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Synthesis, *in-vitro* antibacterial and anticancer screening of novel nicotinonitrile-coumarin hybrids utilizing piperazine citrate

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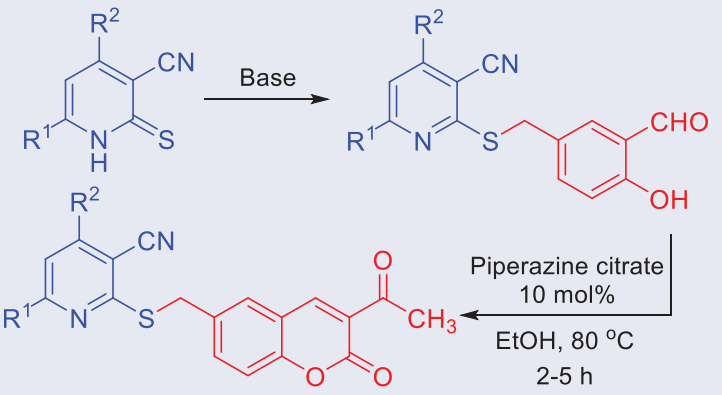
ABSTRACT

An efficient, low-cost, high yield% and eco-friendly synthetic procedure is designed for the synthesis of novel series of nicotinonitrile-coumarin hybrid molecules bearing several aryl and/or heteroaryl moieties. Our strategy includes the synthesis of a novel series of 2-hydroxybenzaldehydes, bearing nicotinonitrile moiety, followed by its Knoevenagel reaction with ethyl acetoacetate in the presence of an organo-base. The above reaction is studied in different reaction conditions. The optimized conditions are performing the reaction in ethanol at 80 °C in the presence of 10 mol% of piperazine citrate (1:1). The *in-vitro* antibacterial activities of the nicotinonitrile-coumarins were evaluated against different Gram-positive and negative bacterial strains. Moreover, the *in-vitro* cytotoxicity of nicotinonitrile-coumarins were estimated against different eukaryotic cell lines. Compounds **5a** and **5b** exhibited the most promising antibacterial agents among the novel series. The structures of novel series of the target nicotinonitrile-coumarin hybrid molecules were confirmed by considering their elemental analyses and spectral data.

GRAPHICAL ABSTRACT



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Supplemental data for this article can be accessed on the publisher's website.

Introduction

Coumarins or 2*H*-chromen-2-ones are natural occurring heterocyclic derivatives that used as effective components in inhibition and healing of many diseases. These derivatives act as antimicrobial,[1] anti-inflammatory,[2] anticancer,[3] anti-Alzheimer disease[4,5] and anti-Parkinson agents.[6] Warfarin and dicoumarol are examples of chromene derivatives which considered as essential oral anticoagulant drugs.[7] Many publications reported the synthesis of coumarins. They can be prepared *via* Wittig,[8] Heck,[9] Perkin and[10] Pechmann reactions.[11,12] In addition, Reformatsky,[13] and Knoevenagel[14–16] reactions can be used as efficient synthetic routes for the preparation of coumarins.

Despite the presence of many attempts to design synthetic routes for the preparation of coumarin derivatives, still there is a considerable need for developing of efficient, low-cost, high yield and eco-friendly procedure for the synthesis of such biologically active heterocyclic derivatives.

Piperazine and its derivatives were used as efficient catalysts in the synthesis of various heterocyclic derivatives. Several 2-amino-3-cyano-4*H*-pyrans derivatives were prepared *via* the Michael addition reactions which catalyzed by piperazine.[17,18] Moreover, pyrano[2,3-*d*]pyrimidinone derivatives were prepared by the one pot reaction of aldehydes, malononitrile and (thio)barbituric acid which catalyzed by piperazine-1,4-diium dihydrogen phosphate.[19] Piperazine-containing polymers can be used in the catalysis for Knoevenagel condensation reactions.[20,21] Chiral 2,5-dibenzylpiperazine was used in the catalytic asymmetric Michael addition of aldehydes to nitroalkenes.[22] In addition, piperazine can be incorporated in many heterogeneous catalysts which used to catalyze several reactions. For example, KG-60-piperazine catalyzes the synthesis of 2-aminothiophenes *via* Gewald three-component reaction.[23] Also, reduced graphene oxide supported piperazine catalyzes Knoevenagel condensation and Michael addition reactions as well as Knoevenagel-Michael addition one-pot process.[24]

Piperazine citrate is a well-known drug for its wide applications as an anthelmintic. It used in the treatment and control as well as prevention of several worm infections as threadworm, roundworm and pinworm in both humans and animals.[25,26] Piperazine citrate causes paralysis of the parasites that lead to diseases by their invasion of the host body. Therefore, it leads to remove the harmful parasites and inhibit the spread of diseases. For example, it helps in the treatment of chicken naturally infected with gastrointestinal helminths.[27] Also, piperazine citrate can be used in the treatment of ascaris lumbricoides infestation.[28] Piperazine citrate also helps in the treatment of bacterial infections.[29]

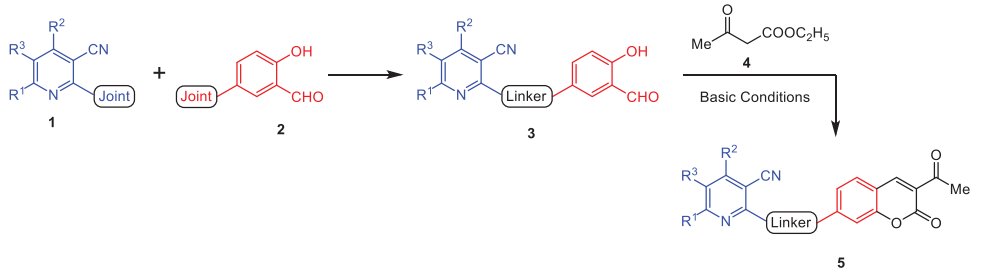
Considering the broad biological importance of nicotinonitriles,[30–35] our research group has exerted many efforts in the field of synthesis of several nicotinonitriles which have been manifested in the publication of many scientific papers.[14,36–39] The work in this study is aimed to study the utility of piperazine citrate, as a green catalyst, in the synthesis of novel series of nicotinonitrile-coumarin hybrid molecules bearing several aryl and/or heteroaryl moieties. The *in-vitro* antibacterial activities of the nicotinonitrile-coumarin hybrid molecules were evaluated against different Gram-positive and negative bacterial strains. Moreover, the *in-vitro* cytotoxicity of nicotinonitrile-coumarins were estimated against different eukaryotic cell lines.

Results and discussion

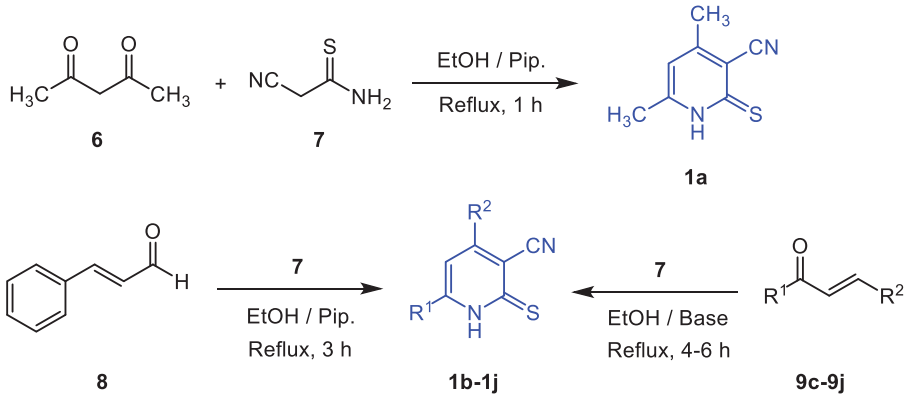
Chemistry

A simple synthetic route was designed for the preparation of novel nicotinonitrile-coumarin hybrid molecules **5** using 2-hydroxybenzaldehydes **3**, incorporating nicotinonitrile moiety, as building blocks. The reaction of nicotinonitriles **1** with 2-hydroxybenzaldehydes **2** in appropriate reaction conditions afforded a novel series of 2-hydroxybenzaldehyde-nicotinonitrile hybrids **3** *via* an appropriate linker. Then, these derivatives reacted with ethyl acetoacetate **4** in the presence of basic conditions to afford the desired nicotinonitrile-coumarin hybrid molecules **5** (Scheme 1).

Thus, the formation of numerous nicotinonitriles was our first step to our aim. In this study, we investigate the synthetic potential of pyridine-2(1*H*)-thiones as effective examples of nicotinonitriles. The most common synthetic route to pyridine-2(1*H*)-thione derivatives includes the cyclocondensation of 1,3-dicarbonyl or α,β-unsaturated carbonyl compounds with 2-cyanothioacetamide. For example, the reaction of acetylacetone **6** with 2-cyanothioacetamide **7**, in boiling ethanol in presence of piperidine, afforded 4,6-dimethyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile **1a**[40] (Scheme 2). Also, 6-phenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile **1b**[41] and pyridine-2(1*H*)-thiones **1c–1j**[42–44] were synthesized by the reaction of 2-cyanothioacetamide with cinnamaldehyde **8** or the appropriate chalcones **9c–9j**[42,44–48] in basic



Scheme 1. General synthetic approach for the synthesis of nicotinonitrile-coumarin hybrids molecules **5**.

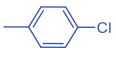
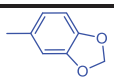
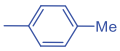
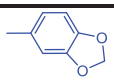
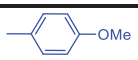
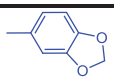
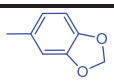
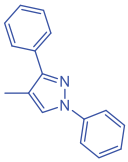
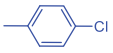
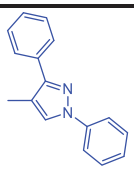
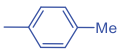
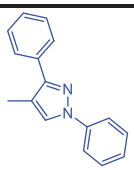
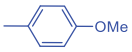
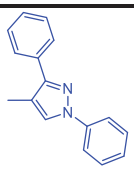


Scheme 2. Synthesis of pyridine-2(1*H*)-thione derivatives **1a–1j**.

medium, respectively (Scheme 2, Table 1). Among the ten pyridine-2(1*H*)-thione derivatives studied in this work, five new pyridine-2(1*H*)-thiones were prepared. Therefore, compounds **1c–1e**, **1h** and **1i**, bearing benzo[*d*][1,3]dioxole or 1,3-diphenyl-1*H*-pyrazole moiety, were prepared by the above procedure (see experimental section).

Next step was the synthesis of novel series of 2-hydroxybenzaldehydes **3**, incorporating pyridine moiety. Formerly, pyridine-2(1*H*)-thione **1a** was stirred with an equivalent amount of potassium hydroxide in ethanol at room temperature for 10 min. Then, an

Table 1. Synthesis of pyridine-2(1*H*)-thiones **1** and 2-hydroxybenzaldehydes **3**.

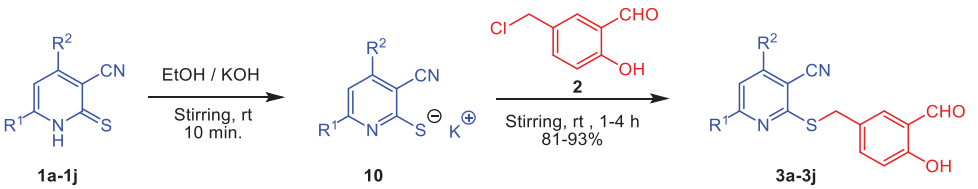
				Pyridine-2(1*H*)-thione 1		2-Hydroxy-benzaldehyde 3	
Entry	**Cpd. 1(3)**	**R^1**	**R^2**	**Yield%**	**m.p. (°C)**	**Yield%**	**Time (h)**
1	**a**	—Me	—Me	94	263-265[41]	97	1
2	**b**		—H	65	245-247[42]	93	1.5
3	**c**			70	244-246*	92	3
4	**d**			79	232-234*	91	2.5
5	**e**			77	218-220*	89	2.5
6	**f**			80	240-242[43]	86	3
7	**g**			72	273[44]	84	3
8	**h**			70	254*	81	4
9	**i**			75	> 300*	84	3.5
10	**j**			86	> 300[45]	84	3

*New pyridine-2(1*H*)-thione derivatives.

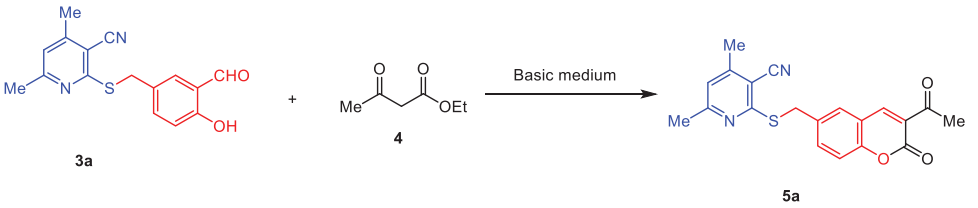
equivalent amount of 5-(chloromethyl)−2-hydroxybenzaldehyde **2**[49] was added with continuous stirring. After one hour of stirring, TLC analysis of the reaction mixture showed the presence of a sole reaction product. The reaction most likely to proceed *via* a simple nucleophilic substitution reaction between thiolate anion **10** and benzyl chloride derivative **2** to give 2-((3-formyl-4-hydroxybenzyl)thio)−4,6-dimethylnicotinonitrile **3a** in 97% yield (Scheme 3). The structure of the isolated product **3a** was established by its elemental analysis and spectral data. The IR spectrum of **3a** shows the presence of OH, CN and CO groups at ῡ 3433, 2233 and 1678 cm^{-1}, respectively. Its mass spectrum gave a molecular ion peak at $m/z = 298$. Its ^{1}H-NMR spectrum revealed six singlet signals at δ 2.37, 2.52, 4.44, 7.10, 10.22 and 10.66 ppm assigned to two CH_3 protons in addition to SCH_2, pyridine-H5, CHO and OH protons, respectively. In addition, it reveals two doublet signals at δ 6.93 and 7.58 ppm and one singlet signal at δ 7.75 ppm corresponding to aromatic H3, H4 and H6 protons, respectively. Its ^{13}C-NMR spectrum reveals the presence of five signals at δ 20.0, 24.6, 32.9, 115.4 and 191.4 ppm corresponding to two CH_3, SCH_2, CN and C=O carbons, respectively, in addition to 11 signals corresponding to pyridine and aromatic carbons (see experimental section).

Similarly, the pyridine-2(1*H*)-thione derivatives **1b–j** were reacted with compound **2** to afford the corresponding 2-hydroxybenzaldehydes **3b–3j**, bearing nicotinonitrile moiety in 81–93% yield (Scheme 3, Table 1).

Subsequently, Knoevenagel synthesis of novel coumarin derivatives **5** was carried out using 2-hydroxybenzaldehydes **3** and ethyl acetoacetate **4**. For example, 2-hydroxybenzaldehyde derivative **3a** was reacted with ethyl acetoacetate **4** in ethanol at reflux in the presence of piperidine as an organo-base to give the corresponding 2-(((3-acetyl-2-oxo-2*H*-chromen-6-yl)methyl)thio)−4,6-dimethylnicotinonitrile **5a** in 45% yield (Scheme 4; Entry 1, Table 2). The structure of **5a** was confirmed *via* elucidation of its elemental analysis and spectral data. The structure of the isolated product **5a** was established by its elemental analysis and spectral data. The IR spectrum of **5a** shows the presence of CN and two CO groups at ῡ 2224, 1722, 1678 cm^{-1}, respectively. Its mass spectrum gave a molecular ion peak at $m/z = 364$. The mass fragmentation pattern of



Scheme 3. Synthesis of 2-hydroxybenzaldehydes, bearing nicotinonitrile moiety **1a–1j**.



Scheme 4. Synthesis of nicotinonitrile-coumarin hybrid molecule **5a**.

Table 2. Optimization of the reaction conditions for products **5a**.

Entry	Solvent	Catalyst	Temp. (°C)	Time (h)	Yield (%)
1	EtOH	Piperidine	80	5	45
2	EtOH	Piperidine	rt	8	Traces
3	Dioxane	Piperidine	110	6	26
4	EtOH	Et_3N	rt	8	Traces
5	EtOH	Et_3N	80	5	38
6	Dioxane	Et_3N	110	5	19
7	EtOH	Morpholine	80	5	35
8	Dioxane	Morpholine	110	6	21
9	DMF	Morpholine	150	6	27
10	EtOH	Piperazine citrate*	80	2	92
11	Water	Piperazine citrate*	rt	8	Traces
12	EtOH	Piperazine citrate*	rt	8	40
13	Dioxane	Piperazine citrate*	110	8	83

*25 mol% of piperazine citrate (1:1) was used.

5a was shown in supplemental file. The ^{1}H-NMR spectrum of **5a** revealed five singlet signals at δ 2.33, 2.43, 2.56, 4.55 and 7.08 ppm assigned to two CH_3 protons in addition to $COCH_3$, SCH_2 and pyridine-H5 protons, respectively. Moreover, it reveals two doublet signals at δ 7.35 and 7.41 ppm and two singlet signals at δ 7.80 and 8.58 ppm corresponding to four chromene-H8, H7, H5 and H4 protons, respectively. Its ^{13}C-NMR spectrum reveals the presence of five signals at δ 20.0, 24.6, 32.9, 115.5 and 195.2 ppm corresponding to two CH_3, SCH_2, CN and $CH_3C{=}O$ carbons, respectively, in addition to 14 signals corresponding to pyridine and chromene carbons (see experimental section).

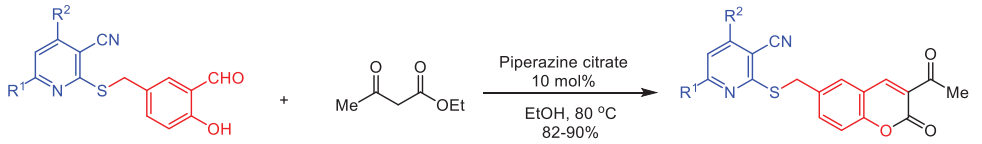
Then, we investigate the optimized reaction condition for this formation of **5a**. We repeated such reaction using different organo-bases and solvents as shown in Table 2. The use of piperidine or triethylamine as a catalyst gave only traces of **5a** when the reaction was carried out in ethanol at room temperature for long reaction times (Entries 2 and 4, Table 2). Then again, **5a** was prepared in 19–38% yields using piperidine, triethylamine or morpholine in different solvents such as ethanol, dioxane or dimethylformamide (DMF) at reflux for long reaction times (Entries 3 and 5–9, Table 2).

The isolation of **5a** in such low yields utilizing the traditional organo-bases stimulated our interest to investigate catalytic potential of new catalysts. Then, we explored the using of piperazine citrate (1:1) as an eco-friendly basic catalyst for the formation of **5a**. Therefore, 25 mol% of piperazine citrate was used to catalyze the above reaction in the presence of water, ethanol or dioxane as a solvent and at different temperatures (Entries 10–13, Table 2). The best yield (95%) was to carry out the reaction in ethanol and at 80 °C for two hours (Entry 10, Table 2). The minimum mol% of piperazine citrate, required to catalyze the formation of **5a**, was studied as shown in Table 3. Different mol% of piperazine citrate were used (Entries 1–6, Table 3). Obviously, the optimum conditions for the formation of **5a** was (Entry 5, Table 3), in which 10 mol% of piperazine citrate was used as an organo-base in ethanol at 80 °C for two hours.

The optimized conditions were applied to Knoevenagel condensation of 2-hydroxybenzaldehydes **3b–j** with ethyl acetoacetate **4**. A structurally divergent range of novel nicotinonitrile-coumarin hybrid molecules **5b–5j**, were prepared in good yields in 82–90% yields (Scheme 5, Table 4).

Table 3. Optimization of the mol% of piperazine citrate (1:1). All entries were carried out using ethanol as solvent and at 80 °C.

Entry	Mol%	Time (h)	Yield (%)
1	1	5	Traces
2	2	5	Traces
3	5	4	39
4	8	4	74
5	10	2	95
6	15	2	94
7	20	2	94



Scheme 5. Synthesis of nicotinonitrile-coumarin hybrid molecules **5b–5j**.

Table 4. Synthesis of nicotinonitrile-coumarin hybrid molecules **5b–5j** using 10 mol% of piperazine citrate as a catalyst.

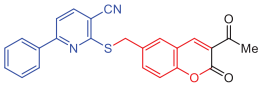
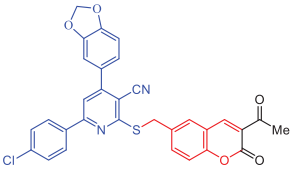
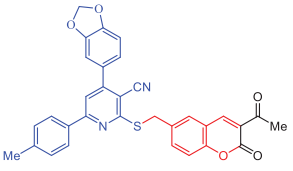
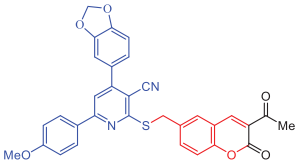
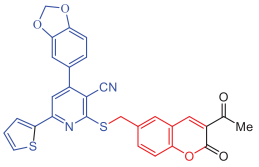
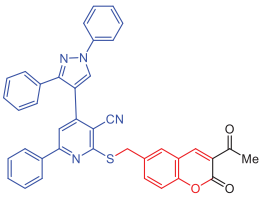
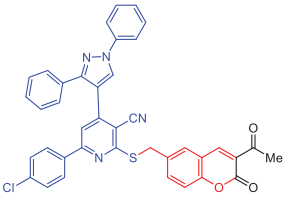
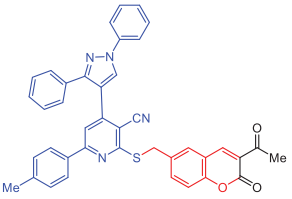
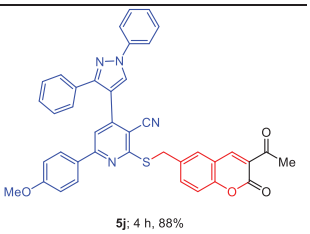
 **5b**; 2 h, 90%	 **5c**; 3 h, 87%	 **5d**; 3 h, 85%
 **5e**; 3.5 h, 84%	 **5f**; 4 h, 88%	 **5g**; 4 h, 86%
 **5h**; 5 h, 82%	 **5i**; 4.5 h, 84%	 **5j**; 4 h, 88%

Table 5. The *in-vitro* antibacterial activities of the novel nicotinonitrile-coumarins against the tested bacterial strains.

	Inhibition zones (mm)				
	Gram-negative bacteria			Gram-positive bacteria	
Compound	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*S. mutans*
5a	44.2 ± 1.0	47.3 ± 1.0	45.0 ± 1.0	38.6 ± 0.7	45.4 ± 1.0
5b	38.6 ± 0.6	31.5 ± 0.6	40.8 ± 1.0	43.6 ± 1.0	40.9 ± 1.0
5c	22.8 ± 0.5	19.1 ± 0.5	21.0 ± 0.5	18.5 ± 0.5	22.6 ± 0.6
5d	38.9 ± 1.0	36.6 ± 1.0	35.5 ± 0.7	33.6 ± 0.6	35.8 ± 0.7
5e	37.9 ± 0.7	43.2 ± 1.0	39.8 ± 1.0	37.7 ± 0.8	40.6 ± 1.0
5f	27.5 ± 0.6	21.3 ± 0.6	29.9 ± 0.7	33.3 ± 1.0	28.9 ± 0.7
5g	21.2 ± 0.5	15.4 ± 0.5	20.4 ± 0.5	15.9 ± 0.5	17.8 ± 0.5
5h	22.9 ± 0.6	17.8 ± .5	19.9 ± 0.5	17.8 ± 0.5	19.8 ± 0.5
5i	23.7 ± 0.6	19.0 ± 0.5	25.4 ± 0.6	20.8 ± 0.6	22.6 ± 0.6
5j	28.7 ± 0.7	31.7 ± 0.7	35.5 ± 1.0	27.4 ± 0.7	23.8 ± 0.6
Ciprofloxacin	25.2 ± 0.6	22.5 ± 0.6	27.3 ± 0.7	24.5 ± 0.6	26.0 ± 0.5

Antimicrobial activities were screened in triplicate and the average reading of the zone of inhibition was determined in mm ± SEM; Inactive (−ve) = (inhibition zone < 8 mm).

Biology

Antibacterial screening

The *in-vitro* antibacterial activities of the novel nicotinonitrile-coumarin hybrids were evaluated against three Gram-negative (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*) as well as two Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) bacterial stains. The values of inhibition zones and MIC were to determine the strength of antibacterial activities using Ciprofloxacin as a standard drug.[50–52] The results of inhibition zones and MIC values were listed in Tables 5 and 6, respectively.

The nicotinonitrile-coumarin derivative **5a** exhibit the most potent antibacterial activities with MIC values of 1.9, 3.9, 3.9, 3.9 and 7.8 µg/mL against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Staphylococcus aureus* bacterial stains, respectively, when compared with Ciprofloxacin. Also, compound **5b** exhibited potent antibacterial activities (MIC values = 3.9–15.6 µg/mL) against the tested bacterial stains.

With regards to nicotinonitrile-coumarin derivatives **5c–5f** bearing benzo[*d*][1,3]dioxole moiety, compounds **5d**, **5e** and **5f** exhibited stronger antibacterial activities (MIC values in the range of 7.8–62.5 µg/mL) when compared with compound **5c** (MIC values in the range of 125–250 µg/mL) against the tested bacterial stains.

With regards to nicotinonitrile-coumarin derivatives **5g–5j**, bearing 1,3-diphenyl-1*H*-pyrazole moiety, compound **5j** exhibited stronger antibacterial activities (MIC values in the range of 15.6–62.5 µg/mL) when compared with compounds **5h and 5i** (MIC values in the range of 62.5–250 µg/mL) against the tested stains. In addition, compound **5g** exhibited the least antibacterial activities with MIC values in the range of 250–500 µg/mL when compared to Ciprofloxacin.

Cytotoxicity against eukaryotic cells

Cytotoxicity against cancer cell lines. The *in-vitro* cytotoxic activity of the most potent nicotinonitrile-coumarins **5a**, **5b**, **5d**, **5e**, **5f** and **5j** was evaluated using neutral red

Table 6. MIC values of the tested antibacterial nicotinonitrile-coumarins.

	MIC (μg/mL)				
	Gram-negative bacteria			Gram-positive bacteria	
Compound	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*S. mutans*
5a	3.9	1.9	3.9	7.8	3.9
5b	7.8	15.6	7.8	3.9	7.8
5c	125	125	250	250	125
5d	7.8	7.8	15.6	15.6	15.6
5e	7.8	3.9	7.8	7.8	7.8
5f	31.2	62.5	31.2	15.6	31.2
5g	250	500	250	500	500
5h	125	250	250	250	250
5i	62.5	125	62.5	125	125
5j	31.2	15.6	15.6	31.2	62.5
Ciprofloxacin	31.2	31.2	31.2	31.2	31.2

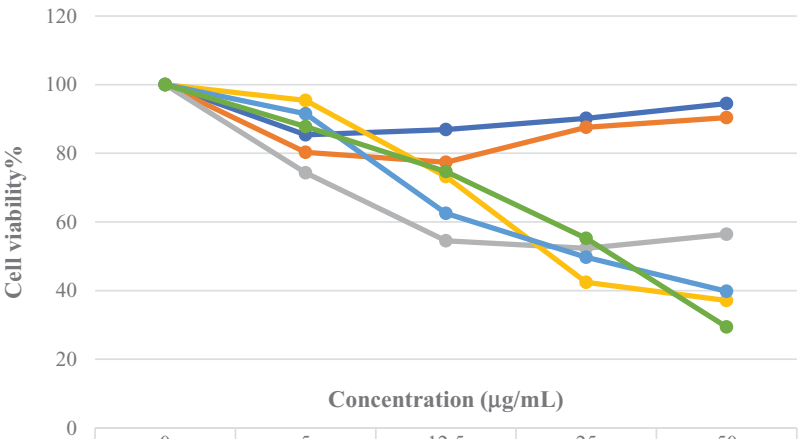
Table 7. The cell inhibition% and IC_{50} values of some novel nicotinonitrile-coumarins against each of MCF-7, Caco2 and HEPG2 cell lines using Doxorubicin as a standard drug.

	IC_{50} in μg/mL (in μM)		
Compound	MCF-7	Caco2	HEPG2
5d	44.74 ± 0.4 (81.94)	29.62 ± 0.5 (54.25)	37.87 ± 0.5 (69.36)
5e	33.17 ± 0.3 (59.02)	35.53 ± 0.4 (63.22)	45.94 ± 0.5 (81.74)
5f	34.33 ± 0.4 (63.81)	38.55 ± 0.4 (71.65)	30.59 ± 0.3 (56.86)
5j	32.58 ± 0.4 (49.36)	29.79 ± 0.3 (45.13)	30.81 ± 0.4 (46.68
Doxorubicin	17.58 ± 0.3 (32.37)	25.17 ± 0.4 (54.86)	20.90 ± 0.3 (38.49)

uptake assay against each of the human breast carcinoma cell line (MCF-7), colon cancer cell line (Caco2) and liver hepatocellular carcinoma cell line (HEPG2) (see Table 7 and Figures 1–3). The results of cytotoxic activity are expressed as the concentration of the tested coumarin required to inhibit 50% of cell growth (IC_{50}, μM).[50,53,54] All the tested coumarins were tested at four different concentrations (5, 12.5, 25 and 50 μg/mL) in comparison with Doxorubicin was used as a standard drug with IC_{50} = 17.58 ± 0.3, 25.17 ± 0.4 and 20.90 ± 0.3 μg/mL against MCF-7, Caco2 and HEPG2, respectively (Table 7).

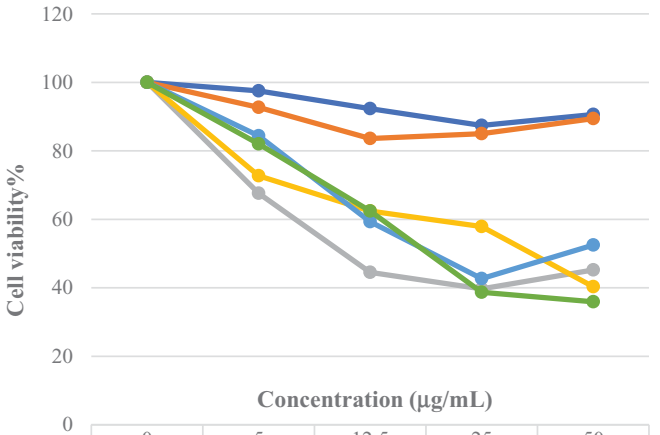
With regards to compounds **5a** and **5b**, Figures 1–3 reveals that the viability of each of MCF-7, Caco2 and HEPG2 cells are greater than 70% (lowest value established by the ISO 10993-5[55] to consider a material as non-cytotoxic). Thus, both compounds **5a** and **5b** are the least cytotoxic antibacterial agents of the tested coumarins.

The coumarins **5d**, **5e**, **5f** and **5j** exerted cytotoxic effects in dose-dependent manners. Compounds **5d** and **5j** exhibited the best cytotoxic activities with IC_{50} values of 29.62 ± 0.5 and 29.79 ± 0.3 μg/mL against Caco2 cell line, respectively, while compounds **5f** and **5j** exhibited the best cytotoxic activities with IC_{50} values of 30.59 ± 0.3 and



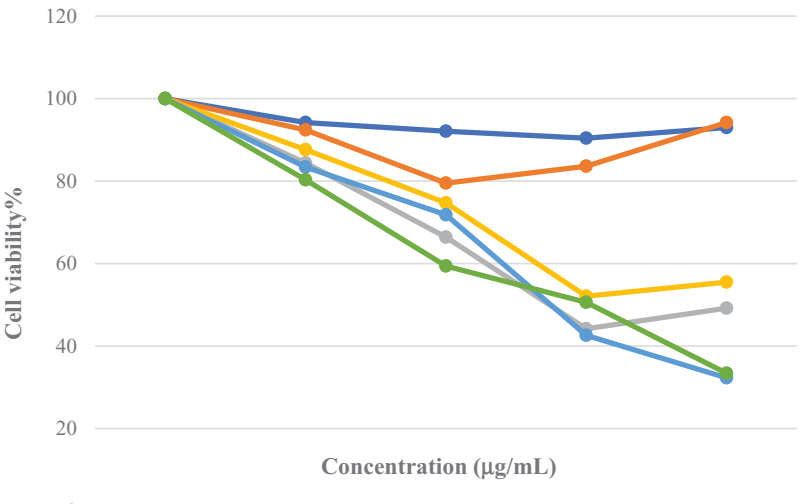
	0	5	12.5	25	50
5a	100	85.4	86.9	90.2	94.5
5b	100	80.3	77.4	87.6	90.4
5d	100	74.3	54.5	52.3	56.4
5e	100	95.4	73.2	42.4	37.1
5f	100	91.5	62.5	49.7	39.8
5j	100	87.8	74.7	55.2	29.4

Figure 1. Cell viability% after treatment with some novel nicotinonitrile-coumarins and Doxorubicin for 24 h on the human breast carcinoma cell line (MCF-7).



	0	5	12.5	25	50
5a	100	97.5	92.3	87.4	90.6
5b	100	92.7	83.6	85	89.4
5d	100	67.6	44.5	39.7	45.2
5e	100	72.7	62.4	57.9	40.3
5f	100	84.4	59.3	42.7	52.5
5j	100	82	62.4	38.7	35.9

Figure 2. Cell viability% after treatment with some novel nicotinonitrile-coumarins and Doxorubicin for 24 h on the human colon cancer cell line (Caco2).



	0	5	12.5	25	50
5a	100	94.2	92.1	90.4	93
5b	100	92.4	79.5	83.6	94.2
5d	100	84.4	66.4	44.2	49.2
5e	100	87.6	74.7	52.1	55.5
5f	100	83.4	71.8	42.6	32.3
5j	100	80.3	59.4	50.6	33.4

Figure 3. Cell viability% after treatment with some novel nicotinonitrile-coumarins and Doxorubicin for 24 h on the human liver hepatocellular carcinoma cell line (HEPG2).

30.81 ± 0.4 µg/mL against HEPG2 cell line, respectively, when compared with Doxorubicin. The rest of tested compounds exhibited decreased cytotoxic activities against both Caco2 and HEPG2 cell lines. On the other hand, all the tested coumarins exhibited dose-dependent cytotoxic activities with IC_{50} values in the range of 32.58–44.74 µg/mL against MCF-7 cell line (Table 7). In general, the results of cytotoxic activity of the nicotinonitrile-coumarins against each of MCF-7, Caco2 and HEPG2 cell lines are consistent with previous publications which reported that nicotinonitriles as well as coumarins possess promising anticancer activities.[3,56–59]

Cytotoxicity against normal cell line. The cytotoxicity activities of coumarin hybrids **5a**, **5b** and **5e** were examined using the human breast epithelial cell line MCF-10A. All hybrids were evaluated at four different concentrations (5, 12.5, 25 and 50 µg/mL). For comparison, Doxorubicin was used as a standard drug.

Figure 4 revealed that Doxorubicin considered as a cytotoxic agent at concentration 12.5 µg/mL or higher, as the viability of the MCF-10A cells became less than 70%.[55] Compounds **5a** and **5b** were found to be non-cytotoxic agents to MCF-10A cells at concentrations up to 50 µg/mL. Thus, both coumarin hybrids **5a** and **5b** are less harmful against MCF-10A cells than Doxorubicin.

Moreover, compound **5e** can be considered as toxic to MCF-10A cells at concentration equal to or higher 25 µg/mL. This concentration is higher than its MIC values against all the tested strains of bacteria.

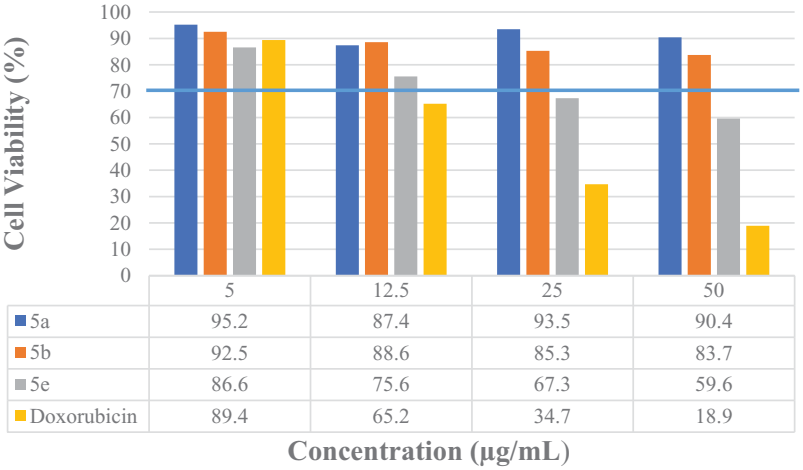


Figure 4. Cell viability after treatment with **5a**, **5b**, **5e** and Doxorubicin for 24 h on MCF-10A cell line.

Table 8. Comparison between the physicochemical parameters of orally active compounds and these of **5a**, **5b** and **5e**.

Parameter	Orally active cpd	**5a**	**5b**	**5e**
Molecular weight	≤500	364.4	412.4	562.6
H-bond donors*	≤5	0	0	0
H-bond acceptors**	≤10	5	5	8
CLog p***	≤5	3.87	4.78	6.1
No. of violations	–	0	0	2

*No. of O or N atoms with one or more hydrogen atoms; **No. of O or N atoms; ***calculated log p (CLog p) is estimated *via* ChemDraw Professional 16.0.0.82.[60]

Cytocompatibility and not cytotoxicity is essential for an antibacterial agent since it would be in contact with the infected tissues and their neighboring eukaryotic cells. Both compounds **5a** and **5b** are the best antibacterial agents. Both are non-cytotoxic, against all the tested eukaryotic cells, at concentrations higher than their MIC values against different tested bacterial strains. Moreover, coumarin **5e** is another promising antibacterial agent. It can be considered nontoxic to all the tested cell lines at concentration equal to or lower 12.5 µg/mL (its cell viability% greater than 70%). This concentration is lower than its MIC values (3.9–7.8 µg/mL) against all the tested bacterial strains. These results revealed that **5a**, **5 b** and **5e** are less harmful against eukaryotic cells than Doxorubicin.

Drug-like

Lipinski stated the Rule of five as an easy tool to predict the drug likeness. Rule of five defines four important physicochemical parameters orally active compounds required for an orally active compound as follows: its molecular weight, its number of hydrogen bond donors, its number of hydrogen bond acceptors and its octanol-water partition coefficient log P (Table 8).[61,62] These parameters are related to acceptable aqueous solubility and intestinal permeability and include the initial steps in oral bioavailability.

The most potent nicotinonitrile-coumarins **5a**, **5b** and **5e** were examined against the mentioned physicochemical parameters. Both **5a** and **5b** are considered as drug-like as both fulfill all the mentioned physicochemical parameters. On the other hand, **5e** fulfilled only two parameters as its H-bond donors are less than five as well as its H-bond acceptors are less than ten. However, it has two violations as its molecular weight more than 500 g/mole and its Clog P value is greater than 5 (see Table 8).

Conclusion

An efficient and eco-friendly synthetic procedure is designed for the synthesis of novel series of nicotinonitrile-coumarin hybrid molecules bearing several aryl and/or heteroaryl moieties. The proposed strategy includes the alkylation reaction of the appropriate pyridine-2(1*H*)-thiones with benzyl chloride derivative in the presence of mild base to prepare a novel series of 2-hydroxybenzaldehydes, bearing nicotinonitrile moiety followed by their Knoevenagel reaction with ethyl acetoacetate in ethanol at 80 °C in the presence of 10 mol% of piperazine citrate (1:1). The structures of novel series of 2-hydroxybenzaldehydes as well as the target nicotinonitrile-coumarin hybrid molecules were confirmed by considering their elemental analyses and spectral data.

Experimental

All organic solvents were acquired from commercial sources and used as received unless otherwise stated. Piperazine citrate (1:1) was acquired from Boc Sciences (CAS 14396-16-8). All other chemicals were acquired from Merck and used without further purification. The melting points were measured on a Stuart melting point apparatus and are uncorrected. IR spectra were recorded on a Smart iTR, which is an ultra-high-performance, versatile Attenuated Total Reflectance (ATR) sampling accessory on the Nicolet iS10 FT-IR spectrometer. ^{1}H-NMR spectra were recorded on Varian Mercury at 300 MHz spectrophotometer using TMS as an internal standard and DMSO-d_6 as solvent and chemical shifts were expressed as δ ppm units. Elemental analyses were carried out on a EuroVector instrument C, H, N analyzer EA3000 Series. Mass spectra were recorded on a GC-MS-QP1000EX spectrometer using inlet type at 70 eV.

General procedure for the synthesis of 2-((3-formyl-4-hydroxybenzyl)thio) nicotinonitrile derivatives 3

A mixture of pyridine-2(1*H*)-thiones **1** (5 mmol) and potassium hydroxide (5 mmol) in ethanol (15 mL) was stirred at room temperature for 10 minutes. Then, 5-(chloromethyl)−2-hydroxybenzaldehyde **2** was added and the stirring was continued. The reaction time was monitored by TLC analyses of the reaction mixture. The product was collected by filtration, washed with each of water and cold ethanol, dried and recrystallized from the proper solvent.

2-((3-Formyl-4-hydroxybenzyl)thio)-4,6-dimethylnicotinonitrile (3a)

Colorless solid; m.p. 114–116 °C (ethanol, 97%); IR (υ cm^{-1}): 3433 (OH), 2233 (CN), 1678 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.37 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 4.44 (s, 2H, SCH_2), 6.93 (d, 1H, Ar-H3), 7.10 (s, 1H, pyridine-H5), 7.58 (d, 1H, Ar-H4), 7.75 (s, 1H, Ar-H6), 10.22 (s, 1H, CHO), 10.66 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6): δ 20.0, 24.6 (2 CH_3), 32.9 (SCH_2), 104.2 (pyridine-*C*3), 115.4 (*C*N), 117.8 (Ar-*C*), 120.9 (pyridine-*C*5), 122.3, 129.2, 129.8, 137.6 (Ar-*C*), 152.9 (pyridine-*C*4), 160.3 (pyridine-*C*6), 160.7 (Ar-*C*), 161.8 (pyridine-*C*2), 191.4 (*C*=*O*); MS (*m*/*z*): 298 (M^+, 84.2%), 297 (100%), 281 (22.3%), 269 (14%), 136 (4.6%); Anal. for $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.73; N, 9.39; found: C, 64.69; H, 4.88; N, 9.24%.

General procedure for the synthesis of 2-(((3-acetyl-2-oxo-2H-chromen-6-yl)methyl)thio)nicotinonitrile derivatives 5

A mixture of 2-hydroxybenzaldehydes **3** (5 mmol) and ethyl acetoacetate **4** (5 mmol) in ethanol (15 mL) in the presence of piperazine citrate (10 mol%) was heated at 80 °C. The reaction time was monitored by TLC analyses of the reaction mixture. The reaction mixture was cooled, filtrated, washed with each of water and cold ethanol and the reaction product was recrystallized from the proper solvent.

2-(((3-Acetyl-2-oxo-2H-chromen-6-yl)methyl)thio)-4,6-dimethylnicotinonitrile (5a)

Pale yellow solid; m.p. 140–142 °C (dioxane/ethanol, 95%); IR (υ cm^{-1}): 2224 (CN), 1722, 1678 (2 CO); ^{1}H-NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.56 (s, 3H, $COCH_3$), 4.55 (s, 2H, SCH_2), 7.08 (s, 1H, pyridine-H5), 7.35 (d, 1H, chromene-H8), 7.41 (d, 1H, chromene-H7), 7.80 (s, 1H, chromene-H5), 8.58 (s, 1H, chromene-H4); ^{13}C-NMR (DMSO-d_6): δ 20.0, 24.6 (2 CH_3), 32.9 (SCH_2), 104.3 (pyridine-*C*3), 115.5 (*C*N), 116.7, 118.1 (chromene-*C*8, *C*4a), 120.7 (pyridine-*C*5), 125.0, 130.5, 134.9, 135.2 (chromene-*C*3, *C*5, *C*4, *C*6), 146.8 (chromene-*C*7), 152.9 (pyridine-*C*4), 158.0 (chromene-*C*8a), 160.5 (pyridine-*C*6), 161.8 (pyridine-*C*2), 162.0 (chromene-*C*O), 195.2 (*C*=*O*); MS (*m*/*z*): 364 (M^+, 69.5%), 349 (12.2%), 321 (100%), 293 (7.2%), 268 (1.5%); Anal. for $C_{20}H_{16}N_2O_3S$: C, 65.92; H, 4.43; N, 7.69; found: C, 65.76; H, 4.51; N, 7.74%.

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References

[1] Patel, D.; Kumari, P.; Patel, N. B. *In Vitro* Antimicrobial and Antimycobacterial Activity of Some Chalcones and Their Derivatives. *Med. Chem. Res.* **2013**, *22*, 726–744. DOI: 10.1007/s00044-012-0073-3.

[2] Timonen, J. M.; Nieminen, R. M.; Sareila, O.; Goulas, A.; Moilanen, L. J.; Haukka, M.; Vainiotalo, P.; Moilanen, E.; Aulaskari, P. H. Synthesis and Anti-inflammatory Effects of a

Series of Novel 7-Hydroxycoumarin Derivatives. *Eur. J. Med. Chem.* **2011**, *46*, 3845–3850. DOI: 10.1016/j.ejmech.2011.05.052.

[3] Riveiro, M. E.; De Kimpe, N.; Moglioni, A.; Vazquez, R.; Monczor, F.; Shayo, C.; Davio, C. Coumarins: Old Compounds with Novel Promising Therapeutic Perspectives. *CMC.* **2010**, *17*, 1325–1338. DOI: 10.2174/092986710790936284.

[4] Anand, P.; Singh, B.; Singh, N. A. A Review on Coumarins as Acetylcholinesterase Inhibitors for Alzheimer's Disease. *Bioorg. Med. Chem.* **2012**, *20*, 1175–1180. DOI: 10.1016/j.bmc.2011.12.042.

[5] Wong, K. Y.; Duchowicz, P. R.; Mercader, A. G.; Castro, E. A. QSAR Applications during Last Decade on Inhibitors of Acetylcholinesterase in Alzheimer's Disease. *MRMC.* **2012**, *12*, 936–946. DOI: 10.2174/138955712802762365.

[6] Matos, M. J.; Viña, D.; Janeiro, P.; Borges, F.; Santana, L.; Uriarte, E. New Halogenated 3-Phenylcoumarins as Potent and Selective MAO-B Inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5157–5160. DOI: 10.1016/j.bmcl.2010.07.013.

[7] Gomez-Outes, A.; Luisa Suarez-Gea, M.; Calvo-Rojas, G.; Lecumberri, R.; Rocha, E.; Pozo-Hernández, C.; Terleira-Fernandez, I. A.; Vargas-Castrillón, E. Discovery of Anticoagulant Drugs: A Historical Perspective. *CDDT.* **2012**, *9*, 83–104. DOI: 10.2174/1570163811209020083.

[8] Zhao, D. G.; Zhou, A. Y.; Du, Z.; Zhang, Y.; Zhang, K.; Ma, Y. Y. Coumarins with α-Glucosidase and α-Amylase Inhibitory Activities from the Flower of *Edgeworthia gardneri*. *Fitoterapia* **2015**, *107*, 122–127. DOI: 10.1016/j.fitote.2015.10.012.

[9] Barancelli, D. A.; Salles, A. G.; Jr, Taylor, J. G.; Correia, C. R. D. Coumarins from Free Ortho-Hydroxy Cinnamates by Heck-Matsuda Arylations: A Scalable Total Synthesis of (*R*)-Tolterodine. *Org. Lett.* **2012**, *14*, 6036–6039. DOI: 10.1021/ol302923f.

[10] Augustine, J. K.; Bombrun, A.; Ramappa, B.; Boodappa, C. An Efficient One-Pot Synthesis of Coumarins Mediated by Propylphosphonic Anhydride (T3P) *via* the Perkin Condensation. *Tetrahedron Lett.* **2012**, *53*, 4422–4425. DOI: 10.1016/j.tetlet.2012.06.037.

[11] Popova, S. A.; Shevchenko, O. G.; Chukicheva, I. Y.; Kutchin, A. V. Synthesis and Biological Evaluation of Novel Coumarins with *Tert*-Butyl and Terpene Substituents. *C&B.* **2019**, *16*, e1800317. DOI: 10.1002/cbdv.201800317.

[12] Zambare, A. S.; Kalam Khan, F. A.; Zambare, S. P.; Shinde, S. D.; Sangshetti, J. N. Recent Advances in the Synthesis of Coumarin Derivatives *via* Pechmann Condensation. *Curr. Org. Chem.* **2016**, *20*, 798–828. DOI: 10.2174/1385272820666151026224227.

[13] Ross, N. A.; MacGregor, R. R.; Bartsch, R. A. Synthesis of β-Lactams and β-Aminoesters via High Intensity Ultrasound-Promoted Reformatsky Reactions. *Tetrahedron* **2004**, *60*, 2035–2041. DOI: 10.1016/j.tet.2004.01.002.

[14] Mekky, A. E. M.; Sanad, S. M. H. Synthesis of Novel Bis(Chromenes) and Bis(Chromeno[3,4-*c*]Pyridine) Incorporating Piperazine Moiety. *Synth. Commun.* **2019**, *49*, 1385–1395. DOI: 10.1080/00397911.2019.1595658.

[15] Shakil, M. R.; Meguerdichian, A. G.; Tasnim, H.; Shirazi-Amin, A.; Seraji, M. S.; Suib, S. L. Syntheses of ZnO with Different Morphologies: Catalytic Activity Toward Coumarin Synthesis via the Knoevenagel Condensation Reaction. *Inorg. Chem.* **2019**, *58*, 5703–5714. DOI: 10.1021/acs.inorgchem.9b00053.

[16] Vekariya, R. H.; Patel, H. D. Recent Advances in the Synthesis of Coumarin Derivatives via Knoevenagel Condensation: A Review. *Synth. Commun.* **2014**, *44*, 2756–2788. DOI: 10.1080/00397911.2014.926374.

[17] Yousefi, M. R.; Goli-Jolodar, O.; Shirini, F. Piperazine: An Excellent Catalyst for the Synthesis of 2-Amino-3-Cyano-4H-Pyrans Derivatives in Aqueous Medium. *Bioorg. Chem.* **2018**, *81*, 326–333. DOI: 10.1016/j.bioorg.2018.08.026.

[18] Mobinikhaledi, A.; Foroughifar, N.; Moghanian, H.; Keshavarzi, N. Piperazine Catalyzed Convenient Synthesis of 4*H*-Pyran Derivatives from α,α′-Bis(Substituted-Benzylidene) Cycloalkanones and Malononitrile under Reflux Conditions. *J. Saudi Chem. Soc.* **2015**, *19*, 399–403. DOI: 10.1016/j.jscs.2012.05.001.

[19] Darvishzad, S.; Daneshvar, N.; Shirini, F.; Tajik, H. Introduction of Piperazine-1,4-Diium Dihydrogen Phosphate as a New and Highly Efficient Dicationic Brönsted Acidic Ionic Salt for the Synthesis of (Thio)Barbituric Acid Derivatives in Water. *J. Mol. Struct.* **2019**, *1178*, 420–427. DOI: 10.1016/j.molstruc.2018.10.053.

[20] Munirathinam, R.; Huskens, J.; Verboom, W. Piperazine-Containing Polymer Brush Layer as Supported Base Catalyst in a Glass Microreactor. *J. Flow Chem.* **2014**, *4*, 135–139. DOI: 10.1556/JFC-D-14-00020.

[21] Wen, X. Polymeric Sulfonate of Piperazine as an Inexpensive and Recyclable Catalyst for Knoevenagel Condensation. *Indian J. Chem.* B. **2006**, *45*, 762–765. DOI: 10.1002/chin.200628080.

[22] Barros, M. T.; Faisca Phillips, A. M. Chiral Piperazines as Efficient Catalysts for the Asymmetric Michael Addition of Aldehydes to Nitroalkenes. *Eur. J. Org. Chem.* **2007**, *2007*, 178–185. DOI: 10.1002/ejoc.200600731.

[23] Rezaei-Seresht, E.; Tayebee, R.; Yasemi, M. KG-60-Piperazine as a New Heterogeneous Catalyst for Gewald Three-Component Reaction. *Synth. Commun.* **2013**, *43*, 1859–1864. DOI: 10.1080/00397911.2012.674607.

[24] Rodrigo, E.; Alcubilla, B. G.; Sainz, R.; Fierro, J. G.; Ferritto, R.; Cid, M. B. Reduced Graphene Oxide Supported Piperazine in Aminocatalysis. *Chem. Commun.* **2014**, *50*, 6270–6273. DOI: 10.1039/c4cc02701a.

[25] Dash, G. K.; Suresh, P.; Sahu, S. K.; Kar, D. M.; Ganapaty, S.; Panda, S. B. Evaluation of *Evolvulus Alsinoides Linn*. for Anthelmintic and Antimicrobial Activities. *J. Nat. Remedies* **2002**, *2*, 182–185. DOI: 10.18311/jnr/2002/146.

[26] Greenberg, B. L.; Gilman, R. H.; Shapiro, H.; Gilman, J. B.; Mondal, G.; Maksud, M.; Khatoon, H.; Chowdhury, J. Single Dose Piperazine Therapy for *Ascaris Lumbricoides*: An Unsuccessful Method of Promoting Growth. *Am. J Clin. Nutr.* **1981**, *34*, 2508–2516. DOI: 10.1093/ajcn/34.11.2508.

[27] Chege, H. W.; Kemboi, D. C.; Bebora, L. C.; Maingi, N.; Mbuthia, P. G.; Nyaga, P. N.; Njagi, L. W. Efficacy of Piperazine Citrate, Levamisole Hydrochloride and Albendazole in the Treatment of Chicken Naturally Infected with Gastrointestinal Helminths. *Livestock Res. Rural Develop* **2017**, *29*, Article 98.

[28] Yetim, I.; Ozkan, O. V.; Semerci, E.; Abanoz, R. Rare Cause of Intestinal Obstruction, *Ascaris Lumbricoides* Infestation: Two Case Reports. *Cases J.* **2009**, *2*, Article 7970. DOI: 10.4076/1757-1626-2-7970.

[29] Mohan, N. R.; Sreenivasa, S.; Manojkumar, K. E.; Rao, T.; Thippeswamy, B. S.; Suchetan, P. A. Synthesis, Antibacterial, Anthelmintic and anti-Inflammatory Studies of Novel Methylpyrimidine Sulfonyl Piperazine Derivatives. *J. Braz. Chem. Soc.* **2014**, *25*, 1012–1020. DOI: 10.5935/0103-5053.20140073.

[30] Gouda, M. A.; Abd El-Ggani, G. E.; Berghot, M. A.; Khalil, A. E. G. M. Synthesis and Antioxidant Activity of Some Novel Nicotinonitrile Derivatives Bearing a Furan Moiety. *J. Heterocyclic Chem.* **2019**, *56*, 2036–2045. DOI: 10.1002/jhet.3584.

[31] Al-Refai, M.; Ibrahim, M.; Al-Fawwaz, A.; Geyer, A. Synthesis and Characterization of New 4-Aryl-2-(2-Oxopropoxy)-6-(2, 5-Dichlorothiophene) Nicotinonitrile and Their Furo[2,3-*b*] Pyridine Derivatives: Assessment of Antioxidant and Biological Activity. *Eur. J. Chem.* **2018**, *9*, 375–381. DOI: 10.5155/eurjchem.9.4.375-381.1792.

[32] Fekry, R. M.; El-Sayed, H. A.; Assy, M. G.; Shalby, A.; Mohamed, A. S. Synthesis and Anticancer Activity of Some Novel Fused Nicotinonitrile Derivatives. *Org. Chem. Curr. Res.* **2016**, Article 1000171. DOI: 10.4172/2161-0401.1000171.

[33] Krauze, A.; Baumane, L.; Sile, L.; Chernova, L.; Vilums, M.; Vitolina, R.; Duburs, G.; Stradins, J. Synthesis, Cardiovascular Activity, and Electrochemical Oxidation of Nitriles of 5-Ethoxycarbonyl-2-Methylthio-1,4-Dihydropyridine-3-Carboxylic Acid. *Chem. Heterocycl. Compd.* **2004**, *40*, 876–887. DOI: 10.1023/B:COHC.0000044570.13567.74.

[34] Tirzite, D.; Krauze, A.; Zubareva, A.; Tirzitis, G.; Duburs, G. Synthesis and Antiradical Activity of 5-Acetyl-2-Alkylthio-4-Aryl-6-Methyl-1,4-Dihydropyridine-3-Carboxylic Acid Nitriles. *Chem. Heterocycl. Compd.* **2002**, *38*, 795–800.

[35] El-Hashash, M. A.; El-Bordany, E. A.; Marzouk, M. I.; El-Naggar, A. M.; Nawar, T.; El-Sayed, W. M. Novel Nicotinonitrile Derivatives Bearing Imino Moieties Enhance Apoptosis and Inhibit Tyrosine Kinase. *ACAMC*. **2019**, *18*, 1589–1598. DOI: 10.2174/1871520618666180510112614.

[36] Sanad, S. M. H.; Abdel Fattah, A. M.; Attaby, F. A.; Elneairy, M. A. A. Pyridine-2(*1H*)-Thiones: Versatile Precursors for Novel Pyrazolo[3,4-*b*]Pyridine, Thieno[2,3-*b*]Pyridines and Their Fused Azines. *J. Heterocycl. Chem.* **2019**, *56*, 651–662. DOI: 10.1002/jhet.3444.

[37] Sanad, S. M. H.; Abdel Fattah, A. M.; Attaby, F. A.; Elneairy, M. A. A. Synthesis and Characterization of Novel Bis(Pyridine-2(1*H*)-Thiones) and Their Bis(2-Methylsulfanylpyridines) Incorporating 2,6-Dibromophenoxy Moiety. *Can. J. Chem.* **2019**, *97*, 53–60. DOI: 10.1139/cjc-2017-0721.

[38] Sanad, S. M. H.; Hefny, M. I. M.; Ahmed, A. A. M.; Elneairy, M. A. A. Synthesis of Novel Bis[(5-Cyanopyridin-6-yl)Sulfanyl]Butanes, Bis(2-*S*-Alkylpyridines) and Bis(3-Aminothieno[2,3-*b*]Pyridines) Incorporating 2,6-Dibromophenoxy Moiety. *J. Heterocyclic Chem.* **2018**, *55*, 2046–2054. DOI: 10.1002/jhet.3239.

[39] Sanad, S. M. H.; Hawass, M. A. E.; Ahmed, A. A. M.; Elneairy, M. A. A. Efficient Synthesis and Characterization of Novel Pyrido[3',2':4,5]Thieno[3,2-*d*]Pyrimidines and Their Fused [1,2,4]Triazole Derivatives. *J. Heterocyclic Chem.* **2018**, *55*, 2823–2833. DOI: 10.1002/jhet.3352.

[40] Dyachenko, I. V.; Dyachenko, V. D. Synthesis of 4, 6-Dimethyl-2-Thioxo-1, 2-Dihydropyridine-3-Carbonitrile by Condensation of Cyanothioacetamide with Acetaldehyde and 1-(Prop-1-en-2-yl) Piperidine. *Russ. J. Org. Chem.* **2016**, *52*, 32–36. DOI: 10.1134/S1070428016010061.

[41] Rodinovskaya, L. A.; Sharanin, Y. A.; Shestopalov, A. M.; Litvinov, V. P. Cyclization Reactions of Nitrils. 29. Regioselective Synthesis of 6-Aryl-3-Cyano-2(1*H*)-Pyridinethiones and the Corresponding Selenones and Their Characteristics. *Chem. Heterocycl. Compd.* **1988**, *24*, 658–665. DOI: 10.1007/BF00475603.

[42] Sanad, S. M. H.; Ahmed, A. A. M.; Mekky, A. E. M. Efficient Synthesis and Molecular Docking of Novel Antibacterial Pyrimidines and Their Related Fused Heterocyclic Derivatives. *J. Heterocyclic Chem.* **2020**, *57*, 590–605. DOI: 10.1002/jhet.3789.

[43] El-Emary, T. I.; Bakhite, E. A. Synthesis and Biological Screening of New 1,3-Diphenylpyrazoles with Different Heterocyclic Moieties at Position-4. *Pharmazie* **1999**, *54*, 106–111.

[44] Mekky, A. E. M.; Sanad, S. M. H. Synthesis, Characterization, and Antimicrobial Evaluation of Novel Thiohydrazonates and Pyrazolo[3,4-b]Pyridines. *Polycycl. Aromat. Comp.* 2019. DOI: 10.1080/10406638.2019.1631194.

[45] Cardia, M. C.; Sanna, M. L.; Meleddu, R.; Distinto, S.; Yañez, M.; Viña, D.; Lamela, M.; Maccioni, E. A Novel Series of 3,4-Disubstituted Dihydropyrazoles: Synthesis and Evaluation for MAO Enzyme Inhibition. *J. Heterocyclic Chem.* **2013**, *50(S1)*, E87–E92. DOI: 10.1002/jhet.1072.

[46] Schwartz, M. A.; Rose, B. F.; Holton, R. A.; Scott, S. W.; Vishnuvajjala, B. Intramolecular Oxidative Coupling of Diphenolic, Monophenolic, and Nonphenolic Substrates. *J. Am. Chem. Soc.* **1977**, *99*, 2571–2578. DOI: 10.1021/ja00450a027.

[47] Bratenko, M. K.; Chornous, V. A.; Vovk, M. V. 4-Functionally Substituted 3-Heterylpyrazoles: IV. 1-Phenyl-3-Aryl(Heteryl)-5-(4-Pyrazolyl)-2-Pyrazolines. *Russ. J.Org. Chem* **2001**, *37*, 556–559.

[48] Brahmbhatt, D. I.; Kaneria, A. R.; Patel, A. K.; Patel, N. H. Synthesis and Antimicrobial Screening of Some 3-[4-(3-Aryl-1-Phenyl-1*H*-Pyrazol-4-yl)-6-Aryl-Pyridin-2-yl] and 4-Methyl-3-Phenyl-6-[4-(3-Aryl-1-Phenyl-1*H*-Pyrazol-4-yl)-6-Aryl-Pyridin-2-yl]Coumarins. *Indian J. Chem. Section B* **2010**, *49*, 971–977.

[49] Guieu, S.; Rocha, J.; Silva, A. M. Synthesis of Unsymmetrical Methylenebisphenol Derivatives. *Synlett* **2013**, *24*, 762–764. DOI: 10.1055/s-0032-1318394.

[50] Muthukumar, T.; Sambandam, B.; Aravinthan, A.; Sastry, T. P.; Kim, J. H. Green Synthesis of Gold Nanoparticles and Their Enhanced Synergistic Antitumor Activity

Using HepG2 and MCF7 Cells and Its Antibacterial Effects. *Process Biochem.* **2016**, *51*, 384–391. DOI: 10.1016/j.procbio.2015.12.017.

[51] Goldfeder, Y.; Zaknoon, F.; Mor, A. Experimental Conditions That Enhance Potency of an Antibacterial Oligo-Acyl-Lysyl. *Antimicrob. Agents Chemother.* **2010**, *54*, 2590–2595. DOI: 10.1128/AAC.01656-09.

[52] Paudel, B.; Bhattarai, H. D.; Lee, J. S.; Hong, S. G.; Shin, H. W.; Yim, J. H. Antibacterial Potential of Antarctic Lichens Against Human Pathogenic Gram-Positive Bacteria. *Phytother. Res.* **2008**, *22*, 1269–1271. DOI: 10.1002/ptr.2445.

[53] Hemaiswarya, S.; Doble, M. Combination of Phenylpropanoids with 5-Fluorouracil as anti-Cancer Agents against Human Cervical Cancer (HeLa) Cell Line. *Phytomedicine* **2013**, *20*, 151–158. DOI: 10.1016/j.phymed.2012.10.009.

[54] Mendoza-Ferri, M. G.; Hartinger, C. G.; Mendoza, M. A.; Groessl, M.; Egger, A. E.; Eichinger, R. E.; Mangrum, J. B.; Farrell, N. P.; Maruszak, M.; Bednarski, P. J.; et al. Transferring the Concept of Multinuclearity to Ruthenium Complexes for Improvement of Anticancer Activity. *J. Med. Chem.* **2009**, *52*, 916–925. DOI: 10.1021/jm8013234.

[55] ISO 10993-5:2009. Biological Evaluation of Medical Devices-Part 5: Tests for *in vitro* cytotoxicity. https://www.iso.org/standard/36406.html (accessed May 22, 2018).

[56] El-Sayed, N. S.; Shirazi, A. N.; El-Meligy, M. G.; El-Ziaty, A. K.; Rowley, D.; Sun, J.; Nagib, Z. A.; Parang, K. Synthesis of 4-Aryl-6-Indolylpyridine-3-Carbonitriles and Evaluation of Their Antiproliferative Activity. *Tetrahedron Lett.* **2014**, *55*, 1154–1158. DOI: 10.1016/j.tetlet.2013.12.081.

[57] Gomha, S. M.; Khalil, K. D. A Convenient Ultrasound-Promoted Synthesis of Some New Thiazole Derivatives Bearing a Coumarin Nucleus and Their Cytotoxic Activity. *Molecules* **2012**, *17*, 9335–9347. DOI: 10.3390/molecules17089335.

[58] Abbas, I.; Gomha, S. M.; Elaasser, M.; Bauomi, M. Synthesis and Biological Evaluation of New Pyridines Containing Imidazole Moiety as Antimicrobial and Anticancer Agents. *Turk. J. Chem.* **2015**, *39*, 334–346. DOI: 10.3906/kim-1410-25.

[59] Abdallah, M. A.; Gomha, S. M.; Abbas, I. M.; Kazem, M. S.; Alterary, S. S.; Mabkhot, Y. N. An Efficient Synthesis of Novel Pyrazole-Based Heterocycles as Potential Antitumor Agents. *Appl. Sci.* **2017**, *7*, 785. DOI: 10.3390/app7080785.

[60] ChemDraw Professional 16.0.0.82. 2017. https://www.perkinelmer.com/uk/product/chemdraw-professional-chemdrawpro.

[61] Lipinski, C. A. Lead-and Drug-like Compounds: The Rule-of-Five Revolution. *Drug Discov. Today Technol.* **2004**, *1*, 337–341. DOI: 10.1016/j.ddtec.2004.11.007.

[62] Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3–25. DOI: 10.1016/S0169-409X(96)00423-1.