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RESEARCH ARTICLE

Synthesis and characterization of some hydroxypyridone derivatives and their evaluation as antimicrobial agents

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Abstract

The synthesis, *in vitro* antimicrobial activities of some novel hydroxy pyridines supported with various pharmacophores is described. Twenty-six out of the tested 58 compounds exhibited variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacteria. The tested compounds revealed better activity against the Gram positive rather than the Gram negative strains. The synthesized hydroxypyridones have shown very significant inhibitory effect against *Staphylococcus aureus* and *Bacillus subtilis*. Twelve compounds namely; **5d, 5f, 6a, 6b, 8b, 18b, 18c, 19c, 21d, 22b, 22d** and **23d** were able to produce appreciable growth inhibitory activity against *Candida albicans* when compared to Clotrimazole. Among these, **22d** proved to be the most potent antifungal agent.

Keywords: Synthesis, hydroxypyridones, antibacterial, antifungal

Introduction

For the last 10 years, we have established a research program aiming at the synthesis and characterization of different heterocyclic ring systems endowed with potential chemotherapeutic activities[1–11]. A wide range of biological activities have been ascribed to pyridine derivatives including antimicrobial[12,13], antitubercular[14,15], antiamoebic[16], antiparasitic[17,18], antimalarial[19] and antiviral[20,21] activities. Particular interest has been given to pyrones and pyridine ring systems[3,6,8,10] which have been subjected to various chemotherapeutic investigations either as antimicrobial, antiviral or anticancer agents. The results revealed that some of the newly synthesized derivatives exhibited promising broad spectrum antitumor and antimicrobial activities. The substitution pattern of the target compounds include various functionalities such as the amino, derived imine, ureido, thioureido and sulfonamido groups that are reported to contribute to various chemotherapeutic activities. It was considered also of interest to cyclize some of the thioureido derivatives to the corresponding thiazolidinones and thiazolines which are known for their potential chemotherapeutic activities. Such structure hybridization was planned with the hope of adding some synergism to the anticipated bioactivities of the target molecules. The variation in the nature and size of substituents at the prementioned functionalities was thought to be of interest as it would offer variable electronic, lipophilic and steric environment that would influence the targeted biological activities. In view of the above-mentioned facts, and in continuation of our interest in studying the synthesis and biological properties of some heterocyclic ring systems as novel structure leads that might be of use in designing new potent and selective chemotherapeutic agents, it was thought worthwhile to synthesize and investigate anticancer and antimicrobial activities of some novel hydroxypyridine derivatives. The target compounds have been subjected to the NCI *in vitro* disease-oriented

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human cells screening panel assay[22–24] and *in vitro* antibacterial and antifungal screening[25] tests.

Experimental

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard (Chemical shifts in δ, ppm). $CDCl_3$ was used as the solvent however, in case of insoluble compounds $CDCl_3$ and few drops of DMSO-d_6 was used. Splitting patterns were designated as follows: *s*: singlet; *m*: multiplet. Mass spectra were measured on a GCM-Q 1000 Ex spectrometer. The IR and mass spectra of some compounds are recorded in Supplementary File S8. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow-up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminium sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254. The starting compounds 4-hydroxy-6-methylpyran-2-one (**1**) and 3-hydroxy-2-methylpyran-4-one (**16**) have been purchased from ACROS Organics Geel-Belgium.

1-amino-4-hydroxy-6-methyl-1H-pyridin-2-one (2)

A solution of 4-hydroxy-6-methyl-2-pyrone **1** (1.26 g, 10 mmol) in ethanol was refluxed with hydrazine hydrate (0.75 mL, 15 mmol) for 1h. The reaction mixture was then cooled, poured into ice cold water; the solid which separated was recrystallized from ethanol as needles. Physicochemical and analytical data are listed in Supplementary File S1. ^{1}H &^{13}C-NMR spectral data are recorded in Supplementary Files S4 and S7, respectively.

N-(4-hydroxy-6-methyl-2-oxo-2H-pyridin-1-yl) substituted sulfonamides (3)

A mixture of the 1-Amino-4-hydroxy-6-methyl-1H-pyridin-2-one **2** (1.4 g, 10 mmol) and appropriate sulfonyl chloride (10 mmol) in pyridine (10 mL) was heated under reflux for 4 h. After cooling to room temperature, the reaction mixture was poured on crushed ice and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol.

N^1-(4-hydroxy-6-methyl-2-oxo-2H-pyridin-1-yl)-N^3-substituted urea (4) and thiourea derivatives (5)

A mixture of **2** (10 mmol) and anhydrous K_2CO_3 (20 mmol) in dry acetone (25 mL) was stirred and treated with the appropriate isocyanate or isothiocyanate (12 mmol). After the mixture was stirred and refluxed for 18 h, acetone was removed under pressure, and the solid mass dissolved in water and acidified with 2 N HCl. The crude product was purified by recrystallization from ethanol as needles.

4-Hydroxy-6-methyl-1-(4-oxo-3-substituted thiazolidin-2-ylideneamino)-1H-pyridin-2-one (6)

A mixture of **5** (10 mmol), ethyl bromoacetate (10 mmol) and sodium acetate (20 mmol) in absolute ethanol (30 mL) was refluxed for 2 h. The reaction mixture was then filtered while hot, concentrated and allowed to cool. The product obtained was recrystallized from ethanol as needles.

1-(3-substituted-4-phenyl-3H-thiazol-2-ylideneamino)-4-hydroxy-6-methyl-1H-pyridin-2-one (7)

A solution of the corresponding thiourea derivative **5** (10 mmol) in absolute ethanol (25 mL) was refluxed with phenacyl bromide (10 mmol) and sodium acetate (20 mmol) for 2 h. The reaction mixture was then cooled and poured into water; the precipitated thiazoline derivative was recrystallized from ethanol as needles.

1-(Arylideneamino)-4-hydroxy-6-methyl-1H-pyridin-2-one (8)

1-Amino-4-hydroxy-6-methyl-1H-pyridin-2-one **2** (1.4 g, 10 mmol) was refluxed with the appropriate aldehyde (10 mmol) for 3 h. The reaction mixture was then concentrated and allowed to cool. The product obtained was recrystallized from ethanol as needles.

4-Hydroxy-6-methyl-1-(4-oxo-2-substituted thiazolidin-3-yl)-1H-pyridin-2-one (9)

A mixture of the appropriate arylidene derivative **8** (10 mmol) and thioacetic acid (15 mmol) in dry dioxan (25 mL) was refluxed for 12 h, cooled then poured onto aqueous K_2CO_3. The obtained solid was recrystallized from ethanol as needles.

p-(4-Hydroxy-6-methyl-2-oxo-2H-pyridin-1-yl) benzenesulfonamide (10)

A solution of 4-hydroxy-6-methyl-1H-pyridin-2-one **2** (2.5 g, 20 mmol) in ethanol (20 mL) was refluxed with *p*-hydrazinobenzenesulfonamide hydrochloride (4.9 g, 22 mmol) for 4 h. On concentration, the separated product was filtered, washed with cold ethanol and recrystallized from a mixture of ethanol as needles. Physicochemical and analytical data are listed in Supplementary File S2. ^{1}H & ^{13}C-NMR spectral data are recorded in Supplementary Files S5 and S7, respectively.

p-(4-Hydroxy-6-methyl-2-oxo-2H-pyridin-1-yl)benzenesulfonylureas (11a,b; X = O) and benzenesulfonylthioureas (11c; X = S)

A mixture of the pyridine derivative **10** (10 mmol) and anhydrous K_2CO_3 (20 mmol) in dry acetone (25 mL) was stirred and treated with the appropriate isocyanate (12 mmol). After the mixture was stirred and refluxed

for 18 h, acetone was removed under pressure, and the solid mass dissolved in water and acidified with 2 N HCl. The crude product was purified by recrystallization from ethanol as needles.

4-hydroxy-6-methyl-1H-pyridin-2-one (12)

To an ice-cooled stirred solution of 1-Amino-4-hydroxy-6-methyl-1H-pyridin-2-one **2** (1.4g, 10 mmol) in acetic acid (15 mL), was added dropwise, a solution of sodium nitrite (1.05 g, 15 mmol) in water (5 mL) over a period of 2 h. Stirring was maintained for further 2 h, and the reaction mixture was then poured into water; the precipitated solid product was filtered, washed with water, dried and recrystallized from ethanol as needles.

1-Substituted sulfonyl-4-hydroxy-6-methyl-1H-pyridin-2-one (13)

A mixture of the 4-hydroxy-6-methyl-1H-pyridin-2-one **12** (1.1 g, 10 mmol) and appropriate sulfonyl chloride (10 mmol) in pyridine (10 mL) was heated under reflux for 4 h. After cooling to room temperature, the reaction mixture was poured on crushed ice and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol.

1-Substituted carbamoyl-4-hydroxy-6-methyl-1H-pyridin-2-one (14) and 1-substituted thiocarbamoyl-4-hydroxy-6-methyl-1H-pyridin-2-one (15)

A mixture of 4-hydroxy-6-methyl-1H-pyridin-2-one **12** (1.1g, 10 mmol) and anhydrous K_2CO_3 (20 mmol) in dry acetone (25 mL) was stirred and treated with the appropriate isocyanate or isothiocyanate (12 mmol). After the mixture was stirred and refluxed for 18 h, acetone was removed under pressure, and the solid mass dissolved in water and acidified with 2 N HCl. The crude product was purified by recrystallization from ethanol as needles.

4-(3-Hydroxy-2-methyl-4-oxo-4H-pyridin-1-yl) benzenesulfonamide (17)

A solution of 3-hydroxy-2-methylpyran-4-one **16** (2.5 g, 20 mmol) in ethanol (20 mL) was refluxed with *p*-hydrazinobenzenesulfonamide hydrochloride (4.9 g, 22 mmol) for 4 h. On concentration, the separated product was filtered, washed with cold ethanol and recrystallized from a mixture of ethanol as needles. Physicochemical and analytical data are listed in Supplementary File S3. ^{1}H & ^{13}C-NMR spectral data are recorded in Supplementary Files S6 and S7, respectively.

N-Arylidene-4-(3-hydroxy-2-methyl-4-oxo-4H-pyridin-1-yl)benzenesulphonamides (18)

4-(3-Hydroxy-2-methyl-4-oxo-4H-pyridin-1-yl)benzenesulfonamide **17** (2.8 g, 10 mmol) was refluxed with the appropriate aldehyde (10 mmol) for 3 h. The reaction mixture was then concentrated and allowed to cool. The product obtained was recrystallized from ethanol as needles.

3-Hydroxy-2-methyl-1-[4-(4-oxo-2-phenylthiazolidine-3-sulfonyl)phenyl]-1H-pyridin-4-one (19)

A mixture of the appropriate arylidene derivative **18** (10 mmol) and thioacetic acid (15 mmol) in dry dioxan (25 mL) was refluxed for 12 h, cooled then poured into aqueous K_2CO_3. The obtained solid was recrystallized from ethanol as needles.

p-(3-Hydroxy-2-methyl-4-oxo-4H-pyridin-1-yl) benzenesulfonylureas (20) and thioureas (21)

A mixture of the 4-pyrone derivative **17** (10 mmol) and anhydrous K_2CO_3 (20 mmol) in dry acetone (25 mL) was stirred and treated with the appropriate isocyanate (12 mmol). After the mixture was stirred and refluxed for 18 h, acetone was removed under pressure, and the solid mass dissolved in water and acidified with 2 N HCl. The crude product was purified by recrystallization from ethanol as needles.

4-(3-Hydroxy-2-methyl-4-oxo-4H-pyridin-1-yl)-*N*-(4-oxo-3-substituted thiazolidin-2-ylidene) benzenesulphonamide (22)

A mixture of the thiourea derivative **21** (10 mmol), ethyl bromoacetate (10 mmol) and sodium acetate (20 mmol) in absolute ethanol (30 mL) was refluxed for 2 h. The reaction mixture was then filtered while hot, concentrated and allowed to cool. The product obtained was recrystallized from ethanol as needles.

N-(3-substituted-4-phenyl-3H-thiazol-2-ylidene)-4-(3-Hydroxy-2-methyl-4-oxo-4H-pyridin-1-yl) benzenesulphonamide (23)

A solution of the corresponding thiourea derivative **21** (10 mmol) in absolute ethanol (25 mL) was refluxed with α-bromoacetophenone (10 mmol) and sodium acetate (20 mmol) for 2 h. The reaction mixture was then cooled and poured into water; the precipitated thiazoline derivative was recrystallized from ethanol as needles.

Biological activity

In vitro *antibacterial and antifungal activities*

Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/mL) was placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (NRRL B-14819) and *Micrococcus luteus* (ATCC 21881) as examples of Gram positive bacteria and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (clinical isolate) as examples of Gram negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (recultured) fungal strains were utilized as representatives for fungi. Ampicillin trihydrate and Clotrimazole were used as standard antibacterial and antifungal agents, respectively.

DMSO alone was used as control at the same above-mentioned concentration. The plates were incubated at 37°C for 24 h for bacteria and for 7 days for fungi. Compounds that showed significant growth inhibition zones (≥14 mm) using the two-fold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

Minimal inhibitory concentration measurement

The micro-dilution susceptibility test in Müller–Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively[25]. Stock solutions of the tested compounds, Ampicillin trihydrate and Clotrimazole were prepared in DMSO at concentration of 800 μg/mL followed by two-fold dilution at concentrations of (400, 200,.....6.25 μg/mL). The microorganism suspensions at 10^6 CFU/mL (Colony Forming Unit/mL) concentrations were inoculated to the corresponding wells. Plates were incubated at 36°C for 24–48 h and the MICs were determined. Control experiments were also done.

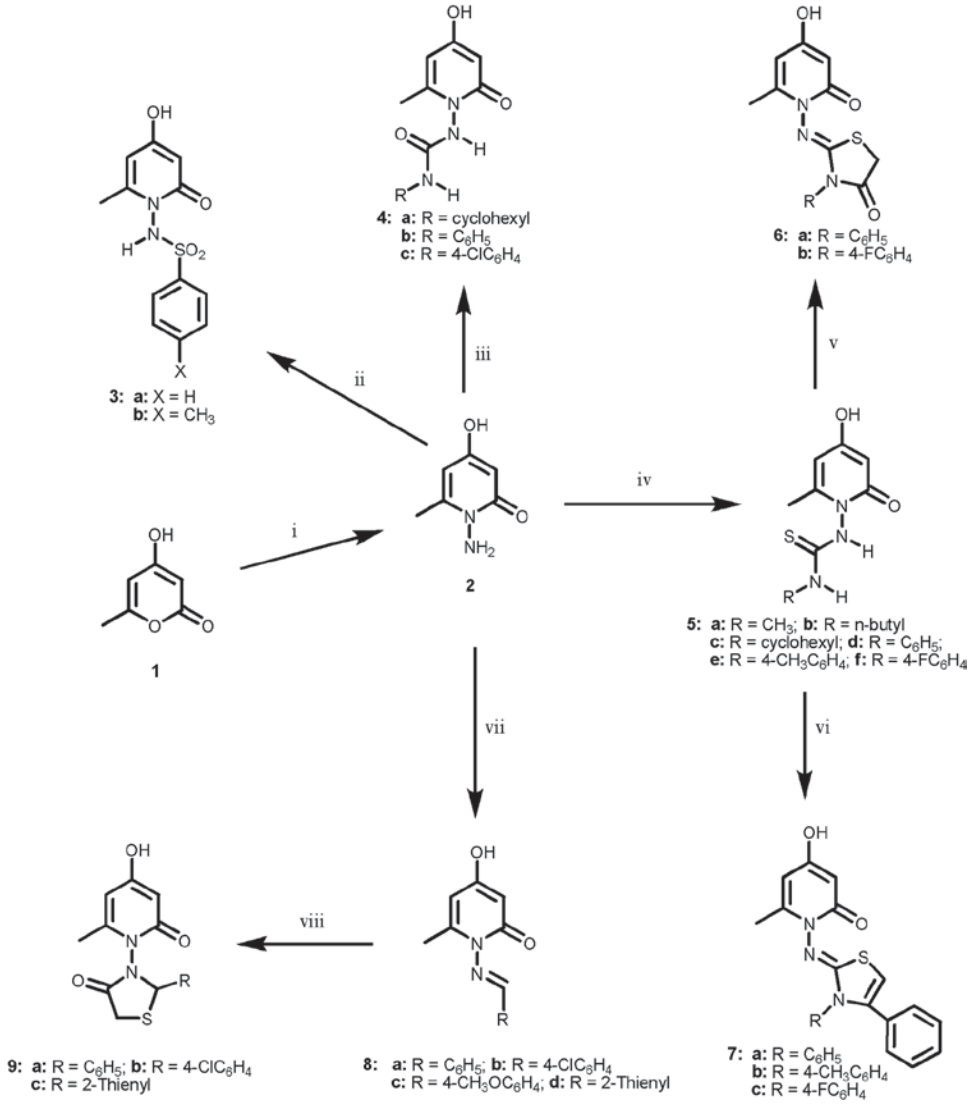
Preliminary in vitro *anticancer screening*

Out of the newly synthesized compounds, 20 derivatives namely; **5c,d,f, 6a,b, 7c, 8b, 9b, 11c, 15a, 18b, 19b, 20b, 21a,b,d, 22b,d** and **23b,d;** were selected by the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay to be evaluated for their *in vitro* antitumor activity. Primary *in vitro* one-dose anticancer assay was performed using the full NCI 60 cell panel in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda. These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48 h continuous drug exposure protocol was used, and a Sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth.

Results and discussion

Chemistry

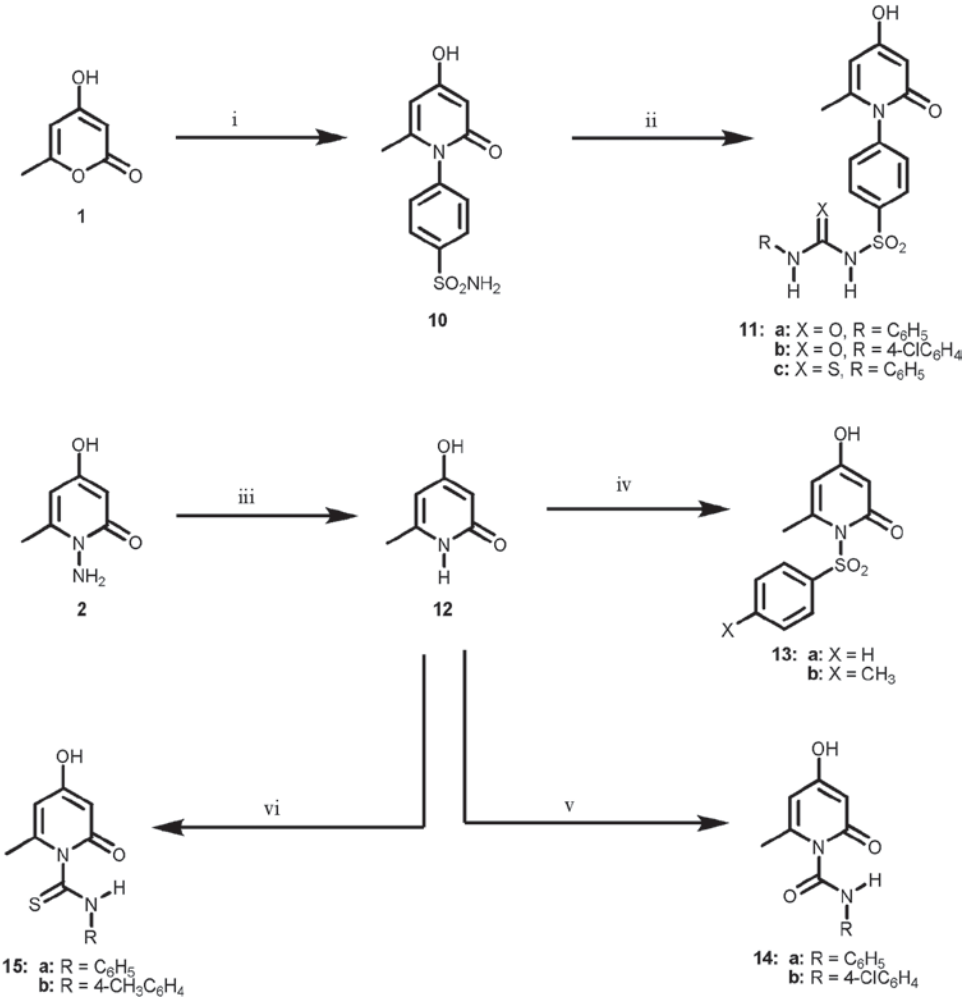
The synthetic strategies adopted for the preparation of the intermediate and target compounds are described in Schemes 1–3. In Scheme 1, the key intermediates 4-hydroxy-6-methylpyran-2-one **1** was reacted with hydrazine hydrate to afford the desired 1-amino-4-hydroxy-6-methylpyridin-2-one **2** which was utilized as the key intermediate in this part. The IR spectrum revealed strong carbonyl absorption at 1651 cm^{-1} as well as two bands at 2873–2936 cm^{-1} and 3121–3279 cm^{-1} for the OH and NH_2 groups, respectively. The ^{1}H-NMR spectrum displayed two singlets at δ 5.88 and δ 5.48 for H-3 and H-5, respectively (Supplementary File S4). The structure was further confirmed from its ^{13}C-NMR spectra (Supplementary File S7). Reaction of the amine **2** with benzenesulfonyl chloride and *p*-toluenesulfonyl chloride in pyridine gave the corresponding *N*-substituted benzenesulfonylamino derivatives **3**. Moreover, condensation of **2** with substituted isocyanates or isothiocyanates in pyridine as alkaline medium, afforded the corresponding substituted ureido and thioureido analogs **4** and **5**. The IR spectra of these compounds showed beside the 2H-pyran carbonyl absorption at 1652–1663 cm^{-1}, two bands at 1310–1366 cm^{-1} and 1163–1178 cm^{-1} due to SO_2N function as well as distinguishable urea carbonyl absorption at 1665–1672 cm^{-1} for **4** and thiourea carbonyl absorption at 1125–1155 cm^{-1} in case of compound **5**. The structures were further confirmed from their ^{1}H-NMR and ^{13}C-NMR spectra (Supplementary Files S4 and S7). Cyclization of the thiouriedo derivatives **5** with ethyl bromoacetate in the presence of anhydrous sodium acetate afforded the corresponding thiazolidin-4-ones **6**. The IR of these compounds was characterized by a strong band at 1720–1735 cm^{-1} for thiazolidine carbonyl, whereas, their ^{1}H-NMR spectra revealed a new singlet at δ 4.02–4.11 ppm attributed to the oxothiazolidine H-5 (Supplementary File S4). The ^{13}C-NMR spectrum of **6a** exhibited the C-5 of the thiazolidine at δ 32.7 (Supplementary File S7). Analogously, reacting the same derivatives **5** with phenacyl bromide under similar reaction conditions resulted in the formation of the desired 1,3-thiazolines **7**. Their ^{1}H-NMR spectra showed a broad singlet at δ 6.29–6.55 ppm due to thiazoline H-5 proton. Finally, condensation of the key intermediate **2** with different aldehyde gave corresponding 1-(arylideneamino)-4-hydroxy-6-methylpyridin-2-ones **8**. The IR spectra of these arylidenes **8** revealed strong 2-pyridone carbonyl absorption at 1655–1660 cm^{-1}, their ^{1}H-NMR spectra revealed a new singlet at δ 7.92–8.16 ppm attributed to the CH=N proton (Table 4). Reaction of arylidene derivatives **8** with mercaptoacetic acid yielded the corresponding thiazolidinone derivatives **9**. Their IR spectra revealed two strong absorptions at 1655–1664 cm^{-1} and 1720–1735 cm^{-1} for the 2H-pyran and thiazolidine carbonyls, respectively. The ^{1}H-NMR and ^{13}C-NMR spectra of the above prepared compounds were recorded in Supplementary Files S4 and S7. Reaction of the 4-hydroxy-6-methylpyran-2-one **1**, with 4-aminobenzenesulfonamide gave the target 1-(4-aminosulfonylphenyl)-4-hydroxy-6-methylpyridin-2-one **10**. The IR spectrum revealed two bands at 1151 cm^{-1} and 1324 cm^{-1} for the SO_2N function and a strong carbonyl absorption at 1669 m^{-1} as well as two bands at 2768–2845 cm^{-1} and 3287–3365 cm^{-1} for the OH and NH_2 groups, respectively. Reaction of **10** with different isocyanates and isothiocyanates in the presence of pyridine afforded the corresponding substituted ureido and thioureido derivatives (**11**; X = O or S), respectively. The IR spectra of these compounds showed beside the 2H-pyran carbonyl absorption at 1669–1673 cm^{-1} two bands at 1324–1352 cm^{-1} and 1167–1179 cm^{-1} due to SO_2N function as well as distinguishable urea carbonyl absorption at 1665–1672 cm^{-1} for (**11**; X = O) and thiourea carbonyl absorption at 1126–1115 cm^{-1} in case of compound (**11**; X = S). The structures were further confirmed from their ^{1}H-NMR and ^{13}C-NMR spectra (Supplementary Files S5 and S7). However, reaction



Scheme 1. Reagents and reaction conditions: (i) ethanol, $NH_2NH_2{\cdot}H_2O$, ref lux, 1 h; (ii) arylsulfonyl chloride, pyridine, ref lux, 4 h; (iii) anhyd. K_2CO_3, dry acetone, RNCO, ref lux, 18 h; (iv) anhyd. K_2CO_3, dry acetone, RNCS, ref lux, 10 h; (v) ethyl bromoacetate, Na acetate, ethanol, ref lux 2 h; (vi) ethanol, phenacyl bromide, Na acetate, ref lux 2 h; (vii) approp. aldehyde, ref lux, 3 h; (viii) thioacetic acid, dry dioxan, ref lux 12 h.

of 1-amino-4-hydroxy-6-methylpyridin-2-one **2** with nitrous acid yielded the corresponding 4-hydroxy-6-methylpyridin-2-one **12**, which was employed as the key intermediate in this part. The IR spectrum of **12** exhibited strong carbonyl absorption at 1751 cm^{-1} as well as two bands at 2860 cm^{-1} and 3292 cm^{-1} for the OH and NH_2 groups, respectively. Reaction of **12** with benzenesulfonyl chloride or *p*-toluenesulfonyl chloride in the presence of pyridine, leads to the formation of the *N*-substituted benzenesulfonyl derivatives **13** (X = H or CH_3). Furthermore, condensation of the 2H-pyran-2-one **12** with isocyanate and isothiocyanate derivatives in pyridine afforded the corresponding 1-substituted carbamoyl and thiocarbamoyl derivatives **14** and **15**, respectively. The IR spectra of these compounds showed beside the 2H-pyran carbonyl absorption at 1662–1669 cm^{-1} a characteristic urea carbonyl absorption at 1654–1658 cm^{-1} for (**14**; X = O) and a thiourea carbonyl absorption at 1122–1136 cm^{-1} in case of compound (**15**; X = S). The ^{1}H-NMR and ^{13}C-NMR spectra of the above prepared compounds were recorded in Supplementary Files S5 and S7.

Likewise, amination of the target compound 3-hydroxy-2-methylpyran-4-one **16** with sulfanilamide afforded the corresponding 1-(4-aminosulfonylphenyl)-3-hydroxy-2-methylpyridin-4-one **17**, which was utilized as a key intermediate in Scheme 3. The IR spectrum of **17** revealed two bands at 1318 cm^{-1} and 1155 cm^{-1} for the SO_2N group and a strong carbonyl absorption at 1653 m^{-1} as well as two bands at 2868–2924 cm^{-1} and 3325–3372 cm^{-1} for the OH and NH_2 groups, respectively. Condensation of the 4-aminosulfonyl derivative **17** with different aldehydes gave the corresponding azomethines **18**. The IR spectra of these arylidenes showed strong carbonyl absorption at 1652–1656 cm^{-1}, while their ^{1}H-NMR spectra revealed a new singlet at δ 7.97–8.26 ppm attributed to the CH=N proton. Reaction of the arylidene derivatives **18** with mercaptoacetic acid afforded the



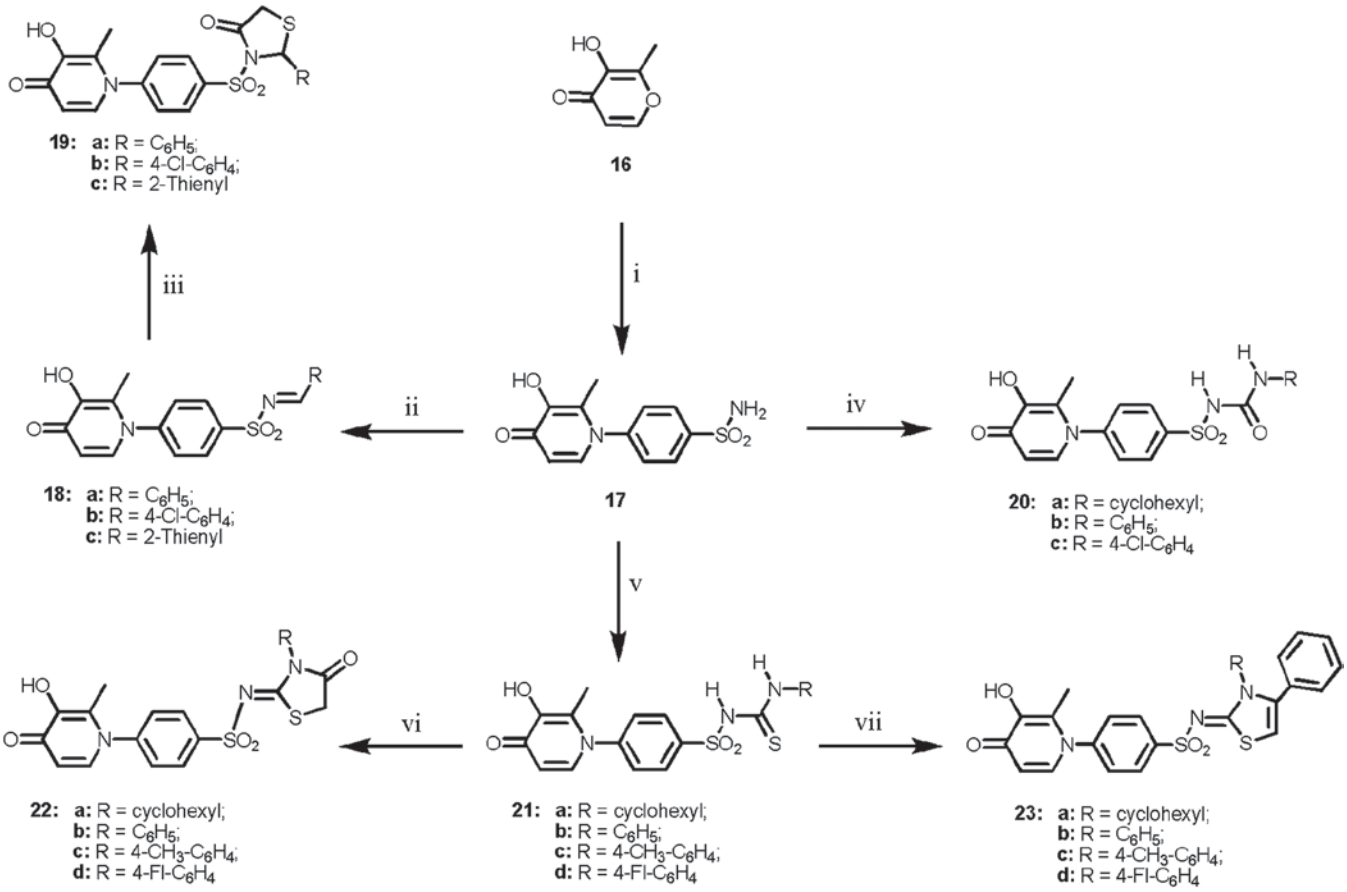
Scheme 2. Reagents and reaction conditions: (i) ethanol, sulfanilamide, ref lux, 4 h; (ii) anhyd. K_2CO_3, dry acetone, approp. RNCO or RNCS, ref lux, 10–18 h; (iii) $NaNO_2/CH_3COOH$, stirring, 2 + 2 h; (iv) arylsulfonyl chloride, pyridine, ref lux, 4 h; (v) anhyd. K_2CO_3, dry acetone, RNCO, ref lux, 18 h; (vi) anhyd. K_2CO_3, dry acetone, RNCS, ref lux, 10 h.

corresponding thiazolidinone derivatives **19**. Their IR spectra revealed two strong carbonyl absorption bands at 1658–1662 cm^{-1} and 1720–1735 cm^{-1} for the 4H-pyran and thiazolidinone moieties, respectively. Moreover, reaction of **17** with isocyanate and isothiocyanate derivatives in pyridine, gave the corresponding substituted sulfonylureido and sulfonylthioureido analogs **20** and **21**, respectively. The IR spectra of these compounds showed beside the 4H-pyran carbonyl absorption at 1651–1656 cm^{-1}, two bands at 1315–1377 cm^{-1} and 1168–1182 cm^{-1} due to SO_2N function as well as distinguishable urea carbonyl absorption at 1660–1667 cm^{-1} for **20** and thiourea carbonyl absorption at 1122–1165 cm^{-1} for compound **21**. Cyclization of the sulfonylthioureido derivatives **21** with ethyl bromoacetate in the presence of anhydrous sodium acetate afforded the corresponding thiazolidin-4-ones **22**. The IR of these compounds displayed a carbonyl band at 1718–1722 cm^{-1}, whereas, their ^{1}H-NMR spectra revealed a broad singlet at δ 4.11–4.18 ppm attributed to the oxothiazolidine H-5. The ^{13}C-NMR spectra of **22** exhibited the C-5 of the thiazolidine moity at δ 32.5–32.9 (Supplementary File S7). Analogously, reacting the same derivatives **21** with phenacyl bromide under similar reaction conditions resulted in the formation of the desired 1,3 -thiazolines **23**. Their ^{1}H-NMR spectra showed a new singlet at δ 6.25–6.34 ppm due to the thiazoline H-5 proton. The ^{1}H-NMR and ^{13}C-NMR spectra of **1–23** are recorded in Supplementary Files S6 and S7.

Biological evaluation

In vitro *antibacterial and antifungal activities*

The antibacterial activity of the synthesized compounds revealed that 26 out of the tested 58 compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains. In general, most of the compounds showed better activity against the Gram positive rather than the Gram negative bacteria. Among the Gram positive bacteria, *S. aureus* and *B. subtilis* showed relatively high sensitivity towards the tested compounds. In this view, compounds **6b** and **22d** were equipotent to Ampicillin (MIC 6.25 μg/mL) against *S. aureus,* whereas the analogs **5f, 6a 15a, 18b, 22b** and **23d** (MIC 12.5 μg/mL) were 50% less active than Ampicillin. Moreover, compounds **5c, 5d, 7a, 8b,**



Scheme 3. Reagents and reaction conditions: (i) sulfanilamide, ethanol, ref lux, 4 h; (ii) RCHO, ethanol, ref lux, 3 h; (iii) $HSCH_2COOH$, dry dioxan, ref lux, 12 h; (iv) RNCO, anhyd. K_2CO_3, dry acetone, ref lux 18 h; (v) RNCS, anhyd. K_2CO_3, dry acetone, ref lux 10 h; (vi) ethyl bromoacetate, anhyd. NaOAc, abs. ethanol, ref lux, 2 h; (vii) phenacyl bromide, anhyd. NaOAc, abs. ethanol, ref lux, 2 h.

9b, 11c, 18c and **21d** (MIC 25 μg/mL) showed 25% of the activity of Ampicillin against the same organism. With regard to the activity against *B. subtilis*, the best activity was displayed by compound **22d,** which was equipotent to Ampicillin (MIC 12.5 μg/mL), whereas the analogs **6b, 7a, 11c, 14b, 15a, 18b, 21d** and **22b** (MIC 25 μg/mL) which represented half the potency of Ampicillin. The analogs **5c, 5d, 5f, 6a, 8a, 9b, 18c, 19c, 21b** and **23d** (MIC 50 μg/mL) exhibited 25% of the potency of Ampicillin against the same species. *Micrococcus luteus* proved to be the least sensitive Gram positive microorganism towards most of the tested compounds. Only two compounds namely; **6b** and **22d** (MIC 25 μg/mL) exhibited moderate growth inhibitory effect towards *M. luteus* which was 50% of the activity of Ampicillin (MIC 12.5 μg/mL).

On the other hand, investigation of the antibacterial potency against the three tested Gram negative strains revealed that most of the active compounds showed better growth inhibitory activity against *E. coli* when compared with the other two strains. Compound was equipotent to Ampicillin (MIC 6.25 μg/mL), whereas the analogs **6b, 15a** and **21d** produced noticeable growth inhibitory activity (MIC 12.5 μg/mL) which was 50% of the activity of Ampicillin. Moreover, compounds **5f, 6a, 14b, 18b, 19c** and **23d** (MIC 25 μg/mL), exhibited moderate activity against the same organism. Meanwhile, the tested *P. aeruginosa* and *K. pneumoniae* strains proved to be weakly sensitive to most of the tested compounds. Among these, compound **22d** showed moderate growth inhibitory profile against *P. aeruginosa* (MIC 25 μg/mL), which was about 50% of the activity of Ampicillin (MIC 12.5 μg/mL).

Concerning the antifungal activity of the tested compounds, the results revealed that eleven compounds namely; **5d, 5f, 6a, 6b, 8b, 18b, 18c, 19c, 21d, 22b, 22d** and **23d** were able to produce appreciable growth inhibitory activity against *C. albicans* (MIC values 12.5–100 μg/mL, respectively) when compared to Clotrimazole (MIC 6.25 μg/mL). Among these, compound **22d** proved to be the most potent antifungal agent with MIC value 12.5 μg/mL and is 50% of Clotrimazole. The analogs **6b, 18b, 19c** and **21d** exhibited recognizable antifungal activity (MIC 25 μg/mL) that represented 25% of the standard's activity. It has been found that all of the tested compounds lacked antifungal activity against *A. niger* (Table 1).

A further investigation into the structures of the active compounds revealed that, the tested compounds belong to two main series namely; 4-hydroxy-6-methyl-2-oxo-1-substituted-2H-pyridines **2–15** (Schemes 1 and 2) and 3-hydroxy-2-methyl-4-oxo-1-substituted-4H-pyridines **17–23** (Scheme 3).

Table 1. Minimal inhibitory concentrations (MIC, μg/mL) of the active newly synthesized compounds.

Compound number	*S. aureus*	*B. subtilis*	*M. luteus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
3b	100	100	100	–	–	–	–
4c	50	100	100	100	–	–	–
5c	25	50	100	50	–	–	–
5d	25	50	50	50	–	100	100
5f	12.5	50	50	25	100	100	100
6a	12.5	50	50	25	100	100	50
6b	6.25	25	25	12.5	50	50	25
7a	25	25	100	50	100	–	–
8a	50	50	100	100	–	–	–
8b	25	100	50	50	–	–	100
9b	25	50	100	100	–	–	–
10	100	100	–	–	–	–	–
11b	50	100	100	50	–	–	–
11c	25	25	50	50	100	–	–
14b	50	25	100	25	50	–	–
15a	12.5	25	50	12.5	50	100	–
17	50	100	–	–	–	–	–
18b	12.5	25	50	25	50	50	25
18c	25	50	50	50	100	100	50
19c	50	50	100	25	50	100	25
20c	50	100	100	50	100	–	–
21b	50	50	100	100	100	–	–
21d	25	25	50	12.5	50	100	25
22b	12.5	25	50	50	100	100	50
22d	6.25	12.5	25	6.25	25	50	12.5
23d	12.5	50	50	25	100	–	100
A	6.25	12.5	12.5	6.25	12.5	12.5	–
C	–	–	–	–	–	–	6.25

(–): Totally inactive (MIC ≥ 200 μg/mL).
A: Ampicillin trihydrate (Standard broad spectrum antibiotic).
C: Clotrimazole (Standard broad spectrum antifungal agent).

Within the first series (Scheme 1), the obtained data in Table 1 showed that the parent pyridine-2-one **1** was totally inactive against all the tested microorganisms. However, the antimicrobial potential of the derived compounds is obviously dependant on the nature of the substituent of the amino function at position-1. In this respect, sulfonylation of this amino group as seen in compound **3b** (X = CH_3) which was weakly active against the three tested Gram positive strains. Derivatization of the N^1-amino substituent into an ureido functionality yielded a mildly active compound **4c** (R = 4-Cl-C_6H_4). However, the preparation of substituted thioureido derivatives led to three active compounds **5c, 5d** and **5f** with a better antimicrobial profile, among which the analog **5f** (R = 4-F-C_6H_4) revealed broad spectrum of antimicrobial activity against all the tested organisms, with a remarkable activity against *S. aureus* (MIC 12.5 μg/mL) and *E. coli* (MIC 25 μg/mL), together with an appreciable antifungal activity against *C. albicans* (MIC 25 μg/mL). Cyclization of the thioureido functionality into thiazolidine derivatives (**6a,b**) resulted in a remarkable improvement in the overall antimicrobial profile. Compound **6a** (Y = H) showed two-fold increase in the activity against *S. aureus*, *E. coli* and *C. albicans* when compared with the parent **5d**, whereas the analog **6b** (Y = F) revealed a distinctive antimicrobial activity against all the tested organisms. It showed equipotent activity to Ampicillin against *S. aureus* (MIC 6.25 μg/mL) and 50% of its activity against *B. subtilis, M. luteus*, *E. coli* and *P. aeruginosa* (MIC 12.5 μg/mL), together with an appreciable antifungal activity against *C. albicans* (MIC 25 μg/mL). Meanwhile, cyclization of the thioureido function into thiazolines as in **7a** (Y = H) led to a limited change in the overall antimicrobial activity when compared with the parent **5d**.

On the other hand, condensation of the N^1-amino group with different aryl and hetaryl aldehydes afforded two active azomethines **8a,b** among which the analog **8b** (R = 4-Cl-C_6H_4) showed better activity than **8a** (R = C_6H_5), especially against *S. aureus, M. Luteus, E. coli* and *C. albicans*. However, cyclization into a thiazolidine ring as in **9b** (R = 4-Cl-C_6H_4) did not offer a noticeable improvement in the overall antimicrobial potential over the parent azomethine.

The compounds presented in Scheme 2, the N^1-benzenesulfonamido analog **10** showed weak activity only against the Gram positive *S. aureus* and *B. subtilis*.

Derivatization of the sulfonamido group into sulfonylureido functionality as in **11b** (X = O, R = Cl) resulted in a limited enhancement in the anti-Gram positive activity and an observable improvement in the activity against *E. coli*. On the contrary, the sulfonylthioureido **11c** (X = S, R = H) exhibited better spectrum of activity against the Gram positive and Gram negative strains except *K. pneumoniae* when compared with the parent sulfonamide **10**. On the other hand, in spite of the inactivity of the key intermediate pyridone **12**, introducing a benzenesulfonyl moiety at position-1 as in **13a,b** did not offer any advantage. However, incorporating a substituted carbamoyl counterpart as in **14b** (R = Cl) resulted in an increase in antimicrobial activity against *B. subtilis* and *E. coli*. Whereas, introducing a thiocarbamoyl moiety at position-1 led to a potentially active compound **15a** (R = H) which exhibited 50% of the activity of Ampicillin against *S. aureus*, *B. subtilis* and *E. coli,* however with no antifungal efficacy.

Shifting to the second series of pyridines **17–23** illustrated in Scheme 3, the key intermediate sulphonamide **17** showed mild activities only against the Gram positive *S. aureus* and *B. subtilis* (MIC 50 and 100 μg/mL, respectively). Condensation of the N^1-sulfonamido group with different aryl and hetaryl aldehydes resulted into two active azomethines **18b,c,** among which the analog **18b** (R = 4-Cl-C_6H_4) showed better activity than **18c** (R = 2-thienyl), against *S. aureus* and *B. subtilis* which was 50% of that of Ampicillin, and *E. coli*. It has also exhibited a remarkable antifungal activity against *C. albicans* which was 25% of that of Clotrimazole. However, cyclization into a thiazolidine ring as in **19c** (R = 2-thienyl) led to a decrease in the Gram positive efficacy and a two-fold increase in the activity against Gram negative organisms when compared to the parent **18c**. Furthermore, derivatization of the parent sulphonamide **17** into sulfonylureido function gave rise to a moderately active compound **20c** (R = 4-Cl-C_6H_4) with MIC range of 50–100 μg/mL. However, bioisosteric replacement of the sulfonylureido function with a sulfonylthioureido as in **21a–d** resulted in a remarkable improvement in the antimicrobial profile. Among these, the analog **21d** (R = 4-F-C_6H_4) exhibited an improvement in the antibacterial potentials against both Gram positive and Gram negative organisms (*B. subtilis* and *E. coli*) together with an appreciable antifungal activity against *C. albicans* (25% of the activity of Clotrimazole). Cyclization of the former sulfonylthioureido compounds into thiazolidine derivatives (**22a–d**) resulted in a remarkable enhancement in the overall antimicrobial potential. Among these, compound **22d** (R = 4-F-C_6H_4) proved to be equipotent to Ampicillin against *S. aureus*, *E. coli* and *B. subtilis* (MIC values 6.25, 6.25 and 12.5 μg/mL), and 50% of its activity against *M. luteus* and *P. aeruginosa* (MIC 25 μg/mL), together with a remarkable antifungal activity against *C. albicans* (MIC 25 μg/mL) which represents 50% of that of Clotrimazole. Finally, cyclization of the sulfonylthioureido function into thiazolines **23d** (R = 4-F-C_6H_4) offered a limited enhancement in the activity against the Gram positive strains when compared with the parent **21d**.

Preliminary in vitro *anticancer screening*

Out of the newly synthesized compounds, twenty derivatives **5c,d,f, 6a,b, 7c, 8b, 9b, 11c, 15a, 18b, 19b, 20b, 21a,b,d, 22b,d** and **23b,d**, were selected by the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay to be evaluated for their *in vitro* antitumor activity. Effective one-dose assay has been added to the NCI 60 Cell screen in order to increase compound throughput and reduce data turnaround time to suppliers while maintaining efficient identification of active compounds. All compounds submitted to the NCI 60 Cell screen, are now tested initially at a single high dose (10 μM) in the full NCI 60 cell panel including leukaemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. However, the data obtained from the one-dose assay revealed that none of the tested compounds showed significant antitumor activity and consequently, none of them was promoted to the 5-dose assay.

Conclusion

The results revealed that twenty six out of the tested fifty eight compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains. In general, most of the tested compounds revealed better activity against the Gram positive than the Gram negative bacteria. Among the Gram positive bacteria tested, two strains namely; *S. aureus* and *B. subtilis* showed high sensitivity towards the tested compounds. However, most of the active compounds displayed better antibacterial activity against *E. coli* when compared with *P. aeruginosa* and *K. pneumoniae*. Moreover, eleven compounds were able to produce appreciable growth inhibitory activity against *C. albicans* fungus when compared with Clotrimazole. However, all of the tested compounds lacked antifungal activity against *A. niger*.

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Declaration of interest

The authors report no conflicts of interest.

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