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Research Article

Binding Expedient of 2-carbamido-1,3-indandione to Nucleic Acids: Potential Fluorescent Probe

Nina Stoyanova[1], Nadezhda Markova[2]*, Ivan Angelov[2], Irena Philipova[2] and Venelin Enchev[1]

[1]Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

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ABSTRACT

Fluorescent and computational methods were used to elucidate the binding expedient of 2-carbamido-1,3-indandione (CAID) tautomers to nucleotides. The dependence of the fluorescence emission of CAID loaded nucleic acids sequences to compound concentration, temperature and time variation was investigated. It was found that the subject compound binds to nucleic acids but does not intercalate. According to our quantum-chemical calculations on the conjugation between CAID and nucleotides, the binding in the formed complexes may be through hydrogen bonds. Two possible types of complexes were considered—CAID to the phosphate group and CAID to the nucleobase. To estimate the binding affinity, the interaction energies of the formed complexes were calculated. Tautomer 2-carboamide-1-hydroxy-3-oxo-indane is preferred in the formation of complexes, and the phosphate group complexes were more stable. Generally, the guanosine and deoxyguanosine monophosphate complexes were the most preferred regardless of the complex type. Because of the lack of cytotoxic effect on untransformed cell lines of mouse embryo fibroblasts Balb/c 3T3 according to our previous report (Markova et al, (2017) Bulg Chem Commun, 49D, 221–226) and the affinity to nucleic acids, we can suggest that the subject compound could be suitable to be used as a novel type of fluorescent biomarker.

INTRODUCTION

Malignant and infectious diseases (including COVID-19) account for a large proportion of morbidity and mortality in the world. The early detection, intervention and expedient treatment is mandatory for a favorable prognosis. Modern imaging methods are powerful tool in medicine. As part of optical molecular imaging techniques, the fluorescence spectroscopy is promising technique for provision of more precise molecular and physiological information. The diagnosis of infectious and other diseases is most often performed by biomarker detection in laboratories using conventional tests (1). There are many shortcomings in the diagnosis of viral infections, including COVID-19, due to the low sensitivity and specificity of the diagnostic tests, as well as the long time to obtain the result. The search and development of new compounds suitable for biomarkers and therapeutics is an important challenge for the scientific community worldwide.

In the most cases, fluorescent labels are distributed randomly and nonhomogenously in the cells and tissues. Therefore, the intensity of the observed fluorescence signal depends not only on the local concentration, but also on the intensity of lighting, medium, optical path length, bleaching and other processes. That is why fluorescence probes with significant spectral shift after binding to the observed object are preferred. It should also be noted that a good fluorescent marker should form hydrogen bonds with biomolecules, and the formed complex is stabilized in solution. There are many biomarkers that bind spontaneously to DNA and exhibit higher emission (2). Some of the most used biomarkers Hoechst 33258, Hoechst 33342 and Hoechst 34580 have NH group and bind to DNA via bifurcated hydrogen bonds and/or electrostatic interactions (3). Unlike TAMRA and DAPI, which damage the cell membranes and the DNA structure, these markers are suitable for application on live-cell cultures because they do not damage them and have lower cytotoxicity.

2-Substituted-1,3-indandiones are biologically active compounds which are used in the medicine and biology (4). Studies on their biological activity indicate the presence of anticoagulant (5), anti-inflammatory (6), antibacterial (7), protein binding (4), antineoplastic (8,9) and neuroprotective action (10). 2-Carbamido-1,3-indanedione (CAID), also known as 1,3-dioxo-2-indane-carboxamide, belongs to the group of 2-substituted indan-1,3-dione. The compound was synthesized by Horton and Murdock (11) in 1960. In solid state, it exists as yellow-orange needle-like crystals with melting point in the range of 180–220°C. The initially proposed structure (11) was later corrected by Enchev et al. (12) It has been found that in solid state and in solution, CAID coexists in two tautomeric forms: 2-(hydroxylaminomethylidene)-indan-1,3-dione, (CAIDA), and 2-carboamide-1-hydroxy-3-oxo-indane, (CAIDB), shown in Fig. 1. In 2007, CAID derivatives were synthesized (13), and it was found that some of them crystallize in two tautomeric forms with different colors of the crystals.

The photophysical properties of CAID have recently been investigated as well as its possible use as a sunscreen and biomarker (14). It exhibits very good photostability and absorbs in the visible area (400–450 nm), UVA (320–400 nm), UVB (290–320 nm) and UVC (200–290 nm) spectrum. CAID is also suitable to be used as a biomarker for biologically relevant molecules because it absorbs and fluoresces in very wide spectral

*Corresponding author email: nadya@orgchm.bas.bg (Nadezhda Markova)

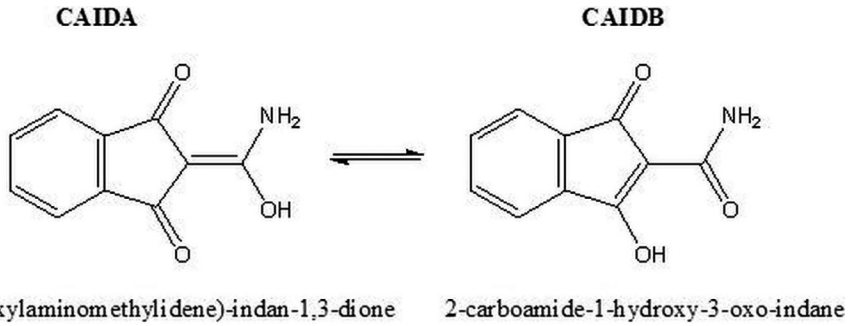

Figure 1. Two tautomeric forms of 2-carbamido-1,3-indanedione.

range and has high quantum yield. A hypsochromic shift by 25–30 nm of the fluorescence band of CAID-tagged DNA sequences was observed after addition of the compound to the DNA sequences. This suggests that CAID could bind to DNA via hydrogen bonds. Fluorescence and microscopic analysis of CAID in Balb/c3T3 cell cultures (15) shows that 2-carbamido-1,3-indanedione has ability to penetrate through cell membranes. The microscopic analyses indicate that the compound is not toxic for the cell lines used. 2-Carbamido-1,3-indanedione also interacts with serum albumin (4); thus, it could be a fluorescence marker not only for DNA and RNA but also for other biomolecules. For live-cell probing penetration and cytotoxicity, as well as side effects on cellular function are an important aspect that should be taken into account when developing novel fluorescent probes. Such a study related to the interaction of the title compound with nucleic acids and the resulting biological applications has not yet been performed. Therefore, it would be useful to examine the binding affinity of the two stable tautomers of 2-carbamido-1,3-indanedione to nucleotides. Such study would explain important aspects of the binding mode of the compound in relation to its biomarker properties.

MATERIALS AND METHODS

Synthesis of 1,3-dioxo-2-indane-carboxamide. 1,3-Dioxo-2-indane-carboxamide was synthesized via a two-step procedure (11). Thus, the condensation of diethyl phthalate with acetonitrile in the presence of sodium methoxide afforded 1,3-dioxo-2-indancarbonitril in 91% yield after flash column chromatography. The desired amide was obtained by subsequent hydrolysis of the corresponding nitrile with concentrated sulfuric acid. Recrystallization from methanol gave 1,3-dioxo-2-indane-carboxamide in 64 % yield. The structure of the obtained compound was proved by ^{1}H and ^{13}C NMR spectra in DMSO-d_6. NMR spectra were recorded on a Bruker Avance II + 600 spectrometer, operating at 600.13 MHz for ^{1}H and 150.92 MHz for ^{13}C, using a 5 mm dual probe head. The measurements were carried out at ambient temperature (293 K). Sample concentration of 0.01 M for ^{1}H and saturated solution for ^{13}C NMR spectra were used. The chemical shifts are identical with these presented in paper (12): ^{1}H NMR: (DMSO-d_6, 600 MHz): δ 9.08 (br, 1H, NH), 8.32 (br, 1H, NH), 7.65–7.63 (m, 2H, arom.), 7.607.50 (m, 2H, arom.) ppm. ^{13}C NMR: (DMSO-d_6, 150.9 MHz): δ 191.13 (C-1, C-3), 167.50 (C-10), 138.21 (C-8, C-9), 133.22 (C-5, C-6), 120.93 (C-4, C-7), 92.27 (C-2) ppm. The ^{1}H and ^{13}C NMR spectra of CAID in DMSO-d_6 are presented in Figures S1 and S2 in the Supporting Information.

Computational details. The quantum-chemical calculations were performed using Gaussian09 software package (16). Geometric optimization of the tautomers CAIDA and CAIDB; the nucleotides AMP, GMP, UMP and CMP; the deoxynucleotides dAMP, dGMP, TMP and dCMP; and the complexes of the tautomers with nucleotides/deoxynucleotides (XMP-CAID or CAID-XMP) were carried out at B3LYP/6-31 + G(d,p) computational level without symmetry constraints. There are two possible ways of association between CAID and the nucleotides: (i) to the nitrogen base denoted as XMP-CAID and (ii) to the phosphate group denoted as CAID-XMP (see Figs. 3 and 4). The local minima were verified by establishing that the Hessians had zero eigen values. The values of Gibbs free energies (ΔG) were calculated for a temperature of 298.15 K.

Spectral measurements. The solvent used for the spectrophotometric and spectrofluorimetric investigations and analyses were purchased form Sigma-Aldrich, Germany as commercial products of analytical grade, and were used without further purification. 2 mL TRIS buffer (tris hydroxymethyl aminomethane) at pH = 7.2 was used for preparing of the solutions. The Ethidium bromide (Sigma-Aldrich, Germany) in concentration 20 μM was used to prepare the solutions required for the study. Double stranded DNA (dsDNA) and RNA sequences, purchased from Metabion, Germany, in concentrations 40 and 200 μg mL^{-1}, respectively, were used in the experiments. The concentration of the title compound varies from 10 to 200 μM in different experiments. A stock solution of CAID in dimethyl sulfoxide (DMSO) (10 mM) was prepared and stored in the dark and diluted prior the measurements. The dye-solution in microliter volume was used to obtain the desired ratio of the DNA/CAID or RNA/CAID complexes. The final DMSO concentration in the experimental volume did not exceed 0.5%. At this concentration, DMSO does not affect the observed spectra and the tested biological objects. The preparations of the complexes for investigation were carried out in a thermostated (37°C) quartz cell at constant stirring. The fluorescence spectra of DNA/EthBr/CAID and DNA/CAID were recorded after excitation at 500 and 330 nm, respectively, while those of RNA/EthBr and RNA/CAID—after excitation at 500 and 350 nm, respectively. The influence of temperature on fluorescence spectra of dsDNA/CAID (λ_{ex} = 330 nm) recorded at room temperature (initially and after 24 h of the experiment), after heating (25–90°C for 1.5 h) and after cooling for 4 h was studied.

Fluorescence measurements were performed using 10 mm path-length quartz cuvettes on a Perkin Elmer LS55 spectrofluorimeter at room temperature or after heating the cuvette holder to specific, digitally controlled temperature. The spectra were recorded, and the data were analyzed and graphically represented by means of computer program Origin 8.0 (Microcal Software, Inc., Northampton, MA).

RESULTS AND DISCUSSION

Fluorescent probes (fluorophores) as molecules that absorb light of a specific wavelength and emit light of a different, typically longer wavelength are administered to investigate biological samples. The role of these probes is to attach to target molecule and act as a marker for analysis with fluorescence microscopy. The fluorophores are distributed randomly and unevenly in cells and tissues, and this way the intensity of the observed fluorescence signal depends on different factors as local concentration, illumination intensity, surroundings and other processes. In order to avoid the influence of these factors, fluorescent probes that exhibit a significant spectral shift upon reaction or binding to the investigated object are preferred for use. Furthermore, a required

feature for fluorescent probes is the absence of photobleaching, as well as to show two or more well-separated emission bands in organic solvents. Such characteristics of fluorescence signals are often manifested by compounds with native tautomerism.

Excited-state intramolecular proton transfer (ESIPT) is very effective for designing dual-band fluorescent signal probes. ESIPT leads to the formation of two tautomeric forms in the excited state of the probe (17), which have well-separated emission maxima. According to our previous investigations (12,14), 2-carbamido-1,3-indanedione is exactly such compound. CAID can exist in gas phase and solution in two tautomeric forms, CAIDA and CAIDB, close by energy (Fig. 1). Two tautomeric forms exist both in the ground and in the first excited state. Due to its molecular structure, CAID could act as a proton donor in hydrogen bonding interactions. Moreover, this compound demonstrates the appropriate spectral ranges of absorption and fluorescence, and the high fluorescence quantum yield (0.78 in saline buffer) which are important advantages for probing biomolecules of high importance for biology and medicine (14).

To elucidate the possibility of CAID binding to nucleic acids and its use as a fluorescent probe in biological investigations, fluorescence spectra of DNA and RNA sequences loaded with CAID (DNA/CAID or RNA/CAID) were recorded. The dependence of the CAID fluorescence to the temperature, the time and the compound concentration was studied. Several different fluorescent measurements were performed to investigate the possibility of conjugation between CAID and DNA sequences. The fluorescence spectra of DNA, DNA loaded with 20 μM ethidium bromide (DNA/EthBr) and DNA/EthBr + CAID (50, 100 or, 200 μM CAID) were recorded over time and are presented in Fig. 2.

Double stranded DNA (dsDNA) was diluted in 2 mL TRIS buffer at pH = 7.2 to achieve concentration 40 μg mL^{-1}. The DNA/EthBr (EthBr as intercalating agent) was used as a target for verification of the title compound capability for DNA labeling as well as to clarify the possibility and manner of binding of CAID to DNA in various concentrations of CAID (50–200 μM). As can be seen from Fig. 2, even at ten-fold higher concentrations of the test substance, the fluorescence spectrum of the ethidium bromide does not change. The value of the peak maximum is lower ca. 4% compared with the peak of the pure EthBr in dsDNA at the conditions described above. Insert in Fig. 2 shows dependence of the F/F_0 to CAID concentration (F_0 and F are the measured fluorescence intensities of DNA/EthBr without and with CAID, respectively). We can conclude that CAID does not displace the intercalating agent and interacts with dsDNA in a different manner, probably.

The fluorescence spectra of dsDNA/CAID (λ_{ex} = 330 nm) were recorded initially and after 24 h of the experiment at room temperature. The solution was heated gradually from 25 to 90°C

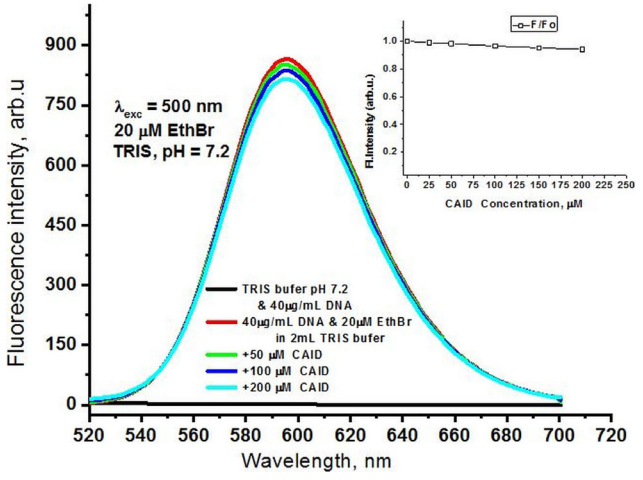

Figure 2. Fluorescence spectra of DNA, DNA/EthBr and DNA/EthBr + CAID at different compound concentrations.

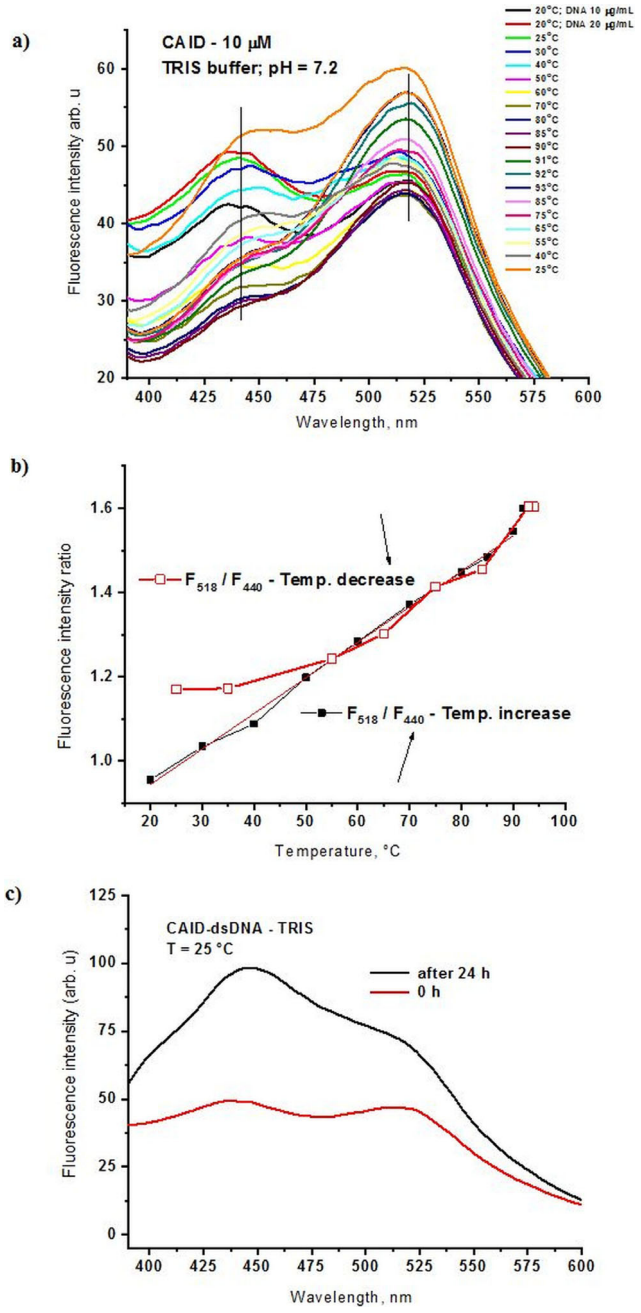

Figure 3. Fluorescence spectra of DNA/CAID after heating and cooling (a), dependence of the fluorescent intensities ratio, F_{518}/F_{440}, to the temperature (b) as well as fluorescence spectra of DNA/CAID at 0 h and after 24 h of the measurements (c).

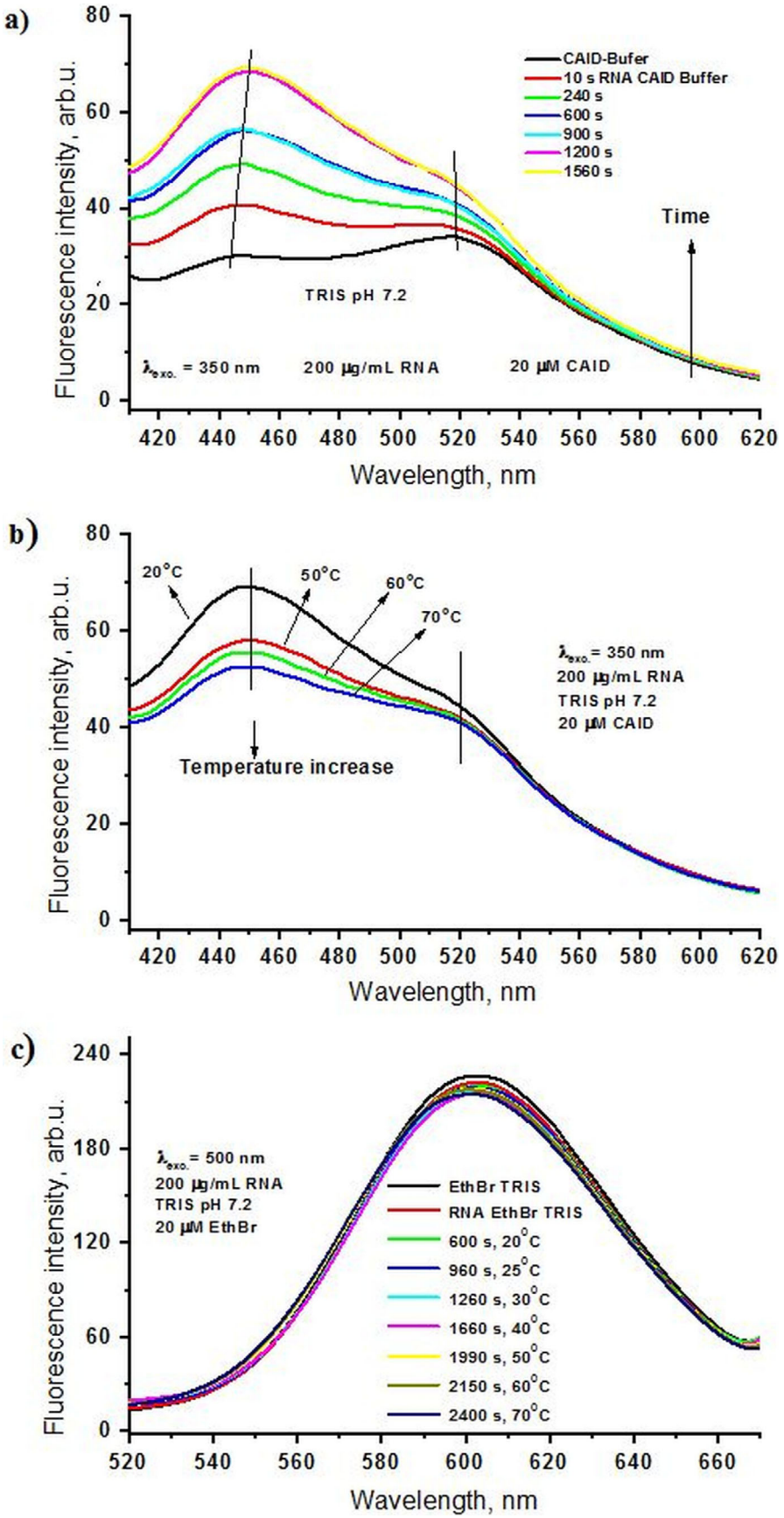

Figure 4. Fluorescence spectra of RNA/CAID over time (a) and at different temperatures (b). Fluorescence spectra of RNA/EthBr over time and at different temperatures (c).

for 1.5 h and was cooled for 4 h. The spectra are presented in Fig. 3. Two maxima at 440 and 518 nm were observed.

At the starting point of the experiment, the two peaks seem approximately the same (Fig. 3a–c). The intensity of the maxima increases with incubation time and that one at 440 nm becomes more intense in the end of measurement. The intensity of the maxima changes in different manner during the experiments. The fluorescent intensities ratio, F_{518}/F_{440}, increases during the heating while at cooling it decreases on the same pattern up to 55°C (Fig. 3b). In the interval 55–25°C, the intensity of the maximum at 518 nm does not change substantially unlike the one at 440 nm (Fig. 3a). After the sample was cooled to room temperature, it was left for up to 24 h and then a fluorescence spectrum was recorded. As a result, the maximum at 440 nm gets more intensive than the one at 518 nm (Fig. 3c). It should be noted that there is no shifting in both of the fluorescent maxima, and the peak at 518 nm is more defined. This fluorescent behavior is probably due to the existence of the two CAID tautomers in ground state and their fluorescence after excitation at 330 nm (14).

To investigate the binding expedient of CAID to RNA, several fluorescent experiments were performed. The fluorescence spectra of RNA/CAID in TRIS buffer at pH = 7.2 were scanned. We have measured the emission of the solution at different temperatures and over time (Fig. 4a,b). The dependence of the CAID fluorescence intensity on the incubation time is presented in Fig. 4a. The intensity increases, and the slight red shift of the maximum were observed. This is due to the fact that time is needed for RNA to undergo conformational changes and to bind to CAID through hydrogen bonds, probably. It is seen that there is similarity of the fluorescent spectra of DNA/CAID and RNA/CAID

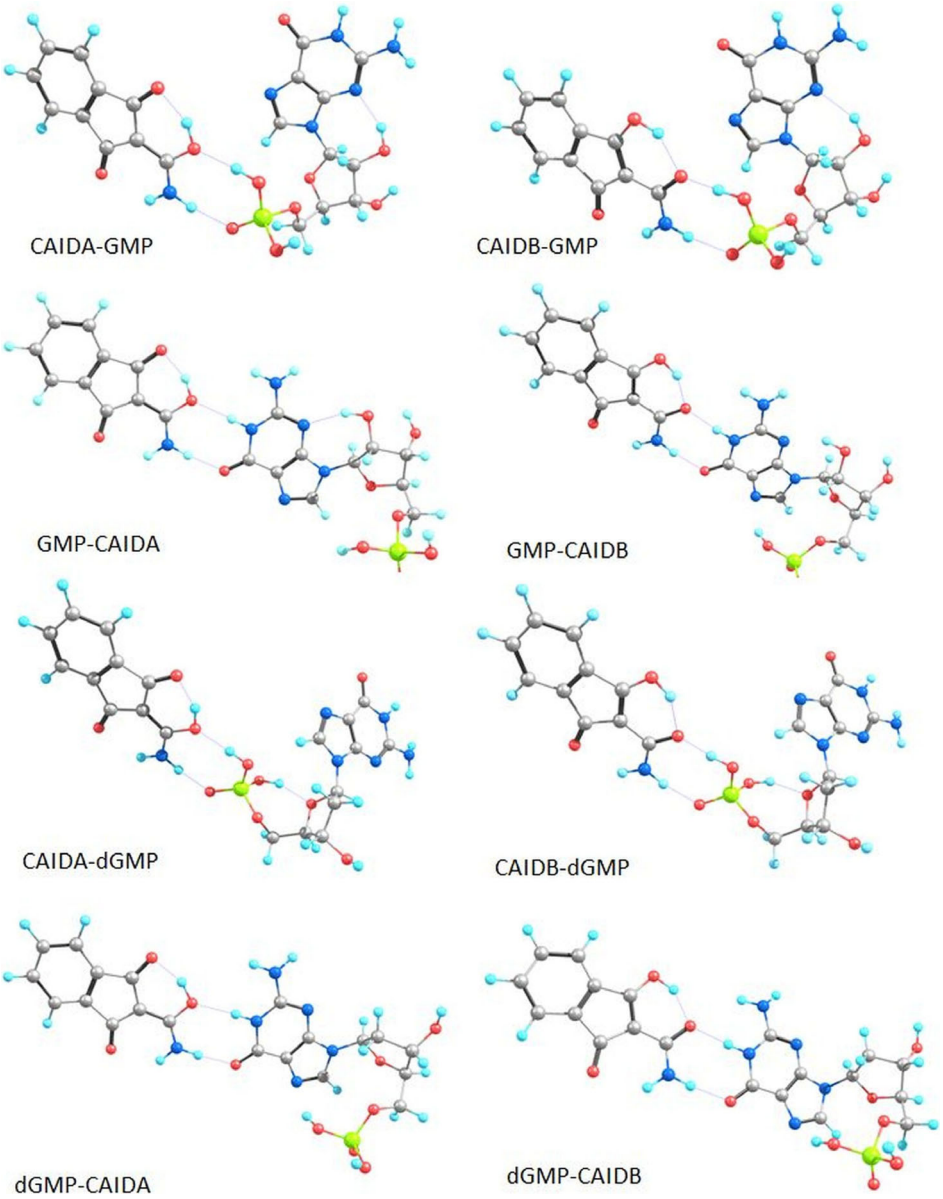

Figure 5. Complexes of the two tautomers, CAIDA and CAIDB, of 2-carbamido-1,3-indanedione with guanosine monophosphate (GMP) and deoxyguanosine monophosphate (dGMP), optimized at B3LYP/6-31+G(d,p) level.

shown in Figs. 3c and 4a, respectively, due to the disruption of the double-helix DNA structure after heating.

Fluorescence spectra at different temperatures were recorded (Fig. 4b). The fluorescence emission decreases when the temperature increases. Such behavior could be explained by the rupture of hydrogen bonds between the test molecule and RNA by heating.

Fluorescence spectra of RNA/EthBr over time and at different temperatures were scanned. The experiment was performed to study the behavior and binding possibility of CAID to RNA compared with EthBr. As can be seen from Fig. 4c, changes in the fluorescence emission were not observed. Therefore, the ethidium bromide as an intercalating agent interacts with RNA in a different manner from that one of CAID under changing medium conditions. Thus, it can be concluded that CAID is suitable for RNA containing biological objects detection. The fluorescent properties of conjugation between CAID and nucleic acid sequences were studied for evaluation of the potential for applying of CAID as a fluorescent probe in biological investigations.

To clarify the affinity of CAID conjugation to nucleic acids, quantum-chemical calculations at B3LYP/6-31+G(d,p) level were performed. We started by modeling hydrogen bonded complexes between the two tautomers of CAID and all possible RNA and DNA nucleotides with purine and pyrimidine nitrogen containing nucleobases: uridine monophosphate (UMP), thymidine monophosphate (TMP), guanosine and deoxyguanosine monophosphates (GMP and dGMP), cytidine and deoxycytidine monophosphates (CMP and dCMP) as well as adenosine and deoxyadenosine monophosphates (AMP and dAMP). There are two possible sites of association of CAID to nucleotide by hydrogen bonding—to the nucleobases (XMP-CAID) or to the phosphate group (CAID-XMP). 2-carbamido-1,3-indanedione takes part in hydrogen bonding by -NH2 and =O (-OH) groups. The complexes formed between guanosine, deoxyguanosine, cytidine and deoxycytidine monophosphates and CAID tautomers are shown in Figs. 5 and 6. The structures of all other complexes are presented in Figures S3 and S5 in the Supporting Information.

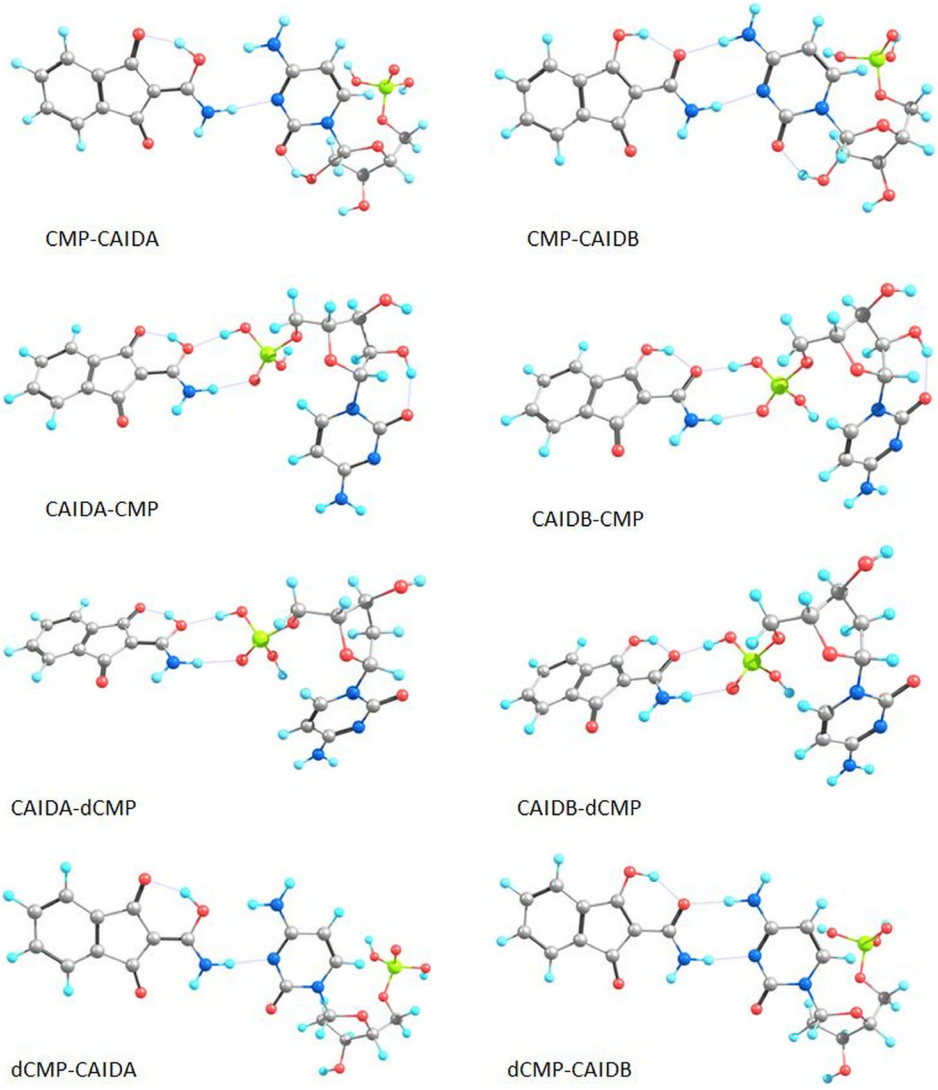

Figure 6. Complexes of the two tautomers, CAIDA and CAIDB, of 2-carbamido-1,3-indanedione with cytidine monophosphate (CMP) and deoxycytidine monophosphate (dCMP), optimized at B3LYP/6-31+G(d,p) level.

All species were optimized at the B3LYP/6-31+G(d,p) level of theory. The relative total energies (ΔE_t) as well as the relative Gibbs free energies (ΔG_{298}) of all CAIDA and CAIDB dimers in the gas phase are presented in Table 1.

According to the computed energies and energy differences, the nucleotides form more stable complexes with tautomer CAIDB when the hydrogen bonding is realized to their phosphate group. The free energy differences between dimers vary from 0.62 to 5.52 kcal mol^{-1}, and in all cases CAIDB complexes are more stable than CAIDA ones. When CAID is hydrogen bounded to the nitrogen base of the nucleotides, CAIDB complexes with GMP, dGMP, UMP and TMP are again preferred structures. According to our results, the structures of the CAID dimers of the other nucleotides are isoenergetic, with a slight predominance of CAIDA complexes.

In order to evaluate the possibility of intermolecular hydrogen bonds formation between CAID and nucleotides, the energies of interaction (E_{int}) were calculated by eq. (1).

$$E_{int} = E_{CAID} + E_{XMP} - E_{complex} \quad (1)$$

E_{XMP}, E_{CAID} and $E_{complex}$ are the E_t energies, calculated at B3LYP/6-31+G(d,p) level, for each nucleotide molecule, CAID tautomer and its nucleotide complex, respectively. The results for ΔE_{int} of all dimers are presented in Table 1. When CAID tautomers are attached to the phosphate group of RNA nucleotides, the most stable complexes are those formed between CAIDB and pyrimidine base nucleotides: CAIDB-UMP and CAIDB-CMP. The interaction energies are 17.67 and 17.51 kcal mol^{-1}, respectively. The picture is slightly different in DNA complexes: the CAIDB complexes are again more stable than those of CAIDA, but the complexes CAIDB-dGMP and CAIDB-dCMP have the highest interaction energy. When the hydrogen bonding is realized with the nitrogen base of the nucleotides, the most stable structures are dimers of CAIDB with GMP and dGMP: ΔE_{int} of the complexes formed are 17.10 and 14.99 kcal mol^{-1}, respectively.

As a summary, the 2-carbamido-1,3-indanedione could form stable complexes with nucleotides, and tautomer B is the preferred structure. Generally, the strongest complexes with CAIDB form the G and dG nucleotides, and the hydrogen bonds are situated preferably in the phosphate group region. The TMP dimers have the lowest interaction energy, and therefore, form the weakest complexes with CAID.

Table 1. Interaction energies, ΔE_{int}, energy differences, ΔE_t and ΔG_{298} (in kcal mol^{-1}), for the complexes of the tautomers CAIDA and CAIDB with nucleotides and deoxynucleosides calculated at B3LYP/6-31+G(d,p) level.

Complex type	ΔE_{int}	ΔE_t	ΔG_{298}	Complex type	ΔE_{int}	ΔE_t	ΔG_{298}
To the phosphate group				To the phosphate group			
CAIDA-GMP	9.59	4.57	5.52	CAIDA-dGMP	13.33	3.12	2.47
CAIDB-GMP	15.65	0.00	0.00	CAIDB-dGMP	17.91	0.00	0.00
CAIDA-AMP	10.42	3.60	2.46	CAIDA-dAMP	12.06	2.92	1.77
CAIDB-AMP	15.48	0.00	0.00	CAIDB-dAMP	16.43	0.00	0.00
CAIDA-UMP	12.4	3.78	2.49	CAIDA-TMP	10.46	3.99	3.16
CAIDB-UMP	17.67	0.00	0.00	CAIDB-TMP	15.91	0.00	0.00
CAIDA-CMP	12.62	3.42	0.62	CAIDA-dCMP	12.62	3.39	2.31
CAIDB-CMP	17.51	0.00	0.00	CAIDB-dCMP	17.47	0.00	0.00
To the nitrogen base				To the nitrogen base			
GMP-CAIDA	9.82	5.78	3.76	dGMP-CAIDA	11.78	1.75	1.20
GMP-CAIDB	17.1	0.00	0.00	dGMP-CAIDB	14.99	0.00	0.00
AMP-CAIDA	8.81	0.58	0.00	dAMP-CAIDA	9.16	0.46	0.00
AMP-CAIDB	10.88	0.00	0.28	dAMP-CAIDB	11.08	0.00	0.04
UMP-CAIDA	9.58	1.24	0.26	TMP - CAIDA	6.63	1.54	0.67
UMP-CAIDB	12.25	0.00	0.00	TMP - CAIDB	9.63	0.00	0.00
CMP-CAIDA	10.41	1.63	0.04	dCMP-CAIDA	10.76	1.40	0.00
CMP-CAIDB	13.5	0.00	0.00	dCMP-CAIDB	13.62	0.00	0.67

CONCLUSION

Quantum-chemical and fluorescent analyses were performed to investigate the binding expedient of 2-carbamido-1,3-indandione tautomers (CAIDA and CAIDB) to nucleotides. Such a study on the binding ability of the title compound to DNA and RNA and the resulting biological applications has not yet been performed. According to our fluorescence experiments, CAID does not act as an intercalating agent. The fluorescent behavior of the compound shows that there is association between CAID and DNA and RNA, and the binding is realized by hydrogen bonds, probably. To elucidate the possibility of conjugation of CAID tautomers to NA, two types of complexes were considered—when the compound is attached to the nitrogen base and to the phosphate group of the monophosphate. The calculated interaction energies of the formed complexes indicate that tautomer CAIDB formed more stable complexes than tautomer CAIDA. The preferred nucleotide moiety for hydrogen bonding is the phosphate group. In general, guanosine and deoxyguanosine monophosphate form the most stable complexes depending on the complex type. As a summary, it can be concluded that CAID has binding affinity to nucleic acids and does not show a cytotoxic effect on untransformed cell lines Balb/c 3T3. Thus, we could suggest the subject compound, 2-carbamido-1,3-indandione as a new reliable fluorescent biomarker with future wide application in medicine and biology as well.

Acknowledgements—The calculations were performed on the AVITOHOL supercomputer at the Institute of Information and Communication Technologies at the Bulgarian Academy of Sciences.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. ^1H NMR spectra of CAID in DMSO-d_6.

Figure S2. ^{13}C NMR spectra of CAID in DMSO-d_6.

Figure S3. Complexes of the two tautomers of 2-carbamido-1,3-indanedione with cytidine monophosphate and deoxycytidine monophosphate, optimized at B3LYP/6-31+G(d,p) level.

Figure S4. Complexes of the two tautomers of 2-carbamido-1,3-indanedione with adenosine monophosphate and deoxyadenosine monophosphate, optimized at B3LYP/6-31+G(d,p) level.

Figure S5. Complexes of the two tautomers of 2-carbamido-1,3-indanedione with uridine monophosphate and thymidine monophosphate, optimized at B3LYP/6-31+G(d,p) level.

Table S1. Total energies, E_t and interaction energies, ΔE_{int} and ΔH_{int} (in kcal mol^{-1}), for nucleotides and deoxynucleosides as well as their complexes with the tautomers CAIDA and CAIDB calculated at B3LYP/6-31+G(d,p) level.

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