Efficient and regioselective one-step synthesis of 7-aryl-5-methyl- and 5-aryl-7-methyl-2-amino-[1,2,4]triazolo[1,5-a]pyrimidine derivatives.

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Abstract

Two facile and efficient one-step procedures for the regioselective synthesis of 7-aryl-5-methyl- and 5-aryl-7-methyl-2-amino-[1,2,4]triazolo[1,5-a]pyrimidines have been developed, via reactions of 3,5-diamino-1,2,4-triazole with variously substituted 1-aryl-1,3-butanediones and 1-aryl-2-buten-1-ones, respectively. The excellent yield and/or regioselectivity shown by the reactions decreased when ethyl 5-amino-1,2,4-triazole-3-carboxylate was used. Being the [1,2,4]triazolo[1,5-a]pyrimidine a privileged scaffold, the procedure herein reported may be useful for the preparation of biologically active compounds. In this study, the preparation of a set of compounds based on the [1,2,4]triazolo[1,5-a]pyrimidine scaffold let to the identification of compound 20 endowed with a very promising ability to inhibit influenza virus RNA polymerase PA-PB1 subunits heterodimerization.
Introduction

[1,2,4]triazolo[1,5-\(\alpha\)]pyrimidine is a privileged structure with numerous chemical and biological applications. Beside to their great versatility in the interactions with metal ions, [1,2,4]triazolo[1,5-\(\alpha\)]pyrimidines showed a wide range of biological activities both in agriculture and in medicine.\(^1\) Examples of biologically active compounds include trapidil (Rocoral\(^\text{\textregistered}\), Fig. 1), a platelet-derived growth factor antagonist that has been used to treat patients with ischemic coronary heart, liver, and kidney disease,\(^1\) and filibuvir (Fig. 1), a non-nucleoside inhibitor of HCV NS5B polymerase that passed stage II clinical trials, although its clinical development program was then suspended.\(^1\) Focusing on the most recent literature, compounds based on the [1,2,4]triazolo[1,5-\(\alpha\)]pyrimidine core have been reported as phosphodiesterase 2 (PDE2a) inhibitors for the treatment of memory disorders,\(^2\) anti-Alzheimer’s disease,\(^2\) anticancer,\(^2\) antimalarial,\(^2\) antitubercular,\(^2\) antileishmanial,\(^2\) antibacterial,\(^2\) antiviral,\(^2\) hypnotic,\(^2\) and CB2 cannabinoid receptor inverse agonists.\(^2\)

![Figure 1. Examples of biologically active compounds with a [1,2,4]triazolo[1,5-\(\alpha\)]pyrimidine scaffold.](image)

We have also been involved in the synthesis of a series of [1,2,4]triazolo[1,5-\(\alpha\)]pyrimidines (compound I and structures II and III, Fig. 1)\(^3\) within our research program on the development of influenza virus (flu) RNA-dependent RNA polymerase (RdRP) PA-PB1 subunits interaction inhibitors.\(^3\) In particular, the synthesis of the
anti-flu compounds entailed the preparation of the key intermediate 2-amino-5-methyl-7-phenyl-[1,2,4]triazolo[1,5-α]pyrimidine (1a) and its isomer 2-amino-7-methyl-5-phenyl-[1,2,4]triazolo[1,5-α]pyrimidine (2a) (Table 1).

A major contribution to the chemistry of 2-amino-[1,2,4]triazolo[1,5-α]pyrimidines has been provided by Desenko and co-workers, who were the first to report on their synthesis,4 and Chernyshev’s research group, who investigated their synthesis further,5a-c their reactivity,5d and their use for the preparation of polycondensed heterocycles.5e-h Nevertheless, the synthesis of 7-aryl-5-methyl- and 5-aryl-7-methyl-2-amino-[1,2,4]triazolo[1,5-α]pyrimidine derivatives has been scarcely explored. In this work, we reported two approaches for their preparation via reaction between 3,5-diamino-1,2,4-triazole and variously functionalized 1-aryl-1,3-butanediones and 1-aryl-2-buten-1-ones, respectively. Both strategies allowed the synthesis of the desired isomer under mild conditions, with high yields, and regioselectively.

**Results and discussion**

The synthetic method known for the regioselective preparation of compound 1a, as well as of some 5,7-diaryl-2-amino-[1,2,4]triazolo[1,5-α]pyrimidines, involves a two-step procedure (Scheme 1) entailing: i) cyclocondensation of 3,5-diamino-1,2,4-triazole (3a) with chalcone 4-phenylbut-3-en-2-one (4a) giving 2-amino-5-methyl-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-α]pyrimidine,4a-c and ii) heteroaromatization using either N-bromosuccinimide (NBS) or Br2.4a However, the overall yields do not exceed 25-30%. Moreover, NBS and Br2 are highly reactive and, thus, this method can only be used to prepare [1,2,4]triazolo[1,5-α]pyrimidines with limited substitutions at the C-5 and C-7 positions. Increased overall yields up to 40-77% were achieved by acetyl protection of the C-2 amino group, by adding Ac2O to the reaction mixture after the completion of cyclocondensation, preventing oxidation of the amino group in the successive step.5a Through this three-step procedure, compound 1a was regioselectively obtained in 77% yield (Scheme 1).
Scheme 1. Known procedures for the synthesis of 1a.

The reaction of 3-amino-1,2,4-triazole bearing different substituents at the C-5 position with unsymmetrically 1,3-diketones where one of the substituents is a methyl group and the other is a group different from methyl, was known from literature to form a mixture of 5-methyl and 7-methyl isomers. The two isomers can be distinguished by NMR on the basis of the chemical shifts of the pyrimidine methyl carbon appearing at 24-25 ppm and 16-17 ppm for the 5-methyl and 7-methyl isomers, respectively. Although 5-methyl isomer is always the main product of the reaction, the ratio of isomers is influenced: i) by the steric hindrance of the substituent on the 1,3-diketone, with a more bulky substituent that gives a higher ratio of 5-methyl isomer, and ii) by the inductive effect of the substituent at the C-5 position of the 3-amino-1,2,4-triazole, which influences the ratio of isomers much more than the inductive effect of the substituent on the 1,3-diketone different from the methyl.

Based on these facts, we hypothesized that, the presumable high nucleophilicity of 3a owing to the presence of a second electron-donating amino groups in the molecule, might led to an efficient synthesis of 2-amino-7-aryl-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidines by cyclocondensation with 1-aryl-1,3-butanediones. To test this idea, we first tried the reaction of 3a with 1-phenyl-1,3-butanedione (5a) in glacial acetic acid at reflux (Table 1, entry 1). Actually, Kreutzberger and Risse reported in 1979 that this reaction condition provided a mixture of isomers 1a and 2a in 2% and 26% yield, respectively. In contrast to what reported by Kreutzberger and Risse who mistakenly inverted the assignment of the structures, we were pleased to find that the
reaction took place rapidly (4 h), highly efficiently, and, more interestingly, highly regioselectively. Indeed, 2-amino-5-methyl-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine (1a) was obtained in 88% yield while its regioisomer 2-amino-7-methyl-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine (2a) was formed only in traces.

To further explore the reaction conditions, the reaction was then carried out in different solvents. In particular, the reaction was repeated in protic and aprotic solvents, i.e. EtOH, DMF, CHCl₃, THF, and acetone (Table 1), in order to understand whether the acid environment of glacial acetic acid could be essential or not for regioselectivity. We also aimed at evaluating the possible influence on tautomerism of 5a, that is well known to be extremely sensitive to solvent effect. In particular, the enolic form of 5a is much greater in nonpolar solvents than in polar or hydrogen-bond donor solvents, since the first ones do not compete with hydrogen-bond formation. For example, in water compound 5a is estimated to be a mixture of both tautomers, with the keto-form being more favoured than the enol one (about 60% and 40%, respectively).

Although data on tautomerism of 5a in glacial acetic acid are not available, it is reasonable to hypothesize that in this solvent the keto-form is predominant over the enol one, while, for example, in CHCl₃ it is known that this compound exists as two kinds of cis-enol forms. No significant reaction was observed in CHCl₃ (entry 2), acetone (entry 3), and THF (entry 4) at reflux after 24 h, because of the insolubility of 3a in these solvents at the used concentrations. On the other hand, the reaction in EtOH (entry 5) and DMF (entry 6) led to the formation of isomer 1a as the main product but in lower yield (57% and 66%, respectively) and much more slowly than in acetic acid. Since the reactions did not go to completion under both conditions after 24 h, we studied the effect of the equiv of 3a (entry 7) as well as the influence of the addition of a base such as triethylamine (entries 8) in DMF. Analogously, the reactions were much more slow and less efficient (69% and 61%, respectively), but, most importantly, they showed a dramatically decreased regioselectivity. Finally, the reaction was carried out in EtOH with the addition of a catalytic amount of acetic acid (entry 9), to verify whether a protic solvent in acid conditions could lead to a similar regioselectivity compared to that obtained with glacial acetic acid. The presence of acetic acid did not influence the outcome of the reaction, meaning that the simple catalytic effect by acid conditions in the condensation reaction is not sufficient to obtain a high regioselectivity.
Thus, the best reaction conditions are treating 3a (1 equiv) and 5a (1 equiv) in acetic acid at reflux for 4 h. Through this one-step procedure, compound 1a was regioselectively obtained in 88% yield.

**Table 1. Optimization of reaction conditions for 1a**

<table>
<thead>
<tr>
<th>Entry</th>
<th>3a (equiv)</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Yield (%)</th>
<th>1a</th>
<th>2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Acetic Acid</td>
<td>4</td>
<td>88</td>
<td></td>
<td>traces</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>CHCl₃</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>3</td>
<td>1</td>
<td>Acetone</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>THF</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>EtOH</td>
<td>24</td>
<td>57</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>DMF</td>
<td>24</td>
<td>66</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>DMF</td>
<td>24</td>
<td>39</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>DMF (1 equiv of Et₃N)</td>
<td>24</td>
<td>48</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>EtOH (0.5 mL of Acetic Acid)</td>
<td>24</td>
<td>62</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

*a The reaction was performed on 1.0 mmol scale of 5a in 2.5 mL of solvent at reflux.  
*b Determined by ¹H NMR.

Utilizing the optimized conditions, we then studied the scope of the reaction (Table 2). Thus, 1-aryl-1,3-butanediones (5b-k) bearing different electron-donating and electron-withdrawing substituents on the phenyl ring were prepared and reacted with 3a. 1-Aryl-1,3-butanediones were in turn synthesized through a
Claisen condensation by reacting aryl-methyl ketones with ethyl acetate in the presence of sodium\textsuperscript{10} (for the synthesis and characterization of compounds 5b-k, see SI).

The results listed in Table 2 show that 1-aryl-1,3-butanediones bearing both electron-donating (entries 2 and 3) and electron-withdrawing (entry 4) substituents on the phenyl ring are suitable substrates for this reaction, reacting smoothly with 3a to give products 1b-d. The effect of the position of electron-withdrawing substituents on the phenyl ring was also studied (entries 5 and 6), and the reaction gave consistently good yields for compounds 1e and 1f. Multiple electron-donating (entry 7) and electron-withdrawing (entry 8) substituents on the 1-aryl-1,3-butanedione phenyl ring gave good yields. Finally, using 1-(naphthalen-1-yl)butane-1,3-dione (entry 9) and 1-(pyridin-4-yl)butane-1,3-dione (entry 10), the reaction gave compounds 1i and 1k in modest yields.

Table 2. Preparation of 2-amino-7-aryl-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidines\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Time (h)</th>
<th>Product</th>
<th>Yield (%)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C\textsubscript{6}H\textsubscript{5}</td>
<td>4</td>
<td>1a</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>p-CH\textsubscript{3}C\textsubscript{6}H\textsubscript{5}</td>
<td>3</td>
<td>1b</td>
<td>93</td>
</tr>
<tr>
<td>3</td>
<td>p-CH\textsubscript{3}SC\textsubscript{6}H\textsubscript{5}</td>
<td>4</td>
<td>1c</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>p-BrC\textsubscript{6}H\textsubscript{5}</td>
<td>3</td>
<td>1d</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>o-ClC\textsubscript{6}H\textsubscript{5}</td>
<td>2</td>
<td>1e</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>m-CF\textsubscript{3}C\textsubscript{6}H\textsubscript{5}</td>
<td>3</td>
<td>1f</td>
<td>86</td>
</tr>
<tr>
<td>7</td>
<td>m,p-Di-CH\textsubscript{3}OC\textsubscript{6}H\textsubscript{5}</td>
<td>3</td>
<td>1g</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>m,p-Di-ClC\textsubscript{6}H\textsubscript{5}</td>
<td>2</td>
<td>1h</td>
<td>83</td>
</tr>
<tr>
<td>9</td>
<td>1-Naphthyl</td>
<td>2</td>
<td>1i</td>
<td>62</td>
</tr>
</tbody>
</table>
The reaction was performed on 1.0 mmol scale of 3a and 1 equiv of 5 in 2.5 mL of glacial acetic acid at reflux.

Isolated yields.

To further explore the scope of the reaction, 5a was reacted with 5-amino-1,2,4-triazole-3-carboxylic acid, characterized by the presence of an electron-withdrawing group at the C-2 position, under the same reaction conditions. Unfortunately, the reaction was accompanied by decarboxylation, thus, it was repeated starting from ethyl 5-amino-1,2,4-triazole-3-carboxylate (6a). The reaction was equally rapid (4 h) and efficient (80% yield) but showed a dramatically decreased regioselectivity, in that ethyl 5-methyl-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-2-carboxylate (7a) and ethyl 7-methyl-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-2-carboxylate (8a) were obtained in the ratio of 3.7:1 (Scheme 2). Of note, although not regioselective, this reaction permitted to obtain derivatives 7a in acceptable yield (63%) with respect to the procedure entailing the reaction of 6a with 4-phenylbut-3-en-2-one (4a) followed by heteroaromatization (Scheme 2), which provided compound 7a in a very lower yield (10%).

Plausible pathways accounting for the formation of compounds 7a and 8a through the reaction of 6a with 5a are speculatively reported in Scheme 3. In particular, an initial direct addition of the amino group at the C(5) position of 6a on the carbonyl carbon C(3) of 5a to give a β-aminovinyl ketone, followed by intramolecular

Scheme 2. Synthesis of 7a via reaction of 6a with 5a and 4a. *Isolated yields.
cyclization of the latter at the nucleophilic N(1) center of 6a on the carbonyl C(1) of 5a would give 7a (Scheme 3a). The same pathway may be responsible for the formation of 1a starting from 3a. On the other hand, compound 8a could be obtained by: i) an initial direct addition of N(1) of 6a on the carbonyl carbon C(3) of 5a followed by intramolecular cyclization of the latter by direct addition of the C(5) amino group of 6a on the carbonyl C(1) of 5a (Scheme 3b); or ii) an initial direct addition of the C(5) amino group of 6a on the carbonyl carbon C(1) of 5a followed by intramolecular cyclization of the latter by direct addition of N(1) of 6a on the carbonyl C(3) of 5a (Scheme 3c).
Scheme 3. Plausible reaction mechanisms toward 1a, 7a, and 8a.
Although a deep investigation of the mechanisms involved in this reaction is beyond the scope of this study, the mechanism reported in Scheme 3b was hypothesized as more likely to occur compared to that in Scheme 3c. Indeed, if the steric hindrance of the phenyl group in 5a was negligible in driving the nucleophilic attack of the amino group, the high regioselectivity observed in the reaction of 3a could not be explained. Thus, the difference in the regioselectivity shown by the reactions of 3a and 6a with 5a might depend on the different nucleophilicity of the two aminotriazoles due to the effect of the substituent. In particular, while the presence of a second electron-donating amino group in 3a might be responsible for a higher nucleophilicity of the C(3) amino group than N(2), the electron-withdrawing ethyl carboxylate moiety in 6a might lead to a smaller difference of nucleophilicity between the C(5) amino group and N(1), resulting in a decreased selectivity in forming 7a and 8a. Moreover, in the acidic conditions used (pH in glacial acetic acid is reported to be 2.4) compound 3a should be almost fully protonated, while about 34% of 6a is in its neutral form according to MoKa predictions,\textsuperscript{11} and this might explain why the nucleophilic attack of N(1) can occur for compound 6a (although this remains the minor pathway). Finally, the presence of two amino groups in 3a with a presumable comparable nucleophilicity would make their initial attack more probable in 3a than in 6a, as also confirmed by lost of regioselectivity observed when one of the two amino groups in 3a was dimethylated.\textsuperscript{6b}

As shown above, the reaction of 3a and 5a under a few different conditions (entries 7 and 8 Table 1) gave a mixture of 1a and 2a, of which, however, the latter is always obtained in very low yield (no more than 30% yield). Thus, we searched for an alternative synthetic procedure to prepare 2-amino-5-aryl-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidine derivatives, taking into account the regioselective cyclocondensation of 3a with chalcones.

The study started by reacting 3a with phenyl-1-propenyl-ketone (9a) in DMF at reflux and by adding Ac\textsubscript{2}O, in order to obtain \(\text{N-}(7\text{-methyl-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl})\text{acetamide}\) to be then heteroaromatized and hydrolysed, to give 2a. After 30 min, the reaction gave a mixture compounds, of which one was the already oxidized \(\text{N-}(7\text{-methyl-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl})\text{acetamide}\) derivative. Thus, the reaction was repeated without adding Ac\textsubscript{2}O, in order to directly obtain 2a (Table 3).
After 30 min, compound 2a was obtained in 44% yield (entry 1), which increased up to 50% yield after 4 h (entry 2).

With these encouraging results, we further explored the reaction conditions. Based on the fact that atmospheric O$_2$ can enhance oxidation of 4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidines, we hypothesized that an higher yield might be achieved by carrying out the reaction in an open flask. Thus, the reaction was initially carried out in different solvents (Table 3), i.e. toluene, dioxane, N-methylpyrrolidone (NMP), and DMF (CHCl$_3$, acetone, and THF have not been used because of the insolubility of 3a), at 110 °C in an open flask. No significant reaction was observed in toluene (entry 3) and dioxane (entry 4) after 24 h, while the reaction in NMP (entry 5) provided compound 2a in 25% yield after 4 h. On the other hand, the reaction in DMF (entry 6) led to the formation of 2a in 57% yield after 2 h. Then, we studied the effect of the equiv of 9a (entry 7), the presence of a base such as Et$_3$N (entry 8), and both of them (entry 9) in DMF, and found that the optimum reaction conditions are treating 3a (2 equiv) and 9a (1 equiv) in the presence of Et$_3$N (1 equiv) in DMF at 110 °C for 2 h (entry 9) in an open flask. Through this one-step procedure, compound 2a was regioselectively obtained in 80% yield.

Table 3. Optimization of reaction conditions for 2a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>3a (equiv)</th>
<th>Et$_3$N (equiv)</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Yield (%)$^b$</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>no base</td>
<td>DM$^c$</td>
<td>0.5</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>no base</td>
<td>DMF$^c$</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>no base</td>
<td>Toluene</td>
<td>24</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ Reaction was carried out in an open flask. $^b$ Isolated yield. $^c$ Reaction was carried out at 110 °C.
<p>| | | | | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>no base</td>
<td>Dioxane</td>
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<tr>
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<td>1</td>
<td>no base</td>
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<tr>
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</tr>
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<td>67</td>
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<td>9</td>
<td>2</td>
<td>1</td>
<td>DMF</td>
<td>2</td>
<td>80</td>
</tr>
</tbody>
</table>

*a* Unless otherwise indicated, the reaction was performed on 1.0 mmol scale of **9a** in 2.5 mL of solvent in an open flask at 110 °C.  
*b* Isolated yields.  
*c* Using the condenser.

With these optimized reaction conditions, we next studied the scope of the reaction (Table 4). Thus, 1-aryl-2-buten-1-ones (**9b-i**) containing different electron withdrawing as well as electron-donating groups on the phenyl ring were synthesized and reacted with **3a**. 1-Aryl-2-buten-1-ones were synthesized through a Witting reaction. In particular, 2-bromoacetophenones were treated with triphenylphosphine in toluene giving triphenylphosphonium bromides,\(^{13}\) which were then reacted with aqueous NaOH in dichloromethane to afford 1-aryl-2-(triphenylphosphorylidene) ethanones, and then with acetaldehyde in a Witting reaction to give the corresponding 1-aryl-2-buten-1-ones\(^{14}\) (for the synthesis and characterization of compounds **9b-i**, see SI).

The results shown in Table 4 highlight that 1-aryl-2-buten-1-ones bearing both electron-donating and electron-withdrawing substituents on the phenyl ring are suitable substrates for this reaction. Indeed, with the exception of 4-nitrophenyl- (entry 4) and 4-pyridinyl- (entry 9) 2-buten-1-ones, which gave derivatives **2d** and **2i** in modest yields, all the other studied 1-aryl-2-buten-1-ones (entries 2, 3, and 5-7) reacted efficiently and regioselectively with **3a** to give 2-amino-5-aryl-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidines in consistently good yields.
Table 4. Preparation of 2-amino-5-aryl-7-methyl-[1,2,4]triazolo[1,5-α]pyrimidines.

![Chemical reaction diagram]

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Time (h)</th>
<th>Product</th>
<th>Yield (%)</th>
<th>b</th>
</tr>
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<tr>
<td>1</td>
<td>C₆H₅</td>
<td>2</td>
<td>2a</td>
<td>80</td>
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</tr>
<tr>
<td>2</td>
<td>p-CH₃C₆H₅</td>
<td>3</td>
<td>2b</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>p-CH₃OC₆H₅</td>
<td>2</td>
<td>2c</td>
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a The reaction was carried out on 1.0 mmol scale of 3a, 0.5 mmol of 9, and 0.5 mmol of Et₃N in 2.5 mL of DMF in an open flask at 110 °C.

b Isolated yields.

To further explore the scope of the reaction, by using the same reaction conditions, 3a was reacted with 4-phenylbut-3-en-2-one (4a) giving isomer 1a regioselectively in 72% yield after 6 h (Scheme 4a). Of note, although in lower yield (72%) than the reaction of 3a with 5a (88% yield), the reaction of 3a with 4a under these conditions is an alternative one-step procedure for the regioselective synthesis of 1a, which was obtained with comparable yield to that of the known three-step procedure (77% yield).5a Finally, by using the same reaction conditions, ethyl 5-amino-1,2,4-triazole-3-carboxylate (6a) was also reacted with both 4a and 9a. Surprisingly, isomers 7a and 8a were obtained in traces and 19% yield, respectively, after 24 h (Scheme 4b).
All the compounds herein reported, with the exception of 1a, were not described previously and their structures were fully characterized by spectra data of $^1$H NMR, $^{13}$C NMR, and HRMS. It is worthwhile to underline that all the products obtained from both the procedures were purified by simple crystallization without the involvement of chromatography.

[1,2,4]triazolo[1,5-α]pyrimidines as anti-flu compounds

As mentioned above, we have recently identified a series of potent anti-flu compounds based on the [1,2,4]triazolo[1,5-α]pyrimidine scaffold that act by inhibiting flu RdRP PA-PB1 subunits interaction.\textsuperscript{3a} In particular, within the optimization of compound I (Fig. 1) we prepared a large series of analogues, along with a few derivatives characterized by the oxidized [1,2,4]triazolo[1,5-α]pyrimidine scaffold (structure II and III, Fig. 1).\textsuperscript{3a} Among them, derivatives 10 and 11 (Table 5) showed a good ability to inhibit flu replication (EC$_{50}$ = 42 and 25 μM, respectively) at non toxic concentrations (CC$_{50}$ > 250 μM). Compound 11 also inhibited PA-PB1 interaction with a comparable IC$_{50}$ (26 μM).

In order to add structure-activity relationship insights, we decided to exploit the scaffolds herein synthesized by preparing an additional set of compounds to study the effect of modifications on the C-5, C-7, and C-2
positions of the [1,2,4]triazolo[1,5-\(\alpha\)]pyrimidine core. Thus, 2-amino derivatives 1e, 1f, 2c, 2d, and 2e variously functionalized at the C-5 or C-7 position were reacted with benzoyl chloride in pyridine at 80 °C providing target derivatives 12-16 (Table 5). To study the C-2 position, target compounds 19 and 20 (Table 5) were prepared starting from 2-carboxylate scaffolds 7a and 8a, which were hydrolyzed to give intermediates 17 and 18, chlorinated, and then reacted with 2-aminobenzamide in CH\(\text{Cl}_2\) in the presence of DIPEA.

The synthesized compounds were first evaluated for the ability to inhibit the physical interaction between fluA PA and PB1 subunits by ELISA including the Tat-PB1\(1-15\) peptide\(^{15}\) as a positive control of inhibition. In parallel, for all the synthesized compounds the antiviral activity was tested by plaque reduction assays (PRA) in Mardin-Darby canine kidney (MDCK) cells infected with a reference fluA virus, the A/PR/8/34 strain. Ribavirin (RBV), a known broad-spectrum inhibitor of RNA viruses polymerase,\(^{16}\) was also included. To exclude that the observed antiviral activities could be due to toxic effects on the target cells, the compounds were also tested by MTT assays in MDCK cells.

As shown in Table 5, derivatives 12-16, which were functionalized on the phenyl ring at the C-5 or C-7 position, although nontoxic, resulted unable to inhibit the viral growth at low micromolar concentrations. Nevertheless, \(p\)-nitrophenyl and \(p\)-chlorophenyl derivatives 15 and 16 showed a good ability to interfere with PA-PB1 heterodimerization (IC\(\text{so} = 25\) and 40 \(\mu\)M, respectively). The lack of antiviral activity was shown also by compound 19, in which however the presence of the benzamide moiety at the C-2 position led to increase of about 15 folds the anti-PA-PB1 activity (IC\(\text{so} = 11\) \(\mu\)M) with respect to the strict analogue compound 10 (IC\(\text{so} = 160\) \(\mu\)M). The best and most balanced results was achieved with compound 20, which showed both the ability to inhibit viral replication and PA-PB1 heterodimerization at non toxic concentrations. In particular, derivative 20 showed a slightly decreased anti-flu activity (EC\(\text{so} = 31\) \(\mu\)M) but an enhanced ability to inhibit PA-PB1 complex formation (IC\(\text{so} = 11\) \(\mu\)M) with respect to its analogue 11 (IC\(\text{so} = 25\) \(\mu\)M), and even better than the reference PB1\(1-15\)–Tat peptide (IC\(\text{so} = 41\) \(\mu\)M).

**Table 5.** Synthesis and biological activity of [1,2,4]triazolo[1,5-\(\alpha\)]pyrimidine derivatives.
### Compd R R’ ELISA PA-PB1 Interaction Assay IC₅₀, μMᵃ PRA in MDCK cells EC₅₀, μMᵇ Cytotoxicity (MTT Assay) in MDCK cells CC₅₀, μMᶜ

<table>
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<tr>
<th>Compd</th>
<th>R</th>
<th>R’</th>
<th>IC₅₀, μMᵃ</th>
<th>EC₅₀, μMᵇ</th>
<th>CC₅₀, μMᶜ</th>
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<td>&gt;100</td>
<td>&gt;250</td>
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<td>163 ± 20</td>
<td>&gt;100</td>
<td>&gt;250</td>
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<tr>
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<td>160 ± 1</td>
<td>92 ± 5</td>
<td>&gt;250</td>
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<td>25 ± 2</td>
<td>92 ± 5</td>
<td>&gt;250</td>
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<tr>
<td>16</td>
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<td>40 ± 4</td>
<td>99 ± 2</td>
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### Compd R R’ ELISA PA-PB1 Interaction Assay IC₅₀, μMᵃ PRA in MDCK cells EC₅₀, μMᵇ Cytotoxicity (MTT Assay) in MDCK cells CC₅₀, μMᶜ

<table>
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<th>Compd</th>
<th>R</th>
<th>R’</th>
<th>IC₅₀, μMᵃ</th>
<th>EC₅₀, μMᵇ</th>
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<td>20</td>
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<td>7 ± 1</td>
<td>31 ± 10</td>
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**Tat-PB1₁₋₁₅ peptide** 35 ± 4 41 ± 5 >100

**RBV** 10 ± 2 >250
Compounds activity in ELISA PA–PB1 interaction assays. The IC\textsubscript{50} value is defined as the compound concentration that reduces the PA–PB1 interaction by 50%. Antiviral activity of the compounds against the fluA A/PR/8/34 strain in plaque reduction assays. The EC\textsubscript{50} value represents the effective compound concentration required to reduce virus plaque formation by 50%. Citotoxicity of the compounds in MTT assays. The CC\textsubscript{50} value represents the compound concentration resulting in 50% inhibition of MDCK cell viability. All the reported values represent the means ± SD of data obtained from at least three independent experiments in duplicate.

Conclusions

In summary, two facile and efficient one-step procedures for the regioselective synthesis of 7-aryl-5-methyl- and 5-aryl-7-methyl-2-amino-[1,2,4]triazolo[1,5-a]pyrimidines have been developed. These procedures have proven to be suitable for 1-aryl-1,3-butanediones and 1-aryl-2-buten-1-ones with different substitution patterns on the phenyl ring, permitting to obtain 2-amino-[1,2,4]triazolo[1,5-a]pyrimidines variously functionalized at the C-5 and C-7 positions, respectively. The synthesized derivatives may be useful for the preparation of biologically active compounds. In this study, they have been used for the synthesis of a set of [1,2,4]triazolo[1,5-a]pyrimidine derivatives as anti-flu compounds. From this study, derivative 20 emerged as a new potential antiviral compound endowed with a very good ability to inhibit flu RNA polymerase complex formation.

Experimental

Material and methods

Commercially available starting materials, reagents, and solvents were used as supplied. Compounds 4-phenylbut-3-en-2-one (4a), 1-phenyl-1,3-butanedione (5a), and phenyl-1-propenyl-ketone (9a) were purchased from Alfa Aesar and Apollo Scientific. Synthesis of 1-aryl-1,3-butanediones (5b-k) and 1-aryl-2-buten-1-ones (9b-i) was reported in the SI. Compound 5-amino-1,2,4-triazole-3-carboxylate (6a) was synthesized as reported in literature.\textsuperscript{17} Hydrolysis of compounds 8a and 9a to [1,2,4]triazolo[1,5-a]pyrimidine-2-carboxylic acid 16 and 17, respectively, was carried out as previously reported by us.\textsuperscript{3a}
All reactions were routinely monitored by TLC on silica gel 60F254 (Merck) and visualized by using UV or iodine. Flash column chromatography was performed on Merck silica gel 60 (mesh 230-400). After extraction, organic solutions were dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated with a Büchi rotary evaporator at reduced pressure. Yields are of purified product and were not optimized. HRMS spectra were registered on Agilent Technologies 6540 UHD Accurate Mass Q-TOF LC/MS, HPLC 1290 Infinity. Purities of compounds 12-16, 19, and 20 were determined by UHPLC on Agilent Technologies 6540 UHD Accurate Mass Q-TOF LC/MS, HPLC 1290 Infinity with DAD detector and evaluated to be 100% pure. HPLC conditions to assess the purity of final compounds were as follows: column, Phenomenex AERIS Widepore C4, 4.6mm × 100 mm (6.6 μm); flow rate, 0.85 mL/min; acquisition time, 10 min; DAD 254 nm; oven temperature, 30 °C; gradient of acetonitrile in water containing 0.1% of formic acid (0–100% in 10 min). $^1$H NMR and $^{13}$C NMR spectra were recorded on Bruker Avance DRX-400MHz using residual solvents such as dimethylsulfoxide (δ = 2.48) or chloroform (δ = 7.26) as an internal standard. Chemical shifts were recorded in ppm (δ) and the spectral data are consistent with the assigned structures. The spin multiplicities are indicated by the: symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and bs (broad singlet).

**General procedure for the synthesis of 2-amino-7-aryl-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidines (1a-k).** A mixture of the appropriate 1-aryl-1,3-butanedione (5a-k) (1 mmol) and 3a (1 mmol) in glacial acetic acid (2.5 mL) was refluxed until no starting material was detected by TLC (2-5h). After cooling, the reaction mixture was poured into ice/water and neutralized with 10% NaOH, obtaining a precipitate that was filtered and crystallized by EtOH/DMF.

**5-Methyl-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (1a).** White crystals. $^1$H NMR (400 MHz, DMSO-d$_6$) δ: 2.52 (s, 3H, CH$_3$), 6.32 (s, 2H, NH$_2$), 7.14 (s, 1H, H-6), 7.56-7.57 (m, 3H, aromatic CH), 8.10-8.12 (m, 2H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) δ: 24.1, 107.3, 128.4, 129.1, 130.3, 131.0, 143.8, 155.7, 161.6, 167.1; HRMS: m/z calcd for C$_{12}$H$_{11}$N$_5$ 226.1093 (M + H$^+$), found 226.1021 (M + H$^+$).

**5-Methyl-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (1b).** White crystals. $^1$H NMR (400 MHz, DMSO-d$_6$) δ: 2.38 and 2.51 (s, each 3H, CH$_3$), 6.32 (s, 2H, NH$_2$), 7.15 (s, 1H, H-6), 7.37 and 8.06 (d, J = 8.0 Hz, each 2H,
aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 21.1, 24.1, 106.9, 127.4, 129.0, 129.1, 141.2, 143.8, 155.8, 161.5, 167.1; HRMS: $m/z$ calcd for C$_{13}$H$_{12}$N$_5$ 240.1250 (M + H$^+$), found 240.1249 (M + H$^+$).

5-Methyl-7-(4-(methylthio)phenyl)[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (1c). Light yellow crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.51 and 2.54 (s, each 3H, CH$_3$), 6.32 (s, 2H, NH$_2$), 7.17 (s, 1H, H-6), 7.41 and 8.13 (d, $J = 8.5$ Hz, each 2H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 14.1, 24.1, 106.7, 124.9, 126.1, 129.5, 142.7, 143.3, 155.8, 161.5, 167.0; HRMS: $m/z$ calcd for C$_{13}$H$_{13}$N$_5$S 272.0971 (M + H$^+$), found 272.0968 (M + H$^+$).

7-(4-Bromophenyl)-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (1d). Light yellow crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.52 (s, 3H, CH$_3$), 6.35 (s, 2H, NH$_2$), 7.19 (s, 1H, H-6), 7.78 and 8.09 (d, $J = 8.5$ Hz, each 2H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 24.1, 107.3, 124.7, 129.5, 131.2, 131.5, 142.6, 155.7, 161.6, 167.1; HRMS: $m/z$ calcd for C$_{12}$H$_{10}$BrN$_5$ 304.0199 (M + H$^+$), found 304.0199 (M + H$^+$).

7-(2-Chlorophenyl)-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (1e). Light yellow powder. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.53 (s, 3H, CH$_3$), 6.31 (s, 2H, NH$_2$), 6.99 (s, 1H, H-6), 7.51-7.65 (m, 4H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 24.1, 109.0, 127.4, 129.5, 130.3, 131.4, 131.9, 132.1, 142.2, 154.9, 161.5, 167.2; HRMS: $m/z$ calcd for C$_{12}$H$_{10}$ClN$_5$ 260.0704 (M + H$^+$), found 260.0702 (M + H$^+$).

5-Methyl-7-(3-(trifluoromethyl)phenyl)[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (1f). Light yellow crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.54 (s, 3H, CH$_3$), 6.38 (bs, 2H, NH$_2$), 7.29 (s, 1H, H-6), 7.82 (t, $J = 7.8$ Hz, 1H, aromatic CH), 7.95 and 8.40 (d, $J = 7.8$ Hz, each 1H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 24.1, 107.9, 125.8 (q, $J_{CF} = 3.03$ Hz), 126.6 (q, $J_{CF} = 2.72.7$ Hz), 127.5 (q, $J_{CF} = 3.03$ Hz), 129.2 (d, $J_{CF} = 32.3$ Hz), 129.7, 131.4, 133.2, 142.2, 155.6, 161.8, 167.0; HRMS: $m/z$ calcd for C$_{13}$H$_{10}$F$_3$N$_5$ 294.0961 (M + H$^+$), found 294.0967 (M + H$^+$).

7-(3,4-Dimethoxyphenyl)-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (1g). Light yellow crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.50 (s, 3H, CH$_3$), 3.84 (s, 6H, OCH$_3$), 6.28 (s, 2H, NH$_2$), 7.11 (d, $J = 8.5$ Hz, 1H, aromatic CH), 7.20 (s, 1H, H-6), 7.77 (d, $J = 1.8$ Hz, 1H, aromatic CH), 7.90 (dd, $J = 1.8$ and 8.5 Hz, 1H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 24.1, 55.7, 55.7, 106.5, 111.2, 112.6, 122.3, 122.9, 143.6, 148.3, 151.1, 155.9, 161.3, 166.9; HRMS: $m/z$ calcd for C$_{14}$H$_{15}$O$_3$N$_5$ 286.1305 (M + H$^+$), found 286.1303 (M + H$^+$).
7-{3,4-Dichlorophenyl}-5-methyl-[1,2,4]triazolo[1,5-α]pyrimidin-2-amine (1h). Light yellow crystals. $^1$H NMR (400 MHz, DMSO-d$_6$) δ: 2.52 (s, 3H, CH$_3$), 6.41 (s, 2H, NH$_2$), 7.27 (s, 1H, H-6), 7.85 (d, $J$ = 8.5 Hz, 1H, aromatic CH), 8.12 (dd, $J$ = 2.0 and 8.5 Hz, 1H, aromatic CH) 8.47 (d, $J$ = 2.0 Hz, 1H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) δ: 24.1, 107.6, 129.3, 130.7, 131.7, 131.0, 131.3, 133.7, 141.1, 155.7, 161.7, 167.1; HRMS: m/z calcd for C$_{12}$H$_9$Cl$_2$N$_5$ 294.0314 (M + H$^+$), found 294.0315 (M + H$^+$).

5-Methyl-7-(naphthalen-1-yl)-[1,2,4]triazolo[1,5-α]pyrimidin-2-amine (1i). White powder. $^1$H NMR (400 MHz, DMSO-d$_6$) δ: 2.56 (s, 3H, CH$_3$), 6.17 (s, 2H, NH$_2$), 7.03 (s, 1H, H-6), 7.36 (d, $J$ = 8.3 Hz, 1H, naphthalene CH), 7.45-7.49 and 7.54-7.58 (m, each 1H, naphthalene CH), 7.62-7.69 (m, 2H, naphthalene CH), 8.03 and 8.12 (d, $J$ = 8.0 Hz, each 1H, naphthalene CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) δ: 24.3, 110.0, 125.2, 125.5 , 126.6, 127.3, 127.9, 128.5, 129.0, 129.8, 130.6, 133.0, 144.2, 155.1, 161.9, 167.1; HRMS: m/z calcd for C$_{16}$H$_{13}$N$_5$276.125 (M + H$^+$), found 276.1247 (M + H$^+$).

5-Methyl-7-(pyridin-4-yl)-[1,2,4]triazolo[1,5-α]pyrimidin-2-amine (1k). White crystals. $^1$H NMR (400 MHz, DMSO-d$_6$) δ: 2.54 (s, 3H, CH$_3$), 6.42 (s, 2H, NH$_2$), 7.30 (s, 1H, H-6), 8.10 and 8.80 (d, $J$ = 4.6 Hz, each 2H, pyridine CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) δ: 24.2, 107.7, 123.0, 137.7, 141.1, 150.1, 155.6, 161.8, 167.2; HRMS: m/z calcd for C$_{11}$H$_{10}$N$_6$ 227.1046 (M + H$^+$), found 227.1044 (M + H$^+$).

Ethyl 5-methyl-7-phenyl-[1,2,4]triazolo[1,5-α]pyrimidine-2-carboxylate (7a) and ethyl 7-methyl-5-phenyl-[1,2,4]triazolo[1,5-α]pyrimidine-2-carboxylate (8a). The title compounds were prepared through the general procedure for the synthesis of 2-amino-5-methyl-[1,2,4]triazolo[1,5-α]pyrimidines by replacing 3a with ethyl 5-amino-1,2,4-triazole-3-carboxylate (6a),$^{15}$ and were separated by flash chromatography eluting with CHCl$_3$/acetone (9:1). 7a: white solid (63% yield); $^1$H NMR (400 MHz, DMSO-d$_6$) δ: 1.31 (t, $J$ = 7.0 Hz, CH$_2$CH$_3$), 2.69 (s, 3H, CH$_3$), 4.38 (q, $J$ = 7.0 Hz, CH$_2$CH$_3$), 7.63-7.65 (m, 4H, H-6 and aromatic CH), 8.07-8.09 (m, 2H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) δ: 14.1, 24.8, 61.7, 112.2, 128.7, 129.3, 129.5, 131.7, 146.7, 155.6, 155.9, 160.0, 166.9; HRMS: m/z calcd for C$_{15}$H$_{14}$N$_4$O$_2$ 283.1196 (M + H$^+$), found 283.1196 (M + H$^+$); 8a: white solid (17% yield); $^1$H NMR (400 MHz, DMSO-d$_6$) δ: 1.35 (t, $J$ = 7.0 Hz, CH$_2$CH$_3$), 2.84 (s, 3H, CH$_3$), 4.41 (q, $J$ = 7.0 Hz, CH$_2$CH$_3$), 7.59-7.60 (m, 3H, aromatic CH), 8.10 (s, 1H, H-6), 8.25-8.27 (m, 2H, aromatic CH); $^{13}$C NMR
General procedure for the synthesis of 2-amino-5-aryl-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidines (2a-i). To a mixture of the appropriate 1-aryl-2-buten-1-ones (9a-i) (0.5 mmol) and 3a (1 mmol) in dry DMF (2.5 mL), dry Et$_3$N (0.5 mmol) was added and the reaction mixture was heated at 110 °C until no starting material was detected by TLC (2-3h). After cooling, the reaction mixture was poured into ice/water, obtaining a precipitate that was filtered and crystallized by EtOH/DMF.

**7-Methyl-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2a).** Light yellow crystals. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 2.64 (s, 3H, CH$_3$), 6.41 (s, 2H, NH$_2$), 7.49-7.54 (m, 3H, aromatic CH), 7.59 (s, 1H, H-6), 8.13-8.15 (m, 2H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$: 17.1, 104.7, 127.0, 128.9, 130.4, 136.7, 145.4, 155.1, 156.8, 167.5; HRMS: m/z calcd for C$_{12}$H$_{11}$N$_5$O 226.1093 (M + H$^+$), found 226.1088 (M + H$^+$).

**7-Methyl-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2b).** Light yellow crystals. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 2.36 and 2.63 (s, each 3H, CH$_3$), 6.38 (s, 2H, NH$_2$), 7.32 (d, $J = 6.0$ Hz, each 1H, aromatic CH), 7.55 (s, 1H, H-6), 8.05 (d, $J = 6.0$ Hz, each 1H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$: 17.4, 21.3, 104.7, 127.2, 129.8, 134.2, 140.6, 145.7, 155.4, 157.2, 167.7; HRMS: m/z calcd for C$_{13}$H$_{13}$N$_5$O 240.1250 (M + H$^+$), found 240.1251 (M + H$^+$).

**5-(4-Methoxyphenyl)-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2c).** Yellow crystals. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 2.62 (s, 3H, CH$_3$), 3.82 (s, 3H, OCH$_3$), 6.34 (s, 2H, NH$_2$), 7.06 (d, $J = 8.8$ Hz, 2H, aromatic CH), 7.51 (s, 1H, H-6), 8.11 (d, $J = 8.8$ Hz, 2H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$: 17.0, 55.4, 104.0, 114.0, 128.6, 129.0, 145.2, 155.1, 156.7, 161.2, 167.3; HRMS: m/z calcd for C$_{13}$H$_{13}$N$_5$O 256.1199 (M + H$^+$), found 256.1193 (M + H$^+$).

**7-Methyl-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2d).** Yellow crystals. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 2.66 (s, 3H, CH$_3$), 6.54 (s, 2H, NH$_2$), 7.71 (s, 1H, H-6), 8.33 and 8.38 (d, $J = 8.9$ Hz, each 2H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$: 17.3, 105.7, 124.2, 128.3, 142.7, 146.0, 148.4, 154.2, 155.1, 167.9; m/z calcd for C$_{12}$H$_{10}$N$_6$O$_2$ 271.0944 (M + H$^+$), found 271.0944 (M + H$^+$).
5-(4-Chlorophenyl)-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2e). Yellow crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.64 (s, 3H, CH$_3$), 6.45 (s, 2H, NH$_2$), 7.57-7.61 (m, 3H, aromatic CH and H-6), 8.17 (d, $J$ = 8.4 Hz, 2H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 17.1, 104.6, 128.8, 129.0, 135.2, 135.5, 145.6, 155.0, 155.4, 167.6; HRMS: $m/z$ calcd for C$_{12}$H$_{10}$ClN$_5$ 260.0704 (M + H$^+$), found 260.0681 (M + H$^+$).

5-(3-Bromophenyl)-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2f). Light brown crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.63 (s, 3H, CH$_3$), 6.42 (s, 2H, NH$_2$), 7.47 (t, $J$ = 7.8 Hz, 1H, aromatic CH), 7.61 (s, 1H, H-6), 7.67 and 8.12 (d, $J$ = 7.8 Hz, each 1H, aromatic CH), 8.29 (s, 1H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 17.3, 105.2, 122.6, 126.2, 129.7, 131.4, 133.3, 139.1, 146.1, 155.2, 155.4, 167.7; HRMS: $m/z$ calcd for C$_{12}$H$_{10}$BrN$_5$ 304.0199 (M + H$^+$), found 304.0194 (M + H$^+$).

5-(2-Fluorophenyl)-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2g). Yellow crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.66 (s, 3H, CH$_3$), 6.49 (s, 2H, NH$_2$), 7.33-7.51 (m, 3H, aromatic CH and H-6), 7.52-7.57 (m, 1H, aromatic CH), 7.97 (dt, $J$ = 1.5 and 7.8 Hz, 1H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 17.1, 108.2 (d, $J$ = 10.1 Hz), 116.5 (d, $J$ = 22.2 Hz), 125.0, 125.1 (d, $J$ = 22.2 Hz), 130.9 (d, $J$ = 3.0 Hz), 132.1 (d, $J$ = 8.0 Hz), 145.2, 153.4, 154.9, 160.0 (d, $J$ = 251.4 Hz), 167.6; HRMS: $m/z$ calcd for C$_{12}$H$_{10}$FN$_5$ 244.0999 (M + H$^+$), found 244.0997 (M + H$^+$).

5-(2,4-Difluorophenyl)-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2h). Light yellow crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.65 (s, 3H, CH$_3$), 6.42 (s, 2H, NH$_2$), 7.24-7.29 (m, 2H, aromatic CH), 7.31 (s, 1H, H-6), 7.41-7.47 and 8.02-8.08 (m, each 1H, aromatic CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 17.1, 104.9 (t, $J_{C-F}$ = 26.7 Hz), 107.9 (d, $J_{C-F}$ = 10.1 Hz), 112.4 (dd, $J_{C-F}$ = 3.0 and 21.2 Hz), 122.0 (dd, $J_{C-F}$ = 4.0 and 13.1 Hz), 132.4 (dd, $J_{C-F}$ = 4.0 and 11.1 Hz), 145.3, 152.5, 154.9, 160.3 (dd, $J_{C-F}$ = 13.1 and 251.4 Hz), 163.2 (dd, $J_{C-F}$ = 13.1 and 252.5 Hz), 167.3; HRMS: $m/z$ calcd for C$_{12}$H$_9$F$_2$N$_5$ 262.0905 (M + H$^+$), found 262.0899 (M + H$^+$).

7-Methyl-5-(pyridin-4-yl)-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (2i). Yellow crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.68 (s, 3H, CH$_3$), 6.56 (s, 2H, NH$_2$), 7.73 (s, 1H, H-6), 8.07 and 8.73 (dd, $J$ = 1.6 and 4.6 Hz, each 2H, pyridine CH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 17.2, 105.2, 120.9, 143.7, 145.9, 150.5, 154.0, 155.0, 167.8; HRMS: $m/z$ calcd for C$_{13}$H$_{10}$N$_6$ 227.1046 (M + H$^+$), found 277.1041 (M + H$^+$).
General procedure for the synthesis of compounds 12-16 by amidation. A solution of benzoyl chloride (2.0 mmol) in dry pyridine (5 mL) was added dropwise to a solution of the appropriate [1,2,4]triazolo[1,5-\(a\)]pyrimidine-2-amine (1e, 1f, 2c, 2d, or 2e) (1.0 mmol) in dry pyridine (15 mL), and then the reaction mixture was maintained at 80 °C overnight. After cooling, it was poured into ice/water, obtaining a precipitate that was filtered and purified as described below.

\(N\)-(7-(2-chlorophenyl)-5-methyl-[1,2,4]triazolo[1,5-\(a\)]pyrimidin-2-yl)benzamide (12). The title compound was prepared starting from 1e and purified by flash chromatography eluting with CH\(_2\)Cl\(_2\)/MeOH (98:2) in 71% yield as white solid. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 2.67 (s, 3H, CH\(_3\)), 7.34 (s, 1H, H-6), 7.46-7.50 and 7.57-7.58 (m, each 2H, aromatic CH), 7.64 (t, \(J = 7.2\) Hz, 1H, aromatic CH), 7.70-7.72 (m, 2H, aromatic CH), 7.96 (d, \(J = 7.5\) Hz, 2H, aromatic CH), 11.30 (s, 1H, NH); \(^13\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\): 24.6, 112.0, 127.5, 128.0, 128.3, 129.6, 129.7, 131.5, 132.1, 132.3, 132.3, 133.4, 143.9, 154.0, 160.1, 164.6, 164.8.; HRMS: \(m/z\) calcd for C\(_{19}\)H\(_{14}\)ClN\(_5\)O 364.0966 (M + H\(^+\)), found 364.0965 (M + H\(^+\)).

\(N\)-(5-methyl-7-(3-(trifluoromethyl)phenyl)-[1,2,4]triazolo[1,5-\(a\)]pyrimidin-2-yl)benzamide (13). The title compound was prepared starting from 1f and purified by flash chromatography eluting with CH\(_2\)Cl\(_2\)/MeOH (98:2) in 45% yield as white solid. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 2.67 (s, 3H, CH\(_3\)), 7.48-7.52 (m, 2H, aromatic CH), 7.57-7.61 (m, 1H, aromatic CH), 7.63 (s, 1H, H-6), 7.87 (t, \(J = 7.8\) Hz, 1H, aromatic CH), 7.99-8.01 (m, 3H, aromatic CH), 8.51 (d, \(J = 7.9\) Hz, 1H, aromatic CH), 8.59 (s, 1H, aromatic CH), 11.34 (s, 1H, NH); \(^13\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\): 24.6, 112.0, 127.5, 128.0, 128.3, 129.6, 129.7, 131.5, 132.1, 132.3, 132.3, 133.4, 143.9, 154.0, 160.1, 164.6, 164.8.; HRMS: \(m/z\) calcd for C\(_{20}\)H\(_{14}\)F\(_3\)N\(_5\)O 398.1229 (M + H\(^+\)), found 398.1227 (M + H\(^+\)).

\(N\)-(5-(4-methoxyphenyl)-7-methyl-[1,2,4]triazolo[1,5-\(a\)]pyrimidin-2-yl)benzamide (14). The title compound was prepared starting from 2c and purified by flash chromatography eluting with CH\(_2\)Cl\(_2\)/acetone (8:2) in 62% yield as white solid. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 2.78 and 3.84 (s, each 3H, CH\(_3\)), 7.11 (d, \(J = 8.8\) Hz, 2H), 7.50-7.54 (m, 2H, aromatic CH), 7.60 (d, \(J = 7.2\) Hz, 1H, aromatic CH), 7.84 (s, 1H, H-6), 8.02 (d, \(J = 7.4\) Hz, 2H, aromatic CH), 8.22 (d, \(J = 8.8\) Hz, 2H, aromatic CH), 11.34 (s, 1H, NH); \(^13\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\): 17.4,
55.8, 106.6, 114.8, 128.4, 128.8, 129.4, 132.5, 147.7, 154.5, 159.4, 160.5, 162.1, 165.2; HRMS: m/z calcd for C_{20}H_{17}N_{5}O_{2} 360.1461 (M + H^+), found 360.1460 (M + H^+).

*N-(7-methyl-5-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)benzamide* (15). The title compound was prepared starting from 2d and purified by flash chromatography eluting with CH_{2}Cl_{2}/acetone (8:2) in 64% yield as white solid. \(^1\)H NMR (400 MHz, DMSO-d_{6}) \(\delta\): 2.82 (s, 3H, CH\_3), 7.50-7.54 (m, 2H, aromatic CH), 7.60 (t, \(J = 7.2\) Hz, 1H, aromatic CH), 8.01-8.03 (m, 3H, H-6 and aromatic CH), 8.39 and 8.46 (d, \(J = 8.8\) Hz, each 2H aromatic CH), 11.47 (s, 1H, NH); \(^{13}\)C NMR (101 MHz, DMSO-d_{6}) \(\delta\): 17.2, 107.6, 124.1, 128.1, 128.4, 128.6, 132.2, 133.6, 142.0, 148.4, 148.7, 153.9, 156.7, 160.9, 164.8; HRMS: m/z calcd for C_{19}H_{14}N_{5}O_{3} 375.1206 (M + H^+), found 375.1201 (M + H^+).

*N-(5-(4-chlorophenyl)-7-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)benzamide* (16). The title compound was prepared starting from 2e and purified by flash chromatography eluting with CH_{2}Cl_{2}/acetone (8:2) in 52% yield as white solid. \(^1\)H NMR (400 MHz, DMSO-d_{6}) \(\delta\): 2.78 (s, 3H, CH\_3), 7.48-7.52 (m, 2H, aromatic CH), 7.57-7.63 (m, 3H, aromatic CH), 7.90 (s, 1H, H-6), 8.00 and 8.23 (d, \(J = 8.2\) Hz, each 2H, aromatic CH), 11.38 (s, 1H, NH); \(^{13}\)C NMR (101 MHz, DMSO-d_{6}) \(\delta\): 17.1, 106.8, 128.1, 128.4, 129.1, 129.1, 132.1, 133.6, 135.0, 136.0, 147.9, 153.9, 158.0, 160.5, 164.8; HRMS: m/z calcd for C_{19}H_{13}ClN_{5}O_{3} 366.1167 (M + H^+), found C_{19}H_{14}ClN_{5}O_{3} 364.0966 (M + H^+), found 364.0963 (M + H^+).

**General procedure for the synthesis of compounds 19 and 20 by amidation.** To a solution of the appropriate [1,2,4]triazolo[1,5-a]pyrimidine-2-carboxylic acid (17\(^{2a}\) or 18\(^{2a}\)) (2 mmol) in well dry CH_{2}Cl_{2} (20 mL), oxalyl chloride (12 mmol) was added and after 30 min dry DMF (2 drops) was added. After 2 h, the reaction mixture was evaporated to dryness to give a residue that was dissolved in well dry CH_{2}Cl_{2} and added of the appropriate aniline (2 mmol) and DIPEA (2 mmol). The reaction was maintained at rt until no starting material was detected by TLC (4h for 19 and 1h for 20). The work up of the reaction and compound purification are reported below.

*N-(2-carbamoylphenyl)-5-methyl-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-2-carboxamide* (19). The reaction mixture was evaporated to dryness and treated with ice/water obtaining a precipitate that was filtered and purified by flash chromatography eluting with CH_{2}Cl_{2}/MeOH (98:2), to give 19 in 52% yield; \(^1\)H-
NMR (DMSO-\textit{d}_6) \begin{align*}
\delta: 2.70 & \text{ (s, 3H, CH}_3\text{), 7.20 and 7.57 (t, } J = 7.5 \text{ Hz, each 1H, aromatic CH), 7.69-7.62 (m, 2H, aromatic CH), 8.25-8.19 (m, 2H, aromatic CH), 8.33 (bs, 1H, CONH}_2\text{), 8.66 (d, } J = 8.2 \text{ Hz, 1H, aromatic CH), 13.14 (s, 1H, NH);} \end{align*}

\begin{align*}
\text{13C NMR} (101 \text{ MHz, DMSO-\textit{d}_6}) \delta: 25.0, 111.9, 120.5, 120.8, 123.6, 128.9, 129.0, 129.4, 129.8, 132.0, 132.6, 138.7, 146.8, 155.8, 157.4, 158.6, 167.0, 170.6; \text{ HRMS: } m/z \text{ calcd for C}_{20}H_{16}N_6O_3 373.1414 (M + H}_+\text{), found 373.1411 (M + H}_+\text{).}
\end{align*}

\begin{align*}
N-(2\text{-carbamoylphenyl})-7\text{-methyl-5-phenyl-[1,2,4]triazolo[1,5-\text{a}]pyrimidine-2-carboxamide} \ (20). \end{align*}

The reaction mixture was filtered and the precipitate was washed with \text{Et}_2\text{O, and then purified by flash chromatography eluting with CH}_2\text{Cl}_2/\text{MeOH (98:2), to give 20 in 59% yield as white solid.} \begin{align*}
\text{1H NMR (400 MHz, DMSO-\textit{d}_6)} \delta: 2.88 & \text{ (s, 3H, CH}_3\text{), 7.22 (t, } J = 7.5 \text{ Hz, 1H, aromatic CH), 7.57-7.60 (m, 4H, aromatic CH), 7.78 (bs, 1H, CONH}_2\text{), 7.86 (d, } J = 7.8 \text{ Hz, 1H, aromatic CH), 8.10 (s, 1H, H-6), 8.38 – 8.25 (m, 3H, aromatic CH and CONH}_2\text{), 8.73 (d, } J = 8.3 \text{ Hz, 1H, aromatic CH), 13.16 (s, 1H, NH);} \end{align*}

\begin{align*}
\text{13C NMR} (101 \text{ MHz, DMSO-\textit{d}_6}) \delta: 17.0, 108.7, 120.4, 120.6, 123.3, 127.7, 128.7, 129.2, 131.6, 132.3, 135.8, 138.6, 149.1, 154.9, 157.2, 158.9, 161.2, 170.4; \text{ HRMS: } m/z \text{ calcd for C}_{20}H_{16}N_6O_3 373.1414 (M + H}_+\text{), found 373.1413 (M + H}_+\text{).}
\end{align*}

\textbf{Biological assays}

\textbf{Compounds and peptide.} RBV (1-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) was purchased from Roche. Each test compound was dissolved in 100\% DMSO. The PB1\textsubscript{1–15}–\text{Tat peptide was synthesized and purified by the Peptide Facility of CRI\textit{I} Biotechnology Center (University of Padua, Padua, Italy). This peptide corresponds to the first 15 amino acids of PB1 protein fused to a short sequence of HIV Tat protein (amino acids 47–59), which allows the delivery into the cell.\textsuperscript{18}

\textbf{Cells and virus.} Mardin-Darby canine kidney (MDCK) cells were grown in Dulbecco’s modified Eagle’s medium (DMEM, Life Biotechnologies) supplemented with 10\% (v/v) fetal bovine serum (FBS, Life Technologies) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin, Life Technologies). The cells were maintained at 37 \^\circ\text{C in a humidified atmosphere with 5\% CO}_2. Influenza virus strain A/PR/8/34 (H1N1, Cambridge lineage) was kindly provided by P. Digard (Roslin Institute, University of Edinburgh, United Kingdom).

\textbf{PA-PB1 interaction enzyme-linked immunosorbent assay (ELISA).} The PA–PB1 interaction was detected as described,\textsuperscript{3c} with some modifications.\textsuperscript{19} Briefly, 96-well microtiter plates (Nuova Aptca) were coated with 400
ng of 6His--PA_{239–716} for 3 h at 37 °C and then blocked with 2% BSA (Sigma) in PBS for 1 h at 37 °C. The 6His--PA_{239–716} protein was expressed in *E. coli* strain BL21(DE3)pLysS and purified as already described. After washing, 200 ng of GST-PB1_{1–25}, or of GST alone as a control, in the absence or the presence of test compounds at various concentrations, were incubated in serum-free DMEM O/N at room temperature as described. Escherichia coli-expressed, purified GST and GST-PB1_{1–25} proteins were obtained as previously described. After washing, the interaction between 6His--PA_{239–716} and GST-PB1_{1–25} was detected with a horseradish peroxidase-coupled anti-GST monoclonal antibody (GenScript) diluted 1:4,000 in PBS supplemented with 2% FBS. Following washes, the substrate 3,3′,5,5′tetramethylbenzidine (TMB, KPL) was added and absorbance was measured at 450 nm by an ELISA plate reader (Tecan Sunrise™). Values obtained from the samples treated with only DMSO were used to set as 100% of PA–PB1 interaction.

**Cytotoxicity assay.** Cytotoxicity of compounds was tested in MDCK cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method, as previously reported. Briefly, MDCK cells (seeded at density of 2 x 10^4 per well) were grown in 96-well plates for 24 h and then treated with serial dilutions of test compounds, or DMSO as a control, in DMEM supplemented with 10% FBS. After incubation at 37 °C for 48 h, 5 mg/mL of MTT (Sigma) in PBS was added into each well and incubated at 37 °C for further 4 h. Successively, a solubilization solution was added to lyse the cells and incubated O/N at 37 °C. Finally, optical density was read at the wavelength of 620 nm on a microtiter plate reader.

**Plaque reduction assay (PRA).** The antiviral activity of test compounds against influenza A virus was tested by PRA as previously described. MDCK cells were seeded at 5 x 10^5 cells/well into 12-well plates, and incubated at 37°C for 24 h. The following day, the culture medium was removed and the monolayers were first washed with serum-free DMEM and then infected with the flu A/PR/8/34 strain at 40 PFU/well in DMEM supplemented with 1 μg/mL of TPCK-treated trypsin (Worthington Biochemical Corporation) and 0.14% BSA and incubated for 1 h at 37 °C. The influenza virus infection was performed in the presence of different concentrations of test compounds or solvent (DMSO) as a control. After virus adsorption, DMEM containing 1 μg/mL of TPCK-treated trypsin, 0.14% BSA, 1.2% Avicel, and DMSO or test compounds was added to the
cells. At 48 h post-infection, cells were fixed with 4% formaldehyde and stained with 0.1% toluidine blue. Viral plaques were counted, and the mean plaque number in the DMSO-treated control was set at 100%.

Conflicts of interest
There are no conflicts of interest to declare.

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Notes and references


