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SYNTHESIS AND EFFICIENCY OF NEW PYRIDINE, CHROMENE AND THIAZOLE CONTAINING COMPOUNDS AS ANTIMICROBIAL AND ANTIOXIDANT AGENTS

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ABSTRACT. The versatile scaffold, *N'*-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) was utilized in the production of new pyridine, chromene and thiazole derivatives as antimicrobial and antioxidant agents. The synthetic strategy involves the treatment of precursor **3** with various arylidene-malononitrile and 3-aryl-2-cyanoacrylate compounds to furnish substituted pyridines **5** and **7**. The interaction of **3** with salicylaldehyde and/or phenyl isothiocyanate followed by cyclization with chloroacetone produced the corresponding 2-imino-2*H*-chromene-3-carbohydrazide and (thiazol-2-ylidene-acetyl)-salicylic acid hydrazide compounds **8** and **9**, respectively. The structural features of the synthesized compounds were confirmed by using spectroscopic methods such as (IR, ^{1}H NMR, ^{13}C NMR and MS). The new pyridine, chromene and thiazole products showed potent antioxidants and antimicrobial activities. The thiazole derivative **9** exhibited the highest anti-bacterial and antifungal activities against *S. aureus* (75.0%) and *B. subtilis* (73.9%) and *C. albicans* (66.6%). The combination between salicylic acid hydrazide and thiazole moieties in the hybrid **9** indicated the best antioxidant activity (87.9%).

KEY WORDS: Salicylic hydrazide, Arylidene-malononitrile, Pyridine, Thiazole, Antioxidant

INTRODUCTION

The modern chemical industry is interested in producing nitrogen-, oxygen- and/or sulfur-containing heterocyclic molecules due to their specific biological and pharmacological activities. Pyridine is the principal component in enormous natural and synthetic compounds of pharmaceutical interest [1, 2]. Therefore, the interest in the preparation and application pyridine-containing compounds is still desirable and continuous [3, 4]. Many pyridine compounds have been described and characterized by their miscellaneous biological activities, such as anti-tumor [5], antituberculosis [6], antibacterial [7], and antihepatitis [8]. The synthesis of pyridine ring system relies upon one of two approaches: the condensation of carbonyl compounds [9-11] or cycloaddition reactions [12-14]. There are exceptions, such as ring expansion from 5-membered rings, but these approaches are generally low-yielding, narrow in applicability, or both. Also, thiazoles are one of the most active heterocyclic compounds which is characterized by a wide range of activities, such as antibacterial [15], antifungal [16], anti-inflammatory [17], antidiabetic [18], antioxidant [19], anticancer [20], antiviral [21-23], and antiprotozoal activities [24]. There are numerous protocols for construction of the thiazole ring system [25], the most broadly used synthetic routes are Hantzsch's Synthesis (the reaction of α-haloketone or aldehyde with thioamide), Gabriel Synthesis (the reaction of α-aminonitriles with dithioacid or esters, carbon disulfide, carbonyl sulfide, and isothiocyanates separately), and Tcherniac's Synthesis (α-thiocyanatoketone on aqueous acid (HCl) or aqueous alkali-induced cyclization afforded 2-hydroxy-4-aryl/alkylthiazole, while cyclization in dry ethereal HCl produced 2-chloro-4-aryl/alkylthiazole). Chromene compounds are one of the most demanded compounds due to their unique pharmacological activity [26, 27]. Chromene scaffolds display tremendous medicinal properties such as antimicrobial [28-30], antioxidant [31], antileishmanial [32], vascular-

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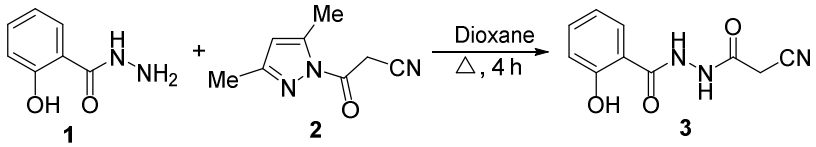
disrupting [33], and blood platelet anti-aggregating effects [34]. The most common synthetic approaches for the synthesis of chromene are ring cyclization by a reaction of resorcinol and different 2-arylidene malononitriles, ring cyclization via nitrophenyl-boronic acid and DBU catalyst, unexpected [4+2] annulation of alkynyl thioethers with alcohols (o-hydroxybenzyl) [35] and/or metal free protocols [36].

Antimicrobial agents are a general term that is mainly concerned with antibiotics such as antibacterial and antifungal [37]. Although antibiotics aren't always effective, they are utilized to treat some bacterial infections, either by killing bacteria or preventing their spread. Antibiotics are useless against viral diseases like colds and flu, as well as the majority of coughs and sore throats. Plants and animals maintain a complex system of overlapping antioxidants to prevent oxidant stress, such as glutathione [38, 39].

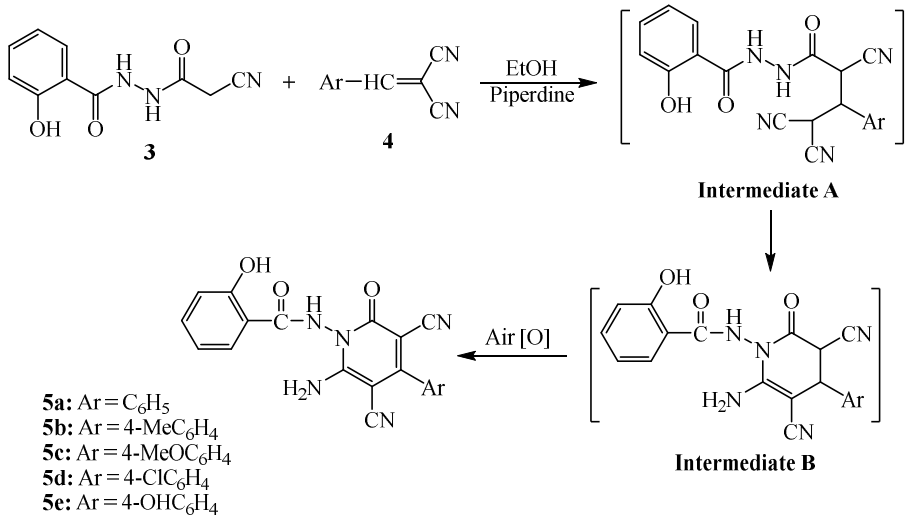
RESULT AND DISCUSSION

The precursor, salicylic acid hydrazide (**1**), was produced for the reaction of methyl salicylate with hydrazine in refluxing ethanol [40] (Scheme 1). Salicylic acid hydrazide (**1**) was treated with 1-(cyanoacetyl)-3,5-dimethylpyrazole (**2**) [41] in dioxane to form the highly versatile precursor, *N*′-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**), in 68.3% yield. The IR absorptions at 3337, 3266 and 3192 cm^{-1} are attributed to the N-H and O-H groups. The nitrile and carbonyl groups were observed at the expected absorptions at 2264 and 1689 cm^{-1}, respectively. The ^{1}H NMR spectrum displayed singlet at δ 3.58 ppm for two protons (-CH_2-). The aromatic protons resonate as multiplet and doublet signals (δ 7.00-8.05 ppm). The singlet signals for the protons of N-H, N-H, and O-H groups were found at δ 10.02, 10.28 and 10.71 ppm.



Scheme 1. Synthesis of *N*′-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**).

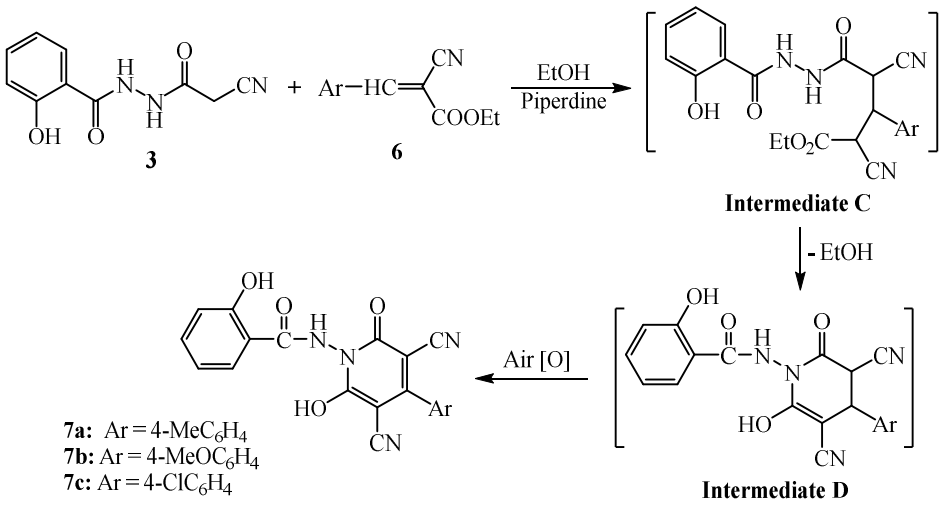
The synthetic plan of this work is based on utilization of the highly versatile *N*′-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) in the building of new heterocyclic ring systems such as pyridine, thiazole, and/or chromene. Thus, the reaction of *N*′-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) with several arylidene-malononitrile derivatives **4** proceeded by heating in ethanol and piperidine to furnish the corresponding *N*-(6-amino-4-aryl-3,5-dicyano-2-oxopyridin-1(2*H*)-yl)-2-hydroxybenzamides **5a-e** (Scheme 2). The formation of pyridine ring system was started by Michael-type addition to form the intermediate **A**, which undergoes cyclization *via* intramolecular addition of N-H on the nitrile group. Air oxidation (loss of hydrogen molecule) of the produced intermediate **B** afforded the target pyridinone derivatives **5a-c**. The structures of the produced pyridinone compounds **5a-c** were elucidated by their spectroscopic analyses. The IR spectrum of pyridone **5c** displayed the expected absorptions of the nitrile and carbonyl groups at 2209 and 1659 cm^{-1}, respectively. The ^{1}H NMR spectrum of pyridone **5c** exhibited singlet at δ 3.84 ppm (-OCH_3), multiplet and doublet signals (δ 7.14-8.10 ppm) for the aromatic protons, and two singlet signals at δ 8.20 and 11.28 ppm for the protons of N-H and O-H groups. The ^{13}C NMR spectrum of compound **5e** displayed 18 signals for twenty carbon atoms. The carbon signals for the carbonyl groups resonate at δ 157.06 and 161.08 ppm.



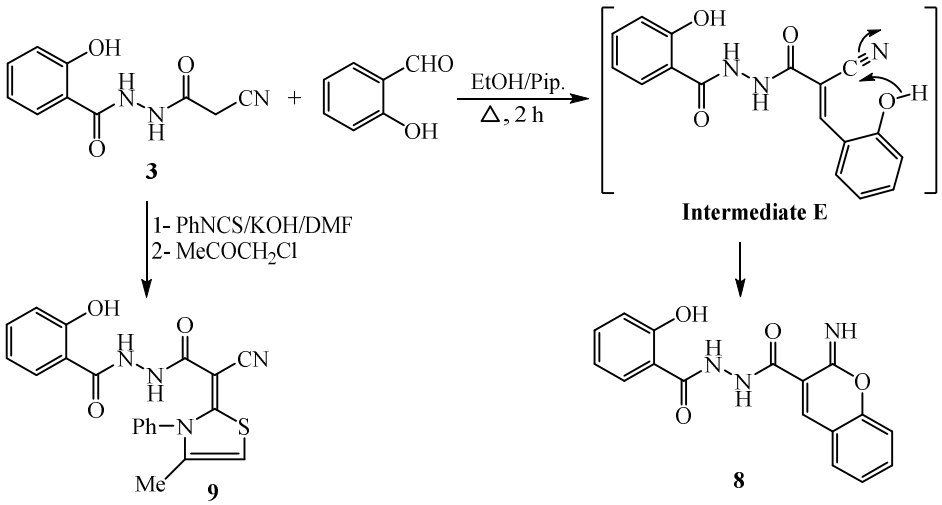
Scheme 2. Synthesis of *N*-(6-amino-4-aryl-3,5-dicyano-2-oxopyridin-1(2*H*)-yl)-2-hydroxybenzamides **5a-e**.

On the other hand, heating *N*'-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) with 3-aryl-2-cyanoacrylatecompounds **6** in ethyl alcohol and piperidine afforded the corresponding 1-(2-hydroxybenzamido)-6-hydroxypyridine compounds **7** (Scheme 3). The formation of 6-hydroxypyridinecompounds **7a-c** start through a Michael-type addition to produce the intermediate **C**, which underdoes loss of EtOH molecule. Air oxidation of the intermediate **D** that formed yielded the corresponding 6-hydroxypyridine compounds **7**.The IR spectrum of **7c** indicated absorptions for the N-H and O-H groups at 3367, 3340 and 3193 cm^{-1}. The nitrile and carbonyl functions were absorbed at 2224, and (1734, 1668), respectively. The ^1H NMR spectrum of **7c** showed multiplet and doublet signals in the region from δ 6.91 to 7.93 for eight aromatic protons. The protons of two hydroxyl groups resonate as singlet signals at δ 10.56 and 11.74 ppm.

The reaction of *N*'-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) with salicylaldehyde has been performed by heating in ethanol and piperidine to produce the corresponding 2-imino-2*H*-chromene-3-carbohydrazide compound **8** (Scheme 4). The reaction mechanism starts by Knoevenagel-type condensation of methylene group (compound **3**) with aldehyde function from salicylaldehyde. The formed intermediate E undergoes intramolecular cyclization between nitrile and hydroxyl groups to produce chromene ring system **8**. The IR spectrum displayed absorptions at 3444, 3350, 3199 cm^{-1} for the N-H and O-H groups. The absorption of carbonyl group was observed at 1690 cm^{-1}. The ^1H NMR spectrum showed multiplet and doublet-doublet signals (δ 6.92 to 7.91 ppm) for the aromatic protons. The four singlet signals at δ 8.66, 8.86, 11.20 and 12.06 ppm were attributed to the protons of 3N-H and O-H groups. The introduction of thiazole ring system to the precursor *N*'-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) was accomplished through the use of phenyl isothiocyanate in DMF and solid potassium hydroxide, followed by cyclization of the intermediate thiourea with chloroacetone to furnish the target, *N*'-(2-cyano-2-(4-methyl-3-phenylthiazol-2(3*H*)-ylidene)acetyl)-2-hydroxybenzohydrazide (**9**). The IR absorptions of (N-H and O-H) are observed at 3345 and 3179 cm^{-1}. The nitrile and carbonyl groups are observed at 2178 (C≡N) and 1652 cm^{-1} (C=O). The ^1H NMR spectrum indicates the proton of thiazole-C5 as singlet at δ 5.84 ppm.



Scheme 3. Synthesis of 1-(2-hydroxybenzamido)-6-hydroxypyridine derivatives **7a-c**.



Scheme 4. Synthesis of salicylic acid hydrazide-chromene and salicylic acid hydrazide-thiazole hybrids **8** and **9**.

Antimicrobial activity

In Table 1, some of the synthesized pyridine, chromene and thiazole compounds showed good inhibition to bacterial and fungal growth especially thiazole compound **9** that showed the highest activity indices (52.0, 69.5, 75.0 and 73.9%) against *E. coli* (52.0%), *P. aeruginosa* (69.5%), *S. aureus* (75.0%) and *B. subtilis* (73.9%). The chromene compound **8** showed weak effect on some

types of bacteria mainly *E. coli* and *P. aeruginosa*, while had activity index values of 54.2 and 60.9% against *S. aureus* and *B. subtitles*, respectively. For *C. albicans* had 51.8% index value. Pyridine compound **7c** displayed activity index values of 36, 52.2, 54.2 and 52.2% while had activity index value of 33.3% for *candida albicans*. Compound **7b** had activity index value of (28, 47.8, 37.5 and 43.5%) against *E. coli*, *P. aeruginosa* and, *S. aureus* and *B. subtilis* and 29. 6% for *C. albicans*. Pyridine compounds **5a**, **5b** and **5e** have no effect on Gram-negative bacterium stain *(E. coli*). For compound **5a**, there is no effect on both *E. coli* and *S. aureus* while had low index value activity 8.7% against *P. aeruginosa* and *B. subtitles* and 7.4% against *C. albicans.* For compound **5c**, the activity index values were 16.0, 30.4, 20.8 and 21.7% against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtitles,* respectively, while had be 14.8% for *C. albicans*. Compound **5d** had activity index values of 24, 43.5, 33.3 and 39.7% against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtitles* while had 25.9% against *C. albicans*. According to compound **5e** had activity index values of 13.0, 25.0 and 30.4% against *P. aeruginosa*, *S. aureus* and *B. subtilis* but had 18.5% against *C. albicans.* While pyridine compounds **5b** and **7a** (substituted with methyl group at the phenyl group) had no effect on both antimicrobial (anti-bacterial and anti-fungal).

$$\% \text{ Activity Index} = \frac{\text{Zone of inhinition by test compound (diameter)}}{\text{Zone of inhinition by by standard (diameter)}} \text{ X } 100$$

Table 1. Antimicrobial activity of the produced pyridine, chromene and thiazole compounds.

Compounds	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
	Inhibition zone, mm (activity index %)		Inhibition zone, mm (activity index %)		Inhibition zone, mm (activity index %)
1	NA	NA	NA	NA	2 (7.4%)
5a	NA	2 (8.7%)	NA	2 (8.7%)	2 (7.45)
5b	NA	NA	NA	NA	NA
5c	4 (16.0%)	7 (30.4)	5 (20.8)	5 (21.7%)	4 (14.8%)
5d	6 (24.0%)	10 (43.5%)	8 (33.3)	9 (39.1)	7 (25.9)
5e	NA	3 (13%)	6 (25.0%)	7 (30.4%)	5 (18.5%)
7a	NA	NA	NA	NA	NA
7b	7 (28.0%)	11 (47.8%)	9 (37.5%)	10 (43.5%)	8 (29.6%)
7c	9 (36.0%)	12 (52.2%)	13 (54.2%)	12 (52.2%)	9 (33.3%)
8	6 (24.0%)	10 (43.5%)	13 (54.2)	14 (60.9%)	14 (51.8%)
9	13 (52.0%)	16 (69.5%)	18 (75.0)	17 (73.9%)	18 (66.6%)
Ampicillin	25 (100.0%)	23 (100.0%)	24 (100.0%)	23 (100.0%)	-
Clotrimazole	-	-	-	-	27 (100%)

NA = No activity.

The MIC (minimum inhibitory concentration) for the potent pyridine, chromene and thiazole compounds **7c**, **8**, and **9** was assessed and the results are presented in Table 2.

Antioxidant activity

The newly synthesized pyridine, chromene and thiazole compounds were tested for their antioxidant activity by using both (*DPPH* and *ABTS* Assay). The results (Table 3) for *ABTS* assay indicated that: the thiazole compound **9** give the best antioxidant property (87.9%). The increasing in antioxidant activity of compound **9** may be because of the combination between salicylic acid hydrazide and thiazole moieties. For IC_{50} values of the synthesized products in relation to *DPPH* inhibition. The concentration of antioxidant obligatory to decrease the initial *DPPH* concentration by 50% (IC_{50}) is the parameter that applied to indicate the antioxidant activity. According to the *ABTS* method, the IC_{50}values of compounds **7c**, **8** and **9** were 89.14, 102.63 and 33.27, respectively. The quantities of the *ABTS* test showed marginally lower scavenging capacity in

relation to the *DPPH* method (Table 4) while IC_{50} values in the *DPPH* Method were 50.46 (compound **7c**), 63.35 (compound **8**) and 19.58 (compound **9**). The tough antioxidant commotion of the synthesized products is record often connected with great content of total phenols. The extant effects determined that the antioxidant activity of thiazole compound **9** is very close to the standard antioxidant (ascorbic acid).

Table 2. MIC (μM) of compounds **7c**, **8** and **9**.

Compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
7c	16	8	8	16	32
8	>100	64	32	32	16
9	4	4	2	4	1
Ampicillin	0.5	2	1	4	-
Clotrimazole	-	-	-	-	2

Table 3. Anti-oxidant activity of the produced pyridine, chromene and thiazole compounds.

Method	*ABTS* Abs.(control)-Abs.(test)/Abs.(control) x 100	
Compounds	Absorbance of samples	% inhibition
Control of *ABTS*	0.505	0
Ascorbic-acid	0.060	88.1%
1	0.449	11.1
5a	0.444	12.1
5b	0.453	10.3
5c	0.431	14.6
5d	0.405	19.8
5e	0.426	15.6
7a	0.402	20.4
7b	0.392	22.4
7c	0.378	25.1
8	0.369	26.9
9	0.061	87.9

Table 4. IC_{50} for *DPPH* and *ABTS* techniques.

Compound	*ABTS* IC_{50} (μM)	*DPPH* IC_{50} (μM)
7c	89.14±0.48	50.40±0.36
8	102.63±0.66	63.35±0.45
9	33.27±0.29	19.58±0.12
Ascorbic acid	31.32±0.24	20.16±0.15

EXPERIMENTAL

A Thermo Scientific Nicolet iS10 FTIR spectrometer was used to determine the infrared spectra (KBr discs). JEOL's NMR spectrometer was used to measure ^{1}H NMR (500 MHz) and 13C NMR (125 MHz) spectra with DMSO-d_6 as the solvent. The mass analyses were performed using a Thermo Scientific Focus/DSQII quadrupole GC/MS system. On a EuroVector instrument analyzer (EA3000 Series), elemental analyses were performed. Commercial vendors were used to get all chemicals and solvents (Fluka and Arcos).

*Synthesis of N'-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**1**).* Salicylic acid hydrazide (**1**) (0.02 mol, 3.04 g) was added to a solution of 1-(cyanoacetyl)-3,5-dimethylpyrazole (**2**) (0.02 mol, 3.26

g) in 40 mL dioxane. The mixture was refluxed for 4 h. The solid that formed was collected and dried to obtain the desired product. Buff crystals, yield 68.3%, mp 201-202°C. IR (ν, cm^{-1}):3337, 3266, 3192, (N-H and O-H), 2264 (C≡N), 1689 (C=O). ^{1}H NMR (δ, ppm): 3.58 (s, 2H), 7.00-7.04 (m, 2H), 7.36-7.39 (m, 1H), 8.05 (d, 1H, J = 6.50 Hz), 10.02 (s, 1H), 10.28 (s, 1H), 10.71 (s, 1H, OH). ^{13}C NMR (δ, ppm): 29.25, 118.22, 119.10, 120.84, 123.19, 129.06, 132.65, 159.36, 160.43, 167.58. Anal. calcd. for $C_{10}H_9N_3O_3$: C, 54.79; H, 4.14; N, 19.17%. Found: C, 54.94; H, 4.20; N, 19.08%. MS: *m/z* 219 [M^+, 77.12%].

Preparation of N-(6-amino-4-aryl-3,5-dicyano-2-oxopyridin-1(2H)-yl)-2-hydroxybenzamide derivatives ***5a-e***

To a suspension of *N*'-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) (0.002 mol, 0.44 g) in 30 mL absolute ethanol, the required arylidene-malononitrile derivative **4** (0.002 mol) and 0.2 mL piperidine were added. The mixture was refluxed for 4 h and the solid that formed was collected and dried to give the target pyridine derivatives **5a-e**.

*N-(6-Amino-3,5-dicyano-2-oxo-4-phenylpyridin-1(2H)-yl)-2-hydroxybenzamide (**5a**).* White crystals, yield 62.4%, mp 239-240ºC. IR (ν, cm^{-1}): 3452 (broad for NH_2, N-H and O-H), 2210 (C≡N), 1663 (C=O). ^{1}H NMR (δ, ppm): 6.98-7.02 (m, 2H), 7.36-7.40 (m, 1H), 7.54 (s, 5H), 8.09-8.11 (dd, 1H, J = 7.50, 1.50 Hz), 8.20 (s, 1H, NH), 11.25 (s, 1H, OH). ^{13}C NMR (δ, ppm): 77.26, 85.22, 113.93, 116.05, 118.47, 120.15, 121.74, 126.58, 127.88, 128.87 (2C), 129.35 (2C), 133.75, 134.06, 156.05, 158.42, 159.64, 160.56. 161.38. Anal. calcd. for $C_{20}H_{13}N_5O_3$:C, 64.69; H, 3.53; N, 18.86%. Found: C, 64.58; H, 3.48; N, 18.92%. MS: *m/z* 371 [M^+, 54.38%].

*N-(6-Amino-3,5-dicyano-2-oxo-4-(4-tolyl)pyridin-1(2H)-yl)-2-hydroxybenzamide (**5b**).* White crystals, yield 48.8%, mp 242-243 ºC. IR (ν, cm^{-1}): 3450 (broad for NH_2, N-H and O-H), 2211 (C≡N), 1662 (C=O). ^{1}H NMR (δ, ppm): 2.39 (s, 3H, CH_3), 6.99-7.01 (m, 2H), 7.33-7.38 (m, 3H), 7.43 (d, 2H, J = 8.00 Hz), 8.08-8.10 (dd, 1H, J = 7.00, 1.50 Hz), 8.19 (s, 1H, NH), 11.26(s, 1H, OH). ^{13}C NMR (δ, ppm): 21.14, 84.47, 77.09, 113.88, 115.93, 118.22, 119.87, 121.50, 128.06, 128.70 (2C), 129.41, 133.95 (2C), 134.39, 136.56, 156.48, 158.64, 160.04, 160.73, 161.31. Anal. calcd. for $C_{21}H_{15}N_5O_3$: C, 65.45; H, 3.92; N, 18.17%. Found: C, 65.28; H, 3.98; N, 18.30%. MS: *m/z* 385 [M^+, 36.95%].

*N-(6-Amino-4-(4-anisyl)-3,5-dicyano-2-oxopyridin-1(2H)-yl)-2-hydroxybenzamide (**5c**).* Buff solid, yield 54.5%, mp 232-233 °C. IR (ν, cm^{-1}): 3453 (broad for NH_2, N-H and O-H), 2209 (C≡N), 1659 (C=O). ^{1}H NMR (δ, ppm): 3.84 (s, 3H), 6.99-7.02 (m, 2H), 7.09 (d, 2H, J = 9.00 Hz), 7.37-7.40 (m, 1H), 7.50 (d, 2H, J = 9.00 Hz), 8.10 (d, 1H, J = 6.50 Hz), 8.20 (s, 1H, NH), 11.28 (s, 1H, OH). ^{13}C NMR (δ, ppm): 55.61, 78.08, 83.81, 113.90, 114.68 (2C), 116.09, 117.97, 120.33, 122.06, 124.38, 127.58, 130.45 (2C), 134.35, 156.48, 157.80, 158.21, 159.51, 160.67, 161.72. Anal. calcd. for $C_{21}H_{15}N_5O_4$: C, 62.84; H, 3.77; N, 17.45%. Found: C, 62.96; H, 3.71; N, 17.53%. MS: *m/z* 401 [M^+, 40.08%].

*N-(6-Amino-4-(4-chlorophenyl)-3,5-dicyano-2-oxopyridin-1(2H)-yl)-2-hydroxybenzamide (**5d**).* White crystals, yield 52.8%, mp 242-243 °C. IR (ν, cm^{-1}): 3344, 3218 (NH_2, N-H and O-H), 2214 (C≡N), 1656 (C=O).^{1}H NMR (δ, ppm): 6.99-7.02 (m, 2H), 7.32-7.46 (m, 3H), 7.60 (d, 2H, J = 9.00 Hz), 7.94 (d, 1H, J = 6.50 Hz), 8.09 (s, 1H), 11.21 (s, 1H). ^{13}C NMR (δ, ppm): 76.96, 84.37, 113.71, 116.63, 118.84, 119.37, 120.95, 127.56, 128.68 (2C), 129.75 (2C), 130.22, 132.93 135.02, 155.85, 156.11, 157.32, 160.44, 161.20. Anal. calcd. for $C_{20}H_{12}ClN_5O_3$: C, 59.20; H, 2.98; N, 17.26%. Found: C, 59.38; H, 2.90; N, 17.14%. MS: *m/z* 405 [M^+, 18.72%].

*N-(6-Amino-3,5-dicyano-4-(4-hydroxyphenyl)-2-oxopyridin-1(2H)-yl)-2-hydroxybenzamide (**5e**).* Buff crystals, yield 45.2%, mp 258-260 °C. IR (ν, cm^{-1}): 3453 (broad for NH_2, N-H and O-

H), 2213 (C≡N), 1633 (broad, C=O). ^{1}H NMR (δ, ppm): 7.00-7.02 (m, 2H), 7.38-7.40 (m, 1H), 7.53-7.55 (m, 4H), 8.09-8.11 (dd, 1H, *J* = 8.00, 2.00 Hz). ^{13}C NMR (δ, ppm): 76.85, 84.23, 113.64, 116.58, 117.03, 117.97, 119.61, 127.47, 128.43 (2C), 128.59 (2C), 129.58, 131.89, 135.68, 152.21, 155.55, 155.67, 157.06, 161.08. Anal. calcd. for $C_{20}H_{13}N_5O_4$:C, 62.02; H, 3.38; N, 18.08%. Found: C, 61.85; H, 3.45; N, 18.18%. MS: *m/z* 387 [M^+, 29.44%].

Synthesis of N-(4-aryl)-3,5-dicyano-6-hydroxy-2-oxopyridin-1(2H)-yl)-2-hydroxybenzamide ***7a-c***

To a suspension of *N*'-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) (0.002 mol, 0.44 g) in 30 mL absolute ethanol, each derivative of ethyl 3-aryl-2-cyanoacrylates **6** (0.002 mol) and 0.2 mL piperidine was added. The mixture was refluxed for 4 h and the solid that formed was collected and dried to give the target 6-hydroxypyridine derivatives **7a-c**.

*N-(3,5-Dicyano-6-hydroxy-2-oxo-4-(4-tolyl)pyridin-1(2H)-yl)-2-hydroxybenzamide (****7a****).* Yellow solid, yield 44.5%, mp 268-269 °C. IR (*v*, cm^{-1}): 3348, 3294, 3188 (N-H and O-H), 2220 (C≡N), 1664 (C=O). ^{1}H NMR (δ, ppm): 2.35(s, 3H, CH_3), 7.00-7.03 (m, 2H), 7.34-7.38 (m, 3H), 7.42(d, 2H, *J* = 8.00 Hz), 8.07-8.09 (dd, 1H, *J* = 7.00, 1.50 Hz), 10.61(s, 1H), 11.47(s, 1H). ^{13}C NMR (δ, ppm): 21.17, 64.57, 73.29, 113.86, 115.13, 117.96, 118.43, 120.62, 128.91, 129.40 (2C), 130.79, 132.38 (2C), 133.84, 138.66, 159.51, 160.44, 161.24, 162.75, 171.02. Anal. calcd. for $C_{21}H_{14}N_4O_4$:C, 65.28; H, 3.65; N, 14.50%. Found: C, 65.41; H, 3.60; N, 14.42%. MS: *m/z* 386 [M^+, 61.04%].

*N-(4-(4-Anisyl)-3,5-dicyano-6-hydroxy-2-oxopyridin-1(2H)-yl)-2-hydroxybenzamide (****7b****).* Yellow solid, yield 48.1%, mp 281-282 °C. IR (*v*, cm^{-1}): 3335, 3281, 3174 (N-H and O-H), 2218 (C≡N), 1658 (C=O). ^{1}H NMR (δ, ppm): 3.83 (s, 3H, OCH_3), 6.98-7.02(m, 2H), 7.08 (d, 2H, *J* = 9.00 Hz), 7.37-7.40 (m, 1H), 7.52 (d, 2H, *J* = 9.00 Hz), 7.96 (d, 1H, *J* = 6.50 Hz), 10.68 (s, 1H), 11.55 (s, 1H). ^{13}C NMR (δ, ppm): 55.67, 65.35, 74.06, 113.72, 114.05 (2C), 114.83, 118.02, 118.60, 120.43, 126.33, 129.22, 130.19 (2C), 133.75, 159.15, 159.86, 160.58, 161.07, 161.93, 170.61. Anal. calcd. for $C_{21}H_{14}N_4O_5$:C, 62.69; H, 3.51; N, 13.92%. Found: C, 62.54; H, 3.58; N, 13.80%. MS: *m/z* 402 [M^+, 47.16%].

*N-(4-(4-Chlorophenyl)-3,5-dicyano-6-hydroxy-2-oxopyridin-1(2H)-yl)-2-hydroxybenzamide (****7c****).* Buff solid, yield 31.6%, mp 230-231 °C. IR (*v*, cm^{-1}): 3367, 3340, 3193 (N-H and O-H), 2224 (C≡N), 1734, 1668 (C=O). ^{1}H NMR (δ, ppm): 6.91-6.96 (m, 2H), 7.43(t, 1H, *J* = 7.50 Hz), 7.56 (d, 2H, *J* = 8.00 Hz), 7.84 (d, 2H, *J* = 8.00 Hz), 7.93 (d, 1H, *J* = 9.00 Hz), 10.56 (s, 1H), 11.74(s, 1H). ^{13}C NMR (δ, ppm): 64.71, 73.41, 113.90, 115.04, 117.87, 118.56, 120.70, 128.78 (2C), 129.05, 130.32 (2C), 132.53, 133.76, 134.28, 159.43, 160.39, 161.69, 162.54, 170.55. Anal. calcd. for $C_{20}H_{11}ClN_4O_4$: C, 59.05; H, 2.73; N, 13.77%. Found: C, 58.89; H, 2.80; N, 13.64%. MS: *m/z* 408 [M^+, 15.31%], 406 [M^+ + 2, 43.84%].

*Synthesis of N'-(2-Hydroxybenzoyl)-2-imino-2H-chromene-3-carbohydrazide (****8****).* A mixture of *N*'-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) (0.002 mol, 0.44 g), salicylaldehyde (0.002 mol, 0.24 mL) and two drops of piperidine in ethanol (30 mL) was refluxed for 4 h. The solid that formed upon cooling was collected to produce the imino-chromene compound **10**. Orange crystals, yield 80.2%, mp 204-205 °C. IR (*v*, cm^{-1}): 3444, 3350, 3199 (N-H and O-H), 1690 (C=O). ^{1}H NMR (δ, ppm): 6.92-6.98 (m, 4H), 7.29-7.32 (m, 1H), 7.42-7.46 (m, 1H), 7.54-7.56 (dd, 1H, *J* = 7.50, 2.50 Hz), 7.86-7.91 (dd, 1H, *J* = 7.50, 2.50 Hz), 8.66 (s, 1H, NH), 8.86 (s, 1H, NH), 11.20 (s, 1H, OH), 12.06 (s, 1H, NH). ^{13}C NMR (δ, ppm): 115.32, 116.44, 117.85, 118.08, 120.87, 122.94, 123.49, 128.78, 129.26, 130.73, 133.65, 139.57, 153.61, 159.52, 159.84, 162.20, 166.09. Anal. calcd. for $C_{17}H_{13}N_3O_4$: C, 63.16; H, 4.05; N, 13.00%. Found: C, 63.28; H, 4.12; N, 12.91%. MS: *m/z* 323 [M^+, 22.68%].

*Preparation of N'-(2-cyano-2-(4-methyl-3-phenylthiazol-2(3H)-ylidene)acetyl)-2-hydroxybenzohydrazide (**9**)*. A 100 mL conical flask was supplied by *N*'-(2-cyanoacetyl)-2-hydroxybenzohydrazide (**3**) (0.44 g, 0.002 mol) in 20 mL DMF and solid KOH (0.12 g, 0.002 mol). The mixture was stirred for 6 h after addition phenyl isothiocyanate (0.24 mL, 0.002 mol). After that chloroacetone (0.18 g, 0.002 mol) was added and stirring was continued for additional 6 h. After dilution with 50 mL ice-water, the solid was recovered and recrystallized from ethanol. Pink solid, yield 39.2%, mp 224-225 °C. IR (ν, cm^{-1}): 3345, 3179 (N-H and O-H), 2178 (C≡N), 1652 (C=O). ^{1}H NMR (δ, ppm): 1.88 (s, 3H, CH_3), 5.84 (s, 1H, thiazole-H), 6.98-7.48 (m, 8H), 7.95 (d, 1H, J = 9.00 Hz), 10.16 (s, 1H, NH), 10.72 (s, 1H, OH), 11.86 (s, 1H, NH). ^{13}C NMR (δ, ppm): 13.97, 79.71, 111.45, 114.76, 118.11, 120.85, 121.47, 125.38, 126.33 (2C), 128.94, 129.64 (2C), 133.26, 133.72, 139.60, 159.50, 160.34, 162.08, 170.19. Anal. calcd. for $C_{20}H_{16}N_4O_3S$:C, 61.21; H, 4.11; N, 14.28%. Found: C, 61.03; H, 4.19; N, 14.30%. MS: *m/z* 392 [M^+, 21.30%].

Screening of antibacterial and antifungal activity

The effect of antibacterial can well-known by testing the amalgamated pyridine, chromene, and thiazole compounds with four types of bacteria (*E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*) and one type of fungi (*C. albicans*) by using the Agar Plate diffusion method. The investigated bacteria and fungi were grown in Muller-Hinton (MH) Media at 37 °C for 24 hours before being injected with 20L of 1 106 cfu/mL of the investigated microorganism in melted MH Media at 50 °C. Well was emptied into 9 cm diameter plates and left to solidify. The studied product was softened in DMSO to get a concentration of 5 mg/mL, and 100 mL was added to the conforming wells. The paper discs were saturated with the expected concentration of the complex solution before being placed in petri dishes containing nutritional agar media (agar 20 g + yeast extract 3 g + peptone 5 g), and each one was replicated three times [42,43].

Determination the minimum inhibitory concentration (MIC). In vitro, for antibacterial efficiency to examined the blended Product as reported by CLSI, 2015 and also in vitro for antifungal activity according to (CLS I M27 – A3 and CLS M38 –A2 Methods) (CLSI 2008). Two fold for the dynamic compound, make a serial dilution by weighted of 0.01 mL MH Broth for antibacterial and yeast peptone dextrose for antifungal assay [42]. For every filter paper which cut as circle and can be consider as well in occulated with 0.01 of the diluted bacterial suspension (5 x 10^6 cfu/mL) [43]. Ampicillin's antibacterial activity was calculated and utilized as a positive control [44].

Antioxidant assay

ABTS (5 mL of 7 mm) was mixed with 88 mL of 140 mm potassium persulfate to make the ABTS reagent. To measure the scavenging activity, 100 mL of ABTS reagent was combined with 100 mL of sample in a 96-well microplate and incubated at 25 °C for 6 m before being diluted with water (1:44 v/v). After incubation, the absorbance was measured at 734 nm using an Elisa reader. A 100 percent methanol control was also used as a control. Extrapolation from regression analysis yielded the IC_{50} ABTS values.

$$\%\, Inhibition = \frac{(A)control - (A)sample}{(A)control} \times 100$$

The DPPH assay was performed three times, and the average values were used. A freshly prepared methyl alcohol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (0.004 percent w/v) was prepared and stored in the dark at 10°C.The test chemical was made into a methanol solution. A volume of 3 mL of DPPH solution was added to a 40 mL aliquot of the methanol solution. A UV-visible spectrophotometer was used to take instantaneous absorbance values. The decline in absorbance at 515 nm was monitored constantly, with data collected at 1 min intervals

until the absorbance levelled off (16 min). All of the tests were repeated three times, and the results were averaged. The DPPH radical's percentage inhibition (PI) was estimated using the formula: PI = [{(AC- AT)/ AC} x 100]. Where AC = control absorbance at t = 0 min and AT = sample absorbance + DPPH at t = 16 min [45].

CONCLUSION

A series of new pyridine, chromene and thiazole compounds was synthesized through the reactions of *N′*-(2-cyanoacetyl)-2-hydroxybenzohydrazide with various arylidene-malononitriles, 3-aryl-2-cyanoacrylates salicylaldehyde and (phenyl isothiocyanate / chloroacetone). The structures of these synthesized compounds were confirmed by multiple spectroscopy techniques and screened for antimicrobial and antioxidant activity. The thiazole compound**9** was the most potent of the tested compounds and showed the highest anti-bacterial and antifungal activities against *S. aureus* (75.0%) and *B. subtilis* (73.9%) and *C. albicans* (66.6%). The combination between salicylic acid hydrazide and thiazole moieties in the hybrid **9** indicated the best antioxidant activity (87.9%).

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