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SYNTHESIS AND BIOLOGICAL PROPERTIES OF NOVEL CATIONIC FLUORESCENT DYE

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Abstract— **The synthesis of a new cationic fluorescent 4-allylamino-N-sulfadiazine-1,8-naphthalimide quaternised with 1-bromopropane has been described. The characterisations of the prepared compound 3 and its intermediates (compounds 1 and 2) were carried out by DSC, FTIR, ^{1}HNMR and ^{13}CNMR techniques. Its photophysical characteristics in organic solvent DMF have been determined using absorption and fluorescence spectroscopy. The newly synthesized compounds have been tested in vitro for its antimicrobial activity against bacteria and fungi. The results obtained suggest that the newly synthesized compounds are suitable in designing new effective antimicrobial preparations.**

Index Terms **— synthesis, naphthalimide dyes, antimicrobial activity, photophysical properties.**

I. INTRODUCTION

1,8-Naphthalimides and their 4-substituted derivatives are well known as typical intramolecular charge transfer chromophore which have been investigated in a large variety of areas due to their strong absorption and emission in the visible region, high photostability and large Stokes shift [1–3]. They can also be used as yellow daylight fluorescent pigments, fluorescent dichroic dyes in liquid crystal displays and fluorescent brighteners in detergents, textiles, papers, plastics and paints [4-6]. Also, naphthalimides with a naphthalene framework and cyclic double imides moiety have been widely investigated as anticancer agents, antimicrobial agents etc. They have been found to possess large clinical potentiality in the treatment of cancers acting by intercalating deoxyribonucleic acid (DNA) [7,8]. This special mechanism has attracted much interest in exploiting other medicinal potentialities of naphthalimides especially their antibacterial and antifungal behaviors. In addition, some of the known structures with microbiological activity are the compounds containing quaternary nitrogen atom which are used in many fields, such as water treatment, medicine and healthcare products, food applications, and textile products. The presence of a quaternary ammonium group enables the biostatic activity of dye because of the positive charge at the N-atom inflicts a variety of detrimental effects on microbes, including damage to cell membranes, denaturation of proteins and disruption to the cell structure [9]. In this paper we report the synthesis and photophysical characteristics of a new cationic fluorescent naphthalimide dye and its intermediates (Figure 1). The novel compounds characterized with DSC, FTIR, ^{1}HNMR and ^{13}CNMR techniques. The antimicrobial activity of dyes in soluble state has been also investigated.

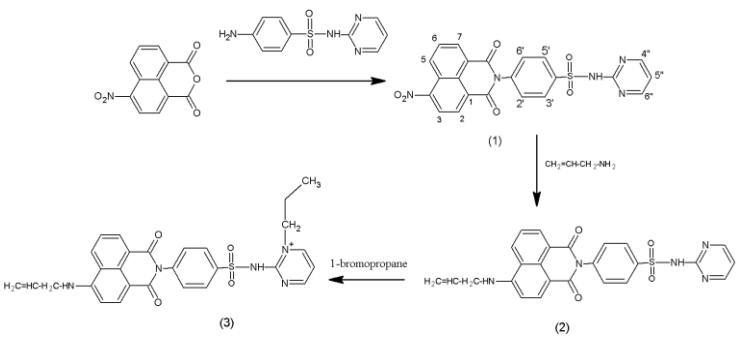

Fig 1. Synthesis of novel compound and its intermediates

II. EXPERIMENTAL PART

Material and methods

The synthesis methods of 5-nitroacenaphthene, 4-nitro-1,8-naphthalic anhydride are reported in the literature [2,4,5]. Acenaphthene, sulfadiazine and 1-bromopropane in pure analysis grade were used as obtained from Merck and Aldrich companies. All organic solvents (Aldrich, Merck) used in this study were of spectroscopic grades. The FTIR spectra were recorded on a Perkin Elmer Spectrum One spectrophotometer on a KBr disc. The ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker spectrometer operating at 400 MHz. The melting points were determined by means of a PerkinElmer Pyris 6 differential scanning calorimeter (DSC). The UV–Visible absorption spectra were recorded on a Cecil 9200 double beam spectrophotometer in DMF solvent. The fluorescence spectra were taken on a Perkin Elmer LS50B spectrofluorimeter.

Synthesis 4-nitro-N-sulfadiazine-1,8-naphthalimide

(Compound 1)

A solution of sulfadiazine (0.01 mol) with 4-nitro-1,8-naphthalic anhydride (0.01 mol) in 100 ml of glacial acetic acid was stirred under reflux for 48 h separately. The crude product which precipitated while cooling down, filtered off, washed with water and treated with 50 ml of 5% aqueous sodium carbonate. The precipitation was filtered off, washed with water and dried. The product was recrystallized with acetic acid and the crystals were creamy.
Yield: 56%; Melting Point (M.P.): 315-316ºC; FTIR (KBr), cm^{-1}: 3100 (N–H str. secondary amino group); 3010 (C–H str. Ar); 1712, 1678 (C=O str. carbonyl groups); 1533, 1349 (NO_2 str. unsym. and sym.); 1238, 1162 (SO_2 str. unsym. and sym.). ^{1}HNMR (400 MHz, DMSO) ppm: 7.07-7.09 (t, 1H, *J*=4.8, C-5”); 7.62-7.64 (d, 2H, C-3’, C-5’); 8.13-8.15 (3H, C-6, C-2’, C-6’); 8.54-8.55 (d, 2H, C-4”, C-6”); 8.58-8.59 (d, 2H, C-2, C-5); 8.61-8.63 (d, 1H, *J*=7.2, C-7); 8.74-8.76 (d, 1H, *J*=8.8, C-3); 12.2 (S, 1H, NH); ^{13}CNMR (DMSO) δ: 116.2, 123.3, 123.3, 123.7, 124.7, 124.7, 127.5, 128.7, 129.2, 129.5, 130.2, 130.6, 132.2, 139.8, 141.2, 149.8, 157.5, 158.9, 158.9, 162.7, 162.7, 163.5.

Synthesis of 4-allylamino-N-sulfadiazine-1,8-naphthalimide

(Compound 2)

To a solution of compound **1** in DMF, allylamine was added at room temperature separately. After 24 h, the final stage of reaction was controlled by TLC in a solvent system n-hexane: acetone (1:1). The resulting solution was poured into 300 ml of water. The precipitation was filtered off, washed with water and dried. Recrystallization from ethanol offered the compound **2** as orange crystals.
Yield: 74%; Melting Point (M.P.): 201-204ºC; FTIR (KBr), cm^{-1}: 3385 (N–H str. secondary amino group); 3083 (CH=) 2939 (C–H str. aliphatic) 1699, 1659 (C=O str. carbonyl groups); 1239, 1162 (SO_2 str. unsym. and sym.). ^{1}HNMR (400 MHz, DMSO) ppm: 3.1 (S, 1H, allyl NH); 3.42 (2H, allyl NCH2); 4.06 (2H, allyl =CH2); 5.22 (1H, allyl CH=); 7.03-7.08 (d, 1H, *J*=4.6, C-3); 7.13-7.18 (t, 1H, *J*=5.6, C-5”); 7.5-7.53 (2H, C-3’, C-5’); 7.61-7.84 (2H, C-2’, C-6’); 8.06-8.15 (t, 1H, *J*=8.7, C-6); 8.18-8.26 (d, 2H, C-4”, C-6”); 8.38-8.58 (3H, C-2, C-5, C-7); 11.25 (S, 1H, NH); ^{13}CNMR (DMSO) δ: 56.4, 109.5, 111.5, 114.3, 115.8, 121.1, 121.3, 121.3, 124.8, 124.8, 125.4, 126.5, 127.1, 130.2, 133.8, 134.2, 137.5, 137.9, 140.7, 151.4, 156.8, 156.8, 162.9, 169.7, 169.7.

Quaternization of compound 2 with 1-bromopropane,

(compound 3)

The compound **2** (0.036 mmol) and excess 1-bromopropane (2.67 mmol) was mixed in dimethylformamide for 48 h at temperature of 50°C. Quaternized compound **3** was evaporated by rotary instrument and the product was precipitated with tetrahydrofuran (THF). The crude dye was recrystallized with ethanol and yellow solid was obtained.
Yield: 55%; Melting Point (M.P.): 198ºC; FTIR (KBr), cm^{-1}: 3265 (N–H str. secondary amino group); 3020 (CH=) 2910 (C–H str. aliphatic) 1678, 1640 (C=O str. carbonyl groups); 1258, 1140 (SO_2 str. unsym. and sym.). ^{1}HNMR (400 MHz, DMSO) ppm: 1.13-1.19 (7H, N^{+}-$\underline{CH_2}$-$\underline{CH_2}$-$\underline{CH_3}$); 3.25 (S, 1H, allyl NH); 3.65 (2H, allyl NCH2); 4.12 (2H, allyl =CH2); 5.4 (1H, allyl CH=); 6.98-7.02 (d, 1H, *J*=4.6, C-3); 7.21-7.23 (t, 1H, *J*=5.6, C-5”); 7.65-7.68 (2H, C-3’, C-5’); 7.86-7.96 (2H, C-2’, C-6’); 8.11-8.21 (t, 1H, *J*=8.7, C-6); 8.22-8.28 (d, 2H, C-4”, C-6”); 8.45-8.49 (3H, C-2, C-5, C-7); 11.1 (S, 1H, NH); ^{13}CNMR (DMSO) δ: 11.3, 22.5, 48.6, 52.5, 108.6, 112.7, 115.3, 116.4, 122.3, 123.3, 123.3, 125.4, 125.4, 126.2, 127.1, 128, 131.1, 133.2, 135.1, 137.8, 138.2, 141.3, 152.4, 157.1, 158.4, 163.2, 170.3, 170.3.

Antimicrobial activity test

The newly synthesized dyes were investigated for antimicrobial activity by the conventional agar well-diffusion method against Gram-positive bacteria and Gram-negative bacteria and Candida albicans fungi. Muller Hinton agar (MHA) medium was purchased from Merck Company. The wells (6 mm diameter) were made in the medium which was streaked with a saline suspension of the overnight bacterial agar culture with a turbidity microorganism equivalent to a 0.5 McFarland. 100 µl of each compound with concentration equal to 1 mg/ml in DMSO (20% V/V) were added in each well and the plates were subsequently incubated at 37 ˚C for 24 h. Zone of

inhibition produced by each compound measured in millimeters (mm) after 24 h. Also, MIC of dyes were determined by broth micro dilution method using Muller-Hinton Broth medium (Merck, Germany) against the test organisms [10].

III. RESULTS & DISCUSSION

Chemical structure properties

The synthetic route for the preparation of cationic compound **3** is outlined in Figure 1. 4-nitro-N-sulfadiazine-1,8-naphthalimide has been obtained by nitration of anhydride naphthalic. The FTIR spectra showed the elimination of anhydride group (1800 cm^{-1}) in 4-nitro-1,8-naphthalic anhydride in products which considers to be a proof of reaction completion. The anhydride group of 4-nitro-1,8-naphthalic anhydride in final product disappears. Allylation of the nitro group was carried out with allylamine at room temperature for 24 h in DMF solution. The obtained product 4-allylamino-N-sulfadiazine-1,8-naphthalimide was isolated by pouring the reaction mixture into water and filtering the precipitate. This compound has an $-NR_2$, which react with 1-bromopropane to obtain the final cationic dye as a yellow precipitate.

The data of ^{1}HNMR compounds **2** and **3** confirmed the presence of the singlet peaks in regions 3.1 and 3.25 ppm which are related to the proton of amine in $NH\text{-}CH_2\text{-}CH{=}CH_2$ group in compounds **2** and **3**, respectively. Also, the presence of the peaks in regions 1.13 to 1.19 ppm which are related to the protons of aliphatic carbons in $N^+\text{-}\underline{CH_2}\text{-}\underline{CH_2}\text{-}\underline{CH_3}$ group in compound **3**.

Photophysical characteristics

The photophysical data of dyes solutions in DMF is shown in Table 1. The absorption band of these compounds **2** and **3** were in the range of 426 to 434 nm. The maximum wavelengths of naphthalimide dyes are related to the electron donating and accepting power of substituents in C-4 position and carbonyl groups, respectively. In compounds **2** and **3** the electron donor group is amino which is a strong donor group. The fluorescent characteristic of compounds **2** and **3** were measured in DMF. Compounds **2** and **3** show intense yellow-green fluorescence due to the charge transfer in the naphthalimide units from the electron donating amino group at C-4 position to the accepting carbonyl groups. The emission of compounds **2** and **3** is in the visible region at about 527 and 527 nm, respectively.

Antimicrobial activity

The newly synthesized compounds were tested in vitro for antimicrobial activity against four bacterial and candida albicans fungi and are represented in Table 1 and 2. Highest inhibition activity was observed against tested fungi strains (zones of inhibition 22 and 24 mm for compounds **2** and **3**, respectively). The MIC of synthesized dyes against the studied microbial cultures was determined by broth micro dilution method. As it can be seen in Table 1, the MIC of compounds **2** and **3** was in the range 62.5–500 µg/ml and 31.25-500 µg/ml, respectively, indicating good antimicrobial potential. As it can be seen in Table 1, the inhibitory activity of antimicrobial dyes against the Gram-positive bacteria was higher than that against the Gram-negative bacteria [11] These observations may be attributed to the structural differences between the bacteria. For the Gram-negative bacteria, the main component of the cell walls is a rigid network composed of three macromolecular concentric shells, while the Gram-positive bacteria have a network that is only one molecule thick, together with up to 25% (mass) of lipoprotein and lipopolysaccharide [11]. Therefore, the Gram-negative bacteria represent greater resistance to mechanical rupture than the Gram-positive cells. Also, the results illustrated that compound **3** has a higher antimicrobial efficacy than the compound **2** as a result of the quaternary ammonium salts in its molecular structure. The positive electrical charges allow the dye molecules to adsorb readily into the microbial surfaces and then penetrate the cell membrane, followed by the destruction of the cell membranes and the leakage of the cell inclusion body. Simultaneously, bacterial enzyme systems are destroyed, causing bacteria to die [4].

TABLE I. ANTIMICROBIAL ACTIVITIES (INHIBITION ZONES AND MIC) OF

Compound	**Antibacterial bacteria Activity**					
	S. aureus		***M. luteus***		***B. subitilis***	
	Zone inhibition (mm)	MIC	Zone inhibition (mm)	MIC	Zone inhibition (mm)	MIC
2	16	62.5	16	125	14	250
3	17	31.25	17	62.5	13	250

SYNTHESIZED DYES AGAINST GRAM POSITIVE BACTERIA

TABLE II. ANTIMICROBIAL ACTIVITIES (INHIBITION ZONES AND MIC) OF

Compound	**Antibacterial bacteria Activity**		**Antifungal Activity**	
	E.coli		***C. albicans***	
	Zone inhibition (mm)	MIC	Zone inhibition (mm)	MIC
2	10	500	22	125
3	14	500	24	125

SYNTHESIZED DYES AGAINST GRAM NEGATIVE BACTERIA AND FUNGI

IV. CONCLUSION

In this study we reported the synthesis and characterization of a new cationic naphthalimide derivative (compound **3**) having quaternary ammonium group and its intermediates. Photophysical characteristics of the synthesized dyes have been determined in DMF solvent. Compounds **2** and **3** showed intense yellow-green fluorescence. The antibacterial and antifungal activities of the synthesized dyes were evaluated against three bacteria and Candida albicans. The results showed good inhibitory activity of the synthesized dyes against the tested bacteria and fungi cultures. Compound **3** has a higher antimicrobial efficacy than the compound **2** as a result of the quaternary ammonium salts in its molecular structure.

REFERENCES

[1] K. Kawai, K. Kawabata, S. Tojo and T. Majima, "Synthesis of ODNs Containing 4-Methylamino-1,8-naphthalimide as a fluorescence probe in DNA," Bioorg. Med. Chem. Lett., vol. 12 pp.2363–2366, September 2002.

[2] H. Shaki, K. Gharanjig, S. Rouhani and A. Khosravi, "Synthesis and photophysical properties of some novel fluorescent dyes based on naphthalimide derivatives," J Photoch Photobio A Chem., vol 216 pp.44–50, November 2010.

[3] P. Alaei, S. Rouhani, K. Gharanjig and J. Ghasemi, "A new polymerizable fluorescent PET chemosensor of fluoride (F-) based on naphthalimide-thiourea dye," Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 90, 85–92, May 2012.

[4] H. Shaki, K. Gharanjig, S. Rouhani, A. Khosravi and J. Fakhar, "Synthesis and application of some novel antimicrobial monoazonaphthalimide dyes: synthesis and characterisation," Color. Technol. Vol. 128, pp. 270-275, August 2012.

[5] A. Khosravi, S. Moradian, K. Gharanjig and F. Afshar Taromi, **"**Synthesis and spectroscopic studies of some naphthalimide based disperse azo dyestuffs for the dyeing of polyester fibres**,"** *Dyes* Pigm**.,** vol. 69, pp. 79-92**,** 2006.

[6] V.B. Bojinov and I.K. Grabchev, "Novel functionalized 2-(2-hydroxyphenyl)-benzotriazole–benzo [de] isoquinoline-1,3-dione fluorescent UV absorbers Synthesis and photostabilizing efficiency," J Photoch Photobio A Chem., vol. 172 , pp. 308–315, January 2005.

[7] Y. L. Luo, K. Baathulaa, V. K. Kannekanti, Ch.H. Zhou, and G. X. Cai, Sci China Chem., vol. 58, pp. 483–494, 2015.

[8] M. F. Braña and A. Ramos, "Naphthalimides as Anticancer Agents: Synthesis and Biological Activity," Curr Med Chem. , vol. 1, pp. 237–255, November 2001.

[9] D. Staneva, E. Vasileva-Tonkova, M. S. I. Makki, T. R. Sobahi, R. M. Abdel-Rahman, A. M. Asiri and I. Grabchev, "Synthesis, photophysical and antimicrobial activity of new water soluble ammonium quaternary benzanthrone in solution and in polylactide film," J Photoch Photobio B Bio, vol. 143, pp. 44–51, January 2015.

[10] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 7th ed, 2006, 26(2): pp.14-16.

[11] S. Liu, J. Ma and D. Zhao, "Synthesis and characterization of cationic monoazo dyes incorporating quaternary ammonium salts," Dyes Pigm., vol 75, pp. 255-262, December 2007.