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Synthesis and cytotoxicity of 7,8-dihalopyrido[1,2-*a*]benzimidazole-6,9-dione and its 1,2,3,4-tetrahydro analogue

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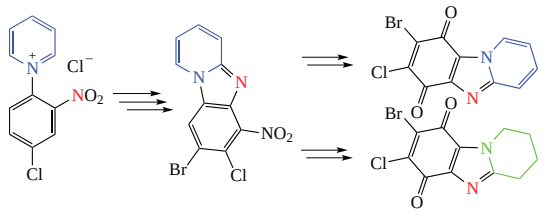
An efficient synthesis of 8-bromo-7-chloropyrido[1,2-*a*]-benzimidazole-6,9-dione and its 1,2,3,4-tetrahydro analogue from *N*-(4-chloro-2-nitrophenyl)pyridinium chloride has been accomplished. The study for antitumour activity against cancer cell lines such as A549, SH-SY5Y, Hep-2, HeLa and MCF-7 revealed that the cytotoxic effect of the compounds obtained was several times higher than that of Mitomycin C.



Keywords: pyrido[1,2-*a*]benzimidazole-6,9-diones, 2-bromo-3-chloro-1,4-benzoquinones, intramolecular reductive reactions, heterocyclization, flow-through catalytic hydrogenation, prooxidant activity, cytotoxicity.

Targeted chemotherapy is among the efficient methods for the drug treatment of malignant tumors. Its fundamental difference from other methods is that the toxicity to healthy body cells is reduced significantly. Imidazole-fused quinones (Figure 1) are known as promising antitumor drugs[1–7] possessing various mechanisms of their action. Some of them, such as the commercial anticancer drug Mitomycin C and its benzimidazole-containing analogues, are activated in the cell upon reduction of the quinone ring with DT-diaphorase whose level in the tumor cells is much higher than in normal cells. The resulting heterocyclic hydroquinone would undergo cross-linking with the DNA nitrogenous bases in transformed cells.[8] This leads to the suppression of their division, and consequently to a halt in tumor growth. Over time, the affected tissue zones die off and are replaced by healthy cells. The cytotoxicity of other antitumour agents is due to the formation of reactive oxygen species. 1,2,3,4-Tetrahydropyrido[1,2-*a*]benzimidazole quinone (PBI, see Figure 1) has a similar prooxidant effect.[7] This compound had more than a 300-fold greater cytotoxicity for human cancer cell lines under hypoxic conditions than the clinically used bioreductive drug Mitomycin C.

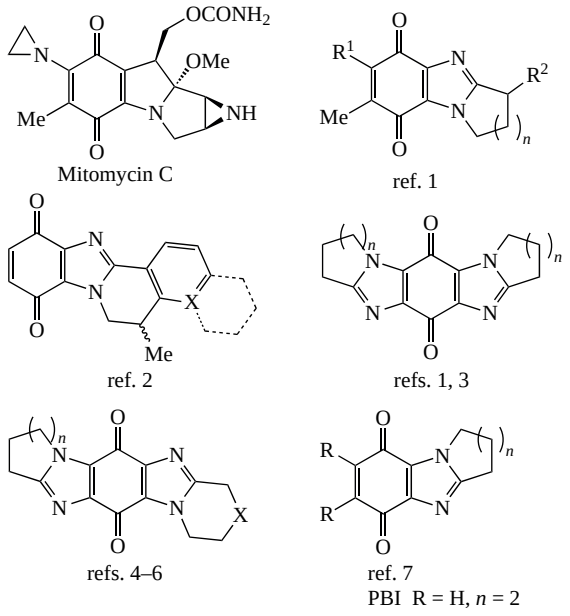
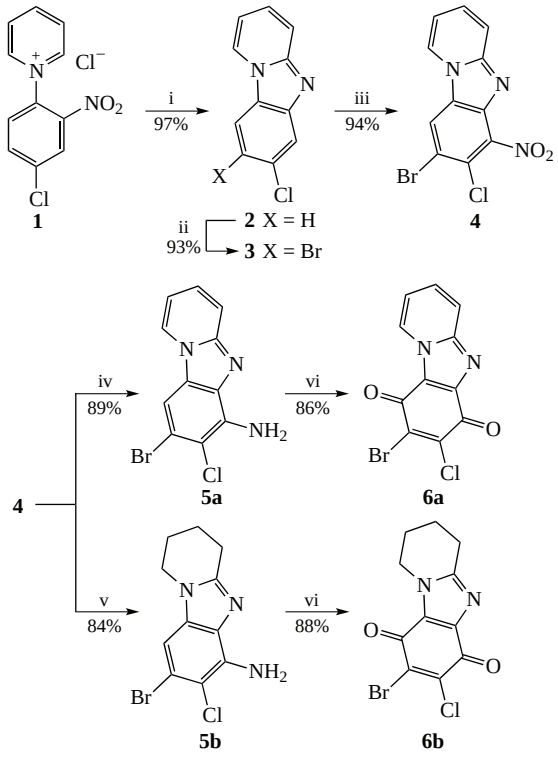


Figure 1 Structures of Mitomycin C and its benzimidazole analogues with antitumor activity.

The high demand for new antitumour drugs necessitates the search for new analogous heterocyclic compounds[9] that may exhibit high antitumor activity. Polyhalo derivatives of heterocyclic quinones may be of particular interest since the presence of halogen atoms should favour a significant increase in the antitumor activity.[10] In this study, we developed an efficient protocol for the synthesis of novel dihalogenated benzimidazole-fused *p*-benzoquionone and its tetrahydro analogue (Scheme 1). Our protocol differs from the known one[11] as it allows benzimidazole-fused *p*-quinones containing either same or different halogen atoms to be synthesized.

It seemed reasonable for the synthesis of both products **6a** and **6b** to employ the same starting compound, namely, *N*-(4-chloro-2-nitrophenyl)pyridinium chloride **1**. Its reductive cyclization gave 7-chloropyrido[1,2-*a*]benzimidazole **2** whose consecutive regioselective bromination with NBS/H_2SO_4 system at 30 °C afforded 8-bromo-7-chloropyrido[1,2-*a*]benzimidazole **3**. The following incorporation of nitro group as the second electrophilic particle under uncoordinated orientation circumstances resulted cleanly in 8-bromo-7-chloro-6-nitro derivative **4**. In this transformation, despite the presence of two



Scheme 1 *Reagents and conditions*: i, $SnCl_2 \cdot 2H_2O$, 4% HCl, 90% Pr^iOH, 40 °C, 5 min; ii, NBS, conc. H_2SO_4, 30 °C, 8 h; iii, KNO_3, conc. H_2SO_4, 35 °C, 2 h; iv, 1% Pd/C, Pr^iOH, 60 °C, 20 bar; v, 10% Pd/C, Pr^iOH, 80 °C, 20 bar; vi, KNO_3, conc. H_2SO_4, 20 °C, 9 h.

deactivating substituents in the substrate, the S_EAr process did not require harsh conditions (see Scheme 1). Nitroarene **4** was hydrogenated into the corresponding amine **5a** in a flow reactor equipped with 1% Pd/C cartridge (60 °C, 20 bar). Upon switching to 10% Pd/C cartridge and slight raising the temperature to 80 °C, the reduction of the nitro group was accompanied by hydrogenation of the pyridine ring to produce piperidino-fused benzimidazole amine **5b**. It is of note that arene halogen atoms remained intact under these hydrogenation conditions. The oxidation of amino arenes **5a,b** to the final heterocyclic quinones **6a**,**b** was performed with the KNO_3/H_2SO_4 system that was efficiently used earlier[12] to oxidize a similar heterocyclic compound.

The cytotoxic activity of novel compounds **6a**,**b** (Table 1) was studied by estimating their effect on the cells of different tumour lines, including neuroblastoma (SH-SY5Y), cervical cancer (HeLa), lung adenocarcinoma (A549), epidermal laryngeal cancer (Hep-2) and breast cancer (MCF-7). The cell viability was estimated by the MTT test (for details, see Online Supplementary Materials). The IC_{50} values represent the concentration of the compound required to reduce the average cell viability to 50% of the control value after incubation at 37 °C for 24 h. The commercially available anticancer drug Tamoxifen was used as the positive control to validate the technique.

As one can see from Table 1, compounds **6a**,**b** had a high cytotoxic effect on all the cell lines studied, the IC_{50} range being from 3.76 to 10.60 μM. Their cytotoxic effect was comparable to or several times higher than that of the commercial antitumor drug Mitomycin C.[13–18] Quinone **6b** exhibited a higher cytotoxicity against A549, Hep-2, and especially MCF-7, whereas the other quinone **6a** was slightly more active against HeLa. The cytotoxic effects of quinones **6a**,**b** on SH-SY5Y were comparable.

It is interesting that upon incubation of a healthy adult human skin keratinocyte cell line (HaCaT) with the compounds studied, the IC_{50} value of the toxic effect of **6a** was 15.09 ± 0.25 μM, which is almost 4 times higher than the IC_{50} value on the HeLa line [selectivity index (SI) = 3.61] and 2 times higher than that on the SH-SY5Y line (SI = 2.12). In turn, for heterocyclic quinone **6b**, the IC_{50} on HaCaT was 16.82 ± 0.14 μM (SI = 4.47 for MCF-7; SI = 2.91 for HeLa and SI = 2.84 for Hep-2).

In addition to the studies for determining the cytotoxic profile of the compounds, the possible mechanisms of cytotoxic action were estimated, including the effects on lipid peroxidation and the mitochondrial membrane potential that is a function of mitochondria.

Table 1 Cytotoxicity of compounds **6a**,**b**, IC_{50} (μM).

Compound	SH-SY5Y	HeLa	A549	Hep-2	MCF-7	HaCaT
6a	7.12 ± 0.35	4.18 ± 0.26	10.60 ± 0.08	8.95 ± 0.33	8.17 ± 0.03	15.09 ± 0.25
6b	6.41 ± 0.05	5.79 ± 0.14	6.77 ± 0.02	5.93 ± 0.01	3.76 ± 0.04	16.82 ± 0.14
Tamoxifen	34.01 ± 2.40	22.31 ± 1.09	17.73 ± 0.18	41.20 ± 3.73	28.94 ± 0.53	35.20 ± 3.73
Mitomycin C[a]	40	29.8	>10	~1.5	61.26	>100

[a] Literature data (refs. 13–18).

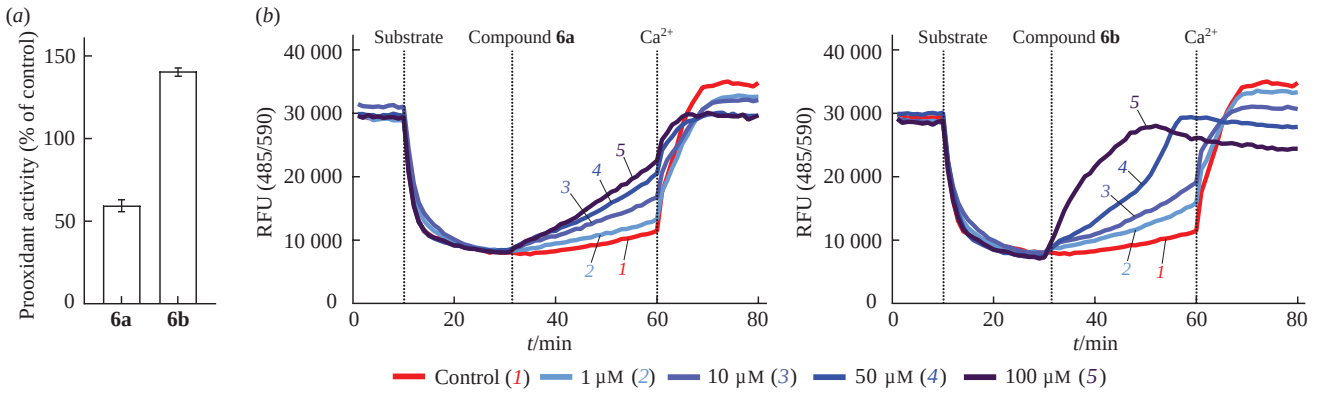


Figure 2 (*a*) Effect of compounds **6a**,**b** (30 μM) on the lipid peroxidation of rat brain homogenate (2 mg ml^{-1}) initiated by 0.5 mM Fe^{II}. Data are presented as the percentage of the prooxidant activity relative to the control (1% DMSO): mean ± SD. (*b*) Effect on the membrane potential of isolated rat liver mitochondria (0.5 mg ml^{-1}). Mitochondria were energized with potassium succinate (5 mM) in the presence of rotenone (1 μM). The concentration of Ca^{2+} was 35 μM.

Both compounds **6a**,**b** were found to be capable of provoking lipid peroxidation in rat brain homogenate, which is characterized by a large accumulation of malonodialdehyde, the final product of membrane lipid destruction, in the system (TBA test) and concentration-dependent depolarization of an isolated mitochondrial membrane (Figure 2) (Safranin A fluorescence measurements, see Online Supplementary Materials).

As a result, it may be assumed that in addition to the intercalating mechanism of antitumor activity of various known[19] quinones, the cytotoxic activity of the 7,8-dihalopyrido[1,2-*a*]-benzimidazole-6,9-diones and their tetrahydro analogues may also be due to the ability of these compounds to cause oxidative damage in the tumor cells and provide a destabilizing effect on the mitochondria. As a result, a cascade of cell deaths through apoptosis is triggered due to the discharge of pro-apoptotic factors from these organelles into the intracellular space.

In summary, the search for new efficient antitumor agents for the chemotherapy of malignant neoplasms among compounds of this class is a relevant and promising approach. The herein developed synthetic protocol may allow for obtaining a wide range of other fused benzimidazole-6,9-dione dihalo derivatives by varying the halogen-containing substrate and the halogenating reagent.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.02.028.

References

1 A. Suleman and E. B. Skibo, *J. Med. Chem.*, 2002, **45**, 1211.
2 E. Moriarty, M. Carr, S. Bonham, M. P. Carty and F. Aldabbagh, *Eur. J. Med. Chem.*, 2010, **45**, 3762.
3 W. G. Schulz and E. B. Skibo, *J. Med. Chem.*, 2000, **43**, 629.
4 V. Fagan, S. Bonham, M. P. Carty and F. Aldabbagh, *Org. Biomol. Chem*., 2010, **8**, 3149.
5 V. Fagan, S. Bonham, M. P. Carty, P. Saenz-Méndez, L. A. Eriksson and F. Aldabbagh, *Bioorg. Med. Chem*., 2012, **20**, 3223.
6 V. Fagan, S. Bonham, P. McArdle, M. P. Carty and F. Aldabbagh, *Eur. J. Org. Chem.*, 2012, 1967.
7 M. Lynch, S. Hehir, P. Kavanagh, D. Leech, J. O'Shaughnessy, M. P. Carty and F. Aldabbagh, *Chem. – Eur. J.*, 2007, **13**, 3218.
8 M. A. Colucci, C. J. Moody and G. D. Couch, *Org. Biomol. Chem.*, 2008, **6**, 637.
9 M. Sweeney, D. Conboy, S. I. Mirallai and F. Aldabbagh, *Molecules*, 2021, **26**, 2684.
10 M. Andrzejewska, L. Yépez-Mulia, R. Cedillo-Rivera, A. Tapia, L. Vilpo, J. Vilpo and Z. Kazimierczuk, *Eur. J. Med. Chem*., 2002, **37**, 973.
11 M. Sweeney, L.-A. J. Keane, M. Gurry, P. McArdle and F. Aldabbagh, *Org. Lett.*, 2018, **20**, 6970.
12 R. S. Begunov, A. A. Sokolov, I. V. Fedyanin, V. O. Sakulina and M. A. Syroeshkin, *Mendeleev Commun.*, 2019, **29**, 184.
13 B. Laffon, N. Fernández-Bertólez, C. Costa, E. Pásaro and V. Valdiglesias, *Food Chem. Toxicol.*, 2017, **102**, 120.
14 M. H. Alkhatiba, W. A. Al-Otaibia and A. N. Wali, *Chem. Biol. Interact.*, 2018, **291**, 72.
15 J.-C. Ko, M.-S. Tsai, S.-H. Weng, Y.-H. Kuo, Y.-F. Chiu and Y.-W. Lin, *Toxicol. Appl. Pharmacol.*, 2011, **255**, 327.
16 M. Fujii, T. Ohnuma, L. Szrajer, J. F. Holland, H. Biller and M. Okabe, *Cancer Invest*., 1993, **11**, 283.
17 W. A. Al-Otaibi, M. H. Alkhatib and A. N. Wali, *Biomed. Pharmacother*., 2018, **106**, 946.
18 Y. Wang, J. Ren, K. Xia, S. Wang, T. Yin, D. Xie and L. Li, *J. Zhejiang Univ., Sci., B*, 2012, **13**, 997.
19 C. Asche, *Mini-Rev. Med. Chem*., 2005, **5**, 449.

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