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RESEARCH ARTICLE

3-Cyano-8-methyl-2-oxo-1,4-disubstituted-1,2,5,6,7,8-hexahydroquinolines: Synthesis and Biological Evaluation as Antimicrobial and Cytotoxic Agents

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Abstract

The synthesis, *in vitro* antimicrobial and cytotoxic activities of some novel hexahydroquinolines supported with various pharmacophores are described. The results revealed that 18 compounds displayed pronounced activity against *Staphylococcus aureus* and *Escherichia coli* bacteria beside a moderate antifungal activity. Compound **25** is the most active candidate with equipotency to ampicillin against *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*, together with an obvious antifungal activity. Additionally, 12 compounds showed remarkable cytotoxic efficiency against human colon carcinoma HT29, hepatocellular carcinoma Hep-G2 and Caucasian breast adenocarcinoma MCF7 cell lines. Among these, the analogs **22** and **25** proved to be the most active cytotoxic members. Collectively, the results would suggest that compounds **22** and **25** could be considered as possible dual antimicrobial-anticancer agents.

Keywords: Synthesis, hexahydroquinolines, antibacterial, antifungal, cytotoxic

Introduction

Over the past two decades, much attention has been focused on addressing the problem of multi-drug resistant (MDR) bacteria and fungi resulting from the widespread use and misuse of classical antimicrobial agents[1]. In addition, fungal infection became a prominent complication and a major cause of mortality in immuno-compromised individuals such as those suffering from tuberculosis, cancer or AIDS and in organ transplant cases[2]. Therefore, there is a real perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through non-traditional mechanisms of action. On the other hand, the increasing number of neoplastic diseases together with the accompanied high mortality rates[3] has stimulated an unprecedented level of research directed towards the search for new structure leads that might be of use in designing novel anticancer drugs. Patients that are subjected cancer chemotherapy are mostly susceptible to microbial infections due to subsequent lack of immunity. Co-administration of multiple drugs for treating cancer disease accompanied with microbial infections might inflect some added health problems especially in patients with impaired liver and/or kidney functions. Therefore, the concept of monotherapy by a single drug which would possess dual utility might be advantageous from both therapeutic and cost-effective stand points.

In recent years, pyridines and some derived fused-ring systems such as quinolines have attracted much attention as versatile chemotherapeutic agents owing to their reported potential antimicrobial activity[4–6], in addition to various antineoplastic[7], antiproliferative[8], and cytotoxic[9] potentials. Furthermore, particular interest has been given to cyanopyridine derivatives as anticancer and antiviral[10] agents.

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During our ongoing studies aimed at the discovery of new structure leads endowed with diverse chemotherapeutic activities, much concern has been given to the antimicrobial and antitumour potentials of some pyridine derivatives[11–14], among which those comprising the 3-cyano-4,6-disubstituted-2(1*H*)-pyridinone scaffold[12] exhibited promising broad spectrum antitumour activity. The obtained results prompted further structure modification of the disubstituted-2(1*H*)-pyridinone scaffold by increasing compounds' lipophilicity via the synthesis of new 1,2,5,6,7,8-hexahydroquinoline analogs. The target compounds were designed to encounter various pharmacophores and functionalities at positin-1 that are believed to be responsible for the biological significance of some relevant antimicrobial and/or anticancer agents such as the formyl, acetyl, nitroso, benzenesulfonyl, thiocarbamoyl and alkyl groups. Moreover, it was considered worthwhile to utilize the N-acetyl derivatives as precursors for the synthesis of the tricyclic fused-ring system tetrahydro[1,2,4]triazolo[4,3-a]quinoline as an interesting structural variation, hoping to improve the anticipated chemotherapeutic activities.

Experimental

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S IR spectrophotometer using the KBr pellet technique. 1H and 13C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard and a mixture of $CDCl_3$ and dimethyl sulfoxide-d6 (DMSO-d_6) as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet; q: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow-up of the reactions and checking the homogeneity of the compounds were made by thin layer chromatography on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

3-Cyano-8-methyl-2-oxo-4-substituted-1,2,5,6,7,8-hexahydroquinolines (4–6)

A one-pot mixture of the appropriate aldehyde **1–3** (10 mmol), 2-methylcyclohexanone 1 (1.12 g, 10 mmol), ethyl cyanoacetate (1.1 g, 10 mmol) and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (50 mL), was refluxed for 6 h. The reaction mixture was allowed to cool, and the formed precipitate was filtered, washed with water, dried and recrystallized. IR (cm^{-1}): 3400-3250 (NH), 2225- 2210 (CN), 1682-1675 (C=O). ^{13}C NMR (δ-ppm) **4:** 15.1 (CH_3), 27.7, 27.9, 34.4, 34.9 (cyclohexyl C), 117.2 (CN), 95.7, 119.5, 132.7, 165.9 (CO), 169.4 (pyridine C), 126.2, 127.7, 128.4, 134.9 (Ar C).

3-Cyano-8-methyl-1-nitroso-2-oxo-4-substituted-1,2,5,6,7,8-hexahydroquinolines (7,8)

To an ice-cooled stirred solution of the appropriate starting material **4** or **5** (10 mmol) in acetic acid (15 mL), was added dropwise a solution of sodium nitrite (1.05 g, 15 mmol) in water (5 mL) over a period of 2 h. Stirring was maintained for further 2 h, and then the reaction mixture was left aside at room temperature for an overnight. The formed solid product was filtered, washed with water, dried and recrystallized from ethanol as needles. IR (cm^{-1}): 2223-2217 (CN), 1675-1667 (C=O pyridone). ^{13}C NMR (δ-ppm) **7:** 15.3 (CH_3), 27.6, 27.7, 30.3, 34.2 (cyclohexyl C), 117.6 (CN), 95.8, 119.2, 133.0, 163.0 (CO), 170.2 (pyridone C), 125.9, 127.8, 128.6, 134.9 (Ar C).

3-Cyano-1-formyl-8-methyl-2-oxo-4-substituted-1,2,5,6,7,8-hexahydroquinolines (9–11)

A solution of the appropriate starting compound **4–6** (3 mmol) in formic acid (5 mL) was heated under reflux for 3 h. The reaction mixture was poured on crushed ice (10 g) and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol. IR (cm^{-1}): 2220-2210 (CN), 1678-1670 (C=O pyridone), 1663-1657 (C=O aldehyde). ^{13}C NMR (δ-ppm) **9:** 15.1 (CH_3), 27.5, 27.7, 34.3, 34.7 (cyclohexyl C), 117.2 (CN), 95.8, 120.0, 133.3, 160.9 (CO) 169.2 (pyridone C), 156.8 (formyl CO), 126.0, 127.5, 128.3, 134.7 (Ar C).

1-Acetyl-3-cyano-8-methyl-2-oxo-4-substituted-1,2,5,6,7,8-hexahydroquinolines (12–14)

To a solution of the appropriate **4–6** (10 mmol) in acetic anhydride (10 mL), was added anhydrous sodium acetate (1.2 g, 15 mmol). The reaction mixture was heated under reflux for 4 h, allowed to cool, and then poured on crushed ice with vigorous stirring. The formed solid product was filtered, thoroughly washed with water, dried and recrystallized from aqueous ethanol. IR (cm^{-1}): 2226-2215 (CN), 1725-1718 (C=O acetyl), 1678-1672 (C=O pyridone). ^{13}C NMR (δ-ppm) **12**: 15.2 (CH_3), 15.7 (CH_3), 27.6, 27.8, 31.9, 34.2 (cyclohexyl C), 117.8(CN), 98.2, 120.0, 133.4, 160.9 (CO), 169.8 (pyridone C), 126.8, 127.9, 128.5, 134.8 (Ar C), 165.3 (CO).

4-Cyano-1,9-dimethyl-5-Substituted-6,7,8,9-tetrahydro [1,2,4]triazolo[4,3-a]quinolines (15–17)

A mixture of the appropriate 1-acetylhexahydroquinoline **12–14** (10 mmol) and hydrazine hydrate 99% (0.9 g, 15 mmol) in ethanol (15 mL) was heated under reflux for 6–8 h. The reaction mixture was allowed to attain room temperature, poured on crushed ice and the precipitated solid product was filtered, washed with water, dried and recrystallized from DMF/water. IR (cm^{-1}): 2220-2215 (CN). ^{13}C NMR (δ-ppm) **15:** 12.3 (CH_3), 22.3 (CH_3), 24.7, 32.4, 29.1, 41.5 (cyclohexyl C), 118.0 (CN), 106.8, 135.8, 147.6, 154.3, 166.9 (pyridone C), 126.9, 128.9, 129.0, 138.2, 160.9 (Ar C).

3-Cyano-8-methyl-2-oxo-1-(substituted sulfonyl)-4-substituted-1,2,5,6,7,8-hexahydro-quinolines (18–20)

A mixture of the appropriate start **4–6** (10 mmol) and benzenesulfonyl chloride (10 mmol, 1.77 g) in pyridine (10 mL), was heated under reflux for 4–6 h. After cooling to room temperature, the reaction mixture was poured on crushed ice and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol. IR (cm^{-1}): 2230-2221 (CN), 1670-1665 (C=O pyridone), 1388-1370 and 1197-1175 (SO_2). ^{13}C NMR (δ-ppm) **18:** 15.1 (CH_3), 27.6, 27.9, 31.3, 34.4 (cyclohexyl C), 95.9, 119.8, 133.0, 163.2 (CO), 169.5 (pyridone C), 117.6 (CN), 125.6, 126.2, 127.7, 128.4, 128, 8, 131.7, 134.9, 139.3 (Ar C).

3-Cyano-8-methyl-2-oxo-1-(substituted-thiocarbamoyl)-4-substituted-1,2,5,6,7,8-hexahydroquinolines (21–26)

A mixture of the appropriate starting compound **4–6** (10 mmol) and the appropriate isothiocyanate (11 mmol) in pyridine (10 mL) was heated under reflux for 6–8 h. After being cooled to room temperature, the reaction mixture was poured on ice cold water and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol as needles. IR (cm^{-1}): 2230-2215 (CN), 1678-1665 (C=O pyridone), 1232-1216 (C=S). ^{13}C NMR (δ-ppm) **22:** 15.7 (CH_3), 27.9, 28.3, 32.4, 35.0 (cyclohexyl C), 117.5 (CN), 98.6, 120.2, 132.9, 162.7 (CO), 168 (pyridone C), 124.5, 125.3, 126.2, 127.7, 128.4, 128.8, 134.9, 139.4 (Ar C), 175.4 (CS).

3-Cyano-1,4-disubstituted-8-methyl-2-oxo-1,2,5,6,7,8-hexahydroquinolines (27–32)

To a solution of the appropriate hexahydroquinoline **4–6** (10 mmol) in pyridine (10 mL), was added the appropriate alkyl halide (10 mmol) and the mixture was heated under reflux for 3–4 h. The reaction mixture was allowed to attain room temperature, poured on ice cold water and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol. IR (cm^{-1}): 2222-2216 (CN), 1675-1668 (C=O pyridone). ^{13}C NMR (δ-ppm) **27:** 15.5 (CH_3), 30.2 (N-CH_3), 27.9, 28.0, 32.4, 34.7 (cyclohexyl C), 117.8 (CN), 95.7, 119.5, 132.9, 161.4 (CO), 169.6 (pyridone C), 126.5, 127.6, 129.2, 134.9 (Ar C).

In vitro antibacterial and antifungal activities

Inhibition-zone (IZ) measurements

All the newly synthesized compounds were evaluated by the agar cup diffusion technique[15] using a 1 mg/mL solution in DMSO. The test organisms utilized were *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633) and *Micrococcus luteus* (ATCC 21881) as examples of Gram-positive bacteria and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumonia* (clinical isolate) as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (recultured) fungal strains.

Minimal inhibitory concentration (MIC) measurement

The MIC of the most active compounds were measured using the twofold serial broth dilution method described by Scott[15]. The MIC values in μg/mL (μM) of the compounds are listed in Table 1.

In vitro MTT cytotoxicity assay

All of the newly synthesized compounds were investigated for their *in vitro* cytotoxic effect via the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method[16,17] against a panel of three human tumour cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma Hep-G2 and colon carcinoma HT29. The results are presented in Table 2 as LC_{50} (μg/mL, μM) which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

Results and discussion

Chemistry

The synthetic strategies adopted for the preparation of the intermediate and target compounds are described in Schemes 1 and 2. In Scheme 1, the key intermediates 3-cyano-8-methyl-2-oxo-4-substituted-1,2,5,6,7,8-hexahydroquinolines **4–6** were synthesized via one-pot multicomponent reaction (MCR) of the appropriate aromatic aldehyde **1–3** and 2-methylcyclohexanone, an excess of ammonium acetate and ethyl cyanoacetate in boiling ethanol. Such type of reactions has received considerable interest since it is easier to perform, gives higher yields and less time consuming, when compared with the traditional two-stepped procedure that involved the formation of the 2-arylidene-6-methylcyclohexanones (chalcones) via *Claisen-Schmidt* condensation followed by cyclocondensation with ethyl cyanoacetate and ammonium acetate. Reacting the starting compounds **4** and **5** with sodium nitrite in the presence of cold acetic acid afforded the corresponding the N-nitroso derivatives **7** and **8**. Whereas, heating compounds **4–6** with formic acid resulted in the formation of the N-formyl derivatives **9–11**. In a similar fashion, warming the starting compounds **4–6** with acetic anhydride in the presence of anhydrous sodium acetate furnished the N-acetyl derivatives **12–14**. In their turn, when the latter compounds were reacted with hydrazine hydrate, the targeted 5,6,7,8-tetrahydro[1,2,4]triazolo[3,4-*a*]quinolines **15–17** were successfully obtained. In Scheme 2, the same hexahydroquinolines **4–6** were utilized as key intermediates for the synthesis of the target compounds **18–32.** In this respect, reacting compounds **4–6** with benzenesulfonyl chloride in a pyridine medium resulted in the introduction of a benzenesulfonyl moiety at position-1 to yield compounds **18–20**. Moreover, condensation of **4–6** with the appropriate isothiocyanate in alkaline medium afforded the corresponding N-arylthiocarbamoyl analogs **21–26.** On the other hand, when compounds **4** were alkylated with

Table 1. Minimal inhibitory concentrations [MIC, μg/mL (μM)] of the tested compounds.

Compound no.	*S. aureus*	*B. subtilis*	*M. luteus*	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*C. albicans*
4	100 (378.3)	100 (378.3)	–[a]	100 (378.3)	–	–	–
5	25 (83.6)	50 (167.3)	–	25 (83.6)	50 (167.3)	–	–
6	50 (169.8)	100 (339.7)	–	25 (84.9)	100 (339.7)	–	–
10	100 (306)	200 (612)	–	100 (306)	200 (612)	–	–
13	25 (73.3)	100 (293.4)	–	25 (73.3)	50 (146.7)	200 (586.8)	100 (293.4)
14	100 (297.3)	100 (297.3)	–	50 (148.6)	50 (148.6)	–	–
16	200 (593.8)	200 (593.8)	–	100 (296.9)	–	–	–
17	200 (601.7)	–	–	200 (601.7)	–	–	–
18	50 (123.6)	100 (247.2)	–	100 (247.2)	100 (247.2)	–	–
19	12.5 (28.5)	25 (57)	100 (228)	6.25 (14.2)	25 (57)	100 (228)	25 (57)
20	25 (57.5)	100 (230)	200 (460)	25 (57.5)	50 (115)	–	100 (230)
22	12.5 (28.8)	50 (115.2)	100 (230.4)	12.5 (28.8)	25 (57.6)	–	25 (57.6)
23	25 (60)	100 (240)	100 (240)	25 (60)	100 (240)	–	50 (120)
25	6.25 (13.3)	25 (53.4)	25 (53.4)	6.25 (13.3)	12.5 (26.7)	50 (106.8)	12.5 (26.7)
26	12.5 (27.6)	50 (110.6)	–	12.5 (27.6)	50 (110.6)	100 (221.2)	12.5 (27.6)
28	50 (141)	100 (282)	–	100 (282)	100 (282)	–	–
30	12.5 (32)	50 (128)	50 (128)	6.25 (16)	25 (64)	100 (256)	50 (128)
32	25 (65)	100 (260)	50 (130)	12.5 (32.5)	25 (65)	200 (520)	100 (260)
Ampicillin	6.25 (18)	12.5 (36)	12.5 (36)	6.25 (18)	12.5 (36)	12.5 (36)	–
Clotrimazole	–	–		–	–		6.25 (18)

[a]MIC > 200 (600) μg/mL (μM).

the appropriate alkyl halide in the presence of sodium hydroxide, the targeted N-alkyl hexahydroquinolines **27–32** were formed, but in low yields. Nevertheless, better yields were obtained when the reaction was carried out in pyridine as a basic solvent. At this stage, it was thought of interest to study the effect of applying different alkylating conditions on such type of compounds. In this respect, compounds **4–6** were treated with the same alkyl halides in the presence of ethanolic silver nitrate[18] or sodium ethoxide[19] as basic catalysts in an attempt to obtain the O-alkyl derivatives **33–35**. Unfortunately, such procedures failed to produce the targeted compounds, where the starting compounds were always separated unreacted.

In vitro antibacterial and antifungal activities

As revealed from MIC data recorded in Table 1, at both μg/mL and μM concentration levels, 18 out of the 29 newly synthesized compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative microorganisms with special pronounced activity against *S. aureus* and *E. coli* bacterial strains. In addition, some members exhibited moderate antifungal activity against *C. albicans*, whereas, all the tested compounds lacked antifungal potential against *Asp. Niger*.

In terms of μg/mL concentration, among the tested Gram-positive bacterial strains, two organisms namely; *S. aureus* and *B. subtilis* showed relative high sensitivity towards the active compounds. In this view, compound **25**

Table 2. Cytotoxic effects LC50; μg/mL (μM)[a] of the active compounds on some human tumour cell lines using the MTT assay.

Compound	HT29[b]	Hep-G2[c]	MCF 7[d]
5	65.8	57.2	46.3
	(220.2)	(191.5)	(155)
6	47.1	26.3	34.1
	(160)	(90)	(116)
11	76.3	–[e]	–
	(237)		
14	41.2	39.4	–
	(122)	(117)	
17	35.8	21.3	41.6
	(108)	(64)	(125)
20	29.6	18.5	25.2
	(68)	(43)	(58)
22	8.4	20.6	12.1
	(19)	(47)	(28)
24	26.3	48.7	64.9
	(70)	(131)	(175)
25	14.9	11.6	9.7
	(32)	(25)	(21)
26	34.9	31.8	–
	(77)	(70)	
29	41.5	–	–
	(133)		
31	54.6	–	–
	(177)		
Doxorubicin[f]	21.1	1.69	2.14
	(40)	(3)	(4)

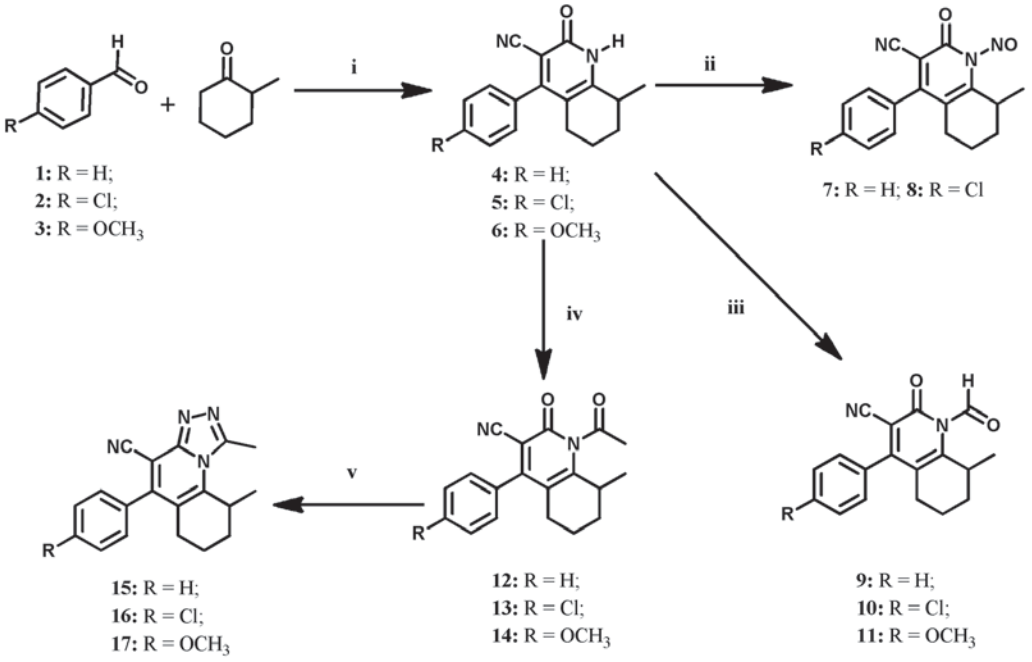
[a]LC_{50}, lethal concentration of the compound which causes death of 50% of cells in 24 h μg/mL (μM).
[b]HT29 (Human colon carcinoma cell line).
[c]Hep-G2 (Human hepatocellular carcinoma cell line).
[d]MCF7 (Human breast cancer cell line).
[e]Totally inactive against this cell line.
[f]Positive control cytotoxic agent.

was equipotent to ampicillin (MIC 6.25 μg/mL) against *S. aureus,* whereas the analogs **19, 22, 26** and **30** (MIC 12.5 μg/mL) were 50% less active than ampicillin. With regard to the activity against *B. subtilis*, compounds **19** and **25** (MIC 25 μg/mL) showed half the activity of ampicillin. Concerning the antibacterial activity against the three tested Gram-negative strains, three analogs namely; **19, 25** and **30** were able to produce a distinctive growth inhibitory profile against *E. coli* (MIC 6.25 μg/mL) which was equipotent to ampicillin, whereas, compounds **22, 26** and **32** (MIC 12.5 μg/mL), were 50% less active than ampicillin against the same organism. Meanwhile, the tested *P. aeruginosa* strain showed moderate sensitivity towards most of the active compounds, particularly compound **25** (MIC 12.5 μg/mL) which was equipotent to ampicillin. On the other hand, only nine compounds **13, 19, 20, 22, 23, 25, 26, 30** and **32** were able to display a noticeable growth inhibitory potential against *C. albicans*, among which compounds **25** and **26** (MIC 12.5 μg/mL) were the most active members when compared with clotrimazole (MIC 6.25 μg/mL).

A close examination of the structures of the active compounds revealed that, their antimicrobial activity is strongly bound to the nature of the substituent of the aryl ring at C_4, together with the substituent linked to position-1 of the ring structure. In general, it could be clearly recognized that potential antibacterial activity was connected with the chlorinated derivatives (R = Cl), whereas moderate activities were displayed by the methoxylated analogs (R = OCH_3). In this context, the key hexahydroquinoline precursors **4–6** showed moderate antimicrobial potential, with particular effectiveness against *S. aureus* and *E. coli*. Nitrosation of position-1 of these compounds resulted in complete abolishment of activity. Introduction of a formyl group furnished one active compound (**10**; R = Cl), which was noticeably less active than the parent compound **5**. Whereas, acetylation (**12–14**) led to a slight improvement in the antimicrobial spectrum, especially **13** (R = Cl), which showed broad antibacterial in addition to some antifungal activities. However, cyclization of the latter N-acetyl compounds into the tricyclic[1,2,4]triazolo[3,4-*a*]quinolines **15–17** resulted in a dramatic reduction in the antimicrobial spectrum and potential. On the other hand, introducing a benzenesulfonyl moiety at position-1 gave rise to three active compounds **18–20**, among which the chloro derivative **19** showed equipotent activity to ampicillin against *E. coli* and 50% of its activity against *S. aureus*, *B. subtilis* and *P.aeruginosa*, together with an appreciable antifungal activity against *C. albicans*. Moreover, incorporating a substituted thiocarbamoyl counterpart as in compounds **21–26**, resulted in an obvious enhancement in both the antimicrobial potential and spectrum. It could be clearly recognized that the activity of these analogs is connected with the aryl substituent (**22, 23, 25** and **26**). Within this series, compound **25** (R = Cl; R^1 = 4-Cl-C_6H_4) was the most active member as it displayed a fourfold increase in the antimicrobial activity against *S. aureus*, *E. coli* and *P. aeruginosa* (MIC 6.25, 6.25 and 12.5 μg/mL, respectively), together with a remarkable antifungal activity (12.5 μg/mL), when compared with the parent **5**. Finally, alkylation of the key intermediates **4–6** with alkyl or arylalkyl groups furnished a series of compounds **27–32** with improved antimicrobial profiles, among which the analogs **28, 30** and **32** (R^2 = benzyl) showed a variable degree of antimicrobial activity, whereas those with the aliphatic substituent (R^2 = CH_3), were totally inactive. When compared with the parent compounds **5** and **6,** the active compounds **30** (R = Cl; R^2 = benzyl) and **32** (R = OCH_3; R^2 = benzyl) revealed an obvious enhancement in the activity against *S. aureus*, *E. coli* and *P. aeruginosa*, together with a moderate antifungal activity.

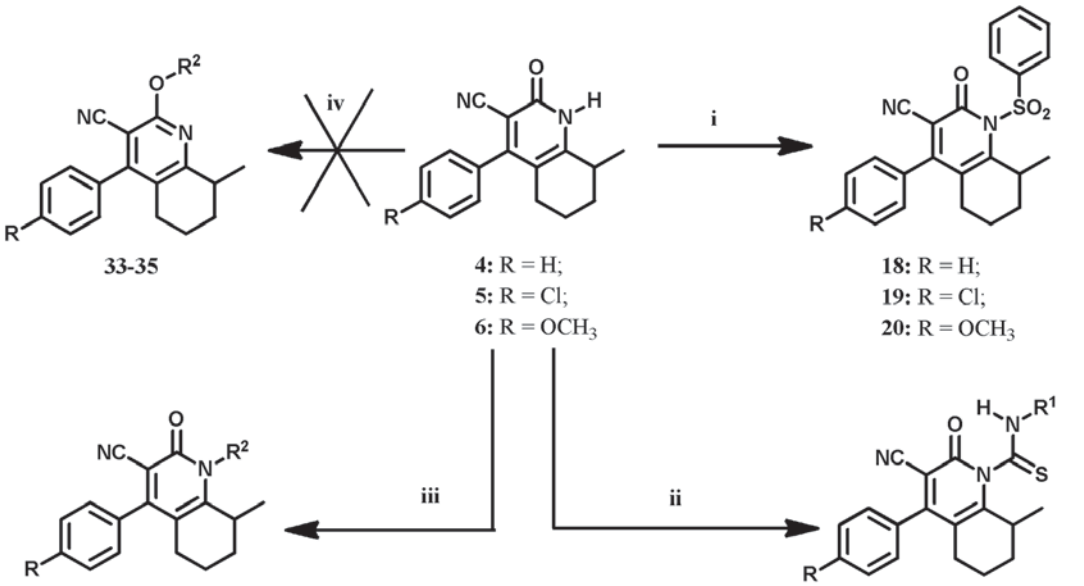
In vitro MTT cytotoxicity assay

At both μg/mL and μM concentration levels, the obtained data in Table 2 revealed that 12 compounds namely; **3, 6, 11, 14, 17, 20, 22, 24, 25, 26, 29** and **31** were able to affect cell viability of the tested tumour cell lines particularly the human colon carcinoma HT29 cell line,



Reagents and reaction conditions: **i:** ethyl cyanoacetate, ammonium actetate, ethanol, reflux, 6h; **ii:** sodium nitrite, acetic acid, zero°C, 2h; **iii:** formic acid, reflux, 3h; **iv:** acetic anhydride, anhyd. sodium acetate, reflux, 4h; **v:** hydrazine hydrate 98%, ethanol, reflux, 6-8h.

Scheme 1. Synthesis of compounds **4–17**.



Reagents and reaction conditions:
i: benzenesulfonyl chloride, pyridine, reflux, 4-6h; **ii:** R^1NCS, pyridine, reflux, 6-8h;
iii: R^2X, pyridine, reflux, 3-4h; **iv:** R^2X, ethanol, silver nitrate or sodium ethoxide, reflux, 3-10h.

Scheme 2. Synthesis of compounds **18–32**.

whereas the rest of the compounds were totally inactive. In addition, the three tested human tumour cell lines exhibited variable degree of sensitivity profiles towards the active compounds. In terms of μg/mL concentration, the human colon carcinoma HT29 cell line showed pronounced sensitivity against compounds **22** and **25** (LC_{50} 8.4 and 14.9 μg/mL, respectively) even higher than that of doxorubicin (LC_{50} 21.1 μg/mL). Moreover, a remarkable cytotoxic potential was displayed by compounds **20** and **24** against the same cell line (LC_{50} 29.6 and 26.3 μg/mL respectively) comparable to that of doxorubicin. The rest of the active compounds showed moderate to weak activity profiles against the same cell line with LC_{50} range of 34.9–76.3 μg/mL. On the other hand, the growth of the human hepatocellular carcinoma Hep-G2 cell line was found to be moderately inhibited by nine of the active compounds with LC_{50} values range of 11.6–48.7 μg/mL, among which compounds **20** and **25** (LC_{50} values 18.5 and 11.6 μg/mL, respectively) revealed the highest cytotoxic activity as compared to doxorubicin (LC_{50} 1.69 μg/mL). Regarding the human breast cancer MCF7, it was proved to be the least sensitive among the tested cell lines, as its growth was inhibited by only seven of the tested compounds. However, a remarkable growth inhibitory potential was shown by compounds **22** and **25** as evidenced from their LC_{50} values (12.1 and 9.7 μg/mL, respectively) when compared to doxorubicin (LC_{50} 2.14 μg/mL).

A close examination of the structure of the active compounds showed that the 4-chloro and 4-methoxy groups at the C-4 aryl ring are the most favourable substituents. Although the key precursors **5** and **6** possessed moderate cytotoxic profiles, yet formylation (**11**) or nitrosation (**7, 8**) of these compounds led to a dramatic reduction or even total loss of activity, respectively. However, acetylation (**14**) and subsequent cyclization into a tricyclic ring system (**17**) resulted in an obvious improvement in the cytotoxic profile, especially against the HT29 and Hep-G2 cell lines. Moreover, sulfonylation as in (**20**), led to a noticeable enhancement in both the cytotoxic spectrum and potential. On the other hand, great improvement of the cytotoxic potential was linked to the introduction of a thiocarbamoyl substituent at position-1. It could be clearly recognized that the chlorinated derivatives (**22, 24, 25** and **26**) showed distinctive cytotoxic activities, whereas **21** and **23** were totally inactive. Among these, the analogs **22** and **25** proved to be the most active members in this study with a broad spectrum of activity against the tested cell lines. Finally, alkylation with a methyl or benzyl groups did not offer any advantage to the cytotoxic profile of such type of compounds. On the contrary, introduction of a methyl group (**29** and **31**) resulted in a marginal enhancement in the activity against HT29 cell line, whereas the effect against the Hep-G2 and MCF7 cell lines was totally lost. Meanwhile, alkylation with a benzyl group led to total abolishment of activity.

Conclusion

Twenty nine novel 3-cyano-8-methyl-2-oxo-1,4-disubstituted-1,2,5,6,7,8-hexahydroquinolines supported with various pharmacophores that are known to contribute to potential chemotherapeutic effects were successfully synthesized and evaluated for their biological activity as antimicrobial agents against eight different microorganisms and/or cytotoxic agents against three different human tumour cell lines at both μg/mL and μM concentration levels. The results revealed that eighteen compounds displayed special pronounced activity against *S. aureus* and *E. coli* bacterial strains, in addition to moderate antifungal activity against *C. albicans*. Compounds **19**, **22**, **25**, **30** and **32** could be considered as the most active broad spectrum antimicrobial members identified in this study. Among these, compound **25** proved to be the most active candidate as it showed equipotency to ampicillin against *S. aureus*, *E. coli* and *P. aeruginosa*, in addition to an obvious antifungal activity comparable with clotrimazole. The best antifungal activity was demonstrated by compounds **25** and **26** which possessed almost half the activity of clotrimazole against *C. albicans.* On the other hand, 12 compounds were able show remarkable cytotoxic efficiency against human colon carcinoma HT29, hepatocellular carcinoma Hep-G2 and Caucasian breast adenocarcinoma MCF7 cell lines. The results revealed that compounds **6, 17, 20, 22, 24** and **25** showed considerable broad spectrum cytotoxic activity against the three tested cell lines, among which the analogs **22** and **25** proved to be the most active members in this study with special effectiveness against the human colon carcinoma HT29 and human breast cancer MCF7 cell lines even higher than that of doxorubicin, the reference standard cytotoxic agent utilized in this test. Finally, the obtained antimicrobial and anticancer data makes such type of compounds a fruitful matrix for further development of more potent and selective cytotoxic and/or antimicrobial agents. At both μg/mL and μM concentration levels, compounds **22** and **25** could be considered as possible dual antimicrobial-anticancer candidates that deserve further investigation and derivatization in order to explore the scope and limitation of its biological activities.

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Declaration of interest

The authors report no conflicts of interest.

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