Journal of Molecular Structure 1150 (2017) 179–187

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Tautomerism and isomerism in some antitrichinellosis active benzimidazoles: Morphological study in polarized light, quantum chemical computations

Kameliya Anichina [a], Anelia Mavrova [a], Denitsa Yancheva [b, *], Jordan Tsenov [b], Rasho Dimitrov [a]

[a] University of Chemical Technology and Metallurgy, 8 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria
[b] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Build. 9, 1113 Sofia, Bulgaria

ARTICLE INFO

Article history:
Received 17 March 2017
Received in revised form
19 August 2017
Accepted 22 August 2017
Available online 24 August 2017

Keywords:
Benzimidazole synthesis
Tautomerism
Polarized light microscopy
DSC
DFT

ABSTRACT

The morphology of the crystal structure of some antitrichinellosis active benzimidazole derivatives including (1*H*-benzimidazol-2-ylthio)acetic acids, [1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-ones, 1*H*-benzimidazol-2-ylthioacetylpiperazines and starting 2-mercapto benzimidazoles, was studied by the use of Polarized Light Microscopy (PLM). Characterization of the crystal phase was complimented by Differential scanning calorimetry analysis (DSC) and spectroscopic data. DFT computations were performed in order to investigate the prototropic tautomerism and the geometry of the molecule of the synthesized compounds.

One distinct type of crystal structure for each one of 5 or 6-methyl-(1H-benzimidazol-2-ylthio)acetic acid **6** was observed by PLM – dendritic and needle-shaped formations. Compound **14**, containing a methyl substituent in the benzimidazole ring crystallized also into two phases; while for the unsubstituted compound **13** a separation of phases does not take place. The influence of the both solvents - chloroform and ethanol on the phase separation and the formation of the crystalline structure of compound **14** was investigated.

The morphological study showed that the cyclization of **6** in the presence of acetic anhydride in pyridine medium led to a mixture of 6-methyl-[1,3]tiazolo[3,2-*a*]benzimidazol-3(2*H*)-one (**10a**) and 7-methyl-[1,3]thiazolo[3,2-*a*]-benzimidazole-3(2*H*)-one (**10b**), which crystallized in the form of fibrils and spherulites respectively.

It was found that a difference in the crystal structures of substituted and unsubstituted benzimidazol-2-thiones, respectively benzimidazol-2-thiol derivatives exists, which may be due not only to the thiol-thione tautomerism but to the prototropic properties of the hydrogen atom in first position of the ring. The calculation results indicated that the thione form is more stable than the thiol tautomer by 51 –55 kJ mol^{-1}. But at the same time ΔG for the two thiol tautomers is below 0.5 kJ mol^{-1}. In solid phase the 5(6)-substituted-1*H*-benzimidazol-2-thiols crystallized in two different crystal structures while the unsubstituted 1*H*-benzimidazol-2-thiol possess one type of crystal structure.

1. Introduction

The benzimidazole heterocyclic system is an essential pharmacophore in modern drug discovery because of the broad spectrum of important biological and pharmacological properties exhibited by benzimidazole compounds, as antimicrobial [1–4], antitumor [5–7], antiallergic [8–10] and antiviral effects [11–15]. Benzimidazole derivatives albendazole, mebendazole, ciclobendazole, febendazole, flubendazole, triclabendazole have achieved significant success in the treatment of many parasitic diseases due to their high efficiency, broad spectrum of antiparasitic activity and low toxicity [16,17].

Well-known specific features of the benzimidazole heterocycle are its prototropic properties, which enable its easy interconversion to different tautomers. The hydrogen atom attached to N-1 of the benzimidazole nucleus readily tautomerizes moving to N-3 atom; consequently, the benzimidazoles may exist in two or even three

* Corresponding author.
E-mail address: deni@orgchm.bas.bg (D. Yancheva).

http://dx.doi.org/10.1016/j.molstruc.2017.08.073
0022-2860/

tautomeric forms. The tautomerism of drug structures plays an essential role in the interaction with biological systems [18–20]. Therefore, after the establishment of the fact that the benzimidazole derivatives have antagonist effect towards purine compounds many theoretical calculations and investigations were performed with the purpose to predict and determined their conformational and tautomeric preferences [18,21–26].

Previously we reported a series of newly synthesized 5(6)-(un) substituted-1*H*-benzimidazol-2-ylthioacetylpiperazine derivatives and also thiazolobenzimidazolones (Scheme 1) [27,28]. Some of these substances exhibited higher activity against *Trichinella spiralis* in vitro in comparison to albendazole [27,28]. The high biological activity of the above mentioned compounds and the existing phenomenon of tautomerism observed in benzimidazole molecules prompted us to explore the morphology of their crystal structure by polarized light microscopy (PLM). It should be pointed out that there are very few data reported about the use of PLM for investigation of the crystal structure of benzimidazole tautomers. PLM analysis is applied extensively in pharmaceutical development and enables observation not only of the simple images of drug substance but also the particle size and shape of the internal structure (morphology) of the crystals of those substances [29,30]. Having in view the range and specificity of the crystallographic properties of pharmaceutical compounds, a PLM research on the synthesized benzimidazole derivatives will contribute to clarify the morphology of their crystals and the possible presence of more than one tautomer. It will allow identifying the distinguishing properties of the tautomeric forms. DSC analysis and spectroscopic data were used to assign the different crystal phases to particular tautomer. The conformational and tautomeric preferences of the molecules were studied by computational methods too. This study will complement the characterization of the 5(6)-(un)substituted-1*H*-benzimidazol-2-ylthioacetylpiperazines and thiazolobenzimidazolones for possible future application as antitrichinellosis agents.

2. Experimental section

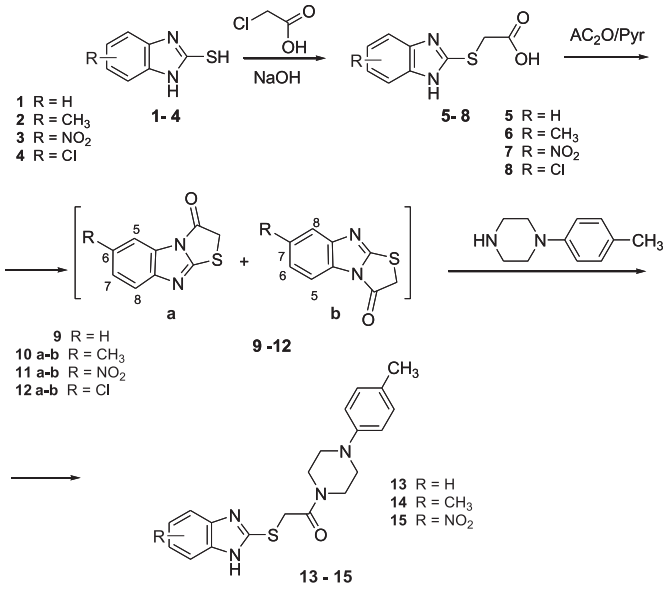
2.1. Materials and methods

Melting points (mp) were determined on an Electrothermal AZ 9000 3MK4 apparatus and were uncorrected. Thin layer chromatography (R_f values) was performed on silica gel 60 plates F_{254} (Merck, 0.2-mm thick) using mobile phase benzene-methanol, 3:1 and benzene-acetone, 8:1, and visualized with ultraviolet light. ^{1}H NMR spectra were obtained on a Bruker Avance II+ 250 MHz and a Bruker Avance II+ 600 MHz NMR instrument using a dual 5-mm probe head and $CDCl_3$ and DMSO-d_6 as solvents. The measurements were carried out at ambient temperature (300 K). Chemical shifts were expressed relative to tetramethylsilan and reported as δ (ppm). Mass spectrometric measurements were performed using an LCQ DECA instrument (Thermo Finnigan, Palo Alto, CA,USA). The compounds solutions (10^{-6} M in CH_3OH) were prepared and directly infused into the ESI source. The ions were produced using spray voltage, capillary voltage and capillary temperature of 4 kV, 8 kV and 220 °C, respectively.

Differential scanning calorimetry studies (DSC) were performed on Perkin Elmer DSC 7 differential scanning calorimeter in an argon atmosphere, in a temperature range from 50 to 250° C and a heating rate of 5° C min^{-1}. The apparatus was calibrated using indium and lead as standards. Samples were in an amount of 7 mg.

2.2. Chemistry

The synthesis of the studied benzimidazole derivatives was performed according to the procedure described in Refs. [27,28]. The methods are given in the Supporting material.



Scheme 1. Synthesis of 1*H*-benzimidazol-2-ylthioacetylpiperazine derivatives.

2.3. Optical polarizing microscopic assay

The morphology of the crystallizing benzimidazole derivatives was studied upon dissolving 2 mg of the tested compound in a 0.1 ml $CHCl_3$ (Sigma–Aldrich) at a room temperature. One drop of the solution was applied on a glass plate and the solvent was allowed to volatilize, after which a cover glass was placed over the sample and the preparation was observed using an Olympus BH2 Polarized Light Microscope, equipped with a camera.

2.4. Calculations

All theoretical calculations were performed using the Gaussian 09 package [31] of programs. Geometry and vibrational frequencies of the studied species were performed by analytical based gradient technique without any symmetry constraint. All the results were obtained using the density functional theory (DFT), employing the B3LYP (Becke's three-parameter non-local exchange [32] and Lee correlation potentials [31]). Incorporation of solvent effect was performed by Integral Equation Formalism of Polarizable Continuum Model (IEFPCM) [33,34].

Tautomeric interconversion in chloroform was simulated by potential energy surface (PES) scans for hydrogen atom migration and rotation around the C–S bond. The moving of H (from S–H bond) to benzimidazolium N was done at regular steps of 0.1 Å. The rotation around C–S bond was carried out in steps of 3.0°. For both migration and rotation, geometry optimization was performed at each step. Transition state was confirmed by optimization of the highest energy geometry identified by the H-atom migration scanning and vibrational analysis. The computations were done at B3LYP/6-311 + G** level of theory.

3. Results and discussion

The synthesis of the studied benzimidazole derivatives was carried out as depicted in Scheme 1.

The preparation of the starting 1*H*-benzimidazole-2-thiols **1**–**4**

was performed by refluxing of ethanol/water solution of sodium hydroxide, carbon disulphide and 4-substituted-1,2-diamino-benzenes. The 5(6)-(un)substituted-1*H*-benzimidazol-2-ylthioacetic acids **5**–**8** were obtained through William's reaction of 5(6)-(un) substituted-1*H*-benzimidazole-2-thiols **1**–**4** with chloroacetic acid in the presence of sodium hydroxide under reflux. 1,3-Thiazolo[3,2-*a*]benzimidazol-3(2*H*)ones **9**–**12** were synthesized by heating 5(6)-(un)substituted-(1*H*-benzimidazol-2-ylthio)acetic acids **5**–**8** with acetic anhydride in pyridine medium on steam bath [28].

The next step comprised in the cleavage of the condensed thiazole ring in the [1,3]-thiazolo[3,2-*a*]benzimidazol-3(2*H*)-ones with an appropriated N-monosubstituted piperazine in ethanol medium. Refluxing for 3 h resulted in the piperazine derivatives of benzimidazol-2-ylthioacetic acids **13**–**15** [27].

The structures of all compounds were established by IR, ^{1}H NMR as well as elemental analysis [27,28]. The data of some ^{1}H NMR, ^{13}C NMR and MS spectra of the synthesized compounds are given as supplementary materials (Figs. 1S–12S). The mass spectral data of thioacetoamides are presented in Table 1.

For compound **14** the 2D heteronuclear ^{1}H/^{13}C correlation spectra (HMQC technique - heteronuclear multiple quantum correlation) were recorded to confirm the assignment of the signals in ^{1}H and ^{13}C spectra and the purity of the compounds (Fig. 9S).

Thus four groups of benzimidazoles were synthesized arising different possibilities for prototropic tautomerism and positional isomerism.

Due to prototropic hydrogen atom at the benzimidazole ring it should be expected the cyclization of thioacetic acid would give two isomers of thiazolobenzimdazolones.

Tanaka and co-workers reported that the cyclization of 5(6)-substituted-(1*H*-benzimidazol-2-ylthio)acetic acid in the eutectic mixture Dewtherm A afforded two positional isomers – 6-th and 7-th isomer [35]. The authors determined their structure by means of ^{1}H NMR spectra. Carrying out the experiment by heating the thioacetic acid with acetic anhydride in pyridine medium they established the presence of the two isomers in the reaction solution through ^{1}H NMR spectroscopy, but they succeeded to isolate only the 6-substituted isomer. Tanaka and co-workers [35] have attributed this to the fact that probably 6-substituted thiazolobenzimdazole is thermodynamically more stable than the 7-substituted isomer.

The formation of only 6-substituted thiazolobenzimdazolones as products was reported for the cyclization of 5(6)-methoxy- [36], 5(6)-methyl- [37], 5(6)-chloro- [38] and 5(6)-nitro-(1*H*-benzimidazol-2-ylthio) acetic acid with acetic anhydride/pyridine [39].

In our study the cyclization of the 5(6)-methyl-(1*H*-benzimidazol-2-ylthio)acetic acid **6** with acetic anhydride was achieved in pyridine medium in accordance with the method described in Ref. [27]. After recrystallization of the crude product we obtained a substance with melting point 166.9 °C and R_f value 0.500 (mobile phase - benzene-acetone = 8:1).

To determine whether the product is a mixture of two positional isomers, we carried out an examination on the morphological crystalline structure in polarized light. The studies demonstrated the existence of two types of crystal structures (Fig. 1), which is a proof that the obtained solid is a mixture of two positional isomers. On Fig. 1a there can be seen clearly distinct bundles of colored needles (fibril structure) – first phase and radial structures with pronounced “Maltese Cross” shape – the second phase. The size of the first phase was larger in comparison to that of the second phase. The substance had a great rate of crystallization. The total crystallization process was completed during the evaporation of the solvent, while the phase which formed a spherulite structure was slightly lagging in time from the phase constituting the needle-shaped crystals. The analysis of the ^{1}H NMR data for the obtained product indicated the presence of two isomers in ratio 1:1.

The two phases were mechanically separated with the help of a microscope and for each of them there was recorded a ^{1}H NMR spectrum (the NMR-spectra are given in Figs. 4S and 5S in the Supplementary material). The chemical shifts δ (ppm) recorded on a Bruker Avance II+ 600 MHz NMR instrument were as follows:

The fibril structure: ^{1}H NMR (DMSO-d_6) δ (ppm): 2.428 (s, 3H, 6-CH_3), 4.599 (s, 2H, CH_2), 7.199(ddd, J = 0.6, 1.6, 8.2 Hz, 1H, 7-H), 7.462 (d, J = 8.2 Hz, 1H, 8-H), 7.686 (m, 1H, 5-H).

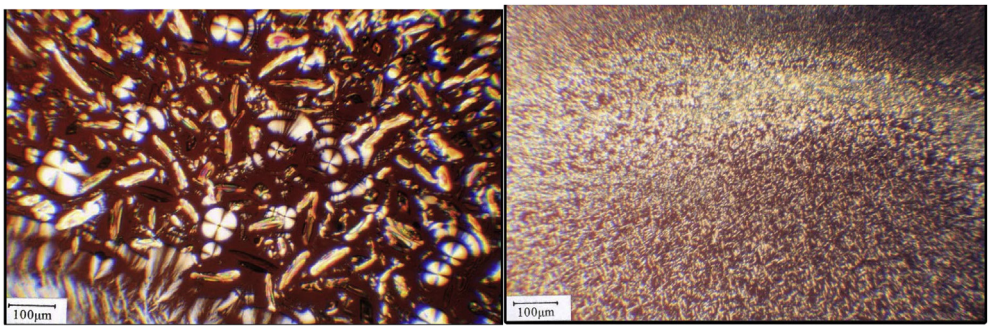
The spherulitic structure: ^{1}H NMR (DMSO-d_6) δ (ppm): 2.410 (s, 3H, 6-CH_3), 4.591 (s, 2H, CH_2), 7.199(dd, J = 0.8, 8.1 Hz, 1H, 6-H), 7.403 (m, 1H, 8-H), 7.729 (d, J = 8.1 Hz, 1H, 5-H).

Differential scanning calorimetry (DSC) studies confirmed the suggestion that the synthesized product is a mixture of 6$^{-\text{the}}$ and the 7$^{-\text{th}}$ isomer, since in the DSC-diagrams two peaks appeared for the melting point, the one at the 120.7° C and the other one at 185.7° C for compounds **10a** and **10b**, respectively (Table 2). The diagrams are given in Fig. 13S in the Supplementary material. When using fractional crystallization with ethanol it was possible to separate the 6- and the 7-isomer.

Based on these results it can be concluded that 6-methyl[1,3]-thiazolo[3,2-*a*]-benzimidazole-3 (2*H*)-one **10a** crystallized in the

Table 1
Multi-stage mass spectrometry (MS^n) data of compounds **13** and **14**.

№	MH^+		MS^2		MS^3		MS^4
13	*m/z* 367	→	*m/z* 177 (100)	→	*m/z* 134	→	*m/z* 119 (100)
14	*m/z* 381	→	*m/z* 205 (26)	→	*m/z* 177 (100)	→	*m/z* 133 (100)
		→	*m/z* 177 (100)	→	*m/z* 134 (100)	→	*m/z* 119 (100)



(a) (b)

Fig. 1. Microscopic pictures of the mixture of 6- and 7-methyl-[1,3]thiazolo[3,2-*a*]-benzimidazole-3(2*H*)-one **10a,b** (a) and 6- and 7-chloro-[1,3]thiazolo[3,2-*a*]-benzimidazole-3(2*H*)-ones **12a,b** (b) in polarized light.

Table 2
Differential scanning calorimetry (DSC) investigations of [1,3]thiazolol[3,2-*a*]-benzimidazole-3(2*H*)-ones.

Compound №	Mp °C	Δ H, J/g	Weight loss on heating, %
9a	183.8	139.6	10
10a	185.7	145.2	4.7
10b	120.7		
11a	228.5	106.7	1.4
11b	223.0		

form of fibrils, and 7-methyl[1,3]thiazolo[3,2-*a*]-benzimidazole-3(2*H*)-one **10b** in the form of spherulites.

The mixture of 6- and 7-chloro[1,3]thiazolo[3,2-*a*]-benzimidazole-3(2*H*)-ones **12**, whose crystal structures are shown in Fig. 1b crystallized in significantly finer formations in comparison to that of 6- and 7-methyl[1,3]thiazolol[3,2-*a*]-benzimidazole-3(2*H*)-ones **10**, the first phase having a markedly acicular nature, and the second, which was localized in a narrow area (the lighter zone in Fig. 1b) forming a multitude of polycrystalline grains.

Compounds **10** and **11**, substituted in the benzimidazole ring, showed presence of two types of crystal structures and in their DSC-diagrams appeared two peaks for the melting points in contrast to the unsubstituted compounds, where the separation of the crystalline phase was not observed and there was only one value of the melting point. In the DSC diagram of compound **9** there is only one peak at 183.4 °C.

Having in view the observation of two crystal phases in the substituted derivatives **10**–**12** we undertook investigation of the precursor compounds.

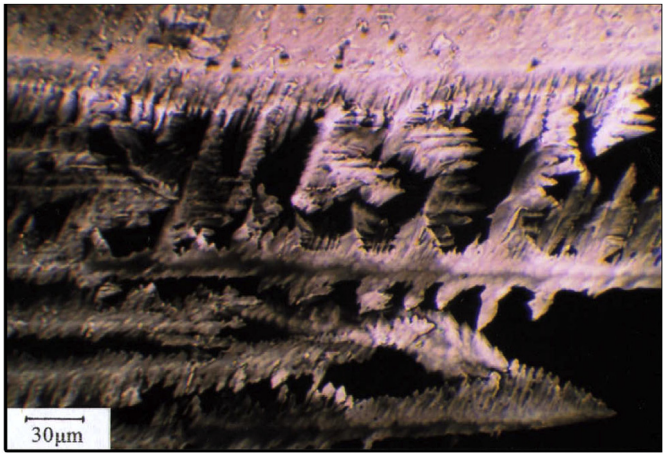


Fig. 2. Microscopic picture of compound 1*H*-benzimidazol-2-thione **1** in polarized light.

Our observation by PLM showed that **1** (Fig. 2) formed a crystalline phase having a dendritic structure with orientation, affected by the formation of the dendrites.

For compound **1** earlier XRD studies revealed that it is crystallizing in thione form [40].

The PLM observation of the benzimidazole derivative 5(6)-methyl-1*H*-benzimidazol-2-thiol **2** showed that the compound builds two kinds of crystal structures (Fig. 3a) - grained polycrystalline layer and a leaf-like structure. The second phase was markedly perfect and imitated elements of spherulites, which at their borders were formed as an amorphous phase that did not crystallized due to the lack of space for the formation of spherulites (Fig. 3b).

Two kinds of crystal structures – dendritic and needle-shaped formations – were observed in polarized light also for 5(6)-methyl-(1*H*-benzimidazol-2-ylthio)acetic acid **6** (Fig. 4a). The survey of that compound revealed that together with the formation of different crystallites, the influence of the "time" factor was very important. The crystal formations obtained in 2 h are shown in Fig. 4b. After 24 h the same sample showed a different appearance – a second crystalline phase with a strong needle character appeared together with the previously mentioned formations (Fig. 4c).

The tests were extended by conducting a microscopic analysis of the crystal structures of the two structurally similar products - **13** and **14**, which differ only by one methyl group as a substituent at the benzimidazole nucleus. For compound **13** characteristic was a continuous process of formation of the crystal structure. The observations were carried out for nearly two months, through which we registered a steady increase in the crystalline phase. As it can be seen from Fig. 5a small spaced apart areas of crystal formations were visible 24 h after the sample preparation. After one month, a homogeneous crystal structure of miniature dendrites was formed, whereat the dendrites were densely arranged on one another in a part of the sample, and with an emphasized loose placement adjacent to the described zone, where the compound was in a small amount (Fig. 5b).

On Fig. 5c there can be seen dendrites of the test compound, well formed in a period of two months. Taking into account that free (empty) areas as in Fig. 5b were not observed, it could be assumed that the crystallization process is fully completed at that time.

The influence of both solvents - chloroform (Fig. 6a) and ethanol (Fig. 6b) on the phase separation and the formation of the crystalline structure of compound **14** was investigated. It is noteworthy, that in each of the two solvents a preventing of phase separation

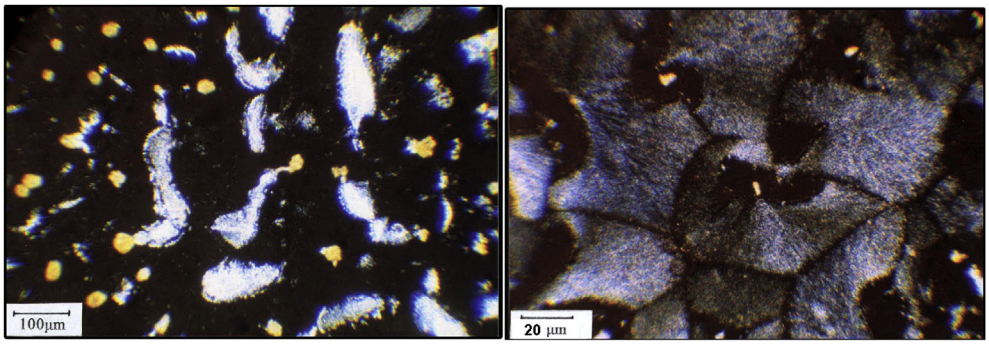


Fig. 3. Microscopic pictures of compound 5(6)-methyl-(1*H*)-benzimidazol-2-thiol **2** in polarized light, scale 100 μm (a) and scale 20 μm (b).

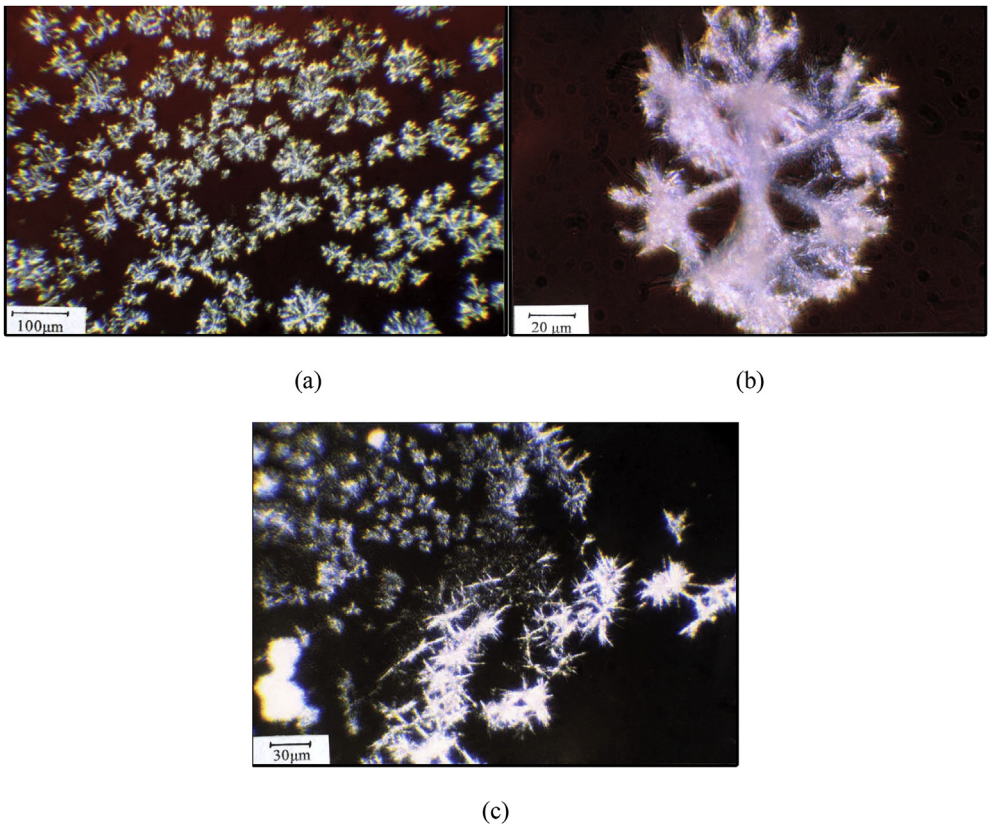


Fig. 4. Microscopic pictures of compound 5(6)-methyl-(1*H*-benzimidazol-2-ylthio)acetic acid **6** in polarized light 2 h after preparation of sample; scale 100 μm (a); scale 20 μm (b); and 24 h after preparation of sample; scale 30 μm (c).

was not observed. In the sample dissolved in ethanol a single phase structure was formed after 2 h, consisting of a great number of spherical particles. But after a period of 72 h already a separation of two phases was observed in the same sample (Fig. 6a). The one phase consisted of coacervates with significant sizes, while in the other remained spherical particles having a size almost equal to the size of the particles generated at the moment of preparation.

In consequence of the use of chloroform, the first phase consisted of a grain structure, while for the second phase there were characteristic well defined, although deformed, spherulites with typical "Maltese Cross" shape (Fig. 6b). Based on these results it could be concluded that irrespectively of the differences in the crystal structures, obtained by the use of various solvents, compound **14,** containing a substituent at the benzimidazole ring, crystallizes into two phases, while for the unsubstituted compound **13** a separation of the phases does not occur.

According to Katritzky [19] there is no clear dividing line between tautomerism and isomerism. The tautomers are simply isomers that interconvert with a relatively low activation energy – below ca 20 kcals. mol^{-1}. The rate of interconversion is controlled by the free energy of activation between the interconverting species. In order to investigate the prototropic tautomerism and the geometry of the synthesized compounds (Scheme 2) DFT computations were performed at B3LYP/6-311 + G** level of theory.

The stability order for the tautomers depicted in Scheme 2 is presented in Table 3 based on the calculated free energies of the species. The energy preferences regarding the positional isomerism in the substituted 1,3-thiazolo[3,2-*a*]benzimidazol-3(2*H*)ones **9**–**12** were estimated by the two isomers (**a** and **b**) of **10**.

As the tautomeric equilibrium in heterocyclic compounds depends strongly on the dielectric constant of the medium and the ability of the solvents to form hydrogen bonds with each one of the tautomers, the optimization of molecular structures **1**–**14** (Scheme 2) was carried out in chloroform and dimethyl sulfoxide. The respective total (G_{tot}) and relative (ΔG) energies are summarized in Table 3. The G_{tot} of compounds **1**–**2** in chloroform and DMSO indicated that the thione form is preferred. In order to estimate the kinetic barrier (free energy of activation) for thione-thiol interconversion, a potential energy scan for H-atom migration in **1a** was carried out (Fig. 17S in Supplementary material). The highest energy geometry identified by the scanning was then optimized to obtain G_{tot} of the transition state (Fig. 18S in Supplementary material). Thus the free energy of activation in chloroform was found to be 122.33 kJ mol^{-1} (29.24 kcal mol^{-1}). On the other hand, thiol **2b** would easily convert into thiol **2c** as according to the calculations the free energy difference between the two forms is within the range 0.69–1.12 kJ mol^{-1}. The thiol **2b** with substituent in position 5 of the benzimidazole ring is more favorable in comparison to thiol **2c** with substituent in position 6. The IR spectra of derivatives **2**–**4**, recorded in KBr disks, showed the simultaneous presence of thione and thiol form (IR-spectra of **2** are given in Figs. 15S and 16S in the Supplementary material). The existence of two different crystalline phases cannot be explained simply by the presence of thiol-thione tautomers, but also with the prototropic properties of the imide hydrogen in the benzimidazole ring.

Taking into consideration the calculation results for the 1*H*-benzimidazol-2-yltioacetic acids **6** and **14**, it should be pointed out that the preferred tautomers are those where the substituent is in position 6-th of the benzimidazole ring. For **6** the free energy difference ΔG between the 6-H (**6a**) and 7-H (**6b**) tautomer is 0.9–1.16 kJ mol^{-1}. The free energy of activation for interconversion between the tautomeric forms of 1H-benzimidazol-2-yltioacetic

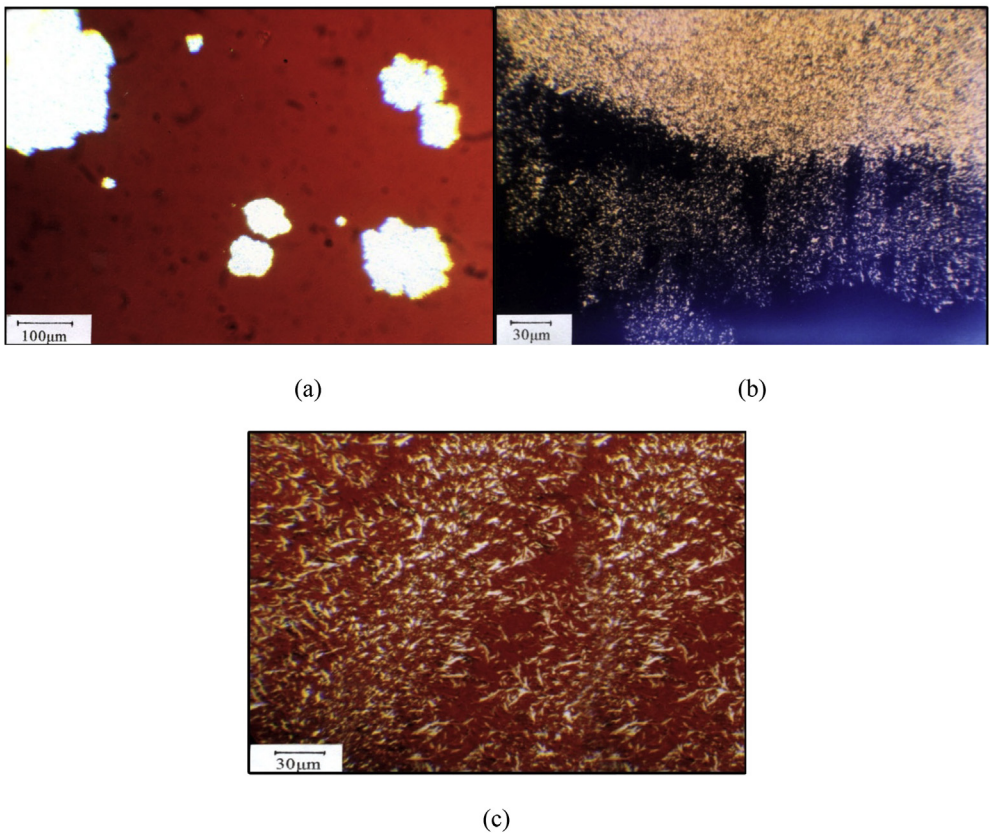


Fig. 5. Microscopic pictures of compound **13** in polarized light 24 h after preparation of the sample (a); 1 month after preparation of the sample (b) and 2 month after preparation of the sample (c).

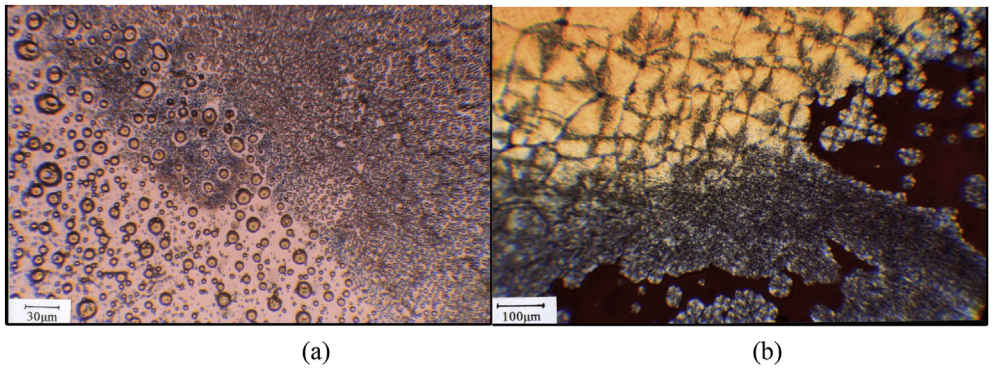
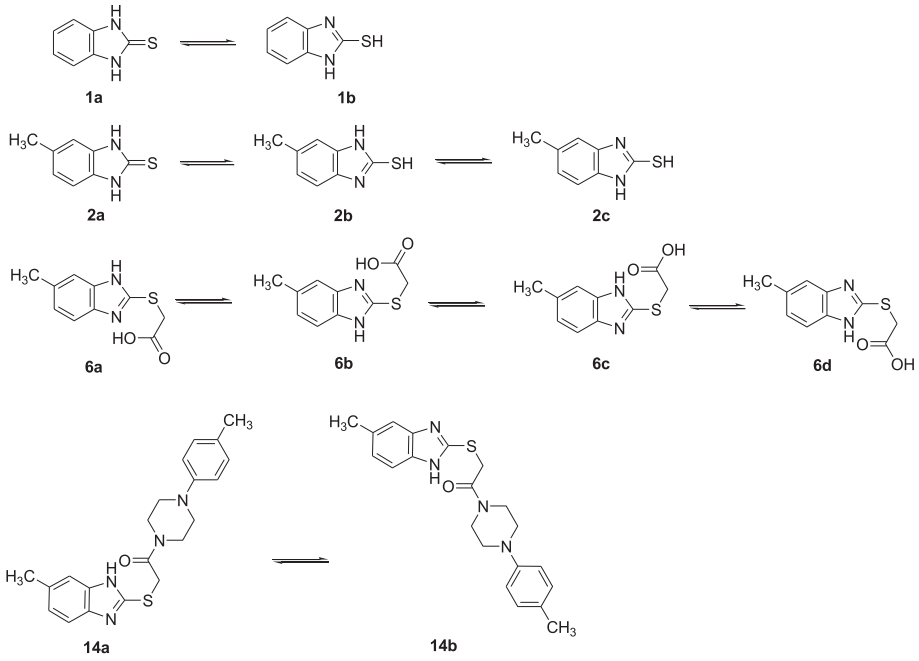


Fig. 6. Microscopic pictures of compound **14** in polarized light by use of ethanol as solvent (a) and chloroform as solvent (b).

acid **6** was modeled by calculating the energy requirements for rotation around the C–S bond and migration of the H-atom from the carboxylic group to the benzimidazolium N-atom in chloroform. H-atom migration to benzimidazolium N-atom is unfavorable and requires 27.56 kJ mol^{-1} (6.59 kcal mol^{-1}) according to the free energy difference ΔG between **6a** and the respective thione/carboxylate form (Fig. 19S in Supplementary material). The rotation is associated to 36.54 kJ mol^{-1} (8.73 kcal mol^{-1}) based on ΔG between **6a** and the highest energy conformer identified by the PES for rotation around the C–S bond (Fig. 20S in Supplementary material). Having in mind the small energy difference between the tautomeric forms of **6** and low activation barrier, it can be concluded that the presence of two crystal structures demonstrated by the PLM analysis is due to the prototropic properties of N1-hydrogen atom in the benzimidazole ring. The molecular structure is stabilized by the formation of a hydrogen bond between the N-atom from the benzimidazole ring and the hydroxy group (Fig. 7a).

Formation of a hydrogen bond C=O···H–N is also possible, but less favorable. Nevertheless, ΔG between the corresponding 6-H (**6c**) and 7-H (**6d**) tautomer remains low 0.34–0.48 kJ mol^{-1}. Similar results were also seen for **14a** and **14b** (ΔG 2.03–3.26 kJ mol^{-1}). In this case the molecular structure is stabilized by C=O···H–N interaction. The molecular configuration of the lower-energy tautomer **14a** is presented in Fig. 7b.

Based on the computational results, non-planar geometry of the investigated compounds was identified. The computed distances from the carbonyl oxygen to the hydrogen atom of 1(*H*)-



Scheme 2. Tautomeric equilibria for 1*H*-benzimidazole derivatives.

Table 3
DFT (B3LYP/6-311 + G**) Total (G_{tot}) and relative (ΔG) free energies of the studied species.

No.		Chloroform		DMSO	
		G_{tot} (a.u.)	ΔG[a] (kJ.mol^{-1})	G_{tot} (a.u.)	ΔG[a] (kJ.mol^{-1})
1	**1a**	−778.127325		−778.131668	
2	**1b**	−778.107821	51.21	−778.111107	53.98
3	**2a**	−817.431217		−817.435139	
4	**2b**	−817.410805	53.59	−817.414078	55.30
5	**2c**	−817.410544	0.69[b]	−817.413651	1.12[b]
6	**6a**	−1045.335400		−1045.340926	
7	**6b**	−1045.335057	0.90	−1045.340483	1.16
8	**6c**	−1045.329259	16.12	−1045.333538	0.48[d]
9	**6d**	−1045.329130	0.34[c]	−1045.333721	18.92
10	**10a**	−968.878772		−968.88235	
11	**10b**	−968.878687	0.22	−968.88190	1.18
12	**14a**	−1507.109035		−1507.115514	
13	**14b**	−1507.108261	2.03	−1507.114274	3.26

[a] $\Delta G = G_{nb} - G_{na}$.
[b] $\Delta G = G_{2c} - G_{2b}$.
[c] $\Delta G = G_{6d} - G_{6c}$.
[d] $\Delta G = G_{6c} - G_{6d}$.

benzimidazole cycle and from the hydroxyl group to the benzimidazolyl nitrogen atom are in the range of 1.75–1.91 Å, hence the possibility for H-bond forming is obvious. The presence of H-bond allows the formation of seven-member-pseudo-cycle, which additionally stabilizes the preferred configuration.

The energy difference between the positional isomers of **10** is almost negligible, with **10a** being slightly more favorable in nonpolar medium, while **10b** being preferred in polar medium.

4. Conclusions

Benzimidazolthiol derivatives were synthesized and the morphology of their crystal structure was investigated microscopically in polarized light. The structures of the compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and MS spectral data and studied by use of Density Functional Theory. As a result, the following conclusions were determined:

- According to DFT calculations the thione form of compounds **1** and **2** is more stable in nonpolar and polar medium than the

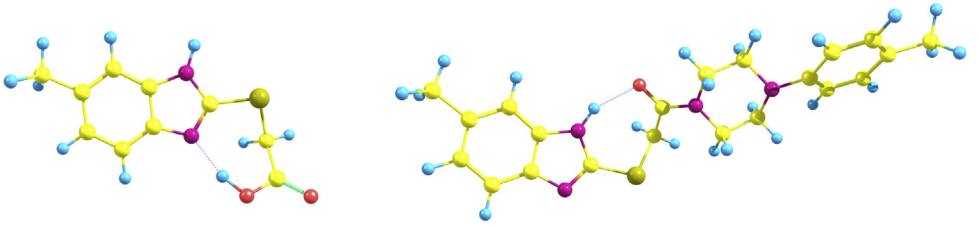


Fig. 7. Hydrogen bonding in tautomer **6a** (a) and tautomer **14a** (b).

thiol form. By the use of polarizing microscopy two different crystal structures were observed in solid phase for the compounds substituted in the benzimidazole ring;

- One distinct type of crystal structure was observed for each one of 5 or 6-methyl-(1H-benzimidazol-2-ylthio)acetic acid **6** - dendritic and needle-shaped formations were determined in polarized light. The derivative unsubstituted in the ring crystallized only in one phase;
- The compound **14**, containing a substituent in the benzimidazole ring crystallized in two phases (independent of the differences in the crystal structures obtained by the use of various solvents), while by the unsubstituted compound **13** a separation of phases does not take place.
- By the use of polarized light microscopy and the differential scanning calorimetry it was confirmed that the cyclization of the thioacetic acids in pyridine medium resulted in a mixture of two isomers 6 - and 7-substituted thiazolobenzimidazolones.
- The results of the examinations through microscopy in polarized light revealed that tautomeric benzimidazoles have a different crystal structure.

Acknowledgement

This study was supported by the Science Fund of the University of Chemical Technology and Metallurgy in Sofia, Grant number N 11252, and by the National Science Fund of Bulgaria, Contract RNF01/0110. The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molstruc.2017.08.073.

References

[1] K.F. Ansari, C. Lal, Synthesis and evaluation of some new benzimidazole derivatives as potential antimicrobial agents, Eur. J. Med. Chem. 44 (2009) 2294–2299.
[2] K. Kumar, D. Awasthi, S.-Y. Lee, I. Zanardi, B. Ruzsicska, S. Knudson, P.J. Tonge, A. Richard, R.A. Slayden, I. Ojima, Novel trisubstituted benzimidazoles, targeting Mtb FtsZ, as a new class of antitubercular agents, J. Med. Chem. 54 (2011) 374–381.
[3] M. Tunçbilek, T. Kiper, N. Altanlar, Synthesis and in vitro antimicrobial activity of some novel substituted benzimidazole derivatives having potent activity against MRSA, Eur. J. Med. Chem. 44 (3) (2009) 1024–1033.
[4] H.-Z. Zhang, G.L.V. Damu, G.-X. Cai, Ch.-H. Zhou, Design, synthesis and antimicrobial evaluation of novel benzimidazole type of fluconazole analogues and their synergistic effects with chloromycin, norfloxacin and fluconazole, Eur. J. Med. Chem. 64 (2013) 329–344.
[5] A.S. Alpan, S. Zencir, I. Zupko, G. Coban, B. Réthy, H.S. Gunes, Z. Topku, Biological activity of bis-benzimidazole derivatives on DNA topoisomerase I and HeLa, MCF7 and A431 cells, J. Enzyme Inhib. Med. Chem. 24 (3) (2009) 844–849.
[6] P. Kamaldeep, A. Sharma, V. Luxami, Synthesis and in vitro antitumor evaluation of primary amine substituted quinazoline linked benzimidazole, Bioorg. Med. Chem. Lett. 24 (2) (2014) 624–629.
[7] A. Sharma, V. Luxami, K. Paul, Synthesis, single crystal and antitumor activities of benzimidazole-quinazoline hybrids, Bioorg. Med. Chem. Lett. 23 (2013) 3288–3294.
[8] H. Nakano, T. Inoue, N. Kawasaki, H. Miyataka, H. Matsumoto, T. Taguchi, N. Inagaki, H. Nagai, T. Satoh, Synthesis and biological activities of novel antiallergic agents with 5-lipoxygenase inhibiting action, Bioorg. Med. Chem. 8 (2) (2000) 373–380.
[9] F. Scaglione, Safety profile of bilastine: 2nd generation H1-antihistamines, Eur. Rev. Med. Pharmacol. Sci. 16 (2012) 1999–2005.
[10] X.J. Wang, M.Y. Xi, J.H. Fu, F.R. Zhang, G.F. Cheng, D.L. Yin, Q.D. You, Synthesis, biological evaluation and SAR studies of benzimidazole derivatives as H1-antihistamine agents, Chin. Chem. Lett. 23 (6) (2012) 707–710.
[11] K.K. Biron, Antiviral drugs for cytomegalovirus diseases, Antivir. Res. 71 (2006) 154–163.
[12] A. Chimirri, S. Grasso, P. Monforte, A. Rao, M. Zappalà, A.M. Monforte, C. Pannecouque, M. Witvrouw, J. Balzarini, E. De Clercq, Synthesis and biological activity of novel 1H,3H-thiazolo[3,4-a]benzimidazoles: non-nucleoside human immunodeficiency virus type 1 reverse transcriptase inhibitors, Antivir. Chem. Chemother. 10 (1999) 211–217.
[13] L. Garuti, M. Roberti, G. Gentilomi, Synthesis and antiviral assays of some benzimidazole nucleosides and acyclonucleosides, Il Farm. 56 (2001) 815–819.
[14] S.K. Kotovskaya, Z.M. Baskakova, V.N. Charushin, O.N. Chupakhin, E.F. Belanov, N.I. Bormotov, S.M. Balakhnin, O.A. Serova, Synthesis and antiviral activity of fluorinated pyrido[1,2-a]benzimidazoles, Pharm. Chem. J. 39 (2005) 574–578.
[15] D.C. Pryde, Th-D. Tran, I. Gardner, H. Bright, P. Stupple, S. Galan, L. Alsop, L. Watson, D.S. Middleton, S. Dayal, M. Platts, E.J. Murray, T. Parkinson, R. Webster, Non-benzimidazole containing inhibitors of respiratory syncytial virus, Bioorg. Med. Chem. Lett. 23 (2013) 827–833.
[16] B.J. Banks, C.J. Dutton, A.C. Goudie, Benzimidazole anthelmintics, US5538990A (1996).
[17] W.S. Campbell, Chemotherapy of Parasitic Diseases, Plenum Press, New York (, 1986, p. 655.
[18] Y. Kasetti, P.V. Bharatam, Tautomerism in drugs with benzimidazole carbamate moiety: an electronic structure analysis, Theor. Chem. Acc. 131 (2012) 1160–1172.
[19] A.R. Katritzky, C.D. Hall, B.E.-D.M. El-Gendy, B. Draghici, Tautomerism in drug discovery, J. Comput. Aided Mol. Des. 24 (2010) 475–484.
[20] Y.C. Martin, Let's not forget tautomers, J. Comput. Aided Mol. Des. 23 (2009) 693–704.
[21] F.F. Ferreira, S.G. Antonio, P.C.P. Rosa, C.O. Paiva Santos, J. Pharm. Sci. 99 (2010) 1734.
[22] L. Houben, R. Ramaekers, L. Adamowicz, G. Maes, Tautomerism and non–planarity of the amino group in 4(7)–aminobenzimidazole: a theoretical and matrix–isolation FT–IR study, Internet Electron. J. Mol. Des. 3 (2004) 163–181.
[23] F. López-Vallejo, J.L. Medina-Franco, A. Hernández-Campos, S. Rodríguez-Morales, L. Yépez, R. Cedillo, R. Castillo, Castillo, Molecular modeling of some 1H-benzimidazole derivatives with biological activity against Entamoeba histolytica: a comparative molecular field analysis study, Bioorg. Med. Chem. 15 (2007) 1117–1126.
[24] F.T. Martins, P.P. Neves, J. Ellena, G.E. Camí, E.V. Brusau, G.E. Narda, Intermolecular contacts influencing the conformational and geometric features of the pharmaceutically preferred mebendazole polymorph, J. Pharm. Sci. 98 (2009) 2336–2344.
[25] C. Öğretir, İ.İ. Öztürk, N.F. Tay, Quantum chemical studies on tautomerism, isomerism and deprotonation of some 5(6)-substituted benzimidazole-2-thiones, ARKIVOC xiv (2007) 75–99.
[26] M.B. Pranzo, D. Cruickshank, M. Coruzzi, M.R. Caira, R. Bettini, Enantiotropically related albendazole polymorphs, J. Pharm. Sci. 99 (2010) 3731–3742.
[27] A. Ts Mavrova, K.K. Anichina, D.I. Vuchev, J.A. Tsenov, P.S. Denkova, M.S. Kondeva, M.K. Micheva, Antihelminthic activity of some newly synthesized 5(6)-(un)substituted-1H-benzimidazol-2-ylthioacetylpiperazine, Eur. J. Med. Chem. 41 (2006) 1412–1420.
[28] A. Ts Mavrova, K.K. Anichina, D.I. Vuchev, J.A. Tsenov, M.S. Kondeva, M.K. Micheva, Synthesis and antitrichinellosis activity of some 2-substituted-[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-ones, Bioorg. Med. Chem. 13 (2005) 5500–5559.
[29] R.A. Carlton, Pharmaceutical Microscopy, Springer Science + Business Media, LLC, 2011.
[30] J.G. Delly, Essential of Polarized Light Microscopy, fifth ed., College of Microscopy, 2008.
[31] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Li, X. Caricato, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A1, Gaussian Inc., Wallingford, CT, 2009.
[32] P.J. Stephens, F.J. Devlin, C.F. Chabalowski, M.J. Frisch, J. Phys. Chem. 98 (1994) 623.
[33] J. Tomasi, M. Perisco, Molecular interactions in solution: an overview of methods based on continuous distributions of the solvent, Chem. Rev. 94 (1994) 2027–2094.
[34] J. Tomasi, B. Mennucci, E.J. Cances, The IEF version of the PCM solvation method: an overview of a new method addressed to study molecular solutes at the QM ab initio level, J. Mol. Struct. (THEOCHEM) 464 (1999) 211–226.
[35] K.M.I. Tanaka, Y. Murakami, Cyclization of [(4- or 5-Substituted-2-benzimidazolyl)thio]acetic acids. Isolation and identification of two possible isomers of substituted thiazolo[3,2-a]benzimidazol-3(2H)-one, Chem. Pharm. Bull. 29 (7) (1981) 1876–1886.
[36] J. Mohan, H.K. Pujary, Heterocyclic systems containing bridgehead nitrogen atom: Part XIV – synthesis of 6-methoxythiazolo[3,2-a]benzimidazol-3(2H)-one & 3-substituted 6-methoxy[3,2-a]benzimidazoles, Indian J. Chem. 10 (1972) 274–276.

[37] H.S. Chaudhary, C.S. Panda, H.K. Pujari, Heterocyclic systems containing bridgehead nitrogen atom: Part VII – synthesis of 6-methyl-, 8-methyl-, 6,7-dimethyl, & 5,8-dimethyl-thiazolo[3,2-a]benzimidazol-3(2H)-one & 2H-7,8-dimethyl-[1,3]thiazolo[3,2-a]benzimidazol-4(3H)-one, Indian J. Chem. 8 (1970) 10.
[38] J. Mohan, V.K. Chadha, H.K. Pujari, Heterocyclic systems containing bridgehead nitrogen atom: Part XV, Synthesis of 6-chlorothiazolo[3,2-a]benzimidazol-3(2H)-one, 2H-8-chloro-[1,3]thiazolo[3,2-a]benzimidazol-4(3H)-one and 3-substituted 6-chlorothiazolo[3,2-a]benzimidazole, Indian J. Chem. 11 (1973) 1119.
[39] V.M. Rao, V.M. Reddy, Synthesis and biological activities of derivatives of mercaptobenzimidazoles. Part-II: same reactions of 2-mercapto-5(or 6)-nitrobenzimidazoles, J. Ind. Chem. Soc. 61 (1984) 89.
[40] G.R. Form, E.S. Raper, T.C. Downie, The crystal and molecular structure of 2-mercaptobenzimidazole, Acta Crystallogr. B32 (1976) 345–348.