Design and Synthesis of new antimicrobial [1,2,4]triazolo[1,5-*c*]pyrimidines

Abderrahim Ben Said [a], Ameur Rahmouni [a], Mejda Daami-Ramadi[b], Anis Romdhane [a] and Hichem Ben Jannet[a,*]

[a]*Laboratoire de Chimie Hétérocyclique, Produits Naturels et Réactivité, Equipe: Chimie Médicinale et Produits Naturels, Faculté des Sciences de Monastir, Université de Monastir, Avenue de l'Environnement, 5019 Monastir, Tunisie.*
[b]*UR13AGR09, Production Horticole Intégrée au Centre Est Tunisien, Centre Régional des Recherches en Horticulture et Agriculture Biologique de Chott-Mariem, Université de Sousse, 4042, Chott-Mariem, Tunisie.*

(Received: 11 April 2016, accepted: 19 November 2016)

Abstract: The synthesis of 14-(aryl)-14*H*-naphtho[2,1-*b*]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine-2- acetonitriles **4a-e** has been accomplished using three steps of reaction using the 2-amino-naphthopyrane-3-carbonitriles **1a-e** as useful key intermediates. Compounds **4a-e** are used as precursors for the preparation of new triazolopyrimidine derivatives **5**, **6**, **7** and **8**. Structures of these compounds were established on the basis of ^{1}H NMR, ^{13}C NMR and HRMS. The synthesized compounds were also screened for their antimicrobial activity.

Key words: Naphtopyrane, Triazolo[1,5-*c*]pyrimidine acetonitrile, Antibacterial activity; Antifungal activity.

INTRODUCTION

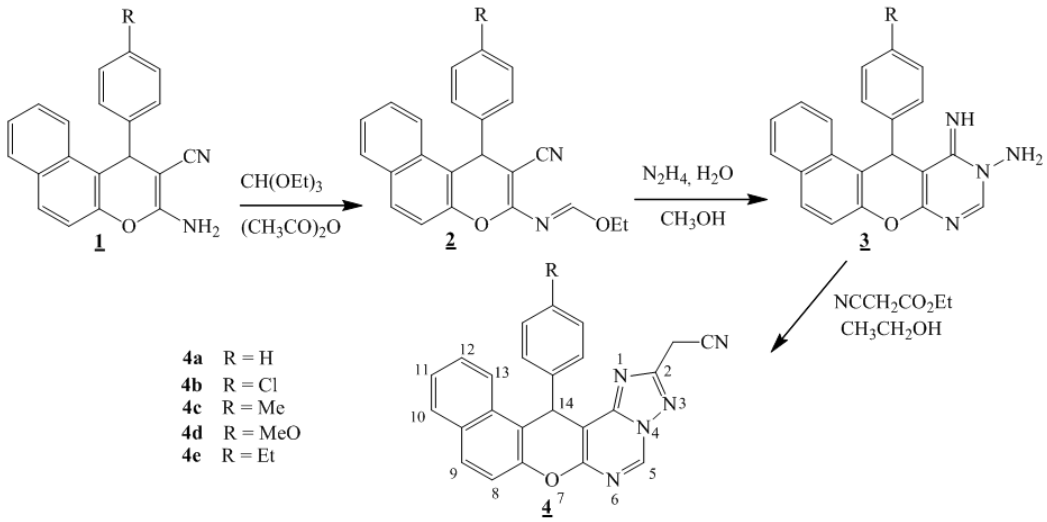
Heterocyclic compounds have drawn special attention in organic chemistry because of their abundance in natural products and their diverse biological properties [1]. Pyrimidine and its derivatives have been recognized as important heterocyclic compounds due to their variety of chemical and biological significance to medicinal chemistry [2,3]. On the other hand, 1,2,4-triazole moiety appears frequently in the structure of various natural products [4] and the synthesis of compounds incorporating this moiety has attracted widespread attention of chemists as well as biologists, mainly due to their diverse biological activities in pharmaceutical and agrochemical fields. A large variety of 1,2,4-triazole derivatives possess anticancer [5], antimicrobial [6], antimycobacterial [7], antiviral [8], anti-inflammatory [9], analgesic [10], antiproloferate and apoptotic properties [11]. Some present-day drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agents) are examples of potent molecules possessing a triazole nucleus [12-15] In view of these findings, it was considered of interest to synthesize new classes of heterocycle systems incorporating the pyrimidine and the 1,2,4-triazole moieties in the hope that they may be biologically active. Herein and as a continuation of our previous work on the synthesis of new 1,2,4-triazolopyrimidines [16,17] we report here the synthesis of 2-cyanomethyl triazolopyrimidines **4a-e** and their utility as building blocks in the synthesis of some novel fused triazolopyrimidines **5**-**8**. The synthesized compounds were also screened for their antimicrobial activity.

RESULT AND DISCUSSION

1. Synthesis

The key intermediates 2-amino-naphto[2,1-*b*] pyrane-3-carbonitriles **1a-e** were prepared according to the literature [20] through a condensation of stoichiometric mixture of malononitrile, aromatic aldehyde and *β*-naphtol in the presence of sodium carbonate. Treatment of **1a-e** with triethyl orthoformate in acetic anhydride at reflux gave the

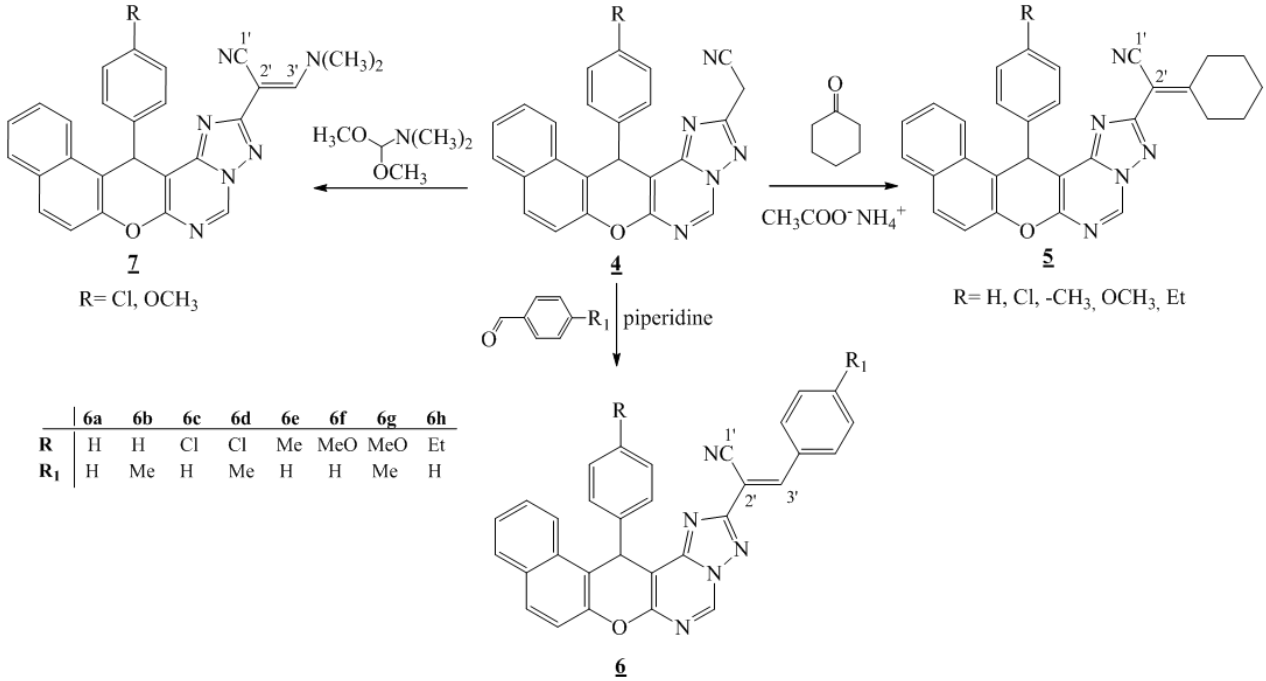
* Corresponding author, e-mail address : hich.benjannet@yahoo.fr , Tel: +216 73 500 279 , Fax: +216 73 500 278



Scheme 1. Synthetic routes of compounds **1-4**

corresponding 2-ethoxy-methyleneamino derivatives **2a-e** and have subjected them to reaction with an aqueous solution of hydrazine in methanol at room temperature to give the naphtopyrano-pyrimidines **3a-e** [21] (scheme **1**). Compounds **3** were further converted into triazolo[1,5-*c*] pyrimidine-2-acetonitriles **4a-e** by treatment with ethyl cyanoacetate under reflux of ethanol in the presence of a few drops of acetic acid (Scheme **1**).

The 2'-cyclohexylidene-2'-(14-aryl-14*H*-naphto[2,1-*b*]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*] pyrimidin-2-yl) acetonitriles **5a-e** were prepared by reacting **4a-e** with cyclohexanone in the presence of ammonium acetate. The reaction was conducted until TLC indicated that the starting materials have been completely converted into products **5a-e**. The structure of these compounds has been characterized by ^{1}H NMR showing a new

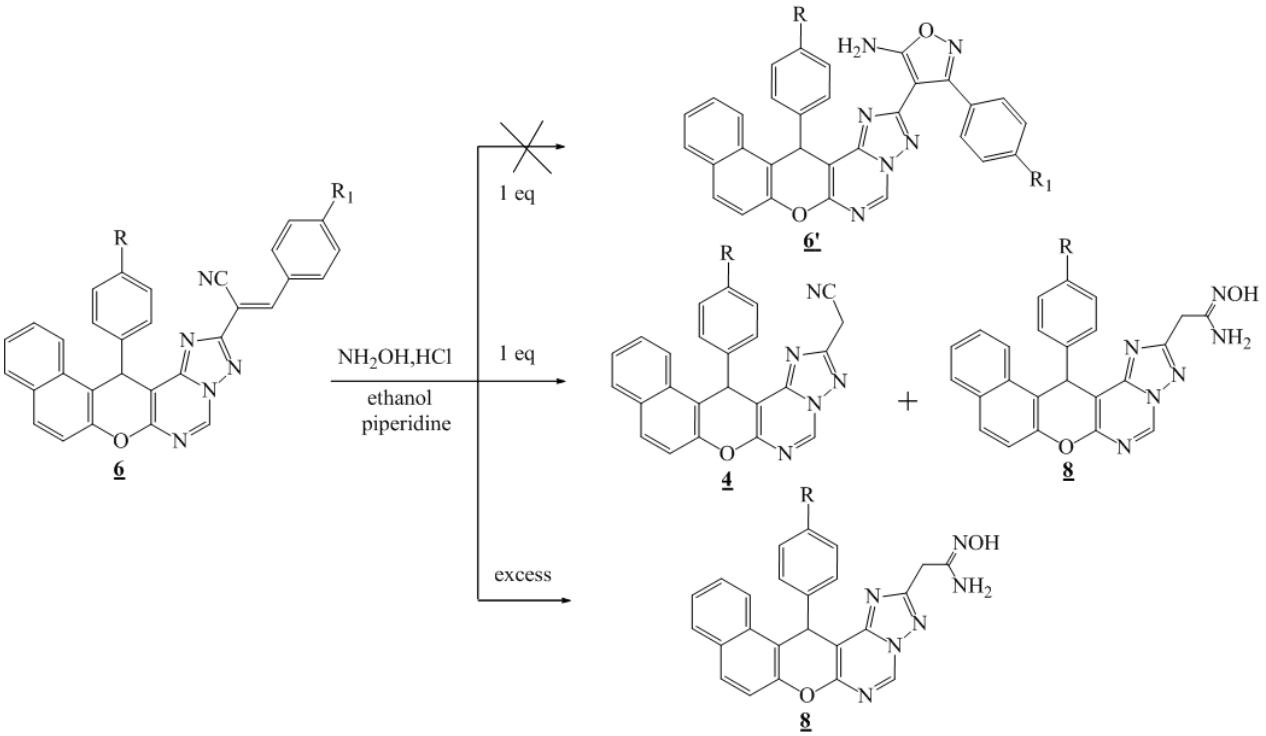


Scheme 2. Synthetic routes of compounds **5-7**

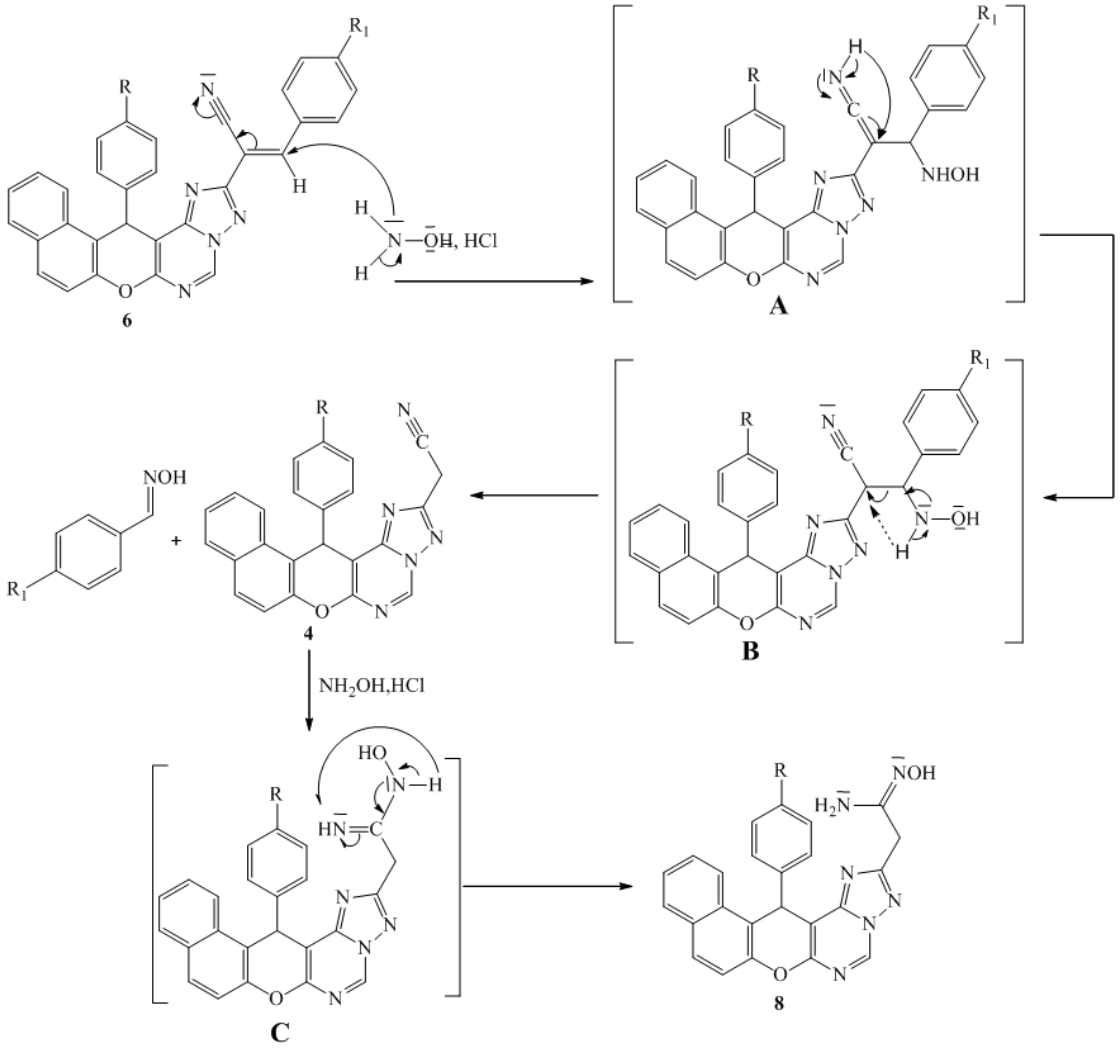
multiplets at the region 1.74-3.94, 2.76-2.89 and 3.09-3.30 ppm attributed to protons of cyclohexyl group. Further, the ^{13}C NMR spectra of **5a-e** exhibited the presence of new signals at 25.3-25.8, 27.7-28.2, 28.1-28.6, 31.2-32.1, 36.1-36.6 and 171.6-172.1 ppm relative to carbons introduced by the cyclohexyl group. Then, the Knoevenagel condensation of **4a-e** with aryl aldehyde, in refluxing ethanol containing a catalytic amount of piperidine furnished the corresponding arylidene derivatives **6a-h**. Further, the ^{1}H NMR spectra of compounds **6** show the disappearance of the singlet relative to the methylene protons (of compounds **4**) and the appearance of new signals, attributable to the ethylenic proton $H_{3'}$ at 8.51-8.59 ppm and protons of the aryl moiety, whose chemical shifts and multiplicities are in agreement with the proposed structure. Analysis of ^{13}C NMR spectra of these compounds show, in addition to the signals relative to arylidene moiety carbons, the disappearance of the signal attributable to the methylene carbon (of compounds **4**). The cyanomethyls **4b,d** reacts with dimethyl-formamide dimethylacetal (DMF-DMA) to yield the corresponding enamines **7b,d** (Scheme 2). The formed acrylonitrile triazolopyrimidines **7b,d** were characterized by their ^{1}H and ^{13}C NMR spectra. In fact, the ^{1}H NMR spectra of compounds **7b,d** show, in addition to the signals relative to protons introduced by the intermediate **4**, the presence of new signals due to the dimethylamine group ($-N(CH_3)_2$) at 3,19-3,28 ppm, the ethylenic proton $H_{3'}$ at 8.32-8.48 ppm and the disappearance of signal related to methylene protons (of intermediate **4**).

Treatment of acrylonitrile triazolopyrimidine **6** with hydroxyle amine hydrochloride was previously reported to effect cyclization and give the amino oxazole **6'** [22,23] The interaction of an equimolar amount of compounds **6** with hydroxyl amine hydrochloride under reflux of ethanol for 8 h did not afford the desined oxazole **6'** but return to the acetonitrile triazolopyrimidines **4** which continue to react with hydroxyl amine hydrochloride to yield the acetimidamide triazolopyrimidines **8** (Scheme 3). While the condensation of **6** with excess of hydroxyl amine hydrochloride in the same conditions gave exclusively the acetimidamide triazolopyrimidines **8** (scheme 3).

In fact, the condensation of the compounds **6** with hydroxylamine hydrochloride led to the non-



Scheme 3. Synthesis route of acetimidamide **8**



Scheme 4. Plausible mechanism for the synthesis of compounds **8**

isolable intermediate A after nucleophilic attack of the NH_2 group on the electrophilic double bond C=C. The tautomerization of A provides the intermediate B which rearranges to release an oxime and forms the corresponding triazolo-pyrimidines acetonitrile **4**.

Finally, the nucleophilic attack of the NH_2 group on the cyano (CN) function provides the intermediate C, which by tautomerization, gives the acetimidamide triazolopyrimidines **8** thermodynamically more stable. The proposed mechanism of the formation of compounds **8a-e** is detailed in Scheme 4.

The structures of compounds **8a-e** have been assigned from their ^{1}H NMR, ^{13}C NMR and mass spectroscopy (HR-ESMS): ^{1}H NMR spectra show the presence of a new signals at 3.43-3.63, 5.52-5.59 and 9.57-9.60 ppm relative to -CH_2-, -NH_2 and -OH protons, respectively. The ^{13}C NMR of these compounds was also in agreement with the proposed structures. In fact, these spectra showed essentially the appearance of new signals at 31.0-33.0 and 165.7-168.2 ppm due to -CH_2- and amidoxime carbons, respectively, and the disappearance of the signal relative to -CN carbon. The ESI-HR-MS showed essentially the correct protonated molecular peak $[M+H]^+$ for all examined compounds **8**.

2. Biological activity

Most of the synthesized heterocycles were screened for their antibacterial and antifungal activities. According to the results given in Table I, most of the tested compounds have displayed good

Table I. Antibacterial activity of compounds **4a-e**, **5a-e**, **6a-h**, **7b**, **7d** and **8a-e**

	Diameters of the inhibition zone (in mm)			
Products	*Pseudomonas syringae pv. syringae*	*Pseudomonas savastanoi*	*Pseudomonas huttiensis*	*Agrobacterium tumefasciens*
4a	-	-	-	-
4b	-	-	-	-
4c	-	-	-	-
4d	-	-	12	-
4e	13	-	-	-
5a	-	-	11.5	-
5b	-	-	11	-
5c	10	-	10.5	-
5d	-	-	9.5	-
5e	-	-	10	-
6a	-	-	10.5	-
6b	-	-	11.5	9
6c	-	-	9.5	10.5
6d	-	-	-	-
6e	-	-	-	-
6g	-	-	10.5	-
6h	-	-	9.5	-
7b	-	-	11.5	-
7d	-	-	-	-
8a	-	-	8.5	-
8b	-	-	10	-
8c	-	-	11.5	-
8d	-	-	-	-
8e	13.25	-	-	-
Ampicilin	21	22	25	18.5
DMSO	-	-	-	-

- no inhibition zone observed.
Ampicilin (antibiotic) tested at 5 mg/mL

antibacterial activity against *P. huttiensis* (IZ = 8.5-12 mm). Similarly, the compounds **4d**, **5a**, **5b**, **6b**, **7b** and **8c** are the most active against *P. huttiensis* (IZ = 11-12 mm). While the compounds **4e** and **8e**, both bearing an ethyl group, exhibited good activity against *P. syringae pv. Syringae* (IZ = 13 and 13.5 mm, respectively). The acrylonitrile derivatives **6b** and **6c** showed moderate activity against *A. tumefasciens* (IZ = 9 and 10.5 mm, respectively). All of the tested compounds have displayed weak antibacterial activity against *P. savastanoi*.

The antifungal results (Table II) revealed that the synthesized compounds showed variable degrees of inhibition against the tested fungi. In fact, the triazolopyrimidine **4b** is the more active compound, as it showed a good antifungal activity against *A. flavus* (IZ = 12 mm), *P. digitatum* (IZ = 11 mm), *A. niger* (IZ = 13 mm), *P. italicum* (*IZ* = 13 mm) and *T. harzianum* (IZ = 14 mm). The Compounds **6a** and **8e** exhibited an important inhibitory activity against *P. digitatum* (IZ = 16 and 14 mm, respectively). However, the

Table II. Antifungal activity of compounds **4a-e**, **5a-e**, **6a-h**, **7b**, **7d** and **8a-e**

Products	Diameters of the inhibition zone (in mm)				
	Aspergillus flavus	*Aspergillus niger*	*Penicillium digitatum*	*Penicillium italicum*	*Trichoderma harzianum*
4a	-	-	-	-	11
4b	12	13	11	13	14
4c	-	11	-	-	-
4d	-	-	-	-	
4e	-	-	10	9	13
5a	8	11	-	10	10
5b	-	11	-	-	-
5c	-	-	-	-	9
5d	-	-	-	-	13
5e	-	12	-	11	-
6a	-	10	16	8	8
6b	8.5	10	-	-	12
6c	-	-	-	-	14
6d	-	-	-	-	-
6e	-	-	-	-	-
6g	-	-	-	-	-
6h	-	8	-	-	11
7b	-	-	-	-	-
7d	-	-	-	-	-
8a	-	-	-	-	10
8b	13	12	-	16	14
8c	9	10	9	-	10
8d	-	9	-	-	-
8e	11.5	-	14	-	11
Carbendazim	32	30	44	37	39
DMSO	-	-	-	-	-

- no inhibition zone observed.
Carbendazim (fungicide) tested at 0.5 mg/mL, Commercial source Bavistin.

compounds **4b**, **4e**, **5d**, **6c** and **8b** possessed good antifungal activity against *T. harzianum,* the inhibition zones varied between 13 and 14 mm. The triazolopyrimidine acetimidamide **8b** is the more active against *P. italicum* (IZ = 16 mm). The compounds **4b**, **5e** and **8b** exhibited good activity against *A. niger* (IZ = 12-13 mm). While compound **8b** was found to be the most active against *A. flavus* (IZ = 13 mm). The remaining compounds showed moderate to weak inhibitory effects.

CONCLUSION

In summary, an efficient method was achieved for the preparation of some new hybrid molecules combined pyranopyrimidines with triazole fonctionalized *via* condensation reactions of methyl cyano precursors **4**, which were prepared in three steps from 2-aminonaphto[2,1-b]pyrane-3-carbonitriles **1**. Structures of all synthesized compounds were elucidated by means of various characterization techniques such as ^{1}H NMR, ^{13}C NMR and HRMS. The study of the antibacterial

and antifungal activity of all new synthesized derivatives showed significant results. In fact, compounds **4d** (OCH_3), **5a** (H), **5b** (Cl), **6b** (R = H, R_1 = Me), **7b** (Cl) and **8c** (CH_3) are the most antibacterial against *P. huttiensis*. While the compounds **4e** (C_2H_5) and **8e** (C_2H_5) exhibited good activity against *P. syringae pv. Syringae.* In the other hand, triazolopyrimidine derivative **4b** is the more antifungal compound, as it showed a good antifungal activity against *A. flavus, P. digitatum, A. niger*, *P. italicum* and *T. harzianum*. However, the compounds **4b** (Cl), **4e** (C_2H_5), **5d** (OCH_3), **6c** (R = Cl, R_1 =H) and **8b** (Cl) possessed good antifungal activity against *T. harzianum.*

EXPERIMENTAL

1. Instrumentation

All reactions were monitored by TLC using aluminum sheets of Merck silica gel 60 F_{254}, 0.2 mm. Melting temperatures were determined on an electrothermal 9002 apparatus and were reported uncorrected. NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C). All chemical shifts were reported as δ values (ppm) relative to residual non-deuterated solvent. Mass spectra were obtained with ESI-TOF (LCT, Waters) using the reflectron mode in the positive ion mode. The starting materials **1**, **2**, **3** and **4** were prepared according to the literature [17].

2. Synthesis

General procedure for preparation of 2'-cyclohexylidene-2'-(14-aryl)-14H-naphto[2,1-b] pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl) acetonitrile (5a-e).

A mixture of 2-cyanomethyl **4a-e** (1 mmol), ammonium acetate (1.2 mmol) and cyclohexanone (1.2 mmol) in dioxane (10 mL) was heated under reflux for 10 h and left to cool to room temperature. The separated crystalline product was filtered off, dried and recrystallized from ethanol.

2'-cyclohexylidene-2'-(14-phenyl)-14*H*-naphtho [2,1-b]pyrano[3,2-e][1,2,4]triazolo[1,5-c] pyrimidin-2-yl) acetonitrile (5a): White solid. Yield: 82%. M.p. 235 °C (Ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.74-1.92 (m, 6H, H-cyclohexyl), 2.76-2.87 (m, 2H, H-cyclohexyl), 3.10-3.30 (m, 2H, H-cyclohexyl), 6.35 (s, 1H, H-pyran), 7.08-7.95 (m, 11H, Ar-H), 9.09 (s, 1H, H-pyrimidine). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 25.3, 27.7, 28.1, 31.6, 36.1, 37.5, 99.2, 102.8, 114.0, 116.1, 117.0, 123.1, 124.7, 126.7, 126.9, 128.0, 128.1, 129.5, 130.4, 131.1, 137.7, 141.9, 148.1, 152.3, 153.2, 162.2, 171.7. HRMS $[M+H]^+$ calcd. for $(C_{30}H_{24}N_5O)^+$: 470.1981, found: 470.1990.

2'-cyclohexylidene-2'-(14-(4-chlorophenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4] triazolo[1,5-c] pyrimidin-2-yl) acetonitrile (5b): White solid. Yield: 79%. M.p. 240 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.74-1.89 (m, 6H, H cyclohexyl), 2.79-2.89 (m, 2H, H cyclohexyl), 3.09 -3.27 (m, 2H, H cyclohexyl), 6.31 (s, 1H, H pyran), 7.15-7.91 (m, 10H, H_{arom}), 9.09 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.3, 27.7, 28.1, 31.7, 36.2, 36.9, 99.2, 102.3, 113.3, 116.0, 117.0, 122.9, 124.8, 127.0, 128.2, 129.4, 129.7, 130.2, 131.1, 132.6, 137.8, 140.3, 148.0, 152.1, 153.1, 162.2, 171.8. HRMS $[M+H]^+$ calcd. for $(C_{30}H_{23}ClN_5O)^+$: 504.1591, found: 504.1580.

2'-cyclohexylidene-2'-(14-(4-methylphenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4] triazolo[1,5-c]pyrimidin-2-yl) acetonitrile (5c): White solid. Yield: 85%. M.p. 254 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.76-1.92 (m, 6H, H cyclohexyl), 2.21 (s, 3H, -CH_3), 2.82-2.88 (m, 2H, H cyclohexyl), 3.15-3.29 (m, 2H, H cyclohexyl), 6.33 (s, 1H, H pyran), 7.01-7.97 (m, 10H, H_{arom}), 9.10 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.9, 25.8, 28.2, 28.6, 32.2, 36.6, 37.5, 99.7, 103.5, 114.6, 116.6, 117.5, 123.6, 125.1, 127.3, 128.4, 128.6, 129.2, 129.9, 130.9, 131.6, 136.9, 138.1, 139.6, 148.5, 152.8, 153.6, 162.7, 172.1. HRMS $[M+H]^+$ calcd. for $(C_{31}H_{26}N_5O)^+$: 484.2137, found: 484.2142.

2'-cyclohexylidene-2'-(14-(4-methoxyphenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4] triazolo [1,5-c]pyrimidin-2-yl) acetonitrile (5d): White solid. Yield: 81%. M.p. 260 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.75-1.93 (m, 6H, H cyclohexyl), 2.82-2.92 (m, 2H, H cyclohexyl), 3.12 -3.32 (m, 2H, H cyclohexyl), 3.68 (s, 3H, -CH_3), 6.31 (s, 1H, H pyran), 6.71-7.95 (m, 10H, H_{arom}), 9.09 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.3, 27.7, 28.1, 31.7, 36.1, 36.6, 54.6, 99.2, 103.1, 113.4, 114.2, 116.1, 117.0, 123.1, 124.6, 126.8, 128.1, 129.1, 129.4, 130.4, 131.1, 134.3, 137.5, 148.0, 152.3, 153.1, 158.1, 162.1, 171.6. HRMS $[M+H]^+$ calcd. for $(C_{31}H_{26}N_5O_2)^+$: 500.2087, found: 500.2083.

2'-cyclohexylidene-2'-(14-(4-ethylphenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4] triazolo[1,5-c]pyrimidin-2-yl) acetonitrile (5e): White solid. Yield: 89%. M.p. 218 °C (ethanol). ^{1}H NMR

($CDCl_3$, 300 MHz): δ 1.14 (t, 3H, -CH_3, *J*= 7.5 Hz), 1.73-1.94 (m, 6H, H cyclohexyl), 2.52 (q, 2H, -$\underline{CH_2}$-CH_3, *J*= 7.5 Hz), 2.82-2.89 (m, 2H, H cyclohexyl), 3.12-3.32 (m, 2H, H cyclohexyl), 6.33 (s, 1H, H pyran), 7.03-7.97 (m, 10H, H_{arom}), 9.10 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 15.1, 25.8, 28.2, 28.3, 28.6, 32.1, 36.6, 37.5, 99.7, 103.6, 114.7, 116.6, 117.5, 123.6, 125.1, 127.3, 128.0, 128.5, 128.6, 129.9, 130.9, 131.6, 138.0, 139.7, 143.2, 148.5, 152.8, 153.7, 162.6, 172.1. HRMS $[M+H]^+$ calcd. for $(C_{32}H_{28}N_5O)^+$: 498.2294, found: 498.2301.

General procedure for preparation of (E)-3'-aryl-2'-(14-aryl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl) acrylonitrile (6a-e).

A mixture of 2-cyanomethyl derivatives **4a-e** (1 mmol) and aryl aldehyde (1 mmol) in ethanol (10 mL) was refluxed for 1h in the presence of few drops of piperidine. After cooling to room temperature, the precipitated product was filtered off and washed several times from ethanol to give **6a-e**.

(E)-3'-phenyl-2'-(14-phenyl-14*H*-naphto[2,1-b] pyrano[3,2-e][1,2,4]triazolo[1,5-c] pyrimidin-2-yl) acrylonitrile (6a): White solid. Yield: 90%. M.p. 280 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.41 (s, 1H, H pyran), 7.10-8.12 (m, 16H, H_{arom}), 8.57 (s, 1H, $H_{3'}$), 9.10 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 37.2, 101.2, 103.0, 114.3, 115.4, 117.0, 123.0, 124.7, 126.8, 126.9, 127.9, 128.1, 128.2, 128.7, 129.5, 129.8, 130.3, 131.2, 131.7, 132.1, 137.8, 141.9, 148.1, 148.6, 152.9, 153.8, 162.9. HRMS $[M+H]^+$ calcd. for $(C_{31}H_{20}N_5O)^+$: 478.1668, found: 478.1656.

(E)-3'-(4-methylphenyl)-2'-(14-phenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acrylonitrile (6b): White solid. Yield: 91%. M.p. 234 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.46 (s, 3H, -CH_3), 6.39 (s, 1H, H pyran), 7.10-8.01 (m, 15H, H_{arom}), 8.51 (s, 1H, $H_{3'}$), 9.08 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.2, 37.2, 99.9, 102.9, 114.3, 115.7, 117.0, 123.0, 124.7, 126.8, 126.9, 127.9, 128.1, 128.2, 129.4, 129.5, 130.0, 130.3, 131.1, 132.1, 137.8, 141.9, 142.7, 148.1, 148.5, 152.9, 153.7, 163.2. HRMS $[M+H]^+$ calcd. for $(C_{32}H_{22}N_5O)^+$: 492.1824, found: 492.1818.

(E)-3'-phenyl-2'-(14-(4-chlorophenyl)-14H-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acrylonitrile (6c): White solid. Yield: 87%. M.p. 254 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.35 (s, 1H, H pyran), 7.18-8.11 (m, 15H, H_{arom}), 8.52 (s, 1H, $H_{3'}$), 9.10 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 36.6, 101.2, 102.4, 113.6, 115.4, 117.0, 122.8, 124.9, 127.0, 128.2, 128.4, 128.7, 129.2, 129.7, 129.8, 130.1, 131.2, 131.7, 132.0, 132.7, 138.0, 140.3, 148.1, 148.5, 152.8, 153.6, 163.0. HRMS $[M+H]^+$ calcd. for $(C_{31}H_{19}ClN_5O)^+$: 512.1278, found: 512.1285.

(E)-3'-(4-methylphenyl)-2'-(14-(4-chlorophenyl) -14*H*-naphtho[2,1-b]pyrano[3,2-e] [1,2,4] triazolo[1,5-c]pyrimidin-2-yl) acrylonitrile (6d): White solid. Yield: 89%. M.p. >300 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.46 (s, 3H, -CH_3), 6.39 (s, 1H, H pyran), 7.17-8.02 (m, 14H, H_{arom}), 8.51 (s, 1H, $H_{3'}$), 9.11 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.2, 36.6, 99.7, 102.3, 113.7, 115.6, 117.0, 122.8, 124.9, 127.0, 128.2, 128.4, 129.2, 129.4, 129.7, 130.0, 130.2, 131.2, 132.0, 132.7, 137.9, 140.3, 142.8, 148.1, 148.7, 152.7, 153.8, 163.2. HRMS $[M+H]^+$ calcd. for $(C_{32}H_{21}ClN_5O)^+$: 526.1435, found: 526.1447.

(E)-3'-phenyl-2'-(14-(4-methylphenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo [1,5-c]pyrimidin-2-yl) acrylonitrile (6e): White solid. Yield: 95%. M.p. 262 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.22 (s, 3H, -CH_3), 6.38 (s, 1H, H pyran), 7.04-8.13 (m, 15H, H_{arom}), 8.57 (s, 1H, $H_{3'}$), 9.11 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.9, 37.3, 101.9, 103.7, 114.9, 115.9, 117.5, 123.5, 125.2, 127.3, 128.2, 128.6, 129.1, 129.4, 129.8, 120.3, 130.9, 131.7, 132.0, 132.7, 137.0, 138.2, 139.5, 148.6, 148.9, 153.5, 154.2, 163.5. HRMS $[M+H]^+$ calcd. for $(C_{32}H_{22}N_5O)^+$: 492.1824, found: 492.1813.

(E)-3'-phenyl-2'-(14-(4-methoxyphenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4] triazolo[1,5-c] pyrimidin-2-yl) acrylonitrile (6f): White solid. Yield: 91%. M.p.: 226 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.69 (s, 3H, -OCH_3), 6.38 (s, 1H, H pyran), 6.76-8.14 (m, 15H, H_{arom}), 8.58 (s, 1H, $H_{3'}$), 9.13 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 36.8, 55.1, 101.9, 103.7, 114.0, 115.0, 115.9, 117.5, 123.5, 125.2, 127.3, 128.6, 129.2, 129.4, 129.9, 130.3, 130.8, 131.7, 132.1, 132.6, 134.7, 138.2, 148.5, 149.0, 153.5, 154.1, 158.6, 163.4. HRMS $[M+H]^+$ calcd. for $(C_{32}H_{22}N_5O_2)^+$: 508.1773, found: 508.1779.

(E)-3'-(4-methylphenyl)-2'-(14-(4-methoxyphenyl) -14*H*-naphtho[2,1-b]pyrano[3,2-e] [1,2,4]triazolo [1,5-c]pyrimidin-2-yl) acrylonitrile (6g): White

solid. Yield: 92%. M.p.: 258 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.47 (s, 3H, -CH_3), 3.69 (s, 3H, -OCH_3), 6.36 (s, 1H, H pyran), 6.76-8.03 (m, 14H, H_{arom}), 8.53 (s, 1H, $H_{3'}$), 9.10 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.7, 36.8, 55.1, 100.4, 103.6, 114.0, 115.0, 116.2, 117.5, 123.5, 125.2, 127.3, 128.6, 129.4, 129.8, 129.9, 130.0, 130.4, 130.8, 131.6, 134.7, 138.1, 143.2, 148.5, 149.0, 153.4, 154.1, 158.6, 163.6. HRMS $[M+H]^+$ calcd. for $(C_{33}H_{24}N_5O_2)^+$: 522.1930, found: 522.1921.

(E)-3'-phenyl-2'-(14-(4-ethylphenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acrylonitrile (6h): White solid. Yield: 90%. M.p. 232 °C (ethanol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.13 (t, 3H, -CH_3, *J*= 7.5 Hz), 2.52 (q, 2H, -$\underline{CH_2}$-CH_3, *J*= 7.5 Hz), 6.40 (s, 1H, H pyran), 7.06-8.14 (m, 15H, H_{arom}), 8.58 (s, 1H, $H_{3'}$), 9.12 (s, 1H, H pyrimidine). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 15.0, 28.2, 37.3, 102.0, 103.7, 115.0, 115.9, 117.5, 123.5, 125.2, 127.3, 128.1, 128.2, 128.6, 129.1, 129.8, 130.3, 130.9, 131.7, 132.0, 132.7, 138.2, 139.7, 143.2, 148.6, 148.9, 153.5, 154.2, 163.5. HRMS $[M+H]^+$ calcd. for $(C_{33}H_{24}N_5O)^+$: 506.1981, found: 506.1990.

General procedure for preparation of (E)-3'-(dimethylamino)-2'-(14-aryl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo[1,5-c] pyrimidin-2-yl) acrylonitrile (7b,d).

A mixture of compounds **4b,d** (10 mmol), N,N-dimethylformamide dimethylacetal (DMF-DMA) (3 mL) in dioxane (25 mL) was stirred at reflux for 8 h. The solvent was evapored and the residue was treated with hexane/CH_2Cl_2 : 9/1 to give compounds **7b,d**.

(E)-3'-(dimethylamino)-2'-(14-(4-chlorophenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e] [1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acrylonitrile (7b): Yellow solid. Yield: 64%. M.p.: 298 °C. ^{1}H NMR (DMSO -d_6, 300 MHz): δ 3.35 (s, 6H, -$N(CH_3)_2$), 6.25 (s, 1H, H pyran), 7.26-8.04 (m, 10H, H_{arom}), 8.08 (s, 1H, $H_{3'}$), 9.40 (s, 1H, H pyrimidine). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.4, 65.7, 100.4, 114.2, 117.5, 118.4, 123.2, 125.1, 127.4, 128.4, 128.6, 130.0, 130.1, 130.2, 131.1, 131.5, 139.1, 141.8, 148.2, 152.1, 153.1, 153.9, 166.4. HRMS $[M+H]^+$ calcd. for $(C_{27}H_{20}ClN_6O)^+$: 479.1387, found: 479.1396.

(E)-3'-(dimethylamino)-2'-(14-(4-methoxyphenyl) -14*H*-naphtho[2,1-b]pyrano[3,2-e] [1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acrylonitrile (7d): White solid. Yield: 71%. M.p.: 282 °C. ^{1}H NMR (DMSO -d_6, 300 MHz): δ 3.27 (s, 6H, (-CH_3)$_2$), 3.69 (s, 3H, -OCH_3), 6.31 (s, 1H, H pyran), 6.75-7.96 (m, 10H, H_{arom}), 8.48 (s, 1H, $H_{3'}$), 9.40 (s, 1H, H pyrimidine). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.0, 54.8, 65.7, 101.3, 113.8, 115.0, 117.4, 118.5, 123.3, 125.0, 127.2, 128.5, 129.1, 129.7, 130.3, 131.0, 135.1, 138.7, 148.0, 152.2, 153.0, 153.8, 157.6, 166.3. HRMS $[M+H]^+$ calcd. for $(C_{28}H_{23}N_6O_2)^+$: 475.1882, found: 475.1894.

Preparation of N'-hydroxy-2'-(14-aryl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acetimidamide (8a-e).

A mixture of compounds **6** (1 mmol), excess of hydroxylamine hydrochloride (10 mmol) and piperidine (10 mmol) in ethanol was refluxed for 20 h. After cooling, the mixture diluted with water and left to stand at room temperature for 1h, the precipitated solid collected by filtration, then recrystallized and washed several times from ethanol to give **8a-e**.

N'-hydroxy-2'-(14-phenyl)-14*H*-naphtho[2,1-b] pyrano[3,2-e][1,2,4]triazolo[1,5-c] pyrimidin-2-yl) acetimidamide (8a): Gray solid. Yield: 65%. M.p. >300 °C (ethanol). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.63 (s, 2H, -CH_2), 5.52 (s, 2H, -NH_2), 6.30 (s, 1H, H_{14}), 7.06-8.11 (m, 11 H, H_{arom}), 9.10 (s, 1H, H_5), 9.59 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 31.0, 36.8, 102.1, 115.0, 117.5, 123.5, 125.1, 126.9, 127.3, 128.1, 128.5, 129.8, 130.2, 131.1, 139.8, 143.1, 147.9, 148.9, 152.3, 153.1, 165.7. HRMS $[M+H]^+$ calcd. for $(C_{24}H_{19}N_6O_2)^+$: 423.1569, found: 423.1579.

N'-hydroxy-2'-(14-(4-chlorophenyl)-14*H*-naphtho [2,1-b]pyrano[3,2-e][1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acetimidamide (8b): Gray solid. Yield: 70%. M.p. >300 °C (ethanol). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.63 (s, 2H, -CH_2-), 5.52 (s, 2H, -NH_2), 6.35 (s, 1H, H_{14}), 7.26-8.08 (m, 10 H, H_{arom}), 9.07 (s, 1H, H_5), 9.59 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 31.0, 36.3, 101.6, 114.4, 117.5, 123.4, 125.2, 127.4, 128.5, 128.6, 130.0, 130.1, 130.2, 131.1, 140.0, 141.9, 147.9, 148.9, 152.2, 153.0, 165.7. HRMS $[M+H]^+$ calcd. for $(C_{24}H_{18}ClN_6O_2)^+$: 457.1180, found: 457.1169.

N'-hydroxy-2'-(14-(4-methylphenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acetimidamide (8c): Gray solid. Yield: 59%. M.p. >300 °C (ethanol). ^{1}H NMR (C_5D_5N-d_5, 300 MHz): δ 1.26 (s, 3H, -CH_3), 3.43 (s, 2H, -CH_2-), 5.59 (s, 2H, -NH_2), 5.71 (s, 1H, H_{14}), 6.20-7.50 (m, 10H, H_{arom}), 7.96 (s, 1H, H_5), 10.80 (s, 1H, -OH). ^{13}C NMR (C_5D_5N-d_5, 75

MHz): δ 22.3, 33.9, 39.3, 105.1, 117.5, 119.6, 125.7, 125.9, 127.1, 129.3, 130.4, 130.7, 131.2, 131.8, 133.1, 133.6, 138.5, 141.0, 142.4, 150.7, 155.0, 155.5, 168.2. HRMS $[M+H]^+$ calcd. for $(C_{25}H_{21}N_6O_2)^+$: 437.1726, found: 437.1724.

N'-hydroxy-2'-(14-(4-methoxyphenyl)-14*H*-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo [1,5-c] pyrimidin-2-yl) acetimidamide (8d): Gray solid. Yield: 66%. M.p. >300 °C (ethanol). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.61 (s, 3H, -OCH_3), 3.63 (s, 2H, -CH_2-), 5.51 (s, 2H, -NH_2), 6.25 (s, 1H, H_{14}), 6.75-8.10 (m, 10 H, H_{arom}), 9.08 (s, 1H, H_5), 9.57 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 31.0, 36.0, 54.9, 102.4, 113.9, 115.2, 117.5, 123.5, 125.0, 127.3, 128.5, 129.1, 129.7, 130.3, 131.1, 135.2, 139.7, 147.8, 149.0, 152.3, 152.9, 158.0, 165.7. HRMS $[M+H]^+$ calcd. for $(C_{25}H_{21}N_6O_3)^+$: 453.1675, found: 453.1685.

N'-hydroxy-2'-(14-(4-ethylphenyl)-14*H*-naphtho [2,1-b]pyrano[3,2-e][1,2,4]triazolo[1,5-c] pyrimidin-2-yl) acetimidamide (8e): Gray solid. Yield: 62%. M.p. >300 °C (ethanol). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.03 (t, 3H, -CH_3, *J*= 8.7 Hz), 2.43 (q, 2H, -$\underline{CH_2}$-CH_3, *J*= 8.7 Hz), 3.61 (s, 2H, -CH_2-), 5.52 (s, 2H, -NH_2), 6.28 (s, 1H, H_{14}), 7.00-8.12 (m, 10H, H_{arom}), 9.09 (s, 1H, H_5), 9.59 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 15.2, 27.5, 31.0, 36.4, 102.3, 115.2, 117.5, 123.5, 125.1, 127.3, 127.9, 128.5, 129.8, 130.2, 131.1, 139.8, 140.4, 142.3, 147.9, 148.9, 152.3, 153.0, 165.7. HRMS $[M+H]^+$ calcd. for $(C_{26}H_{23}N_6O_3)^+$: 451.1882, found: 451.1871.

3. Biological evaluation

All the synthesized compounds have been evaluated for their antibacterial and antifungal activities. For the antibacterial test, four bacterial agents were selected as test microorganisms, namely *Pseudomonas syringae pv syringae, P. savastanoi, P. huttiensis* and *Agrobacterium tumefasciens*. These bacteria were obtained from the Laboratory of phytopathology of the Regional Center of Research in Horticulture and Organic Agriculture (CRRHAB) of Chott-Mariem, Tunisia. They were cultured at 25°C on Nutrient Agar (NA) medium for 48 h before use. Antifungal activity was performed against *Aspergillus flavus, A. niger Penicillium digitatum, p. italicum* and *Trichoderma harzianum*. These fungi were obtained from the Laboratory of Phytopathology of the Regional Center of Research in Horticulture and Organic Agriculture (CRRHAB) of Chott-Mariem, Tunisia. They were cultured at 25°C on potato dextrose agar (PDA) medium one week before use. The screening results were compared with those of ampicillin and carbendazim used as standard references (antibiotic and benzimidazol fungicide) for the antibacterial and the antifungal activity, respectively.

3.1. Antibacterial activity

The purified products were screened for their antibacterial activity by using the agar disc diffusion method Marmonier [18]. NA medium cooled at 45°C was supplemented with a bacterial suspension (10^6 CFU/mL) and poured into Petri plates. After solidification, sterile Whatman paper discs (diameter 6 mm) were placed at the surface of the culture medium and 20 µL (1000 µg/mL) of the product dissolved in DMSO was dropped onto each disc. The negative control plates had no product added to the filter paper whereas in the positive control plates, discs were impregnated with the same volume of ampicillin solution (5 mg/ mL). The treated Petri dishes were incubated at 25°C for 48 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones formed around the discs. The experiment was replicated twice.

3.2. Antifungal activity

The products tested were screened for their antifungal activity using the disc diffusion method [19]. A conidial suspension of the tested fungi was prepared (10^4-10^5 CFU/mL) and added to PDA medium cooled at 45°C and supplemented with streptomycin sulfate (300 mg/ml), and poured uniformly into Petri plates (diameter 90 mm). Sterilized paper discs (6 mm, Whatman No. 1 filter paper) were impregnated with 20 µL (1000 µg/mL) of the product dissolved in DMSO and placed on the culture plates whereas the negative control plates had no product added to the filter paper. In the positive control plates, discs were imbibed with the same volume of a carbendazim suspension (0.5 mg/mL, benzimidazol fungicide, commercial source Bavistin). The diameter of the inhibition zone (mm) around the disc was measured after incubation at 25°C for 4 days and compared with control. The test was performed in triplicate.

Acknowledgement: The authors acknowledge the Ministry of Higher Education, Scientific Research and Technology of Tunisia for its financial support.

REFERENCES

[1] M. Jha, S. Guy, Y. C. Ting, *Tetrahedron Lett.* **2011,** 52, 4337-4341.

[2] R. Sagar, K. Moon-Ju, S. B. Park, *Tetrahedron Lett*. **2008,** 49, 5080-5083.

[3] U. S. Rai, A. M. Isloor, P. Shetty, A. M. Vijesh, N. Prabhu, S. Isloor, M. Thiageeswaran, F. Hoong-Kun, *Eur. J. Med. Chem.* **2010,** 45, 2695-2699.

[4] T. Asami, Y. K. Min, N. Nagata, K. Y. amagishi, S. Takatsuto, S. Fujioka, N. Murofushi, I. Yamaguchi, S. Yoshida, *Plant Physiol*. **2000,** 123, 93-100.

[5] S. M. I. Badr, R. M. Barwa, *Bioorg. Med. Chem.* **2011,** 19, 4506-4512.

[6] M. Amir, H. Kumar, S. A. Javed, *Eur. J. Med. Chem.* **2008,** 43, 2056-2066.

[7] I. Kucukguzel, S. G. Kucukguzel, S. Rollas, M. Kiraz, *Bioorg. Med. Chem. Lett.* **2001,** 11, 1703-1707.

[8] M. Kritsanida, A. Mouroutsou, P. Marakos, N. Pouli, G. S. Papakonstantinou, C. Pannecouque, M. Witvouw, E. DeClercq, *Il Farmaco,* **2002,** 57, 253-257.

[9] M. Amir, S. Kumar, *Acta. Pharm*. **2007**, 57, 31-45.

[10] S. P. Aytac, B. Tozkaparan, F. B. Kaynak, G. Aktay, O. Goktas, S. Unuvar, *Eur. J. Med. Chem*. **2009,** 44, 4528-4538.

[11] K. Sztanke, T. Tuzimski, J. Rzymowska, K. Pasternak, M. K-Szerszen, *Eur. J. Med. Chem*. **2008,** 43, 404-419.

[12] A. M. Isloor, B. Kalluraya, M. Rao, A. M. Rahiman, *J. Saudi Chem. Soc.* **2000,** 4, 265-270.

[13] B. Kalluraya, A. M. Isloor, S. Shenoy, *Indian J.Heterocycl.Chem.* **2001,** 11, 159-162.

[14] B. Kalluraya, A. M. Isloor, F. V. Priya, R. L. Jagadeesha, *Indian J. Heterocycl. Chem.* **2004,** 13, 245-248.

[15] K. Metin, O. Cahit, E. P. Akif, S. Abdurrazak, K. Pelin, K. Mustapha, D. D. Sevgi *Eur. J. Med. Chem.* **2013,** 63, 340-346.

[16] A. Romdhane, J. F. Gallard, M. A. Hamza, H. B. Jannet, *Phosphorus, Sulfur, and Silicon and the Relat. Elem.* **2012,** 187, 612-618.

[17] A. B. Said, A. Romdhane, N. Elie, D. Touboul, H. B. Jannet, *Lett. Org. chem.* **2013,** 10, 185-190.

[18] A. Marmonier, Antibiotiques Technique de diffusion en gélose méthode des disques. *Bactériologie médicale : Techniques usuelles* (SIMEP SA-Paris France) **1987**, p. 237-243.

[19] AL. Barry, C. Thornsberry, Susceptibility test: diffusion test procedures. In: Balows A. Hausler, W.J. Herramann, H.D. Isenberg, H.J. Shadomy, Eds Manual of clinical microbiology, American society for microbiology, Washington, DC, **1991**.

[20] A. H. E Abdel-Wahab, *Pharmaceuticals,* **2012**, 5, 745-757.

[21] M. Kamar, C. Faker, S. Abdelouahid, L. M. C. José, S. Mansour, *Synth. Commun.* **2010,** 40, 3405-3414.

[22] H. Z. Shams, R. M. Mohareb, M. H. Helal, A. E. Mahmoud, *Molecules.* **2011,** 16, 52-73.

[23] S. Bondock, S. Adel, H. A. Etman, F. A. Badria, *Eur. J. Med. Chem.* **2012,** 48, 192-199.