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Synthesis of an Azetyl-Acetonitrile Derivative and an Analog of 2-Aza-Bicyclo. Theoretical Analysis of its Interaction with Phosphodiesterase-4 Enzyme Using a Coupling Model

Figueroa-Valverde Lauro [1,*], Rosas-Nexticapa Marcela [2,*], Alvarez-Ramirez Magdalena [2], Díaz-Cedillo Francisco [3], López-Ramos Maria [1], Mateu-Armad Maria Virginia [2], Cervantes-Ortega Catalina [2], Garcimarrero-Espino Alejandra [2]

1 Facultad de Nutrición, Universidad Veracruzana, Médicos y Odontologos s/n C.P. 91010, Unidad del Bosque Xalapa Veracruz, México
2 Laboratory of Pharmaco-Chemistry, Faculty of Chemical Biological Sciences, University Autonomous of Campeche, Av. Agustín Melgar s/n, Col Buenavista C.P. 24039 Campeche, Camp., México
3 Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional. Prol. Carpio y Plan de Ayala s/n Col. Santo Tomas, D.F. C.P. 11340, México
* Correspondence: lfiguero@uacam.mx (F.V.L.); rosasnm@yahoo.com (R.-N.M.);

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Abstract: Several anti-inflammatory and bronchodilator drugs have been used to treat chronic obstructive pulmonary disease; however, some of these drugs can produce some secondary effects, such as bronchiolitis, emphysema, and others. This study aimed to synthesize an azetyl-acetonitrile derivative (4) and a 2-aza-bicyclo (6) analog to evaluate their theoretical interaction with phosphodiesterase-4 enzyme using 1xmu protein as theorethical model in a docking sever; besides it is important to mention that roflumilast reagent was used as control. The results showed that compounds 4 and 6 could interact with different types of amino acid residues involved in the 1xmu protein surface compared to roflumilast. This phenomenon may be due to differences in the chemical structure of compounds. All these data indicate that compounds 4 and 6 could exert changes in the biological activity of phosphodiesterase-4 enzyme, which may be translated as good candidates for the treatment of chronic obstructive pulmonary disease.

Keywords: azetyl-acetonitrile; 2-aza-bicyclo; phosphodiesterase-4; chronic obstructive pulmonary disease.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the airways that affects the lungs; It is important to mention that this clinical pathology is one of the main causes of morbidity and mortality worldwide [1-10]. There are several risk factors to developed COPD, such as smoking [11], genetics [12], diet [13], alpha-1 protease inhibitor deficiency [14, 15], bronchiolitis [16], emphysema [17], and others. It is noteworthy that several drugs are currently used for treating COPD, such as salbutamol, formoterol and indacaterol [18-20], ipratropium bromide [21], beclomethasone [22, 23], theophylline [24, 25], and roflumilast [26-28]. However, some of these drugs can produce secondary effects, such as

tachycardia [29], cough [30], candidiasis [31], and hypokalemia [32]. In search for new therapeutic alternatives for treating COPD, several compounds have been synthesized: for example, the preparation of a pyrazolopyrimidine derivative from 5-aminopyrazoles and an arylacetoaldehyde as phosphodiesterase-4 inhibitor for the treatment of COPD [33]. In addition, other data showed the synthesis of a thieno[2,3-c]isoquinolin-8-amine analog via the reaction of a pyrimidinone derivative with phosphorus oxychloride to be used as phosphodiesterase-4 inhibitor for the treatment of COPD [34]. Other studies display the reaction of (E)-N′-(3,4-Dimethoxybenzylidene)benzo-hydrazide with methyl iodide to form the compound (E)-N′-(3,4-Dimethoxybenzylidene)-N-methylbenzohydrazide; this compound showed effects as phosphodiesterase-4 inhibitor [35]. On the other hand, a theoretical study showed that a series of polysubstituted quinolines derivatives could be used as phosphodiesterase 4 inhibitors using a pharmacophore model [36]. All these studies indicate the preparation of several compounds with activity on phosphodiesterase 4; however, some protocols use some reagents which as dangerous and require special conditions such as different pH and higher temperatures. Analyzing these data in this research synthesized an azetyl-acetonitrile derivative and 2-aza-bicyclo analog to evaluate the possible activity as phosphodiesterase-4 inhibitors using a docking model.

2. Materials and Methods

2.1. General method.

4-hydroxy-N-prop-2-ynyl-benzamide was prepared using a previously reported method [39]. Other reagents were purchased from Sigma-Aldrich, Co. NMR spectra were determined using a Varian VXR300/5 FT device (300 MHz/$CDCl_3$). It is noteworthy that tetramethylsilane was used as an internal standard. Electron Ionization mass spectrometry (EIMS) was determined using a Finnigan PolarisQ ion trap mass spectrometer. Melting point (m.p.) was determined with an electrothermal-900 model device. The infrared spectrum (IR) was determined on a thermo-scientific iSOFT/IR apparatus. Elemental analysis was determined using a PerkinElmer device (Ser. II CHNS / 02400).

2.2. Chemical synthesis.

2H-azet-1-yl-(4-hydroxyphenyl)methanone (2)

In a round bottom flask (10 ml), 4-Hydroxy-N-prop-2-ynyl-benzamide (200 mg, 0.57 mmol), Copper(II) chloride anhydrous (75 mg, 0.56 mmol), and methanol (5 ml) were stirring for 72 h at room temperature. Then, the solvent was evaporated under reduced pressure, and the product was separated using the methanol:water (2:1) system; yielding 65% of product; m.p. 42-44 ^{0}C; IR (V_{max}, cm^{-1}) 3400, 2138 and 1630: ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ_H: 3.80-6.30 (m, 4H), 5.02 (broad, 1H), 7.00-7.70 (m, 4H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 56.40, 114.60, 122.10, 125.52, 129.74, 134.60, 160.46, 168.40 ppm. EI-MS m/z: 175.06. Anal. Calcd. for $C_{10}H_9NO_2$. C, 68.56; H, 5.18; N, 8.00, O, 18.27. Found: C, 68.52; H, 5.15.

2-[4-[4-(2H-azete-1-carbonyl)phenoxy]phenyl]acetonitrile (3)

In a round bottom flask (10 ml), compound **2** (100 mg, 0.57 mmol), 2-(4-nitrophenyl)acetonitrile (95 mg, 0.58 mmol), potassium carbonate anhydrous (70 mg, 0.50 mmol) in 5 ml of dimethyl sulfoxide was stirring for 48 h at reflux. Then, the solvent was

evaporated under reduced pressure, and the product was purified via crystallization using the methanol:acetone (2:1) system, yielding 44% of product; m.p. 52-54 ^{0}C; IR (V_{max}, cm^{-1}) 2200, 1630 and 1220: ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ_H: 3.60 (m, 2H), 3.80-6.30 (m, 4H), 6.90 (m, 2H), 7.00-7.36 (m, 4H), 7.62 (m, 2H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 23.40, 56.42, 116.94, 117.10, 117.40, 122.12, 122.50, 127.00, 128.34, 129.62, 134.62, 154.64, 162.16, 169.40 ppm. EI-MS m/z: 290.10. Anal. Calcd. for $C_{18}H_{14}N_2O_2$. C, 74.47; H, 4.86; N, 9.65, O, 11.02. Found: C, 74.44; H, 4.83.

(2-[4-[4-[(E)-C-(2H-azet-1-yl)-N-(3-ethynylphenyl)carbonimidoyl]phenoxy]phenyl] acetonitrile (4)

In a round bottom flask (10 ml), compound **3** (200 mg, 0.69 mmol), 3-ethynylaniline (100 µl, 0.88 mmol), boric acid (40 mg, 0.65 mmol) in 5 ml of methanol was stirred for 72 h at room temperature. Then, the solvent was evaporated under reduced pressure, and the product was purified via crystallization using the methanol:acetone (2:1) system, yielding 54% of product; m.p. 82-84 ^{0}C; IR (V_{max}, cm^{-1}) 3350, 2200, 2138 and 1220: ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ_H: 2.94 (s, 1H), 3.66 (m, 2H),3.80-5.50 (m, 3H), 6.92 (m, 1H), 7.00 (m, 2H), 7.08-7.30 (m, 4H), 7.36 (m, 2H), 7.38-7.86 (m, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 23.40, 51.90, 78.20, 84.00, 117.10, 117.40, 121.60, 122.50, 123.94, 124.08, 124.72, 126.80, 128.30, 129.40, 130.50, 130.66, 131.30, 134.04, 135.52, 153.88, 157.10, 157.90, 159.96 ppm. EI-MS m/z: 389.15. Anal. Calcd. for $C_{26}H_{19}N_3O$. C, 80.18; H, 4.92; N, 10.79, O, 4.11. Found: C, 80.14; H, 4.90.

(4-hydroxyphenyl)-[6-[hydroxy(phenyl)methyl]-2-azabicyclo[2.2.0]hexan-2-yl]metha- none (5)

In a round bottom flask (10 ml), compound 2 (100 mg, 0.57 mmol), 1-Phenyl-prop-2-en-1-ol (100 µl, 0.82 mmol), Copper(II) chloride anhydrous (75 mg, 0.56 mmol) and methanol (5 ml) were stirring for 72 h at room temperature. Then, the solvent was evaporated under reduced pressure, and the product was separated using the methanol:water (3:1) system, yielding 55% of product; m.p. 102-104 ^{0}C; IR (V_{max}, cm^{-1}) 3400 and 1630: ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ_H: 1.40-4.00 (m, 7H), 4.26 (m, 1H), 5.02 (broad, 2H), 7.00-7.70 (m, 9H) ppm. The ^{13}C NMR spectra display chemical shifts at 12.65, 29.94, 30.70, 40.44, 54.50, 72.44, 114.30, 126.10, 126.64, 127.12, 128.24, 128.90, 142.12, 160.40, 172.90 ppm. EI-MS m/z: 307.12. Anal. Calcd. for $C_{19}H_{19}NO_3$. C, 73.77; H, 6.19; N, 4.53, O, 15.52. Found: C, 73.74; H, 6.16.

4-[(E)-N-(3-ethynylphenyl)-C-[6-[hydroxy(phenyl)methyl]-2-azabicyclo[2.2.0]hexan-2-yl]carbonimidoyl]phenol (6)

In a round bottom flask (10 ml), compound 5 (200 mg, 0.65 mmol), 3-ethynylaniline (100 µl, 0.88 mmol), boric acid (40 mg, 0.65 mmol) in 5 ml of methanol was stirred for 72 h at room temperature. Then, the solvent was evaporated under reduced pressure, and the product was purified via crystallization using the methanol:water (3:1) system; yielding 66% of product; m.p. 144-146 ^{0}C; IR (V_{max}, cm^{-1}) 3400, 3348 and 2138: ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ_H: 1.00-2.80 (s, 5H), 2.96 (m, 1H), 3.30-3.50 (m, 2H), 4.70 (s, 1H), 6.30 (broad, 2H), 6.8-6.86 (m, 4H), 6.96 (m, 1H), 7.12-7.38 (m, 5H), 7.40-7.86 (m, 3H) ppm. 15.30, 29.70, 39.50, 40.94, 50.10, 74.42, 78.20, 84.00, 118.20, 122.44, 124.10, 126.10, 126.74, 126.80, 127.18, 127.50, 128.30, 130.56, 133.60, 144.10, 153.00, 158.80, 161.70 ppm. EI-MS m/z: 408.18. Anal. Calcd. for $C_{27}H_{24}N_2O_2$. C, 79.39; H, 5.92; N, 6.86, O, 7.83. Found: C, 79.34; H, 5.90

2.3. Pharmacophore evaluation.

The 3D-pharmacophore model for compounds 4 and 6 was determined using LigandScout 4.08 software [37].

2.4. Docking model.

In this study, the interaction of both compounds **4** and **6** with phosphodiesterase-4 protein surface (1xmu) was carried out with Achilles Blind Docking Server [38-40] using roflumilast (phosphodiesterase-4 inhibitor; Figure 1) [41] as control.

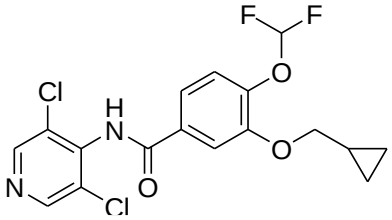

Figure 1. Chemical structure of Roflumilast.

3. Results and Discussion

Some studies have shown synthesizing some phosphodiesterase-4 inhibitors; however, some methods use hazardous reagents requiring special conditions [33-36]. In this study, using a docking model, two compounds such as azetyl-acetonitrile derivative and 2-aza-bicyclo analog, were prepared to evaluate their theoretical activity as phosphodiesterase-4 inhibitors. The first step was achieved by the synthesis of an azete derivative (2); it is important to mention that several reagents have been used to synthesize azete analogs, such as Chloro(1,5-cyclooctadiene)copper(I) dimer [42] cyclopropenyl azide [43], α-(Onitroaryl)benzylphosphonate [44], CopperII chloride [45], Copper(I) iodide [46] and others. In this study, compound 2 (Figure 2) was prepared via an intramolecular reaction of terminal alkyne with an amide group from compound 1 in the presence of Copper(II).

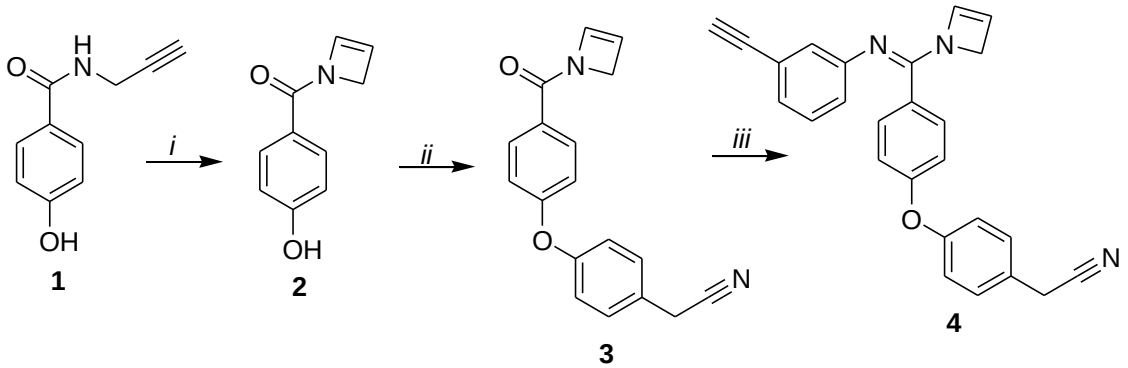

Figure 2. Synthesis of an azetyl-carbonimidoyl-acetonitrile derivative (**4**). *Conditions and reagents*: *i* = Copper(II) chloride, MeOH, room temperature; *ii* = 2-(4-nitrophenyl)acetonitrile, potassium carbonate, dimethyl sulfoxide, reflux; *iii* = 3-ethynylaniline, boric acid, MeOH, room temperature.

The ^{1}H NMR spectrum of **2** displayed several signals at 3.80-6.30 ppm for 1,2-dihydro-azete ring, at 5.02 ppm for the hydroxyl group, and 7.00-7.70 ppm for the phenyl group. The ^{13}C NMR spectra display chemical shifts at 56.40, 122.10, and 134.60 ppm for 1,2-Dihydro-azete ring; at 114.60, 125.52-129.74 and 160.46 ppm for the phenyl group; at 168.40 for amide group. In addition, the mass spectrum from **2** displayed a molecular ion (m/z) at 175.06.

The second stage involves synthesizing an ether derivative (3) using a previously reported method for synthesizing either analog [47]. In this way, compound **3** was prepared from 2-(4-nitrophenyl)acetonitrile and dimethyl sulfoxide in mild conditions (Figure 2). The ^{1}H NMR spectrum of **3** showed some bands at 3.60 ppm for methanol fragment, at 3.80-6.30 ppm for 1,2-dihydro-azete; at 6.90-7.62 ppm for phenyl groups. The ^{13}C NMR spectra display chemical shifts at 23.40 ppm for methanol fragment; at 56.42, 122.12, and 134.62 ppm for 1,2-Dihydro-azete ring; at 116.94-117.10, 122.50-129.62 and 154.64-162.16 ppm for phenyl groups; at 117.40 ppm for acetonitrile group; at 169.44 ppm for amide group. Besides, the mass spectrum from **3** showed a molecular ion (m/z) at 290.10.

The third step was achieved by the synthesis of an imino group involved in compound **4**; it is noteworthy that several procedures have been used for the synthesis of imino groups which are described in the literature [48-51]; nevertheless, in this study boric acid was used as catalyst, because it is not an expensive reagent and special conditions for its use are not required [55]. The ^{1}H NMR spectrum of **4** showed several signals at 2.94 ppm for the alkyne group linked to the phenyl group; at 3.66 ppm for the methanol fragment; at 3.80-3.50 ppm for 1,2-Dihydro-azete ring; at 6.92, 7.38-7.86 ppm for phenyl group linked to alkyne group; at 7.00-7.36 ppm for phenyl group bound to ether group. The ^{13}C NMR spectra showed some bands at 23.40 ppm for the methanol fragment; at 51.90, 123.94, and 135.52 ppm for 1,2-Dihydro-azete ring; at 78.20-84.00 ppm for alkyne group; at 117.10, 121.60-122.50, 128.30, 131.30-134.04 and 157.90-159.96 ppm for phenyl groups bound to ether group; at 117.40 ppm for nitrile group; at 124.08, 124.72-126.80, 130.50-130.66 and 158.88 ppm for phenyl group bound to alkyne group: at 157.10 for imino group. Besides, the mass spectrum from **4** showed a molecular ion (m/z) at 389.15.

The four stages involve the preparation of 2-Aza-bicyclo[2.2.0]hexane derivative (**5**); it is important to mention that there are some protocols for the synthesis of 2-Aza-bicyclo[2.2.0]hexane analogs [52, 53]. In this study, compound **4** reacted with 1-phenyl-prop-2-en-1-ol in the presence of Copper(II) to form **5** (Figure 3). The ^{1}H NMR spectrum of **5** showed several signals at 1.40-4.00 ppm for 2-Aza-bicyclo[2.2.0]hexane fragment; at 4.26 ppm for methylene group linked to hydroxyl and phenyl groups; at 5.02 ppm for hydroxyl groups; at 7.00-7.70 ppm for phenyl groups. The ^{13}C NMR spectra showed some bands at 12.65-54.50 ppm for 2-Aza-bicyclo[2.2.0]hexane fragment; at 72.44 ppm for methanol fragment; at 114.30, 127.12, 128.90 and 160.40 ppm for phenyl group bound to both hydroxyl and amide groups; at 126.10-126.64, 128.24 and 142.12 ppm for phenyl group linked to methanol fragment; at 172.90 ppm for amide group. Additionally, the mass spectrum from **5** showed a molecular ion (m/z) at 309.13.

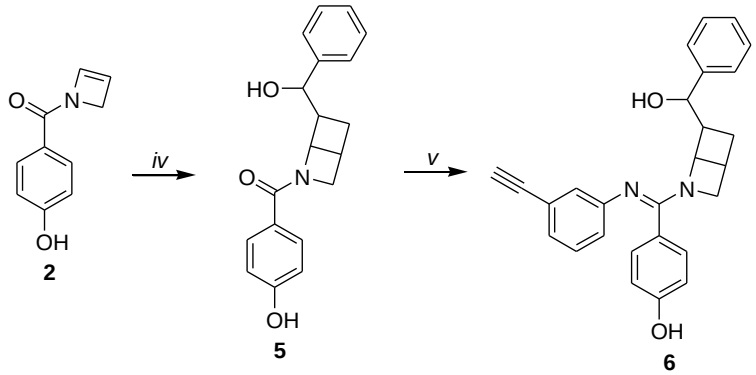

Figure 3. Synthesis of 3-ethynylphenyl-azabicyclo-carbonimidoyl derivative (**6**). *Conditions and reagents*: *iv* = 1-Phenyl-prop-2-en-1-ol, Copper(II) chloride, MeOH, room temperature; *v* = 3-ethynylaniline, boric acid, MeOH, room temperature.

Finally, compound **6** was prepared via a reaction of **5** with 3-ethynylaniline using boric acid as a catalyst. The ^{1}H NMR spectrum of **6** showed several signals at 1.00-2.80 and 3.30-3.56 ppm for 2-Aza-bicyclo[2.2.0]hexane fragment; at 2.96 for the alkyne group; at 4.70 ppm for methanol fragment; at 6.30 ppm for hydroxyl groups; at 6.80-6.86 ppm for phenyl group bound to hydroxyl group; at 6.96 and 7.40-7.86 ppm for phenyl group linked to alkyne group; at 7.12-7.38 ppm for methanol group. The ^{13}C NMR spectra showed some bands at 15.30-50.10 ppm for 2-Aza-bicyclo[2.2.0]hexane fragment at 74.42 ppm for methanol fragment; at 78.20-84.00 ppm for alkyne group; at 118.20, 127.18-133.60 and 161.70 ppm for phenyl bound to hydroxyl group; at 122.44-124.10, 126.80, 128.30-130.56 and 153.00 ppm for phenyl group linked to alkyne group; at 126.10-126.74, 127.50 and 144.10 ppm for phenyl bound to methanol fragment; at 158.80 ppm for imino group Finally, the mass spectrum from **6** showed a molecular ion (m/z) at 418.18.

3.1. Pharmacophore model.

The pharmacophore model provides some elements for the design of new drugs. Analyzing these data, in this study, the LigandScout software [37] was used to develop a pharmacophore model for compounds **4** and **6** (Figure 4). The results showed different functional groups involved in compounds **4** and **6,** which can interact through hydrophobic contacts or as hydrogen bond acceptors or hydrogen bond donors with some biomolecules.

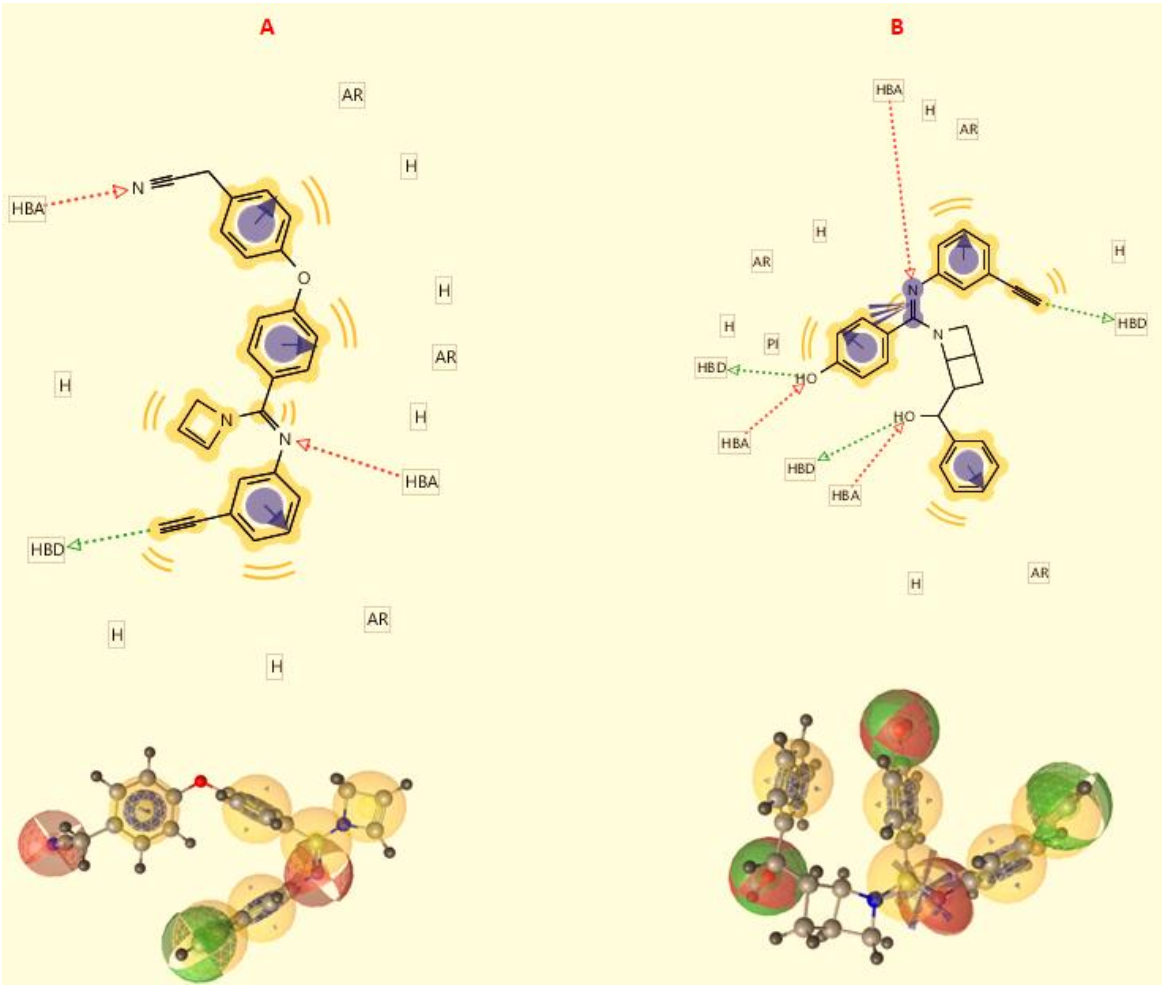

Figure 4. Pharmacophore model from both compounds 4 (A) and 6 (B) using the LigandScout software. The model involves hydrogen bond acceptors (HBA, red) and hydrogen bond donors (HBD, green).

3.2. Theoretical evaluation.

To evaluate the relationship between the chemical structure of compounds 4 and 6 with theoretical activity exerted on phosphodiesterase-4 enzyme, the 1xmu protein was used. It is important to mention that 1xmu protein has been used as a structural basis to evaluate the activity of some drugs as phosphodiesterase 4 inhibitors [54]. In this way, roflumilast has been evaluated as a phosphodiesterase 4 inhibitor using this system [55-57]; for this reason, roflumilast was used as a control in this study in a Docking model. The results showed differences in the interaction of compounds **4** (Figures 5 and 6; Tables 3 and 4) and **6** (Tables 5 and 6) with residues of the amino acid involved in the 1xmu protein surface compared with Roflumilast (Tables 1 and 2). Besides, binding energies from Roflumilast and 1xmu protein surface were higher (-8.00 Å) compared with either compounds **4** (-8.70 Å) or **6** (-9.70 Å). These data showed a higher interaction of both compounds **4** and **6** with 1xmu protein surface; these data suggest both compounds **4** and **6** could exert higher activity on 1xmu protein, possibly due to different functional groups involved in their chemical structure, which could produce changes in chronic obstructive pulmonary disease.

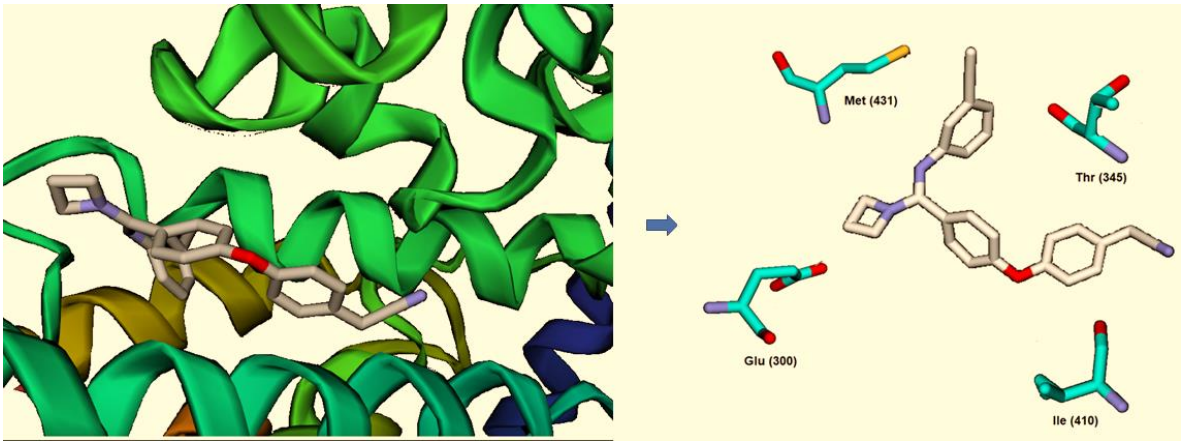

Figure 5. The scheme shows the binding sites of compound **4** with some amino acid residues involved 1xmu protein surface. The visualization was carried out using the Achilles Blind Docking Server [41-43].

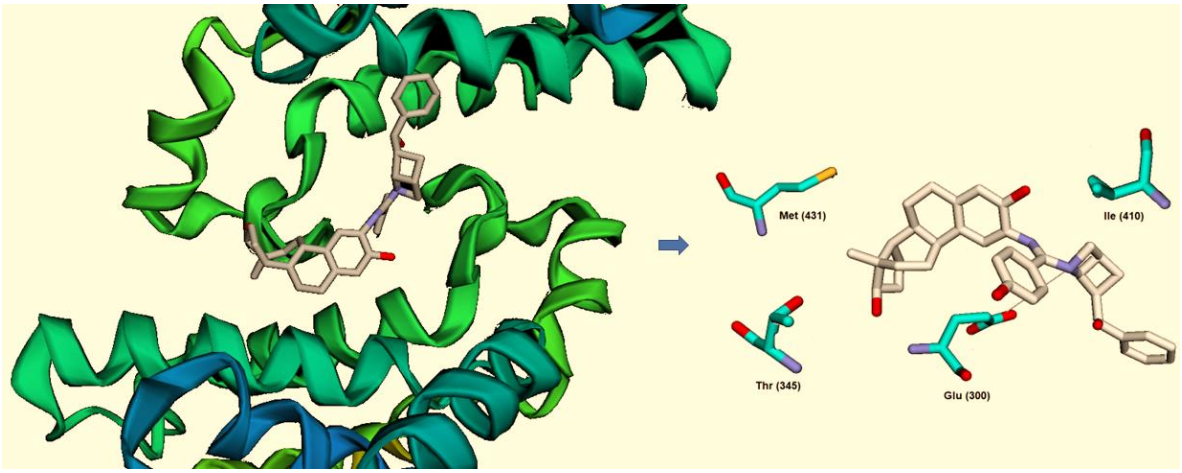

Figure 6. The scheme displays the binding sites of compound **6** with some aminoacid residues involved in 1xmu protein surface. The visualization was carried out using Achilles Blind Docking Server [41-43]

Table 1. Hydrophobic Interactions of Roflumilast with 1xmu protein surface.

Chain	***Residue aminoacids***	***Ligand-Protein distance***
A	Ar (331)	3.78
	Phe (414)	3.98
B	Ile (410)	3.95

Chain	*Residue aminoacids*	*Ligand-Protein distance*
	Met (431)	3.92

Table 2. Hydrogen Interactions of Roflumilast with 1xmu protein surface.

Chain	*Residue aminoacid*	*Distance*
A	Asn (290)	2.22
	Thr (345)	2.89
	Gln (443)	2.09
B	Thr (345)	3.04
	Gln (443)	2.34

Table 3. Hydrophobic Interactions of compound 4 with 1xmu protein surface.

Chain	*Residue aminoacids*	*Ligand-Protein distance*
A	Glu (300)	3.48
	Ile (339)	3.73
	Asp (340)	3.67
	Leu (343)	3.65
	Ala (344)	3.72
	Lys (349)	3.72
	Phe (414)	398
B	Leu (295)	3.81
	Ile (410)	3.95
	Met (431)	3.92

Table 4. Hydrogen Interactions of compound 4 with 1xmu protein surface.

Chain	*Residue aminoacid*	*Distance*
A	Glu (300)	3.27
	Asn (305)	2.56
	Asp (275)	2.89
B	Thr (345)	3.04
	Gln (443)	2.34

Table 5. Hydrophobic Interactions of compound 6 with 1xmu protein surface.

Chain	*Residue aminoacids*	*Ligand-Protein distance*
A	Phe (312)	3.66
	Leu (315)	3.99
	Lys (328)	3.93
	Arg (331)	3.42
	Gln (332)	3.60
	Lys (336)	356
	Phe (414)	398
B	Leu (295)	3.81
	Ile (410)	3.95
	Met (431)	3.92

Table 6. Hydrogen Interactions of compound 6 with 1xmu protein surface.

Chain	*Residue aminoacids*	*Ligand-Protein distance*
A	Asn (290)	3.81
	Arg (331)	3.95
	Thr (345)	2.89
	Gln (441)	2.09
B	Thr (345)	3.04
	Gln (443)	3,22

4. Conclusions

This research presents a facile synthesis of an azetyl-acetonitrile (**4**) derivative and a 2-aza-bicyclo (**6**) analog. Besides, the theoretical study showed that compounds 4 and 6 could exert change in the biological activity of phosphodiesterase-4 enzyme, which may be translated as good candidates for the treatment of chronic obstructive pulmonary disease. However, it is important to mention that to validate this hypothesis, it is necessary to carry out some alternative studies in some biological models.

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None.

Conflicts of Interest

The authors declare no conflict of interest.

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