Med Chem Res
DOI 10.1007/s00044-014-0938-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antibacterial and free radical scavenging potential of synthesized 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-ones

Pawan Kumar Sharma · Prabal Bandyopadhyay · Pratibha Sharma · Ashok Kumar

Received: 22 July 2013 / Accepted: 3 February 2014

Abstract A series of 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one derivatives (**6a**–**m**) was synthesized in quantitative yields and their structures were corroborated on the basis of FT-IR, ^{1}H, ^{13}C NMR, ESI–MS, and elemental analysis data. The synthesized compounds were screened for in vitro antibacterial activities against a representative panel of Gram-positive and Gram-negative bacteria, as well as to evaluate their antioxidant potential using DPPH assay method. Bio-evaluation studies revealed that compounds **6c**, **6d**, **6e**, **6j**, and **6l** exhibited moderate to good antibacterial activity against all the tested bacterial strains. Furthermore, from the antioxidant screening results, it has been observed that compounds **6c**, **6e,** and **6g** manifested profound antioxidant potential (IC_{50} <7.68 μM) in comparison to the standard antioxidant ascorbic acid.

Keywords Flavone · Antibacterial activity · Antioxidant activity · DPPH radical scavenging method

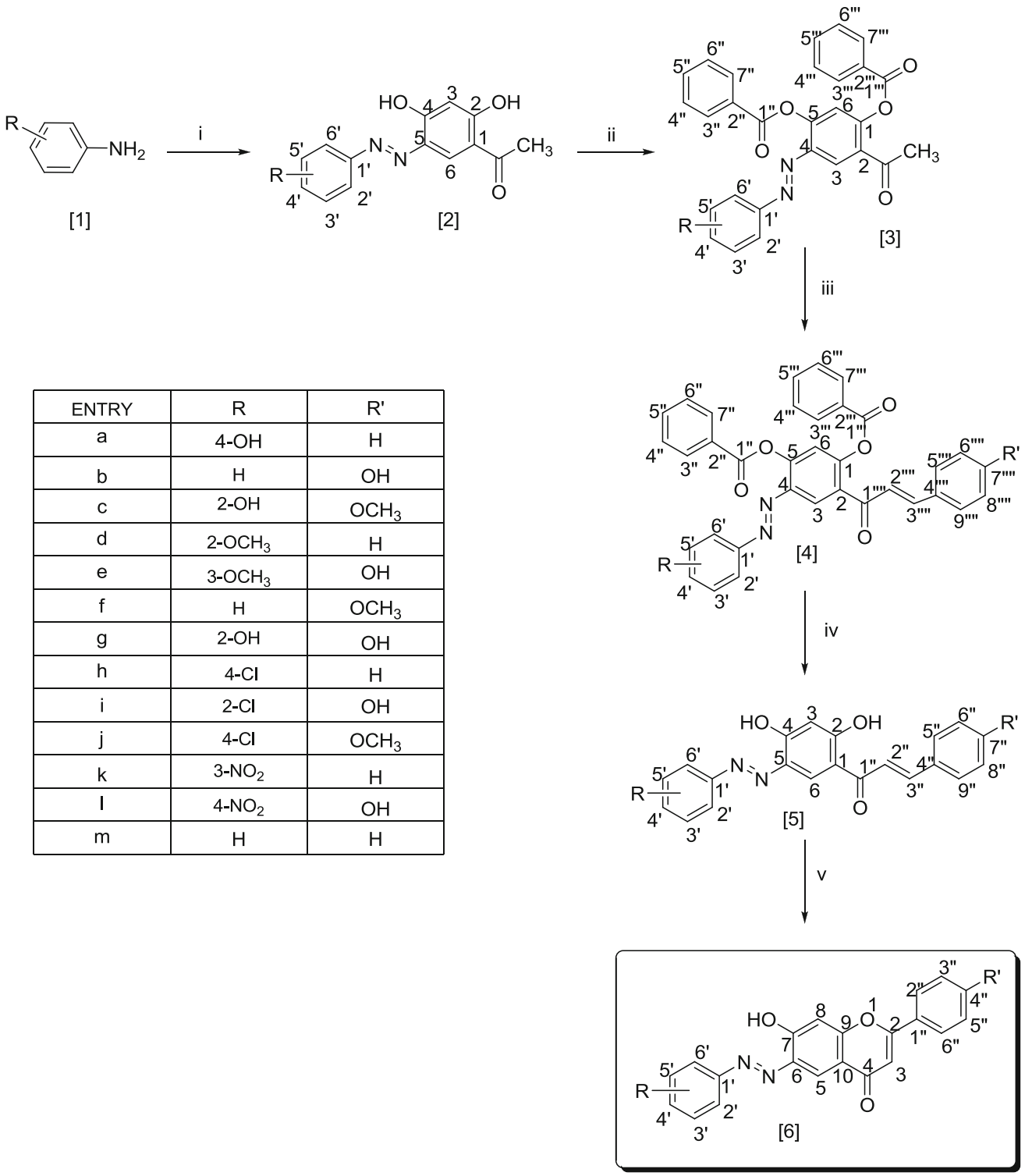
Introduction

Antioxidants have gained a lot of importance due to their potential to act as prophylactic and therapeutic agents in many diseases and also to provide major defense against radical mediated toxicity by protecting the damages caused by free radicals (Valko *et al.*, 2006). The generation of free radicals which is observed during the metabolic process is responsible for wide range of human conditions such as viral infection, myocardial infarction, and neurogenerative diseases. Some free radicals and reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, hydroxyl, and nitric oxide radicals have been found to be responsible for diabetes, neurodegenerative disorders, aging, cancer causing ailments, cardiovascular, autoimmune, and inflammatory diseases (Beckman and Ames, 1998; Halliwell and Gutteridge, 1989). Therefore, minimization of oxidative damage may be an important approach to the primary prevention or treatment of these diseases. In this regard, the role of antioxidants is very well reported in the literature because they may stop the free-radical formation, or interrupt an oxidizing chain reaction (Block, 1992; Rice-Evans *et al.*, 1996). Whence, the development of synthetic compounds, capable of scavenging free radicals, has been a great success in the recent past. Now-a-days, antioxidants that exhibit DPPH radical scavenging activity are increasingly receiving attention (Hossain and Bhattacharya, 2007). It is revealed from the literature survey that flavonoids, being of natural origin (Silva *et al.*, 2000; Teixeira *et al.*, 2005; Siquet *et al.*, 2006) and owing to their inherent antioxidative properties, have been considered as attractive potential antioxidants.

Flavones are privileged structural units that are encountered in a wide range of biologically active and medicinally significant compounds. Recent medicinal chemistry applications of flavones derivatives have been found to be associated with antioxidant (Kang *et al.*, 2008; Manthey *et al.*, 2001), anti-inflammatory (Laupattarakasem *et al.*, 2004), antibacterial (Xu and Lee, 2001; Hamiltonmiller, 1995; Iinuma *et al.*, 1994), antitumor (Han, 1997; Birt *et al.*, 2001), anticancer (Kohen *et al.*, 2007; Blank *et al.*, 2004), antifungal (Li *et al.*, 2002), cardiovascular (Qin *et al.*, 2008; Wang *et al.*, 1996; Tzeng *et al.*, 1997; Liou *et al.*, 1994), anti-osteoporotic (Delcanale *et al.*, 2001; Wang *et al.*, 2005) effect and they are also effective

P. K. Sharma · P. Bandyopadhyay · P. Sharma · A. Kumar (✉)
School of Chemical Sciences, Devi Ahilya University, Takshashila Campus, Khandwa Road, Indore 452001, MP, India
e-mail: drashoksharma2001@yahoo.com

Published online: 16 February 2014



ENTRY	R	R'
a	4-OH	H
b	H	OH
c	2-OH	OCH_3
d	2-OCH_3	H
e	3-OCH_3	OH
f	H	OCH_3
g	2-OH	OH
h	4-Cl	H
i	2-Cl	OH
j	4-Cl	OCH_3
k	3-NO_2	H
l	4-NO_2	OH
m	H	H

Scheme 1 Synthetic pathway for 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one derivatives (**6a–m**). Reagents and conditions: (*i*) $NaNO_2$/HCl, 0–5 °C, 1-(2,4-dihydroxyphenyl)ethanone, 140–150 °C; (*ii*) C_6H_5COCl, NaOH, stirring for 30 min; (*iii*) aromatic aldehyde, KOH/EtOH, Room temp. for 72 h, neutralized with 1:1 cold HCl; (*iv*) 15 % HCl/H_2O reflux, 30 min; and (*v*) NBS (10 mol %)/CH_3CN, Reflux, 20–30 min

in the prevention and treatment of complex diseases such as atherosclerosis, stroke, diabetes, and Alzheimer's disease. Flavones are polyphenolic compounds belonging to the flavonoid group that occur naturally in fruits,

vegetables, nuts, seeds, flowers, and barks (Malikov and Yuldashev, 2002; Huck *et al.*, 2000; Nagao *et al.*, 2002). They are an integral part of the human diet and also the core structure of flavones is also found in several natural products (Awuah and Capretta, 2009; Valla *at el.*, 2008). Development of efficient methods for the construction of the flavones structural framework has thus been an important challenge in organic synthesis. Although the skeleton of phenyl benzopyran-4-one pharmacophore contributed an important role in biological effects of flavonoid derivatives, however the peripheral substituents also elicits their significant role in imparting enhanced antioxidative potential of pharmacophoric entity. It is evident from a number of studies that their antioxidant activity is strongly dependent on their structural features and intrinsically related to the presence of hydroxyl function(s) in the aromatic core structure. A number of methods are available for preparing flavones, chromones, and their analogs (Banerji and Goomer, 1980; Khanna *et al.*, 1992; Looker and Hanneman, 1962; Baker, 1933), including the Allan and Robinson (1924) synthesis. The most common method, however, involves the Baker–Venkataraman rearrangement, wherein an *ortho*-hydroxy acetophenone is benzoylated and the ester is treated with base (pyridine/KOH) to effect an acyl group migration, forming a 1,3-diketone (Mahal and Venkataraman, 1934; Jain *et al.*, 1982; Saxena *et al.*, 1985; Hirao *et al.*, 1984). The ensuing diketone is then cyclized under strong acidic conditions using acetic acid and sulfuric acid to furnish flavones.

Buoyed from these findings and in continuation of our studies on bioactive compounds (Sharma *et al.*, 2012a, b; Sahu *et al.*, 2013; Bandyopadhyay *et al.*, 2011), herein, we have reported the synthesis of a series of 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one derivatives (**6a**–**m**) (Scheme 1) followed by the evaluation of their antibacterial vis-à-vis free radical scavenging potential. Owing to the presence of azo functionality in these compounds, they can be used as chemotherapeutic agents for the treatment of diseases caused by different microorganisms.

Chemistry

A series of these compounds was synthesized containing aryldiazenyl entity, coupled to flavone system. Moreover, it was considered worthy of interest to investigate the influence of substituents as the structural variants on the anticipated biological activities. As per the synthetic pathway depicted in Scheme 1, substituted aniline 1 was diazotized and coupled with 1-(2,4-dihydroxyphenyl)ethanone (prepared by the acylation of resorcinol) to obtain 1-[2,4-dihydroxy-5-(aryldiazenyl)phenyl]ethanone **2**. Compound **2** was then benzoylated to yield 2-acetyl-5-(benzoyloxy)-4-(aryldiazenyl)phenyl benzoate **3**. Further, reaction has been accomplished in ethanolic solution of aromatic aldehyde to obtain 5-(benzoyloxy)-4-(aryldiazenyl)-2-(3-phenylprop-2-enoyl)phenyl benzoate **4**. It was then treated with dilute solution of hydrochloric acid to hydrolyze the ester group in order to yield the compound 1-{2,4-dihydroxy-5-(aryldiazenyl)phenyl}-3-phenylprop-2-en-1-one **5**. The final step of this strategy involves oxidative cyclization of compound using *N*-bromo succinimide (NBS) as catalyst in acetonitrile to give the desired flavones 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one **6** in appreciable yield. It is evident from Table 1 that, the proposed synthetic procedure works well with different substituents. As shown in synthetic pathway (Scheme 1), a variety of substituted anilines and aromatic aldehydes bearing electron-donating and electron-withdrawing substituents were successfully employed to prepare the corresponding flavone derivatives in quantitative yields (66–84 %).

Results and discussion

Spectroscopic characterizations of compounds

All the synthesized compounds were characterized on the basis of their physical, analytical, and spectral data. In general, the FT-IR spectra of compounds (**6a**–**m**) have given confirmation about the vibrational modes of synthesized compounds. The ^{1}H and ^{13}C NMR spectra of all the compounds displayed pertinent signals in their due region. The mass spectra (ESI–MS) of the compounds showed $(M+H)^{+}$ peaks, in agreement with their molecular weight. Elemental analysis results for C, H, and N elements were satisfactory within ± 0.4 % calculated values of the compounds.

In vitro antibacterial activity

All the synthesized compounds were evaluated in vitro against an assortment of three Gram-positive bacteria *Staphylococcus aureus* MTCC 3160, *Bacillus cereus* MTCC 430, and *Arthobacter globiformis* MTCC 4299 and three Gram-negative bacteria *Vibrio cholerae* MTCC 3904, *Escherichia coli* MTCC 1610, and *Shigella dysenteriae* NICED. The antibacterial activity (zone of growth inhibition) of the tested 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one derivatives (**6a–m**) in comparison with that of control drugs ampicillin and ciprofloxacin was determined by Kirby–Bauer disk-diffusion method on Mueller–Hinton agar according to the guidelines of the Clinical Laboratory Standards Institute, 2007, USA (Table 2).

Table 1 List of synthesized 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one derivatives (**6a–m**)

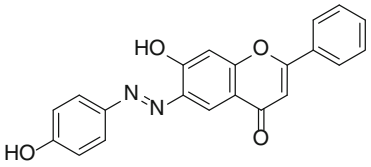
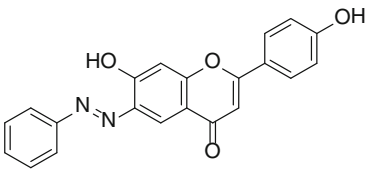
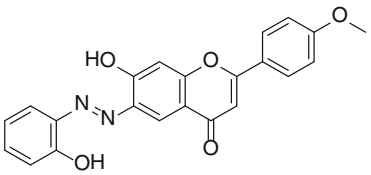
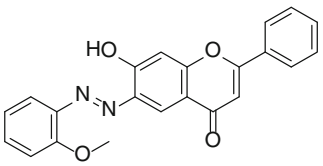
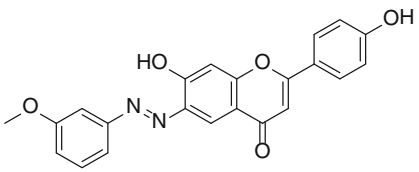
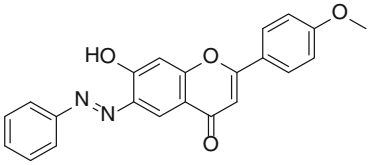
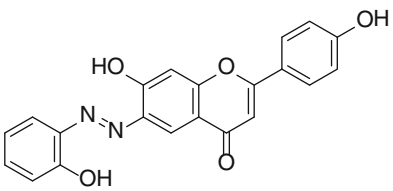
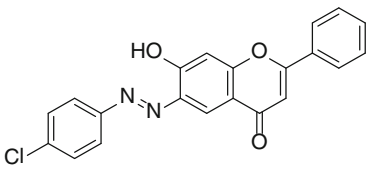
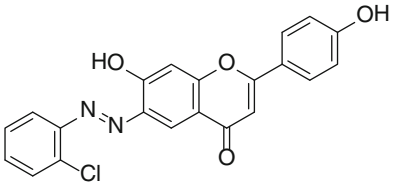
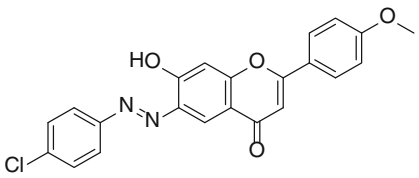
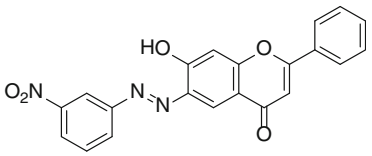
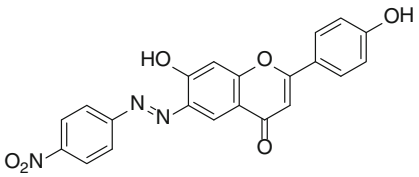
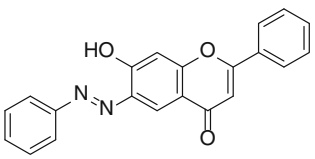
Entry	R	R′	Product (**6**)	Yield (%)[a]
6a	4-OH	H		68
6b	H	OH		70
6c	2-OH	OCH_3		67
6d	2-OCH_3	H		76
6e	3-OCH_3	OH		75
6f	H	OCH_3		73
6g	2-OH	OH		66
6h	4-Cl	H		79

Table 1 continued

Entry	R	R′	Product (**6**)	Yield (%)[a]
6i	2-Cl	OH		74
6j	4-Cl	OCH_3		77
6k	3-NO_2	H		84
6l	4-NO_2	OH		81
6m	H	H		78

[a] Isolated yield

Among all the tested compounds, most of the compounds exhibited moderate to excellent activity. The data also revealed that derivatization of the parent molecule had produced the marked enhancement in the potency of the synthesized analogs as antibacterial agents.

From the data presented in Table 2, it is inferred that the substitution in aryl ring exerted significant influence on the antibacterial profile of the synthesized flavones. Interestingly, compounds **6j** (substituted by *para*-chloro and *para*-methoxy groups) and **6e** (substituted by *meta*-methoxy and *para*-hydroxyl groups) are found to be more active molecules of the series compared to the compounds bearing other electron-donating or withdrawing groups. These compounds (**6j** and **6e**) showed better activity profile (zone of growth inhibition 39.55 and 35.41 mm) against *B. cereus* MTCC 430 as compared to the standard drugs ampicillin (26.0 mm) and ciprofloxacin (28.0 mm). Compound **6c** substituted by *ortho*-hydroxy and *para*-methoxy groups and compound **6l** bearing *para*-nitro group along with *para*-hydroxyl group exhibited excellent efficacy (zone of growth inhibition 33.47, 34.38, 33.25 and 32.38, 33.24, and 32.01 mm) against tested *S. aureus* MTCC 3160, *B. cereus* MTCC 430, and *A. globiformis* MTCC 4299, respectively. The zone of inhibition for compound **6g** (24.85 and 25.97 mm for *S. aureus* MTCC 3160, *B. cereus* MTCC 430) was nearly equal to the control drug ampicillin (25.0 and 26.0 mm), whereas it exhibited slightly lesser activity for *A. globiformis* (20.45 mm) compared to standard drug ampicillin. Interestingly, in case of *S. aureus* MTCC 3160, *B. cereus* MTCC 430, and *A. globiformis* MTCC 4299, a number of compounds (**6c**, **6d**, **6e**, **6f**, **6h**, **6i**, **6j**, and **6l**) displayed inhibitory activity (26.92–39.55 mm) better than that of ampicillin (25.0–29.0 mm) while compounds **6c, 6d, 6e, 6j,** and **6l** have shown better activity than standard drug ciprofloxacin (28.0–31.0 mm). It is evident from Table 2 that compound **6h** displayed effective activity

Table 2 Antibacterial screening data for 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one derivatives (**6a–m**)

Compounds	R	R′	Zone of Inhibition (mm)					
			Gram-positive			Gram-negative		
			SA[b]	BC[c]	AG[d]	EC[e]	VC[f]	SD[g]
6a	4-OH	H	20.90 ± 0.20	–	23.15 ± 0.12	20.13 ± 0.02	19.24 ± 0.17	20.01 ± 0.06
6b	H	OH	21.52 ± 0.28	–	22.05 ± 0.27	20.50 ± 0.16	19.83 ± 0.20	19.97 ± 0.11
6c	2-OH	OCH_3	33.47 ± 0.08	34.38 ± 0.19	33.25 ± 0.13	32.54 ± 0.11	31.79 ± 0.06	32.0 ± 0.21
6d	2-OCH_3	H	30.61 ± 0.36	31.62 ± 0.27	30.16 ± 0.08	29.82 ± 0.05	28.60 ± 0.13	27.67 ± 0.18
6e	3-OCH_3	OH	34.36 ± 0.22	35.41 ± 0.09	34.11 ± 0.14	33.23 ± 0.21	32.17 ± 0.42	33.13 ± 0.24
6f	H	OCH_3	31.20 ± 0.06	32.15 ± 0.46	30.87 ± 0.42	30.36 ± 0.52	–	28.18 ± 0.35
6g	2-OH	OH	24.85 ± 0.18	25.97 ± 0.12	20.35 ± 0.11	–	23.0 ± 0.07	–
6h	4-Cl	H	26.92 ± 0.24	27.82 ± 0.23	24.15 ± 0.04	–	–	23.02 ± 0.34
6i	2-Cl	OH	29.45 ± 0.46	30.20 ± 0.04	–	28.57 ± 0.40	27.72 ± 0.16	–
6j	4-Cl	OCH_3	38.76 ± 0.48	39.55 ± 0.14	37.14 ± 0.22	38.0 ± 0.29	36.96 ± 0.26	37.32 ± 0.18
6k	3-NO_2	H	–	29.91 ± 0.07	–	28.32 ± 0.14	–	32.47 ± 0.23
6l	4-NO_2	OH	32.38 ± 0.35	33.24 ± 0.26	32.01 ± 0.03	31.48 ± 0.22	30.68 ± 0.39	29.09 ± 0.42
6m	H	H	18.0 ± 0.14	19.58 ± 0.23	16.21 ± 0.47	15.34 ± 0.16	13.25 ± 0.31	14.02 ± 0.25
Ampicillin[a]	–	–	25.0 ± 0.07	26.0 ± 0.10	29.0 ± 0.15	37.0 ± 0.13	32.0 ± 0.18	35.0 ± 0.05
Ciprofloxacin[a]	–	–	30.0 ± 0.13	28.0 ± 0.04	31.0 ± 0.09	33.0 ± 0.11	30.0 ± 0.16	32.0 ± 0.14

The tests were repeated in triplicate and the average values along with standard deviation (± SD) were taken to show the inhibitory activity of the synthesized flavone derivatives against the selected sets of bacteria

[a] Antibacterial activity of the synthesized compounds was compared with the standard antibacterial drugs Ampicillin and Ciprofloxacin

[b] SA: *Staphylococcus aureus* MTCC 3160

[c] BC: *Bacillus cereus* MTCC 430

[d] AG: *Arthobacter globiformis* MTCC 4299

[e] EC: *Escherichia coli* MTCC 1610

[f] VC: *Vibrio cholerae* MTCC 3904

[g] SD: *Shigella dysenteriae* NICED

profile (zone of growth inhibition up to 24.15–27.82 mm) against all the three Gram-positive bacteria. Likewise, compound **6k** (substituted by *meta*-nitro group) exhibited antibacterial potency against Gram-negative *E. coli* MTCC 1610 and *S. dysenteriae* NICED and Gram-positive *B. cereus* MTCC 430 with zone of growth inhibition 28.32, 32.47, and 29.91 mm, respectively compared to standard drugs ampicillin (37.0, 35.0, and 26.0 mm) and ciprofloxacin (33.0, 32.0, and 28.0 mm). Compounds **6e** and **6j** demonstrated better activity profile (32.17–36.96 mm) against *V. cholerae* MTCC 3904 as compared to the standard drugs ampicillin (32.0 mm) and ciprofloxacin (30.0 mm). Furthermore, compound **6j** displayed higher inhibitory activity (38 mm) against *E. coli* MTCC 1610 over to the ampicillin (37 mm) and ciprofloxacin (33.0 mm). Compound **6m** exhibited minimum activity profile (zone of growth inhibition 18.0, 19.58, 16.21, 13.25, 14.02, and 15.34 mm) compared to standard drugs ampicillin (25.0, 26.0, 29.0, 32.0, 35.0, and 37.0 mm) and ciprofloxacin (30.0, 28.0, 31.0, 30.0, 32.0, and 33.0 mm) against all Gram-positive bacteria *S. aureus* MTCC 3160, *B. cereus* MTCC 430, and *A. globiformis* MTCC 4299 and Gram-negative bacteria *V. cholerae* MTCC 3904, *S. dysenteriae* NICED, and *E. coli* MTCC 1610, respectively. Compound **6f** displayed better activity (31.20, 32.15, and 30.87 mm) than both standard drugs ampicillin and ciprofloxacin against Gram-positive bacteria *S. aureus* MTCC 3160, *B. cereus* MTCC 430, and *A. globiformis* MTCC 4299. It is also evident from the Table 2 that, compounds **6c**, **6d**, **6e**, **6j**, and **6l** exhibited moderate to good antibacterial activity against all the Gram-positive as well as Gram-negative bacteria.

Further, upon close inspection of results depicted in Table 2, it has also been observed that Gram-positive bacteria were more susceptible toward the newly synthesized series of compounds (**6a–m**) as compared to the Gram-negative bacteria. This may be due to the absence of a unique outer membrane 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 (Scherrer and

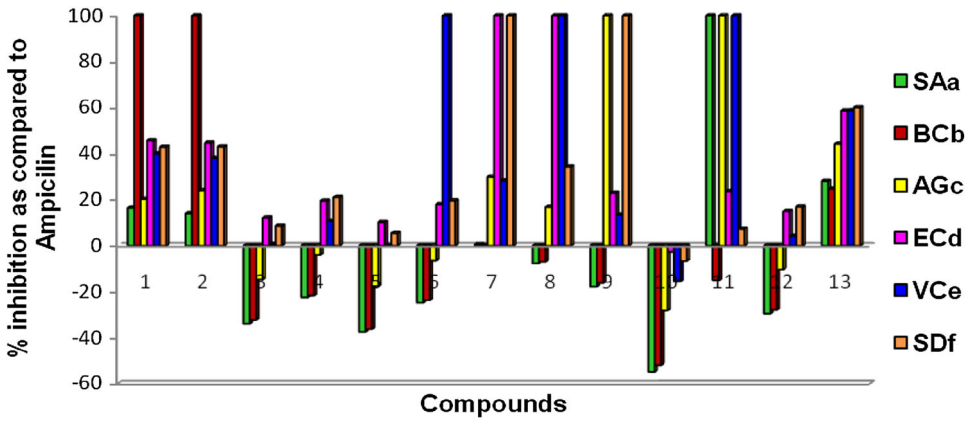


Fig. 1 Percent inhibition (% inhibition) of antibacterial activity for different derivatives of 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one (**6a–m**) as compared to the standard drug Ampicillin. [a]SA: *Staphylococcus aureus* MTCC 3160; [b]BC: *Bacillus cereus* MTCC 430; [c]AG: *Arthobacter globiformis* MTCC 4299; [d]EC: *Escherichia coli* MTCC 1610; [e]VC: *Vibrio cholerae* MTCC 3904; [f]SD: *Shigella dysenteriae* NICED

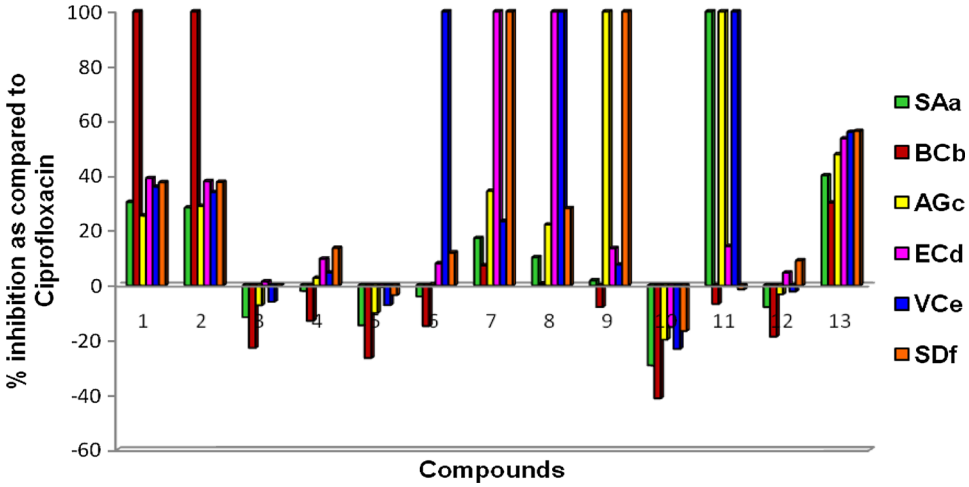


Fig. 2 Percent inhibition (% inhibition) of antibacterial activity for different derivatives of 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one (**6a–m**) as compared to the standard drug Ciprofloxacin. [a]SA: *Staphylococcus aureus* MTCC 3160; [b]BC: *Bacillus cereus* MTCC 430; [c]AG: *Arthobacter globiformis* MTCC 4299; [d]EC: *Escherichia coli* MTCC 1610; [e]VC: *Vibrio cholerae* MTCC 3904; [f]SD: *Shigella dysenteriae* NICED

Gerhardt, 1971), whereas the Gram-negative bacteria possess an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to drug constituents (Betoni *et al.*, 2006). Percent inhibition (% inhibition) of antibacterial activity for different derivatives over control drugs was calculated using following formula:

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

where α and β stands for zone of inhibition of control drugs and synthesized compounds, respectively. The results for the antibacterial activity of the selected synthesized compounds against *S. aureus* MTCC 3160, *B. cereus* MTCC 430, *A. globiformis* MTCC 4299, *V. cholerae* MTCC 3904, *E. coli* MTCC 1610, and *S. dysenteriae* NICED are graphically represented in Figs. 1 and 2.

Further, it is revealed from the results depicted in Figs. 1 and 2 that most of the synthesized compounds gave promising results compared to the reference drugs ampicillin and ciprofloxacin against the tested bacterial strains. The negative % inhibition values of **6c**, **6d**, **6e**, **6f**, **6j**, and **6l** for various bacterial strains are suggestive of the better activity profile of these derivatives as compared to the standard drugs, whereas, the % inhibition +100 indicates that there was no antibacterial activity. The potencies for antibacterial activity were observed in order of **6j** > **6e** > **6c** > **6l** > **6d** > **6f** > **6i** > **6k** > **6h** > **6g** > **6b** > **6a** > **6m**.

In vitro antioxidant activity

In order to explore the antioxidant potential of newly synthesized compounds, the free radical scavenging activity measurements were performed using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay method (Mensor *et al.*, 2001; Blois, 1958; Bondet *et al.*, 1997). various methods are available for the determination of free radical scavenging activity, but the assay employing the stable DPPH has received the maximum attention owing to its accuracy and precision. DPPH is a stable free radical which has an

unpaired valence electron present on nitrogen bridge. Because of this odd electron, DPPH gives a strong absorption band at $\lambda = 517$ nm in visible spectroscopy (deep violet color). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. Thus, antioxidant molecule can quench DPPH free radical (i.e., by providing hydrogen atoms or by electron-donating) and converting them to a colorless/bleached product (i.e., 1,1-diphenyl-2-picrylhydrazine), thereby resulted in a decrease in absorbance. 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. The values of IC_{50}, the effective concentration at which 50 % of the radicals were scavenged, were calculated to evaluate the antioxidant activities. A lower IC_{50} value indicated greater antioxidant activity. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of DPPH, was calculated according to the formula:

$$\% \text{ DPPH radical scavenging activity} = [(A_o - A_t)/A_o] \times 100,$$

where A_t is the absorbance value of the tested compound and A_o is the absorbance value of blank solution without compound, at a particular time. Percentage inhibition after 15, 30, 45, 60, 75, 90, 105, and 120 min was plotted against concentration, to obtain the IC_{50} value. The entire synthesized compounds scavenged DPPH radical (DPPH•) significantly in a concentration-dependent manner. We determined the DPPH radical scavenging activity of all the synthesized compounds (**6a**–**m**), and common antioxidant ascorbic acid was used as the standard for comparing their antioxidant potential. The order of absorbance was good in methanolic solution for all the compounds (**6a**–**m**). Higher absorbance in methanolic solution of DPPH implies better sensitivity. We used a DPPH concentration of 100 μM, in accordance with the requirement of the accuracy of spectrophotometric measurements. The absorbance of DPPH without any additions was stable over 15 min. The DPPH• absorbance decreases due to hydrogen transfer from the antioxidant, thus forming the DPPH-H stable compound. Methanolic DPPH• solutions used at different concentrations were stable (checked over a 120 min period). A significant decrease in the concentration of DPPH• radical due to the scavenging ability of some of the antioxidants was observed.

The stock solution (150 μM) of ascorbic acid and synthesized compounds was diluted to final concentrations of 1, 2, 4, 8, 12, 16, 20, 24, and 30 μM in methanol. Methanolic DPPH solution (1 mL, 100 μM) was added to 2.0 mL of compound and ascorbic acid (AA) solutions of different concentrations taken in separate test tubes and allowed to react at room temperature. After 15 min, the absorbance values were measured at 517 nm and converted into the percentage antioxidant activity. The experiments were done in triplicate. The inhibitory concentration (IC_{50}) value, representing the concentration required to exhibit 50 % antioxidant activity, was extrapolated from the graph plotted with percentage DPPH radical scavenging activity on the y-axis and concentration on the x-axis (Fig. 3). Radical-scavenging activity increases with increasing the concentration of solutions while the absorbance decreases. DPPH scavenging activity of phenolics is positively correlated with the number of hydroxyl groups. Compound **6g** with three hydroxyl groups has lower IC_{50} value as compared to other compounds and ascorbic acid also.

The radical-scavenging activity of synthesized compounds and ascorbic acid was increased with the increase of time (Fig. 4) and the absorbance continued to decrease till a period of 120 min of observation. For the sake of uniformity, a time interval of 15 min was taken for synthesized compounds and ascorbic acid radical scavenging capacity measurements.

It can be seen from Table 3 that, in DPPH assay, compounds **6c**, **6e,** and **6g** displayed better radical-scavenging activities as compared to the commercially available standard antioxidant viz., ascorbic acid.

The structural features of synthesized flavones revealed that as the number of hydroxyl groups increases, the derivatives have a tendency to show better scavenging effects. Thus, compound **6g** exhibited highest radical-scavenging activity as compared to other synthesized compounds due to the presence of three hydroxy groups with minimum IC_{50} value i.e., 1.76 μM. Compounds **6c** and **6e** with IC_{50} values 5.60 and 4.64 μM, respectively, demonstrated better antioxidant profile compared to ascorbic acid (IC_{50} value 7.68 μM) as both these compounds possessed two hydroxy groups which preferred the radical-scavenging activity. Moreover, these compounds also showed better antioxidant activity compared to **6a** and **6b** due to presence of methoxy group (i.e., the presence of the electron-donating OCH_3 group enhanced the stabilization of the resulting nitrogen-centered radical as the number of conjugating structure is more than that without the OCH_3 group) which is absent in the latter cases (compounds **6a** and **6b**). Compounds **6a** and **6b** displayed effective DPPH radical-scavenging activity with IC_{50} values 11.03 and 10.0 μM, respectively. Compounds **6i** and **6l,** which have more than one hydroxy groups, have IC_{50} values 16.66 and 18.32 μM. Also, compound **6l** has stronger electron-withdrawing *para*-nitro group with respect to aryldiazenyl ring which destabilizes the ring with

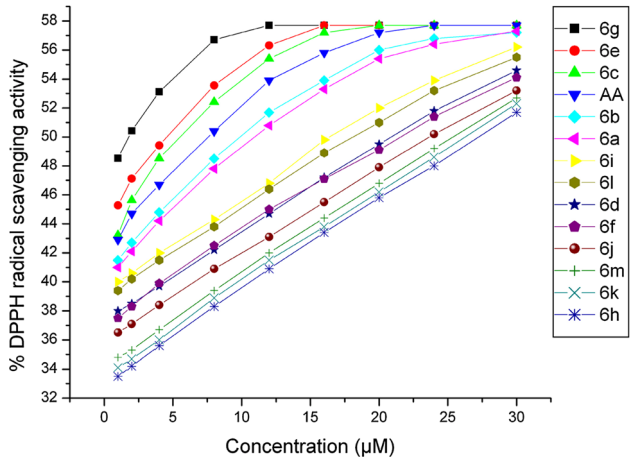


Fig. 3 Graphical representation of percentage (%) DPPH radical scavenging activity for 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one derivatives (**6a**–**m**) and ascorbic acid (AA) at different concentration (μM) after 60 min

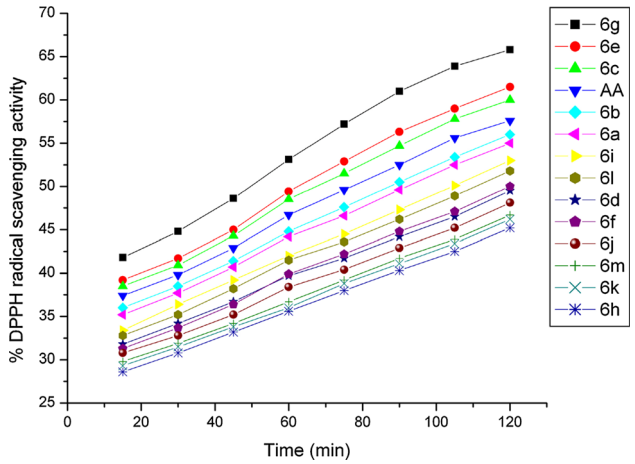
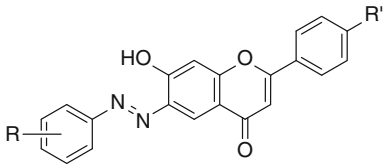


Fig. 4 Graphical representation of percentage (%) DPPH radical scavenging activity for 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one derivatives (**6a**–**m**) and ascorbic acid (AA) at different time interval (minutes) at a concentration of 4 μM

interruption to give more favorable condition for scavenging effect, while in compound **6i**, comparatively less electron-withdrawing *ortho*-chloro group is present. IC_{50} of **6d** and **6f** was found to be 21 and 21.68 μM, respectively, which exhibited lower radical-scavenging activity. Both of these compounds were substituted by methoxy group which preferred radical-scavenging activity, but only single hydroxyl group was present in these compounds. Compound **6j** substituted by chloro and methoxy groups and unsubstituted compound **6m** displayed very low-antioxidant activity-profile with IC_{50} of 23.75 and 25.52 μM, whereas compounds **6h** and **6k** showed minimum

Table 3 Inhibition of DPPH radical by synthesized compounds (**6a**–**m**)



Compound	[a]IC_{50} (μM)	Compound	[a]IC_{50} (μM)
6a	11.03	**6g**	1.76
6b	10.00	**6h**	27.74
6c	5.60	**6i**	16.66
6d	21.00	**6j**	23.75
6e	4.64	**6k**	26.77
6f	21.68	**6l**	18.32
Ascorbic acid[b]	7.68	**6m**	25.52

[a] IC_{50} values were determined by linear regression analysis using at least five different concentrations in triplicate

[b] Antioxidant potential of the synthesized flavones was compared with the standard antioxidant ascorbic acid

scavenging effect with IC_{50} of 27.74 and 26.77 μM, respectively, in this assay. Compound **6k** having nitro group at *meta* position with respect to the aryldiazenyl ring, while in compound **6h** *para*-chloro group was present.

Therefore, in view of the aforementioned findings, the order of antioxidant potencies was found to be **6g** > **6e** > **6c** > **6b** > **6a** > **6i** > **6l** > **6d** > **6f** > **6j** > **6m** > **6k** > **6h**. All the synthesized compounds (**6a**–**m**) with IC_{50} values in the range of 1.76–27.74 μM showed moderate to good radical-scavenging activities in comparison to ascorbic acid as standard which was attributed to the presence of different substituted functional groups attached to aryl diazenyl ring and aryl ring of synthesized flavone derivatives (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. In essence, hydroxyl group substitution in synthesized flavones derivatives, as well as azo functionality attached to the aryl ring of flavonoid structure, appeared to be major contributors to the DPPH radical scavenging activities.

Conclusion

Keeping in view the antibiotic resistance to Gram-positive/Gram-negative pathogens as a problem of major thrust in hospitals, a number of substituted flavones derivatives

(**6a**–**m**) were synthesized as potential antibacterial agents. Conclusively, the antibacterial results based on disk-diffusion method showed that compounds **6c, 6d, 6e, 6j**, and **6l** exhibited moderate to good antibacterial activity against all the tested bacterial strains. The compound **6j** has shown enhanced inhibitory activity compared with standard antibacterial drugs ampicillin and ciprofloxacin against all the Gram-positive and Gram-negative bacteria. It is concluded from the results that substituents on the flavones skeleton are responsible for the enhancement of the antibacterial activity. It has been further deduced that when a methoxy group was present in flavonoid ring, it enhances the activity against *S. aureus MTCC 3160, B. cereus MTCC 430, A. globiformis MTCC 4299, V. cholerae MTCC 3904, E. coli MTCC 1610S. dysenteriae NICED*, and *S. dysenteriae NICED*. These antibacterial results indicated that structural factors, such as type of substitution in the ring and water solubility of the target compounds, could further enhance their inhibitory activities.

Furthermore, evaluation of antioxidant potentials of these compounds suggested that compound **6g** has been found to be a very good antioxidant due to the presence of three hydroxyl groups in its structural armamentarium. The results obtained from this study highlighted that three substituted flavone derivatives (**6c**, **6e**, and **6g**) exhibited potent antioxidant activities (IC_{50} <7.68 μM) as compared to standard antioxidant ascorbic acid, which could be the new addition in list of synthetic antioxidants. 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.

Experimental

General

Reagents were obtained from commercial supplier and used without further purification. Melting points were determined with open capillary tube on a Gallenkamp (variable heater) melting point apparatus and were uncorrected. FT-IR spectra were recorded as KBr pellets on a Bruker Tensor 27 spectrometer with Opus 5.5 software. The ^{1}H and ^{13}C NMR spectra of the synthesized compounds were recorded at 400 and 100 MHz, respectively, using Bruker AVANCE 400 MHz NMR spectrometer in DMSO-d_6 and $CDCl_3$ solvents. The chemical shifts were expressed in δ relative to TMS as internal standard and coupling constants (*J*) in Hz. Spin multiplicities are described as s (singlet), t (triplet), q (quartet), and m (multiplet). Mass analysis was performed on quadruple-time of flight (Q-Tof) mass spectrometer (Micromass, USA) using electrospray ionization (ESI) in positive mode. Elemental analyses (C, H, and N) were carried out on a Vario Micro Cube Elemental Analyzer. TLC is performed using precoated aluminium sheets with silica gel 60 F_{254}. Column chromatography was performed on Merck silica gel 60 (100–200 mesh).

General procedure for the synthesis of 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-ones (**6a**–**m**)

Pertinent aniline derivatives (0.036 M) **1** were diazotized and allowed to couple with 1-(2,4-dihydroxyphenyl)ethanone (prepared by the acylation of resorcinol) at 0–5 °C under stirring to obtain 1-[2,4-dihydroxy-5-(aryldiazenyl)phenyl]ethanone **2**. Compound **2** (0.023 M, 6.56 g) was treated using 10 % sodium hydroxide (10 mL) and benzoyl chloride (0.023 M, 3.8 mL) under mechanical stirring and resulted in the formation of benzoylated product 2-acetyl-5-(benzoyloxy)-4-(aryldiazenyl)phenyl benzoate **3**. Further reaction has been accomplished in ethanolic solution of aromatic aldehyde to obtain 5-(benzoyloxy)-4-(aryldiazenyl)-2-(3-phenylprop-2-enoyl)phenyl benzoate **4**. Synthesized compound **4** (0.007 M, 3.45 g) was refluxed with 15 % HCl (30 mL) solution for half an hour to hydrolyze the ester groups. The contents were poured into ice-cold water and extracted with ethyl acetate (30 mL). The ester layer was dried over anhydrous $MgSO_4$ and the solvent was stripped off by distillation to obtained 1-[2,4-dihydroxy-5-(aryldiazenyl)phenyl]-3-phenylprop-2-en-1-one **5**. The final step of this strategy involves oxidative cyclization of compound **5** using *N*-bromo succinimide (NBS) (10 mol %) as catalyst in acetonitrile solvent to give the desired compound 7-hydroxy-2-aryl-6-aryldiazenyl-4*H*-chromen-4-one **6** in appreciable yield.

Spectral characterization data of representative intermediates (**2**, **3**, **4**, and **5m**) and of all newly synthesized substituted flavones derivatives (**6a**–**m**) are given below:

*1-{2,4-Dihydroxy-5-[(E)-2-phenyldiazen-1-yl]phenyl}ethan-1-one (**2m**)*

Yellowish brown solid (EtOH) (this compound was prepared by coupling of diazotized aniline (0.05 M, 7.02 mL) with 1-(2,4-dihydroxyphenyl)ethanone (0.05 M, 7.60 g) (prepared by the acylation of resorcinol) at 0–5 °C under stirring. After separation and purification of the residue of the reaction, the product **2m** was obtained as a yellowish brown solid); Yield: 78 %; m.p. 116–118 °C; ^{1}H NMR

(DMSO-d_6, 400 MHz): $\delta = 2.64$ (s, 3H, CH_3), 6.89 (s, 1H, H-3), 7.16–7.20 (t, 1H, $J = 7.4$ Hz, H-4′), 7.37–7.39 (d, 2H, $J = 7.4$ Hz, H-2′, H-6′), 7.40–7.44 (t, 2H, $J = 7.4$ Hz, H-3′, H-5′), 7.91 (s, 1H, H-6), 9.86 (s, bs, 1H, OH), 9.95 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 29.2$ (CH_3, $COCH_3$), 102.8 (CH, C-3), 113.6 (C, C-1), 116.1 (C, C-5), 122.3 (CH, C-2′, C-6′), 123.5 (CH, C-6), 128.4 (CH, C-3′, C-5′), 130.0 (CH, C-4′), 151.7 (C, C-1′), 157.2 (C, C-4), 163.6 (C, C-2), 198.4 (CO, $COCH_3$); ESI–MS for $C_{14}H_{12}N_2O_3$: 257 $[M+H]^+$.

*2-Acetyl-5-(benzoyloxy)-4-[(E)-2-phenyldiazen-1-yl]phenyl benzoate (**3m**)*

Reddish brown solid (compound **2m** (0.023 M, 5.89 g) was treated with alkaline 10 % NaOH (10 mL) and benzoyl chloride (0.023 M, 3.41 mL) under mechanical stirring for half an hour in an ice bath. The reaction mixture is then poured in ice-cold water. The solid thus generated was filtered, dried, and recrystallized from methanol and resulted in the formation of benzoylated product **3m** as a dark reddish brown solid); Yield: 64 %; m.p. 122–126 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.62$ (s, 3H, CH_3), 7.07–7.11 (t, 1H, $J = 7.1$ Hz, H-4′), 7.14 (s, 1H, H-6), 7.17–7.19 (d, 2H, $J = 7.1$ Hz, H-2′, H-6′), 7.33–7.37 (t, 2H, $J = 7.4$ Hz, H-5″, H-5‴), 7.39–7.41 (d, 4H, $J = 7.4$ Hz, H-3″, H-7″, H-3‴, H-7‴), 7.46–7.50 (t, 2H, $J = 7.1$ Hz, H-3′, H-5′), 7.85–7.89 (t, 4H, $J = 7.4$ Hz, H-4″, H-6″, H-4‴, H-6‴), 7.92 (s, 1H, H-3); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 28.7$ (CH_3, $COCH_3$), 114.2 (CH, C-6), 121.4 (CH, C-4″, C-4‴), 122.6 (CH, C-3), 123.5 (CH, C-2′, C-6′), 126.4 (C, C-2), 127.7 (C, C-4), 130.3 (CH, C-3″, C-3‴), 133.0 (CH, C-5″, C-5‴), 139.1 (C, C-5), 151.8 (C, C-1′), 154.2 (C, C-1), 164.4 (C, C-1″, C-1‴), 198.6 (CO, $COCH_3$); ESI–MS for $C_{28}H_{20}N_2O_5$: 465 $[M+H]^+$.

*5-(Benzoyloxy)-4-[(E)-2-phenyldiazen-1-yl]-2-[(2E)-3-phenylprop-2-enoyl]phenyl benzoate (**4m**)*

Dark brown solid (EtOH) (a mixture of synthesized compound **3m** (0.0078 M, 3.63 g) and benzaldehyde (0.0078 M, 0.89 mL) in 5 % potassium hydroxide in ethanol (21 mL) was kept at room temperature for 72 h. This was neutralized with 1:1 cold HCl in cold conditions and extracted with ether. The ethereal layer was dried over anhydrous Na_2SO_4 and evaporation of the solvent gave solid product. The obtained solid product was recrystallized from methanol to give compound **4m** as a dark brown solid.); Yield: 71 %; m.p. 121–122 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.88$–6.90 (t, 1H, $J = 7.8$ Hz, H-4′), 6.90–6.94 (d, br, 1H, $J = 16.4$ Hz, H − 2‴), 6.97–7.01 (t, 1H, $J = 7.9$ Hz, H − 7‴), 7.06–7.10 (t, 2H, $J = 7.9$ Hz, H − 6‴, H − 8‴), 7.13–7.15 (d, 2H, $J = 7.9$ Hz, H − 5‴, H − 9‴), 7.16 (s, 1H, H-6), 7.18–7.20 (d, 2H, $J = 7.8$ Hz, H-2′, H-6′), 7.32–7.36 (t, 2H, $J = 7.8$ Hz, H-3′, H-5′), 7.38–7.42 (t, 4H, $J = 7.4$ Hz, H-4″, H-6″, H-4‴, H-6‴), 7.45–7.49 (t, 2H, $J = 7.4$ Hz, H-5″, H-5‴), 7.56–7.60 (d, br, 1H, $J = 16.4$ Hz, H − 3‴), 7.78–7.80 (d, 4H, $J = 7.7$ Hz, H-3″, H-7″, H-3‴, H-7‴), 7.94 (s, 1H, H-3); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 114.3$ (CH, C-6), 120.5 (CH, C − 2‴), 122.0 (CH, C-2′, C-6′), 124.5 (CH, C-3), 125.7 (C, C-2), 127.6 (CH, C − 7‴), 128.1 (C, C-4), 129.3 (CH, C-3′), 130.8 (CH, C-4′), 133.6 (CH, C-5″, C-5‴), 134.2 (C, C − 4‴), 140.7 (C, C-5), 144.1 (CH, C − 3‴), 151.9 (C, C-1′), 155.3 (C, C-1), 164.6 (C, C-1″, C-1‴), 188.1 (C, C − 1‴); ESI–MS for $C_{35}H_{24}N_2O_5$: 553 $[M+H]^+$.

*(2E)-1-{2,4-dihydroxy-5-[(E)-2-phenyldiazen-1-yl]phenyl}-3-phenylprop-2-en-1-one (**5m**)*

Reddish brown solid (synthesized compound **4m** (0.0046 M, 2.54 g) was refluxed with 15 % HCl (30 mL) solution for half an hour to hydrolyze the ester group. The contents were poured in ice-cold water and extracted with ethyl acetate (30 mL). The ester layer was dried over anhydrous $MgSO_4$ and the solvent was stripped off by distillation. The obtained solid product was recrystallized from methanol to give compound **5m** as a reddish brown solid.); Yield: 74 %; m.p. 126–128 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.83$ (s, 1H, H-3), 6.89–6.91 (t, 2H, $J = 8.1$ Hz, H-6″, H-8″), 6.91–6.95 (d, br, 1H, $J = 16.4$ Hz, H-2″), 7.11–7.15 (t, 1H, $J = 8.1$ Hz, H-7″), 7.15–7.19 (t, 1H, $J = 7.5$ Hz, H-4′), 7.22–7.24 (d, 2H, $J = 7.5$ Hz, H-2′, H-6′), 7.38–7.40 (d, 2H, $J = 8.1$ Hz, H-5″, H-9″), 7.41–7.45 (t, 2H, $J = 7.5$ Hz, H-2′,H-6′), 7.56-7.60 (d, br, 1H, $J = 16.4$ Hz, H-3″), 7.97 (s, 1H, H-6), 9.89 (s, bs, 1H, OH), 10.03 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 103.7$ (CH, C-3), 114.6 (C, C-1), 117.3 (C, C-5), 121.4 (CH, C-2″), 122.6 (CH, C-2′, C-6′), 124.8 (CH, C-6), 125.4 (CH, C-7″), 127.0 (CH, C-5″, C-9″), 128.6 (CH, C-6″, C-8″), 130.8 (CH, C-4′), 134.9 (C, C-4″), 144.2 (CH, C-3″), 151.7 (C, C-1′), 158.4 (C, C-4), 164.5 (C, C-2), 188.3 (C, C-1″); ESI–MS for $C_{21}H_{16}N_2O_3$: 345 $[M+H]^+$.

*7-Hydroxy-6-[(E)-2-(4-hydroxyphenyl)diazen-1-yl]-2-phenyl-4H-chromen-4-one (**6a**)*

Reddish brown solid (this compound was prepared by oxidative cyclization of synthesized compound (2*E*)-1-{2,4-dihydroxy-5-[(*E*)-2-(4-hydroxyphenyl)diazen-1-yl]phenyl}-3-phenylprop-2-en-1-one **5a** (0.0054 M, 1.88 g) using *N*-bromo succinimide (NBS) (10 mol %) as a catalyst in acetonitrile solvent. The mixture of compounds was

refluxed for about 20–30 min, and then after cooling, it was poured into ice-cold water. The precipitated solid was collected and recrystallized from methanol was done to achieve the desired compound 7-hydroxy-6-[(*E*)-2-(4-hydroxyphenyl)diazen-1-yl]-2-phenyl-4*H*-chromen-4-one **6a** as a dark reddish brown solid); Yield: 68 %; m.p. 130–132 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,420, 3,052, 1,693, 1,605, 1,495, 1,453; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.78$ (s, 1H, H-3), 6.87–6.89 (d, 2H, $J = 8.1$ Hz, H-2′, H-6′), 7.11 (s, 1H, H-8), 7.19–7.23 (t, 3H, $J = 7.3$ Hz, H-3″, H-4″, H-5″), 7.30–7.32 (d, 2H, $J = 7.3$ Hz, H-2″, H-6″), 7.37–7.39 (d, 2H, $J = 8.1$ Hz, H-3′, H-5′), 8.36 (s, 1H, H-5), 9.47 (s, bs, 1H, OH), 10.02 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 110.6$ (CH, C-8), 118.6 (C, C-10), 121.8 (C, C-6), 126.5 (CH, C-5), 128.9 (CH, C-2″, C-4″, C-6″), 131.7 (CH, C-3″, C-5″), 133.3 (CH, C-2′, C-6′), 138.4 (C, C-1″), 147.6 (C, C-1′), 155.4 (C, C-7), 158.7 (C, C-4′), 160.9 (C, C-9), 164.5 (C, C-2), 179.3 (C, C-4); ESI–MS: 359 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{14}N_2O_4$: C, 70.39; H, 3.94; N, 7.82 %. Found: C, 70.46; H, 4.04; N, 7.71 %.

*7-Hydroxy-2-(4-hydroxyphenyl)-6-[(E)-2-phenyldiazen-1-yl]–4H-chromen-4-one (**6b**)*

Dark reddish brown solid; Yield: 70 %; m.p. 130–131 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,425, 3,060, 1,690, 1,600, 1,505, 1,465; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.79$ (s, 1H, H-3), 6.89–6.91 (d, 2H, $J = 8.2$ Hz, H-3″, H-5″), 7.11 (s, 1H, H-8), 7.16–7.20 (t, 1H, $J = 7.5$ Hz, H-4′), 7.34–7.36 (d, 2H, $J = 8.2$ Hz, H-2″, H-6″), 7.37–7.39 (d, 2H, $J = 7.5$ Hz, H-2′, H-6′), 7.40–7.44 (t, 2H, $J = 7.5$ Hz, H-3′, H-5′), 8.38 (s, 1H, H-5), 9.85 (s, bs, 1H, OH), 10.9 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 109.7$ (CH, C-8), 117.8 (C, C-10), 120.9 (C, C-6), 125.7 (CH, C-5), 128.2 (CH, C-3′, C-5′), 131.5 (CH, C-2″, C-6″), 132.8 (CH, C-4′), 154.5 (C, C-1′), 158.3 (C, C-7), 160.5 (C, C-9), 164.4 (C, C-2), 178.9 (C, C-4); ESI–MS: 359 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{14}N_2O_4$: C, 70.39; H, 3.94; N, 7.82 %. Found: C, 70.47; H, 3.99; N, 7.75 %.

*7-Hydroxy-6-[(E)-2-(2-hydroxyphenyl)diazen-1-yl]-2-(4-methoxyphenyl)-4H-chromen-4-one (**6c**)*

Yellowish brown solid; Yield: 67 %; m.p. 143–144 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,442, 3,062, 2,962, 1,699, 1,608, 1,515, 1,462; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.83$ (s, 3H, OCH_3), 6.78 (s, 1H, H-3), 7.07–7.09 (d, 2H, $J = 8.4$ Hz, H-3″, H-5″), 7.09–7.13 (t, 1H, $J = 7.2$ Hz, H-5′), 7.15 (s, 1H, H-8), 7.17–7.21 (t, 1H, $J = 7.2$ Hz, H-4′), 7.49–7.51 (d, 1H, $J = 7.2$ Hz, H-3′), 7.72–7.74 (d, 1H, $J = 7.2$ Hz, H-6′), 7.75–7.77 (d, 2H, $J = 8.4$ Hz, H-2″, H-6″), 8.37 (s, 1H, H-5), 9.62 (s, bs, 1H, OH), 10.18 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 62.7$ (CH_3, OCH_3), 110.5 (CH, C-8), 118.2 (CH, C-3′), 120.6 (C, C-6), 125.8 (CH, C-5), 128.4 (C, C-1″), 132.2 (CH, C-2″, C-6″), 133.7 (C, C-1′), 138.5 (CH, C-4′), 145.5 (C, C-2′), 155.7 (C, C-7), 158.8 (C, C-4″), 160.2 (C, C-9), 164.6 (C, C-2), 180.1 (C, C-4); ESI–MS: 389 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{16}N_2O_5$: C, 68.04; H, 4.15; N, 7.21 %. Found: C, 68.12; H, 4.06; N, 7.29 %.

*7-Hydroxy-6-[(E)-2-(2-methoxyphenyl)diazen-1-yl]-2-phenyl-4H-chromen-4-one (**6d**)*

Dark brown solid; Yield: 76 %; m.p. 135–137 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,447, 3,045, 2,976, 1,702, 1,603, 1,505, 1,460; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.81$ (s, 3H, OCH_3), 6.76 (s, 1H, H-3), 7.07–7.11 (t, 1H, $J = 7.4$ Hz, H-5′), 7.12 (s, 1H, H-8), 7.17–7.21 (t, 1H, $J = 7.7$ Hz, H-4″), 7.34–7.36 (d, 2H, $J = 7.7$ Hz, H-2″, H-6″), 7.41–7.45 (t, 2H, $J = 7.7$ Hz, H-3″, H-5″), 7.45–7.47 (d, 1H, $J = 7.4$ Hz, H-3′), 7.49–7.53 (t, 1H, $J = 7.4$ Hz, H-4′), 7.82–7.84 (d, 1H, $J = 7.4$ Hz, H-6′), 8.35 (s, 1H, H-5), 9.57 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 63.5$ (CH_3, OCH_3), 111.2 (CH, C-8), 116.7 (C, C-10), 118.3 (C, C-6), 121.8 (CH, C-5′), 126.2 (CH, C-5, C-6′), 128.7 (CH, C-3″, C-5″), 133.6 (C, C-1″), 136.5 (CH, C-4′), 154.7 (C, C-2′), 158.8 (C, C-7), 160.6 (C, C-9), 164.5 (C, C-2), 179.7 (C, C-4); ESI–MS: 373 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{16}N_2O_4$: C, 70.96; H, 4.33; N, 7.52 %. Found: C, 71.05; H, 4.29; N, 7.64 %.

*7-Hydroxy-2-(4-hydroxyphenyl)-6-[(E)-2-(3-methoxyphenyl)diazen-1-yl]-4H-chromen-4-one (**6e**)*

Yellowish brown solid; Yield: 75 %; m.p. 136–138 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,428, 3,049, 2,964, 1,696, 1,608, 1,496, 1,453; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.79$ (s, 3H, OCH_3), 6.81 (s, 1H, H-3), 6.91–6.93 (d, 2H, $J = 7.9$ Hz, H-3″, H-5″), 7.14 (s, 1H, H-8), 7.19–7.21 (d, 1H, $J = 6.8$ Hz, H-4′), 7.34–7.36 (d, 2H, $J = 7.9$ Hz, H-2″, H-6″), 7.45–7.47 (t, 1H, $J = 6.8$ Hz, H-3′), 7.51–7.53 (d, 1H, $J = 6.8$ Hz, H-2′), 7.72 (s, 1H, H-6′), 8.40 (s, 1H, H-5), 9.76 (s, bs, 1H, OH), 10.05 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 63.7$ (CH_3, OCH_3), 110.9 (CH, C-4′), 116.3 (C, C-10), 118.8 (C, C-6), 121.6 (CH, C-2′), 126.4 (CH, C-5), 128.7 (C, C-1″), 133.5 (CH, C-2″, C-6″), 137.9 (CH, C-3′), 154.7 (C, C-1′), 158.7 (C, C-7), 160.9 (C, C-5′), 165.2 (C, C-2), 180.3 (C, C-4); ESI–MS: 389 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{16}N_2O_5$: C, 68.04; H, 4.15; N, 7.21 %. Found: C, 68.13; H, 4.21; N, 7.16 %.

*7-Hydroxy-2-(4-methoxyphenyl)-6-[(E)-2-phenyldiazen-1-yl]-4H-chromen-4-one (**6f**)*

Dark brown solid; Yield: 73 %; m.p. 134–135 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,452, 3,056, 2,983, 1,690, 1,609, 1,513, 1,464; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.82$ (s, 3H, OCH_3), 6.77 (s, 1H, H-3), 6.96–6.98 (d, 2H, $J = 8.1$ Hz, H-3″, H-5″), 7.14 (s, 1H, H-8), 7.15–7.19 (t, 1H, $J = 7.6$ Hz, H-4′), 7.34–7.36 (d, 2H, $J = 8.1$ Hz, H-2″, H-6″), 7.37–7.41 (t, 2H, $J = 7.6$ Hz, H-3′, H-5′), 7.51–7.53 (d, 2H, $J = 7.6$ Hz, H-2′, H-6′), 8.39 (s, 1H, H-5), 9.83 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 63.2$ (CH_3, OCH_3), 110.5 (CH, C-3″, C-5″), 116.7 (C, C-10), 121.3 (C, C-1″), 126.5 (CH, C-2′, C-6′), 128.2 (CH, C-2″, C-6″), 133.8 (CH, C-3′, C-5′), 138.3 (CH, C-4′), 154.5 (C, C-1′), 158.7 (C, C-7), 160.7 (C, C-9), 164.7 (C, C-2), 179.3 (C, C-4); ESI–MS: 373 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{16}N_2O_4$: C, 70.96; H, 4.33; N, 7.52 %. Found: C, 71.07; H, 4.39; N, 7.44 %.

*7-Hydroxy-2-(4-hydroxyphenyl)-6-[(E)-2-(2-hydroxyphenyl)diazen-1-yl]-4H-chromen-4-one (**6g**)*

Reddish brown solid; Yield: 66 %; m.p. 139–140 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,443, 3,042, 1,696, 1,613, 1,518, 1,469; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.79$ (s, 1H, H-3), 6.91–6.93 (d, 2H, $J = 8.0$ Hz, H-3″, H-5″), 7.11-7.15 (t, 1H, $J = 7.5$ Hz, H-5′), 7.16 (s, 1H, H-8), 7.17–7.21 (t, 1H, $J = 7.5$ Hz, H-4′), 7.34–7.36 (d, 2H, $J = 8.0$ Hz, H-2″, H-6″), 7.51–7.53 (d, 1H, $J = 7.5$ Hz, H-3′), 7.72–7.74 (d, 1H, $J = 7.5$ Hz, H-5′), 8.41 (s, 1H, H-5), 9.52 (s, bs, 1H, OH), 10.07 (s, bs, 1H, OH), 10.16 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 110.8$ (CH, C-3″, C-5″), 116.5 (C, C-10), 118.3 (CH, C-3′), 121.8 (CH, C-5′), 124.6 (CH, C-6′), 128.3 (CH, C-2″, C-6″), 133.6 (CH, C-4′), 154.8 (CH, C-2′), 158.7 (C, C-7), 160.2 (C, C-9), 164.5 (C, C-2), 178.8 (C, C-4); ESI–MS: 358 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{14}N_2O_4$: C, 70.58; H, 3.67; N, 7.84 %. Found: C, 70.66; H, 3.56; N, 7.78 %.

*6-[(E)-2-(4-chlorophenyl)diazen-1-yl]-7-hydroxy-2-phenyl-4H-chromen-4-one (**6h**)*

Dark reddish brown solid; Yield: 79 %; m.p. 137–138 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,459, 3,038, 1,691, 1,607, 1,509, 1,460, 776; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.78$ (s, 1H, H-3), 7.15 (s, 1H, H-8), 7.17–7.21 (t, 1H, $J = 7.4$ Hz, H-4″), 7.34–7.36 (d, 2H, $J = 8.2$ Hz, H-3′, H-5′), 7.41–7.45 (t, 2H, $J = 7.4$ Hz, H-3″, H-5″), 7.93–7.95 (d, 2H, $J = 7.4$ Hz, H-2″, H-6″), 8.12–8.14 (d, 2H, $J = 8.2$ Hz, H-2′, H-6′), 8.38 (s, 1H, H-5), 9.81 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 110.3$ (CH, C-8), 117.8 (C, C-10), 120.5 (C, C-6), 124.3 (CH, C-2′, C-6′), 128.8 (CH, C-3″, C-5″), 131.9 (C, C-1″), 133.8 (CH, C-3′, C-5′), 137.5 (C, C-4′), 155.8 (C, C-1′), 159.7 (C, C-7), 161.3 (C, C-9), 165.6 (C, C-2), 180.5 (C, C-4); ESI–MS: 377.5 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{13}ClN_2O_3$: C, 66.94; H, 3.48; N, 7.43 %. Found: C, 66.87; H, 3.59; N, 7.51 %.

*6-[(E)-2-(2-chlorophenyl)diazen-1-yl]-7-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**6i**)*

Reddish brown solid; Yield: 74 %; m.p. 144–145 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,466, 3,049, 1,695, 1,612, 1,508, 1,457, 768; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.81$ (s, 1H, H-3), 6.91–6.93 (d, 2H, $J = 8.2$ Hz, H-3″, H-5″), 7.16 (s, 1H, H-8), 7.34–7.36 (d, 2H, $J = 8.2$ Hz, H-2″, H-6″), 7.41–7.45 (t, 1H, $J = 7.8$ Hz, H-5′), 7.47–7.51 (t, 1H, $J = 7.8$ Hz, H-4′), 7.71–7.73 (d, 1H, $J = 7.8$ Hz, H-3′), 8.12–8.14 (d, 1H, $J = 7.8$ Hz, H-6′), 8.42 (s, 1H, H-5), 9.84 (s, bs, 1H, OH), 10.01 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 111.2$ (CH, C-8), 117.6 (C, C-10), 120.8 (C, C-6), 124.5 (CH, C-6′), 126.7 (CH, C-5′), 128.7 (C, C-2′), 131.5 (CH, C-2″, C-6″), 136.2 (CH, C-4′), 155.7 (C, C-4″), 159.5 (C, C-7), 161.9 (C, C-9), 165.3 (C, C-2), 179.9 (C, C-4); ESI–MS: 393.5 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{13}ClN_2O_4$: C, 64.21; H, 3.34; N, 7.13 %. Found: C, 64.27; H, 3.23; N, 7.24 %.

*6-[(E)-2-(4-chlorophenyl)diazen-1-yl]-7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one (**6j**)*

Reddish brown solid; Yield: 77 %; m.p. 140–141 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,455, 3,043, 2,982, 1,703, 1,610, 1,518, 1,467, 750; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.83$ (s, 3H, OCH_3), 6.79 (s, 1H, H-3), 6.97–6.99 (d, 2H, $J = 8.5$ Hz, H-3″, H-5″), 7.16 (s, 1H, H-8), 7.32–7.34 (d, 2H, $J = 8.5$ Hz, H-2″, H-6″), 7.93–7.95 (d, 2H, $J = 7.9$ Hz, H-3′, H-5′), 8.12–8.14 (d, 2H, $J = 7.9$ Hz, H-2′, H-6′), 8.40 (s, 1H, H-5), 9.77 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 63.9$ (CH_3, OCH_3), 111.5 (CH, C-3″, C-5″), 117.3 (C, C-10), 120.8 (C, C-6), 124.6 (CH, C-2′, C-6′), 126.7 (CH, C-2″, C-6″), 129.8 (CH, C-3′), 137.1 (C, C-4′), 154.3 (C, C-7), 159.4 (C, C-4), 161.3 (C, C-9), 165.7 (C, C-2), 180.1 (C, C-4); ESI–MS: 407.5 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{15}ClN_2O_4$: C,64.95; H, 3.72; N, 6.89 %. Found: C,64.99; H, 3.66; N, 6.97 %.

*7-Hydroxy-6-[(E)-2-(3-nitrophenyl)diazen-1-yl]-2-phenyl-4H-chromen-4-one (**6k**)*

Dark reddish brown solid; Yield: 84 %; m.p. 153–154 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,420, 3,025, 1,692, 1,605, 1,500, 1,453, 1,520, 1,370; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.81$ (s, 1H, H-3), 7.16 (s, 1H, H-8),

7.38–7.40 (d, 2H, $J = 7.1$ Hz, H-2″, H-6″), 7.43–7.47 (t, 1H, $J = 7.1$ Hz, H-3″, H-5″), 7.47–7.49 (t, 1H, $J = 7.1$ Hz, H-4″), 7.91–7.93 (d, 1H, $J = 6.9$ Hz, H-4′), 8.17–8.19 (d, 1H, $J = 6.9$ Hz, H-2′), 8.29–8.33 (t, 1H, $J = 6.9$ Hz, H-3′), 8.41 (s, 1H, H-5), 8.48 (s, 1H, H-6′), 9.69 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 111.6$ (CH, C-8), 118.7 (CH, C-6′), 121.8 (C, C-6), 126.6 (CH, C-4′), 128.3 (CH, C-3″, C-5″), 129.9 (CH, C-2′), 131.7 (C, C-1″), 133.3 (CH, C-3′), 151.3 (C, C-5′), 154.8 (C, C-7), 160.6 (C, C-9), 164.5 (C, C-2), 168.7 (C, C-1′), 180.9 (C, C-4); ESI–MS: 388 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{13}N_3O_5$: C, 65.12; H, 3.38; N,10.85 %. Found: C, 65.06; H, 3.29; N,10.98 %.

*7-Hydroxy-2-(4-hydroxyphenyl)-6-[(E)-2-(4-nitrophenyl)diazen-1-yl]-4H-chromen-4-one (**6l**)*

Dark reddish brown solid; Yield: 81 %; m.p. 150–152 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,426, 3,032, 1,698, 1,612, 1,506, 1,463, 1,531, 1,379; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.81$ (s, 1H, H-3), 6.91–6.93 (d, 2H, $J = 8.3$ Hz, H-3″, H-5″), 7.15 (s, 1H, H-8), 7.34–7.36 (d, 2H, $J = 8.3$ Hz, H-2″, H-6″), 7.40–7.42 (d, 2H, $J = 8.0$ Hz, H-2′, H-6′), 8.26–8.28 (d, 2H, $J = 8.0$ Hz, H-3′, H-5′), 8.39 (s, 1H, H-5), 9.72 (s, bs, 1H, OH), 10.6 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 111.8$ (CH, C-3″, C-5″), 118.9 (C, C-10), 120.9 (C, C-6), 121.7 (CH, C-2′, C-6′), 123.9 (C, C-1″), 126.7 (CH, C-3′, C-5′), 128.4 (CH, C-5), 131.5 (CH, C-2″, C-6″), 151.5 (C, C-4′), 155.2 (C, C-1′), 160.7 (C, C-9), 164.3 (C, C-2), 168.5 (C, C-4″), 180.6 (C, C-4); ESI–MS: 404 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{13}N_3O_6$: C, 62.53; H, 3.25; N, 10.42 %. Found: C, 62.67; H, 3.33; N, 10.31 %.

*7-Hydroxy-2-phenyl-6-[(E)-2-phenyldiazen-1-yl]-4H-chromen-4-one (**6m**)*

Reddish brown solid; Yield: 78 %; m.p. 127–128 °C; FT-IR (KBr) (υ_{max}, cm^{-1}) 3,437, 3,036, 1,696, 1,606, 1,501, 1,457; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.78$ (s, 1H, H-3), 6.89–6.91 (d, 2H, $J = 7.6$ Hz, H-2″, H-6″), 7.09–7.13 (t, 2H, $J = 7.6$ Hz, H-3″, H-5″), 7.14 (s, 1H, H-8), 7.17–7.21 (t, 1H, $J = 7.2$ Hz, H-4′), 7.32–7.34 (d, 2H, $J = 7.2$ Hz, H-2′, H-6′), 7.47–7.51 (t, 2H, $J = 7.2$ Hz, H-3′, H-5′), 7.68–7.72 (t, 1H, $J = 7.6$ Hz, H-4″), 8.38 (s, 1H, H-5), 9.65 (s, bs, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 108.8$ (CH, C-8), 118.6 (C, C-10), 120.7 (C, C-6), 126.3 (CH, C-5), 128.6 (CH, C-2″,C-4″, C-6″), 131.3 (CH, C-3″), 133.1 (C, C-1″), 138.3 (CH, C-4′), 153.7 (C, C-1′), 159.3 (C, C-7), 161.6 (C, C-9), 165.8 (C, C-2), 178.5 (C, C-4); ESI–MS: 343 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{14}N_2O_3$: C, 73.68; H, 4.12; N, 8.18 %. Found: C, 73.66; H, 3.96; N, 8.28 %.

Microbiological method

The following bacterial cultures *S. aureus MTCC 3,160, B. cereus MTCC 430, A. globiformis MTCC 4299, V. cholerae MTCC 3904, E. coli MTCC 1610*, and *S. dysenteriae NICED* were used for the present study to evaluate the antibacterial activity of the synthesized compounds. The antibacterial activity of newly synthesized compounds was evaluated according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2007), USA using the agar disk-diffusion method as well as liquid broth assay.

Kirby–Bauer agar diffusion method (antibacterial assay)

In Kirby–Bauer agar diffusion method, the Mueller–Hinton agar (Hi-Media, Mumbai) was prepared according to the manufacturer's instructions; the media was sterilized at 121 °C for 15–20 min and cooled to 50 °C in heating water bath. Approximately, 20–25 mL of the media was poured on to the 90 mm sterile Petri plates, and it was spreaded in anticlockwise motion. It was then kept for 10–15 min at room temperature for solidification. Plates were inverted and left for overnight in incubator to check the sterility of the plates. Overnight grown cultures (100 µL) of *S. aureus MTCC 3160, B. cereus MTCC 430, A. globiformis MTCC 4299, V. cholerae MTCC 3904, E. coli MTCC 1610*, and *S. dysenteriae NICED* were spreaded over the agar surface of the MHA plates. Whatman no. 1, filter paper disk (approximately 6 mm in diameter) impregnated with different synthesized compounds (0.2 mg/mL in DMSO) were gently placed on the surface of plates using sterile forceps. The plates were inverted and incubated at 37 °C for 18–24 h. The appearance of clear zone of growth inhibition surrounding the disk was measured using antibiotic zone measurement scale (Hi-media) and tabulated. All the tests were performed in duplicate, and the average was taken as the final reading. The standard antibiotics, ampicillin and ciprofloxacin, were used as the positive control whereas DMSO alone as the negative control for all the selected bacteria.

Antioxidant assay

DPPH (0.004 g) was dissolved in MeOH (100 mL) to obtain a concentration of 100 µM. Serial dilutions were carried out with stock solutions (150 µM) of the compounds in methanol to obtain final concentrations of 1, 2, 4, 8, 12, 16, 20, 24, and 30 µM in cuvette. Diluted solutions of compound and ascorbic acid (AA) (2 mL each) were mixed with methanolic DPPH solution (1 mL, 100 µM) and allowed to stand for 120 min for any reaction to occur. The absorbance was recorded at 517 nm on Shimadzu

Pharmaspec UV–Vis spectrophotometer (model-1700). The experiment was performed in triplicate, and the average absorbance was noted for each concentration. The IC_{50} value, which is the concentration of the test compound that reduces 50 % of the initial free radical concentration, was calculated in μM. Ascorbic acid was used as the reference standard, at the same concentrations in methanol as were used for the tested compounds.

Acknowledgments We are grateful to the SAIF, Chandigarh and Director, Defence R&D Establishment, Gwalior for providing facilities for spectro-analytical studies and antibacterial evaluation of the synthesized compounds.

References

Allan J, Robinson R (1924) CCXC—an accessible derivative of chromonol. J Chem Soc Trans 125:2192–2195

Awuah E, Capretta A (2009) Access to flavones via a microwave-assisted, one-pot Sonogashira–carbonylation–annulation reaction. Org Lett 11:3210–3213

Baker W (1933) Molecular rearrangement of some *o*-acyloxyacetophenones and the mechanism of the production of 3-acylchromones. J Chem Soc. doi:10.1039/JR9330001381

Bandyopadhyay P, Sathe M, Ponmariappan S, Sharma A, Sharma P, Srivastava AK, Kaushik MP (2011) Exploration of in vitro time point quantitative evaluation of newly synthesized benzimidazole and benzothiazole derivatives as potential antibacterial agents. Bioorg Med Chem Lett 21:7306–7309

Banerji A, Goomer NC (1980) A new synthesis of flavones. Synthesis 11:874–875

Beckman KB, Ames BN (1998) The free radical theory of aging matures. Physiol Rev 78:547–581

Betoni JEC, Mantovani RP, Barbosa LN, Di stasi LC (2006) A fernandes synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem Inst Oswaldo Cruz 101:387–390

Birt DF, Hendrich S, Wang W (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol Ther 90:157–177

Blank VC, Poli C, Marder M, Roguin LP (2004) Antiproliferative activity of various flavonoids and related compounds: additive effect of interferon-α2b. Bioorg Med Chem Lett 14:133–136

Block G (1992) The data support a role for antioxidants in reducing cancer risk. Nutr Rev 50:207–213

Blois MS (1958) Antioxidant determination by the use of a stable free radical. Nature 181:1199–1200

Bondet V, Brand-Williams W, Berset C (1997) Kinetics and mechanisms of antioxidant activity using the $DPPH^{\bullet}$ Free radical method. Lebensm-Wiss Technol 30:609–615

Clinical and Laboratory Standard Institute (2007) Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement: CLSI document M100-S17. Clinical and Laboratory Standard Institute, Wayne, pp 1987–1988

Delcanale M, Amari G, Armani E, Lipreri M, Civelli M, Galbiati E, Glossi M, Caruso PL, Crivori P, Corrupt PA, Testa B (2001) Novel basic isoflavones as inhibitors of bone resorption. Helv Chim Acta 84:2417–2429

Halliwell B, Gutteridge JMC (1989) Free radicals in biology and medicine, 2nd edn. Clarendon Press, Oxford, pp 416–494

Hamiltonmiller JMT (1995) Antimicrobial properties of tea (*Camellia sinensis* L.). Antimicrob Agents Chemother 39:2375–2377

Han C (1997) Screening of anticarcinogenic ingredients in tea polyphenols. Cancer Lett 114:153–158

Hirao I, Yamaguchi M, Hamada M (1984) A convenient synthesis of 2- and 2,3-substituted 4*H*-chromen-4-ones. Synthesis 11:1076–1078

Hossain SU, Bhattacharya S (2007) Synthesis of *o*-prenylated and ogeranylated derivatives of 5-benzylidene 2,4 thiazolidinediones and evaluation of their free radical scavenging activity as well as effect on some phase II antioxidant/detoxifying enzymes. Bioorg Med Chem Lett 17:1149–1154

Huck CW, Huber CG, Ongania KH, Bonn GK (2000) Isolation and characterization of methoxylated flavones in the flowers of primula veris by liquid chromatography and mass spectrometry. J Chromatogr A 870:453–462

Iinuma M, Tsuchiya H, Sato M, Yokoyama J, Ohyama M, Ohkawa Y, Tanaka T, Fujiwara S, Fujii T (1994) Flavanones with potent antibacterial activity against methicillin-resistant Staphylococcus aureus. J Pharm Pharmacol 46:892–895

Jain PK, Makrandi JK, Grover SK (1982) A facile Baker–Venkataraman synthesis of flavones using phase transfer catalysis. Synthesis 1982:221–222

Kang KA, Zhang R, Piao MJ, Ko DO, Wang ZH, Kim BJ, Park JW, Kim HS, Kim DH, Hyun JW (2008) Protective effect of irisolidone, a metabolite of kakkalide, against hydrogen peroxide induced cell damage via antioxidant effect. Bioorg Med Chem 16:1133–1141

Khanna MS, Singh OV, Garg CP, Kapoor RP (1992) Oxidation of flavanones using thallium(III) salts: a new route for the synthesis of flavones and isoflavones. J Chem Soc Perkin Trans 1:2565–2568

Kohen F, Gayer B, Kulik T, Frydman V, Nevo N, Katzburg S, Limor R, Sharon O, Stern N, Somjen D (2007) Synthesis and evaluation of the antiproliferative activities of derivatives of carboxyalkyl isoflavones linked to N-*t*-Boc-hexylenediamine. J Med Chem 50:6405–6410

Laupattarakasem P, Houghton PJ, Hoult JR (2004) Anti-inflammatory isoflavonoids from the stems of *Derris scandens*. Planta Med 70:496–501

Li XC, Joshi AS, ElSohly HN, Khan SI, Jacob MR, Zhang Z, Khan IA, Ferreira D, Walker LA, Broedel SE, Raulli RE, Cihlar RL (2002) Fatty acid synthase inhibitors from plants: isolation, structure elucidation, and SAR studies. J Nat Prod 65:1909–1914

Liou SS, Teng CM, Ko FN, Lin CN (1994) Synthesis and antiplatelet effects of xanthonoxypropanolamines and related compounds. J Pharm Sci 83:391–395

Looker JH, Hanneman WW (1962) Physical and chemical properties of hydroxyflavones. I. Infrared absorption spectra of monohydroxyflavones and their *o*-methyl and *o*-acetyl derivatives. J Org Chem 27:381–389

Mahal HS, Venkataraman K (1934) Synthetical experiments in the chromone group. Part XIV. The action of sodamide on 1-acyloxy-2-acetonaphthones. J Chem Soc. doi:10.1039/JR9340001767

Malikov VM, Yuldashev MP (2002) Phenolic compounds of plants of the *Scutellaria* L. *genus*. Distribution, structure, and properties. Chem Nat Compd 38:358–406

Manthey JA, Grohmann K, Guthrie N (2001) Biological properties of citrus flavonoids pertaining to cancer and inflammation. Curr Med Chem 8:135–153

Mensor LL, Menzes FS, Leitao GG, Reis AS, Dosantos TC, Coube CS, Leitao SG (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytothe Res 15:127–130

Nagao T, Abe F, Kinjo J, Okabe H (2002) Flavones from the leaves of *Lantana montevidensis Briq.* and consideration of structure-activity relationship. Biol Pharm Bull 25:875–879

Qin CX, Chen X, Hughes RA, Williams SJ, Woodman OL (2008) Understanding the cardioprotective effects of flavonols: discovery of relaxant flavonols without antioxidant activity. J Med Chem 51:1874–1884

Rice-Evans AC, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20:933–956

Sahu V, Sharma P, Kumar A (2013) Synthesis and QSAR modeling 1-[3-methyl-2-(aryldiazenyl)-2*H*-aziren-2-yl]ethanones as potential antibacterial agents. Med Chem Res 22:2476–2485

Saxena S, Makrandi JK, Grover SK (1985) Synthesis of 5- and/or 7-hydroxyflavones using a modified phase transfer-catalysed Baker-Venkataraman transformation. Synthesis 1985:697. doi:10.1055/s-1985-31319

Scherrer R, Gerhardt P (1971) Molecular sieving by the *Bacillus megaterium* cell wall and protoplast. J Bacteriol 107:718–735

Sharma P, Kumar A, Kumari P, Singh J, Kaushik MP (2012a) QSAR modeling of synthesized 3-(1,3-benzothiazol-2-yl)2-phenylquinazolin-4(3*H*)-ones as potent antibacterial agents. Med Chem Res 21:1136–1148

Sharma P, Kumar A, Sharma M, Singh J, Bandyopadhyay P, Sathe M, Kaushik MP (2012b) Synthesis and exploration of QSAR model of 2-methyl-3-[2-(2-methylprop-1-en-1-yl)-1*H*-benzimidazol-1-yl]pyrimido[1,2-a]benzimidazol-4(3*H*)-one as potential antibacterial agents. J Enzyme Inhib Med Chem 27:294–301

Silva FAM, Borges F, Guimarães C, Lima JLFC, Matos C, Reis S (2000) Phenolic acids and derivatives: studies on the relationship among structure, radical scavenging activity, and physicochemical parameters. J Agric Food Chem 48:2122–2126

Siquet C, Paiva-Martins F, Lima JFC, Reis S, Borges F (2006) Antioxidant profile of dihydroxy- and trihydroxyphenolic acids : a structure–activity relationship study. Free Radical Res 40:433–442

Teixeira S, Siquet C, Alves C, Boal I, Marques MP, Borges F, Lima JLFC, Reis S (2005) Structure property studies on the antioxidant activity of flavonoids present in diet. Free Radical Biol Med 39:1099–1108

Tzeng CC, Zhao YL, Chen YL, Liou SS, Wang TC, Chang YL, Teng CM (1997) Synthesis and evaluation of α-methylidene-γ-butyrolactone bearing flavone and xanthone moieties. Helv Chim Acta 80:2337–2344

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160:1–40

Valla C, Baeza A, Menges F, Pfaltz A (2008) Enantioselective synthesis of chromanes by iridium-catalyzed asymmetric hydrogenation of 4*H*-chromenes. Synth lett. doi:10.1055/s-0028-1087359

Wang TC, Chen YL, Tzeng CC, Liou SS, Chang YL, Teng CM (1996) Antiplatelet α-methylidene-γ-butyrolactones: synthesis and evaluation of quinoline, flavone, and xanthone derivatives. Helv Chim Acta 79:1620–1626

Wang SF, Jiang Q, Ye YH, Li Y, Tan RX (2005) Genistein derivatives as selective estrogen receptor modulators: sonochemical synthesis and in vivo anti-osteoporotic action. Bioorg Med Chem 13:4880–4890

Xu HX, Lee SF (2001) Activity of plant flavonoids against antibiotic-resistant bacteri**a**. Phytother Res 15:39–43