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Exploration of antimicrobial and antioxidant potential of newly synthesized 2,3-disubstituted quinazoline-4(3*H*)-ones

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ABSTRACT

A series of 2-(chloromethyl)-3-(4-methyl-6-oxo-5-[(*E*)-phenyldiazenyl]-2-thioxo-5,6-dihydropyrimidine-1(2*H*)-yl)quinazoline-4(3*H*)-ones **9a–j** was synthesized by treating 2-(chloroacetyl)amino benzoic acid with 3-amino-6-methyl-5-[(*E*)-phenyldiazenyl]-2-thioxo-2,5-dihydropyrimidine-4(3*H*)-one **8a–j** and was screened for in vitro antibacterial activities against a representative panel of Gram-positive and Gram-negative bacteria. The compounds were synthesized in excellent yields and the structures were corroborated on the basis of IR, ^{1}H NMR, Mass and elemental analysis data. All the synthesized compounds elicited the potent inhibitory action against all the tested bacterial stains. Furthermore, in order to explore the antioxidant potential of newly synthesized compounds, the free radical scavenging activity measurement were performed by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay method. It is revealed from the antioxidant screening results that the compounds **9c** and **f** manifested profound antioxidant potential.

The quinazolin-4(3*H*)-one structural motifs have attracted a great deal of interest due to their ready accessibility, diverse chemical reactivity and wide gamut of biological activities like antifungal,[1] antitumour,[2] hypotensive,[3] anticancer,[4,5] antiHIV,[6] antiinflammatory,[7] antibacterial,[8] antifolate,[9] and antitumor[10] etc. Quinazoline derivatives acts as powerful inhibitors of epidermal growth factor (EGF) receptors of tyrosine kinase,[11] and some of them show remarkable activity as anticancer,[12] antiviral,[13] and antitubercular agents.[14] Quinazolinones are excellent reservoir of bioactive substances. Several bio-active natural products such as febrifugine and isofebrifugine contain quinazolinone moieties with potential antimalarial activity.

Free radicals and oxygen derivatives are constantly generated in vitro both by accident of chemistry and specific metabolic purposes.[15] The generation of free radicals during the metabolic process is now observed to be responsible for wide range of human conditions such as aging, cancer, atherosclerosis, arthritis, viral infection stroke, myocardial infraction, pulmonary condition, inflammatory bowel disease, neurogenerative disease and others may be produced by reactive oxygen species, for example, hydrogen peroxide scavenging (H_2O_2); hypochlorous acid scavenging (HOCl); hydroxyl radical scavenging (HO radical); peroxyl radical scavenging (ROO radical). The action of free radicals is counteracted by free radicals endogenous or exogenous or synthetic route. Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl, and nitric oxide radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidative agents are effective in the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer.

Nowadays, antioxidants that exhibit DPPH radical scavenging activity are increasingly receiving attention.[16] They have been reported to have interesting anticancer, anti-aging, and antiinflammatory activities. It is revealed from the literature survey that a very little attention has been put in view to explore the antioxidant activity of substituted quinazoline derivatives. In the present study, efforts have been laid down to evaluate the inherent antioxidant property of the synthesized compounds along with the exploration of their antimicrobial potential. Accordingly, syntheses of novel 2-(chloromethyl)-3-(4-methyl-6-oxo-5-[(*E*)-phenyldiazenyl]-2-thioxo-5,6-dihydropyri-midine-1(2*H*)-yl)quinazoline-4(3*H*)-ones derivatives was carried out as potential antioxidants with a view to support new drug development programme to curb various diseases. The model of scavenging of the stable DPPH radical is extensively used to evaluate radical scavenging activities in a very short span of time in comparison to other methods. Compound under investigation reacts with DPPH, which is nitrogen centered radical (Fig. 1) with a characteristic absorption at 517 nm, and convert it to stable diamagnetic molecule 1,1-diphenyl-picryl hydrazine, due to its hydrogen donating ability at a very rapid rate.[17] When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons taken up. Such a change in the absorbance produced in this reaction has been widely applied to test the

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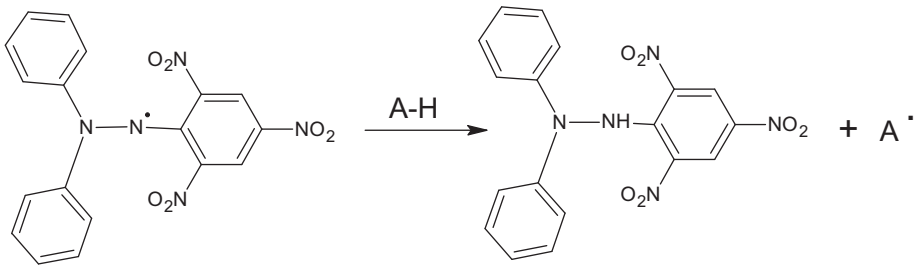
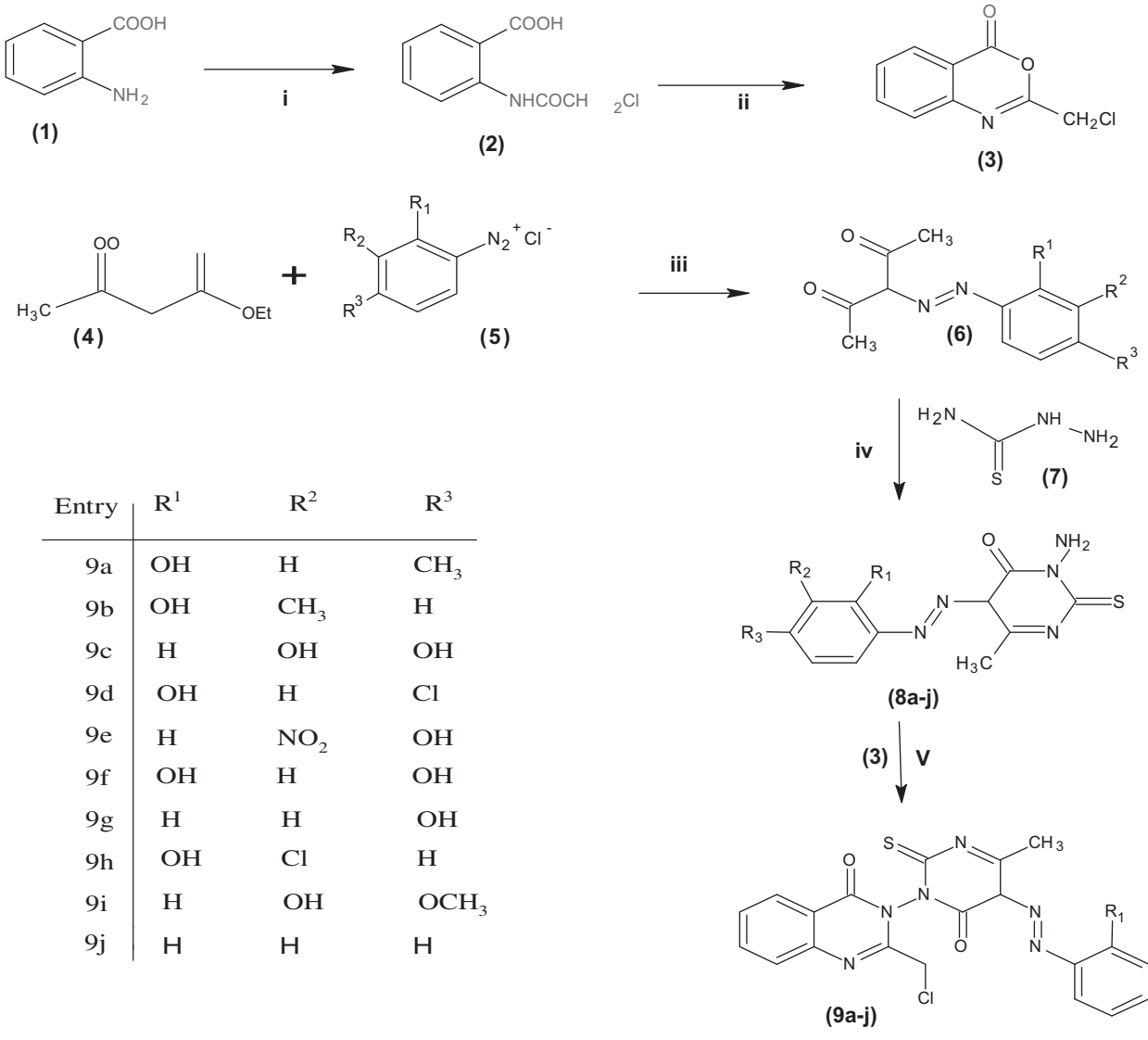


Figure 1. DPPH, chemical structure and its reaction with a scavenger, indicated by A–H.



Entry	R^1	R^2	R^3
9a	OH	H	CH_3
9b	OH	CH_3	H
9c	H	OH	OH
9d	OH	H	Cl
9e	H	NO_2	OH
9f	OH	H	OH
9g	H	H	OH
9h	OH	Cl	H
9i	H	OH	OCH_3
9j	H	H	H

Scheme 1. Synthesis of 2-(chloromethyl)-3-(4-methyl-6-oxo-5-[(*E*)-phenyldiazenyl]-2-thioxo-5,6-dihydropyrimidine-1(2*H*)-yl)quinazoline-4(3*H*)-ones (**9a–j**). Reagants and conditions (i) chloroacetyl chloride, benzene. (ii) H_2SO_4. (iii) Sodium acetate and ethanol at 0-5°C. (iv) Sodium ethoxide. (v) Dry pyridine for 6-10 h.

capacity of numerous molecules to act as free radical scavengers.[18] In this way, the scavenging effects of all the synthesized compounds on the DPPH free radical were evaluated.

Moreover, pyrimidine analogues are used as chemotherapeutic agents for the treatment of diseases resulting from different microorganisms and now there has been a major expansion in the number of pyrimidine derivatives as drugs. The design of pyrimidine analogues as antimetabolites involves changes in the substituents on the ring, isosteric replacement of rings, changes in ring size, attached sugars and phosphate residues, etc. Many useful drugs containing pyrimidine analogues with diverse biological activities have now emerged possessing antifungal, antibacterial,[19–21] anti-inflammatory and antioxidant activities. Thus keeping in view the biological activities displayed by pyrimidine system, it was our endeavor to develop efficacious antimicrobial vis-à-vis antioxidant agents containing pyrimidine ring, coupled to quinazoline

system. Moreover, it was considered worthy of interest to investigate the influence of substituents as the structural variants on the anticipated biological activities.

Buoyed from these findings and in view of the fact that quinazolinones and pyrimidine derivatives possess useful antimicrobial and antioxidant activities, we designed and synthesized a number of 2-(chloromethyl)-3-(4-methyl-6-oxo-5-[(*E*)-phenyldiazenyl]-2-thioxo-5,6-dihydropyrimidine-1(2*H*)-yl)quinazoline-4(3*H*)-ones and evalu ated their antimicrobial and antioxidant potentials.

Anthranilic acid **1** was reacted with chloroacetyl chloride to obtain 2-[(chloroacetyl)amino]benzoic acid[22] **2** in the presence of benzene at room temperature. Compound **2** on treatment with sulphuric acid has been cyclized[23] to yield 2-chloromethyl benzo[*d*][1,3]oxazin-4-one **3**. The synthesis of 3-amino-6-methyl-5-[(*E*)-phenyldiazenyl]-2-thioxo-2,5-dihydropyrimidine-4(3*H*)-one **8** was performed by reaction of ethyl 3-oxo-2-[(*E*)phenyldiazenyl]butanoate **6** with thiosemicarbazide in presence of freshly prepared sodium ethoxide as per the route depicted in Scheme 1. The final step of this route involves condensation between **3** and **8** in dry pyridine for 6–10 h to afford the product **9**. The probable mechanism of the reaction involves the ring opening of 2-chloromethyl benzo[*d*][1,3]oxazin-4-one **3** by the attack of 3-amino-6-methyl-5-[(*E*)-phenyldiazenyl]-2-thioxo-2,5-dihydropyrimidine-4(3*H*)-one **8**. The ring-opening step is followed by the ring closure after the removal of water molecule so as to give the title compound **9**.

All the synthesized compounds were characterized by their physical, analytical and spectral data. In general, the IR spectrum of compound **8** exhibited NH_2 stretching absorption bands at 3427 cm^{-1} (NH_{asym}) and 3252 cm^{-1} (NH_{sym}), whereas the C=S absorption band is visible at 1075 cm^{-1}. Compounds **9a–j** showed disappearance of these bands in their IR spectrum owing to the conversion of free amino group into cyclic nitrogen. Halogen containing compounds **9c** and **d** exhibit characteristic bands at 550–650 cm^{-1} (C–X stretching). The EI-MS and ^1H NMR spectral data of all the synthesized compounds are in confirmation to the structural assignment.

All the synthesized compounds to be examined for antibacterial activity[24] were evaluated in vitro against an assortment of two Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and two Gram-negative bacteria *Pseudomonas aeruginosa and Escherichia coli*. Screening results are summarized in Table 1. The newly generated compounds **9a–j** has exerted significant inhibitory activity against the growth of tested bacterial strains. The data also revealed that derivatization of the parent molecule had produced the marked enhancement in the potency of the synthesized analogues as antibacterial agents. The data presented in Table 1 indicated that the substitution in phenyl ring exerted significant influence on the antibacterial profile. Interestingly, compounds **9a**, **b**, **i**, with methoxy and methyl-substituted ring are found to be more active molecules in the respective series compared to the compounds bearing other electron donating or with drawing groups (**9c**, **d**, **e**, **f**). In general, *meta*-derivatives are more active than the corresponding *para*-substituted derivatives. It has also been observed that the Gram positive bacteria are more susceptible towards the newly synthesized series of quinazoline-4(3*H*)-ones **9a–j**. This may be due to the absence of a unique outer membrane of peptidoglycan in Gram-positive bacteria and hence, the wall of Gram-positive bacteria is permeable to these derivatives. Generally, the Gram-positive bacteria are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier[25] whereas the Gram-negative bacteria possess an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to drug constituents.[26] Percent inhibition (% inhibition) of antibacterial activity for different derivatives over control drug, was calculated using following formula:

$$\%\ \text{inhibition} = (\alpha - \beta)/\alpha \times 100$$

α and β stands for zone of inhibition of control drug and synthesized compounds, respectively.

Further, upon close inspection of results depicted in Figure 2 pertaining to the in vitro screening of synthesized compounds, it has been inferred that all these compounds gives promising results compared to the reference drug ampicillin against the tested bacterial strains. The negative% inhibition values of **9a**, **b**, **i** for some bacterial strains are suggestive of the better activity profile of these derivatives as compared to the standard drug.

Antioxidant assay[27] is based on the measurements of the scavenging ability of compounds towards the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The disappearance of this commercially available radical is measured spectrophotometrically at 517 nm in a methanolic solution. The antioxidant activity was expressed as the 50% inhibitory concentration (IC_{50}) based on the amount of compound required for a 50% decrease of the initial DPPH radical concentration.

As presented in Table 2, the quinazoline-4(3*H*)-ones (**9a–j**) with IC_{50} values in the range of 1.4–8.6 μM showed higher radical-scavenging activities in comparison to ascorbic acid which was attributed to presence of hydroxyl aryl moiety of quinazo-

Table 1
Antibacterial screening data for 2-(chloromethyl)-3-(4-methyl-6-oxo-5-[(*E*)-phenyldiazenyl]-2-thioxo-5,6-dihydropyrimidine-1(2*H*)-yl)quinazoline-4(3*H*)-ones (**9a–j**)

Compound no.	R^1	R^1	R^1	Zone of inhibition (mm)			
				Gram-positive		Gram-negative	
				BS[a]	SA[b]	PA[c]	EC[d]
9a	OH	H	CH_3	33.0	39.0	22.0	25.0
9b	OH	CH_3	H	36.5	44.5	23.5	27.5
9c	H	OH	OH	27.0	31.5	16.0	17.5
9d	OH	H	Cl	29.0	33.0	18.5	20.5
9e	H	NO_2	OH	20.0	29.0	12.0	14.0
9f	OH	H	OH	24.5	30.0	13.5	15.5
9g	H	H	OH	18.5	27.0	10.0	13.5
9h	OH	Cl	H	31.5	33.5	21.5	20.9
9i	H	OH	OCH_3	42.1	47.5	28.0	30.0
9j	H	H	H	16.2	24.5	7.0	11.5
Ampicillin	—	—	—	40.0	36.0	20.0	28.0

[a] BS: *Bacillus subtilis.*
[b] SA: *Staphylococcus aureus.*
[c] PA: *Pseudomonas aeruginosa.*
[d] EC: *Escherichia coli.*

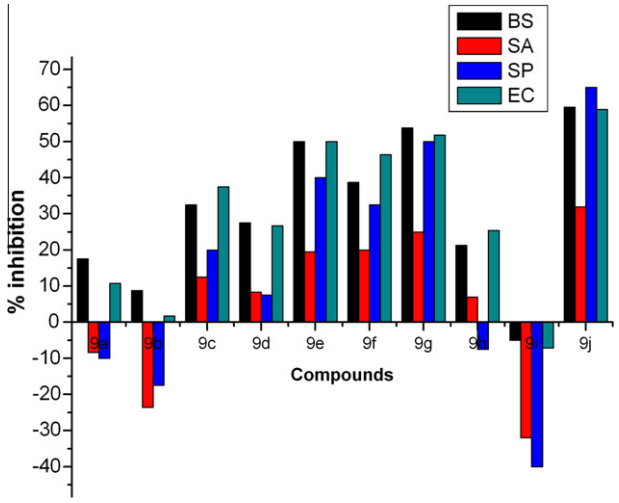
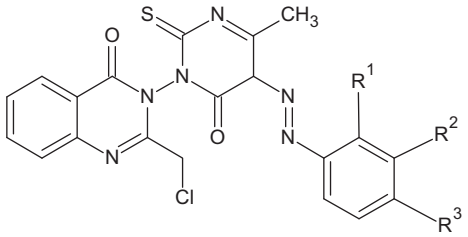


Figure 2. Antibacterial activity for compounds **9a–j** over control drug.

Table 2
Inhibition of DPPH radical by synthesized compound



Compound	[a]IC_{50}	Compound	[a]IC_{50}
9a	5.1	**9f**	2.1
9b	3.5	**9g**	5.4
9c	1.4	**9h**	8.3
9d	7.0	**9i**	3.0
9e	8.6	**9j**	NA
Ascorbic acid	9.2	—	—

NA–not available.
[a] IC_{50} values were determined by linear regression analysis using at least five different concentrations in triplicate.

line-4(3*H*)-ones (that can donate hydrogen atoms) and governs the main factor behind their ability to be scavenged by DPPH. After donating a hydrogen atom, compounds exist in its radical form, and the electron conjugation effect in the structure stabilizes the radical which favors the reaction to occur (Fig. 3).

The difference in activity amongst compounds **9a–j** was due to the difference in the stability of the oxygen centered radical formed in these compounds. Amongst them, compounds **9c** and **f** with two hydroxy substituents showed highest hydrogen donor ability to DPPH radical (IC_{50} values were 1.4 and 2.1 μM, respectively). Since the corresponding IC_{50} values for all synthesized compounds after 60 min were lower than after 30 min, DPPH antiradical scavenging activity was also time-dependent (Fig. 4). The radical-scavenging activity of the tested samples, expressed as percentage inhibition of DPPH, was calculated according to the formula:

$$\text{IC}\ (\%) = [(A_0 - A_t) \setminus A_0] \times 100$$

where, A_t is the absorbance value of the tested sample and A_0 is the absorbance value of blank sample, at a particular time. Percentage

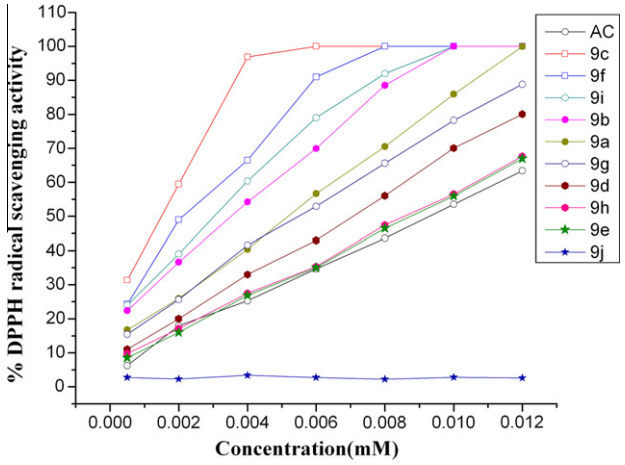


Figure 3. Graphical representation of % DPPH radical scavenging activity of compounds **9a–j** as a function of concentration.

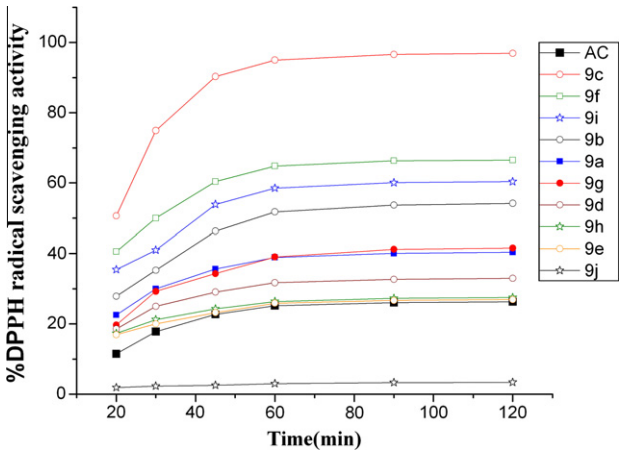


Figure 4. Graphical representation of % DPPH radical scavenging activity of compounds **9a–j** as a function of time.

inhibition after 20, 30, 45, 60, 90, and 120 min was plotted against concentration, and the equation for the line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity.

The entire synthesized compounds scavenged DPPH radical significantly in a concentration-dependent manner. Their comparable scavenging activities were expressed in IC_{50} (concentration required for 50% inhibition of 60 μM DPPH concentration) value. In compound **9c** both the hydroxy groups are *ortho* to each other, therefore, radical ion resulted from the abstraction of either H atom (*ortho*/*meta* hydroxy group with respect to azo group) by DPPH would stabilize by other hydroxy group. Moreover, the radical ion resulted from the abstraction of *para* hydroxyl group would also be stabilized by azo group, *para* to it through extended conjugation. While in compound **9f** both the hydroxy groups are *meta* to each other, therefore the radical ion resulted from the abstraction of H atom would not be stabilized from either hydroxy group. Thus, compound **9c** shows better scavenging effect as compare to compound **9f**. Compound **9i** is less active compared to **9f** due to the presence of single hydroxy group but, also it shows better antioxidant activity compared to **9b** due to presence of methoxy group (i.e., the presence of the electron donating OCH_3 group at the *ortho* position enhanced the stabilization of the resulting oxygen centered radical as the number of conjugating structure is more than that without the OCH_3 group) which is absent in the later case. Compound **9a** shows lesser activity compared to **9b** since it has methyl and hydroxy *meta* to each other (because *meta* positions do not participate in resonance stabilization). Compound **9g** is less active compared to **9a** because of absence of any electron releasing group which otherwise stabilize the radical ion. But it shows higher activity compared to compound **9d** and **h** due to the presence of electron withdrawing chloro groups in them which would destabilize the ring. Also compound **9g** provides better stability of the nitrogen centered radical by extended conjugation resulting in *para* quinonoid structure which has more stabilization than the *ortho* quinonoid structure formed for compound **9d** or **h**. The difference in activity among the compounds **9d** and **h** is due to the different positions of chloro group. Compound **9h** shows lesser radical scavenging activity as it has chloro group *ortho* to hydroxy group, that is, a strong electron with drawing group is situated on *ortho* position which destabilize the radical ion while in compound **9d**, chloro group is situated at *meta* position which therefore neither stabilize nor destabilize the ring, thus latter shows more antioxidant activity compared to former. Similarly, compound **9e** shows lowest activity due to the presence of strong electron withdrawing effect (which would destabilize the ring).

Table 3
Calculated physico-chemical parameters of synthesized quinazoline-4(3*H*)-ones using HyperChem software

Compounds	Heat of formation (kcal/mol)	Hydration energy of molecule (kcal/mol)	Dipole (Debyes)
9a	−117666.43	−13.65	3.99
9b	−117664.86	−11.54	4.32
9c	−120986.71	−23.67	4.10
9d	−121164.63	−31.15	4.88
9e	−131799.40	−31.11	5.72
9f	−120992.19	−18.42	5.00
9g	−114214.4	−17.39	5.05
9h	−121071.49	−12.48	7.40
9i	−124425.14	−18.12	2.78
9j	−106594.35	−9.55	5.14

The physico-chemical parameters, including heat of formation (ΔH_f), dipole, and hydration of molecule were calculated using HyperChem molecular modeling software. Some physico-chemical parameters of the quinazoline-4(3*H*)-ones under investigation were calculated using the HyperChem molecular modeling software (Table 3). A QSAR[28] model could be derived to calculate the redox potential (i.e., a direct measure of the antioxidant property) of phenolic compounds using calculated parameters such as the heat of formation (ΔH_f) of phenoxyl radicals and their corresponding parent phenols. A combination of ΔH_f and other calculated parameters were found to be quite satisfactory for predicting the antioxidant activities, or redox potentials of new phenolic antioxidants. However, a model to predict the antioxidant activity from the data on quinazoline-4(3*H*)-ones and its derivatives is presently being developed.

In conclusion, the synthesized new compounds **9a–j** has exerted significant action on the growth of both Gram-positive and Gram-negative bacteria, which render them as potential antimicrobial agents. Compounds **9c** and **f** are very good antioxidants due to the presence of two hydroxyl groups in them and could be rendered for future antimitotic screening. Hence in view to cater the needs associated with ever increasing demand of newer antibacterial and antioxidant agents, exploration of these findings can envisage these compounds as powerful antibacterial and antioxidative agents.

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Supplementary data

Supplementary data (^{1}H NMR, IR, mass, and elemental analysis) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.031.

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