STUDIA UBB CHEMIA, LXVII, 4, 2022 (p. 303-314)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2022.4.20

ALTERNATIVE PROCEDURES FOR THE GREEN SYNTHESIS OF 3,7-BIS(*N,N*-(2-HYDROXYETHYL)AMINO)PHENOTHIAZINIUM DYE

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ABSTRACT. Two experimental procedures implying mechanochemical and ultrasounds assisted protocols were assessed as alternatives to the classical synthesis of the title MB analogue, based on the substitution of the phenothiazinium tetraiodide with diethanolamine nucleophile. These greener alternatives required a much shorter reaction time and consequently less energy consumption. The sonochemical procedure gave a crystalline reaction product demanding a facile purification. UV-vis spectroscopic analysis emphasized the optical properties of the 3,7-bis(*N,N*-(2-hydroxyethyl)amino)phenothiazinium dye characterized by an intense absorption maxima situated at 663 nm and weak fluorescence emission in aqueous solution (quatum yield 0.7% relative to methylene blue standard) with the emission maxima situated at 685 nm (Stokes shift 484 cm^{-1}). The solid state structure of the dye was determined by X-ray diffraction.

Keywords: *Phenothiazinium dye, mechanochemistry, sonochemistry, XRD, UV-vis absorption spectroscopy*

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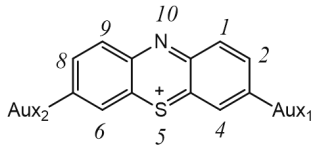
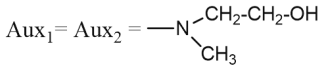
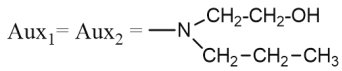
INTRODUCTION

Many synthetic compounds containing the phenothiazine heterocycle core exhibited *in vitro* or *in vivo* biological activities modulated by the introduction of structural appendages such as N-alkyl chains and/or (hetero)aromatic rings which may cause substantial amendments of the pharmacological properties (*e.g*. anti-microbial, anti-viral, anti-inflammatory, anti-oxidant, anti-tumour properties) [1]. Methylene blue (MB) is a phenothiazine derived cationc dye (3,7-*bis*(dimethylamino)phenothiazin-5-ium, figure 1) which display distinct photophysical, electrochemical, and biological properties and it is considered as the model compound for analogues series encompassing various substituents attached at the carbon atoms of the peripheral aromatic rings. [2]. A large number of MB analogues were designed by the systematic variation of the amino-auxochromes grafted in position C3 and C7 of the phenothiazinium chromophore (the oxidized state of the phenothiazine nucleus), thus paving the way towards MB dye congeners useful in modern biomedicine for selective staining, chemotherapeutics or photosensitizers for photoantimicrobial and photo dynamic therapy (PDT) applications [3-5]. By the way of illustration, in figure 1 are depicted the chemical structures of symmetrical (Figure 1a, 1b) or unsymmetrical (Figure 1c) 3,7-disubstituted phenothiazinium dyes bearing symmetrical (Figure 1a, 1c) or non-symmetrical (Figure 1b) secondary amino-auxochrome units comprising short C_1-C_3 alkyl and/or hydroxyalkyl chains imparting higher hydrophilicity.



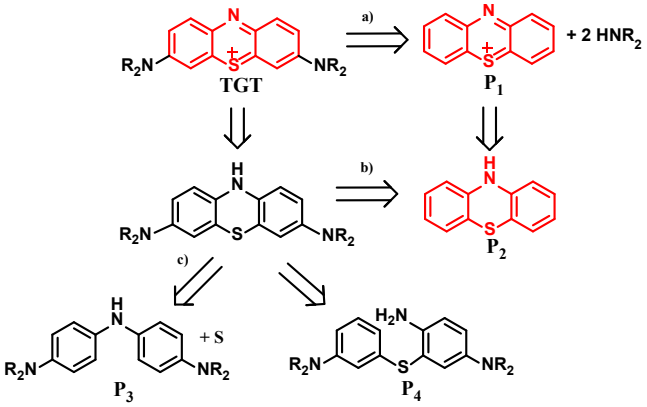
a)
- $Aux_1 = Aux_2 = -N(CH_3)_2$ **MB**
- $Aux_1 = Aux_2 = -N(C_2H_5)_2$
- $Aux_1 = Aux_2 = -N(C_3H_7)_2$
- $Aux_1 = Aux_2 = -N(CH_2\text{-}CH_2\text{-}OH)_2$

b)
- $Aux_1 = Aux_2 =$ 
- $Aux_1 = Aux_2 =$ 

c)
- $Aux_1 = -N(CH_2\text{-}CH_2\text{-}OH)_2$, $Aux_2 = -N(CH_3)_2$
- $Aux_1 = -N(CH_2\text{-}CH_2\text{-}OH)_2$, $Aux_2 = -N(C_3H_7)_2$

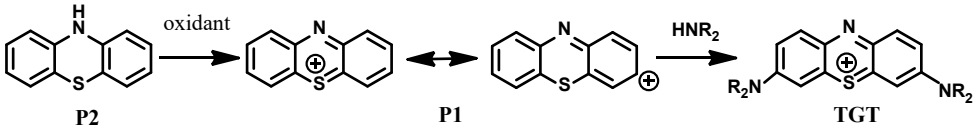
Figure 1. MB analogues: a) symmetrical 3,7-*bis*(dimethylamino)-, 3,7-*bis*(diethylamino)-, 3,7-*bis*(dipropylamino)- 3,7-*bis*(*bis*(2-hydroxyethyl)amino)- [6], b) 3,7-*bis*((2-hydroxyethyl)(methyl)amino)-, 3,7-*bis*((2-hydroxyethyl)(propyl)amino)- [7] c) unsymmetrical 3-dipropylamino-7-(*bis*(2-hydroxyethyl)amino)- [8] phenothiazinium dyes.

A retrosynthetic analysis of target MB analogues (**TGT**) summarized in **Scheme 1** proposes the molecular simplification by C-heteroatom bonds disconnection based on reliable transforms such as: path a) nucleophilic substitution of a phenothiazinium precursor **P1** with amines, path b) coupling of a phenothiazine precursor **P2** with amines path c) ring closure of diphenylamine **P3** or diphenyl sulphide **P4** precursors.



Scheme 1

A literature data survey indicate that the vast majority of the synthetic strategies applied in the preparation of MB analogues are based on the retrosynthetic path a) depicted in scheme 1, following the most straightforward reaction pathway shown in **Scheme 2**.



Scheme 2

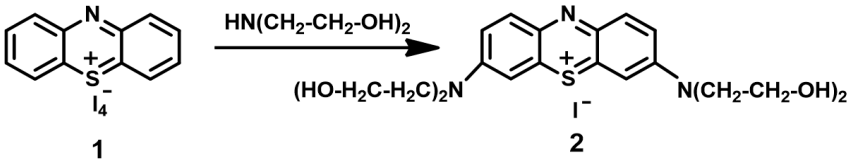
In the first reaction step, phenothiazine precursor **P2** is oxidized to the phenothiazinium cation **P1** which in the second step is subjected to substitution reaction using secondary amine nucleophiles. The success of this strategy is ensured on one hand by the high reactivity of the electron rich phenothiazine core toward oxidation and on the other hand by the regioselectivity of the nucleophilic substitution of the phenothiazinium cation in positions C3 and C7 oriented by the electron withdrawing effect of the thionium salt. The oxidation of the phenothiazine substrate can be achieved by using different reagents, the most widely employed being iodine (generating the phenothiazin-5-ium tetraiodide) followed by bromine (producing phenothiazin-5-ium perbromide). Symmetrical 3,7-disubstituted phenothiazinium dyes were obtained by using an excess of amine nucleophile, while non-symmetrical 3,7-disubstituted phenothiazinium dyes were prepared by a two stages methodology implying successive substitutions using two different amines [2]

The green chemistry philosophy recommends synthetic plans based on environmentally friendly strategies built on a careful selection of reagents and auxiliaries (*e.g.* minimal waste generation, reduced organic solvent use, sustainable feedstocks, effective catalytic processes) and reaction conditions for improved process efficiency. Improved energy efficiency can be attained by switching from a thermal source of energy (which partially dissipate energy in the surrounding area) towards more specific alternative energy inputs which may be beneficially applied (*e.g.* photochemical, microwaves, ultrasounds irradiation) [9] Over the years our research group accumulated knowledge and contributed to the design of new efficient green protocols for the preparation of phenothiazine derivatives by microwaves assisted synthesis (*e.g.* amino- [10], 1,3-dioxanyl- [11], or imino-phenothiazines [12] and sonochemistry [13]); the relevance of ultrasound irradiation and mechanochemical synthesis *versus* classical convective heating procedures was emphasized in the preparation of novel dyes such as (phenothiazinyl)vinyl-pyridinium [14] and MB analogues [15].

In this work, we examine the benefits brought by ultrasound assisted and mechanochemical synthetic procedures recently elaborated by our group, in comparison with classical procedures previously reported for the preparation of 3,7-*bis*(*N,N*-(2-hydroxyethyl)amino)phenothiazinium dye [6, 16], a MB analogue useful as direct dyestuff constituent of hair dye compositions [17]. The optical properties of this dye and its solid-state structure were investigated by UV-vis absorption/fluorescence emission in solution and X-ray diffraction respectively.

RESULTS AND DISCUSSION

The preparation of the symmetrical disubstituted MB analogue **2** is depicted in Scheme 3**.**



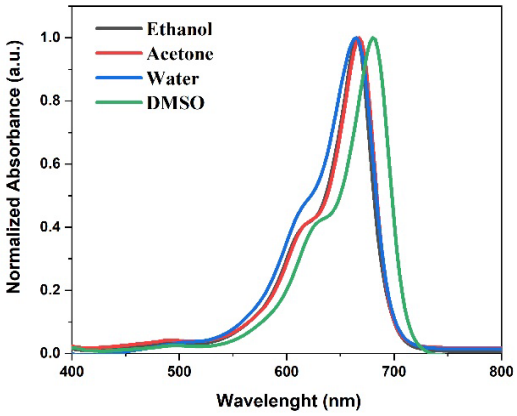
a) methanol, 24h, *r.t.* 40%
b) grinding 30 min 20%
c) methanol, *us*, 1h 26%

Scheme 3

The target 3,7-*bis*(*bis*(2-hydroxyethyl)amino)-phenothiazinium iodide **2** was obtained by subjecting phenothiazinium tetraiodide substrate **1** to a substitution reaction using an excess of diethanolamine nucleophile. Aiming the optimization of the reaction conditions, three alternative experimental procedures were applied: a) classical conditions in homogeneous methanol solution, b) solvent free mechanochemical conditions and c) ultrasound irradiation conditions. Even though the reaction yields obtained by applying mechano- and sonochemical conditions (20-26%) were not superior to the ones afforded by the classical ones (40%), these alternative procedures may be considered “greener” because they require a much shorter reaction time (30-60 minutes in comparison with 24hours) and consequently less energy consumption; moreover, the ultrasounds assisted procedure provided a crystalline reaction product demanding facile purification.

The optical UV-vis absorption properties of **2** recorded in four different solvents (figure 2) show a weak solvatochromism (376 cm^{-1}) upon switching from water to dimethylsulfoxide (DMSO), a solvent with known ability for stabilization by hydrogen bonding.

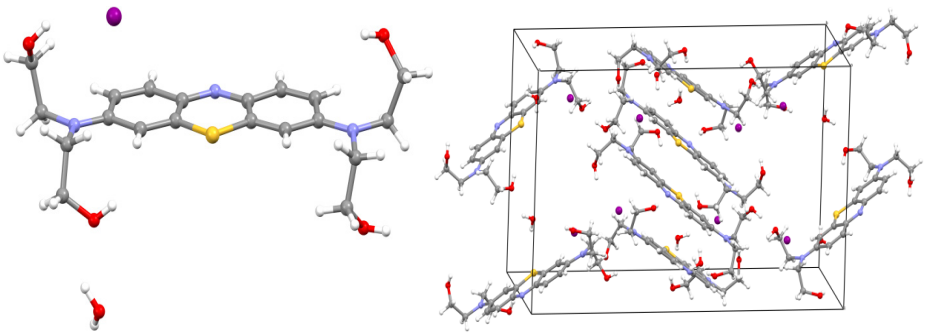
In aqueous solution, upon excitation of **2** with its longest absorption maxima ($\lambda_{max=}$664 nm) was recorded a fluorescence emission band situated at λ_{max}em= 685 nm with 484 cm^{-1} Stokes shift and fluorescence quantum yield 0.7% relative to MB standard (ϕ_F 2%.[18])



Solvent	λ [nm] (ε [cm $^{-1}$ M $^{-1}$])
Ethanol	667 (13272)
Acetone	667 (32202)
Water	664 (56058)
DMSO	681 (35896)

Figure 2. UV-vis absorption bands of **2** in various solvents.

Based on the crystal structure determination by X-ray diffraction, **2** was found to crystallize in the centrosymmetric orthorhombic Pbcn space group. The asymmetric unit is comprised by one 3,7-*bis*(dietanolamino)phenothiazinium cation, one recrystallization water molecule and one iodide anion (Figure 3a). Unit cell is packing eight such asymmetric units which are generated via symmetry operations (Figure 3b).



a) b)

Figure 3. Crystal structure of **2:** a) asymmetric unit; b) unit cell packing

The folding angle between the two aromatic rings of the phenothiazinium core is 178.48° depicting a planar conformation. Such almost planar geometries were reported in literature by single crystal X-ray diffraction on various derivatives which belong to phenothiazine group [19-21].

The supramolecular self-assembled layers of molecules are shaped in a zig-zag fashion with the recrystallization water located between and connecting these layers via O-H···O interactions while the iodide anion is located roughly on the layers (Figure 4). It is worth mentioning that the adjacent molecules (related by a glide plane) are interacting via O-H···N (hydroxyl···thiazine) hydrogen bonds and phenyl···phenyl interactions as well (π···π interactions with a contact distance of 3.292 Å and 3.366 Å respectively) which are contributing to the stabilization of zig-zag layout.

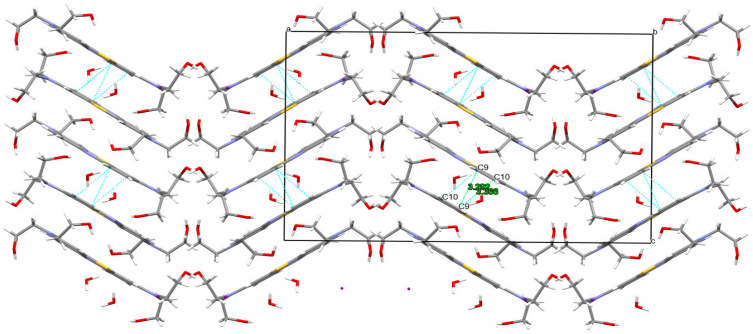


Figure 4. Overall packing perspective seen along *a-axis* displaying zig-zag molecular arrangements

CONCLUSIONS

The 3,7-*bis*(*N,N*-(2-hydroxyethyl)amino)phenothiazinium iodide **2** is a blue dye displaying an intense absorption maxima situated at 663 nm, a weak fluorescence with the emission maxima situated at 685 nm, which crystallize in the centrosymmetric orthorhombic Pbcn space group. The classical synthetic procedures requiring a long reaction time could be amended by greener alternatives such as the hereby described mechanochemical or ultrasounds assisted procedures.

EXPERIMENTAL SECTION

NMR spectra were recorded on Brucker NEO-1 400 MHz instrument. UV-vis absorption spectra were recorded with Perkin Elmer Lambda 35 spectrophotometer. UV-vis emission spectra were recorded on Perkin Elmer LS55 spectrophotometer. HRMS spectra were recorded on Thermo LTQ Orbitrap XL instrument. The powder X-Ray diffraction pattern was recorded on a Bruker D8 Advance diffractometer (X-ray tube operates at 40 kV, 40 mA) which is equipped with a germanium (1 1 1) monochromator and LYNXEYE detector using monochromatic CuKα1 radiation (λ=1.54056 Å).

All chemicals used were of reagent grade.

Starting material Phenazathionium tetraiodide **1** was prepared by the oxidation of phenothiazine with iodine in DCM solution according to the previously reported procedure [22].

Experimental procedures for the synthesis of 3,7-bis(N,N-(2-hydroxyethyl)amino)phenothiazinium iodide **2**

a) Classical synthesis

In a round-bottom flask with a magnetic stirrer and a reflux condenser were introduced 1.5 g (0.002 mol) of phenothiazinium tetraiodide **1** and 40 ml of methanol and then diethanolamine (3ml, 3.27g, 0.03 mol) was added dropwise over 30 minutes. The reaction mixture was stirred for 24 hours at room temperature. After the completion of the reaction, the solvent was removed by vacuum distillation. The solid product was purified by reprecipitation from methanol solution with diethyl ether. The dark precipitate was filtered affording the pure product (yield of 40 %).

b) Mechanochemical synthesis

In a mortar, phenothiazinium tetraiodide **1** (1.5g, 0,002 mol) and diethanolamine (3ml, 3.27g, 0.03 mol) were added and the mixture was grounded with a pestle for 30 minutes. The reaction product was dissolved in methanol and reprecipitated with diethyl ether. The dark precipitate was filtered affording the pure product (yield of 20%).

c) Sonchemical synthesis

Phenothiazinium tetraiodide **1** (1.5 g, 0.002mol) and diethanolamine (3ml, 3.27g, 0.03 mol) were dissolved in ethanol and were introduced into a pear-shaped flask. The flask was equipped with a septum with a syringe needle for degasing the volatile products. After 1 hour of ultrasonating the

solvent was recovered and the product was purified by reprecipitation with methanol and diethyl ether. The dark precipitate was filtered affording the pure product (yield of 26%).

^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 7.81 (d, ^{3}J= 8Hz, 2H), 7.51 (m, 4H), 5.03 (t, ^{3}J= 7Hz, 4H), 3.85 (m, 8H), 3,72 (m, 8H).

^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 154.42, 137.96, 135.44, 133.88, 120.05, 107.5, 59.12, 54,43.

HRMS: calculated for $C_{20}H_{26}NO_4S^+$ 404.13865, found 404.157.

X-Ray powder diffraction

The powder X-Ray diffraction pattern was recorded on a Bruker D8 Advance diffractometer (X-ray tube operates at 40 kV, 40 mA) which is equipped with a germanium (1 1 1) monochromator and LYNXEYE detector using monochromatic CuKα1 radiation (λ=1.54056 Å). The CIF file of **2** has been deposited with the Cambridge Crystallographic Data Centre, having the associated deposition number 2225437.

The crystal structure determination for **2** was carried out from a high-resolution X-ray powder diffraction pattern. The method implies the determination of a structural model by direct-space Monte-Carlo with parallel tempering methods.

The procedure of crystal structure determination from a powder diffraction pattern is a multi-step procedure and requires the use of several computation methods: diffraction pattern indexing; Pawley refinement; space group determination; the search for a structural model and the final Rietveld refinement [23].

Reflex module implemented in Materials Studio software [24] was used for pattern indexing via multiple programs such as TREOR90 [25], DICVOL96 [26], X-cell [27]. The indexing offers multiple possible solutions of unit cells but based on the figure of merit, a common solution of lattice parameters was obtained in all three programs, which belongs to the orthorhombic crystal system and possess the following lattice constants: a=25.49 Å, b=10.91 Å, c=16.28 Å, α=β=γ=90°, V=4531 Å^3. Further the solution was subjected to Pawley refinement, the orthorhombic crystal system being confirmed and the unit cell was found to belong to the Pbcn space group. Based on the molecular composition of the analysed compound, the asymmetric unit would consist of one 3,7-*bis*(dietanolamino)phenothiazinium molecule ($C_{20}H_{26}N_3O_4S$), one water molecule and a iodine anion such that the calculated density is roughly 1.60 g/cm^3. As a next step, the search of structural model was accomplished via Powder Solve module employing

Monte Carlo and parallel tempering optimization. The method is based on molecular translations, rotations and changes in torsion angles in the unit cell. A few million trials per cycle are verified and a comparison between simulated and experimental pattern after each change is carried out such as the match to be as good as possible. Rietveld refinement technique is used in order to refine the structural solution obtained by parallel tempering optimization. A Pseudo-Voight function was used to approximate the diffraction peaks profiles along with the U, V, W parameters of Caglioti's equation [6] were refined. Bragg-Brentano instrument geometry was considered with profile parameters, shift parameters, zero point refined as well. The asymmetry of diffraction lines was refined in a Berar-Baldinozzi approximation. The background was approximated by a polynomial function of order 20 and the preferred orientation parameters were refined considering the March-Dollase correction. As a result, a final comparison between calculated (theoretical) and experimental powder X-ray pattern is shown in Figure 5 and crystallographic details are presented in Table 1.

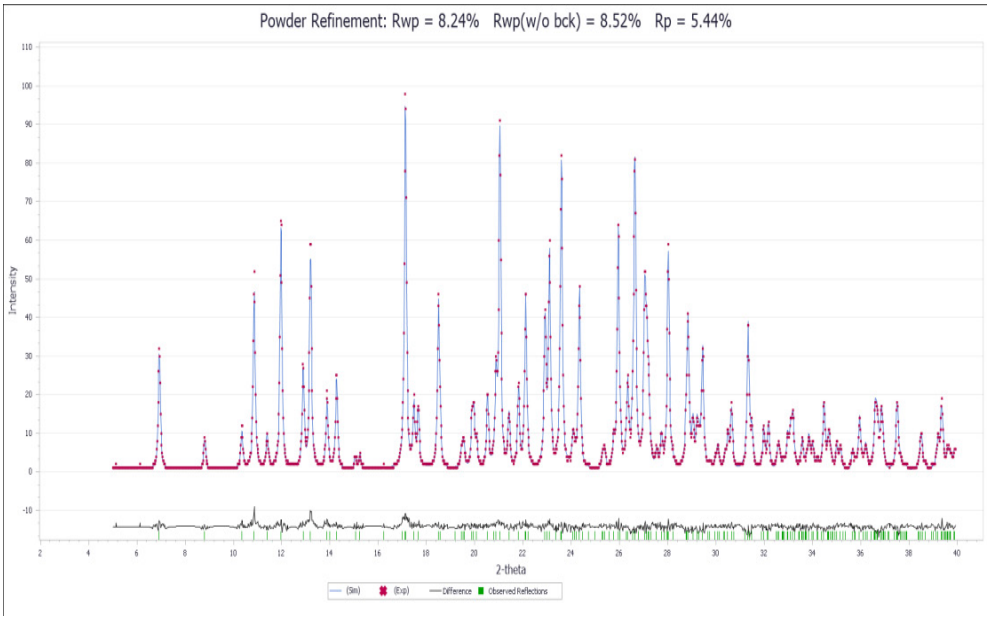


Figure 5. Rietveld refinement highlighting the match between simulated and experimental pattern

Table 1. Crystallographic details for **2** obtained by powder X-ray diffraction analysis

Chemical formula	**$C_{20}H_{28}IN_3O_5S$**
Formula weight (g/mol)	**548.40**
Crystal system	**Orthorhombic**
Space group	**Pbcn (No. 60)**
Z	8
a (Å)	25.50
b (Å)	10.91
c (Å)	16.28
α (°)	90
β (°)	90
γ (°)	90
V (Å^3)	4532
R_{wp} (%)	8.24
ρ_{calc} g/cm^3	1.60

ACKNOWLEDGMENTS

This work was supported by a grant of Romanian Ministry of Research and Innovation, CNCS–UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0142, within PNCDI III.

REFERENCES

1. C. Gopi, M. D. Dhanaraju, *Rev. J. Chem., **2019**, 9(2), 95–126.*
2. P.L. Padnya, A.I. Khadieva, I.I. Stoikov, *Dyes Pigm.*, **2022**, *208*, 110806.
3. M. Wainwright, *Biotechn. Histochem.,* **2003**, *78(3-4)*, 147-155.
4. C. M. L. Francisco, J. M. L. A. Gonçalves, B. S. Brum, T. P. C. Santos, A. Lino-dos-Santos-Franco, D. F. T. Silva, C Pavani, *New J. Chem.*, **2017**, *41*, 14438-14443.
5. M Wainwright, *Photodiagnosis Photodyn. Ther.,* **2005**, *2*, 263-272.
6. G. De Crozals, C. Farre, M. Sigaud, P. Fortgang, C. Sanglar, C. Chaix, *Chem. Commun.*, **2015**, *51*, 4458-4461.

7. A. Gollmer, A. Felgentrager, W. Baumler, T. Maisch, A. Spath*, Photochem. Photobiol. Sci.*, **2015**,*14*, 335-351.
8. S. A. Gorman, A. L. Bell, J. Griffiths, D Roberts, S. B. Brown, *Dyes Pigm.,* **2006**, *71* 153-160.
9. P. Anastas, J. Warner, "*Green Chemistry Theory and Practice*", Oxford Univ. Press, **1998**.
10. L.I. Găină, L.N. Mătărângă-Popa, E. Gal, P. Boar, P. Lönnecke, E. Hey-Hawkins, C. Bischin, R. Silaghi-Dumitrescu, I. Lupan, C. Cristea, L. Silaghi-Dumitrescu, *Eur. J. Org. Chem.*, **2013**, *24*, 5500–5508.
11. L. Gaina, E. Gal, L. Matarânga-Popa, D. Porumb, A. Nicolescu, C. Cristea, L. Silaghi-Dumitrescu, *Tetrahedron*, **2012**, *68(11),* 2465 – 2470.
12. L. Gaina, T. Lovasz, I. A. Silberg, C. Cristea, S. Udrea, *Het. Commun.,* **2001***,* 7(6), 549–554.
13. E. Gal, L. Gaina, H. Petkes, A. Pop, C. Cristea, G. Barta, D. C. Vodnar, L. Silaghi-Dumitrescu, *Beilstein J. Org. Chem.,* **2020**, *16,* 2929–2936.
14. B. Stoean, D. Rugina, M. Focsan, A.-M. Craciun, M. Nistor, T. Lovasz, A. Turza, D.I. Porumb, E. Gál, C. Cristea, L. Silaghi-Dumitrescu, S. Astilean, L. Gaina, *Int. J. Mol. Sci.*, **2021**, *22*, 2985.
15. B. Stoean, L Gaina, C. Cristea, R. Silaghi-Dumitrescu, A. M. V. Branzanic, M. Focsan, E. Fischer-Fodor, B. Tigu, C. Moldovan, A. D. Cecan, P. Achimas-Cadariu, S. Astilean, L. Silaghi-Dumitrescu, *Dyes Pigm.,* **2022**, *205*, 110460.
16. D. Creed, Willie C. Burton, and Newton C. Fawcett, *J. Chem. Soc., Chem. Commun*. **1983**, 1521-1523.
17. A. Lagrange, EP1607393, **2005**.
18. P.T.K. Chin, M.M. Welling, S.C.J Meskers, R.A. Valdes Olmos, H. Tanke, F.W.B. Van Leeuwen *Eur J Nucl Med Mol Imaging,* **2013**, *40*, 1283–1291.
19. W. Li, Q. Huang, Z. Mao, J. Zhao, H. Wu, J. Chen, Z. Yang, Y. Li, Z. Yang, _Y. Zhang, M.P. Aldred, Z. Chi, *Angew.Chem., Int. Ed.,* **2020**, *59*, 3739.
20. L. Marin, A. Bejan, S. Shova, *Dyes Pigm.,* **2020**, *175*, 108164.
21. R.B. Von Dreele, J. Harris, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.,* **1983**, *39*, 170.
22. J. A. Cody, C.S. Larrabee, M. D. Clarck, S. Hlynchuk, J. A. Tatum, *Tet Lett.,* **2012**, *53*, 4896-4899.
23. A. Meden, *Croat. Chem. Acta,* **1998,** *71,* 615–633.
24. Dassault Systèmes BIOVIA, **2014** [Materials Studio], [v8.0.0.843], San Diego: Dassault Systèmes.
25. P-E. Werner, L. Eriksson, M. Westdahl, *J Appl. Cryst*., **1985**, *18,* 367–370.
26. A. Boultif, D. Louer, *J. Appl. Cryst*., **2004**, *37*, 724-731.
27. M. A. Neumann, *J. Appl. Cryst.,* **2003**, *36*, 356-365.