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Anticancer activity of new benzofuroxan–imidazolone hybrids

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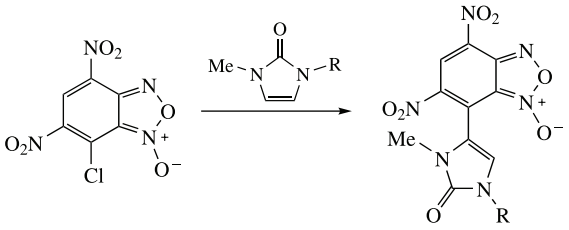
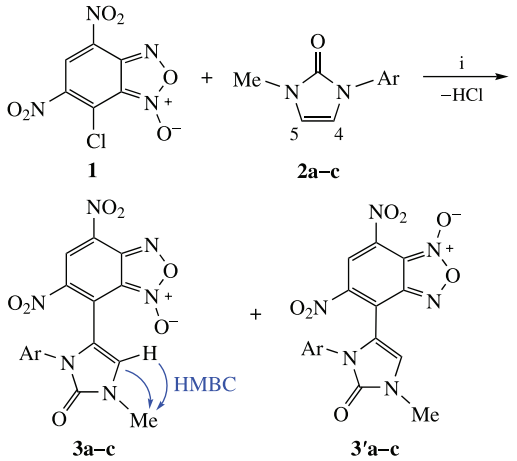
Novel hybrid compounds containing both benzofuroxan and 1*H*-imidazol-2(3*H*)-one moieties were synthesized by an S_NAr reaction between 7-chloro-4,6-dinitrobenzofuroxan and imidazol-2-ones, with the only C^4 regioisomer having been formed. Cytotoxic effects of parent and obtained compounds were estimated on human cancer and normal cells, while two of imidazolones showed higher cytotoxicity in relation to the M-HeLa cancer line compared to the hybrid products. In relation to the normal liver cell line Chang, the tested compounds were found to be non-toxic and can be promising for further development of anticancer agents.



Keywords: benzofuroxans, imidazol-2-ones, hybrid compounds, S_NAr reaction, regioselective synthesis, antitumor activity.

Imidazol-2-ones belong to important heterocyclic compounds exhibiting a wide spectrum of biological activity.[1,2] Various derivatives of imidazol-2-one are antioxidants *in vivo*[3] and are part of many biologically active compounds. The imidazol-2-one scaffold forms the base of the nucleotide antibiotic Nikkomycin X,[4] phosphodiesterase inhibitors with vasodilating and positive inotropic activity Piroximone and Enoximone.[5,6] Some compounds of this class showed memory-improving effect in animal models,[3] anti-inflammatory,[7] antifungal,[8] antibacterial,[9] cytotoxic activities[10,11] and other useful properties.[12–14] The imidazolone core allows one to get range of various receptor antagonists with nanomolar activities.[15]

High biological activity of these compounds prompted us to prepare new substituted imidazol-2-ones, namely, their hybrid derivatives containing benzofuroxan core, taking into account our previous experience in benzofuroxan chemistry.[16,17] One of the most promising areas is the synthesis of benzofuroxan hybrid compounds[18] comprising the introduction of biologically active fragments into the molecules, *e.g.*, ammonium salts,[19] amino acids,[20] or 2-(het)arylpyrrolidine.[17] Recent studies on benzofuroxan derivatives have also shown their significant potential as antitumor compounds.[17] The starting imidazol-2-ones **2a–c** were obtained *via* the reaction of 2,2-dimethoxy-*N*-methylethanamine with aryl isocyanates followed by treatment with trifluoroacetic acid.[21] The synthesis of the target hybrids (Scheme 1) was accomplished *via* the S_NAr reaction between 7-chloro-4,6-dinitrobenzofuroxan **1** and 1*H*-imidazol-2(3*H*)-ones **2a–c** in a 1 : 1 molar ratio in chloroform in the presence of sodium hydrogen carbonate (20% excess) required to neutralize the hydrogen chloride formed during the reaction.† The products were formed as a result of C–C coupling as it was shown earlier for a series of nucleophiles.[22–24] Notably, when we attempted to employ less electrophilic 4,6-dichloro-, 4,6-dichloro-5-nitrobenzofuroxans, no target compounds were formed.



a Ar = Ph, 55%
b Ar = 3-MeC_6H_4, 65%
c Ar = 4-ClC_6H_4, 28%

Scheme 1 *Reagents and conditions*: i, $NaHCO_3$, $CHCl_3$, room temperature, 24 h.

† *Reaction between 7-chloro-4,6-dinitrobenzofuroxan* **1** *and 1*H*-imidazol-2(3*H*)-ones* **2a–c**. To a solution of 7-chloro-4,6-dinitrobenzofuroxan **1** (0.1 g, 0.4 mmol) in $CHCl_3$ (5 ml), a solution of 1*H*-imidazol-2(3*H*)-one **2** (0.4 mmol) in $CHCl_3$ (5 ml) and $NaHCO_3$ (0.04 g, 0.48 mmol) were added at room temperature. The mixture was stirred at room temperature overnight (TLC control, eluent: toluene/ethyl acetate, 2 : 1). After removal of the solvent under reduced pressure, the crude products were purified by column chromatography on 230–400 mesh silica (eluent: toluene/ethyl acetate, 2 : 1) to give target compounds **3a–c**.

Table 1 Cytotoxic effects IC_{50} (μM) of test compounds on the cancer and normal human cell lines.[a]

Test compound	Cancer cell line M-HeLa	Normal cell line Chang liver
1	94.1 ± 8.6	>100
2a	72.6 ± 6.7	>100
3a	>100	>100
2b	60.0 ± 5.7	95.1 ± 8.2
3b	>100	>100

[a] The experiments were repeated three times. The results are expressed as the mean ± standard deviation.

The starting 1*H*-imidazol-2(3*H*)-ones have two reactive sites, namely, the C^4 and C^5 atoms. Thus, the reaction may result in two regioisomeric products. Earlier, the formation of both regioisomers with a predominance of C^5-isomer was reported for the reaction of 1*H*-imidazol-2(3*H*)-ones with methyl iodide.[25] In order to determine the exact substitution site for compounds of type **3**, a complete structure elucidation was carried out for the benzofuroxan **3a** by 2D NMR techniques (COSY, HSQC, HMBC). First, the assignment of the signals in ^1H, ^{13}C and ^{15}N NMR spectra was performed using homo- and heteronuclear correlation experiments. Then, the NOESY experiment was carried out. The presence of NOE between the *N*-methyl group and the proton of the imidazoline ring strongly supports the C^4-substitution (adjacent to the aryl group). In case of C^5-substitution, the occurrence of this NOE is highly unlikely. The presence of the cross-peak between protons of the *N*-methyl group and the $=C^5H$ carbon atom in the $^1H–^{13}C$ HSQC spectrum also suggests substitution at the C^4 carbon atom.

In all cases, the key compounds **3a–c** were formed as mixtures with tautomers **3′a–c** in approximately 1 : 7 ratio, which is in accordance with our previous observations.[26]

Compounds **1**, **2a**,**b**, **3a**,**b** were tested for cytotoxicity against human normal and cancer cell lines at concentrations of 1–100 μM (compound **3c** was not tested due to its instability in solution). Cytotoxic effects were estimated by means of the multifunctional Cytell Cell Imaging system (GE Health Care Life Science, Sweden) using the Cell Viability Bio App which precisely counts the number of cells and evaluates their viability from fluorescence intensity data.[27] The results for IC_{50} data are summarized in Table 1. The parent compounds **2a**,**b** exhibit moderate cytotoxicity to the M-HeLa cancer line, while the hybrid products, in contrast to our expectations, did not reveal antitumor activity. All tested compounds do not show cytotoxic properties against the normal liver cell line.

In conclusion, we have carried out the highly regioselective synthesis of hybrid compounds containing both benzofuroxan and 1*H*-imidazol-2(3*H*)-one moieties when only the C^4-substituted regioisomer was formed. Additionally, the cytotoxicity of compounds has been tested to evaluate their usefulness as novel anticancer agents. The parent compounds **2a**,**b** showed higher cytotoxicity towards the M-HeLa cancer line compared to the hybrid products.

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Online Supplementary Materials

Supplementary data associated with this article (detailed experimental procedures, compound characterization data and copies of NMR spectra) can be found in the online version at doi: 10.1016/j.mencom.2021.11.032.

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