.; Cara Williams, C.; Saiah, E. J Med Chem 2012, 55, 5088.

[37] Cesari, N.; Biancalani, C.; Vergelli, C.; Dal Piaz, V.; Graziano, A.; Biagini, P.; Ghelardini, C.; Galeotti, N.; Giovannoni, M. P. J Med Chem 2006, 49, 7826.

[38] Humne, V. T.; Konda, S. G.; Hasanzadeh, K.; Lokhande, P. D. Chin Chem Lett 2011, 22, 1435.

[39] Cristau, H. J.; Cellier, P. P.; Spindler, J. F.; Taillefer, M. Chem Eur J 2004, 10, 5607.

[40] Salvatore, R. N.; Yoon, C. H.; Jung, K. W. Tetrahedron 2011, 57, 7785.

[41] Russell Buchman, R.; Scozzie, J. A.; Ariyan, Z. S.; Heilman, R. D.; Rippin, D. J.; Pyne, W. J.; Powers, L. J.; Matthews, R. J. J Med Chem 1980, 23, 1398.

[42] Scholl, R.; Keller, A.; Meyer, K.; Penz, H. Justus Liebigs Ann Chem 1934, 512, 30.