Synthesis and antibacterial action of some new isocoumarin derivatives

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ABSTRACT

*With the aim of developing potential antimicrobials, a series of 3,4-disubstituted, 3,4,8 – trisubstituted isocoumarins (3a,b, 5a,b, 10a,b, 13a-f), incorporating nitrogen and oxygen as part of the heteroaromatic ring (piperidine, morpholine, dibenzofuran, coumarin), were synthesized and characterized by IR, ^{1}H NMR, Mass spectroscopy and elemental analysis. In addition their antifungal and antibacterial activities were tested in vitro for their growth inhibitory activities against pathogen fungi, Fusarium pallidoroseum, Chaetonium and against E.Coli, S.Aureus as gram negative and gram positive bacteria. A majority of the compounds were found to be remarkably active against gram negative bacteria. The relationship between the functional group variation and the biological activity of the evaluated compounds is discussed. Antifungal evaluation, in vitro, shows that derivative **6a** (Scheme Ib) displayed the highest antifungal and fungicidal activity against Chaetomium and **13b** (Scheme III) derivative displayed highest antibacterial activity against gram negative bacteria.*

Key words: isocoumarin derivatives, antibacterial activity, antifungal activity.

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INTRODUCTION

High throughput screening of the selected chemical libraries having heterocyclic or carbocyclic ring at their core is one of the most expeditious ways to search for useful medicinal activity. Heterocyclic lactones have long been a mainstay of organic synthesis due to their broad application to organic and medicinal chemistry. The heteroatom improves binding and the rigid cyclic framework, imparts rigidity, enhancing the selectivity, Nitrogen atom being one of them. There are several examples reported in literature where the presence of nitrogen atom in compounds in various form has shown tremendous therapeutic applications. In continuation to our effort to adopt heterocyclization chemistry to high throughput format, we chose to introduce nitrogen atom in isocoumarin moiety in the form of group's such as nitro or amines, to see their effect on the remedial features of isocoumarin.

Since in the last two decades, the incidence of invasive fungal infection has risen sharply. It has become imperative to enlarge the number of antifungal drugs with more potent activity and less toxicity.

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In the course of a medicinal chemistry programme aimed at discovering new heterocyclic lactones endowed with antibacterial and antifungal activities, we have synthesized a series of 3,4-disubstituted isocoumarins, 3,4,8-trisubstituted isocoumarins (Scheme III), aminyl benzoyl isocoumarins (Scheme Ib), coumarinoyl isocoumarins (Scheme II). The presence of aryl, alkyl group, oxygen-nitrogen hetero atoms or nitro group was an important factor to affect antibacterial and antifungal activities.

Isocoumarins are important components in many natural products that exhibit a broad range of biological activities including anti allergic [1-2], antimicrobial [3], immunomodulatory [4-5], cytotoxic [6], antifungal [7], anti-inflammatory [8-9], antiangiogenic [10-11] and differentiation inducing activity against leukemic cells [12]. Isocuomarins are also useful intermediates in the synthesis of a variety of important compounds including isoqunilone alkaloids [13]. Thus a number of methods have been reported in the literature for the synthesis of isocoumarins [14]. In the last 10 years only 3- substituted isocoumarins with no substituent at the 4th position have been synthesized either by traditional approaches [15-18] or by utilizing transition metal catalyzed reactions [19-21]. The synthesis of 3, 4-disubstituted isocoumarins have received considerable attention too [22-24] and recently a number of these compounds have been prepared via catalytic annulation of internal alkynes [24]. However, the catalytic methods are somewhat limited in synthetic scope since they are highly regioselective. But 4-alkyl-3-aroyl isocoumarins are still less known and so its biological activity.

Based on good biological activity in novel heterocyclic system we undertook the synthesis of a new series of compounds incorporating the above mentioned biological active moieties (coumarin, morpholine, piperidine, dibenzofuran etc.) to isocoumarins.

Here, we disclose a short and efficient synthesis of 4- alkyl-3-aroyl, 4-alkyl-3-aroyl-8-nitro, 4-alkyl-3-(4'-aminyl) benzoyl, and coumarinoyl isocoumarin derivatives starting with simple available compounds. The goal of this research was to develop an efficient and cheaper method for the synthesis of an isocoumarin library under mild and more environment friendly conditions with moderate yields.

MATERIALS AND METHODS

Chemistry

The reagents and the solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel GF254. IR were recorded on FTIR Perkin Elmer spectrophotometer and 1H NMR spectra on a Bruker spectrometer (400 MHz) using TMS as internal standard. Mass spectrums were recorded on Thermo Scientific Corporation, DSQ II Mass Spectrometer. All the compounds gave satisfactory elemental analysis. O-acyl benzoic acid **1** and bromo compounds **2** and **5a-f** were prepared by literature method [25-27].

General procedure for the synthesis of 4-Alkyl-3-(4'-aminylyl-benzoyl) isocoumarin 5a-8d

4-Methyl-3-(4'-bromo-benzoyl) isocoumarin (0.1 g, 0.00029 mole) (**3a**), piperidine (0.06 ml, 0.00059 mole) (**4a**), and 3.0 ml DMF were taken in a round bottom flask and refluxed for 8 hrs. After the reaction was over the reaction mixture was poured into ice and the solid obtained was filtered. The crude product was purified by column chromatography using pet. ether-ethyl acetate.

General procedure for the synthesis of 4-alkyl-3-(4'-coumarinoyl) isocoumarin 10a, b

o-acetyl benzoic acid (1 g, 0.006 mole) (**1a**), 3-bromo acetyl coumarin (1.627 g, 0.006 mole) (**9**), K$_2$CO$_3$ (1.76 g, 0.012 mole) and ethyl methyl ketone were taken in a round bottom flask and refluxed

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for 10-12 hrs. Solvent was then removed, water added and extracted with ethyl acetate. Solvent layer was first washed with sod. bicarbonate then with water and dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using pet. ether-ethyl acetate.

General procedure for the synthesis of 4-Alkyl-3-(4'-substituted benzoyl)-8-nitro isocoumarin 13a-f

6-Nitro acetyl benzoic acid (1 g, 0.0047 mole) (**11**), p-hydroxy bromo acetophenone (1.02 g, 0.0047 mole) (**12a**), K_2CO_3 (1.38 g, 0.01 mole) in ethyl methyl ketone was taken in a round bottom flask and refluxed for 10-12 hrs. Solvent was then removed, water added and it was extracted with ethyl acetate. Solvent layer was first washed with sod. bicarbonate then with water and dried finally over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using pet. ether-ethyl acetate.

4-Methyl -3-(4'-piperidin-1-yl-benzoyl) isocoumarin (5a)
86.23% yield; mp: 155^0C; ^{1}H NMR (CDCl$_3$): 1.7 (s,3H, CH$_3$), 3.3 (q, 4H, CH$_2$-N-CH$_2$), 2.8 (m, 6H, CH$_2$-CH$_2$-CH$_2$), 6.9-8.2 (m, 8H, Ar. Protons,): MS (ESI$^+$) m/z 348 [M+H]$^+$ Anal. Calcd (C$_{22}$H$_{21}$NO$_3$): C, 76.08; H, 6.05; N, 4.03 Found: C, 76.00; H, 6.11; N, 3.87.

4-Ethyl -3-(4'-piperidin-1-yl-benzoyl) isocoumarin (5b)
74.68% yield; mp: 130^0C; ^{1}H NMR (CDCl$_3$): 1.2 (t, 3H, CH$_3$), 1.6 (s, 6H, CH$_2$-CH$_2$-CH$_2$), 2.8 (q, 2H, CH$_2$), 3.4 (s, 4H, CH$_2$-N-CH$_2$) , 7.6-7.9 (m, 7H, aromatic protons), 8.42 (d, 1H, C$_8$-H); MS (ESI$^+$) m/z 361 [M]$^+$ Anal. Calcd (C$_{23}$H$_{23}$NO$_3$): C, 76.45; H, 6.37; N, 3.87 Found: C, 76.46; H, 6.77; N, 3.92.

4-Methyl -3-(4'-morpholin-1-yl-benzoyl) isocoumarin (6a)
68.0% yield; mp: 245^0C; ^{1}H NMR (CDCl$_3$): 1.6 (s, 3H, CH$_3$), 3.4 (q, 4H, CH$_2$-N-CH$_2$), 3.9 (q, 4H, CH$_2$-O-CH$_2$), 6.9-8.0 (m, 8H, Ar. Protons); MS (ESI$^+$) m/z 337 [M]$^+$ Anal. Calcd (C$_{20}$H$_{19}$NO$_4$): C, 72.20; H, 5.44; N, 4.01 Found: C, 72.26; H, 5.52; N, 4.31.

4-Ethyl -3-(4'-morpholin-1-yl-benzoyl) isocoumarin (6b)
66.0% yield; mp: 220^0C; ^{1}H NMR (CDCl$_3$): 1.0 (t, 3H, CH$_3$), 2.1 (q, 2H, CH$_2$), 3.2 (q, 4H, CH$_2$-N-CH$_2$), 3.6 (q, 4H, CH$_2$-O-CH$_2$), 6.8-8.4 (m, 8H, Ar. Protons); MS (ESI$^+$) m/z 349 [M-H]$^+$ Anal. Calcd (C$_{21}$H$_{20}$NO$_4$): C, 76.08; H, 6.05; N, 4.03 Found: C, 76.26; H, 5.72; N, 4.31.

4-Methyl -3-(4'-phenylamino -benzoyl) isocoumarin (7a)
68..43% yield; mp: 155^0C; ^{1}H NMR (CDCl$_3$): 2.4 (s,3H, CH$_3$), 3.4 (s, 1H, NH) 7.6-8.45 (m, 13H, Ar. Protons); MS (ESI$^+$) m/z 356 [M+H]$^+$ Anal. Calcd (C$_{23}$H$_{17}$NO$_3$): C, 77.74; H, 4.78; N, 3.94 Found: C, 78.01; H, 5.02; N, 4.11.

4-Ethyl -3-(4'-phenylamino -benzoyl) isocoumarin (7b)
61.85% yield; mp: 145^0C; ^{1}H NMR (CDCl$_3$): 1.1 (t,3H, CH$_3$), 1.9 (q, 2H, CH$_2$) 4.5 (s, 1H, NH) 7.4-8.45 (m, 13H, Ar. Protons); MS (ESI$^+$) m/z 368 [M-H]$^+$ Anal. Calcd (C$_{24}$H$_{19}$NO$_3$): C, 78.04; H, 5.14; N, 3.79 Found: C, 78.23; H, 5.34; N, 4.29.

4-Methyl -3-(4'- tolylamino -benzoyl) isocoumarin (8a)
65.13% yield; mp: 145^0C; ^{1}H NMR δ 1.9 (s, 3H, CH$_3$), 2.4 (s, 3H, CH$_3$), 3.4 (s, 1H, NH), 6.4-7.7 (m, 11H, aromatic protons), 8.0 (d, 1H, C$_8$-H); MS (ESI$^+$): m/z: 368 (M$^+$ - H) ; Anal. Calcd C$_{24}$H$_{19}$NO$_3$ (369.0 g): C, 78.04; H, 5.14; N, 3.79; Found: C, 78.01; H, 5.52; N, 4.11.

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4-Ethyl -3-(4'-tolylamino-benzoyl) isocoumarin (8b)

63.79% yield; mp:138^{0}C; ^{1}H NMR (CDCl$_3$): 1.0 (t, 3H, CH$_3$),2.1 (q, 2H, CH$_2$), 3.2 (s, 1H, NH), 2.4 (s, 3H, CH$_3$) 6.9-8.4 (m, 8H, Ar. Protons); MS (ESI$^+$) m/z 383 [M]$^+$ Anal. Calcd (C$_{25}$H$_{22}$NO$_3$): C, 78.32; H, 5.48; N, 3.65 Found: C, 78.16; H, 5.62; N, 3.88.

4-Methyl-3-(4'-coumarinoyl) isocoumarin (10a)

38.22% yield; mp: 182-185^{0}C; ^{1}H NMR (CDCl$_3$): 1.6 (s, 3H, CH$_3$), 6.8-8.4 (m, 9H, Ar. Protons); MS (ESI$^+$) m/z 333 [M+H]$^+$ Anal. Calcd (C$_{20}$H$_{12}$O$_5$): C, 61.01; H, 5.30 Found: C, 61.01; H, 5.08.

4-Ethyl-3-(4'-coumarinoyl) isocoumarin (10b)

40.02% yield; mp: 200^{0}C; ^{1}H NMR (CDCl$_3$): 1.0 (t, 3H, CH$_3$) , 2.0 (q, 2H, CH$_2$), 7.4-8.2 (m, 9H, Ar. Protons); MS (ESI$^+$) m/z 347 [M+H]$^+$ Anal. Calcd (C$_{21}$H$_{14}$O$_5$): C, 62.40; H, 5.60 Found: C, 62.00; H, 5.36.

4-Methyl-3-(4'-hydroxy benzoyl)-8-nitro isocoumarin (13a)

62.34% yield; mp: 124^{0}C; ^{1}H NMR (CDCl$_3$): 2.3 (s, 3H, CH$_3$), 5.3 (s, 1H, OH), 6.8-8.4 (m, 7H, Ar. Protons); MS (ESI$^+$) m/z 325 [M]$^+$ Anal. Calcd (C$_{17}$H$_{11}$NO$_6$): C, 62.76; H, 3.38; N, 4.30 Found: C, 62.76; H, 3.36; N, 4.37.

4-Methyl-3-(4'-bromo benzoyl)-8-nitro isocoumarin (13b)

61.19% yield; mp: 157^{0}C; ^{1}H NMR (CDCl$_3$): 1.8 (s, 3H, CH), 6.2-8.6 (m, 7H, Ar. Protons); MS (ESI$^+$) m/z 387.9 [M]$^+$ Anal. Calcd (C$_{17}$H$_{10}$NO$_5$Br): C, 52.59; H, 2.57; N, 3.60 Found: C, 52.12; H, 2.45; N, 4.08.

4-Methyl-3-(2',4'-dihydroxy benzoyl)-8-nitro isocoumarin (13c)

60.98% yield; mp: 105-107^{0}C; ^{1}H NMR (CDCl$_3$): 2.0 (s, 3H, CH$_3$), 6.8-8.0 (m, 5H, aromatic protons), 8.5 (d, 1H, C$_7$-H), 9.4 (s, 1H, OH), 9.6 (s, 1H, OH); MS (ESI$^+$) m/z 341 [M]$^+$ Anal. Calcd (C$_{17}$H$_{11}$NO$_7$): C, 59.82; H, 3.22; N, 4.10 Found: C, 60.05; H, 4.00; N, 4.22.

4-Methyl-3-(4'-methoxy benzoyl)-8-nitro isocoumarin (13d)

60.25% yield; mp: 156^{0}C; ^{1}H NMR (CDCl$_3$): 1.7 (-CH$_3$, s, 3H), 4.1 (s, 3H, OCH$_3$), 7.8-8.5 (m, 7H, Ar. Protons); MS (ESI$^+$) m/z 339 [M]$^+$ Anal. Calcd (C$_{18}$H$_{13}$NO$_6$): C, 63.71; H, 3.83; N, 4.12 Found: C, 63.41; H, 3.82; N, 4.33.

4-Methyl-3-(4'-phenyl benzoyl)-8-nitro isocoumarin (13e)

70.37% yield; mp: 76^{0}C; ^{1}H NMR (CDCl$_3$): 2.3(s, 3H, CH$_3$), 7.4-8.4(m, 12H, aromatic protons); MS (ESI$^+$) m/z 386 [M+H]$^+$ Anal. Calcd (C$_{23}$H$_{15}$NO$_5$): C, 71.68; H, 3.89; N, 3.63 Found: C, 71.24; H, 3.82; N, 3.92.

4-Methyl-3-(4'-dibenzofuryl)-8-nitro isocoumarin (13f)

72.04% yield; mp: 100^{0}C; ^{1}H NMR (CDCl$_3$): 2.2 (s, 3H, CH$_3$), 6.8-8.2 (m, 10H, aromatic protons); MS (ESI$^+$) m/z 398 [M-H]$^+$ Anal. Calcd (C$_{23}$H$_{13}$NO$_6$): C, 69.17; H, 3.25; N, 3.50 Found: C, 68.94; H, 3.06; N, 3.47.

Antimicrobial Activity

Antimicrobial activity of the target compounds were tested in vitro against bacterial strains *E. Coli* (gram negative)*, S. Aureus* (gram positive) and fungal strains *F. Pallidoroseum* and *Cheatomium* by using serial agar dilution (cup plate method) [28] and Potato Dextrose Agar medium (Poisoned Food Technique) [29] respectively.

The two microorganisms were cultured in dishes containing agar medium. Four cups (8 mm) were put onto the dishes and each tested compound (0.1ml of 2mg/ml) was then added into the cups under aseptic condition. Then the dishes were incubated at 37^{0}C for 24h. The zone of inhibition of the growth of the bacteria, which was produced by diffusion of the compounds from the cup into the surrounding medium, was measured in milli meters to evaluate the antibacterial activity. Each experiment was repeated twice. DMF was used as a positive control for all the experiments.

The standard fungal culture*, F. pallidoroseum & Chaetonium* were grown on PDA slants at room temperature. Mycelial growth inhibition of *F. pallidoroseum & Chaetonium* was evaluated by the poisoned food technique, where the inhibition in growth of the fungal strain was observed on PDA. The stock solutions (1000ppm) were made from each of the test compounds. The required % concentrations of the compounds (mg/ml) were obtained by mixing the appropriate amount of the stock solution with 20 ml of molten PDA. The amended PDA was poured into Petri dishes and allowed to set.

An inoculum of the fungus obtained from 7 days old axenic culture, grown as above, was placed at the centre of the amended agar medium. Each experiment was performed in triplicate. The diameter of the fungal colony was measured after 4 days, then after 7 days at $26+1^{0}$C and the % inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Growth area in reference - growth area in sample}}{\text{Growth area in reference}} \times 100$$
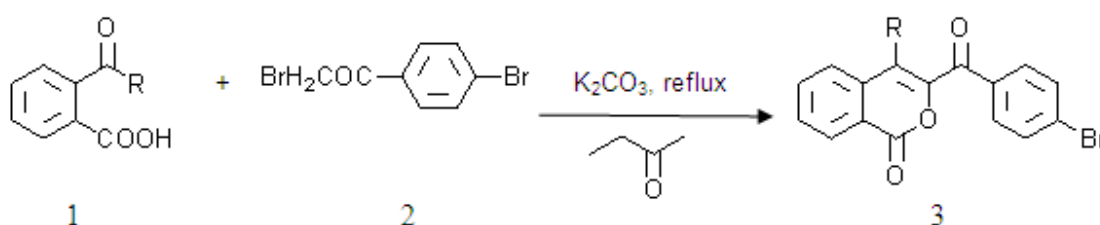
RESULTS AND DISCUSSION

The new isocoumarin derivatives were synthesized in one step process as per scheme (Ia, II, III) condensing   o-acyl benzoic acid with bromoacetophenone derivatives in ethyl methyl ketone in presence of anhy. K_2CO_3 for 10-12 hrs. Appearance of –CO stretching at 1720 - 1768 cm^{-1}, 1537 - 1669 cm^{-1} for aroyl and lactone carbonyl and C-Br stretching at 739 cm^{-1} in IR spectrum and appearance of peak at δ 2.4, δ 1.6, δ 2.7 ppm, δ 6.8 -8.4 ppm in ^{1}H NMR spectrum and comparison of melting points confirmed the structure of compounds Scheme I. Synthesis of aminyl benzoyl isocoumarin derivatives 5a,b-8a,b, Scheme Ib were readily accomplished by refluxing 3 with various primary and secondary amines in presence of DMF as solvent. All the synthesized compounds were purified by column chromatography using petroleum ether- ethyl acetate (80:20) as eluent. Yield of final compounds were in the range of 70 %. In all the compounds of Scheme Ib, appearance of C-N stretching 1238 cm^{-1} in IR and peaks at δ 3.9(-CH$_2$ – O- CH$_2$) ppm , δ 3.6 (-CH$_2$ – N- CH$_2$) ppm, δ 3.4 (-NH), δ 7.3 – 7.9 ppm (aromatic protons) in ^{1}H NMR spectrum followed by elemental analysis and mass spectra confirms the structure (Scheme Ib). In Scheme III, appearance of NO$_2$ stretching at 1344 cm^{-1}, stretching at 3185 cm^{-1} for -OH in IR spectrum and peak at δ 8.4 ppm for proton at C$_7$, which may be probably due to deshielding by nitrogen. Physical data and elemental analysis further confirms the structures of all the compounds. Antimicrobial activity was assessed by the ability of these compounds to inhibit the zone formation induced by *S. Aureus* (gram positive), *E.Coli* (gram negative)

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bacteria and *F. pallidoroseum* and *Chaetomium* fungi. Isocoumarin 3 was selected as lead compound and substitution was carried out in in aroyl group and phenyl ring of isocoumarin moiety. Majority of the synthesized compounds exhibited considerable antibacterial and antifungal activity as evident from their high zone of inhibition given in Table 1 compared to the standard drug Ampicillin.
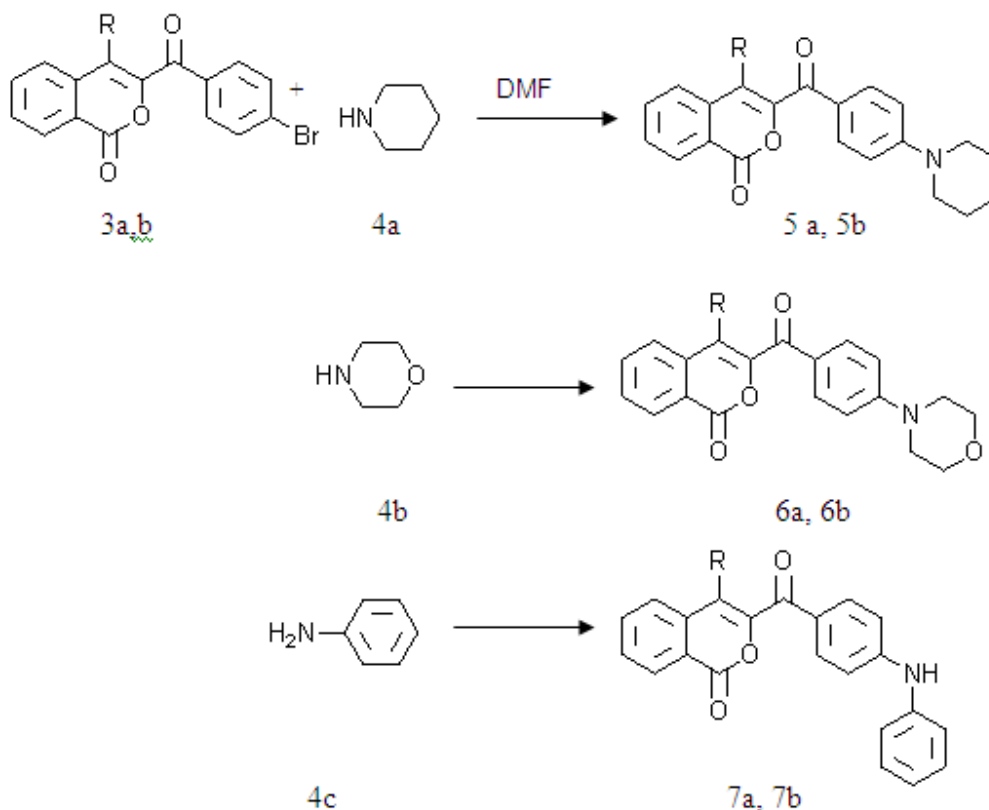
Structure Activity Relationship

A comparison of the compounds activity with that of standard antibiotic ampicillin is effectively presented in (Figure 1, 2). The compounds selected for antibacterial screening were **6a, b, 7a, b, 8a, b, 10a, b** and **13a, b, d**. Activity was moderate with all compounds but was excellent with 10a and 13b against gram positive *S. Aureus*. It is interesting to note that length of alkyl chain did not show any significant activity. Coumarin moiety incorporated in isocoumarin, **10a**, does make a difference and shows highest zone of inhibition. While 4 – bromo substituted aroyl isocoumarin derivative **13b** shows better activity when compared to other groups.

Scheme Ia

Scheme Ib

1a & 3a, R = CH₃, 1b & 3b, R = C₂H₅

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Scheme II

10a , R = CH$_3$

10b, R = C$_2$H$_5$

The same series of compounds were screened against gram negative *E. Coli* bacteria. Significant activity was seen with all the compounds and exhibited even better activity than standard antibiotic ampicillin (Figure 2). 4-Br substituted aroyl isocoumarin shows the excellent activity against same bacteria, giving the conclusion that it is equally effective against both species. Literature survey also supports its role in showing the activity [29]. It is worth noting that in this series, **13d**, (Scheme III), which bears the methoxy group at the C-4 carbon atom of aroyl substitution displayed more potency, than its demethoxylated analogue **13a**. Significant efficacy of methoxy group is also supported in literature [30]. The active entry **13b** was compound with NO$_2$ incorporated in isocoumarin ring and Br at p- position of aroyl group which stressed the importance of this residue in antibacterial activity against E.Coli. Rest all compounds did show better activity than standard antibiotic.

Scheme III

12 a &13a, Ar = ⬡—OH

12b&13b, Ar = ⬡—Br

12c&13c, Ar = ⬡ (HO, OH)

12d&13d, Ar = ⬡—OCH₃

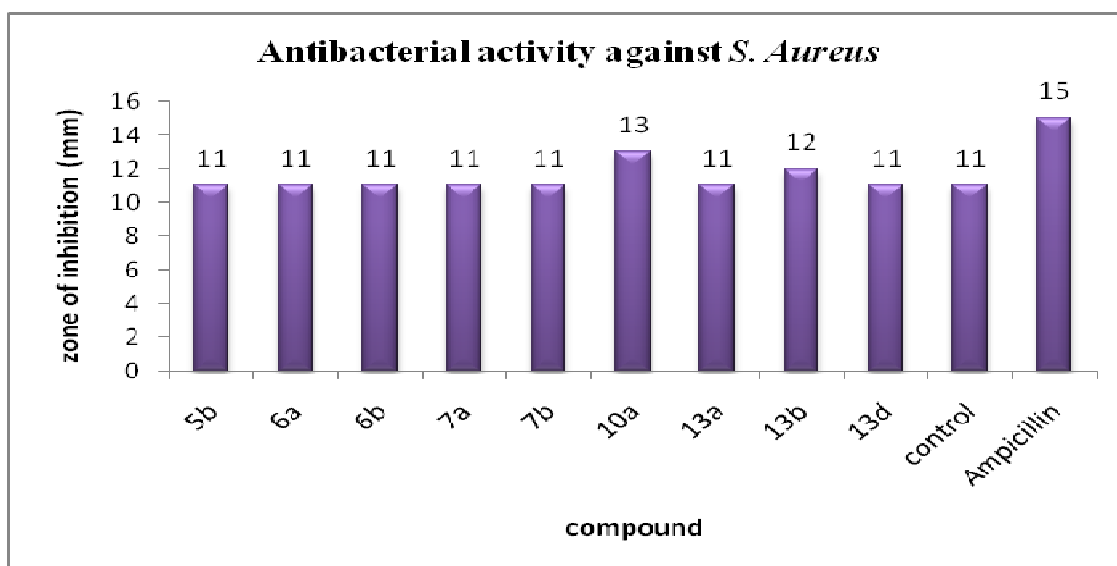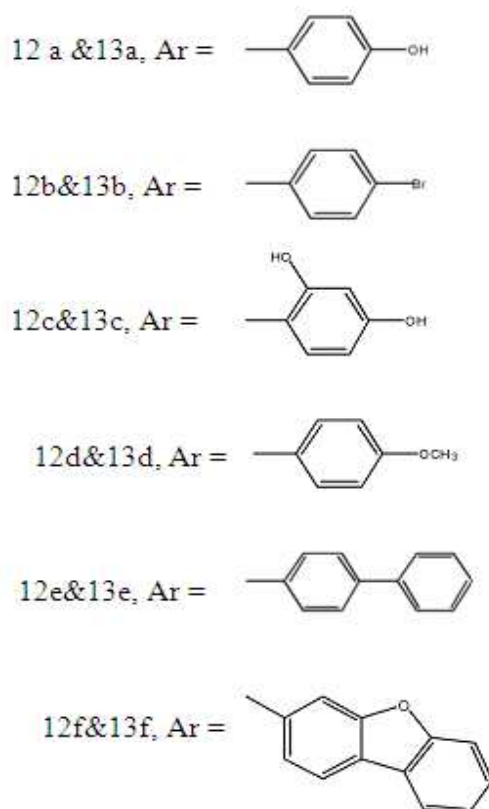12e&13e, Ar = ⬡—⬡

12f&13f, Ar = (dibenzofuran)

Figure: 1

The antimicrobial screening was further extended to antifungal activity. Here, compounds **6a, b, 7a, b, 8a, b, 10a, b** and **13a, b, d** against *Fusaruim Pallidoroseum* and *Chaetomium* was carried out. Comparison of antifungal activity of compounds with the standard drug nystatin against *Chaetomium,* showed that 100% inhibition with **6a** (Scheme Ib) where both N & O heteroatom were present in the form of morpholine moiety at the 4th position of aroyl substitution.
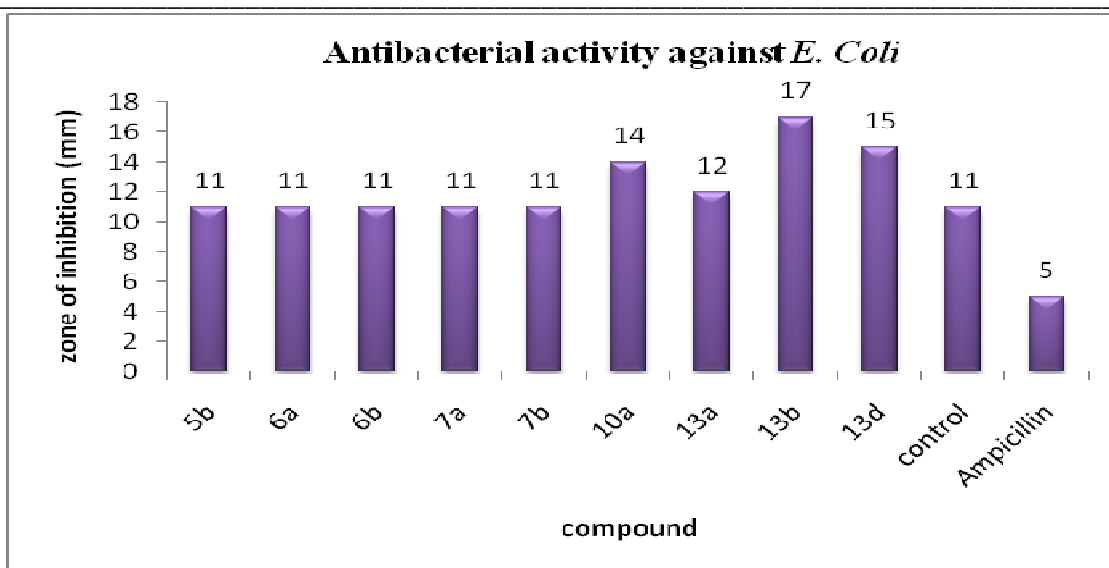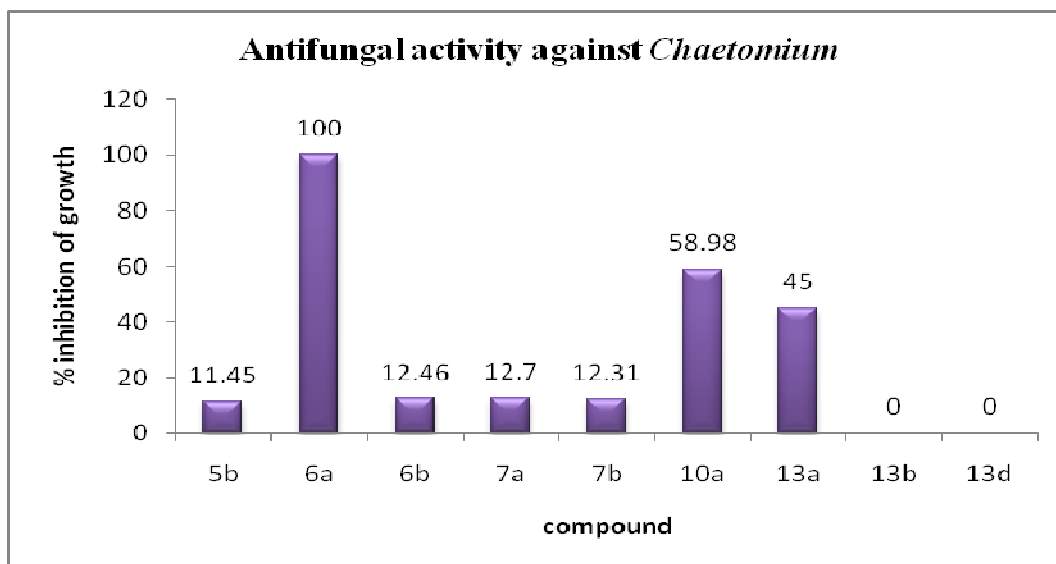
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Figure: 2

Figure: 3

Moderate activity was found with **10a** and **13a** (Scheme II & III). It is worth noting that heteroatoms improve binding and the rigid cyclic framework enhances selectivity. In contrast to antibacterial activity, demethoxylated analogue, **13a**, displayed more potency than methoxylated analogue **13d**. **10a** showed excellent antifungal activity, **13a** was moderate against second fungus, *Fusarium Pallidoroseum*. Rest all compounds activity was insignificant. Presence of heteroatom (**10a**) and OH group at the 4th position of aroyl substitution (**13a**) signifies role of electron density for showing biological activity.
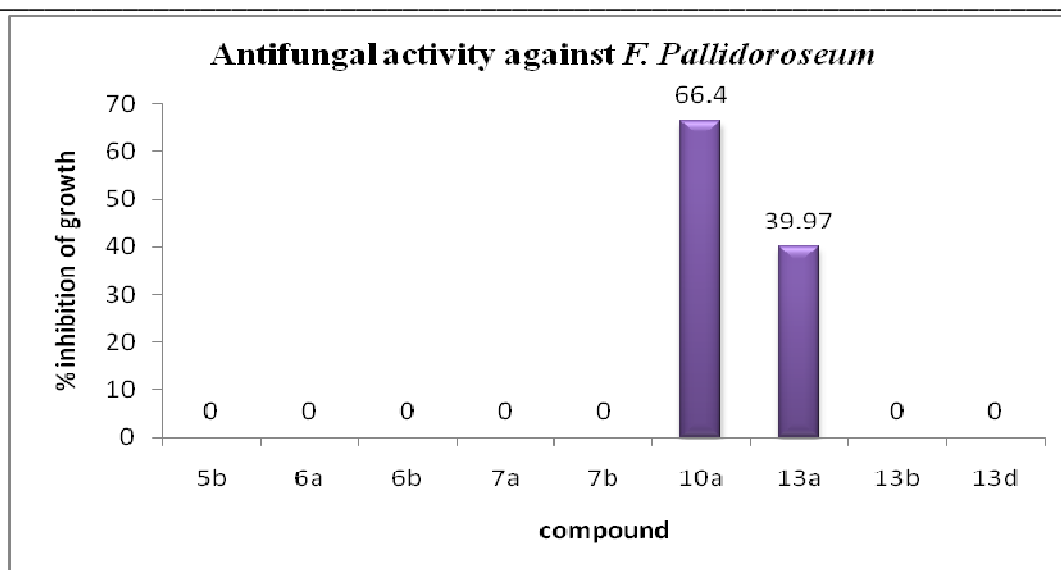
Figure: 4

CONCLUSION

In conclusion, we have synthesized a series of 3,4-disubstituted isocoumarins **3**, aminyl benzoyl isocoumarins **5-8**, coumarinoyl isocoumarins **10** and 3,4,8-trisubstituted isocoumarins **13**. Amongst the potent compounds **10a** have shown significant antifungal activity against *F. pallidoroseum* and *Chaetomium* while **6a** was effective against *Chaetomium*. Compounds **13b**, **13d** exhibited marked antibacterial activity against *E. coli* and compounds **10a**, **13b** were equally effective against *S. aureus*.

Acknowledgement

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