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Synthesis and antimicrobial activity of polyhalobenzonitrile quinazolin-4(3*H*)-one derivatives

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ABSTRACT

A novel series of polyhalobenzonitrile quinazolin-4(3*H*)-one derivatives were synthesized and characterized by NMR, IR, MS, and HRMS spectra. All of the newly prepared compounds were screened for antimicrobial activities against four strains of bacteria (Gram-positive bacterial: *Staphylococcus aureus* and *Bacillus cereus*; Gram-negative bacterial: *Escherichia coli* and *Pseudomonas aeruginosa*) and one strain of fungi (*Candida albicans*). Among the synthesized compounds, 5-(dimethylamino)-8-(2,4,5-trichloroisophthalonitrile) quinazolin-4(3*H*)-one (**7k**) exhibited significant activity towards Gram-positive bacterial, Gram-negative bacterial, and the fungi strains. The MIC (0.8–3.3 μg/mL) and MBC (2.6–7.8 μg/mL) for this compound were close to those of nofloxacin, chlorothalonil, and fluconazole, making it the most potent antimicrobial agents in the series.

Quinazoline is the most commonly encountered heterocyclic compound in medicinal chemistry due to its broad range of pharmacological activities. Many of its derivatives are used as medicines and display antimicrobial,[1] antifungal,[2] anti-HIV,[3] anti-tobacco mosaic virus (anti-TMV),[4] anticancer,[5] anti-tubercular,[6] anti-inflammatory,[7] antidepressant,[8] anticonvul-sant,[8,9] hypolipidemic,[10] analgesic,[11] antiulcer,[12] or immunotropic activities. They also act as inhibitors of protein tyrosine kinase inhibitors, thymidylate synthase,[13] and poly(ADP-ribose) polymerase (PARP).[14] As pesticides, they are used as both insecticides[15] and fungicides. Quinazolinones are an excellent reservoir of bioactive substances. The quinazolin-4(3*H*)-one structural motifs have attracted a great deal of interest due to their accessibility, diverse chemical reactivity, and wide range of previously mentioned biological uses. Several bio-active natural products, such as febrifugine and isofebrifugine,[16] contain quinazolinone moieties with potential antimalarial activity.

On the other hand, organic halide derivatives play important role in drug research. In particular, polyhalobenzonitriles have attracted much attention because of their biological activity. Some polyhalobenzonitriles derivatives are reportedly used as pesticides, for example Bromoxynil (**I**) is used as a herbicide and Chlorothalonil (**II**) is used as a fungicide.[17] Additionally, polyhalobenzonitriles may enhance the activity with other structural fragment and may result in novel biological applications. Our previous reports have explored the antimicrobial (**III**),[18] antitumor (**IV**),[19] and anti-HIV activities of polyhalo-benzonitrile substituted derivatives, and all were found to have good bioactivities (Fig. 1).

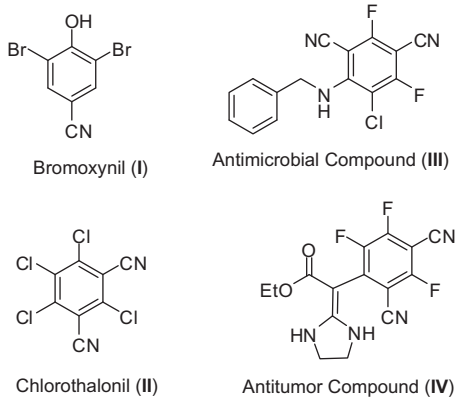

Figure 1. Biologically active polyhalobenzonitrile compounds.

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Prompted by the varied biological activities of quinazolin-4(3*H*)-one derivatives, and understanding the importance of organic halide compounds, we synthesized potential antimicrobial agents containing two biodynamic moieties: a polyhalobenzonitrile and 5-arylamine(or alkylamine)-8-amino-quinazolin-4(3*H*)-one. The newly synthesized compounds, **3m–n**, **4a–l**, **7a–l**, **8a–e**, **9** and **10**, were screened for their in vitro antibacterial and antifungal activity, and we also determined their log*P* and molar refractivity (MR). The bioactive assay showed that most of the tested compounds displayed variable inhibitory effects on the growth of the Gram-positive bacterial strain, Gram-negative bacterial strains, or fungal strain.

The route employed for synthesis of the target compounds is shown in Scheme 1. Compounds **3a–n** were obtained with a 60–80% yield by S_NAr nucleophilic substitution of alkylamine or arylamine with 5-chloro-8-nitro-quinazolin-4(3*H*)-one (**1**) in THF:DMF (3:1) at 80 °C for 12 h.[20] Then compounds **3a–l** were subjected to direct hydrogen reduction in THF to give the intermediates compounds **4a–l**. For the compounds **7a–l**, the deacid reagent promoted the S_NAr nucleophilic substitution of polyhalobenzonitrile **5** or **6** in different solvents, reaction times, and temperatures (Table 1). Finally, we concluded that the best reaction conditions for the target molecules **7a–l** were THF/DMF (7:1) as the solvent and K_2CO_3 as the deacid reagent under 75 °C for 2 h, resulting in an isolated product with a moderate yield (35–70%).

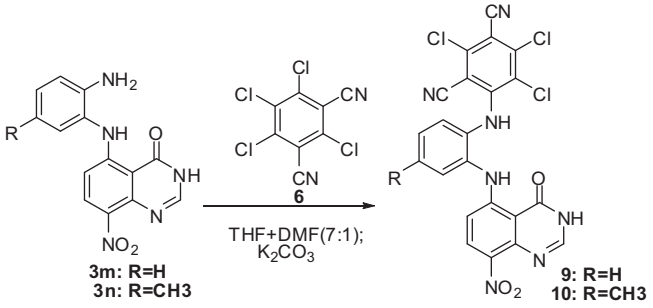
The intermediates (**3m** and **3n**) directly reacted with 2,4,5,6-tetrachloroisophthalonitrile (**6**) to give the polyhalobenzonitrile at the 5-substituted position of quinazolin-4(3*H*)-one (**9** and **10**) (Scheme 2). The structures of all newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, and HRMS studies.[21]

Of all of the compounds, **3m–n**, **4a–l**, **7a–l**, **8a–e**, **9**, and **10** were initially screened for in vitro antimicrobial activity against Gram-positive bacterial strains (*Staphyloccocus aureus* ATCC 6538 [SA] and *Bacillus cereus* ATCC 14579 [BC]), Gram-negative bacterial strains (*Escherichia coli* ATCC 8099 [EC] and *Pseudomonas aeruginosa* ATCC 15442 [PA]), and a fungalstrain (*Candida albicans* ATCC 10231 [CA]) using the disk diffusion assay.[22,23] Standard inoculums ($1–2 \times 10^7$ c.f.u/ml 0.5 Mcfarland standards) were introduced onto the surface of sterile agar plates, and a sterile glass spreader was

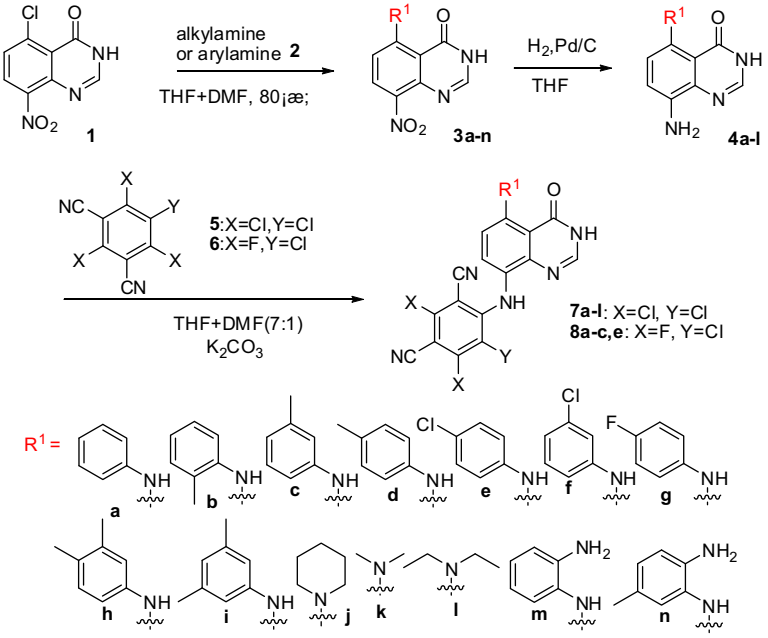
Table 1
Optimization of reaction conditions[a]

Entry	Solvent[b]	Time (h)	*T* (°C)	Alkali[c]	Yield[d] (%)
1	THF	2	25	K_2CO_3	N.R.
2	THF	2	75	K_2CO_3	N.R.
3	DMF	0.5	25	K_2CO_3	<10.0
4	DMF	0.25	75	K_2CO_3	<10.0
5	Methanol	2	25	K_2CO_3	N.R.
6	Methanol	2	75	K_2CO_3	N.R.
7	Toluene	2	25	K_2CO_3	N.R.
8	Toluene	2	75	K_2CO_3	N.R.
9	THF+DMF(1:1)	1	25	K_2CO_3	<10.0
10	THF+DMF(1:1)	1	75	K_2CO_3	<10.0
11	THF+DMF(4:1)	1	25	K_2CO_3	25.5
12	THF+DMF(4:1)	1	75	K_2CO_3	27.4
13	THF+DMF(7:1)	1	25	K_2CO_3	25.0
14	THF+DMF(7:1)	1	75	K_2CO_3	62.4
15	THF+DMF(7:1)	1	75	NaOH	<10.0
16	THF+DMF(7:1)	1	75	NaH	<10.0
17	THF+DMF(7:1)	1	75	*t*-BuOK	<10.0
18	THF+DMF(7:1)	1	75	EtONa	<10.0
19	THF+DMF(7:1)	1	75	Cs_2CO_3	47.2
20	THF+DMF(7:1)	1	75	Et_3N	N.R.

[a] 8-Amino-5-(phenylamino)quinazolin-4(3*H*)-one **4a** (1 mmol) and 2,4,5,6-tetrachloroisophthalonitrile **6** (1 mmol).
[b] The volume of solvent is 5 mL.
[c] Alkali (2 mmol).
[d] Isolated **7a** yield.



Scheme 2. Synthetic pathway of compounds **9** and **10**.



Scheme 1. Synthetic pathway of compounds **7a–l, 8a–c** and **8e**.

used for even distribution of the inoculums. The disks, which measured 6 mm in diameter, were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 121 °C for 1 h. The sterile disks previously soaked in a known concentration (1000 μg/mL) of the test compounds were placed in nutrient agar medium. Solvent and growth controls were performed, using. norfloxacin- and chlorothalonil-soaked disks as positive control, while DMSO-soaked disks were used as negative control. The plates were incubated for 24 h at 37 °C. Susceptibility was assessed on the basis of the diameter of the zone of inhibition against Gram-positive and Gram-negative strains of bacterial and the fungal strain. Inhibition zones (ZOI) were measured and compared to the controls. The bacterial and fungal zones of inhibition values are shown in Table 2.

The inhibition results revealed that the polyhalobenzonitrile substituted compounds **7a–l**, **8a–e**, **9**, and **10** showed moderate to good bacterial and fungal inhibitions, with ZOI up to 19.0 mm. Of these compounds, having polyhalobenzonitrile at the 5-substituted position (such as **7a–l, 8a–e**) or the 8-substituted position (such as **9** and 10) on quinazolin-4(3*H*)-one compounds is beneficial, as both can inhibit the growth of Gram-positive and Gram-negative bacterial strains and the fungal strain. However, non-polyhalobenzonitrile-substituted compounds **3m**, **4c–e** and **4g–k** showed no significant inhibition of bacterial growth and fungal growth for any of the strains tested. However, compounds **3n**, **4a**, **4b**, and **4f** showed some degree of inhibition for the bacterial and fungal strains. This can be explained by the introduction of a polyhalobenzonitrile at the quinazolin-4(3*H*)-one fragment, which can increase the antimicrobial activity of compounds. Noticeably, the compound **7k** (ZOI[BC] = 18.0; ZOI[SA] = 14.3; ZOI[EC] = 19.0; ZOI[PA] = 15.0; and ZOI[CA] = 15.5) had comparably better activity than the other compounds against all the bacterial and fungal strains, and exhibited similar antimicrobial activities as the standard antibiotic drugs, norfloxacin (ZOI[BC] = 18.6; ZOI[SA] = 17.0; ZOI[EC] = 20.0; ZOI[PA] = 16.8 and ZOI[CA] = 21.8) and chlorothalonil (ZOI[BC] = 15.5; ZOI[SA] = 14.0; ZOI[EC] = 18.5; ZOI[PA] = 15.8 and ZOI[CA] = 17.0).

The lipophilicity of the compounds, which is seen as an important parameter related to membrane permeation in biological system, describes the main predictor for the activity.[24] The octanol/water partition coefficient log*P*, which is a measure of hydrophobicity/lipophilicity, was calculated using ChemDraw Ultra 10.0 software integrated with Cambridgesoft Software (Cambridgesoft Corporation).[25] The results obtained are shown in Table 2. The calculated values of log*P* for polyhalobenzonitrile-substituted compounds **7a–l**, **8a–e**, **9**, and **10** were higher than that of the non-polyhalobenzonitrile substituted compounds **3m**, **4c–e**, and **4g–k**. The antimicrobial ZOI observed for polyhalobenzonitrile substituted compounds is higher than that observed for the non-polyhalobenzonitrile substituted compounds.

The molar refractivity (MR), which represents the size and polarizability of a molecule by describing its steric effects, was also calculated (using ChemDraw Ultra 10.0 software) to explain the activity of the synthesized compounds. From the data shown Table 2, it can be inferred that the higher value of molar refractivity favors the activity ratio for quinazolin-4(3*H*)-one derivatives, such as compounds **4a–l** and **7a–l**.

The minimum inhibitory concentration (MIC, μg/mL) of antibacterial activity for compounds **3n**, **4a–b**, **4f**, **7a–k**, **8a–e**, **9**, and

Table 2
Calculated log*P* and molar refractivity (MR) and antimicrobial evaluation of compounds **3m–n**, **4a–l**, **7a–l**, **8a–e**, **9** and **10** y determination of the ZOI

Compound	Log*P*	MR	Zone of inhibition (mm)				
			Gram-positive bacterial		Gram-negative bacterial		Fungal strain
			BC	SA	EC	PA	CA
3m	1.34	77.00	N	N	7.8	N	N
3n	1.80	43.81.	15.0	5.3	14.7	16.8	13.5
4a	1.52	72.77	9.5	N	N	7.0	N
4b	2.01	78.67	6.0	8.0	9.0	8.5	10.0
4c	2.01	78.67	N	N	6.5	N	N
4d	2.01	78.67	N	N	N	N	N
4e	2.08	77.38	N	N	N	N	N
4f	2.08	77.38	6.0	6.5	6.0	6.5	N
4g	1.68	73.18	6.0	N	N	6.0	N
4h	2.50	84.57	N	6.5	N	N	N
4i	2.50	84.57	6.0	N	N	6.3	N
4j	1.08	71.96	N	N	7.8	N	N
4k	0.34	60.04	N	N	8.5	N	N
4l	1.02	69.64	6.0	7.3	6.0	7.5	N
7a	5.53	121.88	11.7	10.0	11.0	11.3	10.3
7b	6.02	127.78	10.5	10.0	12.5	9.0	N
7c	6.02	127.78	11.0	9.7	10.3	10.0	10.0
7d	6.02	127.78	11.0	9.3	10.5	12.0	9.5
7e	6.09	126.49	9.0	8.8	9.0	9.5	9.5
7f	6.09	126.49	9.0	8.5	9.0	8.7	9.0
7g	5.69	122.29	18.0	11.5	9.0	11.0	10.5
7h	6.51	133.68	11.0	8.5	10.8	9.4	10.0
7i	6.51	133.68	10.3	10.0	11.5	10.0	7.0
7j	5.08	121.07	15.0	9.7	9.0	8.7	N
7k	4.35	109.15	18.0	14.3	19.0	15.0	15.5
7l	5.03	118.75	6.0	7.7	6.0	7.5	N
8a	4.73	113.48	6.0	8.9	N	9.5	6.0
8b	5.22	119.38	10.8	10.3	9.7	9.5	7.5
8c	5.71	125.28	10.0	9.8	9.3	10.0	9.5
8e	5.29	118.09	10.4	9.7	9.0	12.0	8.5
9	4.29	130.10	11.2	7.3	7.0	6.7	N
10	4.71	134.52	10.7	9.3	9.0	9.5	7.5
Chlorothalonil	4.33	55.90	15.5	14.0	18.5	15.8	17.0
Norfloxacin	1.37	86.98	18.6	17.0	20.0	16.8	21.8

N: No inhibition zone.

10 were determined using the liquid dilution method.[26,27] Chlorothalonil, norfloxacin and fluconazole were used as a standard drug for the comparison of antibacterial activity (Table 2). Test compounds and positive control drugs were prepared in dimethyl sulfoxide solution at concentrations of 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 μg/mL. Inoculums of the bacterial cultures were also prepared. Inoculums and sterile water were added to series of tubes each containing 1 mL of a test compound solution at the 11 different concentrations listed above. The tubes were incubated for 24 h and carefully observed for the presence of turbidity. The minimum concentration at which no growth was observed was taken as the MIC value (Table 3).

The results obtained for the Gram-positive bacterial and Gram-negative bacterial showed that all the test compounds exhibited weak to good level of antibacterial activity, as determined by the MIC values. Non-polyhalobenzonitrile-substituted compounds (**3n**, **4a**–**b**, and **4f**), 5-(2,4,5-trichloroisophthalonitrile)-substituted quinazolin-4(3*H*)-one (**9** and **10**), and 8-(5-chloro-2,4-difluoro-isophthalonitrile)-substituted quinazolin-4(3*H*)-one (**8a**–**e**) showed reduced antibacterial properties as compared to 8-(2,4,5-trichloro-isophthalonitrile)-substituted quinazolin-4(3*H*)-one (**7a**–**k**). This further indicates that introducing polyhalobenzonitrile substituents, especially 2,4,5-trichloroisophthalonitrile, at 8-substituted quinazolin-4(3*H*)-one can improve their antibacterial activities. For the 8-(2,4,5-trichloro-isophthalonitrile)-substituted quinazolin-4(3*H*)-one (**7a**–**k**), all of these compounds exhibited good inhibition against the Gram-positive bacterial BC and SA. It is worth noting that 5-alkylamine-substituted quinazolin-4(3*H*)-one (**7j** and **7k**) and 5-haloaniline-substituted quinazolin-4(3*H*)-one (**7f** and **7g**) showed much stronger inhibition against Gram-negative bacterial than 5-methylaniline- or 5-aniline-substituted quinazolin-4(3*H*)-one (**7a**–**d, 7h**). Three compounds, 5-(*o*-fuloroaniline)-8-(2,4,5-trichloro-isophthalonitrile) quinazo-lin-4(3*H*)-one (**7g**, MIC[BC] = 0.7 μg/mL; MIC[SA] = 0.8 μg/mL; MIC[EC] = 6.5 μg/mL; MIC[PA] = 1.7 μg/mL), 5-(piperidin-1-yl)-8-(2,4,5-trichloro-isophthalonitrile) quinazolin-4(3*H*)-one (**7j**, MIC[BC] = 0.5 μg/mL; MIC[SA] = 0.7 μg/mL; MIC[EC] = 1.0 μg/mL; MIC[PA] = 1.3 μg/mL), and 5-(dimethylamino)-8-(2,4,5-trichloro-isophthalonitrile) quinazolin-4(3*H*)-one (**7k**, MIC[BC] = 0.5 μg/mL; MIC[SA] = 0.7 μg/mL; MIC[EC] = 1.0 μg/mL; MIC[PA] = 1.3 μg/mL) were identified as having potent antibacterial activity against Gram-positive and Gram-negative bacterial strains, with the same level of antibacterial activity as the standard antibiotic chlorothalonil (MIC[BC] = 0.7 μg/mL; MIC[SA] = 1.3 μg/mL; MIC[EC] = 0.5 μg/mL; MIC[PA] = 1.7 μg/mL) and norfloxacin (MIC[BC] = 0.6 μg/mL; MIC[SA] = 1.2 μg/mL; MIC[EC] = 0.5 μg/mL; MIC[PA] = 0.7 μg/mL).

Compounds **7f**, **7g**, and **7i**–**k** were also examined for antifungal activity by determining the MIC value using the liquid dilution method.[26,27] Chlorothalonil and the antifungal drug fluconazole were used as a positive controls. All five of these test compounds were identified as the most potent antifungal agents against fungal strains, with compound **7k** actually showing more inhibitory activities than the positive control.

The minimum bactericidal concentration (MBC, μg/mL) against Gram-positive bacterial and antifungal activity against fungal strains for compounds **7f**, **7g**, and **7i**–**k** also was determined (Table 3). To determine the minimum bactericidal concentration, 0.1 mL was taken from each tube and spread on agar plates. The number of c.f.u was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed.

All five compounds (**7f**, **7g**, and **7i**–**k**) showed moderate to good antibacterial activity against Gram-positive bacterial and antifungal activity as compared to the positive controls chlorothalonil, norfloxacin, and fluconazole, respectively. Two compounds (**7g** and **7j**) exhibited antibacterial activity that was as strong as chlorothalonil and norfloxacin, but did not show as strong inhibition of fungal activity as fluconazole. Only the compound **7k** (MBC[BC] = 3.3 μg/mL; MBC[SA] = 2.6 μg/mL; MBC[CA] = 7.8 μg/mL) displayed good antifungal activity, which was greater than that observed for the positive control norfloxacin (MBC[BC] = 6.5 μg/mL; MBC[SA] = 5.2 μg/mL), and fluconazole (MBC[CA] = 15.6 μg/mL).

Table 3
Antimicrobial activity (MIC and MBC, [μg/mL]) of compounds **3n**, **4a**, **4b**, **4f**, **7a**–**k**, **8a**–**e**, **9**, **10**

Compound	Minimum inhibitory concentration (MIC) (μg/mL)					Minimum bactericidal concentration (MBC) (μg/mL)		
	Gram-positive bacterial		Gram-negative bacterial		Fungal strain	Gram-positive bacterial		Fungal strain
	BC	SA	EC	PA	CA	BC	SA	CA
3n	20.8	10.4	20.8	20.8	–	–	–	–
4a	125.0	208.0	208.3	250.0	–	–	–	–
4b	166.7	250.0	250.0	416.7	–	–	–	–
4f	N	N	N	N	–	–	–	–
7a	0.5	125.0	250.0	83.4	–	–	–	–
7b	0.5	52.1	125.0	83.4	–	–	–	–
7c	1.0	5.2	104.2	N	–	–	–	–
7d	0.7	10.4	104.2	52.1	–	–	–	–
7e	83.3	4.7	333.3	416.7	–	–	–	–
7f	0.5	0.7	7.8	20.8	1.7	41.7	31.3	31.3
7g	0.7	0.8	6.5	1.7	1.7	6.5	6.5	52.1
7h	0.8	15.6	83.3	N	–	–	–	–
7i	0.7	0.8	7.8	20.8	5.2	31.3	31.3	52.1
7j	0.5	0.7	1.0	1.3	2.6	6.5	4.6	41.7
7k	0.8	3.3	1.3	2.0	0.5	3.3	2.6	7.8
8a	500.0	N	N	500.0	–	–	–	–
8b	52.1	208.3	208.3	208.3	–	–	–	–
8c	291.7	500	500.0	416.7	–	–	–	–
8e	41.7	20.8	208.3	500.0	–	–	–	–
9	41.7	250.0	166.7	125.0	–	–	–	–
10	52.1	500.0	416.7	416.7	–	–	–	–
Chlorothalonil	0.7	1.3	0.5	1.7	0.7	1.7	2.0	7.8
Norfloxacin	0.6	1.2	0.5	0.7	–	6.5	5.2	–
Fluconazole	–	–	–	–	2.0	–	–	15.6

N: No inhibition in MIC or MBC.
–: No test.

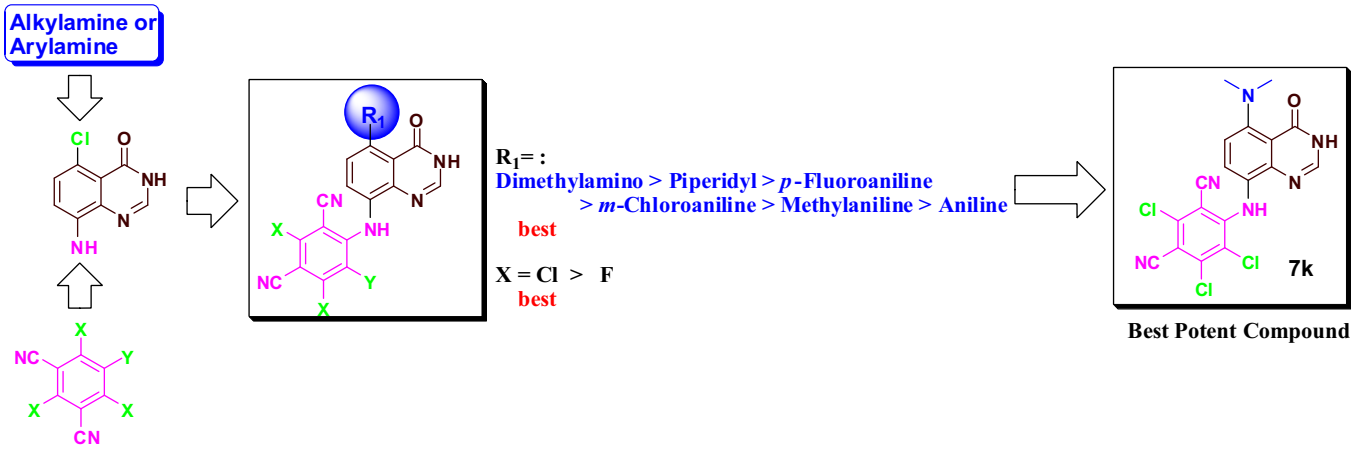


Figure 2. Structure–activity relationship of polyhalobenzonitrile quinazolin-4(3*H*)-oneReferences.

In conclusion, in this study we described an efficient method for the preparation of polyhalobenzonitrile quinazolin-4(3*H*)-one derivatives. All the compounds **7a–l**, **8a–e**, **9**, and **10** were tested for their in vitro antibacterial activity against BC, SA, EC, and PA and for their antifungal activity against *Candida albicans* (ATCC 10231). Most of the synthesized compounds exhibited weak to good activity against Gram-positive and Gram-negative bacterial, as well as the fungal strain. Compounds **7g** (MIC[BC] = 0.7 μg/mL; MIC[CA] = 1.7 μg/mL; MBC[BC] = 6.5 μg/mL; MBC[CA] = 52.1 μg/mL), **7j** (MIC[BC] = 0.5 μg/mL; MIC[CA] = 2.6 μg/mL; MBC[BC] = 6.5 μg/mL; MBC[CA] = 41.7 μg/mL), and **7k** (MIC[BC] = 0.8 μg/mL; MIC[CA] = 0.5 μg/mL; MBC[BC] = 3.3 μg/mL MBC[CA] = 7.8 μg/mL) appeared to be more effective than the other compounds (MIC = 10–500 μg/mL)against the four bacterial and one fungal strains. From a structure–activity relationship, we can conclude that polyhalobenzonitrile substitution improved the biological activity of these compounds against Gram-positive bacterial, Gram-negative bacterial, and fungal strains. The introduction of haloaniline or simple alkylamine into the 5-substituted position of quinazolin-4(3*H*)-one and 2,4,5-trichloro-isophthalonitrile into the 8-substituted position of quinazolin-4(3*H*)-one, both of which make the compound **7k** as the best poten compound for antimicrobial activity in all synthetic derivatives (Fig. 2). These biological effects will be helpful in designing more potent antimicrobial agents.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.068.

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21. *Preparation of 8-amino-5-(phenylamino)quinazolin-4(3H)-one (**4a**). Representative procedure:* In 50 mL round-bottomed flask, compound **3a** (2.8 g, 10 mmol) was dissolved with 20 mL of tetrahydrofuran (THF), then added 0.28 g (w/w) 10% Pd/C, reduced with hydrogen for 24 h. After the completion of the reaction, Pd/C was filtered, and the solvent was evaporated to give a pale yellow powder compound **4a** 2.3 g (yield 92%). *Compound **4a**:* Yellow solid. Mp: 279–281 °C; IR (KBr): 3461, 3371, 3147, 3047, 2912, 1648, 1486, 1250, 1037, 757, 570 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 4.87 (br, 2H, NH_2), 6.65 (d, J = 7.3 Hz, 2H, ArH), 6.82 (d, J = 6.7 Hz, 1H, ArH), 7.18 (t, J = 6.8 Hz, 2H, ArH), 7.26–7.30 (m, 2H, ArH), 7.80 (s, 1H, NCH=N), 9.01 (s, 1H, NH), 11.98 (br, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 115.7, 117.0, 120.0, 122.7, 123.2, 126.2, 129.0, 140.6, 141.1, 143.7, 163.4; HRMS (TOF ES$^+$): m/z calcd for $C_{14}H_{12}N_4O$ $[M+H]^+$, 253.1089; found, 253.1082. *Preparation of 2,4,5-trichloro-6-(4-oxo-5-(phenylamino)-3,4-dihydroquinazolin-8-ylamino)isophthaloni-trile (**7a**). Representative procedure:* In 50 mL round-bottomed flask, Polyhalogenated isophthalonitrile (0.26 g, 1 mmol) was dissolved with 1 mL of DMF and 7 mL of THF, then added the Potassium carbonate (0.14 g, 1 mmol) and compound **4a** (0.252 g, 1 mmol) successively. The mixted solution was stirred at 75 °C for about 1 h by TLC detected. After the completion of the reaction, 50 mL of ethyl acetate was added, and washed with 40 mL of saturated aqueous ammonium chloride solution and 40 mL of brine. The organic phase was dried over anhydrous sodium sulfate, rotary evaporated at vacuum and Silica gel column separate (Petro/AcOEt = 5:1) to get the compound **7a** (yield 60%). *Compound **7a**:* Red solid. Mp: 260–263 °C; IR (KBr): 3464, 3368, 3054, 2240, 1663, 1488, 1320, 1250, 758, 540 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 5.29 (s, 2H, NH), 6.67 (d, J = 8.0 Hz, 2H, ArH), 6.83 (t, J = 7.2 Hz, 1H, ArH), 7.20 (t, J = 7.8 Hz, 2H, ArH), 7.45 (d, J = 8.7 Hz, 1H, ArH), 7.50 (d, J = 8.6 Hz, 1H, ArH), 8.11 (s, 1H, NCH=N), 8.35 (br, 1H, NH); ^{13}C NMR(125 MHz, DMSO-d_6): δ = 112.0, 112.6, 114.8, 115.9, 116.3, 117.8, 119.8, 123.4, 124.6, 126.0, 129.0, 133.8, 139.2, 139.3, 139.6, 142.3, 143.2, 143.3, 143.7,

160.1; HRMS (TOF ES$^+$): m/z calcd for $C_{22}H_{11}C_{l3}N_6O$ [M+Na]$^+$, 502.9958; found, 502.9950.
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