Scaffold Morphing Approach To Expand the Toolbox of Broad-Spectrum Antivirals Blocking Dengue/Zika Replication

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S Supporting Information

ABSTRACT: We have recently discovered a family of 2,6-diaminopurine derivatives acting as DENV inhibitors by targeting an allosteric pocket on the thumb of the viral NS5 polymerase. Although the following target-based optimization allowed conversion of the hits into broad-spectrum DENV/ZIKV inhibitors, no improvement of the antiviral potency was reached. Herein, we applied a phenotypic scaffold-morphing approach to explore additional biologically relevant chemical space around the original hits by converting the flat purine derivatives into more complex chemotypes characterized by a higher degree of saturation. A new microwave-assisted one-pot three-step protocol was also developed to quickly generate chemotypes 6 and 7. Cell-based phenotypic screening allowed identification of promising ant flaviviral agents belonging to different chemotypes. Compound 9d emerged as the most promising broad-spectrum antiviral, being 6 times more potent than ribavirin (RBV) against DENV and 3 times more potent than 7-deaza-2'-C-methyladenosine (7DMA) against ZIKV with good selectivity indexes (>46 and >41, respectively).

KEYWORDS: Scaffold-morphing, furo[3,4-d]pyrimidin-7(5H)-one, broad-spectrum antivirals, dengue, zika

Viral infections still represent a major public health threat due to social and demographic changes in the past 50 years and the consequent spread of pathogens, hosts, vectors, and/or commodities. While viral diseases, which initially affected developing countries, are now spreading worldwide, the available antiviral arsenal is still limited and ineffective against many of these new and re-emerging viruses.¹ The risk of large outbreaks of emerging flaviviruses such as dengue virus (DENV), Zika virus (ZIKV) and West Nile virus (WNV) is thus a global concern since no approved drugs or fully protective vaccines are available yet.² An additional problem is represented by the increased risk of coinfection with different flaviviruses (e.g., DENV, ZIKV, WNV), which may cocirculate within the same vector. This may induce complicated immune responses in infected individuals toward viruses or host proteins (e.g., Guillain–Barré syndrome), thereby exacerbating the course of the disease.³ Although drug-repurposing can serve as a patch to quickly contain the spread of some viruses,^{4,5} there is a growing need for new potent antivirals with a broad-spectrum antiviral activity.

A number of viral and host targets are currently under investigation for the development of new broad-spectrum ant flaviviral agents.⁶ Among the viral targets, NS5 polymerase is one of the most promising and exploited targets, being highly conserved among flaviviruses. A few repurposed

nucleoside derivatives (e.g., RBV and 7DMA) and targeted non-nucleoside inhibitors, both targeting NS5, have been employed to inhibit viral replication (Figure 1).^{7,8} However, the underperformance of target-based approaches in the discovery of innovative drugs should be a reminder that complex cellular functions and physiopathological processes cannot always be deconstructed and easily simplified by individual biological targets to be modulated by drug candidates.⁹ Measurement of the disease-relevant phenotypic response is therefore considered a more successful strategy in identifying new first-in-class drugs and must be supported by new chemical entities (NCEs) with high chemical diversity.^{10,11}

As part of our recent efforts to find innovative ant flaviviral candidates, we initially applied a target-based approach to convert purine-based Src kinase inhibitors (e.g., UPF-2198) into multitarget compounds (e.g., MR-85) (Figure 1). This class of compounds inhibited DENV replication by interacting with an allosteric pocket on the thumb of NS5.¹² In a following

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Figure 1. Repurposed nucleoside inhibitors of NSS polymerase, selected 2,6-diaminopurine hits, and key scaffold I for morphing.

study, we exploited the similarity of the highly conserved allosteric pockets on the DENV and ZIKV NSS thumb to generate new derivatives (e.g., MR-187) that block the replication of both ZIKV and all 4 serotypes of DENV (DENV 1–4).¹³ Although the broad-spectrum activity of compound MR-187 was quite promising, its potency was comparable to that of the original hits and its solubility profile was suboptimal due to the highly conjugated planar structure. Alternatively, hit expansion from MR-85 revealed that, depending on the decoration of the purine core, a hydrophilic morpholine substituent can be introduced at position C2 (MR-186) with only moderate loss of anti-DENV activity (Figure 1). In addition, moving away from the highly rigid and planar 2,6-diaminopurine system may lead to more soluble and potent drug-like candidates.¹⁴

Herein, we envision a scaffold morphing approach as the best strategy to replace the purine core and introduce more flexibility into the final molecules while retaining the key pharmacophore moieties from previous target-based studies. We selected the 2,4-diaminofuro[3,4-*d*]pyrimidin-7(5H)-one **1** (Figure 1) as key scaffold for the morphing approach since it allows expansion and exploration of the biologically relevant chemical space by exploiting the chemical versatility of the lactone ring. Only two papers on the 2,4-diaminofuro[3,4-*d*]pyrimidin-7(5H)-one scaffold have been reported so far in the literature, probably due to the tricky chemical reactivity of this molecule. Britikova et al. were the first to report this scaffold in 1966, although the published structures were not conclusively demonstrated.¹⁵ In 2012, Cantin et al. from AstraZeneca reported the exploitation of this scaffold, resulting

in selective P2X3 antagonists for the treatment of chronic pain.¹⁶ To further explore the versatility of scaffold **1**, we converted the commercial orotic acid **1** into 1,5-dihydrofuro[3,4-*d*]pyrimidine-2,4,7(3H)-trione **2** by treating it with paraformaldehyde in the presence of hydrochloridric acid (Scheme 1). The latter compound was then chlorinated with

Scheme 1^a

^aReagents and conditions: (a) paraformaldehyde, HCl conc, 95 °C, 20 h; (b) R^1PhNH_2 , DIPEA, POCl₃, reflux, 6 h; (c) Et₃N, n-BuOH, 85 °C, 2 h (for **4a**); n-BuOH, r.t., 8 h (for **4b**).

POCl₃ in the presence of DIPEA to give the key intermediate **3** with good overall yield. Extensive studies were conducted to analyze the reactivity of this intermediate toward different nucleophilic amines. The first substitution with primary amines occurs at position C4 and needed heating only in the case of less nucleophilic amines (e.g., p-sulfanilamide), resulting in compounds **4a,b** with good yields. Reaction of **4a,b** with a second amine proves to be quite tricky and may occur at C2 or on the lactone depending on the temperature and amine used. The reaction of **4a** with benzylamine at room temperature gives rise to **5a** with high yields (Scheme 2). The same reaction can also be conducted with morpholine, opening the lactone ring in shorter times (data not shown). Reaction of **5a** with a third amine allowed the introduction of the morpholine moiety at C2 after moderate heating using microwave irradiation. Compound **6a** thus obtained belongs to a first class of scaffold-morphed compounds endowed with a pyrimidine chemotype. The latter compound can be further converted into a second class of scaffold-morphed compounds (5H-pyrrolo[3,4-*d*]pyrimidin-7(6H)-one chemotype) by heating at 170 °C in a sealed tube using microwave irradiation in the presence of TFA, leading to the γ -lactam derivative **7a** in 12% yield. This poor conversion is in line with previous reports on similar compounds.¹⁶ As an alternative synthetic sequence, the morpholine can also be introduced at C2 of intermediates **4a,b**. To avoid any side reactions on the lactone ring, amines must be added dropwise to the preheated reaction mixture to obtain compounds **8a,b** with nearly quantitative yields (Scheme 2). The latter compounds belong to a third class of scaffold-morphed compounds endowed with a furo[3,4-*d*]pyrimidin-7(5H)-one chemotype. Compounds **8a,b** were then used as a starting point to introduce more chemical diversity by having the lactone ring react with nucleophilic amines, as described earlier. However, the lactone moiety of **8a** appeared less reactive than that of **4a**, requiring heating in the

Scheme 2^a

^aReagents and conditions: (a) benzylamine, *n*-BuOH, r.t., 2 days; (b) morpholine, *n*-pentanol, 100 °C, μ W, 10 min; (c) *n*-pentanol, TFA, μ W, 170 °C, 10 min; (d) pre-heated *n*-BuOH, 110 °C, *p*-sulfanilamide (for **8a**) or 3-aminophenol (for **8b**), 2 h; (e) R²NH₂, TFA, *n*-pentanol, reflux, overnight; (f) R²NH₂, TFA, DME, μ W, 170 °C, 10 min.

Scheme 3^a

^aReagents and conditions: (a) R²NH₂, *n*-pentanol, μ W, 80 °C, 15 min; (b) morpholine, *n*-pentanol μ W, 100 °C, 10 min; (c) TFA, μ W, 170 °C, 10 min.

presence of TFA to react with benzylamine. Surprisingly, this reaction did not yield the expected product **6a**, but the related analogue **9a** (pyrimidine chemotype) after amine-mediated opening of the lactone followed by reaction with the solvent in the presence of TFA. This reaction protocol was thus exploited to generate a small collection of highly functionalized derivatives (**9a–e**) by treating **8a,b** with different amines. Alternatively, using DME as solvent and heating in a sealed tube under microwave irradiation at 170 °C in the presence of TFA, side reactions with the alcoholic solvent are avoided and compounds **8a,b** can be converted into the respective γ -lactams **7a–d** with moderate yields.

Next, based on the acquired knowledge on the reactivity of the furo[3,4-*d*]pyrimidin-7(5H)-one scaffold, we aimed at developing a microwave-assisted one-pot procedure to quickly generate functionalized analogues of **6a** and **7a** (Scheme 3). The synthetic sequence that led to these compounds was thoroughly investigated step-by-step to adapt the reaction conditions to a multistep one-pot sequence. In the optimized

protocol, compound **4b** and a primary amine (R²NH₂) were suspended in *n*-pentanol and heated at 80 °C for 15 min in a microwave tube. Morpholine was then added to the reaction mixture (containing intermediates **5**) and irradiated in a microwave at 100 °C for 10 min. The resulting mixture (containing compounds **6**) was divided in two equal portions. To one portion, TFA was added and the reaction was heated at 170 °C for 10 min using a microwave, yielding compounds **7b,e–g** (18–32% yields over three steps). The second portion was purified to obtain compounds **6b–e** (50–65% yields over two steps).

All synthesized compounds were initially evaluated for their ability to inhibit DENV serotype 2 (DENV-2) replication in a virus yield reduction assay using ribavirin (RBV) as reference compound (Table 1). Hit compounds reported in Figure 1 were also included in Table 1 for comparative purposes. The most promising compounds identified from the DENV inhibition screen were subsequently evaluated for their ability to inhibit ZIKV replication, using 7-deaza-2'-C-methyladenosine

Table 1. Inhibitory Effect on DENV/ZIKV Replication

Cpds	DENV-2		ZIKV	
	EC ₅₀ ^a	CC ₅₀ ^b	EC ₅₀ ^a	CC ₅₀ ^b
6a	>100	>100	ND ^c	ND
6b	>115	>115	ND	ND
6c	20.7 (3.1) ^d	64.5	ND	ND
6d	62.1 (1.2)	73.3	ND	ND
6e	>107	>107	ND	ND
7a	21.6 ± 2.6 ^e (>4.8)	>104	>104	>104
7b	100	>120	ND	ND
7c	>97.9	>97.9	ND	ND
7d	12.2 (>8)	>97.9	>97.9	>97.9
7e	70.7	>116	ND	ND
7f	20.6 (>4.9)	>100	ND	ND
7g	>112	>112	ND	ND
8a	>128	>128	ND	ND
8b	>128	>128	ND	ND
9a	1.0 ± 0.7 (>88)	>87.9	>87.9	>87.9
9b	20.7 (1.9)	39.4	57.8 ± 35.2 (>1.7)	>98.9
9c	>83.5	>83.5	ND	ND
9d	1.82 (>46)	>83.5	2.04 (>41)	>83.5
9e	10.9 ± 8.4 (3)	32.8 ± 19.4	13.9 ± 4.0 (3.5)	48.2 ± 35.1
MR-187	10.0 ± 1.9 (6.9)	69.4 ± 12.5	16.7 ± 10.2 (>7.4)	>124
MR-186	40.6 (3)	122	ND	ND
UPF-2198	NA ^f	373	ND	ND
MR-85	7.4 ± 0.7 (>24)	>175	ND	ND
RBV ^g	11.9 ± 4.0 (>34)	>409	ND	ND
7DMA ^h	ND	ND	5.7 ± 2.2 (>64)	>357

^aEC₅₀ values were generated using the virus-yield reduction assay in Vero-B cells (for DENV-2) and in Vero E6 cells (for ZIKV). ^bCC₅₀ values were assessed by the MTS method. ^cND = not determined. ^dSelectivity index: CC₅₀/EC₅₀. ^eValues are the mean of at least three independent experiments. ^fNA = not active. ^gRibavirin. ^h7-Deaza-2'-C-methyladenosine.

sine (7DMA) as reference compound. While derivatives with a furo[3,4-*d*]pyrimidin-7(SH)-one chemotype (**8**) were completely inactive, only a few compounds endowed with a 5H-pyrrolo[3,4-*d*]pyrimidin-7(6H)-one chemotype (**7a,d,f**) exhibited moderate inhibition of DENV replication. The most interesting results were obtained from compounds endowed with a pyrimidine chemotype (**6** and **9**). Within this family, the functionalization in position C5 proved to be quite important for the biological activity: the presence of a free OH group bound to this position through a methylene spacer generally resulted in poorly active (**6c,d**) or inactive compounds (**6a,b,e**). On the other hand, derivatives with the C5 OH group masked as pentyl ether (**9a–e**) were all active against DENV, with the only exception of compound **9c**. Compound **9a** was 12 times more potent than RBV against DENV with a good selectivity index (SI > 88). Compounds **9d,e** inhibited DENV and ZIKV replication with the same efficacy, thus acting as dual DENV/ZIKV inhibitors. Compound **9e** showed a dual DENV/ZIKV activity but a low SI (3 and 3.5, respectively), indicating that this compound may not be selective. Compound **9d** was 6 times more potent than RBV against DENV and 3 times more potent than 7DMA against ZIKV with good SI (>46 and >41, respectively).

Finally, to get an overview on the quality of synthesized inhibitors compared to that of previous reported hits and reference drugs, key molecular descriptors of compounds reported in Table 1 were computed using Datawarrior (Figure 2).¹⁷ Fsp3 was calculated as the number of sp3 hybridized carbons/total carbon count and plotted against the total molecular weight for each molecule.¹⁸ Fsp3 has been shown to

increase as compounds progress to clinical testing and is directly correlated with their molecular complexity and solubility. This increase in compounds' saturation allows in fact exploration of additional biologically relevant chemical space while escaping from the poorly soluble "flatland". Shape index estimates the 3D shape of compounds: values greater than 0.5 suggest the presence of flat scaffolds values lower than 0.5 are suggestive for spherical 3D scaffolds.¹⁹ As shown in Figure 2A, our scaffold-morphing approach allowed conversion of flat purine hits MR-85, -186, 187 (shape index >0.5) into less flat derivatives (**6**, **7**, **9**) showing comparable or increased anti-DENV potencies and more saturated chemotypes (color coded in Figure 2A). As shown in Figure 2B, only derivatives **9** were able to inhibit ZIKV replication, showing a much improved fraction sp3 (Fsp3) and shape index with respect to the active purine hit (MR-187).

Overall, the pyrimidine chemotype **9** represents the best candidate to develop broad-spectrum antinflavivirus agents after a focused optimization to further increase its potency and ADME properties.

In summary, we used a phenotypic scaffold-morphing strategy to discover improved analogues of the flat purine antinflavirals previously identified by our group. A thorough investigation of the furo[3,4-*d*]pyrimidin-7(SH)-one reactivity allowed expansion of the original hits into a collection of new derivatives characterized by three different chemotypes: (i) 5H-pyrrolo[3,4-*d*]pyrimidin-7(6H)-one (**7**); (ii) furo[3,4-*d*]pyrimidin-7(SH)-one (**8**); and (iii) 2,4,5,6-tetrasubstituted pyridines (**6** and **9**). These new compounds were characterized by a higher degree of saturation and a higher molecular

Figure 2. Scatter plot of Fsp3 versus molecular weight. The color and size of the circles represent DENV/ZIKV activity (panels A/B) and shape index, respectively. Chemotypes are color coded, and the most active compounds are numbered. NA/ND = not active/not determined.

complexity, thus allowing exploration of additional biologically relevant chemical space regions. A new microwave-assisted one-pot three-step protocol was also developed to quickly generate functionalized chemotypes **6** and **7**. Phenotypic screening on DENV/ZIKV infected cells allowed identification of promising anti-DENV compounds within derivatives **6**, **7**, and **9**, while only compounds **9** gave access to dual DENV/ZIKV inhibitors. In particular, **9d** represents a promising candidate endowed with low micromolar activity against both DENV and ZIKV, low shape index, and a good Fsp3. Based on the present study, lead optimization strategies are currently ongoing in our lab and will be published in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00583.

Procedures for compound syntheses, and biological evaluations (PDF)

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The authors declare no competing financial interest.

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■ ABBREVIATIONS

DENV, Dengue virus; ZIKV, Zika virus; NSS, Nonstructural protein 5; WNV, West Nile virus; RBV, Ribavirin; 7DMA, 7-Deaza-2'-C-methyladenosine; NCEs, new chemical entities; DIPEA, *N,N*-diisopropylethylamine; TFA, trifluoroacetic acid; μ W, microwaves; DME, dimethoxyethane; SI, selectivity index

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