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ORIGINAL ARTICLE

Synthesis and biological evaluation of some novel tetrahydroquinolines as anticancer and antimicrobial agents

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Abstract

This study reports the synthesis of a series of new 2-amino-3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolines along with some derived fused-ring systems. Ten compounds have shown remarkable cytotoxic activity against human colon carcinoma HT29, hepatocellular carcinoma HepG2 and Caucasian breast adenocarcinoma MCF7 cell lines. Six compounds showed considerable broad-spectrum cytotoxic activity among which two proved to be the most active derivatives. Likewise, seven compounds from the series were found to exhibit significant antimicrobial activity and three of them proved to be the most active candidates. Two alkylthio-pyrimido quinolines are suggested as possible antimicrobial and anticancer candidates in the present series.

Keywords

2-Amino-3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolines, 3H-pyrimido[4,5-b]quinolin-4-ones, antimicrobial agents, cytotoxic agents

History

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Introduction

The ongoing efforts of research on the treatment of malignancy are focused on the discovery of novel products interacting with novel biological targets. Among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, pyridines and fused pyridine ring systems have received much attention since they are proved to be biologically versatile compounds possessing a variety of activities. A wide range of chemotherapeutic activities have been ascribed to pyridine derivatives including antimicrobial[1–3], antiamoebic[1], antiparasitic[4] and antiviral[5,6] activities. As far as the antineoplastic potential is concerned, pyridine derivatives were reported to contribute to a variety of anticancer activity including cytotoxic[7,8], antiproliferative[9] and CDK kinase inhibitory activity[10].

Cyanopyridylureas have been claimed for their properties in treating hyperproliferative and angiogenesis disorders. The 3-cyano-2,6-dihydropyridine is a potent inhibitor of dihydrouracil dehydrogenase and its co-administration with 1-ethoxymethyl-5-fluorouracil enhances the antitumor effect[11]. Furthermore, Piritrexim (2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine), a second-generation antineoplastic drug, having a pyridine ring among their structures was reported to inhibit the enzyme dihydrofolate reductase. Recently, it has been reported that some fused pyridines emerged as potential inhibitors of protein kinase B[12] and HIF 1-α prolyl hydroxylase[13], which play a major role in cancer cell division. During our ongoing studies aimed at the discovery of new structure leads endowed with diverse chemotherapeutic activities, much concern has been raised on the antimicrobial and antitumor potentials of some pyridine derivatives[14–17]. The obtained results prompted further structure modification of the disubstituted-2(1*H*)-pyridinone scaffold by fusing it with a cyclohexane ring leading to the synthesis of new tetrahydroquinoline analogs. The substitution profile of the main tetrahydroquinoline ring was attempted to comprise some counterparts that would confer different electronic, lipophilic and steric environment, which would influence the targeted biological activities. It was, therefore, considered worthwhile to synthesize some tetrahydroquinoline-derived fused-ring systems as interesting structural variation to improve the anticipated chemotherapeutic profile.

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Experimental

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The microwave reactor used was CEM Discover 300 W. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S IR spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as a solvent (chemical shifts in δ, ppm). Splitting patterns were designated as follows: s, singlet; d, doublet; m, multiplet and 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 TLC on silica gel pre-coated aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254. The analysis data are reported in Tables 1 and 2.

Table 1. Physicochemical and analytical data of compounds **1**–**28**.

Compound no.	R	M.p., °C	Yield %	Mol. formula (mol. weight)		Analysis % Calcd.	Analysis % Found
1	C_6H_5	162–164	67	$C_{17}H_{17}N_3$ (263.34)	C	77.54	77.58
					H	6.51	6.67
					N	15.96	16.12
2	4-BrC_6H_4	190–192	54	$C_{17}H_{16}BrN_3$ (342.23)	C	59.66	59.75
					H	4.71	4.83
					N	12.28	12.21
3	4-$CH_3OC_6H_4$	177–179	68	$C_{18}H_{19}N_3O$ (293.37)	C	73.70	73.42
					H	6.53	6.71
					N	14.32	14.09
4	4-$CH_3C_6H_4$	210–212	73	$C_{18}H_{19}N_3$ (277.37)	C	77.95	78.08
					H	6.90	6.72
					N	15.15	15.40
5	2-Thienyl	186–188	81	$C_{15}H_{15}N_3S$ (269.37)	C	66.88	66.97
					H	5.61	5.36
					N	15.60	15.44
6	1-Methyl-pyrrol-2-yl	150–152	50	$C_{16}H_{18}N_4$ (266.35)	C	72.15	72.32
					H	6.81	6.95
					N	21.04	20.88
7	C_6H_5	160–162	59	$C_{18}H_{17}N_3O$ (307.39)	C	74.24	74.32
					H	6.89	6.97
					N	13.67	13.81
8	4-BrC_6H_4	150–152	62	$C_{18}H_{16}BrN_3O$ (370.24)	C	58.39	58.42
					H	4.36	4.45
					N	11.35	11.44
9	4-$CH_3OC_6H_4$	186–188	72	$C_{19}H_{19}N_3O_2$ (321.38)	C	71.01	71.32
					H	5.96	5.73
					N	13.07	12.91
10	4-$CH_3C_6H_4$	137–139	47	$C_{19}H_{19}N_3O$ (305.38)	C	74.73	74.51
					H	6.27	6.47
					N	13.76	13.53
11	2-Thienyl	166–168	63	$C_{16}H_{15}N_3OS$ (297.38)	C	64.62	64.71
					H	5.08	4.89
					N	14.13	14.34
12	4-BrC_6H_4	168–169	42	$C_{19}H_{18}BrN_3O$ (384.27)	C	59.39	59.42
					H	4.72	4.89
					N	10.94	10.85
13	4-$CH_3OC_6H_4$	122–124	41	$C_{20}H_{21}N_3O_2$ (335.40)	C	71.62	71.54
					H	6.31	6.42
					N	12.53	12.70
14	4-$CH_3C_6H_4$	135–137	52	$C_{20}H_{21}N_3O$ (319.40)	C	75.21	75.41
					H	6.63	6.47
					N	13.16	13.25
15	2-Thienyl	182–184	63	$C_{17}H_{17}N_3OS$ (311.41)	C	65.57	65.63
					H	5.50	5.39
					N	13.49	13.21
16	1-Methyl-pyrrol-2-yl	>300	36	$C_{18}H_{20}N_4O$ (308.39)	C	70.11	69.92
					H	6.54	6.69
					N	18.17	18.03
17	C_6H_5	140–142	82	$C_{24}H_{22}N_4S$ (398.53)	C	72.33	72.19
					H	5.56	5.66
					N	14.06	14.14
18	4-BrC_6H_4	140–142	82	$C_{24}H_{21}BrN_4S$ (477.42)	C	60.38	60.46
					H	4.43	4.61
					N	11.74	11.65
19	4-$CH_3OC_6H_4$	134–136	78	$C_{25}H_{24}N_4OS$ (428.55)	C	70.07	69.98
					H	5.64	5.66
					N	13.07	13.15
20	4-CH_3-C_6H_4	134–136	76	$C_{25}H_{24}N_4S$ (412.56)	C	72.78	72.62
					H	5.86	5.66
					N	13.58	13.71
21	2-Thienyl	184–186	73	$C_{22}H_{20}N_4O_2$ (404.55)	C	65.32	65.09
					H	4.98	5.17
					N	13.85	13.56
22	1-Methyl-pyrrol-2-yl	136–138	59	$C_{23}H_{23}N_5S$ (401.54)	C	68.80	68.93
					H	5.77	5.56
					N	17.44	17.24
23	C_6H_5	126–128	85	$C_{25}H_{22}N_4OS$ (426.54)	C	70.40	70.51
					H	5.20	5.09
					N	12.72	12.86

(*continued*)

Table 1. Continued

Compound no.	R	M.p., °C	Yield %	Mol. formula (mol. weight)		Analysis % Calcd.	Found
24	4-BrC_6H_4	126–128	85	$C_{25}H_{21}BrN_4OS$ (505.43)	C	59.41	59.34
					H	4.19	4.09
					N	11.08	11.15
25	4-$CH_3OC_6H_4$	176–178	83	$C_{26}H_{24}N_4O_2S$ (456.57)	C	68.40	68.58
					H	5.30	5.17
					N	12.27	12.09
26	4-CH_3-C_6H_4	102–103	79	$C_{26}H_{24}N_4OS$ (440.57)	C	70.88	71.02
					H	5.49	5.31
					N	12.72	12.45
27	2-Thienyl	194–196	73	$C_{24}H_{20}N_4OS_2$ (432.56)	C	63.86	63.64
					H	4.66	4.83
					N	12.95	13.07
28	1-Methyl-pyrrol-2-yl	152–154	52	$C_{24}H_{23}N_5OS$ (429.54)	C	67.11	66.92
					H	5.40	5.73
					N	16.30	16.18

Table 2. Physicochemical and analytical data of the scheme 2 compounds **29–51**.

Compound no.	R	Y or R_1	M.p., °C	Yield, %	Mol. formula (mol. weight)		Analysis % Calcd.	Found
29	C_6H_5	S	120–122	67	$C_{18}H_{18}N_4S$ (322.43)	C	67.05	66.89
						H	5.63	5.78
						N	17.38	17.21
30	4-BrC_6H_4	S	143–145	52	$C_{18}H_{17}BrN_4S$ (401.32)	C	53.87	53.69
						H	4.27	4.19
						N	13.96	13.78
31	4-OCH_3–C_6H_4	S	207–209	73	$C_{19}H_{20}N_4OS$ (352.46)	C	64.75	64.88
						H	5.72	5.59
						N	15.90	16.07
						S	9.10	8.89
32	4-$CH_3C_6H_4$	S	143–145	52	$C_{19}H_{20}N_4S$ (336.46)	C	67.83	67.02
						H	5.99	6.14
						N	16.65	16.38
						S	9.53	9.61
33	2-Thienyl	S	178–180	71	$C_{16}H_{16}N_4S_2$ (328.46))	C	58.51	58.66
						H	4.91	4.73
						N	17.06	16.87
						S	19.52	19.69
34	C_6H_5	O	222–224	69	$C_{18}H_{18}N_4O$ (306.36)	C	70.57	70.71
						H	5.92	5.69
						N	18.29	18.03
35	4-$CH_3OC_6H_4$	O	234–236	71	$C_{19}H_{20}N_4O_2$ (336.39)	C	67.84	68.06
						H	5.99	6.14
						N	16.66	16.49
36	2-Thienyl	O	250–252	75	$C_{16}H_{16}N_4OS$ (312.39)	C	61.52	61.41
						H	5.16	5.37
						N	17.93	18.07
37	C_6H_5	C_2H_5	146–148	67	$C_{20}H_{22}N_4S$ (350.49)	C	68.54	68.79
						H	6.33	6.14
						N	15.99	16.07
38	C_6H_5	$CH_2COC_6H_5$	138–139	74	$C_{26}H_{24}N_4OS$ (440.57)	C	70.88	71.06
						H	5.49	5.58
						N	12.72	12.44
39	4-BrC_6H_4	CH_3	250–252	75	$C_{19}H_{19}BrN_4S$ (415.35)	C	59.94	60.02
						H	4.61	4.50
						N	13.49	13.52
						S	7.72	7.64
40	4-$CH_3OC_6H_4$	CH_3	146–148	67	$C_{20}H_{22}N_4OS$ (366.48)	C	65.55	65.59
						H	6.05	6.14
						N	15.29	15.17
						S	8.75	8.86
41	4-OCH_3–C_6H_4	$CH_2COC_6H_5$	140–142	76	$C_{27}H_{26}N_4O_2S$ (470.60)	C	68.91	68.67
						H	5.57	5.73
						N	11.91	12.14
42	4-$CH_3OC_6H_4$	C_2H_5	176–178	59	$C_{21}H_{24}N_4OS$ (380.52)	C	66.29	66.12
						H	6.36	6.53
						N	14.72	14.62

Table 2. Continued

Compound no.	R	Y or R_1	M.p., °C	Yield, %	Mol. formula (mol. weight)		Analysis % Calcd.	Found
43	4-$CH_3C_6H_4$	CH_3	183–185	66	$C_{20}H_{22}N_4S$ (350.48)	C	68.54	68.41
						H	6.33	6.50
						N	15.99	13.48
44	4-$CH_3C_6H_4$	$CH_2C_6H_5$	240–242	61	$C_{26}H_{26}N_4S$ (426.59)	C	73.21	73.41
						H	6.14	5.87
						N	13.13	13.34
45	2-Thienyl	CH_3	240–242	61	$C_{17}H_{18}N_4S_2$ (342.48)	C	59.62	59.55
						H	5.30	5.27
						N	16.36	16.34
46	2-Thienyl	C_2H_5	167–169	52	$C_{18}H_{20}N_4S_2$ (356.51)	C	60.64	60.95
						H	5.65	5.71
						N	15.72	15.84
47	2-Thienyl	$CH_2C_6H_5$	>300	63	$C_{23}H_{22}N_4S_2$ (418.58)	C	66.00	66.34
						H	5.30	5.15
						N	13.39	13.17
48	C_6H_5		139–141	81	$C_{18}H_{18}N_4$ (290.37)	C	74.46	74.72
						H	6.25	6.07
						N	19.29	19.18
49	4-BrC_6H_4		240–242	61	$C_{18}H_{17}BrN_4$ (369.26)	C	58.55	58.43
						H	4.64	4.53
						N	15.17	15.21
						S	7.52	7.42
50	4-OCH_3–C_6H_4		121–123	83	$C_{19}H_{20}N_4O$ (320.40)	C	71.23	71.12
						H	6.29	6.40
						N	17.49	17.60
51	4-CH_3–C_6H_4		200–201	78	$C_{19}H_{20}N_4$ (304.40)	C	74.97	75.13
						H	6.62	6.49
						N	18.41	18.56

Compounds **29–33** and **34–36** were also prepared using microwave-assisted synthesis. The yields were about 20–25% much better than the conventional method.

2-Amino-3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolines (1–6)

A mixture of the 2-methylcyclohexanone (1.12 g, 0.01 mol), the appropriate aldehyde (0.01 mol), malononitrile (0.66 g, 0.01 mol) and anhydrous ammonium acetate (6.2 g, 0.08 mol) in absolute ethanol (50 mL) was refluxed for 3–6 h. The reaction mixture was cooled and the resulting precipitate was filtered, washed with water, dried and crystallized with the appropriate solvent. IR (cm^{-1}): 3210–3450 (NH_2), 2214–2226 (CN). ^{13}C NMR (δ ppm) for **1** (R = C_6H_5): 20.8 (CH_3), 21.3, 27.12, 30.5, 36.6 (cyclohexyl C), 117.0 (CN), 91.8, 122.4, 152.9, 157.4, 166.1 (pyridine C), 123.9, 129.6, 132.4, 135.8 (Ar C). **2** (R = 4-BrC_6H_4): 20.2 (CH_3), 21.1, 27.02, 30.9, 36.5 (cyclohexyl C), 116.8 (CN), 89.7, 120.3, 153.1, 157.2, 165.8 (pyridine C), 123.2, 129.8, 132.0, 135.3, (Ar C). **3** (R = 4-$CH_3OC_6H_4$): 20.6 (CH_3), 22.1, 27.6, 31.2, 38.4 (cyclohexyl C), 56.2 (OCH_3), 116.8 (CN), 89.7, 121.3, 152.8, 157.7, 165.3 (pyridine C), 114.2, 127.8, 131.5, 162.4 (Ar C). **4** (R = 4-$CH_3C_6H_4$): 20.2(CH_3), 21.2 (CH_3), 21.4, 27.1, 30.7, 36.5 (cyclohexyl C), 116.9 (CN), 90.1, 120.6, 154.6, 157.2, 165.3 (pyridine C), 128.0, 129.3, 133.4, 138.6 (Ar C). **5** (R = 2-Thienyl): 20.3 (CH_3), 21.5, 28.2, 31.2, 37.81 (cyclohexyl C), 116.8 (CN), 92.3, 122.3, 148.8, 158.5, 165.3 (pyridine C), 127.4, 127.6, 128.8, 136.9, (thiophene ring C). **6** (R = 1-Methyl-pyrrol-2-yl): 21.9 (CH_3), 32.4 (NCH_3), 21.9, 27.28, 31.5, 37.6 (cyclohexyl C), 117.3 (CN), 90.5, 121.8, 153.9, 156.6, 164.8 (pyridine C), 110.9, 112.2, 122.9, 124.1 (pyrrole C).

9-Methyl-5-substituted-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-ones (7–11)

A mixture of the appropriate compound **1–5** (0.01 mol) and formic acid (5 mL) was heated in a boiling water bath for 30 min. After cooling, the reaction mixture was poured onto ice-cold water; the precipitated solid was filtered, washed with water and crystallized from ethanol. IR (cm^{-1}): 3258–3150 (NH), 1695–1715 (C=O). ^{13}CNMR (δ ppm) for **7** (R = C_6H_5): 21.2 (CH_3), 21.4, 27.2, 31.0, 36.4 (cyclohexyl C), 119.6, 126.8, 129.0, 129.1, 135.4, 138.4, 152.2, 162.1, 163.4, 171.5 (ArC), 170.2 (CO). **8** (R = 4-BrC_6H_4): 20.8 (CH_3), 21.6, 27.5, 31.2, 36.8 (cyclohexyl C), 118.6, 126.4, 129.2, 129.3, 136.4, 138.2, 153.2, 162.6, 163.0, 170.3 (ArC), 169.2 (CO). **9** (R = 4-$CH_3OC_6H_4$): 20.7 (CH_3), 21.7, 27.4, 31.8, 39.0 (cyclohexyl C), 56.1 (OCH_3), 115.2, 127.4, 131.5, 135.2, 138.5, 152.6, 162.7, 163.0, 171.0 (Ar C), 170.5 (CO). **10** (R = 4-$CH_3C_6H_4$): 21.0 (CH_3), 22.1 (CH_3), 21.6, 27.3, 30.4, 36.8 (cyclohexyl C), 118.4, 126.5, 129.1, 129.2, 135.6, 138.6, 152.7, 162.4, 163.2, 171.6 (ArC), 169.7 (CO). **11** (R = 2-thienyl): 21.3 (CH_3), 22.2, 27.8, 31.4, 37.6 (cyclohexyl C), 120.3, 122.4, 125.3, 127.4, 135.8, 142.4, 148.4, 162.2, 163.0, 171.7 (Ar C), 170.1 (CO).

2,9-Dimethyl-5-substituted-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-ones (12–16)

A mixture of the appropriate compound **2–6** (0.01 mol), acetic anhydride (5 mL) and concentrated H_2SO_4 (0.5 mL) was heated in a boiling water bath for 10 min then cooled, poured into ice-cold water and treated with 20% NaOH solution until the pH is alkaline (pH 11). The crude solid product was filtered and crystallized from ethanol. IR (cm^{-1}): 3226–3431 (NH), 1707–1715 (C=O). ^{13}CNMR (δ ppm) for **12** (R = 4-BrC_6H_4): 19.9 (CH_3), 20.9 (CH_3), 22.3, 27.4, 31.2, 37.4 (cyclohexyl C), 119.8, 123.5, 129.1, 132.3, 135.2, 137.2, 152.4, 162.4, 164.0, 170.6 (ArC), 168.9 (CO). **13** (R = 4-$CH_3OC_6H_4$): 19.8 (CH_3), 20.7 (CH_3), 21.7, 27.4, 31.8, 39.0 (cyclohexyl C), 56.2 (OCH_3), 114.2, 127.7, 130.5, 135.2,

138.5, 152.6, 162.0, 162.4, 163.9, 171.6 (ArC), 170.2 (CO). **14** (R = 4-$CH_3C_6H_4$): 19.5 (CH_3), 20.1 (CH_3), 21.4 (CH_3), 22.6, 27.6, 30.5, 37.0 (cyclohexyl C), 119.4, 126.3, 129.2, 129.4, 135.5, 138.2, 152.6, 162.3, 163.6, 170.6 (ArC), 169.8 (CO). **16** (1-Methyl-pyrrol-2-yl): 20.0 (CH_3), 21.3 (CH_3), 32.4 (NCH_3), 23.2, 28.1, 31.9, 37.4 (cyclohexyl C), 113.1, 119.6, 120.2, 122.2, 135.6, 147.2, 153.4, 161.4, 164.4, 171.6 (ArC), 170.7 (CO).

4-Imino-9-methyl-3-phenyl-5-substituted-3,4,6,7,8,9-hexahydro-1H-pyrimido[4,5-b]-quinoline-2-thiones (17–22)

A mixture of the appropriate compound **1–6** (0.01 mol), phenyl isothiocyanate (1.35 g, 0.01 mol) in pyridine (15 mL) was refluxed for 2 h. After cooling, the solid was filtered, washed thoroughly with water, dried and recrystallized from acetic acid. IR (cm^{-1}): 3180–3327 (NH), 1629–1615 (C=N), 1195–1210 (C=S). ^{13}CNMR (δ ppm) for **17** (R = C_6H_5): 22.2 (CH_3), 25.4, 31.2, 32.5, 39.4 (cyclohexyl C), 109.8, 124.5, 124.7, 125.5, 126.8, 128.8, 129.1, 138.3, 139.2, 149.6, 156.8, 163.4 (ArC), 166.0 (C=NH), 179.8 (CS). **18** (R = 4-BrC_6H_4): 21.9 (CH_3), 25.3, 31.4, 32.3, 39.2 (cyclohexyl C), 109.5, 123.2, 124.6, 125.3, 126.8, 128.8, 129.3, 132.3, 137.3, 139.4, 148.9, 156.7, 163.4 (ArC), 165.4 (C=NH), 180.5 (CS). **19** (R = 4-$CH_3OC_6H_4$): 22.0 (CH_3), 26.0, 31.8, 32.5, 39.4 (cyclohexyl C), 56.6 (OCH_3), 109.8, 114.4, 124.3, 125.2, 127.6, 128.6, 130.5, 139.7, 148.8, 157.1, 162.3, 163.2 (ArC), 164.7 (C=NH), 178.4 (CS). **20** (R = 4-$CH_3C_6H_4$): 20.8 (CH_3), 22.3 (CH_3), 25.6, 30.8, 32.5, 38.8 (cyclohexyl C), 109.8, 124.1, 124.5, 125.4, 126.6, 128.6, 129.5, 135.4, 139.5, 148.8, 157.1, 163.2 (ArC), 164.9 (C=NH), 179.0 (CS). **22** (1-Methyl-pyrrol-2-yl): 20.6 (CH_3), 32.4 (NCH_3), 25.2, 31.4, 32.1, 38.5 (cyclohexyl C), 108.1, 110.5, 112.2, 124.6, 125.2, 128.8, 129.8, 133.6, 139.6, 145.2, 157.4, 163.4 (ArC), 164.4 (C=NH), 179.6 (CS).

1-Benzoyl-3-(3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolin-2-yl)-thioureas (23–28)

To a solution of the appropriate derivative **1–6** (0.02 mol) in dry acetone (20 mL), a solution of benzoyl isothiocyanate (3.3 g, 0.02 mol) in dry acetone (10 mL) was added. The resultant solution was heated under reflux for 3 h. The reaction mixture was left overnight, concentrated and allowed to cool. The separated crystalline product was filtered, washed with Et_2O and recrystallized from ethanol. IR (cm^{-1}): 3345–3450 (NH), 2210–2220 (CN), 1710–1700 (C=O), 1185–1168 (C=S). ^{13}C NMR (δ ppm) for **23** (R = C_6H_5): 21.7 (CH_3), 25.0, 31.3, 32.4, 39.1 (cyclohexyl C), 91.6, 125.3, 126.8, 127.4, 128.4, 129.1, 129.2, 131.7, 133.5, 138.2, 156.7, 163.2, 166.2 (ArC), 117.6 (CN), 179.5 (CS), 169.9 (CO). **24** (R = 4-BrC_6H_4): 22.1 (CH_3), 25.7, 31.3, 32.4, 37.9 (cyclohexyl C), 91.4, 123.3, 126.8, 127.4, 128.4, 129.1, 131.2, 132.7, 133.5, 137.2, 156.7, 163.2, 166.2 (ArC), 117.2 (CN), 179.1 (CS), 168.7 (CO). **25** (R = 4-$CH_3OC_6H_4$): 21.8 (CH_3), 25.4, 31.5, 32.2, 39.1 (cyclohexyl C), 56.1 (OCH_3), 91.3, 114.6, 125.2, 127.3, 128.6, 130.4, 131.8, 133.5, 156.7, 162.5, 163.2, 166.1 (ArC), 118.0 (CN), 179.8 (CS), 169.5 (CO). **26** (R = 4-$CH_3C_6H_4$): 20.9 (CH_3), 22.4 (CH_3) 25.6, 31.5, 32.3, 38.0 (cyclohexyl C), 91.7, 125.3, 126.8, 127.3, 128.7, 129.7, 131.8, 133.5, 135.2, 138.3, 156.6, 163.4, 165.7 (ArC), 117.5 (CN), 179.2 (CS), 169.4 (CO). **27** (R = 2-thienyl): 21.8 (CH_3), 24.9, 31.1, 32.5, 37.9 (cyclohexyl C), 92.4, 122.7, 125.3, 125.8, 127.2, 127.4, 128.5, 131.8, 133.5, 142.6, 152.4, 163.4, 166.2 (ArC), 118.1 (CN), 180.0 (CS), 168.8 (CO). **28** (1-Methyl-pyrrol-2-yl): 20.6 (CH_3), 32.4 (NCH_3), 25.2, 31.4, 32.1, 38.5 (cyclohexyl C), 92.4, 110.2, 123.1, 125.8, 127.3, 128.5, 131.9, 133.4, 152.2, 163.1, 165.8 (ArC), 117.7 (CN), 179.6 (CS), 169.7 (CO).

General methods for the preparation of 4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-1H-pyrimido [4,5-b]quinoline-2-thiones (29–33) and 4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b] quinoline-2-ones (34–36)

Method A

A solution of the appropriate thioureido derivative **23–27** (0.02 mol) in a mixture of 1N sodium hydroxide (5.5 mL) and EtOH (10 mL) was heated on a boiling water bath for 30 min. The reaction mixture was filtered, if necessary, allowed to cool and rendered acidic with 10% acetic acid. The product was filtered, washed thoroughly with H_2O, dried and crystallized from DMF containing few drops of water. IR (cm^{-1}): 3339–3445 (NH_2), 1158–1172 (C=S).

Method B

A mixture of the appropriate derivative **1–5** (0.001 mol) and thiourea (0.4 g, 0.005 mol) or urea (0.3 g, 0.005 mol) was fused at 260–300 °C using sand bath for 1 h. The reaction mixture was allowed to attain room temperature. The resulting solid product was treated with water, then rubbed with ethanol, filtered and crystallized from DMF containing few drops of water.

Method C

A mixture of the appropriate derivative **1–5** (0.001 mol) and thiourea (0.4 g, 0.005 mol) or urea (0.3 g, 0.005 mol) was heated in a microwave reactor at 250 °C for 15 min. Working up of the reaction was carried out exactly as described under method B. IR (cm^{-1}) for compounds: 3320–3432 (NH), 1695–1708 (C=O). ^{13}C NMR (δ ppm) for **29** (R = C_6H_5): 21.9 (CH_3), 25.3, 31.4, 32.6, 39.2 (cyclohexyl C), 109.9, 124.3, 126.9, 129.1, 138.2, 149.4, 157.1, 163.2, 164.7 (ArC), 182.4 (CS). **30** (R = 4-BrC_6H_4): 22.1 (CH_3), 25.4, 31.2, 32.3, 37.6 (cyclohexyl C), 109.5, 123.4, 124.7, 129.2, 132.3, 137.1, 148.9, 157.3, 163.2, 164.4 (ArC), 183.2 (CS). **31** (R = 4-$CH_3OC_6H_4$): 21.8 (CH_3), 25.3, 31.3, 32.4, 38.7 (cyclohexyl C), 56.0 (OCH_3), 109.7, 114.4, 124.6, 127.8, 130.3, 149.6, 156.9, 162.5, 163.4, 165.3, (ArC), 182.8 (CS). **32** (R = 4-$CH_3C_6H_4$): 20.8 (CH_3), 22.2 (CH_3) 25.4, 31.2, 32.3, 38.2 (cyclohexyl C), 109.9, 124.7, 126.4, 129.8, 135.2, 138.2, 149.5, 157.1, 162.9, 163.7 (ArC), 180.2 (CS). **33** (R = 2-thienyl): 21.9 (CH_3), 25.2, 31.3, 32.3, 38.7 (cyclohexyl C), 110.4, 122.2, 125.3, 126.4, 127.5, 142.5, 145.3, 157.2, 163.4, 164 (ArC), 181.3 (CS). **34** (R = C_6H_5): 21.8 (CH_3), 25.1, 30.8, 31.9, 37.9 (cyclohexyl C), 109.5, 124.4, 126.9, 129.0, 138.2, 149.3, 157.2, 163.3, 164.4 (ArC), 163.2 (CO).

2-Alkylthio-4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinolines (37–47)

To a stirred solution of the proper thione **29–33** (0.02 mol) in a mixture of 1N NaOH (5 mL) and ethanol (2 mL), the selected alkyl or aralkyl halide (0.022 mol) was added. The reaction mixture was stirred at room temperature for 2–3 h and the precipitated product was filtered, washed with aqueous ethanol, dried and crystallized from ethanol. IR (cm^{-1}): 3440–3150 (NH). IR (cm^{-1}) for compounds **38** and **41**: 1680–1685 (ketone C=O). ^{13}C NMR (δ ppm) for **37** (R = C_6H_5; R′ = C_2H_5): 15.8 (CH_3), 21.5 (CH_3), 28.1 (CH_2), 25.2, 31.3, 32.4, 38.4 (cyclohexyl C), 102.8, 126.9, 127.1, 129.0, 135.2, 138.2, 150.0, 157.9, 162.6, 167.3, 167.6 (ArC). **38** (R = C_6H_5; R′ = $CH_2COC_6H_5$): 22.3 (CH_3), 44.2 (CH_2) 24.9, 31.5, 32.4, 38.3 (cyclohexyl C), 102.2, 126.4, 126.7, 128.4, 128.7, 135.3, 129.2, 132.8, 135.3, 137.1, 138.4, 148.9, 158.3, 162.2, 162.8 (ArC), 196.2 (CO). **39** (R = 4-BrC_6H_4; R′ = CH_3): 18.8 (CH_3), 21.7 (CH_3), 25.1, 31.2, 32.3, 38.8

Table 3. ^{1}H NMR data of compounds **1–51**.

Compound	Cyclohexyl H (3 m,7H)	CH_3 (d, $J = 10.0$ Hz)	NH_2[a] (s)	ArH (m)	Others
1	1.56, 2.36, 2.93	1.39	5.03	6.98–7.21 (5H)	
2	1.48, 2.42, 2.85	1.37	5.06	7.32–7.49 (4H)	
3	1.68, 2.39, 2.88	1.36	5.09	6.99–7.26 (4H)	3.85 (s, 3H, OCH_3)
4	1.48, 2.42, 2.85	1.37	5.06	7.12–7.41 (4H)	2.33 (s, 3H, CH_3)
5	1.65, 2.52, 2.97	1.38	5.08	6.80–7.27 (3H)[b]	
6	1.50, 2.45, 2.90	1.35	5.05	6.46–6.68 (3H)[c]	3.52 (s, 3H, NCH_3)
7	1.62, 2.35, 3.07	1.37		7.22–7.42 (5H)	7.89 (s, 1H, NH)
8	1.52, 2.28, 3.12	1.38		7.37–7.49 (4H)	7.91 (s, 1H, NH)
9	1.52, 2.28, 3.12	1.38		6.97–7.26 (4H)	3.91 (s, 3H, OCH_3) 7.91 (s, 1H, NH)
10	1.62, 2.35, 3.07	1.34		6.84–7.32 (4H)	2.37 (s, 3H, CH_3) 7.89 (s, 1H, NH)
11	1.62, 2.26, 2.89	1.39		6.83–7.25 (3H)	8.12 (s, 1H, NH)
12	1.52, 2.20, 2.95	1.36		7.37–7.45 (4H)	2.31 (s, 3H, CH_3) 7.95 (s, 1H, NH)
13	1.62, 2.22, 2.89	1.39		6.83–7.25 (4H)	2.35 (s, 3H, CH_3) 3.87 (s, 3H, OCH_3) 8.12 (s, 1H, NH)
14	1.63–2.58 (6H) 3.08 (1H)	1.36		7.01–7.59 (4H)	2.37 (s, 3H, CH_3) 2.32 (s, 3H, CH_3) 8.14 (s, 1H, NH)
15	1.68–2.43 (6H) 3.10 (1H)	1.37		6.76–7.26 (3H)	2.31 (s, 3H, CH_3) 8.16 (s, 1H, NH)
16	1.52, 2.35, 2.85	1.35		6.48–6.72 (3H)	3.5 (s, 3H, NCH_3) 7.98 (s, 1H, NH)
17	1.62, 2.70, 3.02	1.35		6.92–7.60 (11H)[d]	5.2 (s, 1H, NH)
18	1.67, 2.62, 3.22	1.35		7.31–7.45 (10H)[d]	5.14 (s, 1H, NH)
19	1.55, 2.65, 3.18	1.38		6.87–7.67 (10H)[d]	3.95 (s, 3H, OCH_3) 5.14 (s, 1H, NH)
20	1.67, 2.52, 3.02	1.36		7.07–7.52 (10H)[d]	3.29 (s, 3H, CH_3) 5.07 (s, 1H, NH)
21	1.59–2.49 (6H) 3.20 (1H)	1.40		6.65–7.20 (9H)[d]	5.20 (s, 1H, NH)
22	1.68–2.59 (6H) 3.10 (1H)	1.37		6.42–7.19 (9H)[d]	3.46 (s, 3H, NCH_3) 5.15 (s, 1H, NH)
23	1.72, 2.78, 3.22	1.36		7.10–7.79 (10H)	5.04 (s, 1H, NHCS) 7.95 (s, 1H, NHCO)
24	1.57, 2.60, 3.12	1.39		7.12–7.68 (9H)	5.10 (s, 1H, NHCS) 8.01 (s, 1H, NHCO)
25	1.48, 2.28, 2.48	1.38		6.97–7.26 (9H)	3.89 (s, 3H, OCH_3) 5.14 (s, 1H, NHCS) 8.16 (s, 1H, NHCO)
26	1.68, 2.29, 3.25	1.37		6.84–7.25 (9H)	3.68 (s, 3H, CH_3) 5.14 (s, 1H, NHCS) 8.16 (s, 1H, NHCO)
27	1.69–2.68 (6H), 3.20 (1H)	1.35		6.85–7.76 (9H)	5.18 (s, 1H, NHCS) 7.89 (s, 1H, NHCO)
28	1.52, 2.39, 2.92	1.37		6.52–7.64 (9H)	3.49 (s, 3H, NCH_3) 5.09 (s, 1H, NHCS) 8.03 (s, 1H, NHCO)
29	1.66, 2.51, 2.90	1.36	5.28	7.14–7.42 (5H)	4.21 (s, 1H, NH)
30	1.56, 2.36, 2.93	1.39	5.26	6.98–7.68 (4H)	4.26 (s, 1H, NH)
31	1.59, 2.49, 3.20	1.40	5.29	6.90–7.30 (4H)	3.84 (s, 3H, OCH_3) 4.19 (s, 1H, NH)
32	1.67, 2.52, 3.02	1.38	5.72	7.07–7.57 (4H)	3.29 (s, 3H, CH_3) 4.18 (s, 1H, NH)
33	1.56, 2.44, 3.24	1.41	5.32	6.88–7.30 (3H)	4.30 (s, 1H, NH)
34	1.53, 2.64, 3.05	1.41	5.24	6.97–7.54 (5H)	8.02 (s, 1H, NH)
35	1.57, 2.62, 3.14	1.39	5.35	7.12–7.68 (4H)	3.84 (s, 3H, OCH_3) 7.96 (s, 1H, NH)
36	1.54, 2.26, 3.10	1.38	5.44	6.95–7.28 (3H)	8.10 (s, 1H, NH)
37	1.52, 2.18, 3.12	1.38	5.22	6.94–7.48 (5H)	3.22 (q, 2H, CH_2)[e] 1.23 (t, 3H, CH_3)[e]
38	1.68–2.33, 3.20	1.32	5.32	6.99–7.61 (10H)	4.05 (s, 2H, CH_2)
39	1.57, 2.48, 3.01	1.34	5.44	7.37–7.46 (4H)	2.46 (s, 3H, CH_3)
40	1.48–2.30, 3.23	1.39	5.25	6.99–7.61 (4H)	2.51 (s, 3H, CH_3) 3.88 (s, 3H, OCH_3)
41	1.52, 2.55, 3.04	1.34	5.34	6.99–7.61 (9H)	4.17 (s, 2H, CH_2) 3.85 (s, 3H, OCH_3)
42	1.66, 2.51, 2.98	1.36	5.28	7.01–7.52 (4H)	1.22 (t, 3H, CH_3)[e] 3.25 (q, 2H, CH_2)[e] 3.88 (s, 3H, OCH_3)

Table 3. Continued

Compound	Cyclohexyl H (3 m,7H)	CH_3 (d, $J = 10.0$ Hz)	NH_2[a] (s)	ArH (m)	Others
43	1.65, 2.54, 3.12	1.37	5.65	7.10–7.55 (4H)	2.46 (s, 3H, CH_3) 3.29 (s, 3H, CH_3)
44	1.70, 2.62, 3.09	1.33	5.43	7.17–7.78 (9H)	2.39 (s, 3H, CH_3) 4.17 (s, 2H, CH_2)
45	1.52, 2.24, 3.03	1.32	5.28	7.05–7.26 (3H)	2.51 (s, 3H, CH_3)
46	1.55, 2.26, 3.14	1.33	5.27	6.93–7.28 (3H)	1.26 (t, 3H, CH_3)[e] 3.28 (q, 2H, CH_2)[e]
47	1.62, 2.54, 2.94	1.37	5.47	7.03–7.42 (7H)	4.19 (s, 2H, CH_2)
48	1.64, 2.55, 2.99	1.34	5.66	7.22–7.49 (5H)	8.40 (s, 1H, H-2)
49	1.59, 2.53, 2.96	1.36	5.49	7.36–7.51 (4H)	8.38 (s, 1H, H-2)
50	1.65, 2.63, 3.02	1.35	5.78	6.83–7.36 (4H)	3.74 (s, 3H, OCH_3) 8.39 (s, 1H, H-2)
51	1.63, 2.55, 2.94	1.34	5.62	7.12–7.37 (4H)	2.35 (s, 3H, CH_3) 8.36 (s, 1H, H-2)

[a]D_2O exchangeable protons; [b]Thiophene ring protons; [c]Pyrrole ring protons; [d]ArH + HN =; [e]$J = 8.0$ Hz.

(cyclohexyl C), 102.1, 123.7, 129.1, 132.4, 135.3, 137.1, 148.7, 158.4, 162.4, 167.3, 167.7 (ArC). **40** (R = 4-$CH_3OC_6H_4$; R′ = CH_3): 18.9 (CH_3), 21.9 (CH_3), 25.2, 31.4, 32.2, 38.9 (cyclohexyl C), 56.2 (OCH_3), 102.2, 114.7, 127.8, 130.5, 135.3, 150.1, 158.4, 162.5, 162.6, 167.3 (ArC). **41** (R = 4-$CH_3OC_6H_4$; R′ = $CH_2COC_6H_5$): 21.8 (CH_3), 44.1 (CH_2), 25.4, 31.1, 32.2, 38.6 (cyclohexyl C), 56.1 (OCH_3) 102.1, 114.6, 127.8, 128.4, 128.7, 130.1, 132.8, 135.4, 137.3, 149.5, 158.2, 162.3, 162.6, 167.3, 167.8 (ArC), 196.4 (CO). **42** (R = 4-$CH_3OC_6H_4$; R′ = C_2H_5): 15.5 (CH_3), 21.6 (CH_3), 28.2 (CH_2), 25.4, 31.1, 32.2, 38.6 (cyclohexyl C), 56.1 (OCH_3), 102.3, 114.4, 127.9, 130.4, 135.4, 149.9, 158.0, 162.2, 162.5, 167.2, 167.6 (ArC). **44** (R = 4-$CH_3C_6H_4$; R′ = $CH_2C_6H_5$): 20.9 (CH_3), 22.2 (CH_3), 25.0, 31.5, 32.3, 38.1 (cyclohexyl C), 40.7 (CH_2), 102.5, 124.3, 126.8, 126.9, 127.6, 128.4, 129.7, 135.2, 135.3, 141.2, 149.9, 158.3, 167.4, 167.7 (ArC).

4-Amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-pyrimido[4,5-b]quinolines(48–51)

A mixture of the appropriate intermediate **1–4** (0.01 mol) and formamide (10 ml) was heated under reflux for 2–3 h. The reaction mixture was cooled and the precipitated solid was collected, washed with cold ethanol and crystallized from acetic acid containing few drops of water. IR (cm^{-1}): 3330–3265 (NH_2). **48** (R = C_6H_5): 22.0 (CH_3), 24.7, 31.3, 32.0, 38.4 (cyclohexyl C), 106.8, 126.9, 129.1, 135.3, 138.2, 149.9, 157.3, 158.5, 162.4, 167.7 (ArC). **49** (R = 4-BrC_6H_5): 21.7 (CH_3), 25.3, 31.4, 32.2, 38.6 (cyclohexyl C), 106.1, 123.7, 129.0, 132.3, 135.2, 137.3, 149.7, 157.4, 158.1, 162.4, 167.7 (ArC). **50** (R = 4-$CH_3OC_6H_5$): 21.9 (CH_3), 25.6, 31.4, 32.3, 38.7 (cyclohexyl C), 56.0 (OCH_3), 106.7, 114.4, 127.9, 130.3, 135.4, 149.5, 157.2, 158.3, 162.4, 167.4 (ArC).

Biological activity

In vitro MTT cytotoxicity assay

The synthesized compounds were investigated for their *in vitro* cytotoxic effect via the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[18,19] against a panel of three human tumor cell lines: Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2 and colon carcinoma HT29. The procedures were done in a sterile area using a laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME). Cells were batch cultured for 10 d, then seeded at concentration of 10×10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 °C for 24 h under 5% CO_2 using a water-jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with eight different concentrations of the test compounds (100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 μg/mL). DMSO was employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HepG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10 000 IU/mL penicillin potassium, 10 000 μg/mL streptomycin sulphate and 25 μg/mL amphotericin B), and 1% L-glutamine in 96-well flat bottom microplate at 37 °C under 5% CO_2.

Table 4. Cytotoxic effects (LC_{50}; μM)[a] of the active compounds on some human tumor cell lines using the MTT assay.

Compound no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HepG2	Human breast cancer MCF 7
24	23.6	50.3	49.2
25	28.4	–[b]	–
28	30.3	–	–
30	45.6	43.2	–
33	50.4	47.7	40.4
39	7.9	20.2	2.0
40	10.1	24.6	2.8
42	15.4	37.2	8.7
45	11.5	30.4	3.8
46	18.2	42.6	9.4
Doxorubicin[c]	12.1	1.69	2.14

[a]LC50: Lethal concentration of the compound that causes death of 50% of cells in 24 h (μM).
[b]Totally inactive against this cell line.
[c]Positive control cytotoxic agent.

After 24 h of incubation, the medium was aspirated, 40 μL of MTT salt (2.5 μg/mL) was added to each well and incubated for further 4 h at 37 °C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 μL of 10% sodium dodecyl sulphate in deionized water was added to each well and incubated overnight at 37 °C. The absorbance was then measured using a microplate multiwell reader (Bio-Rad Laboratories Inc., model 3350, Hercules, CA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. Doxorubicin is used as positive control cytotoxic agent. The results of LC_{50} (μM), which is the lethal concentration of the compound causing death of 50% of the cells in 24 h, are presented in Table 4.

Table 5. Minimal inhibitory concentrations [MIC, μg/mL (μM)] of the tested compounds.

Compound no.	*S. aureus*	*B. subtilis*	*M. luteus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
2	50 (146.1)	50 (146.1)	–[a]	50 (146.1)	100 (292.3)	–	–
3	50 (170.4)	100 (340.8)	–	100 (340.8)	–	–	–
4	100 (360.5)	100 (360.5)	200 (721.0)	100 (360.5)	–	–	–
5	50 (185.6)	100 (371.2)	100 (371.2)	200 (742.4)	200 (742.4)	–	–
6	25 (93.8)	100 (375.5)	–	50 (187.7)	100 (375.5)	–	–
12	100 (260.2)	100 (260.2)	100 (260.2)	25 (65.0)	100 (260.2)	–	–
13	100 (298.1)	200 (596.3)	–	100 (298.1)	100 (298.1)	200 (596.3)	–
18	25 (52.3)	50 (104.7)	50 (104.7)	25 (52.3)	50 (104.7)	100 (209.4)	100 (209.4)
19	25 (58.3)	100 (233.3)	–	25 (58.3)	50 (116.6)	200 (466.6)	100 (233.3)
21	50 (123.5)	100 (247.1)	–	100 (247.1)	100 (247.1)	–	–
22	100 (249.0)	100 (249.0)	–	50 (124.5)	50 (124.5)	–	–
24	50 (98.9)	100 (197.8)	100 (197.8)	50 (98.9)	100 (197.8)	50 (98.9)	–
25	50 (109.5)	100 (219.0)	200 (438.0)	100 (219.0)	50 (109.5)	100 (219.0)	–
28	100 (232.8)	100 (232.8)	50 (116.4)	100 (232.8)	200 (405.6)	50 (116.4)	–
30	50 (124.5)	50 (124.5)	100 (249.0)	50 (124.5)	25 (62.2)	100 (249.0)	50 (124.5)
31	100 (283.6)	100 (283.6)	50 (141.8)	50 (141.8)	100 (283.6)	200 (567.2)	100 (283.6)
33	50 (152.2)	100 (304.4)	–	50 (152.2)	200 (608.8)	100 (304.4)	50 (152.2)
39	6.25 (20.0)	25 (80.0)	25 (80.0)	6.25 (20.0)	12.5 (40.0)	50 (160.0)	12.5 (40.0)
40	12.5 (35.6)	25 (71.2)	100 (284.8)	6.25 (17.8)	25 (71.2)	100 (284.8)	25 (71.2)
41	25 (53.1)	100 (212.4)	200 (424.8)	25 (53.1)	50 (106.2)	–	100 (212.4)
42	12.5 (32.8)	50 (131.4)	100 (263.1)	12.5 (32.8)	25 (65.7)	–	25 (65.7)
44	50 (117.2)	100 (234.4)	–	100 (234.4)	100 (234.4)	–	–
45	25 (73.1)	50 (146.1)	50 (146.1)	12.5 (36.5)	100 (292.2)	–	50 (117.2)
46	12.5 (35.0)	50 (140.0)	100 (280.0)	12.5 (35.0)	50 (140.0)	–	12.5 (35.0)
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).

In vitro antibacterial and antifungal activities

All of the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *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. Each 100 mL of sterile molten agar (at 45 °C) received 1 mL of 6 h broth culture and then the seeded agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 mL of the 1 mg/mL solution of the test compounds. The plates were then incubated at 37 °C for 24 h or, in case of *C. albicans*, for 48 h. A control using DMSO without the test compound was included for each organism. Ampicillin trihydrate and clotrimazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial or fungal growth around the discs in mm. The minimal inhibitory concentrations (MIC) of the most active compounds were measured using the twofold serial broth dilution method[20]. The test organisms were grown in their suitable broth: 24 h for bacteria and 48 h for fungi at 37 °C. Twofold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, 12.5 and 6.25 μg/mL. The tubes were then inoculated with the test organisms; each 5 mL received 0.1 mL of the above inoculums and were incubated at 37 °C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values in μg/mL of the prepared compounds are listed in Table 5.

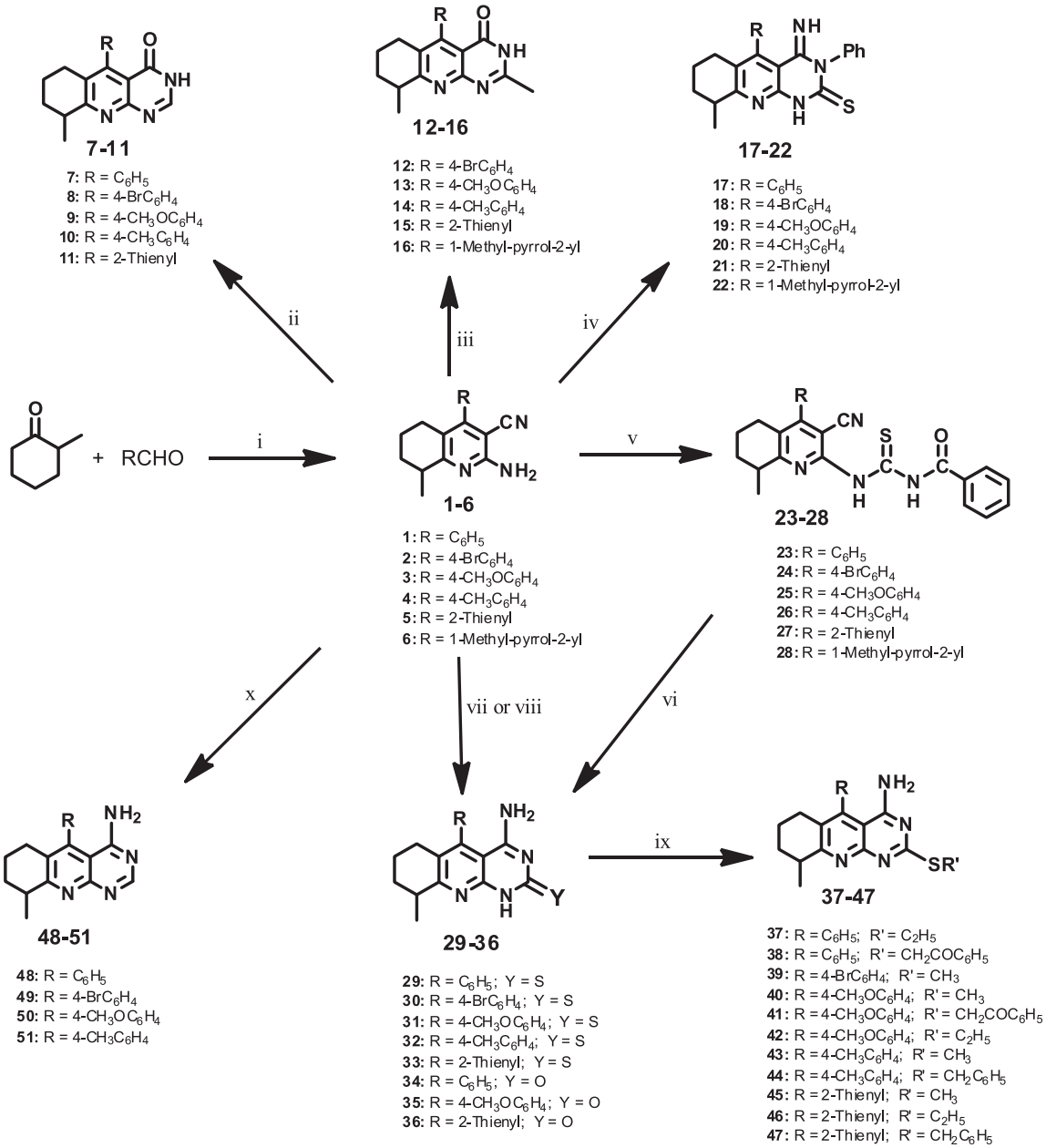
Results and discussion

Chemistry

The synthetic strategies adopted for the preparation of the intermediate and target compounds are described in Scheme 1. The key intermediates in this scheme are the 2-amino-3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolines **1–6**. These compounds were easily synthesized via one-pot multicomponent reaction by treating an appropriate aromatic aldehyde and 2-methylcyclohexanone, with an excess of ammonium acetate and malononitrile in boiling ethanol[21,22]. This reaction is of considerable importance since it is easy to perform, gives high yields, and is less time consuming compared with the traditional two-step procedure that involves the formation of the chalcone via Claisen-Schmidt condensation followed by cyclocondensation with malononitrile and ammonium acetate[23,24].

The IR spectra of these compounds revealed absorption bands at 3210–3450 characteristic for the NH_2 and at 2214–2225 cm^{-1} attributed to the CN group. ^{1}H-NMR of this series showed beside the aromatic protons at δ 7.11–7.42 a doublet of three protons intensity at δ 1.13–1.26 ppm ($J = 10.0$ Hz) for the CH_3 group, a D_2O exchangeable proton singlet of two proton intensity at δ 5.00–5.42 due to the NH_2 group, as well as three multiplets at δ 1.53–1.70, 2.29–2.38 and 2.73–2.84 ppm for the cyclohexyl moiety. The structures were further supported from their ^{13}C NMR spectral data that showed the expected number of aliphatic and aromatic carbons.

Reaction of the 2-aminohydroquinoline derivatives **1–5** with formic acid afforded the targeted 9-methyl-5-substituted-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-ones (**7–11**). The IR spectra of these compounds were characterized by the absence of the CN group absorption and the presence of new sharp absorption bands at 1695–1715 cm^{-1} due to the new C=O groups. Moreover, the ^{1}H-NMR spectra (Table 3) showed beside the aromatic protons a doublet of three protons intensity in the range δ 1.31–1.38 ppm for the CH_3 group as well as three multiplets at δ 1.53–1.70, 2.29–2.38 and 2.83–2.84 ppm corresponding to the protons of the cyclohexyl moiety. Further confirmation of the structure is supported by their ^{13}C NMR spectral data (see Experimental section) that exhibited beside the expected number of aliphatic and aromatic carbons, signals at δ 169.2–170.5 ppm for the CO. Treatment the 2-aminohydroquinoline derivatives **2–6** with acetic anhydride in presence of



Scheme 1. Reagents and reaction conditions: (i) 2-methylcyclohexanone (0.01 mol), approp. aldehyde (0.01 mol) malononitrile (0.01 mol), NH_4OAc (0.08 mol) in Abs. EtOH (50 mL), reflux 3–6 h; (ii) approp. starting compd. (0.01 mol), formic acid (5 mL), heated in boiling water bath 30 min; (iii) approp. starting compd. (0.01 mol), Ac_2O (5 mL), conc. H_2SO_4 (0.5 mL), heated in boiling water bath 10 min; (iv) approp. starting compd. (0.01 mol), PhNCS (0.01 mol) in pyridine (15 mL), reflux 2 h; (v) approp. starting compd. (0.02 mol), PhCONCS (0.02 mol), dry acetone (10 mL), reflux 3 h; (vi) approp. thioureido deriv. (0.02 mol), 1N NaOH (5.5 mL), EtOH (10 mL), heated in boiling water bath 30 min; (vii) approp. starting compd. (0.001 mol), urea or thiourea (0.005 mol), fused at 260–300 °C, sand bath 1 h; (viii) approp. starting compd. (0.001 mol), urea or thiourea (0.005 mol), heated in microwave reactor at 250 °C, 15 min; (ix) approp. thione (0.02 mol), 1N NaOH (5 mL), EtOH (2 mL), alkyl or aralkyl halide (0.022 mol), stirred at r.t., 2–3 h and (x) approp. starting compd. (0.01 mol), $HCONH_2$ (10 mL), reflux, 2–3 h.

few drops of concentrated sulphuric acid gave the corresponding 2,9-dimethyl-5-substituted-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b] quinolin-4-ones **12–16**. The IR spectra of these pyrimidoquinolin-4-one derivatives lacked the CN bands that were present in the starting quinolines and instead exhibited a carbonyl absorption bands at 1707–1715 cm^{-1}. The ^{1}H-NMR spectra showed new singlets at δ 2.45–2.31 ppm due to the new CH_3 group introduced as well as the three multiplets for cyclohexyl moiety at δ 1.51–1.75, 2.24–2.36 and 2.72–2.88 ppm. Likewise, their ^{13}C NMR spectral data exhibited beside the expected number of aliphatic and aromatic carbons, a new CH_3 singlet at δ 19.5–20.2 ppm in addition to a CO signal at δ 168.9–170.7. Reacting the starting aminohydroquinolines **1–6** with the phenyl isothiocyanate in the presence of pyridine gave 4-imino-9-methyl-3-phenyl-5-substituted-3,4,6,7,8,9-hexahydro-1H-pyrimido[4,5-b] quinoline-2-thiones **17–22**. Their IR spectra revealed two characteristic absorption bands at 1615–1629 cm^{-1} and 1195–1210 cm^{-1} due to the (C=N) and (C=S) groups, respectively. The ^{1}H-NMR spectra showed along with multiplets of the cyclohexyl moiety a doublet of three protons at δ 1.34–1.42 ppm for the CH_3 group. The structures of the above pyrimido[4,5-*b*]quinoline-2-thiones **17–22** were further substantiated by their ^{13}C NMR spectra that exhibited two characteristic signals at 178.0–180.0 and δ 164.4–166.0 ppm for the CS and C=NH groups, respectively, in addition to the expected number of aliphatic (cyclohexyl) and aromatic carbons. On the other hand, when compounds **1–6**

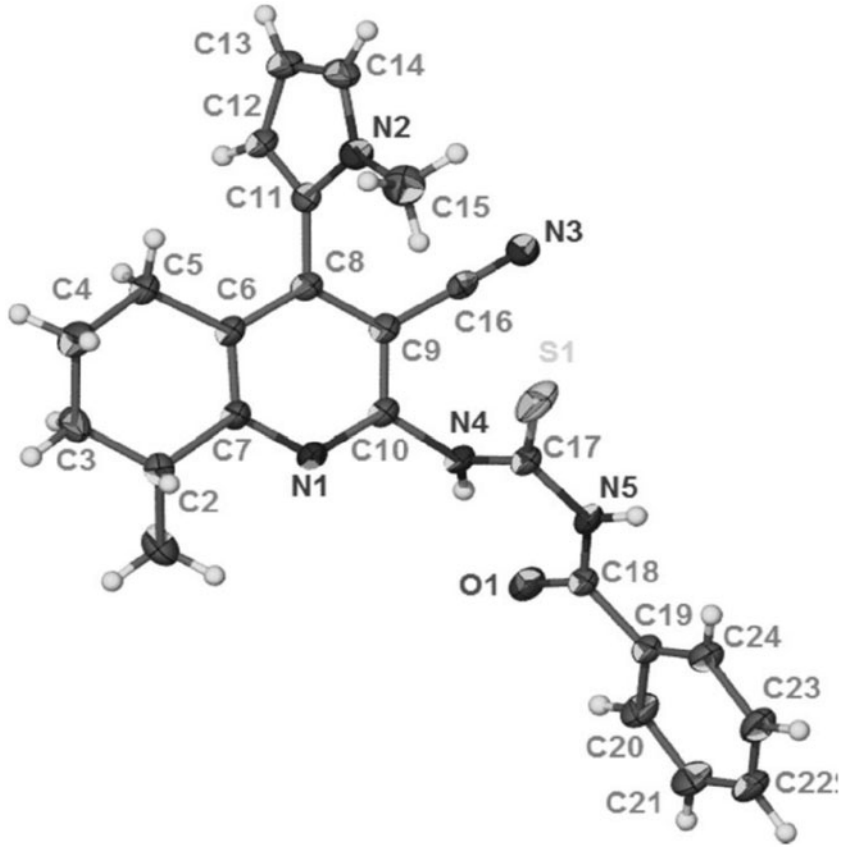


Figure 1. 1-Benzoyl-3-[3-cyano-8-methyl-4-(1-methyl-1*H*-pyrrol-2-yl)-5,6,7,8-tetrahydroquinolin-2-yl]thiourea.

were reacted with benzoyl isothiocyanate in dry acetone, the corresponding substituted thioureido derivatives **23–28** were formed in good yields. The IR spectra of these compounds maintained the characteristic CN absorptions 2210–2220 cm^{-1} in addition to the two new bands at 1700–1710 and 1168–1185 cm^{-1} attributed to the C=O and C=S groups, respectively. The ^{13}C NMR spectra of these compounds showed three signals at δ 117.2–118.1, 179.1–180.3 and 168.7–169.9 ppm for the CN, CS and C=NH groups, respectively. Moreover, the structure of the quinoline derivative **28** was also supported by X-ray crystallography[25] (Figure 1). Cyclization of compounds **23–27** was done by heating them with sodium hydroxide to form 4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinoline-2-thiones **29–33**. It is worth mentioning that direct condensation of **1–5** either with thiourea or urea was attempted as another efficient procedure for a one-step synthesis of the target compounds **29–33** and **34–36**, respectively. The IR spectra of these derivatives (**29–33**) were characterized by the disappearance of the cyano and the carbonyl absorptions and the appearance of broad absorption bands at 3339–3445 cm^{-1} due to amino group as well as a CS absorption at 1158–1172 cm^{-1}. The ^{1}H-NMR spectra showed beside the aromatic protons at δ 6.78–7.42, a doublet of three protons at intensity of δ 1.33–1.36 ppm for the CH_3 group. Further confirmation for the structure was done from their ^{13}C NMR spectral data that exhibited beside the expected number of aliphatic and aromatic carbons, a signal at δ 162.3–163.5 or at δ 182.4–184.6 for the CO (in compounds **29–33**) and CS (in compounds **34–36**), respectively. Thioakylation of the 2-thione derivatives **29–33** could be affected by treating these compounds with a variety of alkyl or aralkyl halides in the presence of sodium hydroxide to produce the alkylthio derivatives **37–47**. Their IR spectra exhibited broad absorption bands at 3262–3338 cm^{-1} due to the amino group. The ^{1}H-NMR spectra showed beside the aromatic protons at δ 6.84–7.62, a doublet of three protons with intensity at δ 1.30–1.35 ppm for the CH_3 group, three multiplets for the cyclohexyl moiety as well as a broad singlet at δ 7.89–8.12 for the NH_2 group, in addition the methylthio derivatives **39**, **40**, **43** and **45** that exhibited a singlet at δ 2.45–2.48 for the S-CH_3 group while the ethylthio derivatives **37**, **42** and **46** afforded a triplet of three proton intensity at δ 1.23–1.26 ($J = 8.0$ Hz) and a quartet of two proton intensity at δ 2.96–3.21 ($J = 8.0$ Hz) due to the CH_3 and CH_2 groups, respectively. Further confirmation for the structure was achieved by their ^{13}C NMR spectral data that lacked the CS signals that existed in the original compounds and exhibited instead an S-CH_3 signal at δ 18.5–19.2 in case of the S-CH_3 derivatives and two signals at δ 15.5–15.8 and δ 27.8–28, in case of the S-ethyl compounds. Furthermore, heating the key intermediates **1–4** with formamide resulted in the formation of the targeted 4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-pyrimido[4,5-b]quinolines **48–51**. The IR spectra of these pyrimido[4,5-*b*] quinolines derivatives were characterized by the disappearance of the cyano groups absorptions and the appearance of broad absorption bands at 3265–3330 cm^{-1} due to the amino group. Their ^{1}H-NMR spectra showed beside the aromatic protons at δ 6.77–7.45, a distinct doublet for three protons at δ 1.33–1.37 ppm for the CH_3 group along with three multiplets at of the cyclohexyl moiety and a broad singlet at δ 7.92–8.22 for the NH_2 group. Further structural assignments were done by their ^{13}C NMR spectral data.

In vitro MTT cytotoxicity assay

Twenty-one analogs (**1**, **4**, **5**, **7**, **8**, **11**, **12**, **15**, **16**, **24**, **25**, **28**, **30**, **33**, **39**, **40**, **42**, **45**, **46**, **48** and **49**) were selected to be evaluated for their *in vitro* cytotoxic effect via the standard MTT method against a panel of three human tumor cell lines: Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2 and colon carcinoma HT29. The results of LC_{50} (μM), which is the

lethal concentration of the compound causing death of 50% of the cells in 24 h, are presented in Table 4.

The obtained data revealed that the three tested human tumor cell lines exhibited variable degree of sensitivity profiles towards ten of the tested compounds: **24**, **25**, **28**, **30**, **33**, **39**, **40**, **42**, **45** and **46**. Among these, compounds **39**, **40** and **45** showed strong activity against the human colon carcinoma HT29 cell line with LC_{50} values of 7.9, 10.1 and 11.5 μM, respectively. Moreover, a remarkable cytotoxic potential was displayed by compounds **42** and **46** against the same cell line (15.4 and 18.2 μM). Compounds **24**, **25** and **28** revealed almost similar cytotoxicity profile against colon carcinoma HT29 with LC_{50} values of 23.6, 28.4 and 30.3 μM, respectively. However, compounds **30** and **33** were able to exhibit moderate activity against the same cell line with LC_{50} value range of 45.6 and 50.4 μM. Furthermore, the growth of the human hepatocellular carcinoma HepG2 cell line was found to be moderately inhibited by eight of the active compounds **24**, **30**, **33**, **39**, **40**, **42**, **45** and **46** with LC_{50} values ranging from 20.2 to 50.3 μM. Among these, the highest cytotoxic activity was displayed by compounds **39** and **40** (LC_{50} values of 20.2 and 24.6 μM, respectively). On the other hand, human breast cancer MCF 7 was proved to be the least sensitive among the cell lines tested as it was affected by only seven of the test compounds. However, an outstanding growth inhibition potential was shown by compounds **39**, **40**, **42**, **45** and **46** as evidenced from their LC_{50} values (2.0, 2.8, 8.7, 3.8 and 9.4 μM, respectively). The remaining two active compounds, namely **24** and **33**, showed moderate to mild activity against the same cell line with LC_{50} values of 49.2 and 40.4 μM, respectively. Further interpretation of the results revealed that compounds **39**, **40**, **42**, **45** and **46** showed considerable broad spectrum of cytotoxic activity against the three tested human tumor cell lines. In particular, compound **39** proved to be the most active member in this study with a broad spectrum of activity against the tested cell lines, with special effectiveness against the human colon carcinoma HT29 and human breast cancer MCF 7 cell lines (LC_{50} values 7.9 and 2.0 μM, respectively). A close examination of the structure of the active compounds showed that the 4-bromophenyl counterpart at position 4 of the tetrahydroquinoline skeleton is the most favourable substituent when compared with other analogs. Moreover, the thioalkyl substituent at position 2 as in compounds **39**, **40**, **42**, **45** and **46** was responsible for the high activity displayed by these analogs. Thiomethyl analogs, **39** and **40**, displayed better profile when compared with a thioethyl derivative as in case of compound **42**. Although compound **30** showed some activity because it carries a 4-bromophenyl substituent, but it lacks optimal activity owing to the presence of 2-thione and/or the absence of a thioalkyl substituent at position 2. Substitution at position 2 with a thiourea moiety or cyclization of these thiourea derivatives resulted in moderate to weakly active compounds **24**, **25**, **28**, **30** and **33** whereas cyclization of the intermediate quinoline derivatives (**1**–**6**) with formic acid, acetic anhydride and formamide led to complete abolishment of the cytotoxic activity.

In vitro antibacterial and antifungal activities

As revealed from MIC data recorded in Table 5 [MIC expressed in both concentration units μg/mL (μM)], 24 of the 51 newly synthesized compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative microorganisms with 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 activity against *Aspergillus niger*.

Among the tested Gram-positive bacterial strains, two organisms, namely *S. aureus* and *B. subtilis*, showed relatively high sensitivity towards the tested compounds. In this view, compound **39** was almost equipotent to ampicillin (MIC 6.25 μg/mL) against *S. aureus*, whereas the analogs **40**, **42** and **46** (MIC 12.5 μg/mL) were 50% less active than ampicillin. Compounds **39** and **40** showed half the activity of ampicillin (MIC 25 μg/mL) against *B. subtilis*. However, these two compounds were able to produce a distinctive growth inhibitory profile against *E. coli* that was equipotent to ampicillin (MIC 6.25 μg/mL), whereas compounds **42**, **45** and **46** showed 50% less activity than ampicillin (MIC 12.5 μg/mL) against the same organism. Meanwhile, the tested *P. aeruginosa* strain showed moderate sensitivity towards most of the active compounds, particularly compound **39** (MIC 12.5 μg/mL) which was equipotent to ampicillin.

Eleven compounds **18**, **19**, **30**, **31**, **33**, **39**, **40**, **41**, **42**, **45** and **46** have displayed a significant growth inhibitory potential against *C. albicans*, among which compounds **39** and **46** (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 related to the nature of the substituents at positions C-2 and C-4, together with the nature of the rings fused with the quinoline system. In general, it could be seen that potential antibacterial activity was connected with the electron-withdrawing group ($R = 4\text{-}BrC_6H_4$) at C-4, whereas moderate activity was displayed by the electron-donating ($R = 4\text{-}CH_3OC_6H_4$) group at the same carbon. In this context, hexahydroquinoline, the key precursors **2**, **3**, **4**, **5** and **6** showed moderate antimicrobial potential, with particular effectiveness against *S. aureus* and *E. coli*. Tetrahydropyrimido[4,5-b]quinolin-4(3H)-ones **7**–**11** obtained as a result of formic acid-induced cyclization led to the complete abolishment of the activity. However, acetic anhydride-derived cyclized products introduced a methyl group at position 2. In these series, two compounds, **12** and **13,** were found to retain antimicrobial activity where the substituent R at position 4 is either 4-bromophenyl or 4-methoxyphenyl, although these two compounds are noticeably less active than the parent compounds **2** and **3**. On the other hand, phenylisothiocyanate-derived cyclization gave rise to four active compounds **18**, **19**, **21** and **22** among which the bromo-derivative **18** was the most active one. Furthermore, reaction of the key intermediates, hexaquinoline derivatives with benzoylisothiocyanate, produced a series of compounds, out of which **24** and **25** showed slightly improved antimicrobial profiles when compared with **2** and **3**. The cyclization of the thiourea derivatives, **23**–**27**, to the corresponding 2-thiones, **29**–**33**, however, did not change the biological profile significantly. Moreover, alkylation of the quinoline-2-thiones **29**–**33** resulted in an obvious enhancement of the antimicrobial activity as well as the spectrum as can be seen in **39**, **40**, **42**, **45** and **46**. It could be clearly recognized that the activity of these analogs is connected with the aryl substituent in position 4 as well as to the substituent linked to position 2. Within this series, compound **39** ($R = 4\text{-}Br\text{–}C_6H_4$) 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 compound **2**.

Conclusion

The present paper describes the synthesis of 2-amino-3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolines as well as derived fused-ring systems. Ten compounds showed remarkable cytotoxic efficiency against human colon carcinoma HT29,

hepatocellular carcinoma Hep-G2 and Caucasian breast adenocarcinoma MCF7 cell lines. Compounds **24**, **39**, **40**, **42**, **45** and **46** showed considerable broad-spectrum cytotoxic activity among which **39** and **40** proved to be the most active derivatives. A close examination of the structure of the active compounds showed that the 4-bromophenyl counterpart of the tetrahydroquinoline skeleton is the most favourable substituent when compared with other analogs. Moreover, the thioalkyl substituent at position 2 as in compounds **39**, **40**, **42** and **45** was responsible for the high activity displayed by these analogs. However, the cyclization of the intermediate quinoline derivatives (**1**–**6**) with formic acid, acetic anhydride and formamide led to complete abolishment of activity. Likewise compounds **18**, **19**, **39**, **40**, **42**, **45** and **46** were found to exhibit moderate antimicrobial activity and compounds **39**, **40** and **46** proved to be the most active candidates. Compounds **39** and **40** could be considered as possible antimicrobial and anticancer candidates that deserve further investigation and derivatization in order to explore the scope and limitation of their biological activities.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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