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Properties of a new fluorescent coumarin derivatization reagent employing molecular modelling techniques

Evagelos Gikas[a,b,*], Maria Parissi-Poulou[a], Michael Kazanis[a], Andreas Vavagiannis[a]

[a]Division of Pharmaceutical Chemistry, Department of Pharmacy, University of Athens, Panepistimiopolis, 157 71 Athens, Greece
[b]GAIA Research Center, The Goulandris Natural History Museum, 13 Levidou Str., Kifissia 14562 Greece

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Abstract

Coumarins are widely employed in chemical analysis due to their fluorescent properties. The conformation and the electronic properties of a newly synthesized derivatization reagent namely BrMOZPhC (4-bromomethyl-coumarin-7-phenyl[6,7-b](9,6)oxazine) was studied. The molecule was found to possess completely planar structure, which enhances the fluorescent quantum yield. Furthermore it exhibits a high degree of aromaticity, as can be depicted by the localization of the HOMO and LUMO orbitals as well as by the charge distribution between the ground and the excited state. The bond connecting the heterocyclic system of the molecule with the phenyl ring is also of significant importance because despite the fact that imposes the only degree of rotational freedom to the molecule, has been found to possess partial double bond character. The properties of the molecule were studied employing both semi-empirical and ab initio calculations whereas its fine electronic structure was explored using Bader's AIM theory.

Keywords: Coumarin; Fluorescence; Semi-empirical; Ab-initio

1. Introduction

The coumarin group consists of molecules of great versatility, containing both natural products (psoralen [1], angelicin etc.) and substances used in the pharmaceutical industry as antibiotics (novobiocin [2]), antiviral agents [3], anticoagulants (warfarin), sensitizers in phototherapy [4] as well as in the chemical industry as optical brighteners [5] and laser dyes [6]. Their photochemical properties dominate their utility. Electron donor or acceptor groups or their combinations at positions 4 and 7 of the coumarin nucleus result in bathochromic shifts whereas extension of the aromatic or conjugated system of the molecule causes pronounced enhancement of fluorescence intensity.

Numerous studies have explored the possibility of substituting the ether oxygen of the lactone with other heteroatoms (N at carbostyril derivatives), or a carbon atom (e.g. incorporating N to the 8th position giving benzopyridine derivatives or at the 3rd position affording benzoxazinone derivatives [7,8]) in order to enhance the fluorescent quantum yield.

Considerable effort has been made the past years towards the development of new and ultra sensitive derivatization reagents exhibiting intense fluorescence. These substances act as staining compounds or molecular probes in the biology field as well as HPLC fluorescent derivatization reagents. Among them are BrMOZPhC (4-bromomethyl-coumarin-7-phenyl[6,7-b](9,6)oxazine) [9] and BrMOZC [10] (Figs. 1B and 2, respectively) have been reported as probes for the determination of carboxylic acids by HPLC and fluorescence detection.

The aforementioned molecules were designed on the basis of extending the π bond system of and also delocalizing the unshared pairs of electrons of the oxygen atoms forming the oxazine and pyran nuclei. A stepwise procedure was used for the design of the two target molecules using the 7-hydroxycoumarin nucleus as lead compound. Many fluoregenic reagents are based on the last, among them being 7-methoxy-4-bromomethylcoumarin (BrMMC) [11–16] and its acetoxy analogue 7-acetoxy-4-bromomethylcoumarin

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* Corresponding author. Address: GAIA Research Center, The Goulandris Natural History Museum, 13 Levidou Str., Kifissia 14562 Greece. Tel.: +30 2108015870; fax: +30 2108080674.

E-mail addresses: vgigas@otenet.gr, bioan@gnhm.gr (E. Gikas).

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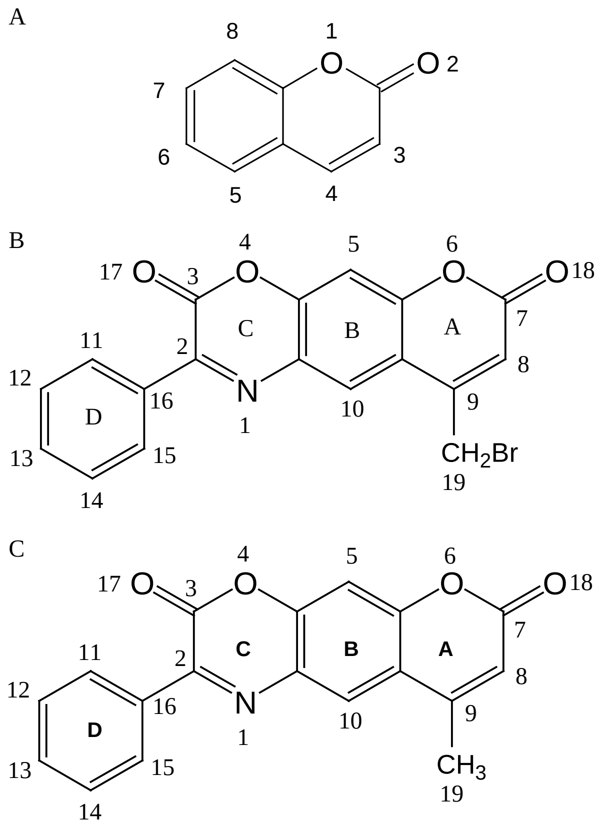
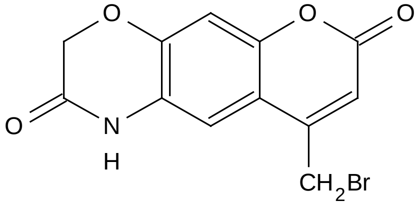


Fig. 1. Structures of the two reported coumarin probes BrMOZC (I) and BrMOPhC (II).

Br-MAC [17,18]. Based on the 7-hydroxycoumarin structure a second electron donor was added at the 6th position with the prospect of enhancing the dipole moment of the molecule affording the 4-bromomethyl-6,7-dimethoxycoumarin (Br-MDMC) [19]. The excitation energy lost through rotational relaxation imposed by the two methoxy groups of Br-MDMC, can be compensated by their fusion in one heterocyclic ring leading to a new reagent with enhanced fluorescence the 4-bromomethyl-6,7-methylendioxycoumarin (Br-MDC) [20].

A series of 7-aminocoumarin derivatives have also appeared in the literature. A common feature of these molecules is the alkylation of the amine moiety, which enhances its electron donor properties. Among the 7-aminocoumarin derivatives exceptional place occupy the luminarins, which are derivatives of piperidinobenzopyran-2-one as well as a series of other piperidinocoumarins [21]. The rotational freedom of the alkyl substituents in this class of molecules is restricted, in the same manner as in as the case of their oxygen bearing counterparts, by the fusion of the exocyclic nitrogen to a heterocyclic ring. Molecules of this type exhibit also enhanced fluorescence intensity compared to their alkylamino counterparts. This is mainly due to the aforementioned restriction of the rotational relaxation.



BrMOZC *(I)*

Fig. 2. The coumarin nucleus (A), the BrMOZPhC molecule (B), along with its methyl counterpart (C). The latter has been employed for the computational chemistry experiments. The numbering of (B) and (C) are arbitrary assigned by the Hyperchem software and do not correspond to the numbering of (A).

Extending also the aromatic system of the nucleus, using essentially a naphthalene instead of a benzene substructure fused to the pyran-2-one afforded molecules as the 4-bromomethyl-7,8-benzocoumarin (Br-MDC). Extension of the π double bond system has also been attempted to the pyayn-2-one ring as happens in the 3-[4-(bromomethyl)-phenyl]-7-dimethylaminocoumarin (Br-MPAC) [22]. Structurally analogous to the coumarins is the 3-bromomethyl-7-methoxy-1,4-benzoxazin-3-one (Br-MB) [23,24] (where the C4 of the benzopyran-2-one nucleus is substituted by N), which exhibits more intense fluorescence than the corresponding coumarins. This can be attributed to the fact that the nitrogen atom can accommodate charge in the excited state, increasing thus the charge separation between the ground and the excited singlet state.

The present work describes the geometry and the electronic properties of the newly synthesized BrMOZPhC probe. This molecule has been applied to the determination of aliphatic fatty acids using HPLC with fluorescence detection. The overall design of the molecule has been supported by the results of the computational chemistry experiments reported here.

2. Computational details

The ground state geometry of the molecule has been optimized in order to achieve a reasonable starting geometry by molecular mechanics using the MM+ force field as implemented in Hyperechem 5.02 [25]. Geometry optimization was performed with the Polak-Ribierre algorithm using 0.1 kcal/mol convergence limit. The initial geometry found, was used as input for further geometry optimization at the semi-empirical level of theory using the PM3 and the AM1 Hamiltonians. The Polak-Ribbiere algorithm was also used with a convergence limit of 0.1 kcal/mol. The optimization was repeated until an energy minimum has been reached. Experiments were carried out using the Hyperchem 5.02 and the ArgusLab v.4 [26]. For assessing the electronic properties of the molecule more accurately, single point calculations using singly excited configuration interaction experiments were used. A total of 20 orbitals (10 ground and 10 excited) were specified in order to evaluate the molecular properties