Microwave-Mediated Synthesis of \(N\)-allyl/Propargyl Derivatives: Enzymatic Analysis as a Potential Factor Xa (FXa) Inhibitor, Theoretical and Computational Molecular Docking

Fabián Santana-Romo, Yorley Duarte, Francisco Castillo, Miguel A. Maestro, and Flavia C. Zacconi

Abstract—Potential FXa inhibitors were developed by a rational design in the first instance, focusing on a key pharmacophore, present in gold standard drugs Rivaroxaban and Apixaban. The phenyl lactam scaffold filling one of the subsites in the catalytic site, S4 pocket, with significant aromatic \(\pi-\pi\) stacking interactions, allows this frame to be invariably located with accuracy, because of the six membered lactam ring attached to an aromatic benzene ring. We anticipated that the addition of an alkyne unit would enable the exploration of the first section of S1 pocket, in this way producing a better molecule optimizing binding between our potential inhibitor and the active site of FXa. A fluorimetric assay was performed to determine IC\(_50\) values on the proposed molecules. All these findings were rationalized by docking energy delta and theoretical structure, vibrational and reactivity analysis to formulate a more accurate explanation about which structure has is the optimal inhibitor for this therapeutic target in the blood coagulation cascade.

Index Terms—FXa inhibitors, molecular docking, theoretical calculations, in vitro fluorimetric assay, synthetic inhibitors, anticoagulants.

I. INTRODUCTION

The World Health Organization (WHO) reported that thrombotic diseases had increased by up to 25 % in human population [1]–[3]. Relevant diseases in the thrombosis pathway include acute coronary syndrome (ACS) [4], venous thromboembolism (VTE) [5], deep vein thrombosis (DVT) [6] which promotes chronic leg pain, edema, and ulcers: all of which lead to his can trigger strokes whose travel through arterial circulation. Giving rise to serious diseases like acute myocardial infarction (AMI) or ischemic stroke [7].

Clotting is a sequential process that involves the interaction of coagulation factors, 14 in total [8], [9]. The majority of them are coagulation enzymes, zymogens and activated forms, which may be important for the pathogenesis of cardiovascular disorders cited above [10]–[13]. The trypsin-like serine protease FXa is an essential component of the prothrombinase complex, it plays a key role in haemostasis, due to it is the point of convergence of the blood coagulation cascade which catalyzes the production of thrombin and leads to clot formation and wound closure [14], [15].

Based on their main role into the blood coagulation cascade, the serine proteases thrombin and FXa have become a focus of interest for the development of novel anticoagulant drugs. FXa direct inhibition will offer newer and safer ways to fight against the undesired clot production in the normal blood coagulation process [16]–[19].

Nowadays, investigation has been focused on novel anticoagulants drugs as direct oral anticoagulants (DOACs) targeting a specific enzyme or coagulation step in the coagulation cascade [20]–[22]. Over the past years, several DOACs targeting FXa have been developed. Rivaroxaban, Apixaban, and Edoxaban are used in clinical practice for prevention and treatment of thrombotic diseases [23]–[25].

Recent synthetic compounds with aliphatic linear scaffolds like \(N\)-propargyl moiety which is an important structural feature of various natural products and pharmaceutical agents, those have exhibited a broad range of biological activities including the inhibition of catalytic site of enzymes with wide or narrow entry way. In addition to the potential to generate a triazole/isoxazole ring that increases the interactions with the amino acid residues, by performing a common 1,3-dipolar cyclization reaction [26]–[31].

In this article we introduce the phenyl lactam scaffold as main core moiety, with the variation of one heteroatom in position 4 respect to the nitrogen atom in the aliphatic ring to determine the possible relationship to the bioactivity against FXa. Carbon, sulfur, oxygen, and a protected nitrogen will be used as the atom variations in the lactam ring. Also, the addition of a propargyl moiety to the terminal aromatic amine group is performed to analyze the possible inhibition activity improvement. Finally, molecular docking and chemical properties from the theoretical calculations of each structure will be evaluated.

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In this article we introduce the phenyl lactam scaffold as main core moiety, with the variation of one heteroatom in position 4 respect to the nitrogen atom in the aliphatic ring to determine the possible relationship to the bioactivity against FXa. Carbon, sulfur, oxygen, and a protected nitrogen will be used as the atom variations in the lactam ring. Also, the addition of a propargyl moiety to the terminal aromatic amine group is performed to analyze the possible inhibition activity improvement. Finally, molecular docking and chemical properties from the theoretical calculations of each structure will be evaluated.

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II. METHODOLOGY

A. General Synthetic Remarks

All the reactions requiring anhydrous conditions were carried out under inert atmosphere and the solvents were appropriately dried before use. Chemical reactions were placed on a microwave Synthesis Reactor Monowave 200, Anton Paar. All reactions were monitored by thin layer chromatography (TLC). Column chromatography purifications were performed by using silica gel. The melting points of solid derivatives were measured using an Electrothermal IA9100 digital melting point apparatus (Staffordshire, UK). NMR spectra were recorded on Bruker Electrothermal IA9100 digital melting point apparatus (Staffordshire, UK). NMR spectra were recorded on Bruker Advance III HD 400 NMR spectrometer (400 MHz) with CDCl$_3$ as a solvent. The nuclear magnetic resonance (NMR) data were reported in $\delta$ (ppm) from tetramethylsilane (TMS). IR spectra were recorded in KBr pellet, on a BRUKER VECTOR 22 spectrophotometer.

a) General procedure for the synthesis of anilines derivatives (7-10)

The synthetic strategy adopted for the microwave-promoted synthesis was according to the Ullmann-Goldberg type coupling reaction variation methodology as it follows, 2-piperidinone (1), 4-Boc-piperazinone (2), thiomorpholine-3-one (3) and morpholin-3-one (4) (1.2 equiv.), CuI (2 equiv.) within the stoichiometry of the reaction, with 2-fluoro-4-iodoanilines (5) (1 equiv.) in dry toluene (PhMe) in the presence of N,N’-dimethylethlenediamine (DMEDA)[32] (6) (2 equiv.) as a ligand, and dry K$_2$PO$_4$ (2 equiv.) as the base (Scheme 1).

The equipment (Synthesis Reactor Monowave 200, Anton Paar) irradiates with power (6–7 bar, 850 watts) at 110 °C for 1 hour (1 h) for 5, 6, 7, and 2 hours (2 h) for 8.

The crude reaction mixture was prepurified by using 0.5-1 cm thick of silica gel and Celite 545 on a Büchner funnel with ethyl acetate as the mobile phase. The solvent was removed by evaporation on a rotary evaporator. Then each mixture was appropriately dried before use. Chemical reactions were carried out under inert atmosphere and the solvents were appropriately dried before use. Chemical reactions were placed on a microwave Synthesis Reactor Monowave 200, Anton Paar. All reactions were monitored by thin layer chromatography (TLC). Column chromatography purifications were performed by using silica gel. The melting points of solid derivatives were measured using an Electrothermal IA9100 digital melting point apparatus (Staffordshire, UK). NMR spectra were recorded on Bruker Advance III HD 400 NMR spectrometer (400 MHz) with CDCl$_3$ as a solvent. The nuclear magnetic resonance (NMR) data were reported in $\delta$ (ppm) from tetramethylsilane (TMS). IR spectra were recorded in KBr pellet, on a BRUKER VECTOR 22 spectrophotometer.

b) General procedure for the synthesis of N-alllyl/propargyl aniline derivatives (10-13)

The microwave-promoted synthesis of bimolecular nucleophilic substitution for propargylation of anilines (7-10) were performed by adding each aniline (1 equiv.), K$_2$CO$_3$ as base (1.5 equiv.), and 3 mL of dry acetonitrile into a microwave G-30 vessel. Then the mixture was stirred at 500 rpm under nitrogen inert atmosphere for 20 minutes. At the same time in a 3 mL common vessel weigh (0.1 equiv.) of KI as halogen exchange reagent and the propargyl bromide solution 80 % wt in toluene (11) was added (1 equiv.) accompanied by 1 mL of dry acetonitrile. After that, pour the contents into the G-30 vessel (Scheme 2). (Synthesis Reactor
Monowave 200, Anton Paar) irradiates with power (7-8 bar, 850 watts) at 160 °C for 30 minutes. The crude reaction mixture was pre purified by using 0.5-1 cm tick of silica gel and Celite 545 on a Büchner funnel with ethyl acetate as the mobile phase. The solvent was removed by evaporation on a rotary evaporator. Then each mixture was purified by flash column chromatography using Silica gel 60 as stationary phase and eluted with n-hexane:ethyl acetate 80:20.

Scheme 2. Synthesis of N-propargyl-anilines derivatives.

1-(3-Fluoro-4-(prop-2-yn-1-ylamino)phenyl)piperidin-2-one (12)

Brown solid; 69% yield; m.p. 121-122 °C; IR (KBr) ν/cm⁻¹ 3332, 3021, 2954, 2923, 2862, 1621, 1535, 1411, 1350, 1257, 1219, 1180, 1103, 1010, 979, 926, 864, 802, 756; ¹H NMR (400 MHz, CDCl₃) δ 6.94 – 6.84 (m, 2H), 6.74 (t, J = 9.0 Hz, 1H), 4.24 (s, 1H), 3.90 (s, 2H), 3.53 (t, J = 5.7, 1.0 Hz, 2H), 3.43 (s, 2H), 2.99 (td, J = 5.8, 1.2 Hz, 2H), 2.23 (td, J = 2.5, 0.5 Hz, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 167.0, 152.4, 150.0, 149.9, 134.5, 131.3, 121.6, 112.7, 80.3, 71.7, 68.5, 64.1, 50.0, 33.1; ¹⁹F NMR (376 MHz, CDCl₃) δ -133.50; ESI-HRMS (m/z): calcd. for C₁₉H₁₃FN₂O [M+H]⁺: 246.1168, found 246.1077.

-tert-butyl 4-(3-Fluoro-4-(prop-2-yn-1-ylamino)phenyl)-3-oxopiperazine-1-carboxylate (13)

Brown solid; 72% yield; m.p. 138-140 °C; IR (KBr) ν/cm⁻¹ 3340, 3240, 2862, 2113, 1689, 1535, 1481, 1327, 1249, 979, 948, 871, 817, 771, 632; ¹H NMR (400 MHz, CDCl₃) δ 6.97 – 6.87 (m, 2H), 6.75 (t, J = 8.9 Hz, 1H), 4.30 (s, 1H), 4.18 (s, 2H), 3.91 (s, 2H), 3.71 (dd, J = 6.1, 4.4 Hz, 2H), 3.61 (dd, J = 6.4, 4.2 Hz, 2H), 2.20 (td, J = 2.4, 0.8 Hz, 1H), 1.45 (d, J = 1.1 Hz, 9H, 3 CH₃); ¹³C NMR (101 MHz, CDCl₃) δ 165.8, 153.7, 152.2, 149.8, 133.1, 121.7, 112.7, 101.8, 80.8, 80.2, 71.7, 50.0, 48.2, 40.4, 33.0, 28.3(3 CH₃); ¹⁹F NMR (376 MHz, CDCl₃) δ -133.49; ESI-HRMS (m/z): calcd. for C₁₉H₁₃FN₂O [M+H]⁺: 347.1645, found 347.1597.

-(3-Fluoro-4-(prop-2-yn-1-ylamino)phenyl)thiomorpholine ne-3-one (14)

Brown solid; 70% yield; m.p. 147-148 °C; IR (KBr) ν/cm⁻¹ 3356, 3240, 2113, 1874, 1658, 1527, 1419, 1280, 1211, 1188, 1026, 964, 910, 864, 817, 763, 702, 648; ¹H NMR (400 MHz, CDCl₃) δ 6.99 – 6.89 (m, 2H), 6.78 (t, J = 9.0 Hz, 1H), 4.23 (s, 1H), 3.96 (s, 2H), 3.90 (td, J = 5.7, 1.0 Hz, 2H), 3.43 (s, 2H), 2.99 (td, J = 5.8, 1.2 Hz, 2H), 2.23 (td, J = 2.5, 1.2 Hz, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 167.0, 152.4, 132.7, 112.3, 113.4, 112.7, 80.3, 71.8, 52.7, 33.2, 30.6, 26.8; ¹⁹F NMR (376 MHz, CDCl₃) δ -133.55; ESI-HRMS (m/z): calcd. for C₁₉H₁₃FN₂O [M+H]⁺: 264.0733, found 264.0744.

4-(3-Fluoro-4-(prop-2-yn-1-ylamino)phenyl)morpholin-3-one (15)

Brown solid; 67% yield; 139-141 °C; IR (KBr) ν/cm⁻¹ 3348, 2985, 2924, 1843, 1666, 1535, 1427, 1381, 1280, 1226, 1180, 1103, 1033, 995, 941, 887, 864, 833, 802, 772, 687, 625; ¹H NMR (400 MHz, CDCl₃) δ 7.03 – 6.94 (m, 2H), 6.78 (t, J = 8.9 Hz, 1H), 4.29 (s, 2H), 3.94 (s, 1H) 3.97 (t, J = 5.1 Hz, 2H), 3.95 – 3.94 (s, 2H), 3.67 (t, J = 5.1 Hz, 2H), 2.23 (td, J = 2.5, 0.5 Hz, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 166.8, 152.3, 149.9, 134.5, 131.3, 121.6, 112.7, 80.3, 71.7, 68.5, 64.1, 50.0, 33.1; ¹⁹F NMR (376 MHz, CDCl₃) δ -133.50; ESI-HRMS (m/z): calcd. for C₁₉H₁₃FN₂O [M+H]⁺: 248.0961, found 248.0903.

B. Computational Methods

The Gaussian 09 computational package was used to perform ground-state geometry optimization calculations, vibrational frequency calculations and reactivity parameters calculations, without symmetry constraints, employing Becke’s three-parameter hybrid functional combined with Lee–Yang–Parr correlation functional B3LYP. A 6-311+G(d,p) basis set was also employed [33].

C. Biological Evaluation of FXa Inhibition

By using SensoLyte® Rh110 Factor Xa Assay Kit “Fluorimetric”. FXa (bovine purified enzyme) activity was measured. Upon FXa protease cleavage, this substrate generates the Rh110 (rhodamine 110) as free fluorophore which can be detected at excitation/emission of 490 nm/520 nm. The fluorescence reading from the substrate control well must be used as the background fluorescence. All fluorescence readings ought to be expressed in relative fluorescence units (RFU).

All compounds were evaluated in vitro for their FXa enzyme inhibitory activity using Rivaroxaban (gold standard inhibitor) and Gabexate mesylate (FXa kit inhibitor) as the positive control. As a priority test, all the synthesized compounds were screened at 100 µM to obtain a preliminary result regarding the potential inhibitory activity of the novel molecules. This assay was performed by contrasting the curves of the positive and negative controls, thus determining all those curves that are close to or below half the average of the maximum value reported in the controls.

D. Molecular Docking

All computational calculations were performed using the Schrödinger’s Small-Molecule Drug Discovery Suite. The initial setup of FXa enzyme for calculations was prepared using the Protein Preparation Wizard of Schrödinger [34]. The FXa crystal structure complexed with Apixaban (PDB ID 2P16) [35] was used for docking experiments. The docking calculations using rigid-receptor and flexible-ligand were performed with Glide through the Single Precision (SP) mode [36]. The docking poses for each ligand were analysed using the E_mode score and their interaction with residues at binding sites (Table I).
### TABLE I: DOCKING SCORES FOR FXa BINDING STUDY

| Compound | Glide score (kcal/mol) | Glide E<sub>model</sub> (kcal/mol) |
|----------|------------------------|-------------------------------|
| 9        | -6.89                  | -45.65                        |
| 14       | -5.84                  | -45.10                        |
| Apixaban | -11.41                 | -119.92                       |

E<sub>model</sub> is a specific combination of Docking Score, CvdW is the non-bonded interaction energy between the ligand and the receptor) and the internal torsional energy of the ligand conformer.

\[
\text{CvdW} = \text{Coul} + \text{vdW} \tag{1}
\]

### III. RESULTS AND DISCUSSION

#### A. Synthetic Steps

Design, synthesis and development of potential novel anticoagulants were covered in this investigation. Microwave-mediated synthesis of novel N-allyl/propargyl aniline derivatives, eight novel compounds, were obtained from a lactam ring with different heteroatoms in position 4 and 2-fluoro-4-iodoaniline as starting materials. The purified products were obtained in moderate to good yields (Table II). The isolated and purified compounds were fully characterized by ¹H-NMR, ¹³C-NMR, ¹⁹F-NMR, IR and MS techniques. Moreover, X-ray diffraction analysis for most active compounds was performed.

### TABLE II: DERIVATIVES YIELD

| Compound | Yield (%) |
|----------|-----------|
| 7        | 50        |
| 8        | 86        |
| 9        | 73        |
| 10       | 89        |
| 12       | 69        |
| 13       | 72        |
| 14       | 70        |
| 15       | 67        |

#### B. Theoretical Calculations

A theoretical description of a chemical system is a powerful tool to understand a reaction or system behavior. In order to understand the structure and energetic behavior of compounds 9 and 14, the optimization and a first approach to reactivity indexes (electronic chemical potential (µ), chemical hardness (η) and electrophilicity (ω)) were calculated.

**a) Structural analysis**

Structural parameters were optimized and compared with crystal data for both systems (Table III and Table IV). Both structures have been well refined, with final R indices [I>2σ(I)] of R₁=0.0381, wR₂=0.1060 for Molecule 9 and R₁=0.0263, wR₂= 0.0749 for Molecule 14. These bond angles show a good correlation between calculated and experimental bond distances, suggesting good robustness for the central structure for the systems that is not changed when the propargyl moiety is added.

### TABLE III: COMPARISON BETWEEN CALCULATED AND EXPERIMENTAL SELECTED BOND ANGLES FOR COMPOUND 9

| Bond (Å) | CALC | EXP |
|----------|------|-----|
| S(1)-C(1)| 1.826| 1.798|
| S(1)-C(3)| 1.824| 1.796|
| N(1)-C(2)| 1.377| 1.356|
| N(1)-C(4)| 1.477| 1.479|
| N(1)-C(5)| 1.440| 1.436|
| O(1)-C(2)| 1.220| 1.232|
| F(1)-C(7)| 1.364| 1.363|
| N(2)-C(8)| 1.388| 1.380|

### TABLE IV: COMPARISON BETWEEN CALCULATED AND EXPERIMENTAL SELECTED BOND ANGLES FOR COMPOUND 14

| Bond (Å) | CALC | EXP |
|----------|------|-----|
| S(1)-C(2)| 1.824| 1.821|
| S(1)-C(3)| 1.827| 1.826|
| N(1)-C(1)| 1.377| 1.350|
| N(1)-C(4)| 1.478| 1.475|
| N(1)-C(5)| 1.440| 1.437|
| O(1)-C(1)| 1.220| 1.235|
| F(1)-C(7)| 1.363| 1.366|
| N(2)-C(8)| 1.382| 1.386|
| N(2)-C(11)| 1.458| 1.457|

### Table V: Vibrational Comparison between Systems

| Vib. (cm⁻¹) | Molecule 9 | Molecule 14 |
|-------------|------------|-------------|
| v(C=O)      | 1719.1     | 1717.6      |
| v(CH₂)      | 3010.7     | 2916.4      |
| v₂(NH₂)     | 3581.6     | 3333.0      |
| v₂(NH₂)     | 3684.9     | 3433.3      |
| v(=CH)      | 3475.4     | 3240.4      |
| v(NH)       | 3620.5     | 3356.1      |

*From propargyl moiety.
b) Vibrational analysis
The vibrational analysis shows a considerable difference between calculated and experimental vibrations but can clearly differentiate both molecules. The symmetrical and asymmetrical vibrations for -NH₂ disappear when reacted to the propargyl derivative, and a -CH and -NH vibration appear. Calculated and experimental vibration frequencies are shown in Table V.

c) Reactivity indexes
The electronic chemical potential (μ) characterizes the tendency to escape of electrons from the equilibrium, indicating the difference in an electronic cloud. This index is quantified by the finite difference approximation and the Koopman’s theorem, and its associated to the ionization potential (I) and the electron affinity (A), and to the energies of Frontier Molecular Orbitals (FMOs) [37].

\[
\frac{I + A}{2} = LUMO + HOMO \tag{2}
\]

The chemical hardness is a measure of the resistance of a chemical species to change its electronic configuration, and it is associated together with electronegativity, to the stability of the systems and the resistance of a molecule to exchange electron density. In order to quantify this system, the same approximations as for electronic chemical potential were made [38].

\[
\frac{I - A}{2} = \frac{LUMO - HOMO}{2} \tag{3}
\]

The electrophilicity index indicates the trend to accept electrons in an equilibrium system, giving information when comparing two molecules. As a higher index value, the more electrophilic character has the molecule, as a lower index value, a more nucleophilic character has the molecule. It is also associated with the energy stabilization of a molecule when it acquires an additional quantity of electron density from the environment[39]. This index considers the tendency of an electrophile to acquire electron density given by μ, and the resistance of the molecule to give electron density, by η, giving the expression:

\[
\Omega = \frac{\eta^2}{\mu} \tag{4}
\]

C. FXa Inhibition Assay
The assay showed that several of the novel compounds do not exhibit inhibitory activity against FXa at 100 µM, only compound 9 displayed FXa moderate inhibition and 14 showed FXa inhibition higher than 50% in the screening assay. The data obtained are correlated by demonstrating that both thiomorpholine-3-one derivatives, 9 and 14, have showed the highest activity against FXa (Table VII). Dose response curves indicate an IC₅₀ value of 72.14 µM for 9 and 33.50 µM for 14. These values proved that the addition of the propargyl group to the original disubstituted aniline increases the FXa activity inhibition, so that as a projection for the development of new triazole or isoxazole derivatives through 1,3-dipolar reactions.

| Compound | FXa Inhibition (%) | IC₅₀ (µM) |
|----------|---------------------|-----------|
| 7        | 23.4                | -         |
| 8        | 20.9                | -         |
| 9        | 31.8                | 72.14     |
| 10       | 17.4                | -         |
| 12       | 20.9                | -         |
| 13       | 20.9                | -         |
| 14       | 84.3                | 33.50     |
| 15       | 20.8                | -         |

*Tested at 100 µM
D. Molecular Docking

Molecular docking studies of Apixaban to FXa enzyme have shown that the 4-methoxy group is oriented to the bottom of the active site, near waste A190 and C191. The pyrazole moiety showed two interaction of Hydrogen bond with G216 and E146 residues, which contributed to high affinity to FXa. Finally, the phenyl lactam scaffold is appropriately positioned in a hydrophobic pocket in the middle to Y99, W215 and F174 residues (Fig. 2, Apixaban is shown in orange). According to the results presented here, phenyl lactam scaffold was retained as the main core in the new set of molecules.

To enhance the binding affinity and investigate the effect of the substituents of new molecules to FXa enzyme, a series of halogens, as well as an amine and propynyl-amine group were introduced into the phenyl moiety. Furthermore, to increase the affinity of the lactam moiety, a series of heteroatoms were incorporated into this scaffold. The docking results for molecules 9 and 14 (the most active molecules of the series) indicated that the thiomorpholine-one group doesn’t present any different interaction compared to lactam scaffold of Apixaban, showing that the change of heteroatom does not improve the affinity to FXa enzyme in this region. Furthermore, the phenyl moiety of molecules 9 and 14 are correctly positioned into the hydrophobic pocket, showing π-π stacking interactions with Y99 and F174 residues (Figure 2, molecule 9 in green and 14 in blue).

These docked poses agree with the glide score, Glide E_{model} and biological FXa activity reported for molecules 9, 14 and Apixaban, which indicate that Apixaban binds much better to the FXa enzyme than the other evaluated molecules.

![Fig. 2. Molecular interaction of FXa enzyme bound to Apixaban (orange), compound 14 (blue), and compound 9 (green).](image)

IV. CONCLUSION

We have proposed a simple approach to the development of potential FXa inhibitors. We did this by designing and analyzing the main core of gold standard inhibitors. These novel derivatives were produced by a simple two step-microwave-mediated coupling synthesis, which were tested against FXa obtaining as a result that the heteroatom sulfur has a preponderant role in the pharmacophore fitting in the pocket S4 of the catalytic site. Consequently, derivatives 9 and 14 projecting the structural evolution of the inhibition of the human coagulation cascade, considering as a base scaffold for the exploration of the S1 pocket by the addition of an alkyne. The purpose of this terminal structure is to provide the potential to convert it into an isoxazole or triazole moiety through a 1,3-dipolar cycloaddition reaction.

Molecular docking results indicate the potential interactions between each synthesized compound and the FXa enzyme. Furthermore, the phenyl moiety of 9 and 14 is correctly positioned into the hydrophobic pocket, showing π-π stacking interactions with Y99 and F174 residues.

Structural analysis shows a good correlation between calculated and experimental parameters, where the propargyl moiety does not affect the distances of selected bonds. Vibrational analysis shows a slight difference between calculated and experimental although these results are in agreement when comparing the change in selected vibrations when the propargyl moiety is added.

The energetic analysis shows that the FMOs have a slight difference between molecules 9 and 14, thus the difference between reactivity parameters is also slight. The FMOs diagram shows no difference between both systems, demonstrating that the binding analysis difference comes from the structural added propargyl moiety, and not from the orbital composition.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author FCZ designed the study, managed the literature searches and wrote the manuscript. Author FSR carried out the synthesis, purification and characterization of all compounds and wrote the manuscript. Authors YD and FC carried out the molecular docking and theoretical calculations respectively. Author MAM carried out the X-Ray analysis. All authors read and approved the final manuscript.

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