Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 41 / Number 3 / September 2021 / pp. 145-151

Synthesis, Antibacterial Activity and Photophysical Properties of Bis-benzamide derivatives

Bis-benzamid Türevlerinin Sentezi, Antibakteriyel Aktivitesi ve Fotofiziksel Özellikleri

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Received date : 10.09.2021
Accepted date : 15.10.2021

ABSTRACT

In this study, N,N'-(dodecane-1,12-diyl)bis(2,4-dichlorobenzamide) and N,N'-(dodecane-1,12-diyl)bis(4-bromobenzamide) as new bis-benzamides were synthesized by reaction of 1,12-diaminododecane and two different acyl chloride compounds in 88% and 92% yield, respectively. Their molecular structures were characterized using 1H NMR, 13C NMR and FT-IR techniques. Antibacterial activities of the synthesized compounds were screened against *Staphylococcus* aureus ATCC 25923 and *Escherichia* coli ATCC 25922 strains. Moreover, photophysical properties of the products in CH_2Cl_2 and $CHCl_3$ were investigated using UV-vis spectroscopy. The compound **3a** exhibited positive solvatochromism about 31 nm by increasing of solvent polarity.

Keywords: Antibacterial activity, Bis-benzamide, Photophysical property

ÖZET

Bu çalışmada yeni bis-benzamid türevleri olarak, 1,12-diaminododekan bileşiğinin ve iki farklı açil klorür bileşiği ile tepkimesi sonucunda N,N'-(dodekan-1,12-diil)bis(2,4-diklorobenzamid) ve N,N'-(dodekan-1,12-diil)bis(4-bromobenzamid) bileşikleri sırasıyla %88 ve %92 verimle sentezlendi. Moleküler yapıları 1H NMR, 13C NMR ve FT-IR teknikleri kullanılarak karakterize edildi. Sentezlenen bileşiklerin antibakteriyel aktiviteleri *Staphylococcus* aureus ATCC 25923 ve *Escherichia* coli ATCC 25922 suşlarına karşı tarandı. Ayrıca, CH2Cl2 ve CHCl3 içindeki ürünlerin fotofiziksel özellikleri UV-vis spektroskopisi kullanılarak incelendi. Bileşik **3a**, çözücü polaritesinin artmasıyla yaklaşık 31 nm'de pozitif solvatokromizm gösterdi.

Anahtar Kelimeler: Antibakteriyel aktivite, Bis-benzamid, Fotofiziksel özellik

1. Introduction

Bis-benzamides represent a group of compounds that are widely used in many fields such as bioactive compounds in medicinal chemistry [1] and anion recognition sensors in analytical chemistry [2-5]. It is known that bis-benzamides exhibit various pharmacological activities such as potential metal chelators inhibiting redox activity in Alzheimer's disease [6], anticancer [1, 7, 8], antiproliferative [9], tyrosyl-DNA phosphodiesterase I−topoisomerase I inhibitors [10], anti-HIV [11] and anti-HCV activities [12] as well as α1-AR receptor [13], acetylcholinesterase [14], Kirsten rat sarcoma 2 viral oncogene homolog−PDEδ [15], thioredoxin reductase [16], glutaminase and glutamate dehydrogenase inhibitors [17]. In addition, methylene-linked bis-phenylbenzimidazoles which stabilize telomeric DNA/RNA hybrids have been reported [18]. Furthermore, bis-benzamides are also useful as intermediate compounds for synthesis of pharmacologically active compounds such as amidoximes [19], lycopodium alkaloids (+)-flabellidine and (−)-lycodine [20]

In this study, the synthesis and antibacterial activity of bis-benzamide derivatives were reported. Additionally, investigation of photophysical properties of the products was also reported.

2. Materials and Methods

2.1. Materials and Instrumentation

The reagents used were purchased high grade from commercial Merck or Aldrich, and commercially available solvents were used without further purification. The Fourier Transform Infrared Spectroscopy (FT-IR) technique was used for spectroscopic characterization via Varian Scimitar Series FT-IR spectrophotometer using horizontal ATR. The Nuclear Magnetic Resonance (NMR) spectra and decoupling experiments were performed using Bruker Ultrashield Plus Biospin GmbH (at 400 MHz). Chemical shifts were given in parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard. $DMSO_{d6}$ was used as solvent for the NMR measurements. The following abbreviations were used; s = singlet, d = doublet, d = doublet of doublets, t = triplet and m = multiplet. Melting points were determined on a Stuart SMP3 hot stage apparatus and were uncorrected.

2.2. Synthesis of the Bis-benzamides, 3a, 3b

Bis-benzamides were synthesized used a literature method [19]. A solution of pyridine (2.2 mmol) in dichloromethane (15 mL) was dropwise added to a stirred solution of 1,12-diaminododecane 1 (1.0 mmol) in dichloromethane (25 mL). After 15 minute, a solution of corresponding acyl chloride in dichloromethane (25 mL) was dropwise added to a stirred mixture at room temperature. The reaction was observed to be complete after three hours by thin layer chromatography and then the solvent was evaporated under reduced pressure. The crude solid mixture was washed with water followed by diethyl ether.

2.2.1. N,N'-(dodecane-1,12-diyl)bis(2,4-dichlorobenzamide), 3a

White crystals. Yield, 0.48 g, 88%. m.p.: 133 – 135 ºC. IR (cm^{-1}): υ_{max} 3279 (N-H), 3055, 2919, 2851, 1643 (C=O), 1587, 1539, 1471. ^{1}H NMR (400 MHz, $DMSO_{d6}$): δ 8.44 (t, 2H, *J* = 5.3 Hz, N–H), 7.67 (d, 2H, *J* = 1.8 Hz, Ar–H), 7.47 (dd, 2H, *J* = 8.2 Hz, 1.8 Hz, Ar–H), 7.41 (d, 2H, *J* = 8.2 Hz, Ar–H), 3.22-3.17 (m, 4H, $C1H_2$ and $C12H_2$), 1.52 – 1.45 (m, 4H, $C2H_2$ and $C11H_2$), 1.34 – 1.23 (m, 16H, $C3H_2$, $C4H_2$, $C5H_2$, $C6H_2$, $C7H_2$, $C8H_2$, $C9H_2$ and $C10H_2$). ^{13}C NMR (100 MHz, $DMSO_{d6}$): δ 165.2 (2 x C=O), 136.2 (2 x Cq), 134.2 (2 x Cq), 131.1 (2 x Cq), 130.1 (2 x C), 129.0 (2 x C), 127.3 (2 x C), 39.0 (2 x C), 28.99 (2 x C), 28.96 (2 x C), 28.8 (2 x C), 28.7 (2 x C), 26.3 (2 x C).

2.2.2. N,N'- (dodecane-1,12-diyl) bis (4-bromobenzamide), 3b

White crystals. Yield, 0.52 g, 92%. m.p.: 188 – 190 ºC. IR (cm^{-1}): υ_{max} 3226 (N-H), 3052, 2918, 2847, 1626 (C=O), 1589, 1533, 1480. ^{1}H NMR (400 MHz, $DMSO_{d6}$): δ 8.50 (t, 2H, *J* = 5.3 Hz, N–H), 7.77 (d, 4H, *J* = 8.5 Hz, Ar–H), 7.66 (d, 4H, *J* = 8.5 Hz, Ar–H), 3.25-3.20 (m, 4H, $C1H_2$ and $C12H_2$), 1.53 – 1.46 (m, 4H, $C2H_2$ and $C11H_2$), 1.30 – 1.21 (m, 16H, $C3H_2$, $C4H_2$, $C5H_2$, $C6H_2$, $C7H_2$, $C8H_2$, $C9H_2$ and $C10H_2$). ^{13}C NMR (100 MHz, $DMSO_{d6}$): δ 165.1 (2 x C=O), 133.8 (2 x Cq), 131.2 (4 x C), 129.3 (4 x C), 124.6 (2 x Cq), 28.97 (2 x C), 28.95 (4 x C), 28.7 (2 x C), 26.4 (2 x C).

2.3. Antibacterial Activity of the Products

Disk agar diffusion method was used to evaluate the antibacterial properties (AMP) of the products [21] against *Staphylococcus aureus* (*S. aureus*) ATCC

25923 and *Escherichia coli* (*E. coli*) ATCC 25922 strains. *S. aureus* or *E. coli* culture were inoculated to Triptic Soy Broth (TSB) then incubated at 37 ºC for 24 h. Activated culture was transferred to TSB again and incubated at 37 ºC for 5 h. Approximately 450 µl of this culture were transferred to TSB and the absorbance of the culture was set to 0.08-0.1 ABS at 625 nm (~ 10^{6-7} cfu/ml). For the spread plate technique, 0.1 ml of this activated culture was transferred to Triptic Soy Agar (TSA) and spread homogeneously. Five sterile disks (6 mm filter paper disk) were placed on the agar surface. Sample solutions (15 µl) were inoculated on the discs; concentrations were 500, 250, 125, 62.5 and 31.25 µg/ml, respectively. DMSO was used as negative control; ampicillin (Bioanalyse LTD) was used as positive control. Then medium inoculated at 37 ºC for 24 h. Antibacterial effect around the disks was evaluated according to clear zone.

2.4. Determination of Photophysical Properties of the Products

The stock solutions of **3a and 3b** were prepared in 0,01 M concentration in CH_2Cl_2 and $CHCl_3$ for the determination of absorption maxima and absorbance. Absorption spectra were recorded in a Shimadzu 1800 spectrophotometer at 25 °C. The measurements were accomplished in thermostated quartz cuvettes (path length ł = 1 cm).

3. Results and Discussion

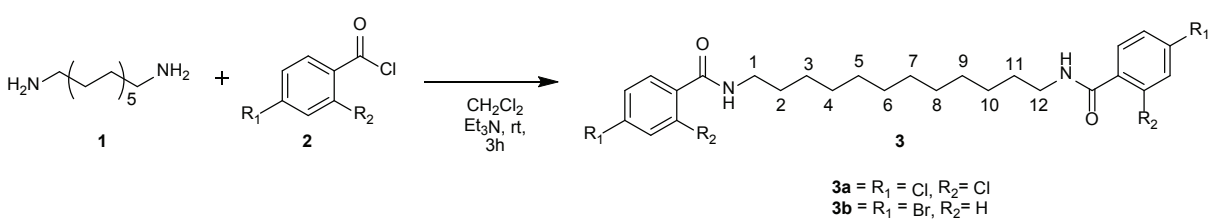
3.1. Synthesis and Characterization

The bis-benzamide derivatives *N,N'*-(dodecane-1,12-diyl)bis(2,4-dichlorobenzamide) **3a** and N,N'-(dodecane-1,12-diyl)bis(4-bromobenzamide) **3b** were synthesized using a literature method by reaction of 1,12-diaminododecane 1 and 2,4-dichlorobenzoyl chloride and 4-bromobenzoyl chloride, respectively, in dichloromethane at room temperature, in 88% and 92% yield, respectively (Scheme 1). The structures of the products **3a and 3b** were characterized by using FT-IR and 1H/13C NMR techniques. Representative FT-IR, 1H NMR and 13C NMR spectra for the compound **3a** are given in Figure 1, Figure 2 and Figure 3, respectively.

In the FT-IR spectra of the compounds **3a and 3b**, amide N-H and aromatic C-H stretching peaks were observed at 3279 and 3226 cm^{-1}, 3055 and 3052 cm^{-1}, respectively. In addition, the aliphatic C-H stretching peaks for **3a and 3b** were observed at 2919 and 2851 cm^{-1} and 2918 and 2847 cm^{-1}, respectively. The carbonyl (C=O) groups peaks were observed at 1643 $cm^{-1}$and 1626 cm^{-1}, respectively. In the ^{1}H NMR spectra of **3a and 3b**, the aromatic protons were observed in the range of 7.77-7.41 ppm, depending on the substituent group. The multiplet peaks observed in the range of 3.25–3.17 and 1.53 – 1.45 ppm was attributed to the protons of $C1H_2$ / $C12H_2$ and $C2H_2$ and $C11H_2$, respectively. In the ^{13}C NMR spectra of **3a and 3b**, the peak observed at 165.2 ppm and 165.1 ppm was attributed to the carbon of the C=O groups. The peaks of carbons C1 and C12 in the aliphatic chain were observed at 39.0 ppm for compounds 3a. However, since the peaks of C1 and C12 carbons in the aliphatic chain shifted below the solvent peaks in the spectra of **3b**, the shift values could not be determined exactly.

3.2. Antibacterial Properties

Antibacterial activity studies of the bis-benzamide derivatives **3a, 3b** was performed against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 strains using disk agar diffusion method, and Ampicillin was used as reference drug. The compounds **3a and 3b** exhibited antibacterial activity against *S. aureus* with a zone of 8.3 mm and 7.4 mm, respectively, at 31.25 µg/ml, while the reference drug ampicillin exhibited antibacterial activity against mentioned bacteria with a zone of 13.8 mm at 10 µg (Table 1). In addition, the compounds **3a and 3b** exhibited antibacte-



Scheme 1. Synthesis of bis-benzamide derivatives **3a**, **3b**

ISSN: 2458 - 8806

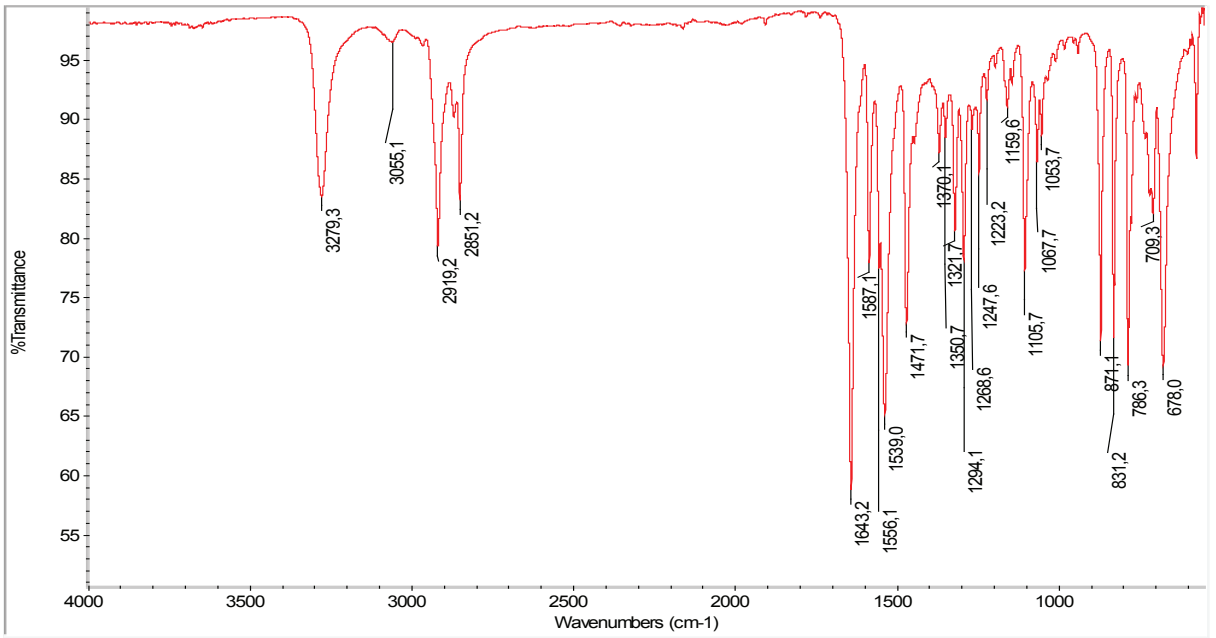


Figure 1. Representative FT-IR spectrum for the compounds **3a**

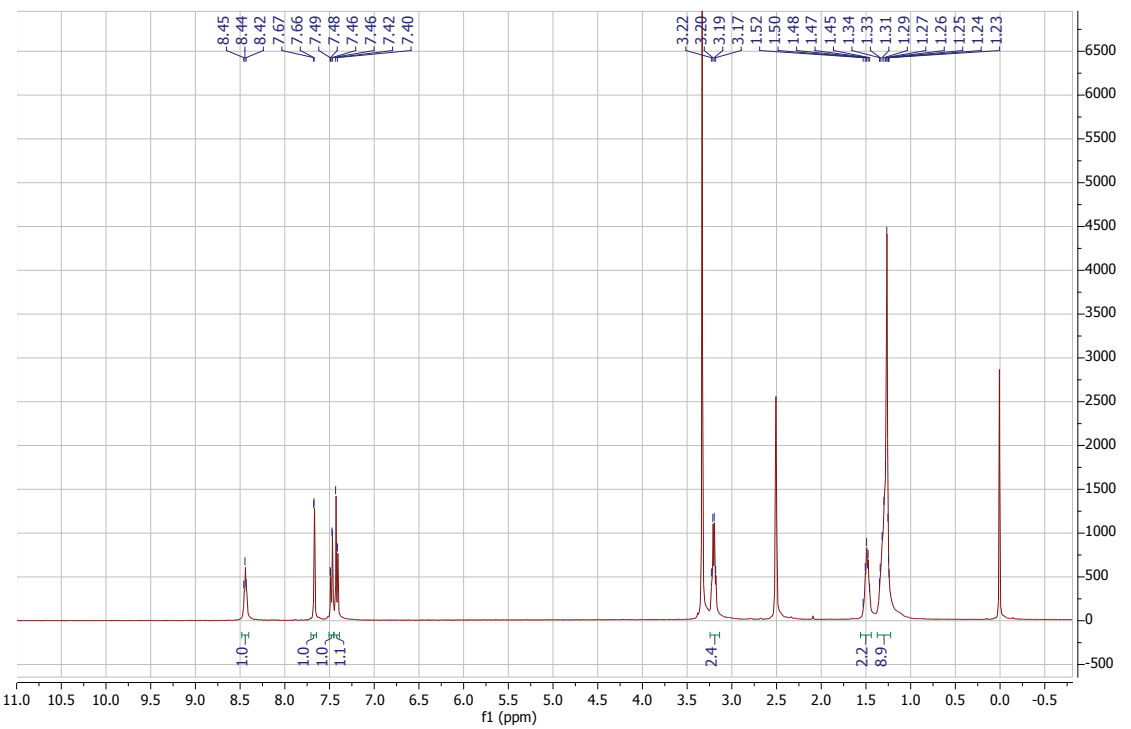


Figure 2. Representative ^{1}H NMR spectrum for the compounds **3a**

rial activity against *E. coli* with a zone of 7.5 mm and 7.3 mm, respectively, at 31.25 µg/ml, while the reference drug ampicillin exhibited antibacterial activity against mentioned bacteria with a zone of 14.9 mm at 10 µg (Table 1). As a result, it can be said that bis-benzamide derivatives **3a, 3b** have moderate antibacterial activity against both bacterial strains compared to ampicillin.

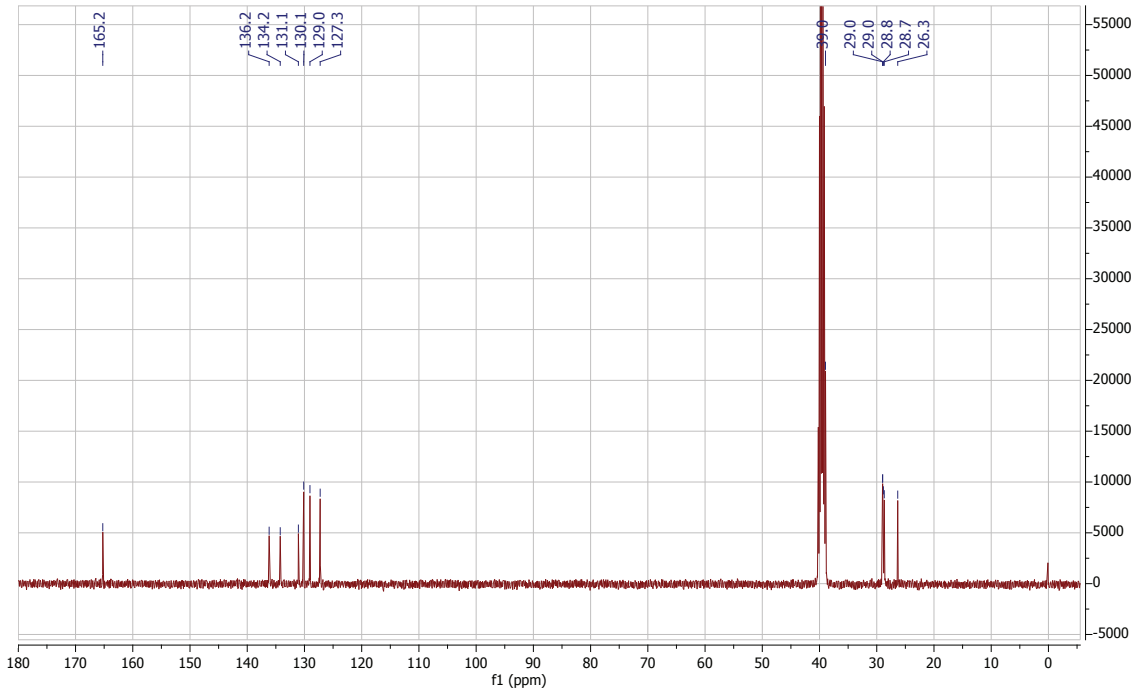


Figure 3. Representative ^{13}C NMR spectrum for the compounds **3a**

Table 1. Antibacterial activity of bis-benzamide derivatives **3a**, **3b**

	Bacteria / inhibition zone diameter (mm)	
Compound	S. aureus ATCC 25923	E. coli ATCC 25922
3a	8.3	7.5
3b	7.4	7.3
Ampicillin	13.8	14.9
DMSO	-	-

3.3. Photophysical Properties

Molecules, that change their spectral behaviour (colour, bathocromic / hypsochromic shift etc.) in response to surrounding solvent polarity have great potential in understanding of the interactions of a molecule with its microenvironment [22, 23]. Therefore, solvatochromism is observed when the absorption/fluorescence spectra changes due to the dissolution of the same substance in various solvents. Such studies are important because they involve important information regarding the microenvironment dependence of a drug's potential to reach a target receptor or the recognition of a chemical species by fluorogenic probes [24, 25]. The absorption spectra of **3a** was obtained in solvents of different polarities and hydrogen bonding abilities; namely, polar protic (chloroform), polar aprotic (dichloromethane) while **3b** didn't have any absorption peak in the range of 300-800 nm. To conduct in-depth evaluation regarding the influence of polarity of compounds **3a** behaviour $E_T(30)$, Dimroth–Reichardt polarity indexes were used [chloroform: 39.1, dichloromethane 40.7] since the compounds have very limited solubility in other solvents such as MeOH, Toluene, Acetonitrile [26]. **3a** exhibited positive solvatochromism about 31 nm while a bathochromic shift (or red shift) was observed with increasing solvent polarity by changing the chloroform to dichloromethane [24-26]. On the other hand, **3a** is soluble in polar aprotic solvent

ISSN: 2458 - 8806

such as THF and DMSO but no peak in the absorption spectra at 0.01 M concentration as seen example inset of blue peak of **3a** in THF (Figure 4).

Conclusion

In summary, we synthesized of bis-benzamide derivatives by reaction of 1,12-diaminododecane and 2,4-dichlorobenzoyl chloride and 4-bromobenzoyl chloride, respectively, in dichloromethane. The compounds exhibited antibacterial activity against in a zone range of 7.3-8.3 mm at 31.25 µg/ml *S. aureus* and *E. coli* strains. Interestingly, **3a** showed positive solvatochromism about 31 nm while a bathochromic shift was observed with increasing solvent polarity

Acknowledgements

This work was funded by Mersin University Research Fund (Project No: BAP 2020-1-AP5-4097).

References

1. Wei G, Huang L, Jiang Y, Shen Y, Huang Z, Huang Y, Sun X, Zhao C: Lenvatinib-zinc phthalocyanine conjugates as potential agents for enhancing synergistic therapy of multidrug-resistant cancer by glutathione depletion. Eur. J. Med. Chem. 2019, 169: 53-64.
2. Lopéz-Martínez LM, García-Elías J, Ochoa-Terán A, Ortega HS, Ochoa-Lara K, Montaño-Medina CU, Yatsimirsky AK, Ramírez J, Labastida-Galván V, Ordoñez M: Synthesis, characterization and anion recognition studies of new fluorescent alkyl bis (naphthylureylbenzamide)-based receptors. Tetrahedron 2020, 76: 130815.
3. García-Elías J, Ochoa-Terán A, Yatsimirsky AK, Ortega HS, Ochoa-Lara K, López-Martínez LM, Castro- Riquelme CL, Garcí ÁL, Madrigal-Peralta D, Labastida-Galván V, Ordoñez M: Synthesis and anion recognition studies of new oligomethylene bis (nitrophenylureylbenzamide) receptors. RSC Adv. 2019, 9: 39147-39162.
4. Pina-Luis G, Ochoa-Teran A, Rivero IA: Solid phase synthesis of N-alkyl-bis-o-aminobenzamides for metal ion sensing based on a fluorescent dansyl platform. J. Comb. Chem. 2009, 11: 83-90.
5. Winstanley KJ, Smith DK: Ortho-substituted catechol derivatives: the effect of intramolecular hydrogen-bonding pathways on chloride anion recognition. J. Org. Chem. 2007, 72: 2803-2815.
6. Nguyen M, Meunier B, Robert A: Catechol-Based Ligands as Potential Metal Chelators Inhibiting Redox Activity in Alzheimer's Disease. Eur. J. Inorg. Chem. 2017, 2017: 3198-3204.

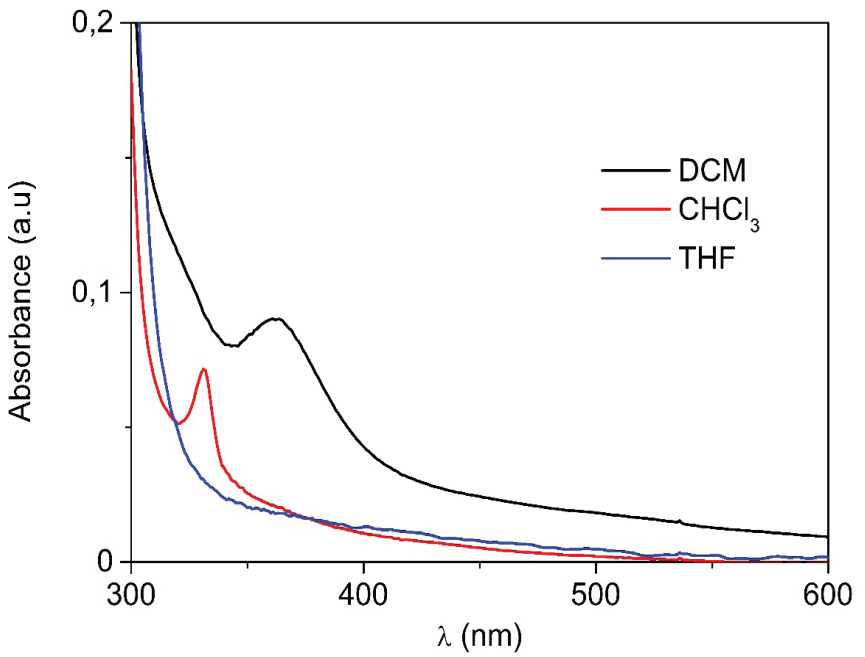


Figure 4. The absorption spectra of **3a** (0.01 M) in various solvent. The lines indicate the solution of **3a** in solvent respectively (red-chloroform, black-dichloromethane and blue- tetrahydrofuran)

7. Wang J, Hong G, Li G, Wang W, Liu T: Novel homo-bivalent and polyvalent compounds based on ligustrazine and heterocyclic ring as anticancer agents. Molecules 2019, 24: 4505.

8. Filatov AS, Knyazev NA, Shmakov SV, Bogdanov AA, Ryazantsev MN, Shtyrov AA, Starova GL, Malchanov AP, Larina AG, Boitsov VM, Stepakov AV: Concise synthesis of tryptanthrin spiro analogues with in vitro antitumor activity based on one-pot, three-component 1,3-dipolar cycloaddition of azomethine ylides to cyclopropenes. Synthesis 2019, 51: 713-729.

9. Dodo K, Minato T, Hashimoto Y: Structure–activity relationship of bis-galloyl derivatives related to (−)-epigallocatechin gallate. Chemical and Pharmaceutical Bulletin 2009, 57: 190-194.

10. Nguyen TX, Morrell A, Conda-Sheridan M, Marchand C, Agama K, Bermingam A, Stephen AG, Chergui A, Naumova A, Fisher R, O'Keefe BR, Pommier Y, Cushman M: Synthesis and biological evaluation of the first dual tyrosyl-DNA phosphodiesterase I (Tdp1)–topoisomerase I (Top1) inhibitors. J. Med. Chem. 2012, 55: 4457-4478.

11. Al-Masoudi NA, Al-Haidery N, Faili NT, Pannecouque C: Amino acid derivatives. Part 5. Synthesis and anti-HIV activity of new sebacoyl precursor derived thioureido-amino acid and phthalimide derivatives. Arkivoc 2010, 2010: 185-195.

12. Rivero-Buceta E, Carrero P, Doyagüez EG, Madrona A, Quesada E, Camarasa MJ, Pérez- Pérez MJ, Leyssen P, Paeshuyse J, Balzarini J, Neyts J, San-Felix A: Linear and branched alkylesters and amides of gallic acid and other (mono-, di- and tri-) hydroxy benzoyl derivatives as promising anti-HCV inhibitors. Eur. J. Med. Chem. 2015, 92: 656-671.

13. Strappaghetti G, Brodi C, Giannaccini G, Betti L: New 4-(4-methyl-phenyl) phthalazin-1 (2H)-one derivatives and their effects on α1-receptors. Bioorg. Med. Chem. Lett. 2006, 16: 2575-2579.

14. García ME, Borioni JL, Cavallaro V, Puiatti M, Pierini AB, Murray AP, Peñéñory AB: Solanocapsine derivatives as potential inhibitors of acetylcholinesterase: synthesis, molecular docking and biological studies. Steroids 2015,104: 95-110.

15. Jiang Y, Zhuang C, Chen L, Lu J, Dong G, Miao Z, Zhang W, Li J, Sheng C: Structural biology-inspired discovery of novel KRAS–PDEδ inhibitors. J. Med. Chem. 2017, 60: 9400-9406.

16. He J, Li D, Xiong K, Ge Y, Jin H, Zhang G, Hong M, Tian Y, Yin J, Zeng H: Inhibition of thioredoxin reductase by a novel series of bis-1, 2-benzisoselenazol-3 (2H)-ones: Organoselenium compounds for cancer therapy. Bioorgan. Med. Chem. 2012, 20: 3816-3827.

17. Zhu M, Fang J, Zhang J, Zhang Z, Xie J, Yu Y, Ruan JJ, Chen Z, Hou W, Yang G, Su W, Ruan BH: Biomolecular interaction assays identified dual inhibitors of glutaminase and glutamate dehydrogenase that disrupt mitochondrial function and prevent growth of cancer cells. Anal. Chem. 2017, 89: 1689-1696.

18. Islam MK, Jackson PJM, Thurston DE, Rahman KM: Methylene-linked bis-phenylbenzimidazoles–a new scaffold to target telomeric DNA/RNA hybrid duplex. Org. Biomol. Chem. 2018, 16: 4424-4428.

19. Berger O, Ortial S, Wein S, Denoyelle S, Bressolle F, Durand T, Escale R, Vial HJ, Vo-Hoang Y: Evaluation of amidoxime derivatives as prodrug candidates of potent bis-cationic antimalarials. Bioorg. Med. Chem. Lett. 2019, 29: 2203-2207.

20. Azuma M, Yoshikawa T, Kogure N, Kitajima M, Takayama H: Biogenetically inspired total syntheses of Lycopodium alkaloids, (+)-flabellidine and (−)-lycodine. J. Am. Chem. Soc. 2014, 136: 11618-11621.

21. Barry AL, Thornsberry C: Susceptibility Tests: Diffusion Test Procedures, Manual of Clinical Microbiology, (Ballows A, Hausler WJ, Herrman KL, Isenberg HD, Shadomy HJ.), Washington DC, 1117-1125, (1991).

22. Bani-Yaseen AD: Solvatochromic and fluorescence behavior of sulfisoxazole. J. Fluoresc. 2011, 21: 1061-1067.

23. Miotke MM, Marek J: Solvatochromism of antiinflammatory drug–naproxen sodium. J. Mol. Liq. 2017, 230: 129-136.

24. Yalcin E, Matković M, Jukić M, Obrovac LG, Piantanida I, Seferoğlu Z: Novel fluorene/fluorenone DNA and RNA binders as efficient non-toxic ds-RNA selective fluorescent probes. Tetrahedron 2018, 74: 535-543.

25. Yalcin E, Erkmen C, Taskin-Tok T, Caglayan MG: Fluorescence chemosensing of meldonium using a cross-reactive sensor array. Analyst, 2020, 145: 3345-3352.

26. Reichardt C: Solvatochromic dyes as solvent polarity indicators. Chem. Rev. 1994, 94: 2319-2358.

ISSN: 2458 - 8806