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Identification of [1,2,3]Triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones as Novel Inhibitors of Chikungunya Virus Replication

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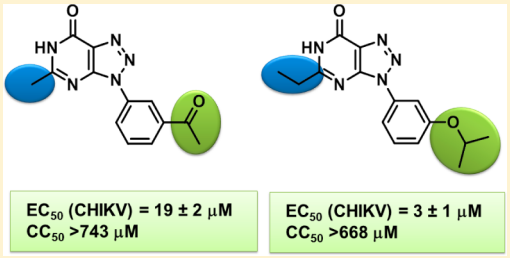
Supporting Information

ABSTRACT: Chikungunya virus (CHIKV) is a re-emerging Alphavirus that is transmitted to humans by *Aedes* mosquitoes. Currently, there are still no drugs or vaccines available for the treatment or prevention of this disease. Although traditionally restricted to Africa and Asia, the adaptation of the virus to *Aedes albopictus*, a mosquito species with an almost worldwide distribution, has contributed to the geographical spread of this virus in the past decade. Here, we report on a new family of compounds named [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones that inhibit CHIKV replication in the low micromolar range with no toxicity to the host (Vero) cells. The most potent compound in this series has an EC_{50} value below 1 μM with no cytotoxicity detected up to 668 μM, therefore affording a selectivity index greater than 600. Interestingly, the compounds have little or no antiviral activity on the replication of other members of the Togaviridae family. The exploration and study of this class of selective inhibitors of CHIKV replication will contribute to deeper insights into the CHIKV life cycle and may be a first step toward the development of a clinical drug candidate.



INTRODUCTION

Chikungunya virus (CHIKV) is an insect-borne viral disease that was first described during an outbreak in southern Tanzania in 1952.[1] Taxonomically, CHIKV is a RNA virus of the Alphavirus genus of the Togaviridae family and belongs more specifically to the Semliki Forest virus (SFV) complex. CHIKV is transmitted to humans by virus-carrying *Aedes* mosquitoes. CHIKV infection causes an illness with symptoms that are similar to those of dengue fever: first, there is an acute febrile phase that lasts for two to five days, followed by a prolonged arthralgic disease that affects the joints of the extremities.[2] Most patients fully recover, but in 10% of the cases, joint pain may persist for several months or even years. Occasional cases of eye, neurological, and heart complications have been reported, as well as gastrointestinal complaints.[3,4]

During the past 50 years, several re-emergences of CHIKV have occurred in Africa and Asia. Starting in February 2005, a major outbreak of CHIKV infections occurred in the islands of the Indian Ocean, more in particular in the French territory La Réunion. There were 270 000 estimated cases, nearly half of the island population, and 237 reported deaths.[5] Subsequently, this epidemic spread to other regions of India and Southeast Asia, and transmission was reported for the first time in Europe, in a localized outbreak in northeastern Italy.[6] Cases have now been reported in more than 40 countries.[7] Interestingly, these outbreaks have been related to the ability of the virus to adapt to a new vector, *Aedes albopictus*.[8,9] As this mosquito species has a wider distribution than the traditional vector *Aedes aegypti*, this could contribute to a broader spread of the virus. In December 2013, the first autochthonous CHIKV cases were confirmed on the Caribbean island of St. Martin. Since then, CHIKV has been and is currently still spreading to neighboring countries,[10] and it is also possible that CHIKV will be able to spread to the American mainland in the near future.

There are no specific drugs to prevent or cure the chikungunya infection. Treatment is primarily directed at relieving the symptoms, more in particular the joint pain by administration of corticosteroids or NSAIDs. Although researchers have recently reported on the development of a new candidate vaccine to protect against CHIKV infection,[11] there is still no approved commercial CHIKV vaccine available.

Up to now, only a few examples of selective inhibitors of CHIKV replication have been described.[12−18] Therefore, there is still an unmet need for new classes of potent and selective inhibitors of CHIKV replication.[19] To this end, a joint screening program was set up between our laboratories to identify such novel compounds. The selective antiviral activity of a structurally diverse collection of chemical compounds from CSIC (Madrid) was evaluated for selective antiviral activity in a

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virus-cell-based assay for CHIKV replication at KU Leuven. One of the chemical samples, coded TP274, portrayed an antiviral activity/cytotoxicity profile that matched our hits selection criteria, which are protection of the host cells from any signs of virus-induced effects without eliciting a significant adverse effect on cell or monolayer morphology and cell metabolism (Figure 1). Chemical validation of this hit sample

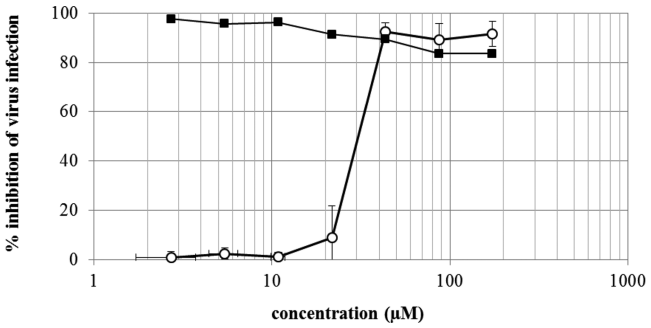


Figure 1. Dose–response curve (in μM) obtained for the chemical sample coded TP274 as identified in a general screening. The anti-CHIKV activity is represented in white circles while the cytotoxic/cytostatic effect is represented in black squares.

by high-performance liquid chromatography–mass spectrometry (HPLC-MS) analysis revealed a 1:1 mixture of two compounds. Both entities were separated by chromatography, structurally characterized, and individually evaluated for anti-CHIKV activity. This revealed that the chloro compound **1**[20] was inactive while the 7-oxo derivative **2** (Figure 2) showed the antiviral activity.

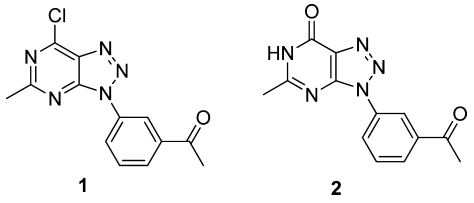


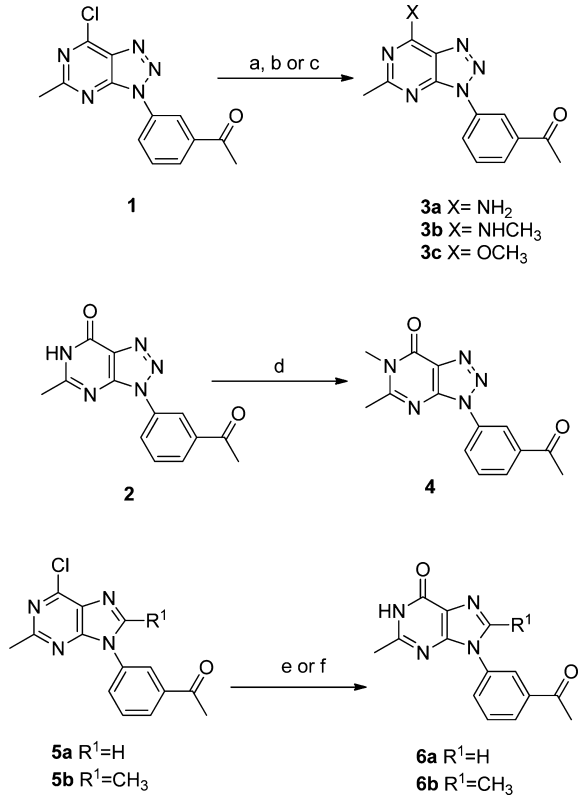
Figure 2. Compounds present in the sample coded TP274.

On the basis of the good activity/toxicity profile of compound **2**, its low molecular weight, and the clear need for new compounds that selectively inhibit CHIKV replication, a synthetic program was initiated to prepare structural analogues and to explore the structure–activity relationship of this compound class in more detail. The synthesis and exploration of the relationship between the compound structure and anti-CHIKV activity is reported here in detail.

RESULTS AND DISCUSSION

Chemistry. On the basis of the structure of compound **2**, our first series of modifications was meant to evaluate the impact on the antiviral activity of small substituents at positions 6 and/or 7 of the [1,2,3]triazolo[4,5-*d*]pyrimidine or replacement of this heterocyclic base by a purine analogue (Scheme 1) while keeping a 3-acetyl substituent at the aryl ring. Thus, reaction of the 7-chloro derivative **1**[20] with ammonia or methylamine in MeOH afforded the 7-amino and 7-methylamino derivatives (**3a** and **3b**, respectively) in good yields. Similarly, treatment of **1** with MeONa in MeOH led to the 7-OMe derivative **3c** in 61% yield. Reaction of **2** with DBU

Scheme 1. Synthesis of Triazolopyrimidines and Analogues[a]



[a]Reagents and conditions: (a) NH_3/MeOH, MW, 70 °C, 30 min (**3a**, 66% yield); (b) $MeNH_2$/MeOH, rt, 2 h (**3b**, 67% yield); (c) MeONa/MeOH, MW, 100 °C, 20 min (**3c**, 61% yield); (d) MeI, DBU, DMA, rt, 16 h (**4**, 86% yield); (e) CH_3COONa, DMF, MW, 120 °C, 1 h (**6a**, 62% yield); (f) HCl/dioxane, MW, 100 °C, 2 h (**6b**, 61% yield).

and MeI in *N*,*N*-dimethylacetamide afforded exclusively the 6-methyl derivative **4** (86% yield). Finally, reaction of the 6-chloropurine **5a**[21] with sodium acetate in DMF afforded the hypoxanthine derivative **6a** (62% yield). Reaction of the 6-chloro-8-methyl purine (**5b**)[21] with 1 N HCl in dioxane yielded the purinone derivative **6b** (61% yield).

The evaluation of this first series of compounds in the virus-cell-based assay (see the Antiviral Activity section) revealed that all these modifications have a deleterious effect on the antiviral activity. Therefore, the next series of compounds kept the [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one as the heterocyclic base and focused on exploring different substituents at the aryl ring. To this end, the approach employed for the synthesis of compound **1** was used, incorporating a final hydrolysis step from the 7-chloro to the keto form, as shown in Scheme 2. Reaction of an equimolar amount of differently substituted anilines (**7**) with 4,6-dichloro-5-aminopyrimidines (**8**) in isobutanol in the presence of HCl at 150 °C for 10 min under microwave irradiation afforded the 4-chloro-5,6-diaminopyrimidines **9a–m** in good to excellent yields, that in many cases were isolated by filtration.[20−22] Then, reaction of **9a–m** with $NaNO_2$ in CH_2Cl_2 in the presence of HCl at room temperature for 30 min afforded the triazolo derivatives **10a–m.** Finally, treatment of **10a–m** with sodium acetate in DMF afforded the [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones **11a–m**. Saponification of the ethyl ester **11m** afforded the carboxylic acid **11n**.

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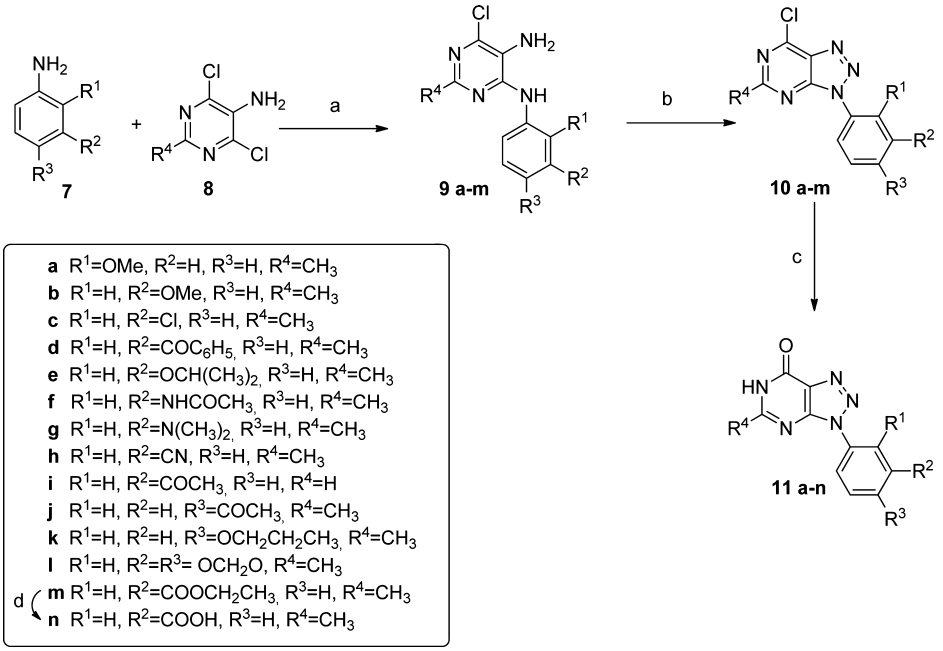
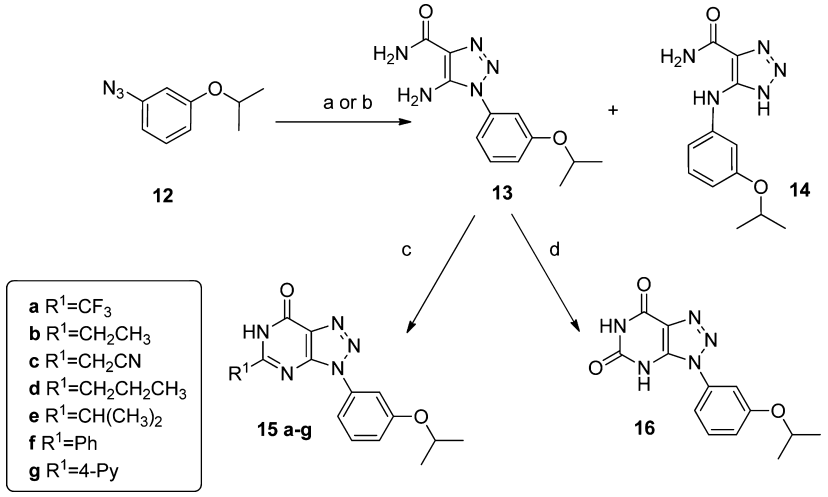
Scheme 2. Triazolopyrimidin-7-ones Differently Substituted at the Aryl Ring[a]



[a]Reagents and conditions: (a) isobutyl alcohol, HCl (cat), MW, 150 °C, 10 min to 1 h (71−98% yield); (b) $NaNO_2$, HCl 1 N/DCM (1:1), rt, 30 min (56−75% yield); (c) CH_3COONa, DMF, MW, 120 °C, 1 h (50−83% yield); (d) KOH 20%, DMF, MW, 100 °C, 30 min (92% yield).

Scheme 3. Synthesis of Triazolopyrimidones from Aminocarboxamides[a]



[a]Reagents and conditions: (a) cyanoacetamide, EtONa, EtOH, reflux, 2 h (**13**, 15% yield and **14**, 68% yield); (b) cyanoacetamide, NaH, DMF, 0 °C, 30 min, then addition of **12** in DMF, rt, 1 h (**13**, 70% yield); (c) R^1COOEt, EtONa, refluxing EtOH, 1−8 h (8−79% yield); (d) *N,N′*-diisopropylcarbodiimide, 90 °C, 16 h (56% yield).

The biological evaluation of compounds **11a−n** showed a number of compounds with antiviral activity, the best of which was the 3-isopropoxyderivative **11e** with an EC_{50} value 1.4-fold better than that of the acetyl derivative **2**. Therefore, the next series of modifications were based on compound **11e**.

The construction of the [1,2,3]triazolo[4,5-*d*]pyrimidine starting from 4,6-dichloro-5-aminopyrimidines, as shown in Scheme 2, has been very convenient for exploring substituents at the aryl ring or even at positions 6 and/or 7 of the heterocyclic base but has serious limitations to incorporate different substituents at position 5 of the heterocycle, since the nature of this substituent is determined by the substituent at position 2 in the parent pyrimidine. A more reasonable pathway to introduce diversity at position 5 of the triazolo[4,5-*d*]pyrimidine could be devised based on a ring-closing reaction performed on 5-amino-1,2,3-triazolo-4-carboxamides with different esters (Scheme 3). The required 5-amino-1,2,3-triazolo-4-carboxamides could be obtained by reaction of arylazides with cyanoacetamide in basic media.[23] This approach has been extensively explored in the literature for 1-benzyl derivatives, but the described examples of 1-aryl derivatives are much scarcer.[24−27] Thus, reaction of 1-azido-3-isopropoxybenzene (**12**, Scheme 3)[28] with cyanoacetamide in the presence of EtONa in refluxing EtOH afforded a mixture of two isomeric compounds, whose ^1H NMR spectra showed significant differences affecting the NH protons. In particular, the minor

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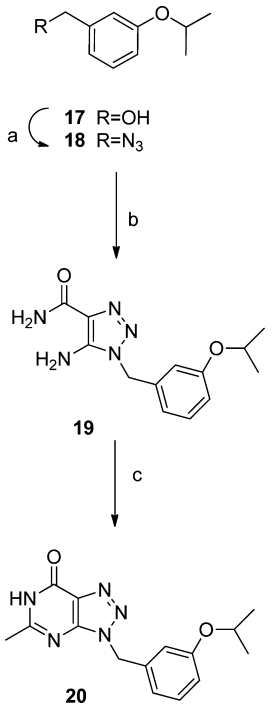
product showed a unique signal at 6.37 ppm corresponding to two protons, while the major isomer showed two well-defined signals at 14.67 and 6.55 ppm, both of them corresponding to a single proton. These compounds were assigned as the expected 5-aminotriazol derivative **13** (15% yield) and the corresponding Dimroth isomer **14** (68%) arising by Dimroth rearrangement of the internal N-aryl group to the more thermodynamically stable exocyclic position.[24,29]

Mechanistic considerations on similar analogues suggest the Dimroth rearrangement is favored by the use of protic solvents.[30] Thus, reaction of **12** with a preformed solution of cyanoacetamide and NaH in DMF afforded the 5-aminotriazol **13** in 70% yield with no detection of the Dimroth isomer. Finally, reaction of **13** with different esters in the presence of EtOH/EtONa afforded a variety of 5-substituted triazolo[4,5-*d*]pyrimidines (**15a−g**) (Scheme 3). It should be mentioned that, under these cyclization conditions, the Dimroth rearrangement was a competing reaction, so the quicker the cyclization, the lower the formation of the Dimroth isomer. In this way, different linear or branched alkyl or aromatic substituents were incorporated at position 5 of the heterocycle. In addition, reaction of the 5-aminotriazol **13** with 1,1′-carbonyldiimidazole in DMF at 90 °C afforded the triazolopyrimidin-5,7-dione **16** in 56% yield.

A similar synthetic scheme was used to prepare a benzylic analogue of compound **11e** incorporating a methylene unit between the aryl ring and the [1,2,3]triazolo[4,5-*d*]-pyrimidinone as shown in Scheme 4. The starting azide **18** was obtained in 89% yield in two steps by reaction of the benzylic alcohol **17** with mesyl chloride and further substitution reaction of the mesyl derivative thus formed with NaN_3.

Scheme 4. Synthesis of the Benzylic Derivative 20[a]



[a]Reagents and conditions: (a) MsCl, DMAP, DMF, rt, 5 h, then addition of NaN_3, rt, 4 h (89% yield); (b) cyanoacetamide, NaH, DMF, 0 °C, 1 h, then addition of **18**, DMF, rt, 1 h (77% yield); (c) CH_3COOEt, EtONa, refluxing EtOH, 8 h (75% yield).

Treatment of **18** with cyanoacetamide in DMF in the presence of NaH afforded the 5-amino-1*H*-1,2,3-triazole-4-carboxamide **19** as the unique compound in 77% yield. Finally, reaction of **19** with ethyl acetate in the presence of sodium ethoxide led to the benzylic derivative **20** in 75% yield.

Antiviral Activity. The compounds were evaluated for their potential inhibitory effect on the in vitro replication of CHIKV in Vero cells (Table 1). Chloroquine was included as a reference compound.[13] The antiviral activity is expressed as the 50% effective concentration (EC_{50}) and the 90% effective concentration (EC_{90}), which is indicative of the concentration of compound that is required to inhibit the virus-induced cytopathic effect on the host cell by 50% and 90%, respectively. The overall antimetabolic effect of the compounds, indicative for the adverse effect on noninfected host cells (combined cytotoxic, cytostatic, and antimetabolic effect), is expressed as

Table 1. Antiviral Evaluation of the Triazolopyrimidines and Analogues against CHIKV in Vero Cells[a]

compd	EC_{50} (μM)[b]	EC_{90} (μM)[c]	CC_{50} (μM)[d]
1	>174	>174	>174
2	19 ± 2	38 ± 16	>743
3a	225 ± 33	309 ± 48	>746
3b	>443	>443	514 ± 55
3c	>441	>441	495 ± 34
4	>441	>441	>706
6a	127 ± 10	161 ± 27	491
6b	>440	>440	>703
11a	348 ± 36	460 ± 13	>777
11b	28 ± 6	179 ± 44	>777
11c	32 ± 11	235 ± 7	>764
11d	23 ± 6	47	>604
11e	12 ± 4	156 ± 43	>704
11f	318	425	206 ± 78
11g	>370	>370	538
11h	131 ± 11	187 ± 21	>793
11i	>490	>490	>784
11j	326 ± 53	>743	>743
11k	169	>701	594 ± 100
11l	>461	>461	>737
11m	202 ± 53	331 ± 64	322
11n	>399	>399	>638
15a	68 ± 4	>147	104 ± 32
15b	3 ± 1	18 ± 18	>668
15c	115 ± 16	137	215 ± 63
15d	>399	>399	>638
15e	280	>638	>638
15f	204 ± 72	>360	277 ± 128
15g	75 ± 19	>144	82 ± 22
16	>435	>435	>696
20	167 ± 10	232 ± 62	>872
chloroquine	11 ± 7	21 ± 18	89 ± 28

[a]All data are mean values ± standard deviation for at least three independent experiments. [b]50% effective concentration or calculated concentration of compound that is required to protect 50% of the cells against cytopathic effects caused by the viral infection. [c]90% effective concentration or calculated concentration of compound that is required to protect 90% of the cells against cytopathic effects caused by the viral infection. [d]50% cytotoxic concentration or calculated concentration of compound that reduces the overall cell metabolic activity (by a combined cytotoxic, cytostatic, and antimetabolic effect) to 50%.

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CC_{50} and is the calculated concentration of compound that reduces the measured metabolic activity of compound-treated cells by 50%. The evaluation of the first series of compounds (**3a−c**, **4**, **6a**, **6b**) revealed that a carbonyl was required at position 7 of the heterocyclic base to elicit antiviral activity, as shown for compound **2**. Those compounds with a NH_2, NHMe, or OMe at position 7 (compounds **3a−c**, respectively) were significantly less active or inactive. Also, the NH at position 6 should be unsubstituted according to the lack of activity of the N-Me derivative **4**. When the triazolopyrimidine in compound **2** is replaced by the analogous imidazopyrimidine (compound **6a**), some antiviral activity could be observed but at EC_{50} values 7-fold higher than that of compound **2**. If, in addition, a methyl group is incorporated at position 8 of the purine (compound **6b**), the antiviral activity is lost.

Regarding the substituents at the aryl ring (compounds **11a−n**), the requirements for antiviral activity are also quite strict. In particular, those compounds with a methoxy (**11b**), a Cl (**11c**), a benzoyl (**11d**), or an isopropoxy (**11e**) at position 3, together with the acetyl of the hit compound **2**, produced the best inhibitory activity. However, when the methoxy substituent is at position 2, the EC_{50} is more than 10-fold higher than when the methoxy is at position 3 (compound **11a** vs **11b**). Additionally, moving the acetyl group from position 3 to position 4 (compound **2** vs **11j**) results in a 17-fold decrease in antiviral efficacy. Therefore, these data stress the importance of the substituent on the aryl ring at position 3. The best EC_{50} values are obtained when this substituent is an isopropoxy (compound **11e**). Interestingly, compound **11i**, with an acetyl at position 3 at the aryl ring but a hydrogen at position 5 of the base, is inactive, indicating the importance of this position of the heterocyclic base in the antiviral activity. This became more obvious with the evaluation of compounds **15a−g** and **16**. For this series, some compounds showed antiviral activity (**15a−c**, **f**, or **g**), although in several cases, this was accompanied by some cytotoxicity according to the CC_{50} values. Interestingly, compound **15b** with an ethyl substituent at position 5 showed very interesting EC_{50} and EC_{90} values, with no cytotoxicity up to 668 μM. Thus, compound **15b** with a selectivity index of 222, is the most potent and selective compound among these triazolopyrimidines and has a better profile than the reference compound chloroquine. Finally, the benzyl derivative **20** was at least 9-fold less potent than its corresponding aryl analogue **2**.

The hit compound **2** and the most potent compound of this series **15b** were evaluated for selective antiviral activity against several CHIKV clinical isolates (Table 2), including the new strain from St. Martin that is currently responsible for an epidemy in the West Indies. On average, compound **15b** was 10 times more active than the original hit compound **2**, regardless of the clinical strain tested, with EC_{50} values in the low micromolar range and a best value of 0.75 μM for CHIK Congo. Interestingly, both compounds were significantly more active by a factor of 4 against the African Congo strain than against the four other strains. Interestingly, antiviral activity was also found for compounds **2** and particularly **15b** against the recently emerged St. Martin strain (Caribbean 2013). No cytotoxicity up to 300 μM was observed in Vero E6 cells using the same experimental settings as the antiviral assay.

In the virus test panel, also other Togaviridae family members such as the Sindbis virus and Semliki Forest virus were included, but no or only marginal antiviral activity was detected. Similarly, a broad-spectrum evaluation of the compound did not reveal any selective antiviral activity against a selection of other viruses (i.e., Coxsackievirus B3 e.a.; data not shown).

Table 2. Antiviral Evaluation of the Triazolopyrimidine 2 and 15b against Different Laboratory Strains and Clinical Isolates of CHIKV, SFV, and SINV in Vero Cells

		compd	
		2	**15b**
species	virus (strain)	EC_{50} (μM)[a]	EC_{50} (μM)[a]
Chikungunya	899 (lab)	19 ± 2	2.6 ± 1
	LR2006-OPY1 (lab)	25	2.6 ± 0.5
	Venturini (Italy 2008)	26 ± 2	1.4 ± 0.01
	Congo 95 (2011)	6.4 ± 0.05	0.75 ± 0.4
	St. Martin (2013)	24 ± 0.5	2.9 ± 0.05
Semliki forest virus	Vietnam (lab)	>464	219
Sindbis virus	HRsp (lab)	>464	69 ± 8

[a]50% effective concentration or calculated concentration of compound that is required to protect 50% of the cells against cytopathic effects caused by the viral infection.

The triazolopyrimidines that were the subject of this study are confirmed to be highly selective inhibitors of CHIKV replication and not of related and unrelated viruses, which points toward a mechanism of action that involves a highly specific target in the CHIKV life cycle.

CONCLUSIONS

Here, we report that several 3-aryl-[1,2,3]triazolo[4,5-*d*]-pyrimidin-7(6*H*)-ones are potent and selective inhibitors of CHIKV replication. These compounds are easily accessible in two to three synthetic steps using two complementary approaches: (1) starting from 6-chloropyrimidine-4,5-diamines, treatment with sodium nitrite and hydrolysis of the 7-chloro to the keto form; or (2) starting from arylazides that react with cyanoacetamide followed by a ring-closing reaction with different esters. The structural requirements for anti-CHIKV activity have been clearly established both at the level of the heterocyclic base and the aryl substitution. On the basis of these modifications, compound **15b** was identified as a potent and selective inhibitor of CHIKV replication with a better profile than the reference compound chloroquine and a selectivity index greater than 200. Interestingly, this class of compounds specifically targets CHIKV among the members of viruses of the Togaviridae family. Further optimization of the antiviral activity is ongoing, and mechanism of action studies are being carried out to identity the molecular target of this novel family of anti-CHIKV compounds.

EXPERIMENTAL SECTION

Chemistry Procedures. Melting points were obtained on a Reichert-Jung Kofler apparatus and are uncorrected. The elemental analysis was performed with a Heraeus CHN-O-RAPID instrument. The elemental compositions of the compounds agreed to within ±0.4% of the calculated values. For all the tested compounds, satisfactory elemental analysis was obtained supporting greater than 95% purity. Electrospray mass spectra were measured on a quadrupole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC/MS HP 1100). Compounds containing a Cl showed the typical Cl isotopic pattern in the MS spectra. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INNOVA 300 operating at 299 MHz (^{1}H) and 75 MHz (^{13}C), respectively, and a Varian INNOVA-400 operating at 399 MHz (^{1}H) and 99 MHz (^{13}C), respectively.

Analytical TLC was performed on silica gel 60 F_{254} (Merck) precoated plates (0.2 mm). Spots were detected under UV light (254

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nm) and/or charring with ninhydrin. Separations on silica gel were performed by preparative centrifugal circular thin-layer chromatography (CCTLC) on a Chromatotron® (Kiesegel 60 PF_{254} gipshaltig (Merck)), with layer thicknesses of 1 and 2 mm and flow rates of 4 or 8 mL/min, respectively. Flash column chromatography was performed with silica gel 60 (230−400 mesh) (Merck).

Microwave reactions were performed using the Biotage Initiator 2.0 single-mode cavity instrument from Biotage (Uppsala). Experiments were carried out in sealed microwave process vials utilizing the standard absorbance level (400 W maximum power). The temperature was measured with an IR sensor on the outside of the reaction vessel.

HPLC-MS Analysis of the Sample TP274. The sample coded TP274 was analyzed through a HPLC Waters 2695 instrument connected to a Waters Micromass ZQ spectrometer and a photodiode array detector. The column used was a Sunfire C-18 (150 mm × 19 mm × 5 μm), and the flow rate was 1 mL/min. Solvents used were CH_3CN for bottle A and H_2O (0.1% HCOOH) for bottle B. The gradient used was from 10% A to 100% A in 10 min. HPLC-MS analysis revealed a 1:1 mixture of two compounds: a peak at 3.52 min with *m/z* 287.6 and Cl isotopic pattern (compound **1**), and a second peak at 5.04 min with *m/z* 269.1 (compound **2**).

*3-(3′-Acetylphenyl)-5-methyl-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (**2**).* A microwave vial was charged with **1**[20] (80 mg, 0.29 mmol) and sodium acetate (70 mg, 0.88 mmol) in anhydrous DMF (1.3 mL) and was irradiated at 120 °C for 1 h. After work-up, the residue was purified by CCTLC in the Chromatothron (dichloromethane/methanol, 30:1) to yield 65 mg (83%) of **2** as a yellow solid. mp 257−259 °C. MS (ES, positive mode): *m/z* 270 $(M + H)^+$. ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.45 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 7.83−8.56 (m, 4H, Ar), 12.79 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 22.1 (CH_3), 27.4 (CH_3), 121.7, 126.9, 129.2 (Ar), 129.3 (C-7a), 136.1, 138.4, 130.8 (Ar), 149.3 (C-3a), 156.2 (C-5), 161.3 (C-7), 197.5 (CO). Anal. ($C_{13}H_{11}N_5O_2$): C, H, N.

*3-(3′-Acetylphenyl)-7-amino-5-methyl-3H-[1,2,3]-triazolo[4,5-d]-pyrimidin-7(6H)-one (**3a**).* A microwave vial containing **1**[20] (100 mg, 0.34 mmol) in a solution of NH_3 in methanol (2.0 M, 4 mL) was sealed and heated in a microwave reactor at 70 °C for 30 min. After cooling, the resulting precipitate was filtered and purified by flash column chromatography (dichloromethane/methanol) to yield 60 mg (66%) of **3a** as a solid. mp: 286−287 °C. MS (ES, positive mode): *m/z* 269 $(M + H)^+$. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.50 (s, 3H, CH_3), 2.69 (s, 3H, $COCH_3$), 7.82 (pt, *J* = 7.9 Hz, 1H, H-5′), 8.10 (d, *J* = 7.8 Hz, 1H, H-6′), 8.17 (s, 1H, NH), 8.45 (d, *J* = 8.1 Hz, 1H, H-4′), 8.50 (s, 1H, NH), 8.70 (s, 1H, H-2′). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 26.7 (CH_3), 27.6 ($CO\underline{C}H_3$), 120.9, 123.8, 126.1 (Ar), 128.6 (C-7a), 130.9, 137.1,138.6 (Ar), 150.3 (C-3a), 156.7 (C-7), 168.0 (C-5), 197.9 (CO). Anal. ($C_{13}H_{12}N_6O$): C, H, N.

*3-(3′-Acetylphenyl)-5-methyl-7-methylamino-3H-[1,2,3]-triazolo-[4,5-d]pyrimidin-7(6H)-one (**3b**).* A solution of **1**[20] (100 mg, 0.34 mmol) in methylamine in methanol (2.0 M, 10 mL) was stirred at room temperature for 2 h. After volatiles removal, the residue was purified by flash column chromatography (dichloromethane/methanol) to yield 64 mg (67%) of **3b** as a solid. mp: 220−221 °C. MS (ES, positive mode): *m/z* 283 $(M + H)^+$. ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.55 (s, 3H, CH_3), 2.68 (s, 3H, $COCH_3$), 3.05 (d, *J* = 4.6 Hz, 3H, $NHC\underline{H}_3$), 7.82 (pt, *J* = 7.9 Hz, 1H, H-5′), 8.10 (d, *J* = 7.8 Hz, 1H, H-6′), 8.45 (d, *J* = 8.0 Hz, 1H, H-4′), 8.70 (s, 1H, H-2′), 8.92 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 27.2 (CH_3), 27.6 ($CO\underline{C}H_3$), 27.8 ($NHCH_3$), 120.9, 124.4. 126.1 (Ar), 128.6 (C-7a), 130.9, 137.1, 138.6 (Ar), 150.3 (C-3a), 156.7 (C-7), 168.0 (C-5), 197.9 (CO). Anal. ($C_{14}H_{14}N_6O$): C, H, N.

*3-(3′-Acetylphenyl)-7-methoxy-5-methyl-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-7(6H)-one (**3c**).* A microwave vial was charged with **1**[20] (100 mg, 0.32 mmol) and sodium methoxide (86 mg, 1.59 mmol) in methanol (4 mL) and was irradiated at 100 °C for 20 min. After volatiles removal, the residue was purified by CCTLC in the Chromatothron (dichloromethane/methanol, 30:1) to yield 55 mg (61%) of **3c** as a white solid. mp: 158−160 °C. EM (ES, positive mode): *m/z* 284 $(M + H)^+$. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.69 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 4.23 (s, 3H, OCH_3), 7.86−8.67 (m, 4H, Ar). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 26.8 (CH_3), 27.6 (CH_3), 55.6 (OCH_3), 125.2, 121.4, 126.6 (Ar), 129.3 (C-7a), 131.1, 136.5, 138.7 (Ar), 151.6 (C-3a), 161.6 (C-7), 168.0 (C-5), 197.8 (CO). Anal. ($C_{14}H_{13}N_5O_2$): C, H, N.

*3-(3′-Acetylphenyl)-5,6-dimethyl-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7(6H)-one (**4**).* To a flask charged with **2** (90 mg, 0.33 mmol) in *N*,*N*-dimethylacetamide (2 mL), iodomethane (27 μL, 0.43 mmol) and DBU (66 μL, 0.44 mmol) were added. The reaction was stirred at room temperature overnight under argon atmosphere. Then, a hexane/diethyl ether (1:1) mixture (10 mL) was added, and the resultant suspension was cooled at −20 °C. The precipitate was dissolved in dichloromethane (20 mL) and washed with saturated aqueous $NaHCO_3$ (15 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (dichloromethane/methanol) to yield 80 mg (86%) of **4** as a yellow solid. mp: 166−168 °C. EM (ES, positive mode): *m/z* 284 $(M + H)^+$. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.66 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 3.57 (s, 3H, CH_3), 7.80−8.57 (m, 4H, Ar). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.2 (CH_3), 26.9 (CH_3), 30.8 (CH_3), 121.0, 126.1, 128.1 (Ar), 128.6 (C-7a), 130.4, 135.6, 138.0 (Ar), 146.8 (C-3a), 155.5 (C-5), 162.1 (C-7), 197.1 (CO). Anal. ($C_{14}H_{13}N_5O_2$): C, H, N.

*9-(3′-Acetylphenyl)-2-methyl-1H-purin-6(9H)-one (**6a**).* To a solution of **5a**[21] (154 mg, 0.54 mmol) in methanol (10.0 mL), sodium methoxide (3.0 mmol) and 2-mercaptoethanol were added. The reaction was heated in a microwave reactor at 100 °C for 90 min. After cooling, the product was isolated by filtration and dried to obtain 89 mg (62%) of **6a** as a yellowish solid. mp: 286−287 °C. MS (ES, positive mode): *m/z* 269 $(M + H)^+$. ^1H NMR (300 MHz, DMSO-d_6): δ 2.22 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 7.67 (pt, *J* = 7.8 Hz, 1H, H-5′), 7.91 (d, *J* = 7.8 Hz, 1H, H-6′), 8.18 (m, 2H, H-2′, H-4′), 8.46 (s, 1H, H-8). Anal. ($C_{14}H_{12}N_4O_2$): C, H, N.

*9-(3-Acetylphenyl)-2,8-dimethyl-1H-purin-6(9H)-one (**6b**).* To a solution of **6b**[21] (63 mg, 0.20 mmol) in dioxane (5.0 mL), a 1 N HCl aqueous solution was added. The reaction was heated in a microwave reactor at 100 °C for 2 h. The mixture was extracted with ethyl acetate (15 mL), and the organic layer was washed with a saturated aqueous Na_2CO_3 solution (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The residue was purified by flash column chromatography (dichloromethane/methanol) to yield 35 mg (61%) of **6b** as a solid. mp: 282−283 °C. MS (ES, positive mode): *m/z* 283 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 7.74 (m, 2H, H-5′, H-6′), 8.01 (dd, *J* = 2.4, 1.1 Hz, 1H, H-2′), 8.10 (ddd, *J* = 5.0, 3.5, 1.7 Hz, 1H, H-4′). Anal. ($C_{15}H_{14}N_4O_2$): C, H, N.

General Procedure for the Reaction of 4,6-Dichloropyrimidines with Substituted Anilines (9a−m). A microwave vial was charged with the corresponding aniline (**7**, 1.0 mmol), the 4,6-dichloropyrimidine (**8**, 1.0 mmol), isobutanol (2.5 mL), and 37% aqueous HCl (0.07 mL/mmol). The reaction vessel was sealed and heated in a microwave reactor at 150 °C for 10−30 min. After cooling, the reaction mixture was worked up as indicated in each case.

*6-Chloro-N⁴-(2′-methoxyphenyl)-2-methylpyrimidine-4,5-diamine (**9a**).* Following the general procedure, a microwave vial was charged with 5-amino-4,6-dichloro-2-methylpyrimidine (200 mg, 1.12 mmol), 2-methoxyaniline (126 μL, 1.12 mmol), isobutanol (2.8 mL), and 37% aqueous HCl (84 μL). After cooling, the product was isolated by filtration and dried to obtain 291 mg (98%) of **9a** as a beige solid. mp: 241−243 °C. MS (ES, positive mode): *m/z* 265 $(M + H)^+$. ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.27 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.58 (s, 2H, NH_2), 6.96 (m, H-6′), 7.14 (m, 2H, H-4′, H-5′), 7.75 (m, 1H, H-3′), 8.68 (s, 1H, NH).

The synthesis of compounds **9b−m** was performed following a similar procedure, and all details and analytical and spectroscopic data are included in the Supporting Information.

General Procedure for the Synthesis of 7-Chloro-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidines (10a−m) Starting from 4,5-Diaminopyrimidines. To a suspension of the corresponding 4,5-diaminopyrimidine (**9a−m**, 1.00 mmol) in dichloromethane (3.5 mL/mmol), $NaNO_2$ (1.05 mmol) and 1 N HCl (3.5 mL/mmol) were

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added. The mixture was stirred at room temperature for 30 min. The reaction was diluted with dichloromethane. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated to dryness.

*7-Chloro-3-(2′-methoxyphenyl)-5-methyl-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**10a**).* Following the general procedure, to a suspension of **9a** (296 mg, 1.12 mmol) in dichloromethane (3.9 mL), $NaNO_2$ (81 mg, 1.17 mmol) and 1 N HCl (3.9 mL) were added. After work-up, 233 mg (75%) of **10a** were obtained as a pale brown solid. mp: 156−158 °C. MS (ES, positive mode): m/z 276 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.34 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 7.15 (ddd, J = 7.6, 1.7, 1.1 Hz, 1H, H-5′), 7.32 (dd, J = 8.5, 1.1 Hz, 1H, H-3′), 7.47 (dd, J = 7.8, 1.7 Hz, 1H, H-6′), 7.62 (ddd, J = 8.4, 7.5, 1.7 Hz, 1H, H-4′).

The synthesis of compounds **10b−m** was performed following a similar procedure, and all details and analytical and spectroscopic data are included in the Supporting Information.

General Procedure for the Synthesis of [1,2,3]Triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (11a−m). To a solution of the corresponding 7-chloro-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine (**10**) (1.00 mmol) in anhydrous DMF (4.5 mL/mmol), sodium acetate (3.00 mmol) was added. The reaction was microwave-irradiated at 120 °C for 1 h. The mixture was dissolved in dichloromethane and washed with brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified as indicated in each case.

*3-(2′-Methoxyphenyl)-5-methyl-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7(6H)-one (**11a**).* Following the general procedure, to a solution of **10a** (170 mg, 0.62 mmol) in anhydrous DMF (2.8 mL), sodium acetate (154 mg, 1.85 mmol) was added. After work-up, the residue was purified by precipitation with dichloromethane/hexane to yield 79 mg (50%) of **11a** as a beige solid. mp: 235−237 °C. MS (ES, positive mode): m/z 258 $(M + H)^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.34 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 7.16 (m, 1H, H-5′), 7.33 (d, J = 8.5, 1H, H-3′), 7.48 (dd, J = 7.8, 1.3 Hz, 1H, H-4′), 7.63 (m, 1H, H-6′), 12.63 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.8 (CH_3), 56.5 (OCH_3), 113.5, 121.1, 123.3, 127.9 (Ar), 129.2 (C-7a), 132.7 (Ar), 150.8 (C-3a), 154.9 (Ar), 156.3 (C-5), 160.7 (C-7). Anal. ($C_{12}H_{11}N_5O_2$): C, H, N.

The synthesis of compounds **11b−m** was performed following a similar procedure, and all details and analytical and spectroscopic data are included in the Supporting Information.

*5-Amino-1-(3-isopropoxyphenyl)-1H-1,2,3-triazole-4-carboxamide (**13**). Procedure A.* To a solution of Na (71 mg, 3.08 mmol) in ethanol (3 mL), cyanoacetamide (130 mg, 1.54 mmol) was slowly added for 30 min. This was followed by the addition of the azide **12**[28] (273 mg, 1.54 mmol) and reflux for 2 h. The mixture was concentrated to dryness. The crude obtained was dissolved in water (10 mL), acidified by addition of acetic acid, and extracted with ethyl acetate (2 × 10 mL). The combined organic phases were washed with a solution of $NaHCO_3$ and brine, dried on Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography (hexane/ethyl acetate, 1:1). The fastest moving fractions afforded 274 mg (68%) of **14** as a yellow solid while the slowest fractions afforded 60 mg (15%) of **13**. Data for ((3′-isopropoxyphenyl)amino)-1*H*-1,2,3-triazole-4-carboxamide (**14**): mp: 212−213 °C. MS (ES, positive mode): m/z 262 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (d, J = 6.0 Hz, 6H, $CH(C\underline{H}_3)_2$), 4.56 (sept, J = 6.0 Hz, 1H, CH), 6.42 (d, J = 7.7 Hz, 1H, H-4′), 6.96 (d, J = 7.7 Hz, 1H, H-6′), 7.13 (t, J = 8.1 Hz, 1H, H-5′), 7.21 (s 1H, H-2′), 7.53 (s, 1H, NH-amide), 7.86 (s, 1H, NH-amide), 8.53 (s, 1H, NH-amine), 14.67 (s, 1H, NH-triazol). Anal. Calcd. for ($C_{12}H_{15}N_5O_2$): C, H, N. Data for **13**: mp: 123−125 °C. MS (ES, positive mode): m/z 262 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.29 (d, J = 6.0 Hz, 6H, $CH(C\underline{H}_3)_2$), 4.70 (hept, J = 5.7 Hz, 1H, CH), 6.37 (s, 2H, NH_2), 7.08 (m, 3H, H-2′, H-4′, H-6′), 7.26 (s, 1H, $CONH_2$), 7.47 (pt, J = 7.9 Hz, 1H, H-5′), 7.62 (s, 1H, $CONH_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.4 (CH_3), 70.4 (CH), 111.6, 116.5, 116.9 (Ar), 122.3 (C-4), 131.3, 136.6 (Ar), 145.3 (C-5), 158.9 (Ar), 165.0 (CO). Anal. ($C_{12}H_{15}N_5O_2$): C, H, N.

Procedure B. To a suspension of NaH (60% in mineral oil, 152 mg, 6.24 mmol) in anhydrous DMF (5.9 mL), a solution of cyanoacetamide (326 mg, 3.88 mmol) in anhydrous DMF (5.9 mL) was added at 0 °C. After 1 h, a solution of **12**[28] (624 mg, 3.52 mmol) in anhydrous DMF (5.9 mL) was slowly added and stirring was continued at room temperature for 1 h. Volatiles were removed. The residue obtained was purified by flash column chromatography (dichloromethane/methanol, 20:1) to yield 641 mg (70%) of **13** as a yellowish solid.

General Procedure for the Synthesis of [1,2,3]Triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (15a−g) from the 5-Amino-1*H*-1,2,3-triazole-4-carboxamide 13. To a solution of Na (5.00 mmol) in ethanol (10 mL/mmol), the carboxamide **13** (1.00 mmol) and the appropriate ester (4.00 mmol) were added. The reaction mixture was heated at reflux for 1−8 h. After cooling, the reaction was concentrated to dryness. The residue was dissolved in water and acidified by addition of acetic acid, and the precipitate thus formed was isolated by filtration and purified as specified.

*5-Ethyl-3-(3′-isopropoxyphenyl)-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7(6H)-one (**15b**).* Following the general procedure, to a solution of Na (44 mg, 1.90 mmol) in ethanol (3.8 mL), **13** (100 mg, 0.38 mmol) and ethyl propionate (176 μL, 1.52 mmol) were added. The reaction mixture was refluxed for 8 h. After work-up, a precipitate was obtained that was purified by CCTLC (dichloromethane/methanol, 20:1) to yield **15b** (60 mg, 53%) as a white solid. mp: 242−244 °C. MS (ES, positive mode): m/z 300 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.23 (t, J = 7.5 Hz, 3H, CH_3), 1.31 (d, J = 6.0 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.71 (q, J = 7.5 Hz, 2H, CH_2), 4.69 (hept, J = 6.0 Hz, 1H, CH), 7.06 (d, J = 8.0, 1H, H-4′), 7.51 (pt, J = 8.1 Hz, 1H, H-5′), 7.58 (d, J = 8.0 Hz, 1H, H-6′), 7.63 (s, 1H, H-2′), 12.70 (s 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.5 (CH_3), 22.1 ($CH(\underline{C}H_3)_2$), 28.1 (CH_2), 70.3 (CH), 109.2, 114.0, 116.6 (Ar), 129.4 (C-7a), 131.1, 136.9 (Ar), 149.1 (C-3a), 156.3 (C-7), 158.5 (Ar), 165.0 (C-5). Anal. ($C_{15}H_{17}N_5O_2$): C, H, N.

The synthesis of compounds **15a**, **c−g** was performed following a similar procedure, and all details and analytical and spectroscopic data are included in the Supporting Information.

*3-(3′-Isopropoxyphenyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7-(6H,4H)-dione (**16**).* To a solution of **13** (120 mg, 0.50 mmol) in DMF (2.5 mL), 1,1′-carbonyldiimidazole (89 mg, 0.55 mmol) was added. The mixture was stirred at 90 °C overnight. Then, it was concentrated to dryness, and the residue was suspended in acetone. The precipitate thus formed was filtered to yield 81 mg (56%) of **16** as a white solid. mp: 286−288 °C. MS (ES, positive mode): m/z 288 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (d, J = 6.0 Hz, 6H, $CH(C\underline{H}_3)_2$), 4.63 (hept, J = 6.1 Hz, 1H, CH), 6.97 (m, 1H, H-4′), 7.31 (s, 1H, H-2′), 7.40 (m, 2H, H-5′, H-6′), 8.34 (s, 1H, N^4H),10.63 (s, 1H, N^6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2 (CH_3), 70.1 (CH), 110.0, 115.3, 116.2 (Ar), 121.0 (C-7a), 130.8 (Ar), 135.2 (C-3a), 136.5 (Ar), 154.3 (C-5), 157.5 (C-7), 158.3 (Ar). Anal. ($C_{13}H_{13}N_5O_3$): C, H, N.

*1-(Azidomethyl)-3-isopropoxybenzene (**18**).* To a solution of **17** (185 mg, 1.11 mmol) in anhydrous DMF (1.4 mL), DMAP (163 mg, 1.34 mmol) and CH_3SO_2Cl (104 μL, 1.34 mmol) were added. The reaction mixture was stirred at room temperature for 5 h. Then, NaN_3 was added and stirring was continued at room temperature for 4 h. It was diluted with water (20 mL) and extracted with diethyl ether (4 × 10 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness to yield 226 mg (89%) of **18** as an oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (d, J = 6.1 Hz, 6H, $CH(C\underline{H}_3)_2$), 4.38 (s, 2H, CH_2), 4.61 (hept, J = 6.0 Hz, 1H, CH), 6.89 (m, 3H, H-2′, H-4′, H-6′), 7.28 (pt, J = 7.7 Hz, 1H, H-5′).

*5-Amino-1-(3′-isopropoxybenzyl)-1H-1,2,3-triazole-4-carboxamide (**19**).* To a suspension of NaH (60% in mineral oil, 37 mg, 1.55 mmol) in anhydrous DMF (1.4 mL) at 0 °C, a solution of cyanoacetamide (79 mg, 0.94 mmol) in anhydrous DMF (1.4 mL) was added. After 1 h, a solution of **18** (196 mg, 0.86 mmol) in anhydrous DMF (1.4 mL) was slowly added and stirring was continued at room temperature for 1 h. Volatiles were removed. The residue obtained was purified by flash column chromatography (dichloromethane/methanol, 20:1) to yield 183 mg (77%) of **19** as a white solid. mp: 171−173 °C. MS (ES, positive mode): m/z 276 $(M + H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.23 (d, J = 6.0 Hz, 6H,

$CH(C\underline{H}_3)_2$), 4.54 (hept, J = 6.0 Hz, 1H, CH), 5.35 (s, 2H, CH_2), 6.38 (s, 2H, NH_2), 6.71 (m, 2H, H-2′, H-6′), 6.83 (d, J = 7.9 Hz, 1H, H-4′), 7.08 (s, 1H, $CONH_2$), 7.23 (pt, J = 8.1 Hz, 1H, H-5′), 7.45 (s, 1H, $CONH_2$).

*3-(3-Isopropoxybenzyl)-5-methyl-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7(6H)-one (**20**).* To a solution of Na (41 mg, 1.80 mmol) in ethanol (3.6 mL), **19** (100 mg, 0.36 mmol) and ethyl acetate (230 μL, 1.45 mmol) were added. The reaction mixture was heated at reflux for 8 h. After cooling, the reaction was concentrated to dryness. The residue was dissolved in water, acetic acid was added, and the precipitate was isolated by filtration to yield 81 mg (75%) of **20** as a white solid. mp: 186−187 °C. MS (ES, positive mode): m/z 300 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.22 (d, J = 6.0 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.41 (s, 3H, CH_3), 4.56 (hept, J = 6.1 Hz, 1H, CH), 5.65 (s, 2H, CH_2), 6.72 (m, 3H, H-2′, H-4′, H-6′), 7.22 (pt, J = 8.1 Hz, 1H, H-5′). ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1 (CH_3), 22.4 (CH_3), 50.0 (CH_2), 69.8 (CH), 115.6, 115.6, 120.1 (Ar), 128.7 (C-7a), 130.6, 137.7 (Ar), 149.7 (C-3a), 156.6 (C-5), 158.3 (C-7), 160.7 (Ar). Anal. ($C_{15}H_{17}N_5O_2$): C, H, N.

Cells and Virus Strains. CHIKV Indian Ocean strain 899 (Genbank FJ959103.1) was generously provided by Prof. S. Günther (Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany). CHIKV strain LR2006_OPY1 (GenBank DQ443544.2) and the clinical isolates Venturini (Italy 2008), Congo 95 (2011), and St. Martin (2013) belong to the collection of viruses at the UMR 190, Marseille, France. Sindbis virus (SINV, strain HRsp, GenBank J02363.1) and the Semliki Forest virus (SFV, Vietnam strain, GenBank EU350586.1) belong to the collection of viruses at the Rega Institute of Medical Research, Belgium. All viruses were propagated in African green monkey kidney cells (Vero cells (ATCC CCL-81)).

Vero cells were maintained in cell growth medium composed of minimum essential medium (MEM Rega-3, Gibco, Belgium) supplemented with 10% fetal bovine serum (FBS, Integro, The Netherlands), 1% L-glutamine (Gibco), and 1% sodium bicarbonate (Gibco). The antiviral assays were performed in virus growth medium that is the respective cell growth medium supplemented with 2% (instead of 10%) FBS. All cell cultures were maintained at 37 °C in an atmosphere of 5% CO_2 and 95−99% humidity.

Antiviral and Antimetabolic Assays. Vero cells were seeded in 96-well tissue culture plates (Becton Dickinson, Aalst, Belgium) at a density of 2.5×10^4 cells/well in 100 μL of assay medium and were allowed to adhere overnight. Next, a compound dilution series was prepared in the medium on top of the cells after which the cultures were infected with 0.01 MOI of CHIKV 899 inoculum in 100 μL of assay medium. A similar setup was used for SINV and SFV. Each assay was performed in multiplicate (at least in 3-fold) in the same test, and assays were repeated independently to assess for interexperiment variability. On day 7 postinfection (p.i.), the plates were processed using the MTS/PMS method as described by the manufacturer (Promega, The Netherlands). The 50% effective concentration (EC_{50}), which is defined as the compound concentration that is required to inhibit virus-induced cytopathic effect by 50%, was determined using logarithmic interpolation. The overall antimetabolic effect of the compounds, which is representative for the cytostatic, cytotoxic, and antimetabolic effect, was evaluated in uninfected cells, also by means of the MTS/PMS method. The 50% cytostatic/cytotoxic concentration (CC_{50}; i.e., the concentration of compound that reduces the overall metabolic activity of the cells by 50%) was calculated using logarithmic interpolation. All assay wells were checked microscopically for minor signs of virus-induced CPE or alterations of the cell or monolayer morphology and were used to distinguish between true hit compounds and mere cytoprotective compounds.

The antiviral activity of the best compound was validated in an assay for CHIKV OPY 1, Venturini, Congo and St. Martin. The amount of each virus in the assay has been calibrated by trial so that the replication growth is at the beginning of the plateau at day 2. One day prior to infection, 5×10^4 Vero E6 cells were seeded in 100 μL of medium (with 2.5% FCS) in each well of a 96-well titer plate. The next day, 2-fold serial dilutions of the compounds, in triplicates, were added to the cells (25 μL/well). Four virus control wells (per virus) were supplemented with 25 μL of medium containing 0.1% DMSO, and four cell control wells were supplemented with 50 μL of medium. After 15 min, 25 μL of a virus mix containing the appropriate amount of viral stock diluted in medium was added to the 96-well plates. Cells were cultivated for 2 days after which 100 μL of the supernatant was collected and transferred to 96 square well plates preloaded with 400 μL of RAV-1 lysis buffer from Macherey Nagel "NucleoSpin 96 virus kit". Viral RNA was extracted (NucleoSpin 96 virus kit running on an Eppendorf epMotion 5075 liquid handler automat) and quantified by real time RT-PCR to quantify the amount of viral RNA, which is representative for the amount of progeny virions that are produced (SuperScript III Platinium one-step RT-PCR with Rox from Invitrogen). The four control wells were replaced by four 2-log dilutions of an appropriate T7-generated RNA standards. IC_{50} values were determined by fitting a sigmoidal curve on values of virus yield inhibition (in %) versus the log values of drug concentration (Kaleidagraph software).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis and spectroscopic data of compounds **9b−m**, **10b−m**, **11b−n**, and **15a**, **15c−g**; elemental analysis of compounds **2**, **3a−c**, **4**, **6a**, **6b**, **11a−n**, **13**, **15a−g**, **16**, and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Schwartz, O.; Albert, M. L. Biology and pathogenesis of chikungunya virus. *Nat. Rev. Microbiol.* **2010**, *8*, 491−500.

(2) Suhrbier, A.; Jaffar-Bandjee, M. C.; Gasque, P. Arthritogenic alphaviruses−An overview. *Nat. Rev. Rheumatol.* **2012**, *8*, 420−429.

(3) Singh, S. K.; Unni, S. K. Chikungunya virus: Host pathogen interaction. *Rev. Med. Virol.* **2011**, *21*, 78−88.

(4) Thiberville, S. D.; Moyen, N.; Dupuis-Maguiraga, L.; Nougairede, A.; Gould, E. A.; Roques, P.; de Lamballerie, X. Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Res.* **2013**, *99*, 345−370.

(5) Staikowsky, F.; Talarmin, F.; Grivard, P.; Souab, A.; Schuffenecker, I.; Le Roux, K.; Lecuit, M.; Michault, A. Prospective study of Chikungunya virus acute infection in the Island of La Réunion during the 2005−2006 outbreak. *PLoS One* **2009**, *4*, e7603.

(6) Thiboutot, M. M.; Kannan, S.; Kawalekar, O. U.; Shedlock, D. J.; Khan, A. S.; Sarangan, G.; Srikanth, P.; Weiner, D. B.; Muthumani, K. Chikungunya: A potentially emerging epidemic. *PLoS Neglected Trop. Dis.* **2010**, *4*, e623.

(7) Tang, B. L. The cell biology of Chikungunya virus infection. *Cell. Microbiol.* **2012**, *14*, 1354−1363.

(8) Vazeille, M.; Moutailler, S.; Coudrier, D.; Rousseaux, C.; Khun, H.; Huerre, M.; Thiria, J.; Dehecq, J.-S.; Fontenille, D.; Schuffenecker, I.; Despres, P.; Failloux, A.-B. Two Chikungunya isolates from the

outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito *Aedes albopictus*. *PLoS One* **2007**, *2*, e1168.
(9) Tsetsarkin, K. A.; Vanlandingham, D. L.; McGee, C. E.; Higgs, S. A single mutation in Chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog.* **2007**, *3*, e201.
(10) Leparc-Goffart, I.; Nougairede, A.; Cassadou, S.; Prat, C.; de Lamballerie, X. Chikungunya in the Americas. *Lancet* **2014**, *383*, 514.
(11) Plante, K.; Wang, E.; Partidos, C. D.; Weger, J.; Gorchakov, R.; Tsetsarkin, K.; Borland, E. M.; Powers, A. M.; Seymour, R.; Stinchcomb, D. T.; Osorio, J. E.; Frolov, I.; Weaver, S. C. Novel Chikungunya vaccine candidate with an IRES-based attenuation and host range alteration mechanism. *PLoS Pathog.* **2011**, *7*, e1002142.
(12) Bassetto, M.; De Burghgraeve, T.; Delang, L.; Massarotti, A.; Coluccia, A.; Zonta, N.; Gatti, V.; Colombano, G.; Sorba, G.; Silvestri, R.; Tron, G. C.; Neyts, J.; Leyssen, P.; Brancale, A. Computer-aided identification, design and synthesis of a novel series of compounds with selective antiviral activity against chikungunya virus. *Antiviral Res.* **2013**, *98*, 12−18.
(13) Bourjot, M.; Delang, L.; Nguyen, V. H.; Neyts, J.; Guéritte, F.; Leyssen, P.; Litaudon, M. Prostratin and 12-*O*-tetradecanoylphorbol 13-acetate are potent and selective inhibitors of chikungunya virus replication. *J. Nat. Prod.* **2012**, *75*, 2183−2187.
(14) Bourjot, M.; Leyssen, P.; Eydoux, C.; Guillemot, J. C.; Canard, B.; Rasoanaivo, P.; Guéritte, F.; Litaudon, M. Chemical constituents of *Anacolosa pervilleana* and their antiviral activities. *Fitoterapia* **2012**, *83*, 1076−1080.
(15) Ozden, S.; Lucas-Hourani, M.; Ceccaldi, P.-E.; Basak, A.; Valentine, M.; Benjannet, S.; Hamelin, J.; Jacob, Y.; Mamchaoui, K.; Mouly, V.; Desprès, P.; Gessain, A.; Butler-Browne, G.; Chrétien, M.; Tangy, F.; Vidalain, P.-O.; Seidah, N. G. Inhibition of chikungunya virus infection in cultured human muscle cells by furin inhibitors: Impairment of the maturation of the E2 surface glycoprotein. *J. Biol. Chem.* **2008**, *283*, 21899−21908.
(16) Lucas-Hourani, M.; Lupan, A.; Desprès, P.; Thoret, S.; Pamlard, O.; Dubois, J.; Guillou, C.; Tangy, F.; Vidalain, P.-O.; Munier-Lehmann, H. A phenotypic assay to identify chikungunya virus inhibitors targeting the nonstructural protein nsP2. *J. Biomol. Screening* **2013**, *18*, 172−179.
(17) Pohjala, L.; Utt, A.; Varjak, M.; Lulla, A.; Merits, A.; Ahola, T.; Tammela, P. Inhibitors of alphavirus entry and replication identified with a stable chikungunya replicon cell line and virus-based assays. *PLoS One* **2011**, *6*, e28923.
(18) Kaur, P.; Thiruchelvan, M.; Lee, R. C. H.; Chen, H.; Chen, K. C.; Ng, M. L.; Chu, J. J. H. Inhibition of chikungunya virus replication by harringtonine, a novel antiviral that suppresses viral protein expression. *Antimicrob. Agents Chemother.* **2013**, *57*, 155−167.
(19) Rashad, A. A.; Mahalingam, S.; Keller, P. A. Chikungunya virus: Emerging targets and new opportunities for medicinal chemistry. *J. Med. Chem.* **2013**, *57*, 1147−1166.
(20) Aguado, L.; Thibaut, H. J.; Priego, E. M.; Jimeno, M. L.; Camarasa, M. J.; Neyts, J.; Pérez-Pérez, M. J. 9-Arylpurines as a novel class of enterovirus inhibitors. *J. Med. Chem.* **2010**, *53*, 316−324.
(21) Aguado, L.; Camarasa, M. J.; Pérez-Pérez, M. J. Microwave-assisted synthesis of 9-arylpurines. *J. Comb. Chem.* **2009**, *11*, 210−212.
(22) Aguado, L.; Canela, M. D.; Thibaut, H. J.; Priego, E. M.; Camarasa, M. J.; Leyssen, P.; Neyts, J.; Pérez-Pérez, M. J. Efficient synthesis and anti-enteroviral activity of 9-arylpurines. *Eur. J. Med. Chem.* **2012**, *49*, 279−288.
(23) Barili, P. L.; Biagi, G.; Livi, O.; Scartoni, V. A facile "one pot" synthesis of 2,9-disubstituted 8-azapurin-6-ones (3,5-disubstituted 7-hydroxy-3H-1,2,3-triazolo[4,5-d]pyrimidines). *J. Heterocycl. Chem.* **1985**, *22*, 1607−1609.
(24) Bertelli, L.; Biagi, G.; Calderone, V.; Giorgi, I.; Livi, O.; Scartoni, V.; Barili, P. L. 1-(1,2,3-Triazol-4-yl)-benzimidazolones, a new series of heterocyclic derivatives. *J. Heterocycl. Chem.* **2000**, *37*, 1169−1176.
(25) Biagi, G.; Giorgi, I.; Livi, O.; Nardi, A.; Scartoni, V. Triazolylbenzimidazolthiones and derivatives of the new 1,2,3-triazolo 1,5-a 1,3,5 benzotriazepine heterocycle. *J. Heterocycl. Chem.* **2002**, *39*, 1293−1298.
(26) Biagi, G.; Giorgi, I.; Livi, O.; Scartoni, V.; Velo, S.; Barili, P. L. New 4-(benzotriazol-1-yl)-1,2,3-triazole derivatives. *J. Heterocycl. Chem.* **1996**, *33*, 1847−1853.
(27) Miyashita, A.; Fujimoto, K.; Okada, T.; Higashino, T. Synthesis of fused pyrimidinones by reaction of aminoarene-carboxamide with esters; Preparation of pyrrolo[2,3-*d*]-, thieno[2,3-*d*]-, isoxazolo[5,4-*d*]-, and 1,2,3-triazolo[4,5-*d*]-pyrimidinones, and quinazolones. *Heterocycles* **1996**, *42*, 691−699.
(28) Hu, M.; Li, J.; Q. Yao, S. In situ "click" assembly of small molecule matrix metalloprotease inhibitors containing zinc-chelating groups. *Org. Lett.* **2008**, *10*, 5529−5531.
(29) Loidreau, Y.; Marchand, P.; Dubouilh-Benard, C.; Nourrisson, M. R.; Duflos, M.; Lozach, O.; Loaëc, N.; Meijer, L.; Besson, T. Synthesis and biological evaluation of *N*-arylbenzo[*b*]thieno[3,2-*d*] pyrimidin-4-amines and their pyrido and pyrazino analogues as Ser/Thr kinase inhibitors. *Eur. J. Med. Chem.* **2012**, *58*, 171−183.
(30) Baines, K. M.; Rourke, T. W.; Vaughan, K.; Hooper, D. L. 5-(Arylamino)-1,2,3-triazoles and 5-amino-1-aryl-1,2,3-triazoles from 3-(cyanomethyl)triazenes. *J. Org. Chem.* **1981**, *46*, 856−859.