RATIONAL DESIGN, SYNTHESIS AND CHARACTERIZATION OF HYBRID MOLECULES WITH PYRAZOLINE, PYRIMIDINE AND THIAZOLIDINE NUCLEI AS POTENTIAL ANTIBACTERIAL AGENTS

Liliana Rocio Guerrero-Villalobos,[1] Fabián Orozco-López[2]

Abstract: In this paper, a set of computational tools were used to design and evaluate molecular structures resulting from the combination of the biologically interesting pyrazoline, aminopyrimidine and thiazolidine nuclei (molecular modification) to obtain new bioactive compounds. Key physicochemical properties were calculated (absorption, distribution, metabolism, excretion and toxicity), to determine the bioavailability of the designed compounds and to perform a preselection of 12 derivatives which were then optimized and studied by molecular docking with the receptor PBP3 (4bjp) from *Escherichia coli*. By these studies, 8 compounds were selected by their binding energies (from -5,36KJ/mol to – 7,05KJ/mol) and significant interactions with the amino acids of the receptor in its active site. In general, the synthesis of the selected compounds was carried out from the α,β-unsaturated carbonyl compounds as precursors. The dihydropyrazole derivatives were obtained from the reaction of chalcones with one equivalent of hydrazine derivatives by one-step cyclocondensations. The pyrimidine series were synthesized starting by the reaction of chalcones and guanidine, giving rise to the corresponding amonopyrimidines, which were then reacted with aromatic and heteroaromatic aldehydes to obtain the acyclic azomethine compounds. The thiazolidine-4-ones were obtained from the aminopyrimidines synthesized above, using three-component cyclocondensation reactions with 2-mercaptoacetic acid and benzaldehyde, in anhydrous toluene or benzene as solvents and using conditions of reflux with Dean- Stark. Finally, assays were carried out aiming to the formation of β-lactam rings, using the Staudinger-type cycloaddition reaction of 2-chloroacetyl chloride with cyclic imines. All the obtained compounds were fully characterized by IR spectroscopy, as well as mono- and bidimensional NMR techniques. The most promising compounds will be evaluated by *in vitro* assays as potential antibacterial agents.

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Keywords: Antibacterials, molecular modification, virtual screening, pyrazolines, aminopyrimidines, thiazolidines.

Introduction

The indiscriminate use of antibiotics due to the growing proliferation of bacterial diseases has generated resistant and highly infectious strains that are often fatal in immunocompromised patients (Cuellar, 2013). This has motivated the scientific community to face new challenges in the development of latest introduced compounds with the widest possible spectrum, low toxicity, greater bioactivity and high selectivity.

In addition, the synthetic methods that could be more versatile, economical and practical, implying both health benefits and the generation of new knowledge in the development of pharmacophores of interest (World Health Organization, 2016). This scenario has made it possible to boost the search and discovery of new antimicrobial agents, which is reflected in the long lists of new antibiotic compounds that were not available due to the high amount of research and development in the different areas of both chemistry and medicine, seeking to address the problem (Vardanyan & Hruby, 2016). Along with this, advances in the understanding of bacterial physiology have established a structure-activity relationship (SAR) (Reguero, Barreto, & Jimenez, 1989), which has been used to modify the chemical structures of antibiotics with the aim of improving their antimicrobial activity, and thus to fight bacteria that have acquired resistance to previous antimicrobials (Amábile-Cuevas, 2003).

In this field, it is important to mention that the process of developing new medicines can take years to reach its final stage and represent an excellent economic investment to ensure its mechanism of action and guarantee its safety and efficacy *in vivo* (Marovac, 2001). Consequently, the current trend in obtaining new medicines seeks to rationalize the development of new therapeutic agents based on the relationship between the chemical structure of the medicine and its biological activity, as well as the rational design of new drugs using current tools of computational chemistry which daily grow in their ability to detect unobvious similarities and differences between pharmacotherapeutic agents (Escalona, Carrasco, & Padrón, 2008).

From this standpoint, the synthesis of heterocyclic compounds is proposed as an alternative to address this problem by means of versatile approaches such as Diversity-Oriented Synthesis - DOS (Spring,

[1] National University of Colombia, Bogotá, Colombia, lrguerrerov@unal.edu.co
[2] National University of Colombia, Bogotá, Colombia, forozcol@unal.edu.co

2003), molecular modification or hybrid pharmacophore (Moreno-Díaz, et al., 2008) and bioisosterism (Navarrete-Vázquez, et al., 2006), in order to circumvent the defence mechanisms and thus enhance pharmacological and pharmacodynamic effect of drugs; a process that throughout several studies have shown new and / or better biological activity.

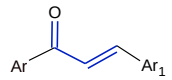
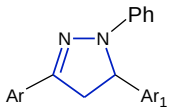
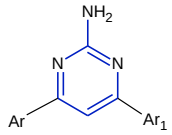
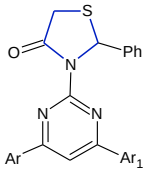
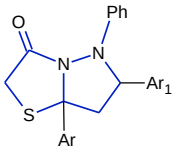
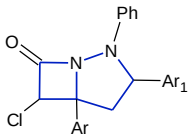
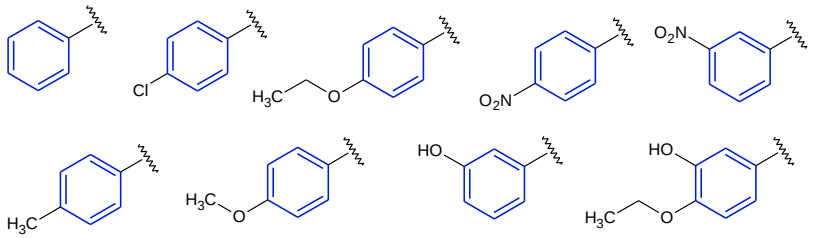
In this respect, this work seeks to apply the methodology of rational *in silico* drug design with the aim of obtaining molecular prototypes with pyrazole, aminopyrimidine, thiazolidonic and ß-lactam nuclei with potential antimicrobial activity; all of them tackled by the derivatization of chalcones as building blocks. In this study, ADMET parameters and virtual screening were considered in order to establish bioavailability and the proper structure-activity relationships, selecting this way the best prototypes to be synthesized by means of computational tools (Cheng et al., 2012), thus contributing to an increase in the chances of success and a decrease in costs (time, infrastructure, experimentation, among others), compared to traditional methods used in the discovery of drugs.

Methods

a. Structures preparation and virtual screening

Several aspects were taken into account. At the outset, the identification and design of the prototypes to be studied was carried out through the molecular modification approach (hybrid pharmacophore), with the fusion of some nuclei of the different chemical structures of the antibiotics (pyrazolines, aminopyrimidines, thiazolidones and β-lactams). To establish the best oral bioavailability profile according to the model developed by Lipinski (Lipinski, 2001), 60 new hybrid structures were designed and were then evaluated through *in silico* studies for the prediction of some relevant ADMET properties. In this study, molecular prototypes (pharmacophoric nuclei) were designed and diversified by the substitutions patterns at the Ar and Ar_1 positions, which sought to enhance biological activity and/or improve their bioavailability (Table 1).

Table 1: Chemical structure of prototypes 1-6(a-j).

					
1	**2**	**3**	**4**	**5**	**6**
Substituents in Ar and Ar_1					
					
Source: Author					

In order to determine the bioavailability and toxicity profile of the prototypes, *in silico* tools were used that allowed the analysis and subsequent pre-selection of candidate compounds to be studied further. The first source of filtration was the prediction and evaluation of oral absorption from the physicochemical properties (Lipinski's rules) since the more distant they are from the parameters of the rule, the more more complicated to overcome the subsequent stages in a possible development of drugs. Table 2 shows the values of the best prototypes, in which only 5 violates one of Lipinski's rules (lipophilicity), showing an excellent bioavailability profile. *Ampicillin* was chosen as blank and was therefore subjected to the same process of evaluation of the bioavailability profile used in the other compounds, to compare the values and to analyse the selection criteria of the compounds in each phase. In that sense, it is observed that most of the scores are considerably higher except **5j**.

Table 2: Predicted ADME properties of the best prototypes.

Compound	MlogP	S+logP	RuleOf5	RuleOf5_Code	MWt	M_NO	T_PSA	HBDH
1i	3.822	4.201	0	<None>	282.341	3	46.53	1
2g	4.669	5.037	1	LP	358.443	4	45.06	1
2j	4.881	5.464	1	LP	372.47	4	45.06	1
3d	4.092	4.007	0	<None>	281.746	3	51.8	2
3h	3.824	4.05	0	<None>	261.328	3	51.8	2
4a	4.093	4.343	0	<None>	453.566	5	55.32	0
4i	3.773	4.387	0	<None>	485.565	7	95.78	2
4j	4.03	4.264	0	<None>	483.592	6	75.55	1
5i	4.642	4.754	1	LP	446.571	5	53.01	1
5j	3.678	2.831	0	<None>	310.42	3	23.55	0
6a	6.027	4.805	1	LP	409.318	3	23.55	0
6c	5.424	5.033	1	LP	418.926	4	32.78	0
Ampicillin	0.656	-1.302	0	<None>	349.411	7	112.73	4

Source: Author

To predict the toxicity of the prototypes that presented the best bioavailability profiles, the compounds were subjected to estimation of *in silico* toxicity from their molecular structure using the free online software admetSAR, which is a database that relates structure-activity (Cheng et al., 2012). In general, for toxicity predictions, the entire series of compounds with $-NO_2$ substituents showed toxicity as possible carcinogenic agents and exhibited significant levels of toxicity in environmental, biological models. Compared with Ampicillin, scores for Rat Acute Toxicity LD_{50} of most of the prototypes resulted similarly, but almost at the limit of the toxicity value, according to the scale of (Hodge & Sterner, 1949) (From moderately toxic to slightly toxic). The prototype **5i** presented the best profile, with the lowest toxicity of the series (Table 3).

With complete ADMET data obtained, it was possible to select the group of molecules to continue to the molecular docking study, since it was necessary to confirm that the potential new drug will be acceptable in terms of efficacy (activity) and safety (Toxicity) for *in vitro* and *in vivo* assays in animals and subsequently, in humans.

Table 3: Results of toxicity prediction for the selected prototypes.

Compound	Rat Acute Toxicity LD_{50}, mg/kg	Fish Toxicity pLC_{50}, mg/L	Tetrahymena Pyriformis Toxicity $pIGC_{50}$, ug/L	AMES Toxicity	Carcinogens
1i	529.14	0.7881	1.4756	Non AMES toxic	Non-carcinogens
2g	800.40	0.9462	0.8867	Non AMES toxic	Non-carcinogens
2j	843.98	0.9254	0.9021	Non AMES toxic	Non-carcinogens
3d	611.98	0.9897	1.0663	Non AMES toxic	Non-carcinogens
3h	555.09	2.1895	0.4607	Non AMES toxic	Non-carcinogens
4a	975.35	1.3660	0.5324	Non AMES toxic	Non-carcinogens
4i	1079.17	1.4308	0.5852	Non AMES toxic	Non-carcinogens
4j	1096.11	1.4392	0.5863	Non AMES toxic	Non-carcinogens
5i	1107.54	1.1490	0.5910	Non AMES toxic	Non-carcinogens
5j	760.65	1.7126	0.2589	Non AMES toxic	Non-carcinogens
6a	977.08	0.9791	0.9481	Non AMES toxic	Non-carcinogens
6c	1000.56	0.8618	0.8056	Non AMES toxic	Non-carcinogens
Ampicillin	545.78	1.7369	0.2178	Non AMES toxic	Non-carcinogens

Source: Author

The group comprised of 12 compounds that presented the best bioavailability profiles were subjected to geometric optimization with the help of the HyperChem v.8.0.10 computational tool, in order to find the lowest energy (most stable) molecular conformation for each candidate, which will allow the study of the atomic and molecular properties of the prototypes. With this study, it was possible to establish that 10 of the molecules are below the energy value observed for the reference compound. Only prototypes **6a** and **6c** have values close to Ampicillin, indicating that these molecules at the conformational level are the least stable and possibly the most reactive.

Table 4: Single point energy and gradient of optimized structures of selected prototypes

Compound	Energy (kcal/mol)	Energy Gradient (kcal/mol)
1i	16.659472	0.009584
2g	12.243122	0.009579
2j	12.008585	0.009787
3d	27.334485	0.009215
3h	21.333536	0.009500
4[a]	30.989200	0.009848
4i	31.256056	0.009958
4j	33.507793	0.009481
5i	28.509365	0.008822
5j	25.530389	0.009847
6a	88.456739	0.009296
6c	89.821765	0.009569
Ampicillin	88.301683	0.09338

Source: Author

With the objective of determining the viability of the interaction of the selected molecules with the catalytic domain of the enzyme transpeptidase PBP3 (4bjp) of *E. coli* (Berman, et al., 2000), and to find the most likely ligand-receptor binding conformation, the group of compounds were subjected to computer–aided molecular coupling analysis. The study of molecular docking was performed using the computational tool Auto Dock 4.2.6, by a rigid-body approach and study was conducted at the active site identified for the enzyme PBP3 and considering eight key amino acid residues: Ser307, Lys310, Ser359, Asn361, Lys494, Thr495, Gly496 and Thr497, responsible for the binding of ß-lactams to the active site of PBPs (Sauvage, et al., 2014).

The different Docking results (Table 5), show which of the selected molecules display binding energy values similar to the reference drug Ampicillin (-6.16 kJ / mol), and which of them have a better interaction with the amino acids of the catalytic site of the enzyme. In the set of results comprising hydrogen bonds, bonding energies and docking conformations of each of the molecules with the active site, it is evident that the prototypes **1i** and **4i** had interactions with several amino acids in the pocket and which of the atoms of the molecule participate in those bindings. It is also shown that **5i** and **5j** have no apparent interaction in the study.

Table 5. Binding energies and interactions with active site residues.

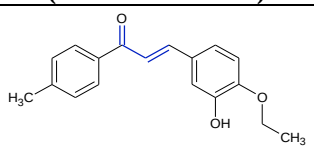
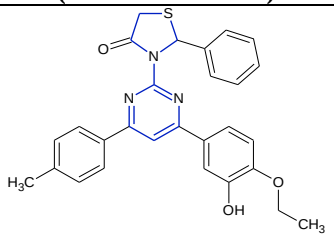
Prototype	Structures and Hydrogen bonds (Residue-interaction)	Binding energy (kJ/mol)	Prototype	Structures and Hydrogen bonds (Residue-interaction)	Binding energy (kJ/mol)
1i	 SER 307:H 1i:31:O THR 497:HN 1i:27:O TYR 347:HH 1i:24:O THR 495:O 1i:39:H SER 359:H 1i:31:O	-5.78	**4j**	 SER 307:O 4j:44:H THR 497:HH 4j:26:O	-6.52

Table 5. Binding energies and interactions with active site residues.

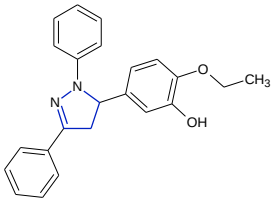
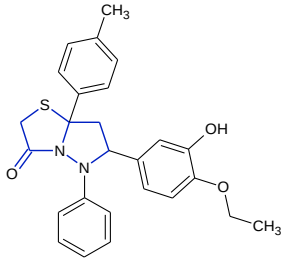
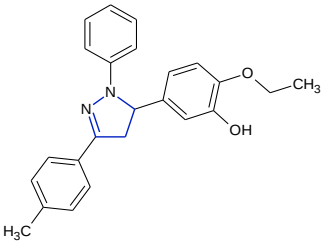
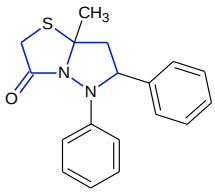
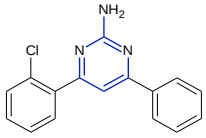
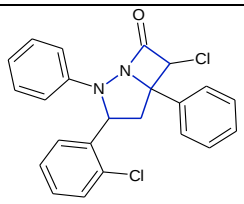
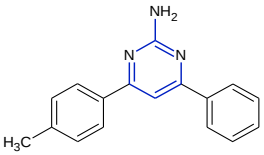
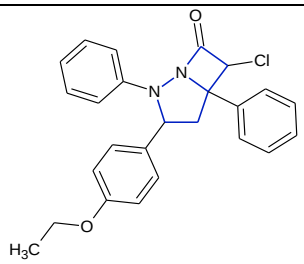
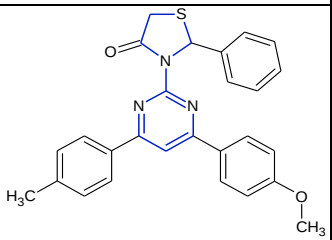
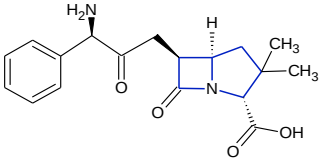
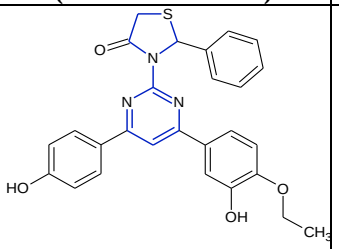
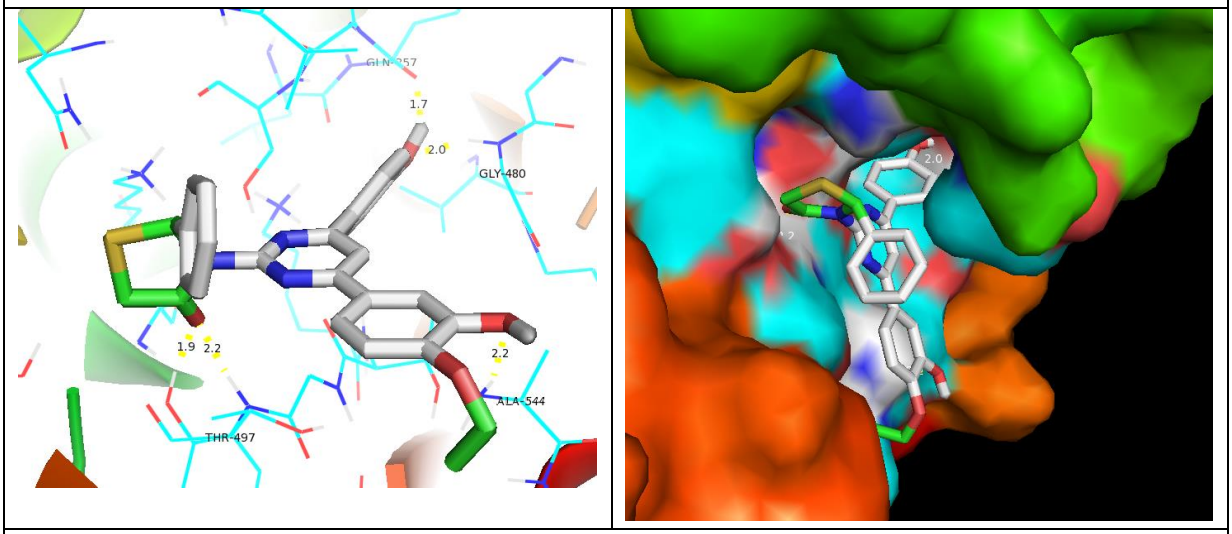
Prototype	Structures and Hydrogen bonds (Residue-interaction)	Binding energy (kJ/mol)	Prototype	Structures and Hydrogen bonds (Residue-interaction)	Binding energy (kJ/mol)
2g	 ALA 544:HN 2g:19:O ALA 544:HN 2g:20:O SER 307:H 2g:2:N THR 497:H 2g:19:O	-7.64	**5i**	 LYS 499: HN 5i:44:O	-5.58
2j	 ASN 361:H 2j:19:O ASN 361:H 2j:20:O GLY 480:HN 2j:26:O	-7.62	**5j**	 NO	1-5.69
3d	 PHE 417:O 3d:31:H ASN 361:O 3d:32:H TYR 419:OH 3d:2:N	-5.55	**6a**	 SER 307:H 6a:37:O SER 359:H 6a:37:O	-6.22
3h	 ASN 361:O 3h:34:H TYR:419:HH 3h:34:O PHE 417:O 3h:35:H	-5.36	**6c**	 THR 497:H 6c:19:O	-7.05
4a	 TYR 419:HH 4a:27:O LYS 499:H 4a:17:O SER 307: O 4a:26:S	-6.84	**AMP.**	 ASN 361:O AMP:42:H SER 397:H AMP:24:O ALA 544:HN AMP:11:O ALA 544:HN AMP:12:O SER 359:H AMP:24:O SER 359:O AMP:43:H	-6.16

Table 5. Binding energies and interactions with active site residues.

Prototype	Structures and Hydrogen bonds (Residue-interaction)	Binding energy (kJ/mol)	Prototype	Structures and Hydrogen bonds (Residue-interaction)	Binding energy (kJ/mol)
4i	 GLY 480:HN 4i:7:O ALA 544:HN 4i:17:O GLN 357:O 4i:40:H TYR 514:HH 4i:27:O THR 497:HN 4i:27:O	-6.31			
Source: Author					

After the enzyme-ligand molecular coupling study, each prototype was visualized and analysed using the PyMOL computational tool (Figure 1), which determined that 10 compounds have interaction with at least one of the residues of the amino acids that are part of the active site of *E. coli* PBP3. Among the compounds that showed interaction with the catalytic site of the enzyme, one is a chalcone (**1i**), two belong to molecules with pyrazoline nuclei (**2g**, **2j**), two to aminopyrimidine derivatives (**3d**, **3h**), three to thiazolidone compounds (**4a**, **4i**, **4j**) and two to derivatives with β-lactam nuclei (**6a**, **6c**). The last two prototypes are still under consideration to pass to the synthesis stage.

Figure 1: Molecular Docking Display of 4i with the active site of the PBP3 receptor (4BJP) of *E. coli*.



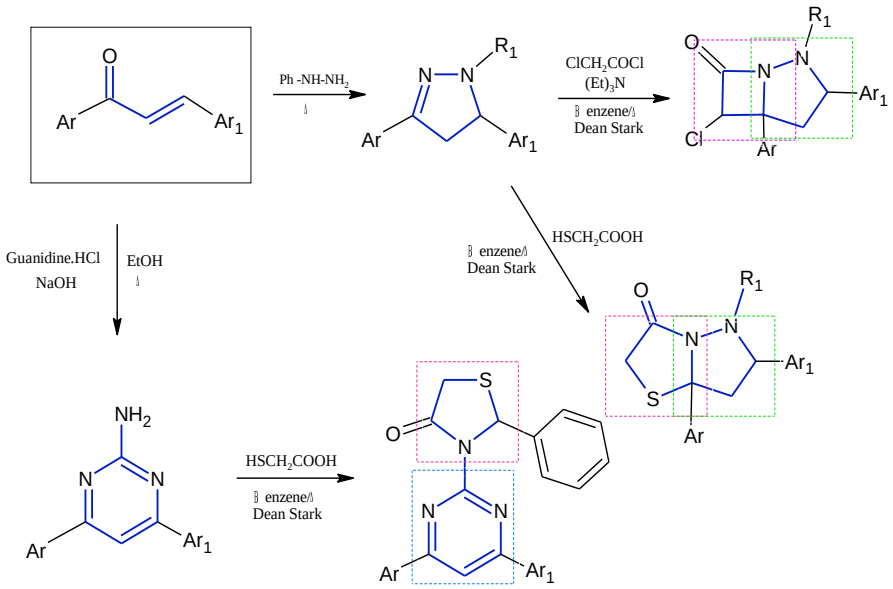
Source: Author

Chemistry

In general, the synthesis of the selected compounds was carried out in the preparation of α, β-unsaturated carbonyl compounds as precursors (chalcones), using the Claisen-Schmidt type cyclocondensation reaction between aromatic aldehydes and the corresponding substituted acetophenones, according to the procedure reported in the literature (Durst & Gokel, 1985). Dihydropyrazole derivatives were obtained from the reaction of chalcones with one equivalent of hydrazine or hydrazine derivatives, by cyclocondensation reactions monitored by TLC. The aminopyrimidine series synthesis was performed by the reaction of chalcones with guanidine to obtain thereby the substituted aminopyrimidine derivatives, which in turn were reacted with aromatic and heteroaromatic aldehydes to obtain the acyclic azomethines. Such as the pyrazolines synthesized,

aminopyrimidine series exhibited high fluorescence properties in solution, making them interesting because of their potential application in molecular materials science.

Figure 2: General scheme of synthesis of the selected hybrid compounds.



Source: Author

In general, thiazolidine-4-ones were obtained from the aminopyrimidines synthesized above, using three-component cyclocondensation reactions with 2-mercaptoacetic acid and benzaldehyde, in anhydrous toluene or benzene as solvents and using conditions of reflux with Dean- Stark trap. Finally, assays were carried out for the formation of β-lactam rings, which were conducted using the cycloaddition reaction of 2-chloroacetyl chloride with a cyclic imine, in our particular case pyrazolines. This reaction is known as a Staudinger cycloaddition; however, although many the reaction conditions were used, the desired product could not be obtained, and the pyrazole compound was obtained instead, leading to the conclusion that there is a possible competition between the oxidation reaction (aromatization) and the cycloaddition (formation β-lactam ring). That is to say that the thermodynamic driving force of the aromatic and more stable pyrazole formation ends up overcoming the formation of the fused β-lactam ring.

All the obtained compounds were fully characterized and confirmed by IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR and two-dimensional techniques.

Table 6: Experimental data of the synthesized compounds

Compound	Ar	Ar_1	Yield %	Tf. (°C)	t. Rx (min)
1i	-pMe	-mOH, -pOEt	92	96-98	180
2g	C6H5-H	C6H5-mOH, -pOEt	62	196-197	180
2j	C6H5-pMe	C6H5-mOH, -pOEt	58	93-95	240
3d	-H	-oCl	85	62-63	8
3h	-H	-pMe	72	98-99	12
4a	-pMe	-pOMe	18	80-82	8
4i	-pOH	-pOH, -pOEt	23	110-113	25
4j	-pMe	-pOEt	22	102-104	15

Source: Author

Conclusions

Following a methodology based on the rational design of *in silico drugs*, it was possible to propose and study a series of prototypes that have in their structure thiazolidone, aminopyrimidine, pyrazoline and β-lactam rings. Out of 60 molecules proposed, it was possible to determine that ten exhibited interactions directly with some of the key amino acids that make up the active site of *E. coli*'s PBP3 enzyme (4BJP). The ten selected prototypes were considered for synthesis phase, from which eight were obtained and are considered promising hits to be evaluated in subsequent *in vitro* tests to corroborate the acceptable profile of bioavailability and potential antibacterial activities observed in the computational studies. The series of aminopyrimidine and pyrazoline derivatives presented a characteristic luminescence that could be used for other purposes, such as bioindicators and chemosensors, as well as in molecular materials science.

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