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# SYNTHESIS AND CYTOTOXIC EVALUATION OF 1,2,3-TRISUBSTITUTED -2-(2-OXOALKYL)-1,2-DIHYDROBENZIMIDAZOLES

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**ABSTRACT**

A new series of 1,2,3-trisubstituted 2-(2-oxoalkyl)-1,2-Dihydrobezimidazoles have been synthesized and evaluated for their cytotoxic activity.

*Keywords: α-Amidoalkylation, Synthesis, Benzimidazole, Cytotoxicity*

## INTRODUCTION

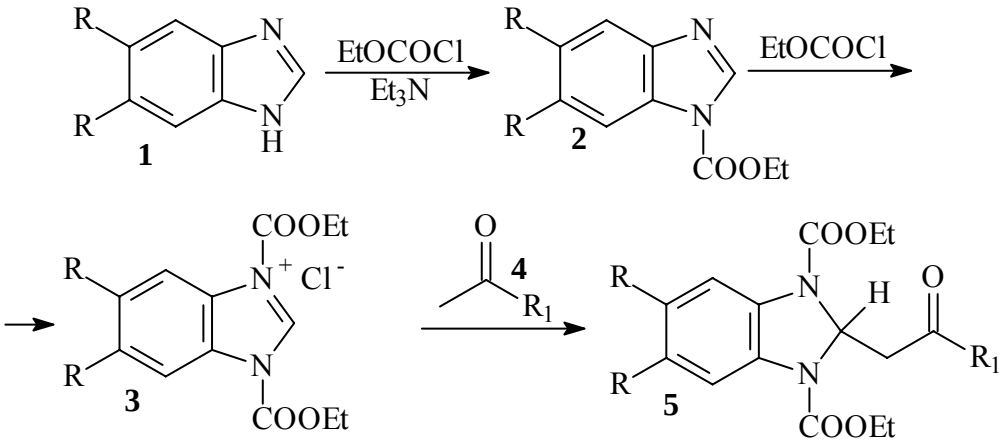
A great number of benzimidazole derivatives have been synthesized and extensively investigated for their biological activity. The continually increasing interest of this class of compounds related to their diverse biological activity.[1,2] The positions 1, 2 and 3 are the reactive centers of the benzimidazole molecule, which dictate the chemistry and activity of benzimidazole derivatives. It is well known that benzimidazoles exhibit antimicrobial[3], antitubercular, anticancer, antihelmintic, anticonvulsant and analgesic activities.

1,2-Dihydrobenzimidazoles with substituents at 1, 2 and 3 positions are not easily accessible with the synthetic routes available in the literature. Several years ago we reported a new approach to 1,2,3-trisubstituted benzimidazoles, using adducts **3** of benzimidazoles **1** and acyl chlorides as α-amidoalkylation reagents toward some ketones.[4] It was found that adducts of benzimidazoles and ethylchloroformate reacted with ketones as acetone, acetophenone or benzalacetone, affording the corresponding 2-(2-oxoalkyl)-1,3-diacyl-2,3-dihydrobenzimidazoles as a result of an intermolecular α-amidoalkylation reaction. The present work describes the synthesis and cytotoxic evaluation of 2-(2-oxoalkyl)-1,3-carboxyethyl-2,3-dihydro benzimidazoles.

**CHEMISTRY**

Herein we report an extension of the previous developed reaction, using successfully adducts **3** as electrophiles in an intermolecular amidoalkylation reaction toward substituted in the aromatic ring acetophenones (**Table 1**, **4e-g**). The reaction conditions were properly modified in comparison with the previously reported[4], allowing better yields of **5**.

The target 1,2,3-trisubstituted 1,2-dihydrobenzimidazoles **5** were prepared by the reaction outlined in **Scheme 1**. To equimolar amounts of benzimidazoles **1** and ethylchloroformate an equimolar amount $Et_3N$ was added to afford the corresponding N-carboxyethylbenzimidazoles **2,** which were isolated and characterized. Treatment of **2** with ethylchloroformate led to adducts **3** which exist in reaction mixture in equilibrium of salt form and covalent structure.




**Scheme 1**

**Table 1**

| Entry | R | $R_1$ | 5 | |
|---|---|---|---|---|
| | | | Yield (%) | Mp ($^0$C) |
| **a** | H | Me | 92 | 55-56 |
| **b** | Me | Me | 75 | 131-132 |
| **c** | H | $C_6H_5$ | 90 | 89-90 |
| **d** | Me | $C_6H_5$ | 70 | 144-145 |
| **e** | H | 4-HO-$C_6H_4$ | 95 | 150-151 |
| **f** | Me | 4-HO-$C_6H_4$ | 98 | 124-124.5 |
| **g** | Me | 4-MeO-$C_6H_4$ | 62 | 161-162 |
| **h** | H | $C_6H_5CH_2$ | 70 | 81-81.5 |
| **i** | H | $C_6H_5CH$=CH | 95 | 93-93.5 |
| **j** | Me | $C_6H_5CH$=CH | 72 | 141-142 |

An amidoalkylation reaction takes place with the chosen ketones **4,** when added in the reaction mixture, leading to the corresponding 2-(2-oxo-2-alkyl/aryl)-1,3-dicarboxyethylbenzimidazoles **5** in good yields (**Table 1**, **5a-j**). The reaction could be

carried out as one-pot reaction, starting with one mole of **1**, two moles of ethylchloroformate and the corresponding ketone **4**.

## EXPERIMENTAL SECTION

### Cell cultures and cytotoxicity assay

Three cell lines: FL (normal human amniotic cells, ATCC CCL-62), RD (human embryonal rhabdomyosarcoma, ATCC CCL-136) and A2058 (human metastatic melanoma, ECACC 91100402) were used in the tests. The cells (density $1.10^5$cells/ml) were plated in cell culture flasks with a growth area of 25 $cm^2$ and cultivated in liquid Dulbecco's Minimal Essential Medium (Serva) supplemented with 10% (v/v) normal calf serum, 100 IU penicillin and 0.1mg/ml streptomycin in Heraeus incubator at 37$^o$C with 5% $CO_2$ in air and high humidity. Twenty four hours later test agent (benzimidazole derivative **5**) was added to give a final concentration of $10^{-4}$ M and the cultures were incubated for another 96 hours. End-point determinations of cell density were carried out every 24 hours by a standard haemocytometer chamber. Cell viability was measured using trypan blue exclusion test[5].

### Biological Results

All synthesized benzimidazole derivatives were evaluated for cytotoxic activity using three (1 normal and 2 tumor) cell lines /one dose ($10^{-4}$ M) assay. Most of the tested compounds exhibited strong non-selective cytotoxic effect (**5a, 5e**) with percentage of cell survival after 96 h treatment ranging between 0% and 10%. Comparison between **5c** and **5e** showed that at least in that case the toxicity is related to the presence of 4-hydroxyphenyl group in C-2 substituent. The structure-activity relation was observed for the pair **5d** -**5b**, but the effect was more pronounced and not tumor-specific as significant reduction of growth of the normal cells was detected also.

The most toxic among the tested compounds was **5a** - even after 24 h incubation no growth of FL, neither of RD or A2058 cells was observed. Replacement of methyl group (**5a**) with phenyl moiety (**5c**) diminished this effect to a great extend.

Comparison of the activities of **5a** with **5b** and **5c** with **5d** would give some insight into the influence of the 5,6-methyl groups on biological activity. However the effect was not consistent, since **5b** was less toxic than **5a**, **5d** had higher activity than **5c**. Overall, these results show that the effect of presence/absence of the two methyl substituents at C-5 and C-6 is variable and depends on the nature of the substituent at C-2.

The most promising for further testing as potential antitumour agent is **5i**. The toxicity of **5i** is quite low for the normal cells, but more than 2 and 10 times higher for RD and A2058 cells, respectively. Attempts to increase this tumour-specific antiproliferative activity via combination of shortening the length of the C-2 substituent chain by one carbon atom, addition of two methyl groups at C-5 and C-6 (**5j**) did not give satisfactory results.

**Supporting Information Available:** Chemical characterization data is available free of charge via the Department of Organic Chemistry.

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