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Article

Similarity-based Virtual Screening to Find Antituberculosis Agents based on Novel Scaffolds

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Abstract: A method is developed to identify molecular scaffolds potentially active against the Mycobacterium tuberculosis complex (MTBC). A structurally heterogeneous set of compounds active against MTBC was used to obtain a structural pattern model based on structural invariants. This model was statistically validated through a Leave-n-Out test. It successfully discriminated between active or inactive compounds over 86% in database sets and was also able to select new active chemical structures in external databases. The selection of new substituted pyrimidines, pyrimidones and triazolo[1,5-*a*]pyrimidines was particularly interesting because these structures could provide new scaffolds in this field. The seven selected candidates were synthesized and six of them showed activity *in vitro*.

Keywords: MTBC; virtual screening; topological indices; linear discriminant analysis; pharmacological activity distribution diagrams; antimicrobial drugs; drug design

1. Introduction

Tuberculosis is one of the deadliest infections in the world, killing almost 1.45 million people annually [1,2]. Most of these deaths occur in poor countries, and a more rational distribution of wealth could prevent them. However, the increasing incidence of drug-resistant MTBC has caused its resurgence in developed countries and makes therapeutic alternatives necessary [3,4]. Although new compounds potentially active against these bacteria are constantly being sought, very few new treatments have been developed in recent years [5-9]. Therefore, more extensive investigations are needed to find new molecular scaffolds capable of generating new inhibitors of mycobacteria.

The α-acetylenic ketones of type A (Scheme 1) have been shown to be highly versatile building blocks. For instance, these conjugated ynones have proven to be very suitable substrates for the synthesis of (E)-3-acylpropenoic acids [10], and of a wide range of heterocyclic systems [11-21], including the synthesis of the natural product L-lathyrine and related analogues [22,23]. Furthermore, when properly functionalized, compounds of type A have also proven to be valuable substrates for combinatorial and parallel synthesis on solid support of highly molecular diverse 2,4,6-trisubstituted pyrimidines [24-26]. These α-acetylenic ketones are produced readily, mainly by direct palladium-catalyzed coupling of acyl chlorides with 1-alkynes (Sonogashira reaction), [28,29] by reaction of alkynylzinc chlorides with acid halides, [30,31] or by reaction of lithium or magnesium acetylides with aldehydes followed by subsequent oxidation with oxalyl chloride in

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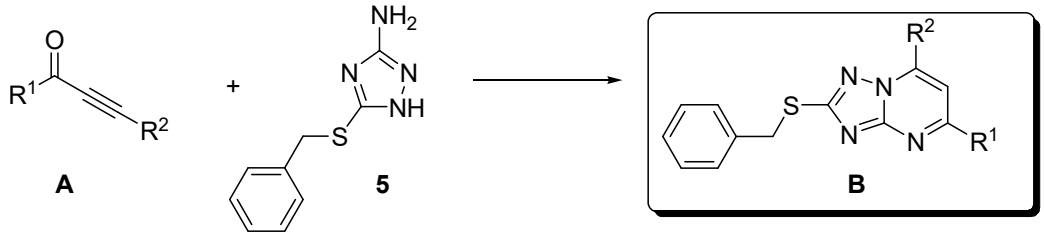
DMSO (Swern oxidation) [32], MnO_2 [12,13,15,33], t-butyl hydroperoxide [34] or with IBX [33-35].

In this paper we have further expanded the synthetic usefulness of these conjugated ynones A, and we wish to report the synthesis of novel 2,5,7-substituted triazolo[1,5-*a*]pyrimidines B by cyclocondensation of different α-acetylenic ketones A with 3-amino-5-benzylsulfanyl-1,2,4-triazole 5 (Scheme 1).



Scheme 1

The triazolo[1,5-*a*]pyrimidine nucleus is of considerable chemical and pharmacological interest. Antibiotic, anticholesteremic, antidiabetic, antiallergic, anti-inflammatory, antipyretic, antiphlogistic, analgesic and anticancer activities have been described for these types of compounds. They also serve in the treatment and prevention of circulatory diseases such as hypertension, heart diseases, stroke, hypercholesterol, arteriosclerosis and are an effective coronary vasodilators and bronchodilators [36-49].

On the other hand, several triazolo[1,5-*a*]pyrimidine derivatives have found applications on the agrochemical industry. 1,2,4-triazolo[1,5-*a*]pyrimidinesulfonamides are used as herbicides, and plant growth inhibitors, and they show activity against acetolactate synthase [46,50,51].

Currently, there are many methods available to synthesize triazolo[1,5-*a*]pyrimidines, mainly they are cyclocondensations between 3-amino-1,2,4-triazoles and 1,3-bifunctional synthons such as 1,3-dicarbonyl compounds or their equivalents [52-59], vinylogous iminium salts [47,60,61], ketene dithioacetals [48,62,63], and 3-ketovinyl compounds [46]. We used α-acetylenic Ketones of types A as 1,3-bifunctional synthons.

Although a few pyrimidines [64], pyrimidones [65] and triazolo[1,5-a]pyrimidines [66] have already been synthetized and tested for anti-TB activity, their substitution patterns and synthesis methods are different from the compounds described here.

The identification of new targets requires knowledge of the specific biochemical pathways of mycobacteria, but many metabolic processes are still unknown and the structure-based design of new anti-TB agents is a complex task [67].

On the other hand, extra-mechanistic virtual screening methodologies have demonstrated their ability to model the presence of activity within structurally heterogeneous groups of compounds in different therapeutic areas [68-77] as well as in predicting toxicological properties [78,79] and drug-like character [80]. In these models, structural similarity is the key. Molecules are characterized through structural invariants, that is, by descriptors that are independent of molecular conformation. Many of them are topological indices (TI) [81-87], which are capable of characterizing most of the molecular structure [88-95].

The aim of this study was to develop new models based on structural invariants in order to screen structural databases to identify new potentially useful scaffolds against MTBC.

2. Results and Discussion

2.1. Antituberculosis activity modelling

A group of 32 compounds with known activity against MTBC has been compiled from various sources [96-98]. A set of 45 compounds with a different pharmacological activity was also used as inactive group. Each compound was characterized by a set of 90

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structural invariants calculated using the DesMol program [99]. The descriptors used with their symbols, definitions and references are shown in table 1. These were used to build a model capable of discriminating between active and inactive antituberculous compounds, as follows.

Linear discriminant analysis functions were calculated with randomly selected subsets of 25 active and 35 inactive compounds by using BMDP New System [103]. Descriptor selection was based on the Fisher-Snedecor F parameter. The variables were introduced step by step in the DF: in each step the variable that added the most to the separation of the groups was entered in the equation, or the variable that contributed the least to improving said separation was eliminated. The classification criterion was the shortest Mahalanobis distance. The discriminant ability of the DF was evaluated by two parameters, Wilks λ, and the percentage of correct classification in each group. The independent variables in this study were the calculated structural invariants, and the discrimination property was the presence of activity against MTBC.

The validation of the selected DF was performed by two methods. One internal leave-*n*-out test in which the program randomly chose and pulled out a ratio of compounds, and used them to evaluate the DF obtained with the rest; and another external test with a previously unused data set.

To choose the optimal ranges of values for this equation, the corresponding Pharmacological Distribution Diagram, PDD [104], was obtained. These diagrams are useful to determine the intervals of the equation in which the probability of finding new candidates is maximum. This is a graph similar to a histogram in which expectancies appear on the ordinate axis. For an arbitrary range of values of a given function, the activity expectancy is $E_a = a/(i+100)$, where a is the percentage of active compounds in the range and i is the corresponding percentage of inactive within the same range. The expectancy of inactivity is similarly defined as $E_i = i/(a+100)$. This plot provides good visualization of the regions of minimal overlap between the active and inactive compounds and helps to select the optimal interval of the DF.

The 90 descriptors and the selected discriminant function were used as filters to select potential candidates in structural databases. For this, the maximum and minimum values of each descriptor were established as thresholds for the 90 variables, while for DF, the optimal interval was taken. Compounds exhibiting all the 90 values within the thresholds and DF value within the optimal interval were selected as candidates.

The following equation DF was obtained by stepwise LDA using the set shown in table 2:

$$DF = 0.88 - 11.99\, J_1 + 4.86\, J_1^{v} - 11.11\, J_3^{v} + 0.81\, {}^{1}D - 0.2\, {}^{4}C_c \quad (1)$$

$N = 60 \qquad \lambda = 0.34 \qquad F = 21.12$

Where N=60 represents 25 anti-MTBC drugs and 35 presumably inactive compounds.

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Table 1. Descriptors used.

Symbol	Name	Definition	Reference
N	Molecular size	Number of non-hydrogen atoms.	**Error! Reference source not found.**
V*k* *k*=3,4	Vertices of degree *k*	Number of atoms having *k* bonds, σ or π, to non-hydrogen atoms.	**Error! Reference source not found.**
R	Ramification	Number of single structural branches.	**Error! Reference source not found.**
W	Wiener path number	Sum of the distances between any two atoms in terms of bonds.	100
L	Length	Maximal distance between atoms in terms of bonds.	**Error! Reference source not found.**
PR*k* *k*=0-3	Pairs of ramifications at distance *k*	Number of pairs of single branches at distance *k* in terms of bonds.	**Error! Reference source not found.**
${}^{k}\chi_{t}$ *k*=0-4 *t*=p,c,pc	Randić-like indices of order *k* and type path (p), cluster (c) and path-cluster (pc)	$${}^{k}\chi_{t}=\sum_{j=1}^{k n_t}\left(\prod_{i\in S_j}\delta_i\right)^{-1/2}$$ δ_i, number of bonds, σ or π, of the atom *i* to non-hydrogen atoms. S_j, *j*th sub-structure of order *k* and type *t*.	**Error! Reference source not found.Error! Reference source not found.Error! Reference source not found.**
${}^{k}\chi_{t}^{v}$ *k*=0-4 *t*=p,c,pc	Kier-Hall indices of order *k* and type path (p), cluster (c) and path-cluster (pc)	$${}^{k}\chi_{t}^{v}=\sum_{j=1}^{k n_t}\left(\prod_{i\in S_j}\delta_i^{v}\right)^{-1/2}$$ δ_i^v, Kier-Hall valence of the atom *i*. S_j, *j*th sub-structure of order *k* and type *t*.	**Error! Reference source not found.Error! Reference source not found.Error! Reference**

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G_k k=1-5	Topological charge indices of order k	$G_k = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \lvert \mathbf{M}_{ij} - \mathbf{M}_{ji} \rvert \delta(k, \mathbf{D}_{ij})$ **M=AQ**, product of the adjacency and inverse squared distance matrices for the hydrogen-depleted molecular graph. **D**, distance matrix. δ, Kronecker delta	**Error! Reference source not found.,Error! Reference source not found.**
G_k^v k=1-5	Valence topological charge indices of order k	$G_k^v = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \lvert \mathbf{M}_{ij}^v - \mathbf{M}_{ji}^v \rvert \delta(k, \mathbf{D}_{ij})$ $\mathbf{M}^v=\mathbf{A}^v\mathbf{Q}$, product of the electronegativity-modified adjacency and inverse squared distance matrices for the hydrogen-depleted molecular graph. **D**, distance matrix. δ, Kronecker delta	**Error! Reference source not found.,Error! Reference source not found.**
J_k k=1-5	Pondered topological charge indices of order k	$J_k = \frac{G_k}{N-1}$	**Error! Reference source not found.,Error! Reference source not found.**
J_k^v k=1-5	Pondered valence topological charge indices of order k	$J_k^v = \frac{G_k^v}{N-1}$	**Error! Reference source not found.,Error! Reference source not found.**
${}^kD_t$ k=0-4 t=p,c,pc	Connectivity differences of order k and type path (p), cluster (c) and path-cluster (pc)	${}^kD_t = {}^k\chi_t - {}^k\chi_t^v$	**Error! Reference source not found.**
E_k k=1-5	Topological charge	$E_k = G_k^v - G_k$	**Error! Reference**

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	differences of order k		**source not found.**
F_k k=1-5	Pondered topological charge differences of order k	$F_k = J_k^v - J_k$	**Error! Reference source not found.**
${}^kC_t$ k=0-4 t=p,c,pc	Connectivity quotients of order k and type path (p), cluster (c) and path-cluster (pc)	${}^kC_t = \frac{{}^k\chi_t}{{}^k\chi_t^v}$	**Error! Reference source not found.**
${}^kQ_t$ k=0-4 t=p,c,pc	Inverse connectivity quotients of order k and type path (p), cluster (c) and path-cluster (pc)	${}^kQ_t = \frac{{}^k\chi_t^v}{{}^k\chi_t}$	**Error! Reference source not found.**
CG_k k=1-5	Topological charge quotients of order k	$CG_k = \frac{G_k}{G_k^v}$	**Error! Reference source not found.**
QG_k k=1-5	Inverse topological charge quotients of order k	$QG_k = \frac{G_k^v}{G_k}$	**Error! Reference source not found.**

Table 2. Classification for each compound by DF.

Active Group				**Inactive Group**			
Compound	DF	Prob.[a] active	Predicted activity[b]	Compound	DF	Prob.[a] inactive	Predicted activity[b]
Streptonicozid	3.14	1.000	+	Butibufen	-3.14	1.000	-
Tobramycin	3.49	1.000	+	Aldicarb	-2.74	1.000	-
Kanamycin	3.88	1.000	+	Antrafenine	-2.55	1.000	-
Amikacin	4.14	1.000	+	Carprofen	-1.89	0.997	-
Dihydrostreptomycin	2.80	0.999	+	Beclobrate	-1.84	0.997	-
Streptomycin	2.32	0.997	+	Benzoctamine	-1.77	0.996	-
Ethambutol	2.23	0.996	+	Carmofur	-1.77	0.996	-
Pyrazinamide	1.97	0.992	+	Aminothiazole	-1.73	0.996	-
Enviomycin	1.92	0.991	+	Acifran	-1.72	0.996	-
Ofloxacin	1.82	0.988	+	Brilliant Blue	-1.72	0.996	-
Moxifloxacin	1.66	0.982	+	Amitraz	-1.68	0.995	-
Gatifloxacin	1.66	0.981	+	Clofibrate	-1.62	0.994	-
Rifampin	1.60	0.978	+	Paraoxon	-1.52	0.992	-

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Azithromycin	1.49	0.971	+	Piroxicam	-1.39	0.989	-
Verazide	1.44	0.967	+	Carmustine	-1.11	0.977	-
Isoniazid	1.20	0.938	+	Ornithine	-1.11	0.977	-
Capreomycin	1.16	0.930	+	Alpidem	-1.10	0.976	-
Sparfloxacin	0.98	0.889	+	Alprazolam	-1.04	0.972	-
Clarithromycin	0.93	0.874	+	Bixin	-1.04	0.972	-
Salinazid	0.92	0.871	+	Benzoic Acid	-1.03	0.971	-
Tuberin	0.91	0.869	+	Amsacrine	-0.98	0.967	-
Clofazimine	0.13	0.432	NC	Azaserine	-0.96	0.965	-
Imipenem	0.09	0.404	NC	Theofibrate	-0.88	0.957	-
Ph-Aminosalicylate	-0.200	0.230	-	Azacosterol	-0.83	0.951	-
PAS	-1.000	0.031	-	Allicin	-0.77	0.943	-
				Prazepam	-0.77	0.943	-
				Camazepam	-0.71	0.933	-
				Aminopromazine	-0.57	0.904	-
				Bromazepam	-0.45	0.870	-
				Carnitine	-0.32	0.824	-
				Acipimox	-0.28	0.809	-
				Buspirone	-0.03	0.677	-
				Azapicyl	0.03	0.640	-
				Acronine	0.11	0.588	-
				Captodiamine	0.28	0.470	NC

[a] Prob., probability; [b] When probability active or probability inactive >0.60, the compound is classified as active "+" or inactive "-", respectively. In any other case, the compound is considerate Non Classified "NC".

The topological descriptors selected in this equation were: the charge indices (J_1, J_1^v, J_3^v); the difference index ($^1D = {}^1\chi - {}^1\chi^v$); and the quotient index ($^4C_c = {}^4\chi_c/{}^4\chi_c^v$), where $^m\chi_t$ and $^m\chi_t^v$ are, respectively, single and valence Randić-Kier-Hall indices of order m and type t. Topological charge-transfer indices, J_1, J_1^v and J_3^v, are measures of the contribution of molecular topological structure to the charge transfer at topological distance 1 and 3, respectively [68,101]. Difference and quotient indices are related to charge distributions within molecular fragments [68]. Thus, ^1D is the net contribution of the heteroatoms to the electronic charge within fragments of order 1 (bonds). The ^4C_c index can be related to electron densities of cluster-type fragments of order 4 (three atoms bonded to a central one) in which there is at least one heteroatom.

Table 2 shows the results of the classification for each one of the compounds included in the LDA.

The linear equation gave good results since most compounds were classified with a probability over 86% (table 2). When the probabilities are between 40% and 60%, the compounds were counted as Non Classified (NC), and finally below 40%, they were considered as inactive. Under this framework, the error percentage in the active set was about 9%, whereas in the inactive was 0%.

The results of the internal validation are illustrated in table 4, with the percentage of correct classification within each group. Five runs were performed. A number of compounds ranging from 9 to 16 were randomly extracted from the training to a test set. Wilks values are shown for each equation. Correct classification percentages are shown for

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training and test sets, for active and inactive compounds. The number of compounds classified as active (+) or inactive (-) appears in parentheses, where (a/b) = number of (+) compounds / number of (-) compounds. Average values are also shown, as well as the performance of DF function.

The results for the training and test groups are within the same range. The mean percentage of success obtained with the training group for DF (table 3) was 87% for active (+) and 96% for inactive (-). For the test it was 80% and 92%, respectively. The results were similar to those obtained with the DF equation, which points out the validity of the LDA equation.

The results of the external validation test are shown in table 4. As can be seen, for the active set there was a misclassified compound, namely ethionamide. The same is found in the opposite group, where glucosamine was misclassified.

Figure 1 shows the PDD obtained from DF. As can be inferred from Figure 1, the optimal range of DF to find active compounds can be established between 0 and 4.5.

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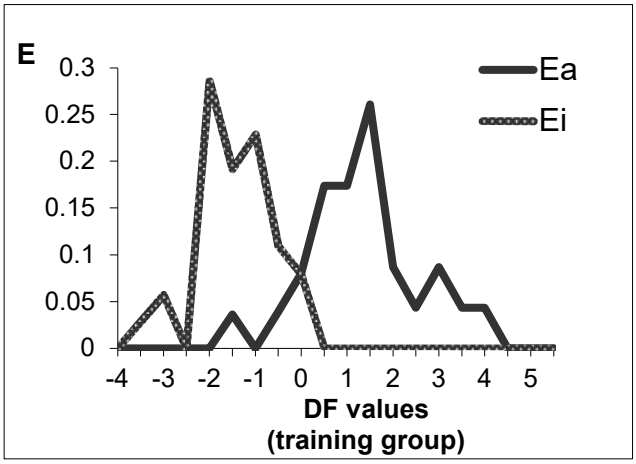
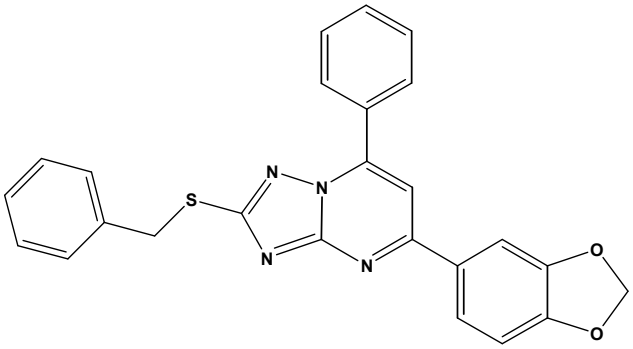


Figure 1. PDD in the training set, obtained by DF equation. E, expectancy of activity or inactivity; black line, active group; grey line, inactive group.

2.2. Similarity-based Virtual screening

A virtual library containing new pyrimidine derivatives generated by combining the chemical scaffolds and substituents depicted in table 5 was screened by the formalism described above. Compounds selected as possible candidates were synthesized and tested *in vitro*.

After building the database containing the substituted pyrimidines, the virtual screening was performed. Seven potentially active molecules were selected. The structures of the selected compounds are presented in figure 2.



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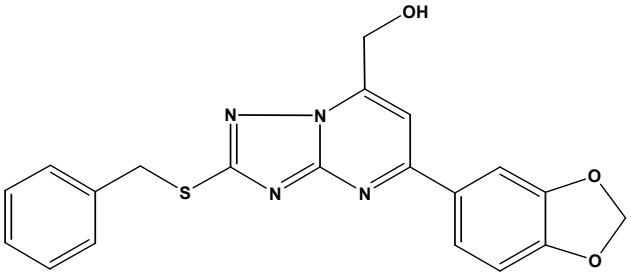

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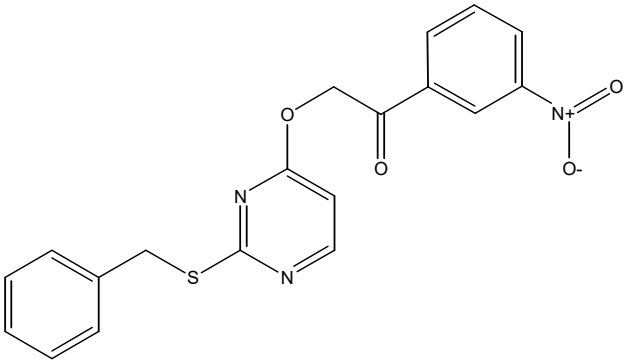

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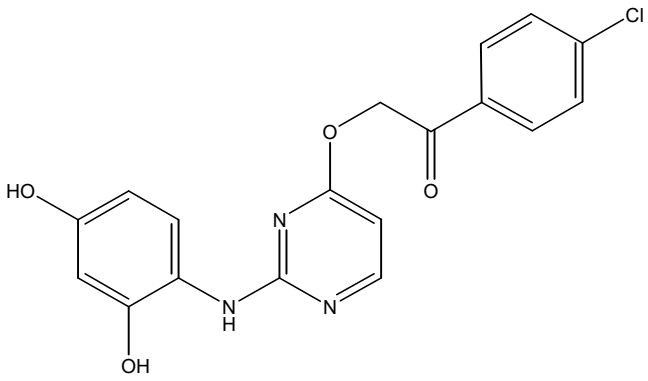

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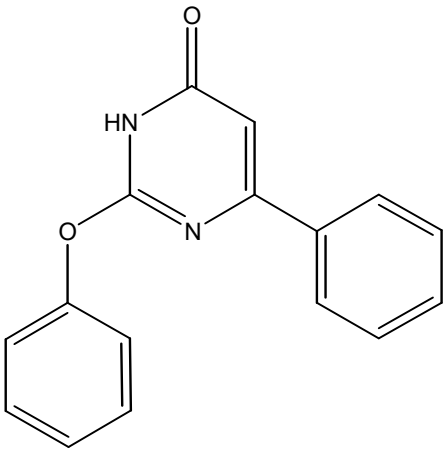

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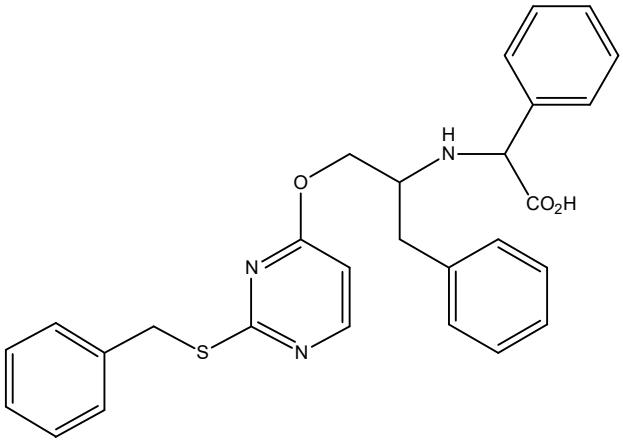

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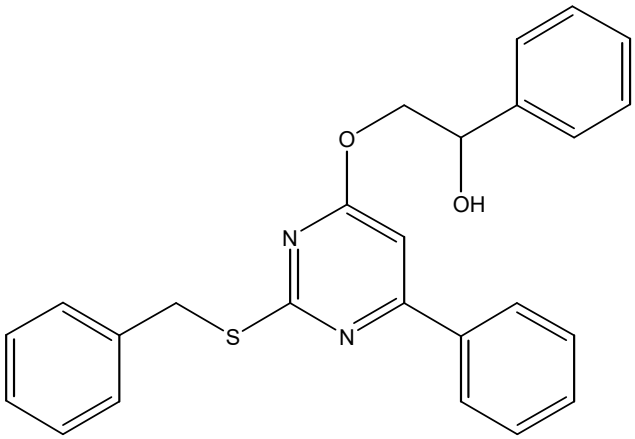

Figure 2. Structures of the selected compounds.

2.3. Chemistry

After building the database containing the substituted pyrimidines, the virtual screening was performed. Seven potentially active molecules were selected. The structures of the selected compounds are presented in figure 2.

Synthesis of 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives. Reaction of the required magnesium acetylides, generated from alkynes **1a-b** and *i*-PrMgCl in dry THF at 0ºC, with piperonal **2**, afforded the expected propargylic alcohols **3a-b**. Oxidation of **3a-b** with MnO_2 afforded α-acetylene ketones **4a** and **4b** in 79% and 37% overall yield, respectively (Scheme 2).

Cyclocondensation of 3-amino-5-benzylsulfanyl-1,2,4-triazole **5** with α-acetylene ketones **4a-b** in dry DMF at 40ºC, gave the corresponding triazolo[1,5-*a*]pyrimidines **6a-b** in moderate yields (73% and 38%, respectively) (Scheme 2).

Finally, deprotection of **6b** in acidic conditions afforded **7** in 77% yield (Scheme 3).

Synthesis of pyrimidine derivatives. Two of us reported on the synthesis of novel 4-alkoxypyrimidines starting from 2-alkylsulfanylpyrimidinones of type **8 Error! Reference source not found.Error! Reference source not found.**. The method is based on a selective *O*-alkylation reaction with bulky aliphatic alcohols using the Mitsunobu conditions (method A, Scheme 4) or in basic medium with sterically demanding agents like α–haloketones (method B, Scheme 4).

Oxidation of the thioether moiety to the corresponding sulfone **10** using *m*-CPBA and nucleophilic displacement by different nucleophiles produced the corresponding highly molecular diverse pyrimidines of type **11**. In addition, when 4-isopropoxipyrimidine **11a** was treated with a 1:1 mixture of H_2SO_4/AcOH at 90 ºC during 15 min. the selective cleavage of the 4-isopropoxy group took place affording to 2-aryloxypyrimidinone **12** (Scheme 5).

On the other hand, the reaction of the deprotected amine **9e** with phenylboronic acid and glyoxylic acid (Petasis reaction) **Error! Reference source not found.**,**Error! Reference source not found.Error! Reference source not found.** gave the desired α-phenyl glycine derivative **13** in moderated yield (Scheme 6).

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Table 3. Results of the *internal validation* for DF.

		Training Group		Test Group	
Run nº		(+)	(-)	(+)	(-)
1	0.28	90%(18/2)	100%(0/26)	60%(3/2)	100%(0/9)
2	0.31	87%(20/3)	96%(1/27)	50%(1/1)	100%(0/7)
3	0.35	86%(19/3)	89%(3/24)	100%(3/0)	75%(2/6)
4	0.33	82%(14/3)	100%(0/27)	88%(7/1)	100%(0/8)
5	0.33	91%(20/2)	96%(1/27)	100%(3/0)	86%(1/6)
Average	-	87%	96%	80%	92%
DF	0.34	84%(21/4)	97%(1/34)	No	No

(a/b) = number of (+) compounds / number of (-) compounds.

Table 4. Results of the *external validation* for DF.

ACTIVE GROUP				INACTIVE GROUP			
Compound	DF	Prob.[a] active	Predicted activity	Compound	DF	Prob.[a] inactive	Predicted activity
Morphazinamide	4.32	1.000	+	Canthaxanthin	-3.46	1.000	-
Neomycin	5.02	1.000	+	Genite	-2.57	1.000	-
Tubercidin	2.85	0.999	+	Altretamine	-1.91	0.997	-
Ciprofloxacin	2.15	0.995	+	Etifoxin	-1.86	0.997	-
Viomycin	1.75	0.986	+	Dichlone	-1.78	0.996	-
Rifabutin	0.73	0.799	+	Feprazone	-1.47	0.991	-
Ethionamide	-1.30	0.014	-	Antipyrine	-0.44	0.868	-
				Benorylate	-0.25	0.792	-
				Chloropal	0.19	0.526	-
				Glucosamine	1.77	0.014	+

[a] Prob., probability; [b] When probability active or probability inactive >0.60, the compound is classified as active "+" or inactive "-", respectively. In any other case, the compound is considerate Non Classified "NC".

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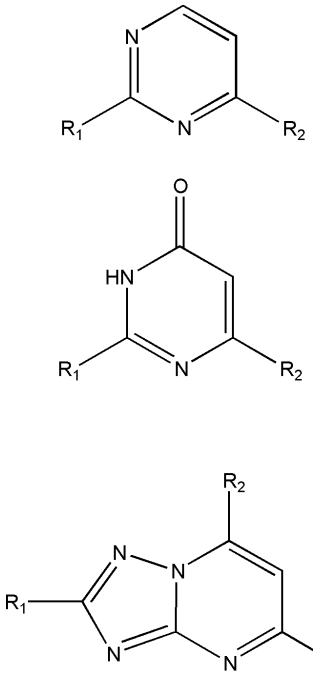
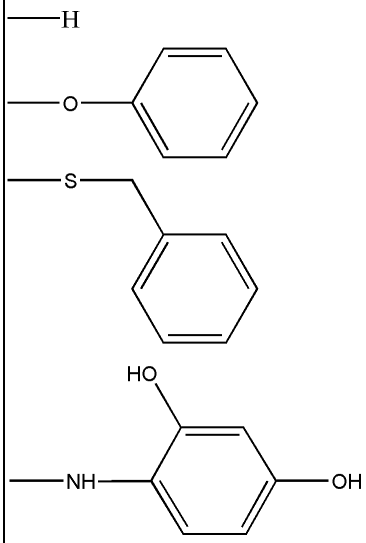
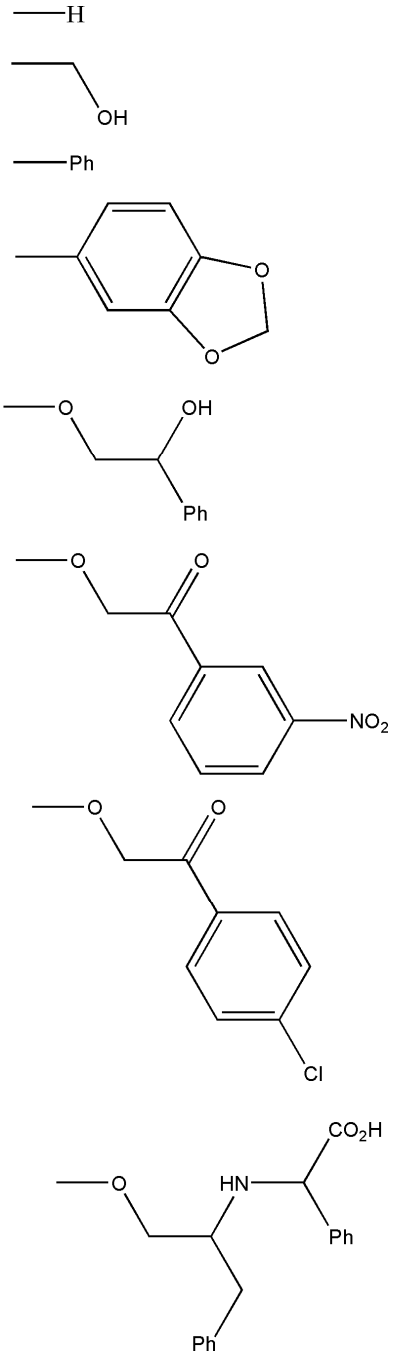
Table 5. Scaffolds and fragments used for the generation of the virtual library.

Chemical scaffolds	R_1	R_2
		

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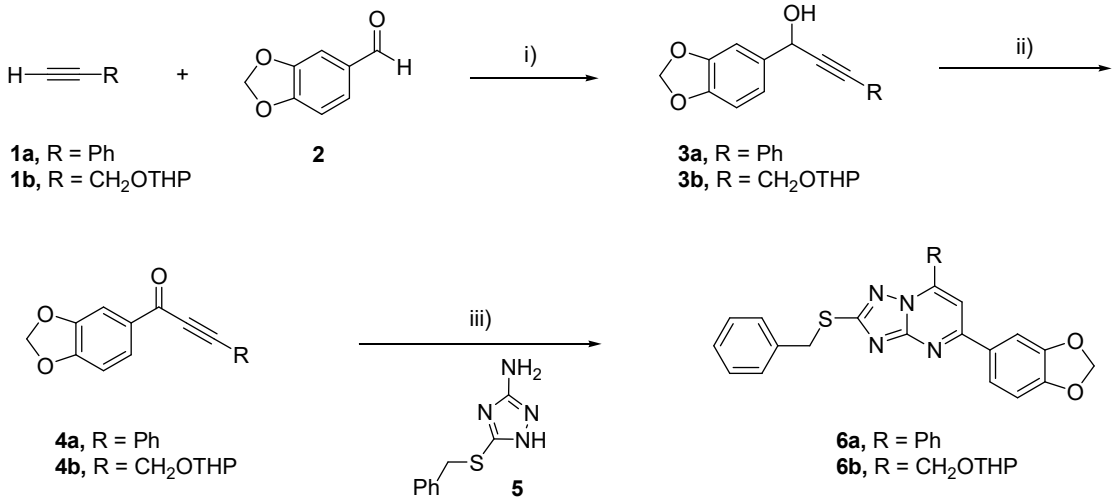
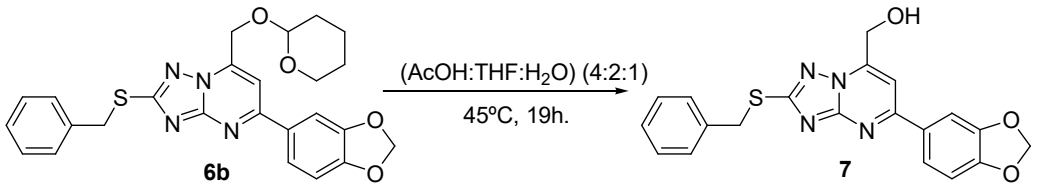
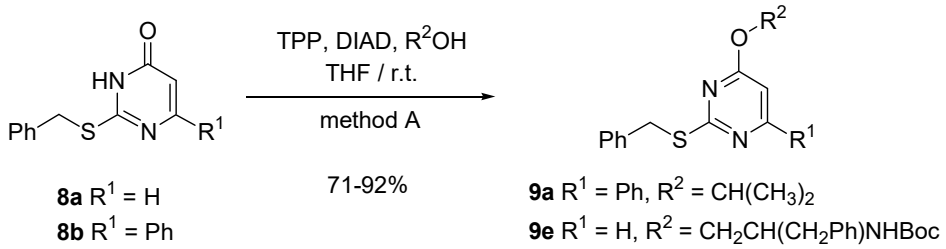


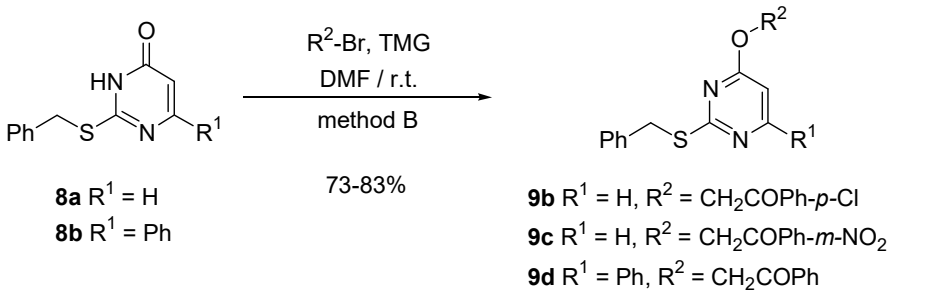
Reagents and conditions: i) *i*-PrMgCl, THF, 0ºC, 5-12h., ii) MnO_2, CH_2Cl_2, 0ºC-r.t., 3-12h., iii) DBU, DMF, $MgSO_4$, 40ºC, 1-2h.

Scheme 2



Scheme 3





Scheme 4

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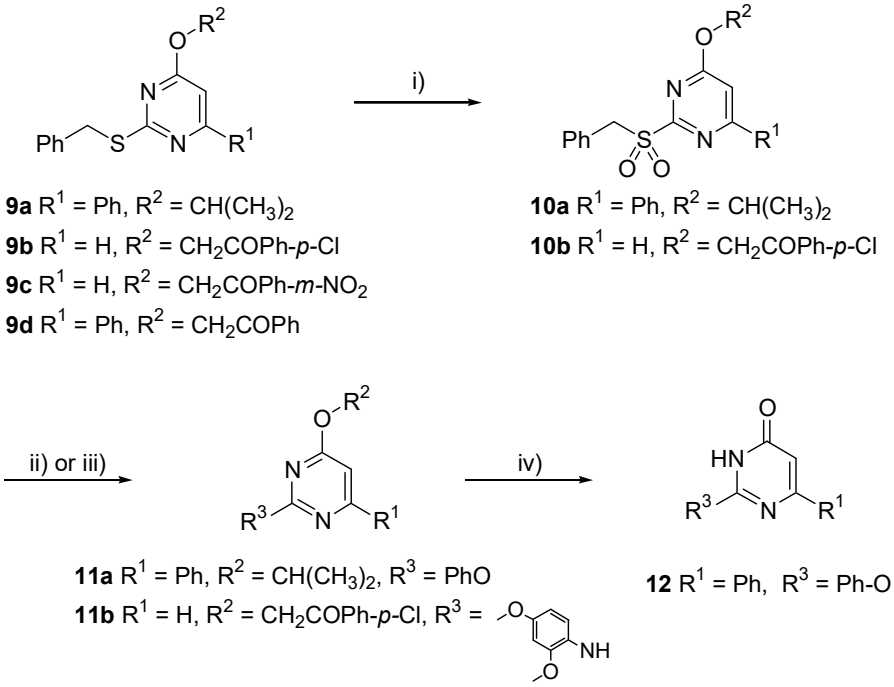
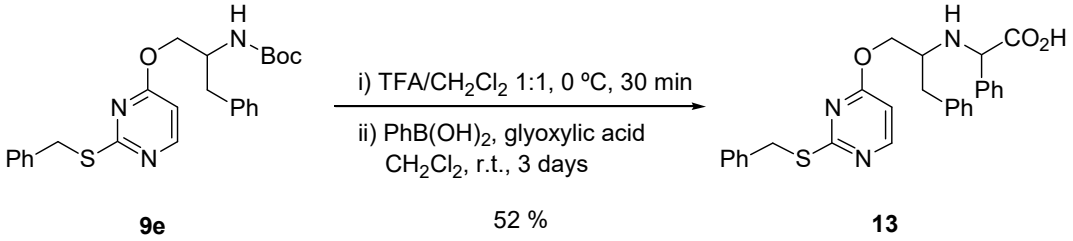
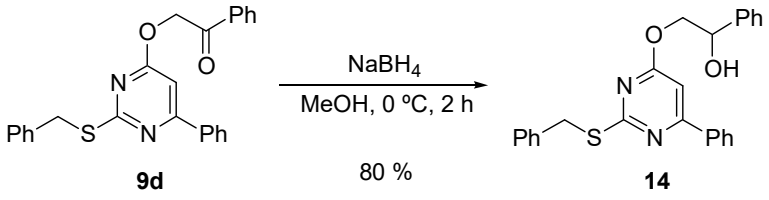


Reagents and conditions: i) *m*-CPBA, CH_2Cl_2, 0ºC to r.t., 2 h; ii) Ar-NH_2, dioxane, reflux, 30 h ; iii) PhOH, Cs_2CO_3, dioxane, 60 ºC, 3 h; iv) H_2SO_4/AcOH 1:1, 90 ºC, 15 min

Scheme 5



Scheme 6



Scheme 7

Finally, we prepared the compound **14** by reducing the carbonyl group of compound **9d** with $NaBH_4$ in MeOH. This reduction afforded the hydroxy derivative **14** in 80% isolated yield (Scheme 7).

Microbiological study. To check the predicted antituberculosis activity of the selected candidates, microbiological tests were performed. The result of *in vitro* susceptibility test of conventional drugs is shown in table 6, and the experimental results of the selected compounds are illustrated in table 7. Two compounds, **7** and **12**, showed

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MIC_{50}=MIC_{90}=32 mg/L, corresponding to 81.5 μM and 121.1 μM, respectively; four compounds, **6a**, **9c**, **11b** and **13**, showed MIC_{90}=64 mg/L against *M. tuberculosis*, which corresponds to 146, 167.8, 160.1, 131.8 μM, respectively; and **14** was inactive at the assayed concentrations. The compound **11b** showed the best MIC_{50} = 80 μM, but its MIC_{90} raised to 160,1 μM. Thus, the results pointed out that **7** was the most active agent, showing molar MIC_{90} four times lower than Ethambutol but within the same magnitude order.

Table 6. Experimental MIC of conventional drugs against MTBC.

Compound	MIC range / mg/L	MIC_{50} / mg/L	MIC_{90} / mg/L	Molecular mass / Da	MIC_{50} / μM	MIC_{90} / μM
Ethambutol	1-8	4.0	4.0	204.3	19.6	19.6
Isoniazid	0.05-0.2	0.05	0.05	137.1	0.4	0.4
Rifampin	0.125-1	0.125	0.5	823.0	0.2	0.6
Streptomycin	0.125-0.5	0.25	0.5	581.6	0.4	0.9

MIC range: minimal and maximal inhibitory values found

Table 7. Experimental MIC of the selected compounds against MTBC.

Compound	MIC range / mg/L	MIC_{50} / mg/L	MIC_{90} / mg/L	Molecular mass / Da	MIC_{50} / μM	MIC_{90} / μM
6a	64	64	64	438.5	146.0	146.0
7	32	32	32	392.4	81.5	81.5
9c	64	64	64	381.4	167.8	167.8
11b	16 - 64	32	64	399.8	80.0	160.1
12	32	32	32	264.3	121.1	121.1
13	32 - 64	64	64	485.6	131.8	131.8
14	>128	>128	>128	414.5	>308.8	>308.8

MIC range: minimal and maximal inhibitory values found

3. Materials and Methods

3.1 Chemical Methods

DMF was dried over activated molecular sieves (4 Å). THF was dried over Na/benzophenone prior use. All the other commercially available chemicals were used as purchased without further purification. Reactions involving magnesium acetylides and synthesis of triazolo[1,5-*a*]pyrimidines were run under a dry Ar atmosphere. Melting points (capillary tube) were measured with an Electrothermal digital melting point apparatus IA 91000 and are uncorrected. IR spectra were recorded on a Mattson-Galaxy Satellite FT-IR. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz respectively, on a Brucker DPX200 Advance instrument with TMS as internal standard. MS spectra were recorded on a VG Quattro instrument in the positive ionization FAB mode, using 3-NBA or 1-thioglycerol as the matrix or in a Thermo Quest 2000 series apparatus for the EI (70eV) mode. Analytical TLC was performed on precoated TLC plates, silica gel 60 F_{254} (Merck). Flash-chromatography (FC) purifications were performed on silica gel 60 (230-400 mesh, Merck).

Synthesis of propargylic alcohols 3a-b. General procedure.

To a cooled (0ºC) solution of the corresponding alkyne **1a-b** in dry THF (2 mL/mmol), *i*-PrMgCl (2 M solution in THF) was added dropwise under Ar. The mixture was stirred at that temperature for 4 hours. Then, a solution of the piperonal **2** (1.3 equiv) in dry THF (1 mL/mmol) was slowly added dropwise over a period of 15 min. The reaction mixture, under Ar, was stirred from 0ºC to r.t. until total consumption of **1a-b** (5-12 h., monitored by TLC). The reaction was quenched with saturated solution of NH_4Cl (3 mL/mmol) at r.t. and the organic solvent was eliminated under reduced pressure. The aqueous layer was extracted with AcOEt (3×3 mL/mmol) and the combined organic layers were dried over

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$MgSO_4$, the solvent was evaporated and the resulting residue purified by flash-chromatography (n-hexane:AcOEt).

1-Benzo[1,3]dioxol-5-yl-3-phenyl-prop-2-yn-1-ol (3a). According to the general procedure described above, reaction between **1a** (4.01 g, 39.26 mmol) and piperonal **2** (7.65 g, 50.9 mmol) afforded 8.02 g (81%) of **3a** as a white solid. M.p.: 60-61ºC. IR (KBr): ν 3439 (br., OH). ^{1}H NMR ($CDCl_3$): δ 7.5-7.4 (m, 2H_{arom}), 7.3 (m, 3H_{arom}), 7.2-7.1 (m, 2H_{arom}), 6.85 (d, 1H_{arom}, J = 8.0 Hz), 6.00 (s, 2H, OCH_2O), 5.63 (s, 1H, CHC≡C), 2.59 (br., 1H, OH). ^{13}C NMR ($CDCl_3$): δ 147.8, 147.6, 134.6 (3s, 3C_{arom}), 131.7, 128.6, 128.3 (3d, 5CH_{arom}), 122.2 (s, C_{arom}), 120.4, 108.1, 107.4 (3d, 3CH_{arom}), 101.2 (t, CH_2), 88.6 (s, C≡CPh), 86.5 (s, C≡CPh), 64.8 (d, CH). MS (FAB$^+$) *m/e*: 253 ([M+1]$^+$, 9). Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.39; H, 4.61%.

1-Benzo[1,3]dioxol-5-yl-3-(tetrahydro-pyran-2-yloxy)-prop-2-yn-1-ol (3b). According to the general procedure described above, reaction between **1b** (0.21 g, 1.48 mmol) and piperonal **2** (0.29 g, 1.92 mmol) afforded the crude of the compound **3b**. The reaction mixture was used in the next step of synthesis without further purification.

Synthesis of α-acetylenic ketones 4a-b. General procedure.

Over a cooled (0ºC), mechanically stirred suspension of MnO_2 (5 equiv) in CH_2Cl_2 (3 mL/mmol), a solution of the propargyl alcohol **3a** or of the reaction mixture **3b** in CH_2Cl_2 (3 mL/mmol) was added dropwise. The reaction mixture was stirred from 0ºC to r.t. until total consumption of **3a-b** (3-12 h., monitored by TLC) and then filtered through a Celite® pad. The solvents were removed under reduced pressure and the resulting residue purified by flash-chromatography (n-hexane:AcOEt).

1-Benzo[1,3]dioxol-5-yl-3-phenyl-propynone (4a). According to the general procedure described above, reaction between **3a** (1.98 g, 7.87 mmol) and MnO_2 (3.84 g, 39.35 mmol) afforded after crystallization of the residue with MeOH:H_2O instead of the chromatographic purification 1.91 g (97%) of **4a** as a yellow solid**.** M.p.: 100-101ºC. IR (KBr): ν 1625 (s, C=O). ^{1}H NMR ($CDCl_3$): δ 7.92 (dd, 1H_{arom}, J = 8.2 Hz, J' = 1.8 Hz), 7.7- 7.6 (m, 3H_{arom}), 7.5-7.4 (m, 3H_{arom}), 6.93 (d, 1H_{arom}, J = 8.2 Hz), 6.10 (s, 2H, OCH_2O). ^{13}C NMR ($CDCl_3$): δ 176.0 (s, C=O), 152.8, 148.1 (2s, 2C_{arom}), 132.9 (d, 2CH_{arom}), 131.9 (s, C_{arom}), 130.6, 128.6, 127.2 (3d, 4CH_{arom}), 120 (s, C_{arom}), 108.2, 107.9 (2d, 2CH_{arom}), 102.1 (t, CH_2), 92.3 (s, C≡CPh), 86.7 (s, C≡CPh). MS (FAB$^+$) *m/e*: 251 ([M+1]$^+$, 100). Anal. Calcd for $C_{16}H_{10}O_3$: C, 76.79; H, 4.03. Found: C, 76.53; H, 4.24%.

1-Benzo[1,3]dioxol-5-yl-4-(tetrahydro-pyran-2-yloxy)-but-2-yn-1-one (4b). According to the general procedure described above, reaction between the reaction mixture of **3b** and MnO_2 (0.72 g, 7.4 mmol) afforded 157 mg (overall yield = 37%) of **4b** as a yellow oil. IR (NaCl): ν 1640 (m, C=O). ^{1}H NMR ($CDCl_3$): δ 7.82 (dd, 1H_{arom}, J = 7.4 Hz, J' = 1.8 Hz), 7.55 (d, 1H_{arom}, J = 1.6 Hz), 6.89 (d, 1H_{arom}, J = 8.2 Hz), 6.08 (s, 2H, OCH_2O), 4.9-4.8 (m, 1H, CH), 4.56 (s, 2H, CH_2OThp), 3.9-3.8 (m, 1H, CH_2), 3.6-3.5 (m, 1H, CH_2), 1.9-1.6 (m, 6H, 3CH_2). ^{13}C NMR ($CDCl_3$): δ 175.6 (s, C=O), 152.9, 148.1 (2s, 2C_{arom}), 131.5 (s, C_{arom}), 127.4 (d, CH_{arom}), 108.2, 107.9 (2d, 2CH_{arom}), 102.1 (t, CH_2), 97.3 (d, CH), 89.6 (s, C≡CCH$_2$), 83.3 (s, C≡CCH$_2$), 62.0, 54.0, 30.1, 25.2, 18.8 (5t, 5CH_2). MS (FAB$^+$) *m/e*: 289 ([M+1]$^+$, 100). Anal. Calcd for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: 66.94; H, 5.76%.

Synthesis of triazolo[1,5-*a*]pyrimidines 6a-b. General procedure.

A mixture of 3-amino-5-benzylsulfanyl-1,2,4-triazole **5**, DBU and anhydrous $MgSO_4$ (5g/g) in dry DMF (3 mL/mmol) was heated at 40ºC under Ar for 30 min. Then, a solution of the corresponding α-acetylenic ketone **4a-b** in dry DMF (3 mL/mmol) was slowly added using a *syringe pump* over a period of 5h. The reaction mixture, under Ar, was stirred at 40ºC until total consumption of **5** (1-2 h., monitored by TLC). The reaction was filtered and the organic solvent was eliminated under reduced pressure. The residue was dissolved with CH_2Cl_2 (15 mL/mmol) and was washed with saturated solution of NH_4Cl (3×3 mL/mmol). The organic layer was dried over $MgSO_4$, the solvent was evaporated and the resulting residue purified by flash-chromatography (n-hexane:AcOEt).

5-Benzo[1,3]dioxol-5-yl-2-benzylsulfanyl-7-phenyl-1,2,4-triazolo[1,5-*a*]pyrimidine (6a). According to the general procedure described above, reaction between **5** (106 mg, 0.52 mmol), **4a** (303 mg, 1.21 mmol, 2.5 equiv), DBU (81 µl, 0.53 mmol, 1.1 equiv) and

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anhydrous $MgSO_4$ (0.5g) afforded 166 mg (73%) of **6a** as a white solid. M.p.: 143-144ºC. ^{1}H NMR (DMSO-d_6): δ 8.3-7.1 (m, 14H_{arom}), 6.21 (s, 2H, OCH_2O), 4.59 (s, 2H, PhCH_2S). ^{13}C NMR (DMSO-d_6): δ 166.4, 159.4, 158.1, 150.1, 148.1, 146.1, 137.7 (7s, 8C$_{arom}$), 131.5 (d, CH_{arom}), 130.1 (s, C$_{arom}$), 129.7, 128.9, 128.4, 128.3, 127.2, 123.0, 108.5, 107.3, 105.6 (9d, 13CH_{arom}), 101.8, 34.4 (2t, 2CH_2). MS (FAB$^+$) *m/e*: 439 ([M+1]$^+$, 100). Anal. Calcd for $C_{25}H_{18}N_4O_2S$: C, 68.48; H, 4.14; N, 12.78; S, 7.31. Found: C, 68.29; H, 4.27; N, 13.01; S, 7.02%.

5-Benzo[1,3]dioxol-5-yl-2-benzylsulfanyl-7-(tetrahydro-pyran-2-yloxymethyl)-1,2,4-triazolo[1,5-*a*]pyrimidine (6b). According to the general procedure described above, reaction between **5** (255 mg, 1.24 mmol), **4b** (698 mg, 2.42 mmol, 2 equiv), DBU (12 µl, 0.082 mmol, 5% mol.) and anhydrous $MgSO_4$ (1.25g,) afforded 225 mg (38%) of **6b** as a yellow solid. M.p.: 62-64ºC. ^{1}H NMR ($CDCl_3$): δ 7.8-6.9 (m, 9H_{arom}), 6.09 (s, 2H, OCH_2O), 5.24 (d, 1H, *J* = 17 Hz, CH_2OThp), 5.01 (d, 1H, *J* = 17 Hz, CH_2OThp), 4.9 (m, 1H, C*H*), 4.60 (s, 2H, PhCH_2S), 4.0-3.9 (m, 1H, CH_2), 3.7-3.6 (m, 1H, CH_2), 1.9-1.7 (m, 6H, 3CH_2). ^{13}C NMR ($CDCl_3$): δ 168.3, 160.1, 155.8, 150.4, 148.5, 146.5, 137.2, 130.7 (8s, 8C$_{arom}$), 129.1, 128.5, 127.4, 122.7, 108.4, 107.8, 102.7 (7d, 9CH_{arom}), 101.7 (t, CH_2), 99.14 (d, *C*H), 62.6, 62.5, 35.6, 30.2, 25.1, 19.3 (6t, 6CH_2). MS (FAB$^+$) *m/e*: 477 ([M+1]$^+$, 100). Anal. Calcd for $C_{25}H_{24}N_4O_4S$: C, 63.01; H, 5.08; N, 11.76; S, 6.73. Found: C, 63.22; H, 4.84; N, 11.92; S, 6.50%.

Synthesis of (5-benzo[1,3]dioxol-5-yl-2-benzylsulfanyl-1,2,4-triazolo[1,5-*a*]pyrimidin-7-yl)-methanol (7). A solution of 5-benzo[1,3]dioxol-5-yl-2-benzylsulfanyl-7-(tetrahydro-pyran-2-yloxymethyl)-1,2,4-triazolo[1,5-*a*]pyrimidine **6b** (99 mg, 0.21 mmol) in (AcOH:THF:H_2O) (4:2:1) (4 mL/mmol, 1 mL) was heated at 45ºC for 19 h. The organic solvent was eliminated under reduced pressure, was added saturated solution of $NaHCO_3$ (3 mL/mmol) and the aqueous layer was extracted with AcOEt (3×3 mL/mmol). The combined organic layers were dried over $MgSO_4$, the solvent was evaporated and the resulting residue purified by flash-chromatography (n-hexane:AcOEt) afforded 64 mg (77%) of **7** as a yellow solid. M.p.: 175-177ºC. IR (KBr): ν 3199 (br., OH). ^{1}H NMR (DMSO-d_6): δ 7.9-7.2 (m, 9H_{arom}), 6.25 (s, 2H, OCH_2O), 6.17 (t, 1H, *J* = 5.5 Hz, CH$_2$O*H*), 5.05 (d, 2H, *J* = 5.5 Hz, CH_2OH), 4.63 (s, 2H, PhCH_2S). ^{13}C NMR (DMSO-d_6): δ 166.6, 159.3, 155.2, 150.5, 150.1, 148.3, 137.7, 130.2 (8s, 8C$_{arom}$), 128.9, 128,4, 127.2, 122.7, 108.7, 107.0, 102.5 (7d, 9CH_{arom}), 101.9, 57.7, 34.4 (3t, 3CH_2). MS (FAB$^+$) *m/e*: 393 ([M+1]$^+$, 100). Anal. Calcd for $C_{20}H_{16}N_4O_3S$: C, 61.21; H, 4.11; N, 14.28; S, 8.17. Found: C, 60.94; H, 4.36; N, 14.09; S, 8.39%.

Method A for the preparation of the alkoxypyrimidines 9a and 9e. Mitsunobu reaction.

A solution of DIAD (1.2 equiv) in dry THF (1 mL/mmol) is added dropwise to a solution of Ph_3P (1.2 equiv), the appropriate 2-benzylsulfanylpyrimidinone **8a-b** (1 equiv) and different alcohols in tetrahydrofuran (2 mL/mmol) at room temperature. The reaction mixture was stirred at room temperature until total disappearance of **8a-b** (TLC monitoring). The solvent was evaporated until dryness and the crude product adsorbed over silica purified by flash chromatography (n-hexane:EtOAc).

2-Benzylsulfanyl-4-isopropoxy-6-phenyl-pyrimidine (9a). According to the general procedure described above, reaction between **8b** (500 mg, 1.70 mmol), TPP (675 mg, 2.55 mmol), and DIAD (0.50 mL, 2.55 mmol) in dry THF (6 mL), afforded after 2 h. 526 mg (92%) of **9a** isolated as colourless solid. M.p.: 81-82ºC. ^{1}H NMR ($CDCl_3$): δ 8.1-8.0 (s, 2H_{arom}), 7.7-7.2 (m, 8H_{arom}), 6.77 (s, 1H, H_{pyrim}), 5.46 (hept, 1H, *J* = 6.2 Hz, C*H*(CH$_3$)$_2$), 4.54 (s, 2H, PhCH_2S), 1.38 (d, 6H, *J* = 6.2 Hz, CH(CH_3)$_2$). ^{13}C NMR ($CDCl_3$): δ 170.8, 169.4, 164.6 (3s, 3C$_{pyrim}$), 138.0, 136.8 (2s, 2C$_{arom}$), 130.5, 128.8, 128.7, 128.5, 128.4, 127.0 (6d, 10CH_{arom}), 99.7 (d, CH_{pyrim}), 69.5 (d, *C*H), 35.4 (t, CH_2), 21.9 (q, 2CH$_3$). MS (EI) *m/e*: 336 ([M]$^+$, 90). Anal. Calcd for $C_{20}H_{20}N_2OS$: C, 71.40; H, 5.99; N, 8.33; S, 9.53. Found: C, 71.51; H, 6.17; N, 8.14; S, 9.25%.

[1-Benzyl-2-(2-benzylsulfanyl-pyrimidin-4-yloxy)-ethyl]-carbamic acid *tert*-butyl ester (9e). According to the general procedure described above, reaction between **8a** (1.00 g, 4.59 mmol), TPP (1.58 g, 5.96 mmol), *N*-Boc-phenylalaninol (1.50 g, 5.96 mmol) and DIAD (1.15 mL, 5.96 mmol) in dry THF (15 mL), afforded after 5 h. 1.47 g (71%) of **9e** isolated as colourless solid. M.p.: 108-110ºC. IR (KBr): ν 3388 (br., NH), 1685 (s, C=O). ^{1}H NMR ($CDCl_3$): δ 8.30 (d, 1H, *J* = 4.8 Hz, H_{pyrim}), 7.5-7.4 (m, 10H_{arom}), 6.48 (d, 1H, *J* = 4.8 Hz,

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H_{pyrim}), 4.81 (br., 1H, N*H*), 4.37 (s, 2H, CH_2), 4.30 (s, 2H, CH_2), 4.25 (br., 1H, C*H*), 2.90 (m, 2H, PhCH_2C), 1.39 (s, 9H, (CH_3)$_3$). ^{13}C NMR ($CDCl_3$): δ 171.3, 168.4 (2s, 2C_{pyrim}), 157.5 (d, CH_{pyrim}), 152.2 (s, *C*=O), 137.8, 137.3 (2s, 2C_{arom}), 129.3, 128.8, 128.5, 127.1, 126.6, 126.4 (6d, 10CH_{arom}), 103.7 (d, *C*H$_{pyrim}$), 79.6 (s, *C*), 64.1 (t, *C*H$_2$), 50.8 (d, *C*H), 37.8, 35.2 (2t, 2*C*H$_2$), 28.3 (q, 3*C*H$_3$). MS (FAB$^+$) *m*/*e*: 452 ([M+1]$^+$, 17). Anal. Calcd for $C_{25}H_{29}N_3O_3S$: C, 66.49; H, 6.47; N, 9.31; S, 7.10. Found: C, 66.30; H, 6.68; N, 9.07; S, 6.08%.

Method B for the preparation of the alkoxypyrimidines 9b-d.

To a solution of the corresponding 2-benzylsulfanylpyrimidinone **8a-b** (1 equiv) in DMF (3 mL per mmol), 1.1 equiv of TMG were added. Then, the corresponding phenacyl bromide (1.1. equiv) was added dropwise. The reaction mixture was until total disappearance of **8a-b** (TLC monitoring). The solvent was evaporated until dryness and the crude product adsorbed over silica purified by flash chromatography (n-hexane:EtOAc).

2-(2-Benzylsulfanyl-pyrimidin-4-yloxy)-1-(4-chloro-phenyl)-ethanone (9b). According to the general procedure described above, reaction between **8a** (600 mg, 2.8 mmol), (4-chloro-phenyl)-acetyl bromide (850 mg, 3.6 mmol) and TMG (0.45 mL, 3.6 mmol) in dry DMF (8 mL), afforded after 6 h. 856 mg (83%) of **9b** isolated as colourless solid. M.p.: 111-112ºC. IR (KBr): ν 1698 (s, C=O). ^{1}H NMR ($CDCl_3$): δ 8.34 (d, 1H, *J* = 5.6 Hz, H_{pyrim}), 7.9-7.3 (m, 9H_{arom}), 6.64 (d, 1H, *J* = 5.6 Hz, H_{pyrim}), 5.54 (s, 2H, CH_2O), 4.25 (s, 2H, PhCH_2S). ^{13}C NMR ($CDCl_3$): δ 191.7 (s, *C*=O), 171.0, 167.6 (2s, 2C_{pyrim}), 157.8 (d, *C*H$_{pyrim}$), 140.3, 137.0, 132.5 (3s, 3C_{arom}), 129.2, 128.5, 128.4, 127.1, (4d, 9*C*H$_{arom}$), 103.8 (d, *C*H$_{pyrim}$), 67.4, 35.1 (2t, 2CH_2). MS (EI) *m*/*e*: 372 ([M+2]$^{+\cdot}$, 19), 370 ([M]$^{+\cdot}$, 52). Anal. Calcd for $C_{19}H_{15}ClN_2O_2S$: C, 61.53; H, 4.08; N, 7.55; S, 8.65. Found: C, 61.32; H, 3.98; N, 7.76; S, 8.46%.

2-(2-Benzylsulfanyl-pyrimidin-4-yloxy)-1-(3-nitro-phenyl)-ethanone (9c). According to the general procedure described above, reaction between **8a** (1.00 g, 4.6 mmol), (3-nitro-phenyl)-acetyl bromide (1.64 g, 5.7 mmol) and TMG (0.72 mL, 5.7 mmol) in dry DMF (15 mL), afforded after 6 h. 1.31 g (75%) of **9c** isolated as colourless solid. M.p.: 97-98ºC. IR (KBr): ν 1705 (s, C=O). ^{1}H NMR ($CDCl_3$): δ 8.74 (s, 1H_{arom}), 8.46 (d, 1H_{arom}, *J* = 8.2 Hz), 8.35 (d, 1H, *J* = 5.6 Hz, H_{pyrim}), 8.24 (d, 1H_{arom}, *J* = 7.8 Hz), 7.71 (t, 1H_{arom}), 7.3-7.2 (m, 5H_{arom}), 6.65 (d, 1H, *J* = 5.6 Hz, H_{pyrim}), 5.60 (s, 2H, CH_2O), 4.26 (s, 2H, PhCH_2S). ^{13}C NMR ($CDCl_3$): δ 191.1 (s, *C*=O), 171.1, 167.4 (2s, 2C_{pyrim}), 158.0 (d, *C*H$_{pyrim}$), 148.4, 137.0, 135.4 (3s, 3C_{arom}), 133.3, 130.2, 128.5, 128.3, 128.0, 127.1, 122.7 (7d, 9*C*H$_{arom}$), 103.8 (d, *C*H$_{pyrim}$), 67.5, 35.0 (2t, 2CH_2). MS (EI) *m*/*e*: 381 ([M]$^{+\cdot}$, 18). Anal. Calcd for $C_{19}H_{15}N_3O_4S$: C, 59.83; H, 3.96; N, 11.02; S, 8.41. Found: C, 59.57; H, 4.11; N, 11.16; S, 8.14%.

2-(2-Benzylsulfanyl-6-phenyl-pyrimidin-4-yloxy)-1-phenyl-ethanone (9d). According to the general procedure described above, reaction between **8b** (500 mg, 1.7 mmol), phenyl-acetyl bromide (415 mg, 2.0 mmol) and TMG (0.26 mL, 2.0 mmol) in dry DMF (5 mL), afforded after 4 hr. 512 mg (73%) of **9d** isolated as colourless solid. M.p.: 134-135ºC. IR (KBr): 1706 (m, C=O). ^{1}H NMR ($CDCl_3$): 8.1-8.0 (m, 4H_{arom}), 7.6-7.5 (m, 6H_{arom}), 7.4-7.1 (m, 5H_{arom}), 7.05 (s, 1H, H_{pyrim}), 5.67 (s, 2H, OCH_2), 4.39 (s, 2H, PhCH_2S). ^{13}C NMR ($CDCl_3$): 192.9 (s, *C*=O), 170.7, 168.9, 165.2 (3s, 3C_{pirim}), 137.5, 136.4, 134.3 (3s, 3C_{arom}), 133.8, 130.7, 128.8, 128.7, 128.6, 128.4, 127.7, 127.1, 127.0 (9d, 15CH_{arom}), 99.1 (d, *C*H$_{pyrim}$), 67.7, 35.2 (2t, 2CH_2). MS (EI) *m*/*e*: 412 ([M]$^+$, 25). Anal. Calcd for $C_{25}H_{20}N_2O_2S$: C, 72.79; H, 4.89; N, 6.79; S, 7.77. Found: C, 72.57; H, 5.00; N, 6.56; S, 8.09%.

Oxidation of 9a,b to Sulfones 10a,b. General Procedure.

To a cooled (0ºC) solution of pyrimidine derivatives **9a**,**b** (1 equiv) in CH_2Cl_2 (5 mL per mmol), 2.5 equiv of *m*-CPBA (60 % purity) were added in small portions. The mixture was then stirred at 0ºC during 2 h., then diluted with CH_2Cl_2 (20 mL per mmol) and washed with aq. satd. $NaHCO_3$ solution (2×5 mL per mmol) and brine (5 mL per mmol). The separated organic layer was dried ($MgSO_4$), filtered and evaporated to give a residue which was purified by flash chromatography using n-hexane/EtOAc.

4-Isopropoxy-6-phenyl-2-phenylmethanesulfonyl-pyrimidine (10a). According to the general procedure described above, the reaction of **9a** (450 mg, 1.34 mmol) and *m*-CPBA (1.15 g, 3.34 mmol) in CH_2Cl_2 (7 mL), afforded to **10a** (432 mg, 88%) isolated as colourless solid. M.p.: 124-125ºC. ^{1}H NMR ($CDCl_3$): δ 8.1-8. 0 (m, 2H_{arom}), 7.6-7.2 (m, 8H_{arom}), 6.99 (s, 1H, H_{pyrim}), 5.65 (hept, 1H, *J* = 6.0 Hz, C*H*(CH_3)$_2$), 4.88 (s, 2H, PhCH_2S), 1.43 (d, 6H,

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J = 6.2 Hz, CH(C*H*$_3$)$_2$). ^{13}C NMR (CDCl$_3$): δ 170.9, 165.5, 164.7 (3s, 3C$_{pyrim}$), 135.0 (s, C$_{arom}$), 131.6, 131.3, 128.8, 128.7, 128.2, 127.2 (6d, 10CH$_{arom}$), 127.1 (s, C$_{arom}$), 106.0 (d, CH$_{pyrim}$), 71.6 (d, CH), 57.3 (t, CH$_2$), 21.7 (q, 2CH$_3$). MS (EI) *m/e*: 368 ([M]$^+$, 67). Anal. Calcd for C$_{20}$H$_{20}$N$_2$O$_3$S: C, 65.20; H, 5.47; N, 7.60; S, 8.70. Found: C, 65.29; H, 5.60; N, 7.48; S, 8.88%.

1-(4-Chloro-phenyl)-2-(2-phenylmethanesulfonyl-pyrimidin-4-yloxy)-ethanone (10b). According to the general procedure described above, the reaction of **9b** (675 mg, 1.82 mmol) and *m*-CPBA (1.31 g, 4.55 mmol) in CH$_2$Cl$_2$ (9 mL), afforded to **10b** (613 mg, 84%) isolated as colourless solid. M.p.: 141-142ºC. IR (KBr): ν 1692 (s, C=O). ^{1}H NMR (CDCl$_3$): δ 8.65 (d, 1H, *J* = 5.8 Hz, H$_{pyrim}$), 7.92 (d, 2H$_{arom}$, *J* = 6.8 Hz), 7.52 (d, 2H$_{arom}$, *J* = 6.8 Hz), 7.3-7.2 (m, 5H$_{arom}$), 7.12 (d, 1H, *J* = 5.6 Hz, H$_{pyrim}$), 5.72 (s, 2H, CH$_2$O), 4.54 (s, 2H, PhCH$_2$S). ^{13}C NMR (CDCl$_3$): δ 190.9 (s, C=O), 169.3, 163.9 (2s, 2C$_{pyrim}$), 158.3 (d, CH$_{pyrim}$), 140.8, 132.2 (2s, 2C$_{arom}$), 131.0, 129.4, 129.2, 128.8, 128.7 (5d, 9CH$_{arom}$), 126.4 (s, C$_{arom}$), 111.4 (d, CH$_{pyrim}$), 68.3, 57.6 (2t, 2CH$_2$). MS (FAB$^+$) *m/e*: 405 ([M+3]$^+$, 24), 403 ([M+1]$^+$, 62). Anal. Calcd for C$_{19}$H$_{15}$ClN$_2$O$_4$S: C, 56.65; H, 3.75; N, 6.95; S, 7.96. Found: C, 56.81; H, 3.54; N, 7.18; S, 8.15%.

***Ipso*-substitution reaction of pyrimidinyl sulfone derivatives 10a,b.**

Synthesis of 4-isopropoxy-2-phenoxy-6-phenyl-pyrimidine (11a). To a solution of phenol (55 mg, 0.57 mmol) in dioxane (2 mL), the Cs$_2$CO$_3$ (210 mg, 0.59 mmol) was added. The reaction mixture was stirred at r.t. for 15-20 min. Then the sulfone 10a (200 mg, 0.54 mmol) was added. After stirring at 60ºC for 3 hours the solvent was removed *in vacuo* and the mixture was acidified with 2 *N* hydrochloric acid and extracted with ethyl acetate. The residue was purified using flash chromatography to give 11a (131 mg, 80 %) as colourless solid. M.p.: 68-69ºC. ^{1}H NMR (CDCl$_3$): δ 8.0-7.9 (m, 2H$_{arom}$), 7.5-7.3 (m, 8H$_{arom}$), 6.84 (s, 1H, H$_{pyrim}$), 5.28 (hept, 1H, *J* = 6.2 Hz, CH(CH$_3$)$_2$), 1.34 (d, 6H, *J* = 6.2 Hz, CH(C*H*$_3$)$_2$). ^{13}C NMR (CDCl$_3$): δ 171.9, 166.4, 165.0 (3s, 3C$_{pyrim}$), 153.2, 136.5 (2s, 2C$_{arom}$), 130.6, 129.1, 128.7, 127.0, 124.8, 121.9 (6d, 10CH$_{arom}$), 98.6 (d, CH$_{pyrim}$), 69.9 (d, CH), 21.8 (q, 2CH$_3$). MS (EI) *m/e*: 306 ([M]$^+$, 24). Anal. Calcd for C$_{19}$H$_{18}$N$_2$O$_2$: C, 74.49; H, 5.92; N, 9.14. Found: C, 74.36; H, 5.75; N, 9.40%.

Synthesis of 1-(4-chloro-phenyl)-2-[2-(2,4-dimethoxy-phenylamino)-pyrimidin-4-yloxy]-ethanone (11b). To a solution of the sulfone **10b** (125 mg, 0.31 mmol) in dioxane (2 mL), the 2,4-dimethoxyaniline (100 mg, 0.62 mmol) was added. The reaction mixture with good stirring was heated at 100ºC until total consumption of the starting material (30 h., TLC monitoring). The solvent was removed under reduced pressure and the residue purified by flash-chromatography (n-hexane:EtOAc) to give **11b** (25 mg, 20 %) as an orange crystalline solid. M.p.: 122-123ºC. IR (KBr): ν 3244 (br., NH), 1705 (m, C=O). ^{1}H NMR (CDCl$_3$): δ 8.20 (d, 1H, *J* = 5.4 Hz, H$_{pyrim}$), 7.96 (d, 2H$_{arom}$, *J* = 8.2 Hz), 7.77 (d, 1H$_{arom}$, *J* = 8.8 Hz), 7.54 (d, 2H$_{arom}$, *J* = 8.2 Hz), 7.30 (s, 1H$_{arom}$), 6.44 (d, 1H$_{arom}$, *J* = 2.4 Hz), 6.36 (d, 1H, *J* = 5.2 Hz, H$_{pyrim}$), 5.90 (d, 1H, *J* = 8.2 Hz, NH), 5.58 (s, 2H, CH$_2$O), 3.83 (s, 3H, CH$_3$O), 3.72 (s, 3H, CH$_3$O). ^{13}C NMR (CDCl$_3$): δ 192.2 (s, C=O), 168.5, 159.6 (2s, 2C$_{pyrim}$), 158.6 (d, CH$_{pyrim}$), 155.4, 149.7, 140.2, 132.9 (4s, 4C$_{arom}$), 129.3, 129.2 (2d, 4CH$_{arom}$), 121.9 (s, C$_{arom.}$), 120.3, 102.7 (2d, 2CH$_{arom}$), 98.6 (d, CH$_{pyrim}$), 98.5 (d, CH$_{arom}$), 67.3 (t, CH$_2$), 55.6, 55.4 (2q, 2CH$_3$). MS (EI) *m/e*: 401 ([M+2]$^+$, 17), 399 ([M]$^+$, 52). Anal. Calcd for C$_{20}$H$_{18}$ClN$_3$O$_4$: C, 60.08; H, 4.54; N, 10.51. Found: C, 60.29; H, 4.48; N, 10.32%.

Removal of the 4-isopropoxy group. Synthesis of 2-phenoxy-6-phenyl-3*H*-pyrimidin-4-one (12). The 4-isopropoxypyrimidine **11a** (87 mg, 0.28 mmol) was added to a mixture of AcOH (0.6 mL) and con. H$_2$SO$_4$ (0.6 mL). The reaction mixture was stirred at 90ºC for 15 min. After cooling, the mixture was neutralised with aq. 5 *N* NaOH and extracted with CH$_2$Cl$_2$ (3 × 5 mL). The combined organic layers were washed with brine and the separated organic layer was dried (MgSO$_4$), filtered and eliminated under reduced pressure to afford the pure pyrimidinone **12** (59 mg, 80 %) as colourless solid. M.p.: 263-264ºC. IR (KBr): ν 3060-2750 (br., NH), 1669 (s, C=O). ^{1}H NMR (DMSO-*d*$_6$): δ 12.75 (br., NH), 7.9-7.8 (m, 2H$_{arom}$), 7.6-7.5 (m, 8H$_{arom}$), 6.74 (s, 1H, H$_{pyrim}$). ^{13}C NMR (DMSO-*d*$_6$): δ 166.0, 161.4, 158.7 (3s, 3C$_{pyrim}$), 151.8, 135.8 (2s, 2C$_{arom}$), 130.6, 129.5, 128.8, 126.6, 125.7, 121.7 (6d, 10CH$_{arom}$), 102.4 (d, CH$_{pyrim}$). MS (EI) *m/e*: 264 ([M]$^+$, 54). Anal. Calcd for C$_{16}$H$_{12}$N$_2$O$_2$: C, 72.72; H, 4.58; N, 10.70. Found: C, 72.47; H, 4.81; N, 10.86%.

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Synthesis of [1-benzyl-2-(2-benzylsulfanyl-pyrimidin-4-yloxy)-ethylamino]-phenyl-acetic acid (13): Petasis reaction.

To a stirred solution of glyoxylic acid monohydrate (74 mg, 0.78 mmol) in dichloromethane (5 mL) was added the primary amine **9e**, previous deprotection of Boc using standard conditions, (275 mg, 0.78 mmol), followed by phenylboronic acid (99 mg, 0.78 mmol). After the flask was purged with nitrogen and sealed, the reaction mixture was stirred vigorously at room temperature for 3 days. The resulting precipitate was isolated by filtration and washed with to dichloromethane to give the pure pyrimidinone **13** (197 mg, 52 %) as colourless solid. M.p.: 134-135ºC. IR (KBr): ν 3217 (br, NH), 1713 (s, C=O). ^{1}H NMR (DMSO-d_6): δ 8.42 (d, 1H, J = 5.8 Hz, H_{pyrim}), 7.9-7.8 (m, 2H, NH + COOH), 7.5-7.2 (m, 15H_{arom}), 6.69 (d, 1H, J = 5.8 Hz, H_{pyrim}), 4.63 (s, 1H, CHCOOH), 4.34 (s, 2H, PhCH_2S), 4.31 (dd, 1H, J = 4.0 Hz, J' = 11.0 Hz, OCH_2), 4.17 (dd, 1H, J = 6.0 Hz, J' = 11.0 Hz, OCH_2), 3.15 (br., 1H, PhCH$_2$CH), 3.02 (dd, 1H, J = 4.8 Hz, J' = 13.6 Hz, PhCH_2CH), 2.85 (dd, 1H, J = 7.5 Hz, J' = 13.6 Hz, PhCH_2CH). ^{13}C NMR (DMSO-d_6): δ 172.6, 170.0, 168.0 (3s, 3C), 158.0 (d, CH$_{pyrim}$), 137.8, 137.7 (2s, 3C_{arom}), 134.1, 130.0, 129.2, 128.8, 128.4, 127.8, 127.3, 127.0, 126.4 (9d, 15CH$_{arom}$), 104.1 (d, CH$_{pyrim}$), 66.9 (t, CH$_2$), 62.4, 55.5 (2d, 2CH), 36.4, 34.2 (2t, 2CH$_2$). MS (FAB$^+$) m/e: 486 ([M+1]$^{\cdot+}$, 28). Anal. Calcd for $C_{28}H_{27}N_3O_3S$: C, 69.25; H, 5.60; N, 8.65; S, 6.60. Found: C, 69.52; H, 5.84; N, 8.48; S, 6.31%.

Synthesis of 2-(2-benzylsulfanyl-6-phenyl-pyrimidin-4-yloxy)-1-phenyl-ethanol (14). To a stirred and cooled (0 ºC) solution of pyrimidinone **9d** (500 mg, 1.21 mmols) in MeOH (6 mL) was added $NaBH_4$ (165 mg, 4.24 mmols) in small portions while stirring (vigorous evolution of gas observed). Stirring was continued for 2 h. at 0ºC. The solution was evaporated to dryness, and the crude residue was partitioned between EtOAc (10 mL) and aq satd NH_4Cl solution (15 mL). The organic layer was separated, washed with H_2O (5 mL), dried ($MgSO_4$), and evaporated to give a residue which was purified by flash chromatography using hexanes/EtOAc to afford pure (**14**) as a colourless solid (403 mg, 80%). M.p.: 140-141ºC. IR (KBr): 3400 (br., OH). ^{1}H NMR ($CDCl_3$): 8.1-8.0 (m, 2H_{arom}), 7.5-7.3 (m, 13H_{arom}), 6.90 (s, 1H, H_{pyrim}), 5.17 (dd, 1H, J = 8.4 Hz, J' = 3.2 Hz, PhCH), 4.53 (s, 2H, PhCH_2S), 4.47 (dd, 1H, J = 11.6 Hz, J' = 8.4 Hz, CH_2O), 4.03 (dd, 1H, J = 11.4 Hz, J' = 3.2 Hz, CH_2O), 2.90 (br., 1H, OH). ^{13}C NMR ($CDCl_3$): 170.9, 169.6, 165.1 (3s, 3C_{pyrim}), 139.8, 137.7, 136.4 (3s, 3C_{arom}), 130.8, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 127.1, 126.2 (9d, 15CH$_{arom}$), 99.1 (d, CH$_{pyrim}$), 72.5 (d, CH), 71.7, 35.4 (2t, 2CH$_2$). MS (EI) m/e: 414 ([M]$^+$, 33). Anal. Calcd for $C_{25}H_{22}N_2O_2S$: C, 72.44; H, 5.35; N, 6.76; S, 7.74. Found: C, 72.62; H, 5.14; N, 6.52; S, 7.46%.

3.2 Microbiological Methods

Thirty-two isolates of *M. tuberculosis* from respiratory (n=21) and non-respiratory (n=11) clinical samples, identified by conventional methods **Error! Reference source not found.** and by DNA hybridization probe (Accuprobe® Gen Probe Inc., San Diego, California) were selected from the laboratory collection of Dept. Microbiología, Hospital Clínico Universitario. Valencia, Spain. Susceptibility to first-line antituberculosis drugs (ethambutol, isoniazid, rifampin and streptomycin) was tested by a fluorometric method (Bactec® MGIT 960, Becton-Dickinson) and by a microdilution method **Error! Reference source not found.**.

The organims were grown in modified Middlebrook 7H9 broth supplemented with 10% OADC enrichment (Difco Laboratories) for seven days at 37ºC. The inoculum size was obtained by dilution of *M. tuberculosis* isolates suspensions in 7H9 broth to yield an absorbance equivalent to that of a MacFarland nº 0.5 standard.

Antimicrobial susceptibility test was performed in 96-well microplates using serial twofold microdilution in 7H9 broth. Initial drug dilutions were prepared in deionized water or, if no soluble, dimethyl sulfoxide. Subsequent twofold dilutions were performed in 150 µL of modified 7H9 broth in the microplates to provide a final test range of 128 to 0.125 mg/L. Ten µL of a suspension of mycobacteria were added to the wells. Plates were covered with Parafilm "M"® (Laboratory Film, American national Can TM), and incubated for 12 days at 37ºC. Starting at 13 day of incubation, 20 µL of Resazurina® (Sigma 2127) with a concentration of 250 mg/L were added to the wells, and the microplates were

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reincubated at 37ºC for an additional period of 48h. MIC_{50} and MIC_{90} were determined as the lowest concentrations of the compounds yielding no visible changes from blue to pink **Error! Reference source not found.**.

4. Conclusions

New chemical scaffolds have been identified that could render new lead drugs in this field, by using easy to calculate descriptors, such as structural invariants. The only substructure common to all the selected molecules is the pyrimidine ring, and the most frequent substituent is the benzylsulfanyl in position 2. The 1,2,4-triazolo[1,5-*a*]pyrimidine system is present in structures **7** and **6a**, which have the same substituted groups in 2 and 5. Only one pyrimidone was selected (**12**). All these structures are proposed as new base structures in order to design new combinatorial synthesis projects.

Although several action mechanisms have been considered in the training group the validity of the approach is not negligible. One possible explanation to this fact could be that the equation retains the structural features involved in all the mechanisms considered. In opinion of the authors, this approach is not able to find new action mechanisms, but it is possible to obtain new unexpected molecular structures acting through the known mechanisms which combine properties of the known compounds. This feature could be useful in order to avoid problems of resistance.

Supplementary Materials: Table with all the thresholds for the descriptors and DF (Eq. 1), Representative spectra.

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