Synthesis and Antibacterial Activity of Different Derivatives of 3(2-aryl methylidene hydrazinyl)-2H-chromene-2-one

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Abstract
In the present study, novel derivatives of 3(2-aryl methylidene hydrazinyl) –2H-chromene-2-one were prepared and evaluated for their in vitro antibacterial activity against strains like S.typhi, Ca. albicans, B.substitis, P.vulgaris, P.aeruginosa, S.aureus, P.syringae, B.pumillus, S.epidermidis, Bordetella bronchiseptica etc. The newly synthesized compounds were characterized by IR, 1H NMR and 13C NMR. The results revealed that all synthesized compounds have a significant biological activity against the tested bacteria. Among the synthesized derivatives IIIA2 (2-nitroaryl methylidene hydrazinyl)-2H-6-Chloro-chromene-2-one, (III B1) (2-chloroaryl methylidene hydrazinyl)-2H-6- bromo-chromene-2-one, (III B2) (2-nitroaryl methylidene hydrazinyl)-2H-6- bromo-chromene-2-one and IIIC1 (2-chloroaryl methylidene hydrazinyl)-2H-6- nitro-chromene-2-one were found to be most effective antibacterial compounds.

Keywords: 3(2-aryl methylidene hydrazinyl) – 2H-chromene-2-one; antibacterial activity; salicylaldehyde; dimethylmalonate.

Introduction
Coumarin derivatives have been shown to possess a remarkably broad spectrum of biological activities including antibacterial [1-3], antifungal [4-7], anti-inflammatory [8], anticoagulant [9-10], antitumour [11-13], antioxidant [14-16] and anti-HIV [17-18]. In Thorne's addition, these compounds are used as fluorescent brightening agents and as dyes for tuning lasers [19-21]. Main representatives of the class are the hydroxyl derivatives, 4- and 7-hydroxycoumarins, also biologically active and very important for the synthesis of other coumarin derivatives. Inspired by the above facts and in continuation of our ongoing research program in the field of synthesis and characterization of different derivatives of 3(2-aryl methylidene hydrazinyl) –2H-chromene-2-one [22], we hereby report the synthesis and antibacterial activity of 3(2-aryl methylidene hydrazinyl) –2H-chromene-2-one derivatives. The structures of all compounds have been confirmed by spectral analysis (IR, 1H NMR and 13C NMR).

Experimental
The purity of the synthesized compounds were checked by thin layer chromatography on silica gel G in various solvent systems using iodine vapours as detecting agent. Melting points were determined by the melting point determination apparatus (TEMPO) in open capillary tubes and are uncorrected. Infra-red spectra were recorded on FTIR Shimedzu-8400S spectrometer using KBr pellets. Proton NMR spectra were recorded on Bruker Avance II 400 NMR Ultra Shield Spectrometer using DMSO-d6 as a solvent and TMS as internal standard. All the synthesized compounds have been screened in vitro for their anti-bacterial activity.

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Chemistry

A series of 3(2-aryl methylidene hydrazinyl)–2H-chromene-2-one has been synthesized. Reaction of substituted salicylaldehydes with dimethyl malonate in the presence of a base (piperidine) furnished the corresponding 2-oxo-2H-chromen-3yl-acetate (Ia–c) which on further reaction with hydrazine hydrate afforded 3-hydrazinyl 2H-chromene-2-one(IIa-c). The compounds (IIa–c) were further reacted with aromatic aldehydes to afford 3(2-aryl methylidene hydrazinyl)–2H-chromene-2-one (III A1-D3). The synthesized compounds 3(2-aryl methylidene hydrazinyl)–2H-chromene-2-one (III A1-D3) were characterized on the basis of their spectral and analytical studies.

General method

The title compounds were prepared in following steps.

2-oxo-2H-chromen-3yl-acetate (Ia–c)

In a 500-ml round-bottomed flask equipped with a reflux condenser are placed (0.02 mol) of salicylaldehyde, (0.03 mol) of ethyl malonate, and 20 ml. of absolute ethanol. To this mixture add 1 ml. of piperidine and 0.5 ml. of glacial acetic acid and the solution is heated under reflux for 3 hr. The mixture was cooled and the solid obtained was separated by filtration and recrystallized from ethanol to give the corresponding compounds.

Synthesis of 3-hydrazinyl-2H-Chromen-2-one (IIa–c)

In a 20ml of hydrazine hydrate (98%) was refluxed 0.02mol of compound (Ia–c) for 2 h. The precipitate formed after cooling was filtered, washed with water, dried and recrystallized from ethanol.

Synthesis of 3(2-aryl methylidene hydrazinyl)-2H-Chromene-2-one (III A1-D3)

For 2-6 h, 0.01 mol of compound (IIa–c) and 0.011 mol of appropriate aromatic aldehydes and 25 ml of ethanol (96%) were refluxed. The solid that separate was filtered and recrystallized from ethanol. By adopting similar type of procedures, and employing equimolar quantities of reactants, different derivatives of 3(2-aryl methylidene hydrazinyl)-2H-chromene-2-one were synthesized. Synthetic pathway for preparation of title compound is shown in Scheme 1. Physical data of synthesized compounds is given in Table 1. Elemental analysis of compounds (III A1-D3) is given in Table 2.

Spectral data

(2-methylaryl methylidene hydrazinyl)-2H-6- Chloro-chromene-2-one (III A1)

FTIR (KBr) (V max cm-1):–1590 (NH2), 1550(N-N), 1100(C-N), 1625(C=N), 1675 (C=C), 850-550(C-Cl), 3030(C-H), 1575 (C-C).1H NMR data - (ppm, 300MHz,TMS, δppm):7.2(m, 8H, Ar-H), 2.4(s, 1H,NH), 2.87(s, 3H, CH3).13C NMR data- (ppm, 100MHz, DMSO) δppm:162 (C=O), 120-128(C=C), 20.9(CH3),131.1(C=N).

(2- nitroaryl methylidene hydrazinyl)-2H-6- Chloro-chromene-2-one (III A2)

FTIR (KBr) (V max cm-1):–1590 (HN2), 1550 (N-N), 1100 (C-N), 1625(C=N), 1675(C=C), 850-550 (C-Cl), 1390-1290(N-O), 1575 (C-C). 1H NMR data - (ppm, 300MHz, TMS)-7.2-8.6(m, 8H, Ar-H), 2.52(s, 1H,NH), 6.43(s, C-H). 13C NMR data- (ppm, 100MHz, DMSO) δppm: 162 (C=O), 120-128(C=C), 20.9 (CH3), 131.1(C=N).

(2- methoxyaryl methylidene hydrazinyl)-2H-6- Chloro-chromene-2-one (III A3)

FTIR (KBr) (V max cm-1):–1590 (NH2), 1550(N-N), 1100(C-N), 1625 (C=N), 1675 (C=C), 850-550(C-Cl), 1395-1440 (C-O-H), 1210-1320 (O-C), 1575(C-C).1H NMR data - (ppm, 300MHz,TMS)-7.4(m, 8H,Ar-H), 2.36(s, 1H,NH), 6.62 (s, C-H), 3.80(s,3H OCH3).13C NMR data- (ppm, 100MHz, DMSO) δppm:162 (C=O), 120-128 (C=C benzene), 20.9 (CH3 aliphatic),131.1(C=N), 52-58 (OCH3)
Scheme 1 Synthesis of 3(2-aryl methylidene hydrazinyl)-2H-Chromene-2-one derivatives

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Table 2 Elemental analysis of compounds (III A1-D3)

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(2- chloroaryl methylidene hydrazinyl)-2H-6- bromo-chromene-2-one (III B1)

FTIR (KBr) (max cm⁻¹): 1590(NH₂), 1550(N-N), 1100(C-N), 1625(C=N), 1675(C=C), 690– 515 (C-Br), 910-630 (C-Cl), 1575 (C-C). 1H NMR data - (ppm, 300MHz, TMS) -7.7(m, 8H, Ar-H), 2.44 (s, 1H, NH), 6.55 (s, C-H). 13C NMR data - (ppm, 100MHz, DMSO) δppm:162 (C=O), 120-128 (C=C), 20.9 (CH₃), 131.1 (C=N)

(2-nitroaryl methylidene hydrazinyl)-2H-6- bromo-chromene-2-one (III B2)

FTIR (KBr) (Vmax cm⁻¹): 1590(NH₂), 1550(N-N), 1100(C-N), 1625(C=N), 1675(C=C), 690– 515 (C-Br), 3030 (C-H), 1575(C-C). 1H NMR data - (ppm, 300MHz, TMS) -7.09(m, 8H, Ar-H), 2.60(s, 1H, NH), 6.57 (s, C-H). 13C NMR data - (ppm, 100MHz, DMSO) δppm:162(C=O), 120-128(C=C), 20.9(CH₃), 131.1(C=N)

(2- methoxyaryl methylidene hydrazinyl)-2H-6- bromo-chromene-2-one (III B3)

FTIR (KBr) (Vmax cm⁻¹): 1590(NH₂), 1550(N-N), 1100(C-N), 1625(C=N), 1675(C=C), 690– 515(C-Br), 1395-1440(C-O-H), 1210-1320(O-C), 1575(C=C). 1H NMR data - (ppm, 300MHz, TMS) -7.16(m,8H, Ar-H), 2.72 (s, 1H, NH), 6.32 (s, C-H), 3.78 (s, OCH₃). 13C NMR data - (ppm, 100MHz, DMSO) δppm:162(C=O),120-128(C=C), 20.9(CH₃), 131.1(C=N), 54-60 (OCH₃)

(2- chloroaryl methylidene hydrazinyl)-2H-6- nitro-chromene-2-one (III C1)

FTIR (KBr) (Vmax cm⁻¹): 1590(NH₂), 1550(N-N), 1100(C-N), 1625 (C=N), 1675(C=C), 1575 (C-C), 1590-1450 (N-O stretching), 910-630 (C-Cl). 1H NMR data - (ppm, 300MHz, TMS) -7.10(m,8H, Ar-H), 2.50 (s, 1H, NH), 6.22 (s, C-H). 13C NMR data - (ppm, 100MHz, DMSO) δppm:162(C=O),120-128(C=C), 20.9(CH₃), 131.1(C=N)

(2- methylaryl methylidene hydrazinyl)-2H-6- nitro-chromene-2-one (III C2)

FTIR (KBr) (Vmax cm⁻¹): 1590(NH₂), 1550(N-N), 1100(C-N), 1625 (C=N), 1675(C=C), 1575 (C-C), 1590-1450(N-O), 900-690(C-H). 1H NMR data - (ppm, 300MHz, TMS) -7.34(m,8H, Ar-H), 2.32 (s, 1H, NH), 6.35 (s, C-H), 2.30-2.38 (s,CH₃). 13C NMR data - (ppm, 100MHz, DMSO) δppm:162(C=O),120-128(C=C), 20.9(CH₃), 131.1(C=N).

3(2- methoxaryl methylidene hydrazinyl)-2H-6- nitro-chromene-2-one (III C3)

FTIR (KBr) (Vmax cm⁻¹): 1590(NH₂), 1550(N-N), 1100 (C-N), 1625 (C=N), 1675 (C=C), 1590-1450(N-O stretching), 1395-1440(C-O-H), 1210-1320(O-C), 1575(C=C). 1H NMR data - (ppm, 300MHz, TMS) -7.25(m, 8H, Ar-H), 2.56 (s, 1H, NH), 6.88 (s, C-H), 3.76(s,3H OCH₃). 13C NMR data - (ppm, 100MHz, DMSO) δppm:162(C=O), 120-128 (C=C), 20.9(CH₃), 131.1(C=N), 52-63 (C-O).
(2-chloroaryl methyldiene hydrazinyl)-2H-6- methoxy-chromene-2-one (III D1)

FTIR (KBr) (V\text{max cm}^{-1}): 1590(HN2), 1550(N-N), 1100 (C-N), 1625 (C=O), 1675(C=C), 1395-1440 (C-O-H), 1210-1320(O-C), 850-550(C-Cl), 910-630(C-Cl), 1575(C-C). 1H NMR data - (ppm, 300MHz,TMS)-7.16(m,8H,Ar-H), 2.44 (s,1H, NH), 6.55 (s, C-H), 3.78 (s,3H OCH3).13C NMR data- (ppm, 100MHz, DMSO) δppm:162(C=O),120-128(C=C),20.9(CH3),131.1(C=N), 160.1 (OCH3)

(2- methylaryl methyldiene hydrazinyl)-2H-6- methoxy-chromene-2-one (III D2)

FTIR (KBr) (V\text{max cm}^{-1}): 1590(HN2), 1550(N-N), 1100(C-N), 1625 (C=O), 1675(C=C), 1395-1440(C-O-H), 1210-1320(O-C), 3030(C-H), 1575(C-C). 1H NMR data - (ppm,300MHz,TMS)-7.35(m,8H, Ar-H), 2.23 (s, 1H,NH), 6.15 (s, C-H), 3.78 (s,3H,OCH3), 2.32 (CH3).13C NMR data- (ppm,100MHz, DMSO) δppm:162(C=O),120-128(C=C),20.9(CH3),131.1(C=N), 58.2 (OCH3).

(2- nitro arylmethylidene hydrazinyl)-2H-6- methoxy-chromene-2-one (III D3)

FTIR (KBr) (V\text{max cm}^{-1}): 1590(HN2), 1550(N-N), 1100(C-N), 1625 (C=O), 1675(C=C), 1395-1440(C-O-H), 1210-1320(O-C), 1590-1450 (N-O stretching), 1575 cm-1 (C=C). 1H NMR data- (ppm, 300MHz,TMS)-7.7(m, 8H, Ar-H), 2.67 (s, 1H,NH), 6.54 (s, C-H), 3.20 (OCH3).13C NMR data- (ppm,100MHz, DMSO) δppm:162(C=O),120-128(C=C),20.9(CH3),131.1(C=N), 56.50 (OCH3).

The Antimicrobial evaluation

The synthesized compounds were evaluated for their in vitro antibacterial activity against bacteria: Salmonella typhi MTCC 8767, Candida albicans MTCC 3958, Bacillus substilis MTCC 1305, Proteus vulgaris MTCC 7299, Pseudomonas aeruginosa MTCC 3542, Staphylococcus aureus MTCC 9542, P. syringae MTCC 6725, Bacillus pumillus MTCC 8964, Staphylococcus epidermis MTCC 7919, Bordetella bronchiseptica MTCC 6837. Antimicrobial activity was assessed by agar disc diffusion technique. Ampicillin and Gentamycin was used as a standard drug for antibacterial Activity. The crude extracts were dissolved in 5 % dimethyl sulphoxide (DMSO). The antimicrobial assays of solvent extracts were performed by agar disc diffusion. The bacterial strain were activated by inoculating a loopful of the strains in the nutrient broth (30 ml), and incubated for 6 h to maintain McFarland standard turbidity (106 cells/ml) for bacterial strains. Later, 0.1 ml of inoculum of bacterial strain was inoculated into the molten Muller Hinton agar (Hi-media), spread uniformly into the Petri plate.

The test sample (40 μl) was introduced-Media and on later the allowed disc to dry. Then (6 the disc mm) of was impregnated on the seeded agar plates. The plates were allowed to stand for 1 h for pre-diffusion of the extract. The plates were prepared in triplicates and incubated at 37°C/24 h for bacterial strain. The antibacterial activity was taken on the basis of diameter of zone of inhibition (mm). All the experiments were done in triplicates Table 3.

Result and Discussion

In this study different novel compounds of 3(2-aryl methyldiene hydrazinyl)-2H-Chromene-2-one (IIIA1-D3) have been synthesized and evaluated for antibacterial activity. We described here a convenient and efficient protocol for the preparation of 3(2-aryl methyldiene hydrazinyl)-2H-chromene-2-one (III A1-D3). All compounds were synthesized according to Scheme 1. At the first stage, condensation of substituted salicylaldehyde was done with dimethyl malonate in the presence of a base (piperidine) to yield 2-oxo-2H-chromen-3yl-acetate. Further 2-oxo-2H-chromen-3yl-acetate was reacted with hydrazine hydrate to furnish 3-hydrazinyl 2H-chromene-2-one. Then these compounds were treated with different aromatic aldehydes to give 3(2-aryl methyldiene hydrazinyl)-2H-chromene-2-one. All the synthesized compounds showed significant antibacterial activity, against bacterial strain, S. typhi, C. albicans, B. substilis, P. vulgaris, P. aeruginosa, S. aureus, P. syringae, B. pumillus, S. epidermidis, Bordetella bronchiseptica as compared to the standard drug Gentamycin and ampicillin. Compound IIIA2, IIIB1, IIIB2, IIIC1 with its electron withdrawing substituents (bromo, Chloro and nitro group) on aromatic rings were found to be the most active against selected bacterial strain.
Table 3 In vitro antibacterial activity of the title compounds (IIIA₁-D₃)

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NA = not active  Diameter of the hole = 5 mm.

Conclusion

In this paper we report the synthesis of some 3(2-aryl methylidene hydrazinyl)-2H-Chromene-2-one (III A₁-D₃) derivatives and the antibacterial evaluation of some of the novel compounds. The preliminary in vitro antibacterial data demonstrated that the compound IIIA₂, IIIB₁, IIIB₂ and IIIC₁ has the most potent activity against all bacteria. This potency could be attributed to the presence of the electron withdrawing groups like nitro, bromo and chloro.

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References


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