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Original article

Synthesis and anti-microbial activity of some 1- substituted amino-4, 6-dimethyl-2-oxo-pyridine-3-carbonitrile derivatives

Rizk E. Khidre [a,c], Ameen A. Abu-Hashem [b,c,*], Mohamed El-Shazly [d]

[a] Chemical Industries Division, National Research Centre, Dokki 12622, Giza, Egypt
[b] Department of Photochemistry, National Research Centre, Dokki 12622, Giza, Egypt
[c] Chemistry department, Faculty of Science, Jazan University, Saudi Arabia
[d] Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

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ABSTRACT

A new series of 1- substituted amino-4,6-dimethyl-2-oxo-pyridine-3-carbonitrile such as hydrazide hydrazones **3a-h**; ethane-1,2-diaminopyridine **6**; phthalimidopyridines **8a**,**b**; hydrazides **10a**,**b**; urea **11a** and thiourea **11b** were synthesized in a good to excellent yield in step efficient process, using 1-amino-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (**1**) as a key intermediate. The antibacterial and antifungal activities of the synthesized compounds were evaluated. The obtained data indicated that the majority of the tested compounds exhibited both antibacterial and antifungal activities, particularly compounds **8a** and **8b** showed a comparable effect to a well known antibacterial and antifungal agents.

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1. Introduction

Hydrazines and their derivatives constitute an important class of compounds that has found wide utility in organic synthesis [1,2]. The chemistry of carbon-nitrogen double bond of hydrazone is becoming the backbone of condensation reaction in benzo-fused *N*-heterocycles [3], also it constitutes an important class of compounds for new drug development [4]. A number of hydrazide hydrazone derivatives have been claimed to possess interesting bioactivity such as anti-microbial [1], antitubercular [5,6], anti-convulsant [7], analgesic [8], anti-inflammatory [9,10], antiplatelet aggregation [11], anticancer [12,13], antifungal [14], antiviral [15], antibacterial [16], and antimalarial [17] activities. Aroylhydrazide hydrazones containing hetero-ring such as pyridine ring have attracted special attention [18,19]. Also in relation to the biologically active compounds containing carbon-nitrogen bond, natural and synthetic compounds with cyanopyridine moiety proved to exhibit significant antibacterial [20] and antifungal [21] activities. Encouraged by these observations and in continuation to our previous work in the synthesis of biologically active compounds [22–25], we synthesized newer pyridine derivatives by facile methods. Antibacterial and antifungal activities of the prepared compounds have been investigated, and the results showed that some of the synthesized compounds have broad spectrum antibacterial and antifungal activities.

2. Results and discussion

2.1. Chemistry

The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1–3. Reaction of 1-amino-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (**1**), which was prepared according to the reported method [26,27], with molar quantity of aryl aldehydes **2a**–**h** namely, 4-flourobenzaldehyde **2a**, thiophen-2-carbaldehyde **2b**, 2-chloroquinoline-3-carbaldehyde **2c**, 2-chloro-6-methyl quinoline-3-carbaldehyde **2d**, 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2e**, tetrazolo[1,5-a] quinoline-4-carbaldehyde **2f**, 7-methyltetrazolo[1,5-a]quinoline-4-carbaldehyde **2g**, and 3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2h** in absolute ethanol containing few drops of glacial acetic acid at reflux temperature to afford hydrazide hydrazones **3a**–**h** in excellent yield.

* Corresponding author. Department of Photochemistry, National Research Centre, Dokki 12622, Giza, Egypt. Tel.: +2 012 521 1700, +966 56 351 0497; fax: +202 33370931.
E-mail address: aminaliabuhashem@yahoo.com (A.A. Abu-Hashem).

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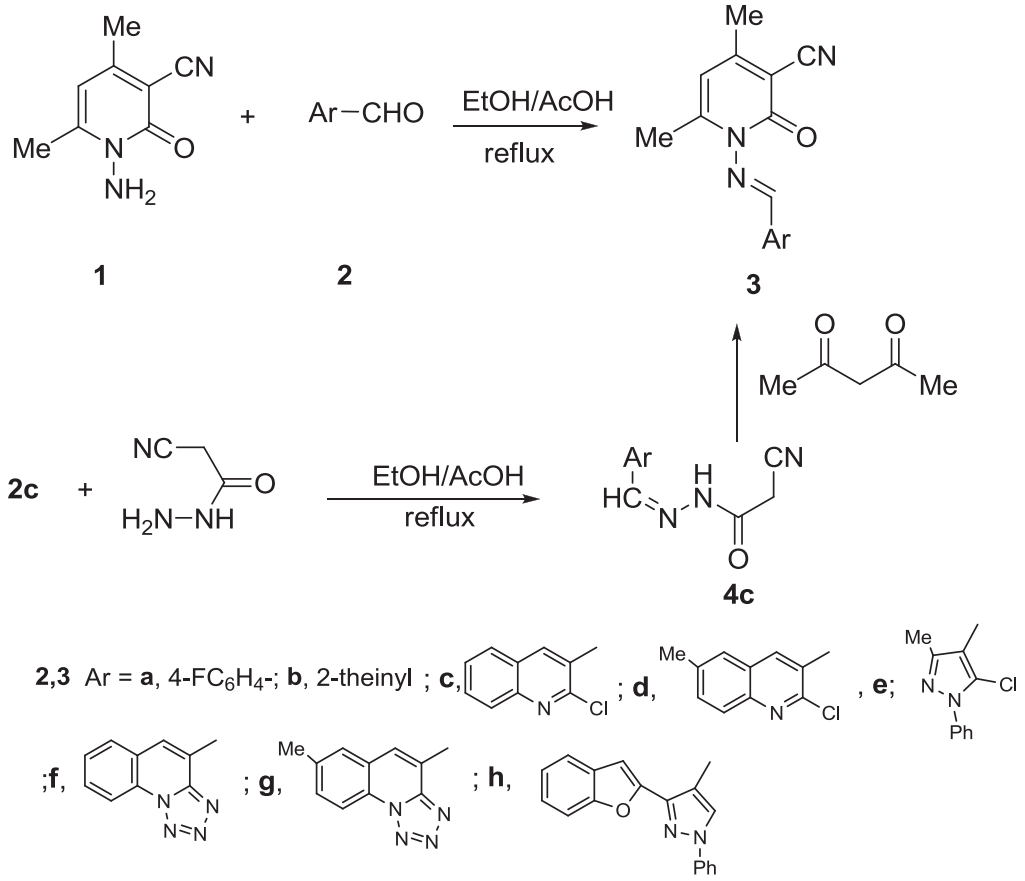


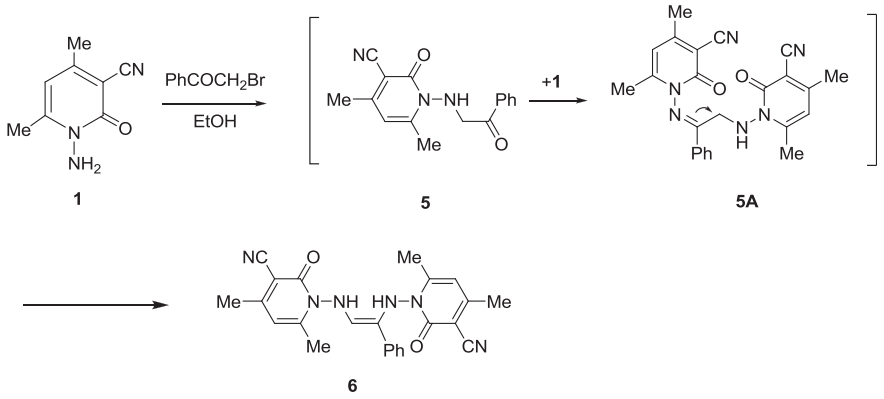
Scheme 1. Synthesis of hydrazide hydrazone derivatives. Reagents and conditions: absolute ethanol, glacial acetic acid (1 mL), reflux, 5–8 h, 75–90% yield.

On the other hand, hydrazide hydrazone **3c** was synthesized, by another route, from the reaction of cyanoacetylhydrazone, which was prepared by the reaction of cyanoacetyl hydrazide with aldehydes, and acetyl acetone [28] e.g. 2-chloroquinoline-3-carbaldehyde **2c**. The reaction of **2c** with cyanoacetyl hydrazide resulted in the formation of *N′*-((2-chloroquinolin-3-yl) methylene)-2-cyanoacetohydrazide which reacted with acetyl acetone in refluxed ethanol to afford **3c** (Scheme 1).

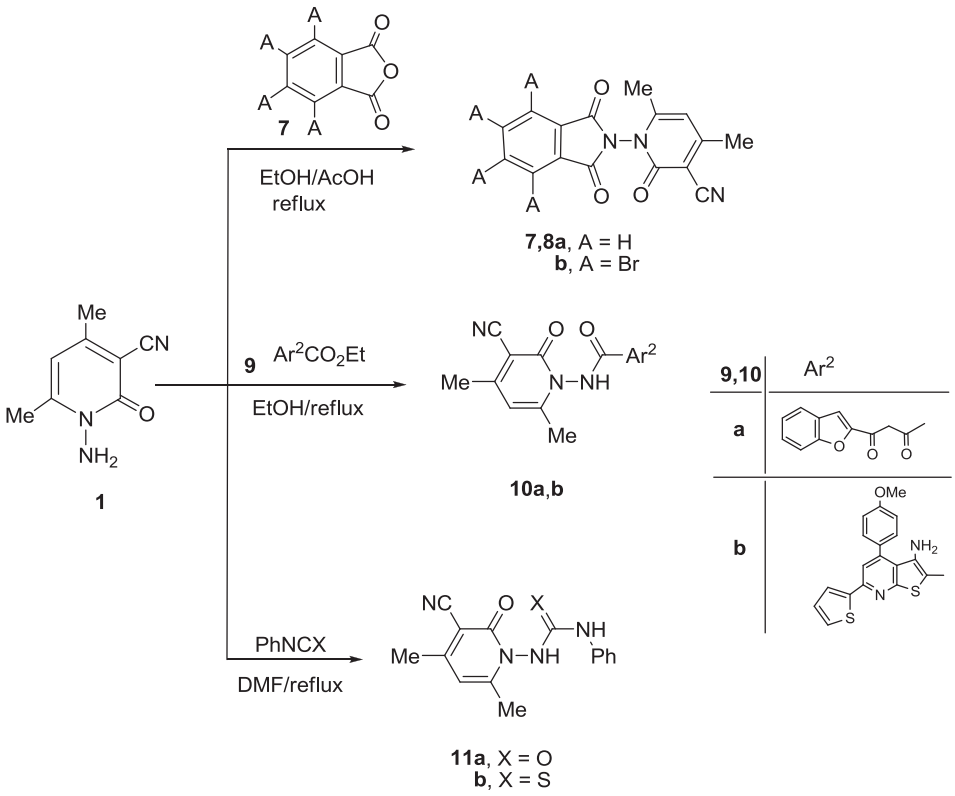
The structure of compounds **3a**–**h** was investigated qualitatively by elemental and spectral analysis. IR spectra of **3a**–**h** exhibited a carbonyl absorption band in the region 1670–1680 cm^{-1} in addition to the absorption bands C=N in the region 1632–1625 cm^{-1} and the absorption band of NH_2 was absent. Their corresponding ^1H NMR spectra showed singlet signal at δ 8.12–8.55 corresponding to CH = N- group of hydrazone. Two highly intense sharp singlets at δ 2.20–2.29 ppm and 2.25–2.32 ppm were detected, indicating the presence of six alkyl protons of two CH_3 substituents. Also the corresponding broad signal of NH_2 was absent.

Compound **6** was prepared by the reaction of 1-amino-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (**1**) with phenacyl bromide in refluxing ethanol yielding **6** as a sole product based on TLC [29–31], *via* intermediates **5** and **5A** as described in Scheme 2. Compound **6** was obtained irrespective if one or 2 equivalents of **1** were being used.

Spectroscopic data (IR, ^1H NMR, MS) and elemental analysis of compound **6** confirmed its structure. The elemental analysis and mass spectrum of the product proved that the reaction proceeded



Scheme 2. Synthesis of ethane-1,2-diaminopyridine. Reagents and conditions: phenacyl bromide (5 mmol), absolute ethanol, reflux, 7 h, crystallization from EtOH/DMF, 77% yield.



Scheme 3. Synthesis of *N*-2-oxo-pyridinyl phthalimide, hydrazides, urea, and thiourea derivatives. Reagents and conditions: A) absolute ethanol, glacial acetic acid (1 mL), reflux, 5–9 h, 70–72% yield; B) aromatic ester (10 mmol), absolute ethanol, reflux, 7–8 h, 65–75% yield; C) phenyl isocyanate or phenyl isothiocyanate (10 mmol), dioxane (30 mL), drops of triethylamine, 6–8 h, 66–68% yield.

in 2:1 M ratio (**1**: phenacyl bromide). ^{1}H NMR of **6** showed singlet signal corresponding to two protons at 6.36 ppm for C_5–H and $C_{5'}$–H present in the two pyridine rings. Two singlet protons signals at 9.21 and 10.39 ppm were detected corresponding to the two NH groups.

Treatment of **1** with molar quantity of phthalic anhydrides **7a** or tetrabromophthalic anhydride **7b** in absolute ethanol containing few drops of glacial acetic acid at reflux temperature, phthalimide derivatives **8a** and **8b** were obtained in 72 and 70% yield, respectively. 1-amino-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (**1**) was reacted with aryl esters **9a**,**b** namely, e.g. ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate **9a** or ethyl 3-amino-4-(4-methoxyphenyl)-6-(thiophen-2-yl)thieno[2,3-b]pyridine-2-carboxylate **9b** in absolute ethanol at reflux temperature to afford the corresponding hydrazides **10a** and **10b**, respectively in 75 and 65% yield. The reaction of **1** with phenyl isocyanate or phenyl isothiocyanate in refluxing DMF afforded substituted urea **11a** or thiourea **11b** as sole products respectively (Scheme 3).

The Structures of compounds **8**, **10**, and **11** were established through, spectroscopic and elemental analyses data. The IR spectra of compound **11a** for example displayed a broad signal band at 3395 cm^{-1} due to 2NH absorptions. Additionally the proton signal in ^{1}H NMR spectra of these groups were observed at 8.98 and 9.45 ppm, respectively.

3. Pharmacological evaluation

3.1. Antibacterial activity

Antibacterial activity of the synthesized compounds was tested using agar well diffusion method. The activity of the tested samples was studied against the *Staphylococcus aureus* (RCMB 000106) and *Bacillus subtilis* (RCMB 000107) (as Gram positive bacteria) and *Pseudomonas aeruginoca* (RCMB 000102), *Escherichia coli* (RCMB 000103) (as Gram negative bacteria). Standard antibiotics, penicillin G and streptomycin were used as standard antibiotics against Gram positive and Gram negative bacteria. Compounds **1**, **3a**, **3f**, **3g**, **3h**, **6**, **8a**, **8b**, **10a**, **10b**, **11a**, and **11b** possessed good anti-microbial activity against *S. aureus* and *B. subtilis* (Table 1). Compounds **8a** and **8b** were the most effective against *S. aureus* with zones of inhibition 24.4 and 26.4 respectively. Also the same compounds (**8a** and **8b**) showed the highest activity against *B. subtilis* with zones of inhibition 25.4 and 26.4 respectively. Compounds **1, 3f**, **3g**, **3h**, **6**, **8a**, **8b**, **10a**, and **11a**, were effective against *E. coli* and only compounds **1** and **3b** showed activity against *P. aeruginoca*. It is obvious that phthalimide derivatives (**8a** and **8b)** exhibited good anti-microbial activity against Gram positive and Gram negative bacteria.

3.2. Antifungal activity

The limited number of available safe antifungal agents is the driving force to search for new candidates. The promising antibacterial activity of the tested compounds has encouraged us to test these compounds against four different pathogenic fungi. The tested fungi were *Aspergillus fumigatus* (RCMB 002003), *Geotrichum candidum* (RCMB 052006), *Candida albicans* (RCMB 005002), *Syncephalastrum racemosum* (RCMB 005003). Itraconazole and clotrimazole were used as standard antifungal agents (Table 2). In general, all the compounds exhibited low to moderate antifungal activity except compounds **8a** and **8b** which were effective against three different fungi. The anti-microbial activity of this class of compounds has been confirmed by other research groups [32–34]. It was proved that other phthalimide derivatives as phthalimido

Table 1
Antibacterial activity of compounds **1**; **3a-h**; **8a,b**; **10a,b**; and **11a,b**.[a,b,c]

Compound	Gram positive bacteria		Gram negative bacteria	
	Staphylococcus aureus (RCMB 000106)[d]	*Bacillus subtilis* (RCMB 000107)	*Pseudomonas aeruginosa* (RCMB 000102)	*Escherichia coli* (RCMB 000103)
1	14 ± 0.5	17 ± 0.5	21.4 ± 0.08	22.8 ± 0.1
3a	14.9 ± 0.03	15.2 ± 0.04	NA[e]	NA
3b	14 ± 0.5	NA	14 ± 1.52	NA
3c	NA	NA	NA	NA
3d	NA	NA	NA	NA
3e	NA	NA	NA	NA
3f	18.6 ± 0.01	17.3 ± 0.03	NA	9.3 ± 0.04
3g	23.2 ± 0.01	24.3 ± 0.03	NA	11.2 ± 0.01
3h	21.2 ± 0.02	23.2 ± 0.04	NA	11.5 ± 0.02
6	15 ± 0.08	13.5 ± 0.2	NA	11.5 ± 0.08
8a	24.4 ± 0.5	25.4 ± 0.1	NA	10.8 ± 0.10
8b	26.4 ± 0.2	26.4 ± 0.1	NA	12.6 ± 0.12
10a	20.4 ± 0.08	21.8 ± 0.1	NA	7.4 ± 0.10
10b	13 ± 0.10	14.2 ± 0.08	NA	NA
11a	7.2 ± 0.08	10.4 ± 0.5	NA	11.4 ± 0.50
11b	15 ± 0.08	13.5 ± 0.2	NA	NA
Penicillin G	29.48 ± 0.82	32.56 ± 0.56	28.32 ± 0.10	33.56 ± 0.78
Streptomycin	25 ± 0. 20	29 ± 0.4	24 ± 0.10	25 ± 0.30

[a] Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (10 mg/mL) concentration of tested samples. The concentration used for the standard antibiotic was (30 μg/mL).
[b] The test was done using the diffusion agar technique. Well diameter: 6.0 mm (100 μL was tested).
[c] Data are expressed in the form of mean ± SD.
[d] RCMB: Regional Center For Mycology And Biotechnology Culture Collection.
[e] NA: No activity.

phosphor esters exhibited also antibacterial and antifungal activity [22].

The presence of intact phthalimide ring is important to the biological activity as opining of the phthalimide ring results in profound decrease in the biological activity. Also it is obvious from our anti-microbial data that compound **8b** showed slightly higher anti-microbial activity compared to **8a** which can be correlated to the presence of bromine substitutions. Several studies have suggested the beneficial effect of bromine substitution as important factor for increasing cellular permeability of many drug candidates [35,36]. The high anti-microbial activity of the prepared phthalimide can be also correlated to the substitution of electron withdrawing groups on the pyridine ring attached to the phthalimide moiety. This finding is supported by previous studies suggesting the positive role of electron withdrawing groups on the biological activity of phthalimide derivatives [37,38].

Despite phthalimide anti-microbial activity, their cytotoxic activity was generally low and extremely restricted to certain structures with specific heterocyclic rings attached to the phthalimide moiety [39,40] or they were totally ineffective as cytotoxic agents [41]. Even compounds with two phthalimide derivatives were also ineffective as cytotoxic agents [42]. The reported anti-microbial activity accompanied with the lack of measurable cytotoxic activity, indicates that phthalimide derivatives act as anti-

Table 2
Antifungal activity of compounds **1**; **3a-h**; **8a,b**; **10a,b**; and **11a,b**.[a,b,c]

Compounds	Fungi			
	Aspergillus fumigatus (RCMB 002003)[d]	*Geotrichum candidum* (RCMB 052006)	*Candida albicans* (RCMB 005002)	*Syncephalastrum racemosum* (RCMB 005003)
1	NA[e]	NA	NA	NA
3a	NA	NA	NA	NA
3b	11 ± 0.2	13 ± 0.05	12 ± 0.08	NA
3c	13.3 ± 0.09	12.4 ± 0.1	10.2 ± 0.05	NA
3d	14.5 ± 0.05	13.2 ± 0.1	12.4 ± 0.05	NA
3e	10.3 ± 0.04	11.5 ± 0.1	13.2 ± 0.02	NA
3f	11.2 ± 0.01	13.4 ± 0.04	11.4 ± 0.03	NA
3g	15.2 ± 0.08	18.4 ± 0.50	15.4 ± 0.1	NA
3h	14.2 ± 0.02	16.5 ± 0.04	15.5 ± 0.02	NA
6	10 ± 0.20	12 ± 0.05	11 ± 0.08	NA
8a	19.2 ± 0.03	21.4 ± 0.04	18.2 ± 0.03	NA
8b	22.5 ± 0.02	23.5 ± 0.20	20.2 ± 0.05	NA
10a	NA	NA	NA	NA
10b	11.1 ± 0.01	14 ± 0.08	10 ± 0.02	NA
11a	9.2 ± 0.08	10.4 ± 0.5	7.4 ± 0.1	NA
11b	10.2 ± 0.2	12 ± 0.05	11 ± 0.08	NA
Itraconazole	28 ± 0.05	27 ± 0.10	26 ± 0.02	22 ± 0.09
Clotrimazole	26 ± 0.10	23 ± 0.30	18 ± 0.1	20 ± 0.2

[a] Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (10 mg/mL) concentration of tested samples. The concentration used for the standard antibiotic was (30 μg/mL).
[b] The test was done using the diffusion agar technique. Well diameter: 6.0 mm (100 μL was tested).
[c] Data are expressed in the form of mean ± SD.
[d] RCMB: Regional Center For Mycology And Biotechnology Culture Collection.
[e] NA: No activity.

microbial agents through a different mechanism other than cytotoxicity. The world wide increase in anti-microbial drug resistance and the promising anti-microbial activity of these phthalimide derivatives are encouraging factors to investigate the possibility of their utilization as future anti-microbial agents.

4. Conclusion

The new ring system compounds prepared in our study seems to have interesting biological activity. Furthermore, the present investigation offers rapid and effective new procedures for the synthesis of the polycondensed new heterocyclic ring systems as hydrazide hydrazone, amino pyrazol, tetrazoloquinolin, pyridinyl phthalimide, phenyl (thio) urea-pyridine derivatives. The feasibility of the synthetic procedures and the excellent yield of the prepared compounds are the main advantages for the developed protocol. The antibacterial and antifungal activities of the prepared compounds were evaluated showing moderate to good activities. Phthalimide derivatives **8a** and **8b** exhibited the highest antibacterial and antifungal activities comparable to standard antibiotics.

5. Experimental

5.1. Chemistry

5.1.1. General procedures

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data (in accordance with the calculated values) were obtained from the microanalytical unit, National Research Centre, Dokki, Giza, Egypt. The IR spectra (KBr) were recorded on a Shimadzu CVT-04 spectrophotometer. The ^{1}H-NMR spectra were recorded at 270 MHz on a Varian EM-360 spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift (δ) values are given in parts per million. The mass spectra were determined using a Varian MAT CH-5 spectrometer (70 eV). 2-chloroquinoline-3-carbaldehyde **2c** [43], 2-chloro-6-methyl quinoline-3-carbaldehyde **2d** [43], 5-chloro-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2e** [44], tetrazolo[1,5-a]quinoline-4-carbaldehyde **2f** [45], 7-Methyltetrazolo[1,5-a]quinoline-4-carbaldehyde **2g** [46], 3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2h** [47], ethyl 4-(benzofuran-2-yl)-2,4-dioxobut anoate **9a** [48], and ethyl 3-amino-4-(4-methoxyphenyl)-6-(thiophen-2-yl)thieno[2,3-b]pyridine-2-carboxylate **9b** [49] were prepared according to the reported procedures.

5.1.2. *N′–((2-chloroquinolin-3-yl) methylene)-2-cyanoacetohydrazide (**4c**)*

To a solution of 2-cyanoacetohydrazide (0.99 g, 10 mmol) in absolute ethanol (30 mL) 2-chloroquinoline-3-carbaldehyde **1** (1.91 g, 10 mmol) was added in the presence of two drops of glacial acetic acid. The reaction mixture was heated under reflux for 1 h then left to cool to room temperature. The solid product formed upon pouring onto ice/water was collected by filtration.

IR (KBr) ν_{max}/cm^{-1} 1620 (C=O), 2185 (CN), 3195 (NH); ^{1}H NMR (DMSO-d$_6$) δ 3.98 (s, 2H, CH$_2$), 7.28–7.92 (m, 5H, Ar–H), 9.02(s, 1H, CH=N), 12.17(s, H, NH, D$_2$O-exchangeable); MS *m/z* (%): 272 (M$^+$, 12), 63(100).

5.1.3. General procedures for the synthesis of hydrazide hydrazone **3a–h**

A mixture of **1** (1.63 g, 10 mmol) and the appropriate aromatic aldehyde derivatives **2a–h** (10 mmol) in mixture of absolute ethanol (30 mL) containing few drops of glacial acetic acid (1 mL) was refluxed till the solid was formed, The reaction mixture was allowed to cool to room temperature, filtered off, washed with ethanol, and crystallized from appropriate solvent to produces **3a–h** in high yields.

5.1.4. *1-[(4-fluoro-benzylidene)-amino]-4,6-dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (**3a**)*

Reaction time 5 h, yellow crystals, mp 191–193 °C (EtOH), yield 90%. IR (KBr, cm^{-1}): ν_{max} 3025 (CH, aryl), 2200 (CN), 1670 (C=O, amide), 1630 (C=N); ^{1}H NMR (DMSO-d_6) ppm: δ 2.29 (s, 3H, CH$_3$), 2.32 (s, 3H, CH$_3$), 6.37 (s, 1H, C$_5$–H, pyridine), 7.35 (2d, 2H, J = 8.62 Hz, 4-F-phenyl), 7.96 (2d, 2H, J = 8.64 Hz, 4-F-phenyl), 8.55 (s, 1H, CH=N–). EI-MS: *m/z* (%) 271 (M$^+$ +2, 32.60), 270 (M$^+$ +1, 44.50), 269 (M$^+$, 75.10), 249 (M$^+$ – F, 100). Anal.Calc. for C$_{15}$H$_{12}$FN$_3$O (269.27): C, 66.91; H, 4.49; F, 7.06; N, 15.60. Found: C, 66.85; H, 4.42; F, 7.10; N, 15.55.

5.1.5. *4,6-dimethyl-2-oxo-1-[(thiophen-2-ylmethylene)-amino]-1,2-dihydro-pyridine-3-carbonitrile (**3b**)*

Reaction time 5 h, yellowish crystals, mp 210–212 °C (MeOH), yields 88%. IR (KBr, cm^{-1}): ν_{max} 3030 (CH, aryl), 2205 (CN), 1674 (C=O, amide), 1627 (C=N); ^{1}H NMR (DMSO-d_6) ppm: δ 2.22 (s, 3H, CH$_3$), 2.27 (s, 3H, CH$_3$), 2.31 (s, 3H, CH$_3$), 6.35 (s, 1H, C$_5$–H, pyridine), 7.25 (t, 1H, J = 7.0 Hz, thiophene), 7.74 (d, 1H, J = 6.95 Hz, thiophen), 7.94 (d, 1H, J = 7.1 Hz, thiophene), 8.44 (s, 1H, CH=N–); EI-MS: *m/z* (%) 259 (M$^+$ +2, 30.20), 258 (M$^+$ +1, 40.60), 257 (M$^+$,78.30). Anal.Calc. for C$_{13}$H$_{11}$N$_3$OS (257.31): C, 60.68; H, 4.31; N, 16.33; S, 12.46. Found: C, 60.61; H, 4.25; N, 16.22; S, 12.40.

5.1.6. *1-[(2-chloro-quinolin-3-ylmethylene)-amino]-4,6-dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (**3c**)*

Reaction times 5 h, yellow crystals, mp 295–297 °C (EtOH), yield 85%. IR (KBr, cm^{-1}): ν_{max} 3040 (CH, aryl), 2210 (CN),1675 (C=O, amide), 1628 (C=N); ^{1}H NMR (DMSO-d_6) ppm: δ 2.23 (s, 3H, CH$_3$), 2.26 (s, 3H, CH$_3$), 6.36 (s, 1H, C$_5$–H, pyridine), 7.75–8.43 (m, 5H, quinoline), 8.54 (s, 1H, CH=N–); EI-MS: *m/z* (%) 338 (M$^+$ + 2, 29.40%), 337 (M$^+$ + 1, 30.10%), 336 (M$^+$, 80.50%). Anal.Calc. for C$_{18}$H$_{13}$ClN$_4$O (336.78): C, 64.19; H, 3.89; Cl, 10.53; N, 16.64; Found: C, 64.11; H, 3.80; Cl, 10.43; N, 16.60.

5.1.7. *1-[(2-chloro-6-methyl quinolin-3-ylmethylene)-amino]-4,6-dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (**3d**)*

Reaction times 8 h, brownish powder, mp 260–262 °C (EtOH), yield 83%. IR (KBr, cm^{-1}): ν_{max} 3035 (CH, aryl), 2220 (CN), 1678 (C=O, amide), 1632 (C=N); ^{1}H NMR (DMSO-d_6) ppm: δ 2.24 (s, 3H, CH$_3$), 2.28 (s, 3H, CH$_3$), 2.36 (s, 3H, CH$_3$), 6.34 (s, 1H, C$_5$–H, pyridine), 7.55–7.78 (m, 4H, quinoline), 8.22 (s, 1H, 1H, CH=N–); EI-MS: *m/z* (%) 352 (M$^+$ + 2, 26.20%), 351 (M$^+$+1, 22.30%), 350 (M$^+$,70.40%). Anal.Calc. for C$_{19}$H$_{15}$ClN$_4$O (350.80): C, 65.05; H, 4.31; Cl, 10.11; N, 15.97; Found: C, 65.15; H, 4.35; Cl, 10.01; N, 15.90.

5.1.8. *1-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-ylmethylene)-amino]-4,6-dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (**3e**)*

Reaction times 7 h, yellowish crystals, mp 212–214 °C (EtOH), yield 80%. IR (KBr, cm^{-1}): ν_{max} 3033 (CH, aryl), 2218 (CN), 1679 (C=O, amide), 1630 (C=N); ^{1}H NMR (DMSO-d$_6$) ppm: δ 2.21 (s, 3H, CH$_3$), 2.25 (s, 3H, CH$_3$), 2.74 (s, 3H, CH$_3$), 6.33 (s,1H, C$_5$–H, pyridine), 7.31–7.55 (m, 5H, phenyl), 8.18 (s,1H, CH=N–); EI-MS: *m/z* (%) 367 (M$^+$ + 2, 36.20), 366 (M$^+$ + 1, 42.10), 365 (M$^+$, 78.50). Anal.Calc. for C$_{19}$H$_{16}$ClN$_5$O (365.82): C, 62.38; H, 4.41; Cl, 9.69; N, 19.14. Found: C, 62.30; H, 4.36; Cl, 9.55; N, 19.10.

5.1.9. *4,6-Dimethyl-2-oxo-1-[(1,2,3,4-tetrazolo[1,5-a]quinolin-6-ylmethylene)-amino]-1,2-dihydro-pyridine-3-carbonitrile (**3f**)*

Reaction times 6 h, yellow crystals, mp 275–277 °C (dioxane), yield 82%. IR (KBr, cm^{-1}): ν_{max} 3037 (CH, aryl), 2215 (CN), 1680 (C=O, amide), 1630 (C=N); ^{1}H NMR (DMSO-d6) ppm: δ 2.20 (s, 3H,

CH_3), 2.27 (s, 3H, CH_3) 6.37 (s, 1H, C_5–H, pyridine), 7.42–7.71(m, 5H, quinoline), 8.15 (s, 1H, CH=N–); EI-MS: *m/z* (%) 345 (M^+ + 2, 30.10), 344 (M^+ + 1, 40.30), 343 (M^+, 75.20). Anal.Calc. for $C_{18}H_{13}N_7O$ (343.34): C, 62.97; H, 3.82; N, 28.56; Found: C, 62.90; H, 3.75; N, 28.49.

*5.1.10. 4,6-Dimethyl-2-oxo-1-[(9-methyl-1,2,3,4-tetrazolo[1,5-a] quinolin-6-ylmethylene)-amino]-1,2-dihydro-pyridine-3-carbonitrile (**3g**)*

Reaction times 8 h, yellowish brown crystals, mp 310–312 °C (DMF), yield 79%. IR (KBr, cm^{-1}): ν_{max} 3030 (CH, aryl), 2216 (CN), 1677 (C=O, amide), 1628 (C=N); ^{1}H NMR (DMSO-d_6) ppm: δ 2.22 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.35 (s, 1H, C_5–H, pyridine), 7.35–7.55 (m, 4H, quinoline), 8.30 (s, 1H, CH=N–); EI-MS: *m/z* (%) 359 (M^+ + 2, 28.40), 358 (M^+ + 1, 47.30), 357 (M^+, 82.30). Anal.Calc. for $C_{19}H_{15}N_7O$ (357.37): C, 63.86; H, 4.23; N, 27.44. Found: C, 63.93; H, 4.28; N, 27.51.

*5.1.11. 1-[(3-benzofuran-2-yl-1-phenyl-1H-pyrazol-4-ylmethylene)-amino]-4,6-dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (**3h**)*

Reaction times 6 h, yellowish brown crystals, mp 255–257 °C (DMF), yield 75%. IR (KBr, cm^{-1}): ν_{max} 3029 (CH, aryl), 2210 (CN), 1675 (C=O, amide), 1625 (C=N); ^{1}H NMR (DMSO-d_6) ppm: δ 2.21 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.36 (s, 1H, C_5–H, pyridine), 7.31–7.94 (m, 9H, *H*-Het & Ar), 8.12 (s,1H, CH=N–); EI-MS: *m/z* (%) 435 (M^+ + 2, 25.30), 434 (M^+ + 1, 40.20), 433 (M^+, 80.10). Anal.Calc. for $C_{26}H_{19}N_5O_2$ (433.46): C, 72.04; H, 4.42; N, 16.16. Found: C, 72.12; H, 4.46; N, 16.12.

*5.1.12. Synthesis of (Z)-1-(2-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-ylamino)-1-phenylvinylamino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (**6**)*

To a solution hydrazides **1** (1.63 g, 10 mmol) in ethanol (50 mL), phenacyl bromide (0.99 g, 5 mmol) was added. The reaction was refluxed for 7 h, then cool to room temperature. The formed solid was filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford compounds **6**. Yellow crystals, yield 77%, mp 235–236 °C. IR (cm^{-1}, KBr): ν_{max} 3320, 3313(2NH), 2218, 2212 (2CN), 1675, 1669 (2C=O, amide); ^{1}H NMR (DMSO-d_6) ppm: δ 2.18 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 5.93 (s, 1H, C=CH–), 6.36 (s, 2H, $C_{5,5'}$-H pyridine), 7.63–7.72 (m, 5H, *H*-Ph), 9.21 (s, 1H, D_2O exchangeable, NH), 10.39 (s, 1H, D_2O exchangeable, NH). EI-MS: *m/z* (%) 428 (M^+ + 2, 10), 427 (M^+ + 1, 15), 426 (M^+, 60). Anal.Calc. for $C_{24}H_{22}N_6O_2$ (426.47): C, 67.59; H, 5.20; N, 19.71. Found: C, 67.51; H, 5.08; N, 19.66.

*5.1.13. General procedures for the synthesis of N-2-oxo-pyridinyl phthalimide **8a,b***

A mixture of **1** (1.63 g, 10 mmol) and phthalic anhydride **7a** or tetrabromophthalic anhydride **7b** (10 mmol) in mixture of absolute ethanol (30 mL) containing few drops of glacial acetic acid (1 mL) was refluxed for ~10 h (TLC), The reaction mixture was allowed to cool at room temperature, filtered off, washed with ethanol, and crystallized from appropriate solvent to produces **8a** and **8b** respectively.

*5.1.14. 1-(1, 3-dioxo-1,3-dihydro-isoindol-2-yl)-4,6-dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (**8a**)*

Reaction times 5 h, yellowish brown powder, mp 155–157 °C (DMF), yield 72%. IR (KBr, cm^{-1}): ν_{max} 3038 (CH, aryl), 2208 (CN), 1710, 1695 (C(1,3) = O), 1677 (C=O, amide); ^{1}H NMR (DMSO-d_6) ppm: δ 2.23 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.35 (s, 1H, C_5–H, pyridine), 7.45–7.83 (m, 4H, *H*-Het). EI-MS: *m/z* (%) 295 (M^+ + 2, 20.50), 294 (M^+ + 1, 45.10), 293 (M^+, 88.20). Anal.Calc. for $C_{16}H_{11}N_3O_3$ (293.28): C, 65.53; H, 3.78; N, 14.33; Found: C, 65.50; H, 3.71; N, 14.30.

*5.1.15. 4,6-dimethyl-2-oxo-1-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)-1,2-dihydropyridine-3-carbonitrile (**8b**)*

Reaction times 9 h, yellow powder, mp 225–227 °C (DMF), yield 70%. IR (KBr, cm^{-1}): ν_{max} 3044 (CH, aryl), 2218 (CN), 1715, 1696 (C(1,3) = O), 1679 (C=O, amide); ^{1}H NMR (DMSO-d_6) ppm: δ 2.22 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); 6.37 (s, 1H, C_5–H, pyridine). EI-MS: *m/z* (%) 610 (M^+ + 2, 30.73), 609 (M^+ +1, 55.20), 608 (M^+, 83.60). Anal.Calc. for $C_{16}H_7Br_4N_3O_3$ (608.86): C, 31.56; H, 1.16; Br, 52.49; N, 6.90. Found: C, 31.50; H, 1.12; Br, 52.42; N, 6.85.

*5.1.16. General procedures for the synthesis of hydrazides **10a,b***

A mixture of **1** (1.63 g, 10 mmol) and ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate **9a** or ethyl 3-amino-4-(4-methoxyphenyl)-6-phenylthieno[2,3-b]pyridine-2-carboxylate **9b** (10 mmol) in absolute ethanol (30 mL) containing few drops of glacial acetic acid (1 mL) was refluxed for ~7 h (TLC), The reaction mixture was allowed to cool at room temperature, filtered off, washed with ethanol, and crystallized from appropriate solvent to produces **10a** and **10b**, respectively.

*5.1.17. 4-(benzofuran-2-yl)-N-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-2,4-dioxobutanamide (**10a**)*

Reaction times 8 h, white powder, mp 149–151 °C (MeOH), yield 75%. IR (KBr, cm^{-1}): ν_{max} 3420 (br., NH), 3035 (CH, aryl), 2218 (CN), 1735, 1720 (2C=O), 1685, 1674 (2C=O, amide). ^{1}H NMR (DMSO-d_6) ppm: δ 2.22 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.82 (s, 2H, CH_2), 6.35 (s, 1H, C_5–H, pyridine), 7.22–7.89 (m, 5H, benzofurane), 9.20 (br., 1H, NH, D_2O exch.). EI-MS: *m/z* (%) 379 (M^+ + 2, 22.10), 378 (M^+ + 1, 30.20), 377 (M^+, 76.60). Anal.Calc. for $C_{20}H_{15}N_3O_5$ (377.35): C, 63.66; H, 4.01; N, 11.14; Found: C, 63.72; H, 4.11; N, 11.18.

*5.1.18. 3-amino-N-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-4-(4-methoxyphenyl)-6-(thiophen-2-yl)thieno[2,3-b]pyridine-2-carboxamide (**10b**)*

Reaction time 7 h, yellow powder, mp 137–139 °C (dioxane), yields 65%. IR (KBr, cm^{-1}): ν_{max}: 3440 (br., NH, NH_2), 3032 (CH, aryl), 2221(CN), 1684, 1676 (2C=O, amide). ^{1}H NMR (DMSO-d_6) ppm: δ 2.21 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 6.33 (s, 1H, C_5–H, pyridine), 7.19–8.53 (m, 9H, *H*-Het & Ar), 6.99. (br., 2H, NH_2, D_2O exch.), 11.51 (br., H, NH, D_2O exch.); EI-MS: *m/z* (%) 529 (M^+ + 2, 12.40), 528 (M^+ + 1, 22.60), 527 (M^+, 79.90). Anal.Calc. for $C_{27}H_{21}N_5O_3S_2$ (527.62): C, 61.46; H, 4.01; N, 13.27; S, 12.15; Found: C, 61.39; H, 3.93; N, 13.18; S, 11.99.

*5.1.19. General procedures for the synthesis of 1-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H) -yl)-3-phenyl(thio)urea (**11a,b**)*

A mixture of compound **1**(1.63 g, 10 mmol), phenyl isocyanate or phenyl isothiocyanate **8b** (10 mmol), in dioxane (30 mL) containing few drops of triethylamine was refluxed for ~7 h (TLC). The solution was evaporated under reduced pressure to ≈1/3 of its volume. The separated precipitate was filtered off, washed with ethanol, dried, and crystallized from dioxane to afford **12a** and **12b**, respectively.

*5.1.20. 1-(3-Cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-3-phenylurea (**11a**)*

Reaction time 6 h, yellow powder, mp 345–347 °C (dioxane), yields 66%. IR (KBr, cm^{-1}): ν_{max}: 3395 (br., 2NH), 3030 (CH, aryl), 2216 (CN), 1681, 1672 (2C=O, amide); ^{1}H NMR (DMSO-d_6) ppm: δ 2.23 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.35 (s,1H, pyridine H-5), 7.24–7.64 (m, 5H, phenyl), 8.98 (br., 1H, NH, D_2O exch.), 9.45 (br., 1H, NH, D_2O exch.). EI-MS: *m/z* (%) 284 (M^+ + 2, 27.50), 283 (M^+ + 1,

16.80), 282 (M^+, 70.10). Anal.Calc. for $C_{15}H_{14}N_4O_2$ (282.30): C, 63.82; H, 5.00; N, 19.85; Found: C, 63.76; H, 4.48; N, 19.80.

*5.1.21. 1-(3-Cyano-4,6-dimethyl-2-oxo-pyridin-1(2H)-yl)-3-phenylthiourea(**11b**)*

Reaction time 8 h, yellowish brown powder, mp 205–207 °C (dioxane), yields 68%. IR (KBr, cm^{-1}): ν_{max}: 3390 (br., 2NH), 3032 (CH, aryl), 2218 (CN), 1675 (C=O, amide 1355 (C=S). ^1H NMR (DMSO-d_6) ppm: δ 2.22 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 6.34 (s, 1H, pyridine H-5), 7.32–7.69 (m, 5H, phenyl), 8.95 (br., 1H, NH, D_2O exch.), 8.55 (br., 1H, NH, D_2O exch.). EI-MS: *m/z* (%) 300 ($M^+ + 2$, 34.40), 299 ($M^+ + 1$, 10.70), 298 (M^+, 68.50). Anal.Calc. for $C_{15}H_{14}N_4OS$ (298.36): C, 60.38; H, 4.73; N, 18.78; S, 10.75. Found: C, 60.31; H, 4.70; N, 18.72; S, 10.68.

5.2. Biology Experiments

This work has been done in, *The Regional Center for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo, Egypt*.

5.2.1. Antibacterial activity

Antibacterial activity was investigated using agar well diffusion method. The activity of the tested samples was studied against the *S. aureus* and *B. subtilis* (as Gram positive bacteria) and Pseudomonas aeruginosa, *E. coli* and *Salmonella typhi* (as Gram negative bacteria). The solution of 10 mg/mL of each compound and standard drug in DMSO was prepared for using against the tested bacteria. Centrifuged pellets of bacteria from a 24 h old culture containing approximately 104–106 CFU (colony forming unit) per mL were spread on the surface of nutrient agar (typetone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1000 mL of distilled water, pH 7.0) which was autoclaved under 121 °C for at least 20 min. Wells were created in medium with the help of a sterile metallic bores and then cooled down to 45 °C. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100 μL of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds was prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 37 °C for 24 h and then the plates were examined for the formation of inhibition zone. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture.

5.2.2. Antifungal activity

Tested samples were screened separately *in vitro* for their antifungal activity against various fungi viz. *A. fumigatus*, *G. candidum*, *C. albicans*, *S. racemosum*, these species were isolated from the infected organs of some patients on *Sabouraud dextrose agar* plates. The culture of fungi was purified by single spore isolation technique. The antifungal activity was done by agar well diffusion method according to the following procedure:

5.2.2.1. Sabouraud dextrose agar plates. A homogeneous mixture of glucose-peptone-agar (40:10:15) was sterilized by autoclaving at 121 °C and 15 lb/cm^2 for 20 min. The sterilized solution (25 mL) was poured in each sterilized Petri dish in laminar flow and left for 20 min to form the solidified sabouraud dextrose agar plate. These plates were inverted and kept at 30 °C in incubator to remove the moisture and to check for any contamination.

5.2.2.2. Antifungal assay. Fungal strain was grown in 5 mL Sabouraud dextrose broth (glucose:peptone, 40:10) for 3–4 days to achieve 105 CFU/mL cells. The fungal culture (0.1 mL) was spread out uniformly on the sabouraud dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5–10 min so that culture is properly adsorbed on the surface of Sabouraud dextrose agar plates. Small wells of size (4 mm × 2 mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100 μL of the tested samples (10 mg/mL) was loaded into the wells of the plates. All compounds was prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 30 °C for 3–4 days and then the plates were examined for the formation of inhibition zone. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus.

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