

SHORT COMMUNICATION article

Study of the diastereoisotopic protons effects on the stability of dorzolamide stereochemistry by spectroscopic methods

Halima Oulad Ali *  , Nasser Belboukhari  , and Khaled Sekkoum  

Bioactive Molecules and Chiral Separation Laboratory, Faculty of Exact Sciences,
Tahri Mohammed University, Istiklal Street PO 417 Bechar, 08000, Bechar, Algeria

* Author to whom correspondence should be addressed

Received: 27-09-2024, Revised: 02-11-2024, Accepted: 07-11-2024, Published: 31-12-2024

Copyright© 2024. This open-access article is distributed under the *Creative Commons Attribution License*, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

HOW TO CITE THIS

Oulad Ali et al. Study of the diastereoisotopic protons effects on the stability of dorzolamide stereochemistry by spectroscopic methods. *Mediterr J Pharm Pharm Sci.* 2024; 4(4): 15-21. [Article number: 176]. <https://doi.org/10.5281/zenodo.14049067>

Keywords: Diastereoisotopic, FTIR, NMR, spectroscopy, UV-visible

Abstract: In scientific and analytical research, Ultraviolet-visible spectroscopy, Fourier Transform Infrared spectroscopy, and Nuclear Magnetic Resonance spectroscopy have widely been used to analyze and characterize various substances. Ultraviolet, infrared, and Nuclear Magnetic Resonance spectroscopy were studied for diastereoisotopic protons of dorzolamide, which is used to treat glaucoma and ocular hypertension. Methanol was used as a solvent in Ultraviolet and we obtained three wavelengths: 203 nm, 253 nm, and 257 nm, where Ultraviolet spectroscopy was employed for stability testing of pharmaceuticals. By analyzing the Fourier Transform Infrared spectrum, we also can determine the presence or absence of specific functional groups in a compound, helping to identify the compound or confirm its structure in analyzing the Nuclear Magnetic Resonance spectrum. It can determine the types and numbers of hydrogen (proton) and carbon atoms in a molecule and their chemical environment, which helps elucidate the molecular structure.

Introduction

Chemically, dorzolamide (DZL) is (4S,6S)-4 (ethylamino)-6-methyl-5,6-dihydro-4H-thieno[2,3-b] thiopyran- 2-sulfonamide 7,7-dioxide hydrochloride (**Figure 1**) [1, 2]. DZL is a carbonic anhydrase inhibitor, which is used for the treatment of glaucoma and ocular hypertension [2, 3]. After topical ocular administration, DZL rapidly reaches the systemic circulation where it distributes preferentially to red blood cells [4]. Glaucoma is a multifactorial optic neuropathy marked by progressive degeneration from the loss of optic nerves and retina ganglion cells. Another strategy to reduce intraocular pressure is to increase the drainage of ocular fluid through the trabecular meshwork pathway (cholinergic agonists and prostaglandin analogs) [4]. Glaucoma is the leading cause of irreversible global blindness; the two drugs are often combined in eye drops for the therapy of glaucoma [5-7]. Clinical studies were conducted using the single stereoisomer (I) with the absolute configuration SS around the two chiral centers of the molecule [8-10]. In order to assess the potential for the *in-vivo* inversion of configuration at one or both chiral centers of (I) and the stereochemistry and an absolute configuration of the

metabolite [4, 11, 12]. A literature scan showed that various studies were reported for the analysis of DZL [1], including spectroscopy of Ultraviolet (UV), Infrared (IR), and Nuclear Magnetic Resonance (NMR). UV-visible spectroscopy is a technique used to study the interaction of light with a sample in the UV and visible regions of the electromagnetic spectrum [13, 14]. It provides information about the electronic transitions of molecules and is commonly used to determine the absorption or transmission of light by a sample at different wavelengths [2, 15]. IR spectroscopy is an analytical technique used to study the molecules in the IR light absorption function. It can be used to analyze the chemical structure and DZL properties, as well as to detect impurities [16]. NMR of DZL is a technique used to analyze the molecular structure of this drug. The NMR allows us to determine the positions and interactions of atoms in the DZL molecule, the chemical liaisons, and the molecular conformations [17]. This study aims to determine the stereochemistry of DZL utilizing UV, FTIR, and NMR spectroscopy. This investigation was used to identify the drug's diastereoisotopic proton.

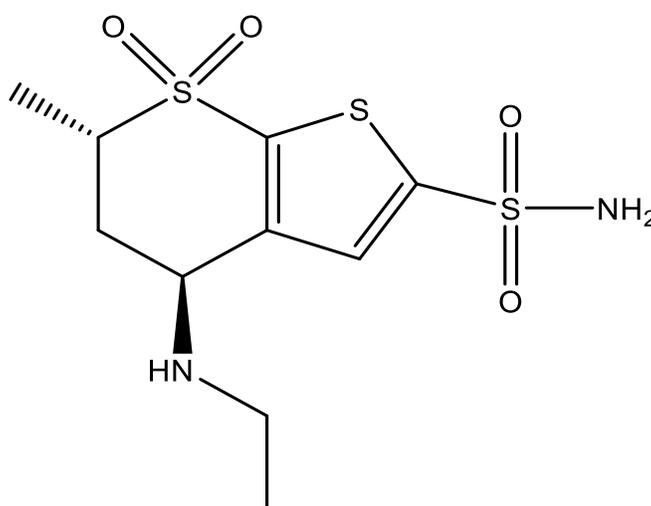


Figure 1: Chemical structure of dorzolamide

Materials and methods

Dorzolamide can be characterized using UV, IR, and NMR spectroscopy methods. The following are the general steps for each technique: The apparatus utilized in this investigation was of the type "SPECORD 50 PLUS" brand, this new dual-beam UV/Vis photometer for the spectral range from 190 to 1100 nm combined. The commercially available eye drops containing a 2.0% solution of sterile DZL HCl (20 mg/ml) were used. Prepared as a solution of DZL in a methanol solvent, a UV spectrophotometer is used to measure the absorbance of the solution in the 200-400 nm wavelength range. Analyze the UV absorption spectrum to identify characteristic peaks and determine the concentration of DZL. The Fourier Transform Infrared spectroscopy (FTIR) "Cary 630 FTIR" equipment was used in this study. Analyze the Chantillon using an IR spectrometer to register the IR absorption spectrometer, then analyze the specter to identify the bands with DZL absorption characteristics reveal information about the molecule structure, and better understanding the chemical structure of organic compounds and making informed decisions about their properties, reactivity and behavior in various environments. We used the software, master nova, this software allows us to automate the process of analyzing and predicting NMR spectra of organic molecules by entering their structural information.

Results and discussion

UV-vis spectroscopy analysis: The specter of absorption UV-Vis of DZL in methanol presents three characteristic bonds of absorption as shown in **Figure 2** and **Table 1**.

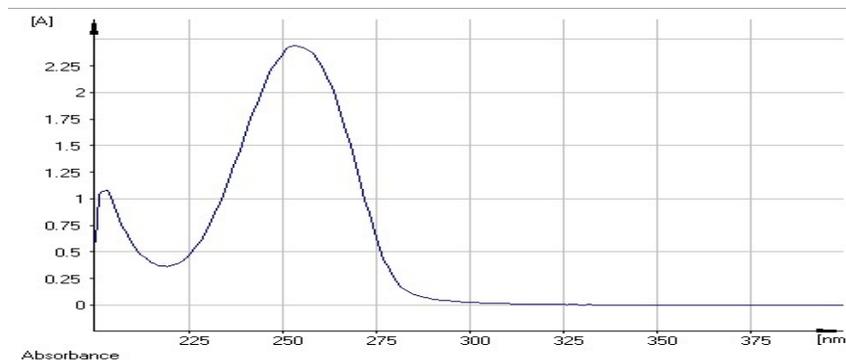


Figure 2: UV- absorbance spectra of dorzolamide

According to the Beer-Lamber absorption law, we can determine the molecular extinction coefficients which give information on the intensities of the absorption bands. The first bond situated at $\lambda_{\max}=203$ nm with an energy of $\Delta E=140.967$ kcal/mol, attributed to the electronic transition $\pi \rightarrow \pi^*$ which corresponds to the double bonds between both C=C. The second bond situated at $\lambda_{\max}=253$ nm an energy of about $\Delta E=113.108$ kcal/mol attributed to the electronic transition $\pi \rightarrow \pi^*$ which corresponds to the double links between both S=O. The third bond situated at $\lambda_{\max}=257$ nm with an energy about $\Delta E=111.348$ Kcal/mol attributed to the electronic transition $n \rightarrow \pi^*$ which corresponds to the double bond and free doublet of heteroromes S=O, C-N.

Table 1: UV-Vis spectroscopic analysis of dorzolamide

	Bond I	Bond II	Band III
λ_{\max}	203	253	257
A	1.079	2.438	2.395
ΔE	140.967 kcal/mol	113.108 kcal/mol	111.348 Kcal/mol

In **Figure 3**, it can be noticed that the electronic transition energy is inversely related to the wavelength, so when the transition energy decreases, the wavelength increases. This observation provides information on the molecule's stability based on the number of existing intramolecular interactions (electronic and structural).

The infrared spectrum presents 8 characteristic absorption bands: Two thin bands are located respectively at 2890 and 2950 cm^{-1} corresponding to the symmetric and asymmetric valence vibrations of the bonds of the CH methyl (**Table 2**). The presence of sulfur in the structure of this product is characterized by a band located around 1166 cm^{-1} corresponding to the valence vibration of the C-S bond. A wide band around 3304 cm^{-1} is attributed to the valence vibration of the N-H intramolecular hydrogen bond. The amine function is characterized by a fine absorption band around 1636 cm^{-1} , corresponding to the deformation vibration. A fine absorption bung is located at 1630 cm^{-1} attributed to the valence vibration of aromatic C=C. The aromaticity of this product is confirmed by the presence of an absorption band around 3010 cm^{-1} corresponding to the valence vibration of aromatic C-H, and an absorption gap is located at 620 cm^{-1} corresponding to the deformation vibration of C-H outside the plane (**Figure 4**).

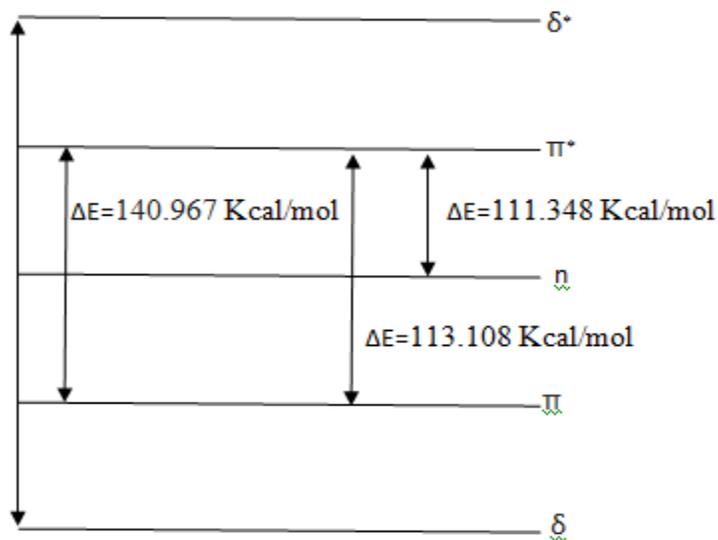


Figure 3: Energy diagram of dorzolamide

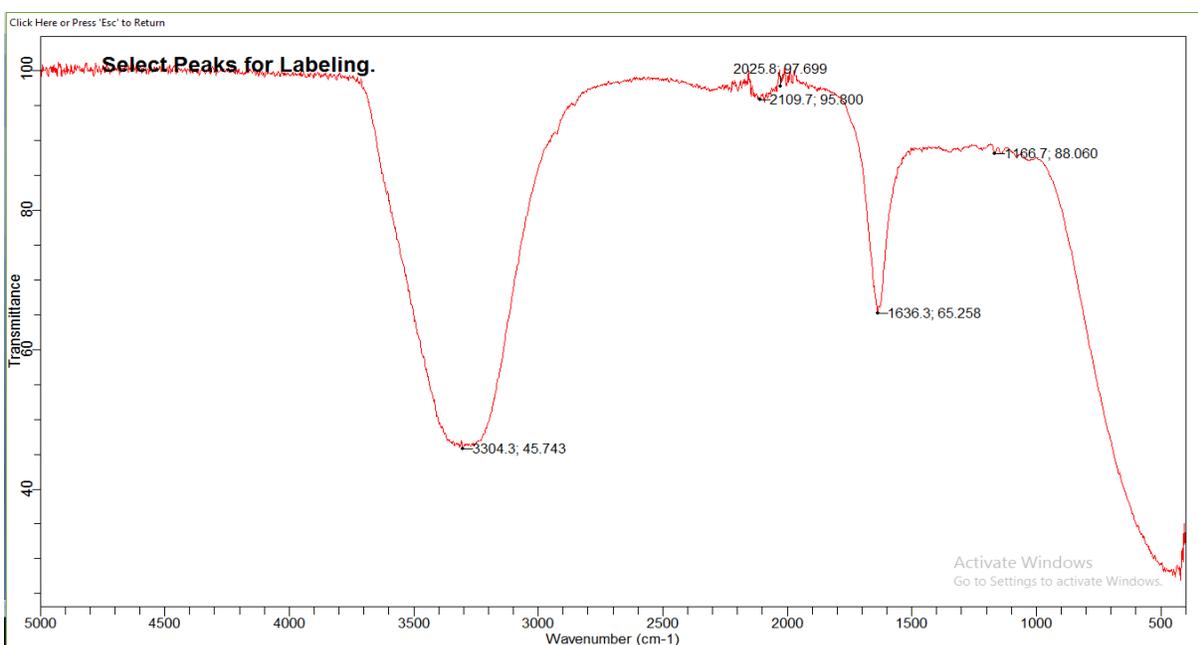


Figure 4: Dorzolamide IR spectrum

Table 2: Result of IR spectroscopic analysis of dorzolamide

	$\nu \text{ (cm}^{-1}\text{)}$	$\delta \text{ (cm}^{-1}\text{)}$	$\gamma \text{ (cm}^{-1}\text{)}$
CH ₃	2890,2950	-	-
C=C aromatic	-	1630	-
C-H aromatic	-	-	620
C-S	1166	-	-
N-H amine	3304	1636	685

*H*¹ NMR spectroscopic analysis: The determinations of the structure of DZL, based on the H NMR spectra indicate the presence of 09 carbon atoms.

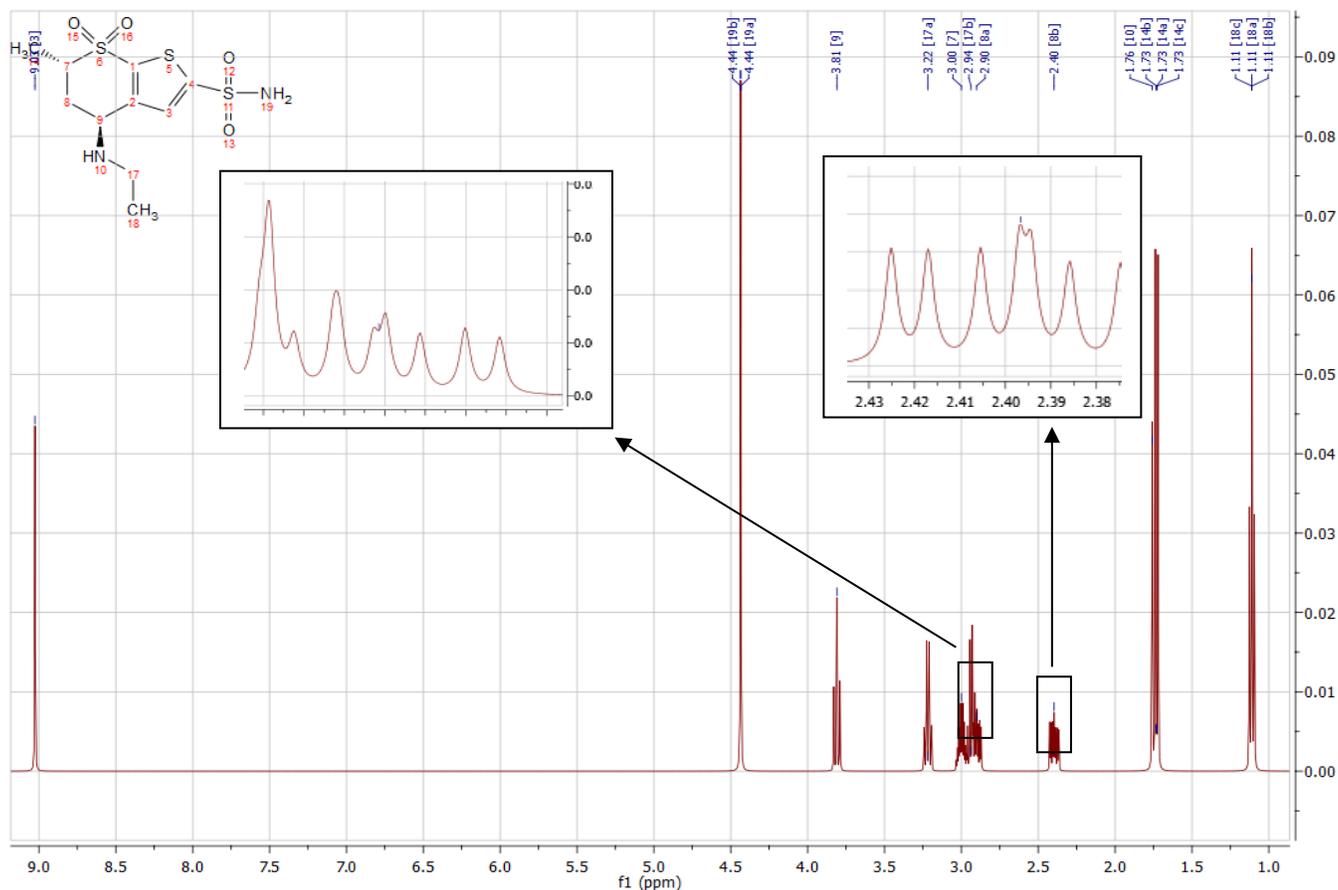


Figure 5: H MNR spectrum of dorzolamide

Table 3: Chemical shifts in H NMR and the attribution of dorzolamide signals

Position	RMN ¹ H
3	9.03(s, 1H)
7	3.00 (s, 1H)
8a	2.90(d, 2H)
8b	2.90(d, 2H)
9	3.81 (s, 1H)
14	1.73(m, 4H)
17a	3.32(s, 1H)
17b	3.32(s, 1H)
18	1.11(m, 3H)
NH	1,76(m, 1H)
NH ₂	4.44(m, 2H)

^1H NMR spectrum analysis of DZL shows the presence of multiple protons corresponding to a group (NH) at 1.76 ppm and 4.44 ppm. The signals at 1.11, 1.73, 3.32, 3.81, 9.03 ppm are attributable respectively to (H-18, H-14, H-17, H-9, H-3). A singular signal of a proton resonating at 3.00 ppm is attributable to the (H-7) presence of methyl. Two multiply signals resonating at 2.90 ppm attributable to (H_{8a} , H_{8b}) ($\text{ddd}, {}^2\text{J}_{ab}$, ${}^3\text{J}_{ac}$, ${}^3\text{J}_{ad}$, 2H), ($\text{ddd}, {}^2\text{J}_{ba}$, ${}^3\text{J}_{bc}$, ${}^3\text{J}_{bd}$, 2H) presence of diastereoisotopic protons.

Conclusion: UV spectroscopy was used to assay the absorption of light by DZL in the ultraviolet range which provides valuable information about the chemical structure and electronic transitions in the pharmaceutical form. The absorption maxima were 203, 253, and 257 nm. IR spectroscopy was used to analyze the infrared radiation absorbed by DZL which allowed the identification of the functional groups, providing information about the molecular structure, such as the presence of carbonyl groups, amines, and aromatic rings. NMR spectroscopy, specifically proton HNMR, was used to analyze the NMR of protons in the DZL molecule which provides information about the connectivity of the atoms in DZL, allowing for the determination of the chemical environment and molecular structure. Thus, by employing these spectroscopic techniques, were able to spectroscopically to assay the diastereoisotopic protons of DZL.

References

1. Dinc E, Buker E. Spectrochromatographic determination of dorzolamide hydrochloride and timolol maleate in an ophthalmic solution using three-way analysis methods. *Talanta*. 2019; 191: 248-256. doi: 10.1016/j.talanta.08.082. 2018
2. Bijari N, Ghobadi S, Mahdiuni H, Khodarahmi R, Ghadami SA. Spectroscopic and molecular modeling studies on binding of dorzolamide to bovine and human carbonic anhydrase II. *International Journal of Biological Macromolecules*. 2015; 80: 189-199. doi: 10.1016/j.ijbiomac.2015.06.028
3. Thangabalan B, Kahsay G, Eticha T. New RP-HPLC method development and validation for dorzolamide in ophthalmic dosage form. *Journal of Analytical Methods in Chemistry*. 2018; 2018: 4596141. doi: 10.1155/2018/4596141
4. Constanzer ML, Chavez CM, Matuszewski BK. Low level determination of dorzolamide and its de-ethylated metabolite in human plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*. 1997; 15(7): 1001-1008. doi: 10.1016/s0731-7085(96)01935-8
5. Erk N. Rapid and sensitive HPLC method for the simultaneous determination of dorzolamide hydrochloride and timolol maleate in eye drops with diode-array and UV detection. *Pharmazie*. 2003; 58(7): 491-493. PMID: 12889534.
6. Tambe S, Jain D, Amin P. Simultaneous determination of dorzolamide and timolol by first-order derivative UV spectroscopy in simulated biological fluid for in vitro drug release testing. *Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy*. 2021; 255: 119682. doi: 10.1016/j.saa.2021.119682
7. Bebawy LI. Application of TLC-densitometry, first-derivative UV-spectrophotometry and ratio derivative spectrophotometry for the determination of dorzolamide hydrochloride and timolol maleate. *Journal of Pharmaceutical and Biomedical Analysis*. 2002; 27(5): 737-746. doi: 10.1016/s0731-7085(01)00529-5
8. Kraimi A, Belboukhari N, Sekkoum K, Aboul-Enein HY. Chiral anticoagulants drugs based on coumarin. *Aditum Journal of Clinical and Biomedical Research*. 2021; 2(1): 1-13. doi: 04.2021/1.1027
9. Serap S. Determination of dorzolamide in ophthalmic solutions using solid-phase extraction by high-performance liquid chromatography. *Acta Pharmaceutica Turcica*. 2000; XLII(2, 3): 98-100. doi: Nil.
10. Scozzafava A, Banciu MD, Popescu A, Supuran CT. Carbonic anhydrase inhibitors: inhibition of isozymes I, II and IV by sulfamide and sulfamic acid derivatives. *Journal of Enzyme Inhibition*. 2000; 15(5): 443-453. doi: 10.3109/14756360009040700

11. Belboukhari N. Enantioseparation of hydroxyflavanones on amylose and cellulose CSPS by HPLC methods. *Journal of Chromatography and Separation Techniques*. 2012; 3(6): 67-68. doi: 10.4172/2157-7064.S1.004
12. Mimouni FZ, Belboukhari N, Sekkoum K, Aboul-Enein HY. Novel Gatifloxacin3-carboxamide derivatives as anti-tumor agents: synthesis, enantioseparation, and molecular docking. *Current Analytical Chemistry*. 2022; 18(10): 1108-1116. doi: 10.2174/1573411018666220819181513
13. Hamache T, Belboukhari N, Sekkoum K. Stereochemical of atorvastatin drug by using spectroscopic. *Analysis, Clinical Trials ,and Case Studies*. 2024; 3(1): 1-4. doi: 10.31579/2835-835X/046
14. Haddad D, Belboukhari N, Sekkoum K, Bouanini M. Determination of the structural parameters of Repaglinide in tablet: an antidiabetic drug, using spectroscopic methods. *Mediterranean Journal of Pharmacy and Pharmaceutical Sciences*. 2024; 4(2): 75-81. doi: 10.5281/zenodo.12601827
15. Addadi K, Sekkoum K, Belboukhari N, Cheriti A, Aboul-Enein HY. Screening approach for chiral separation of β -aminoketones by HPLC on various polysaccharide-based chiral stationary phases. *Chirality*. 2015; 27(5): 332-338. doi: 10.1002/chir.22434
16. Sağlık S. Determination of dorzolamide in ophthalmic solutions using solid-phase extraction by high-performance liquid chromatography. *Acta Pharmaceutica Turcica*. 2000; XLII(2, 3): 98-100. doi: Nil.
17. Supuran CT, Scozzafava A, Sarametb I, Banciu MD. Carbonic anhydrase inhibitors: inhibition of isozymes I, II, and IV with heterocyclic mercaptans, sulfenamides, sulfonamides and their metal complexes. *Journal of Enzyme Inhibition*. 1998; 13(3): 177-194. doi: 10.3109/14756369809028339

Author contribution: NB conceived and designed the study. HOA collected data. HOA & KS analyzed data. HOA & NB performed data analysis and interpretation. HOA & NB drafted and revised the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for its contents.

Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission were completely observed by the authors.

Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.

Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.