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Synthesis of new biologically active triazolo, tetrazolo and coumarinoyl derivatives of isocoumarins

Poonam Koppula[1*] and Nalini Purohit[2]

[1] *School of Science and Education, Navrachana University, Vadodara - 391410*

[2] *Department Of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara – 390002*

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Abstract: With the aim of developing potential antimicrobial agents, a series of triazolo / tetrazolo isoquinolines incorporating both nitrogen and oxygen as part of the hetero aromatic ring were prepared from 4-alkyl-3-aroyl isocoumarins via a number of intermediate steps. Some novel coumarinoyl derivatives of isocoumarins were also synthesized starting from 4-alkyl-3-aroyl isocoumarin and characterized by FTIR, ^{1}H NMR, Mass Spectroscopy and elemental analysis. All the title compounds 7a,b, 8a,b, 10a-g were evaluated for their antibacterial activity against *S. Aureus* (as gram positive), *E. Coli* (gram negative bacteria) and antifungal activity against *Fusarium pallidoroseum & Chaetonium in vitro* and analgesic activity which was tested *in vivo* on mice. Most of the compounds showed appreciable results for all the three activities. The relationship between the functional group variation and biological activity of the evaluated compound is discussed. The new compounds emerged as potential molecule for further development.

Keywords: Isocoumarins; triazolo isoquinolines; tetrazolo isoquinolines; coumarinoyl isocoumarin; antibacterial; antifungal; analgesic activities.

1. Introduction

Carbon containing nitrogen ring system undoubtedly belong to the most important heterocycles in nature as it takes part in many biologically significant reactions and are developed as pharmacologically active compounds or drugs.

Research on a new substance possessing antimicrobial activity has attracted considerable attention owing to the continuous increase in the bacterial resistance[1]. Further, infection caused by various microorganisms pose a serious challenge to the medical community and need for an effective therapy has led to the search for novel antimicrobial agents[2]. The pharmacologically important heterocycles with nitrogen bridge derived from 1,2,4-triazole and tetrazole paved the way toward active research in triazole-tetrazole chemistry. As a result, a variety of new improved compounds are being added to this field every year. A number of attempts were made to improve the activities of the compounds varying the substitution on the triazole and tetrazole nucleus. The need for safe and effective systemic antifungal agents has intensified due to the rapid growth in the number of immuno compromised patients.

1,2,4-Triazole moiety appears frequently in the structure of various natural products[3] and the synthesis of compounds incorporating this moiety has attracted widespread attention of chemists as well as

* Corresponding author: E-mail: poonam.yadav970@gmail.com

biologists, mainly due to their diverse biological activities in pharmaceutical and agrochemical fields[4-17]. A variety 1,2,4-tiazole derivatives possess antibacterial[4,5] ,antifungal[4], anti mycobacterial[12], antiviral[8], anti-inflammatory[9], anticonvulsant[10], antidepressant[11], antitubercular[12], antitumoral[13], antihypertensive[14], analgesic[15], enzyme inhibitor[16], hypoglycemic[17], sedative, hypnotic, antiparasitic, herbicidal, insecticidal and plant growth activities. Thus, several potent drugs possessing triazole nucleus have been applied in medicine, like, Alprazolam (anxiolytic agent, tranquilizer), Anastrozole, Letrozole, Vorozole (antineoplastics, nonsteroidal competitive aromatase inhibitors), Estazolam (hypnotic, sedative, tranquilizer), Etoperidone (antidepressant), Fluconazole, Itraconazole, Terconazole (antifungal agents), Ribavirin (antiviral agent), Benatradin (diuretic), Rilmazafon (hypnotic, anxiolytic, used in the case of neurotic insomnia), Nefazodone (antidepressant, 5-HT2 A-antagonist), Rizatriptan (antimigraine agent)[18] .

Tetrazoles are useful reagents in heterocyclic synthesis and are widely used in ring cleavage/ring closure reactions with electrophilic reagents to form new C–N and N–N bonds[19]. Tetrazoles are regarded as biologically equivalent to the carboxylic acid group, and extensive work on the synthesis of tetrazoles has been carried out in the field of material sciences, pharmaceuticals, explosives, and photography [20]. The synthesis of 5-substituted 1H-tetrazoles from nitriles has received much attention recently, and new preparative methods have appeared for the same [21-22]. Also, tetrazole functional group is much less abundant but the use is increasing due to the excellent properties as a metabolically stable isosteric replacement for the carboxylic acid moiety [23] and as a cis-peptide bond mimetic[24]. Tetrazoles have also been used as precursors to other heterocycles[25] and in high energy compounds[26].

Triazolo and tetrazolo isoquinolines are known to posse's good pharmacological properties large numbers of such compounds have been synthesized by different routes which involve 1-hydrazoisoquinolines as intermediate[27-28]. Synthesis of this intermediate itself is quite difficult and comprises large number of steps.

In view of these observations and continuation of our earlier work on the synthesis and biological activity of isocoumarin derivatives, the present work was undertaken to prepare a series of triazolo and tetrazolo isoquinoline derivative adapting simple steps. A different route was adapted for coumarinoyl derivative of isocoumarin.

Numerous compounds with biological activity have been investigated, however many of them are not suitable for therapeutic use due to their toxic, carcinogenic and mutagenic properties. Nowadays, it is possible to make modifications of active chemical structures, in order to synthesize compounds with improved therapeutic activity and reduced toxicity.

Many naturally occurring organic compounds, as well as synthetic compounds, having oxygen as heteroatom exhibit interesting pharmacological properties.

Oxygen heterocyclic compounds have been shown to inhibit a range of fungi and bacteria[29-32]. It is believed that these cyclic compounds represent a starting point for the exploration of new derivatives possessing a range of antimicrobial activity. Various interesting biological activities are associated with naturally occurring coumarin, isocoumarins and their derivatives which have found extensive application in the field of medicine and agricultural chemistry. Isocoumarins and its derivatives are an important type of oxygen containing aromatic heterocyclic compound, having various functional groups[33-36], through which coumarin moiety can be introduced into the structurally rigid isocoumarin parent compound.

Numerous researchers have focused on the isolation, purification and biological activity of coumarin and isocoumarin separately[37-39], however both moiety together have attracted special attention, as far as biological activity is concerned since both show good antimicrobial activities. However to the best of our knowledge, isocoumarin derivatives having coumarin moiety have not been reported earlier. Based on these findings, we describe the synthesis of some compounds featuring different heterocyclic rings fused onto the isocoumarin moiety with the aim of obtaining more potent pharmacologically active compounds and a different route was adapted for the same.

During this study various new isocoumarins incorporating triazoles, tetrazoles and coumarin moiety were synthesized. The structures of all these compounds were established through spectroscopic techniques. All the compounds were screened for antimicrobial and analgesic activities.

2. Results and discussion

During our studies on isocoumarins we have found that isocoumarins can be easily converted to 1-hydrazinoisoquinoline (Figure 4). This promoted us to carry out synthesis of some triazolo- and tetrazolo – isoquinolines by making use of isocoumarins as starting materials.

Based on good biological activity in novel heterocyclic system, we undertook the synthesis of a new series of compounds incorporating the above mentioned biologically active moieties (triazole, tertazole, coumarin) in to 4-alkyl-3-aroyl isocouamrins (Figure 4 & 7).

The isocoumarins **3** were prepared from o-acyl benzoic acid **1** and 2- bromo acetyl dibenzofuran **2** using the reported method[40] (Figure 4). The isocoumarins **3a-b** showed IR absorption frequencies at 1735, 1600 and 1266 cm^{-1} for ϒ lactone, -C=O and C-O respectively. ^1H NMR signals are obtained at δ 2.1 (s, 3H, CH_3), 7.3-8.0 (m, 10H, aromatic protons), 8.2 (d, 1H, C_8-H) and Mass spectrum showed *m/z* at 355 (M^+ + 1), 340, 311, 264, 174, 160 and 146. The isocoumarins **3a-b** were converted to isoquinolone derivative **4a-b** on reaction with liquor ammonia in ethanol. Simple chemical tests (Functional Group Analysis) revealed the presence of amide group in **4** and nitrogen confirmed by elemental analysis. The resultant isoquinolones **4** were then converted to 1-chloro isoquinolines **5** by treatment with $POCl_3$ - PCl_5. Here, presence of halogen was proved by the absence of amide carbonyl in IR spectra. **5** on treatment with hydrazine afforded the required 1- hydrazinoisoquinolines **6**, and the absence of Cl group was confirmed by elemental analysis. After the confirmation of **6**, two different pathways were carried out, one in which **6** was condensed with HCl / $NaNO_2$ resulting cyclization of hydrazine group into tetrazole derivative **7** in quantitative yield, and in second pathway, **6** was condensed with formic acid which via cyclization resulted in triazole derivative **8**. The IR spectra's of tetrazolo isoquinolines **7** and triazolo isoquinolines **8** showed absorption at 1692, 1645, 1567, 1497 and 1675 1620, 1559, 1494 cm^{-1} for –C=O, C=N, C=C, C-N respectively In both compounds **7** & **8**, the proton at 8th position, ortho to hetero atom, appeared as doublet at δ7.9, which in isocoumarin **3** appeared at δ8.4 showing the absence of oxygen and introduction of nitrogen heterocyclic moiety and finally confirms the formation of tetrazole / triazole isoqunolines.

The syntheses of 4-alkyl-3- coumarinoyl isocoumarins **10a-g** were achieved with an efficient synthetic route outline in (Figure 7). The isocoumarins **9a, b, e** were prepared by condensing different o-acyl benzoic acids such as acetophenone – o – carboxylic acid **1a** and propiophenone – o – carboxylic acid **1b** with p-hydroxy bromoacetophenone **2b** in presence of anhy. K_2CO_3 using ethyl methyl ketone as solvent by reported method[41] Scheme I. **9c** & **9d** were prepared by same method as given above. Here in place of o-acyl benzoic acid, 6-nitro-o-acetyl benzoic acid **1c**[42] was used. **9f** and **9g** were prepared by condensing 2-carboxy benzaldehyde with p-hydroxy bromoacetophenone by reported method[43]. Here bromo derivative was same but 2-carboxy benzaldehyde **1d** was used instead of o-acyl benzoic acid. The isocoumarins formed were then subjected to cyclization by reacting them with ethyl acetoacetate in presence of conc. sulfuric acid which was used as a catalyst and solvent both to get the target compounds **10** in good yield (Pechmann condensation). They showed lactonic absorption at 1706.24 cm^{-1} and carbonyl absorption at 1604.26 cm^{-1}. Characteristic signals in ^1H NMR were obtained at δ 2.3 (s, 3H, C_4' –H), 6.8-8.1 (m, 7H, aromatic protons), 8.2 – 8.4 (d, 1H, C_8-H).

2.1. Antibacterial activity:

Antibacterial activity of newly synthesized compounds was tested in vitro in bacterial strains, *Staphylococcus aureus* and *Escherichia coli* using serial agar dilution (cup plate method).[44] 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 added into the cups under aseptic condition. Then the dishes were incubated at 37^0C for 24h. The zone of inhibition of the growth of the bacteria, which were produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity. Each experiment was repeated twice. DMF was used as a positive control for the experiments and the results were compared against standard drug ampicillin.

Antibacterial results were interpreted in terms of the diameter of the inhibition zone for antibacterial activity. The experiments have revealed that all the isocoumarins synthesized showed good results

against gram negative bacteria i.e *E.Coli* with the zone of inhibition but the activity was found maximum with triazole- isoquinoline derivative. All the compounds showed excellent activity against gram positive bacteria, *S. Aureus* because zone of inhibition for control was zero. The antibacterial data of coumarinoyl derivative **10** indicates a strong and better activity against gram positive organism, which was almost equivalent to the reference drug. However, all the compounds were moderately active against gram negative bacteria. The antibacterial data also revealed that the introduction of the nitro group at 8th position of isocoumarin ring and absence of alkyl groups at the 4th position of the isocoumarin moiety enhances the activity against both the bacteria.

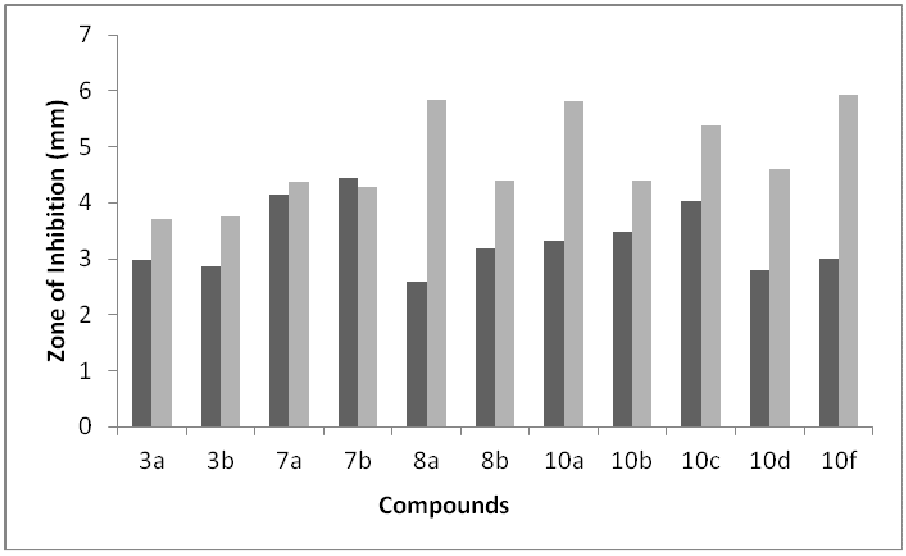

Figure 1. Antibacterial activity: S. Aureus – black; E.Coli – gray

2.2. Antifungal activity:

The title compounds were screened for antifungal activity. It was performed in vitro against fungal strains *Fusarium pallidoroseum and Colletotrichum capsici* using Potato Dextrose Agar Medium (Poisoned Food Technique) [45].

The standard fungal culture *F. pallidoroseum & C. capsici* were grown on PDA slants at room temperature. Mycelial growth inhibition of *F. pallidoroseum & Cheatonium* 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 and then 7 days at 26+1^{0}C and the % inhibition was calculated using the following equation:

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

The antifungal screening data showed appreciable activity of the test compounds. Among test compounds, 8a,b, 10b-d, 10 f showed remarkable activity against chaetonium. 7a,b were found to be moderate and 3a,b were reasonably good.

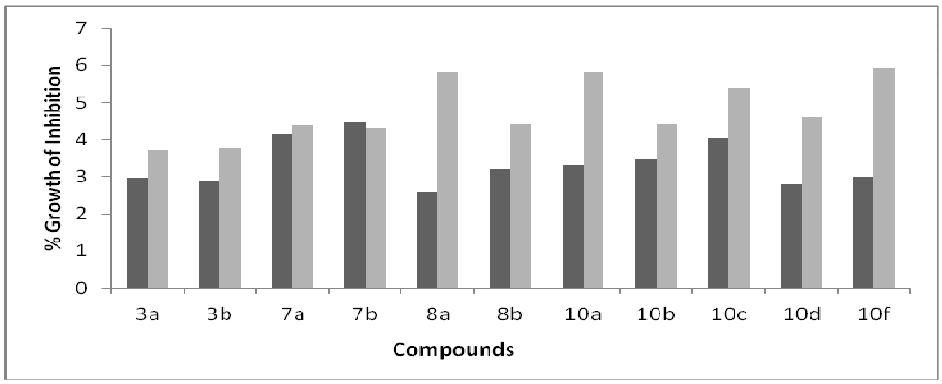

Figure 2. Antifungal Activity: Fusarium Pallidoroseum – black; Chaetonium – gray

2.3. Analgesic activity:

Analgesic activity of the compounds was determined by Tail flick method [46]. Mice of either sex weighing between 20-25 g which shows positive response were selected and divided into different groups with four mice in each group. The first group served as control which received 2% gum acacia. Second group served as standard which received analgin at a dose of 50 mg/kg body weight orally. Rest groups received test compounds at a dose of 50 mg/kg body weight of mouse, orally. The tail of the mouse was dipped (up to 5 cm) in a water bath at 55 ± 0.7^0C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cut-off time being 60 seconds. The first reading was taken immediately after administration of the standard drug and test compounds and afterwards at the intervals of 30 minutes. The response time was recorded.

The results obtained showed that **8a & 8b** having tetrazole nucleus were found to be most effective in reducing the pain, than **7a & 7b** having triazole nucleus**,** which in turn was better than simple isocoumarin moiety **3a & 3b**, when compared to the standard drug . The presence of isocoumarin – coumarin moiety together (Figure 7), enhanced the analgesic activity on comparison with all other compounds of the same series and gave average reaction time comparable to the standard drug. The change in the functional groups such as alkyl chain at the 4^{th} position of the isocoumarin does not make remarkable effect in the analgesic activity.

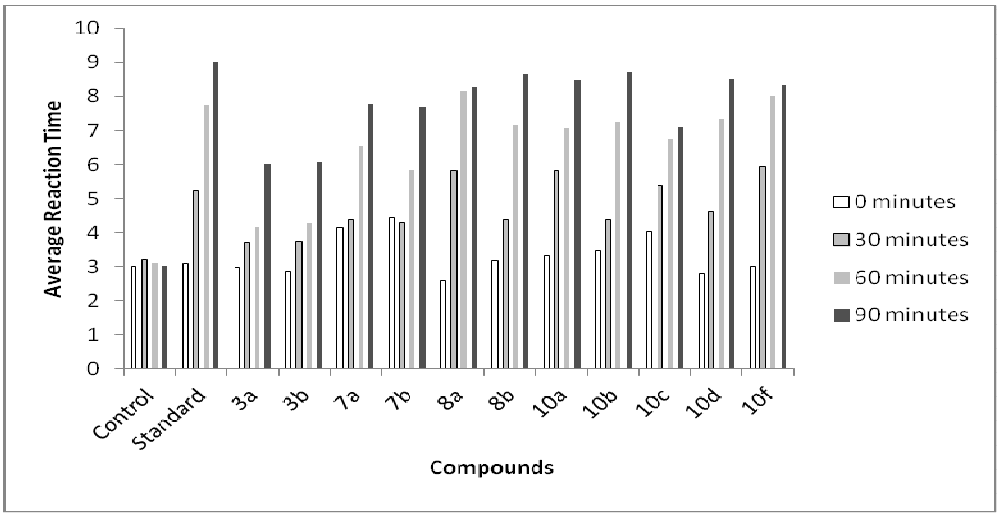

Figure 3. Analgesic Activity: white – response time after 0 minutes; gray with border – response time after 30 minutes; gray without border – response time after 60 minutes; black – response time after 90 minutes

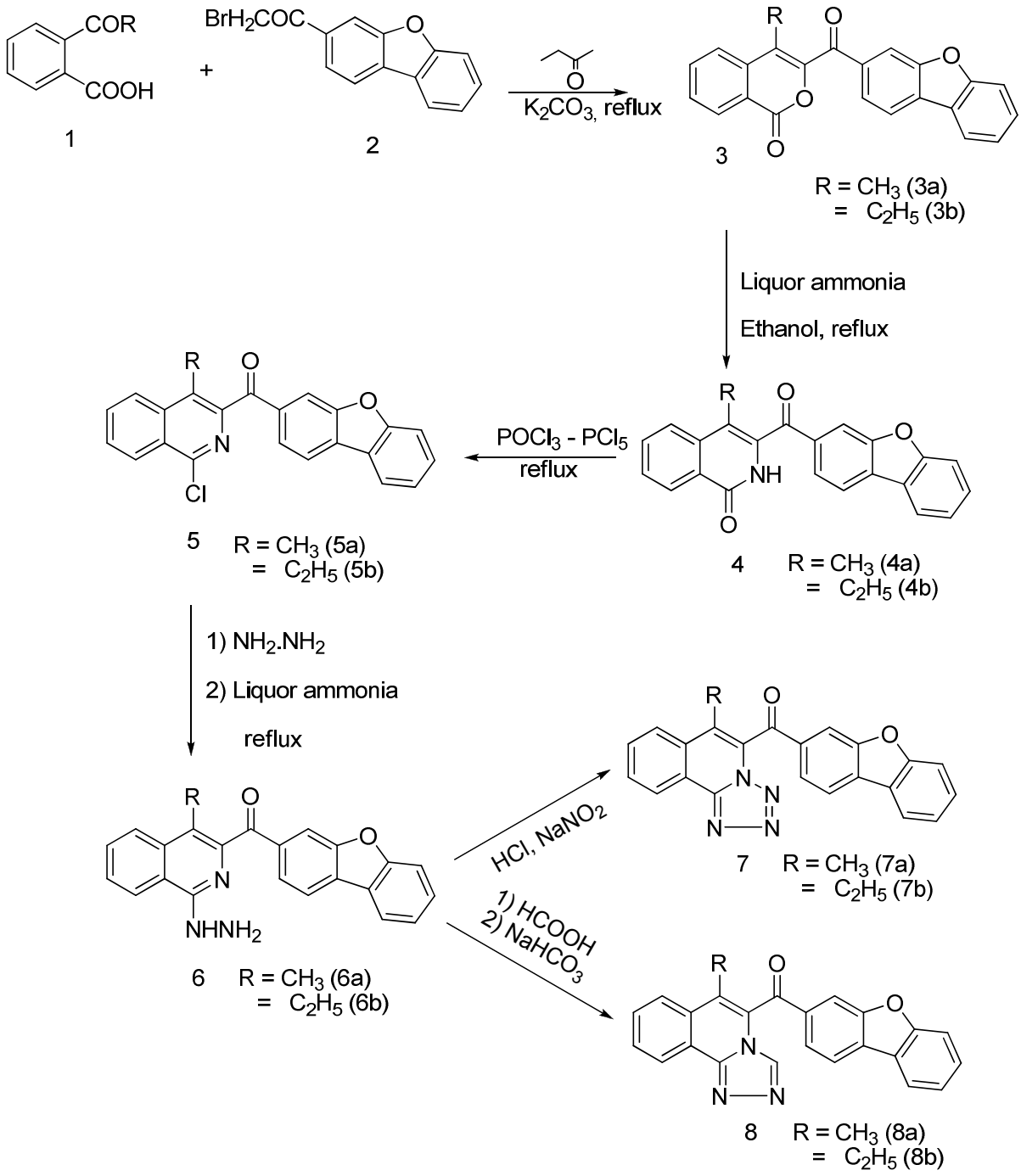

Figure 4. Synthesis of Tetrazolo and Triazolo Isoquinolines

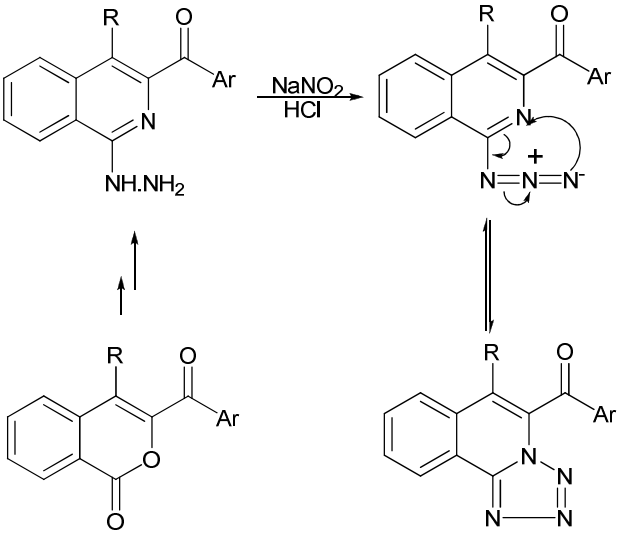

Figure 5. Mechanism;Tetrazole isoqunolines (Tetrazole – Azide Tautomerism)

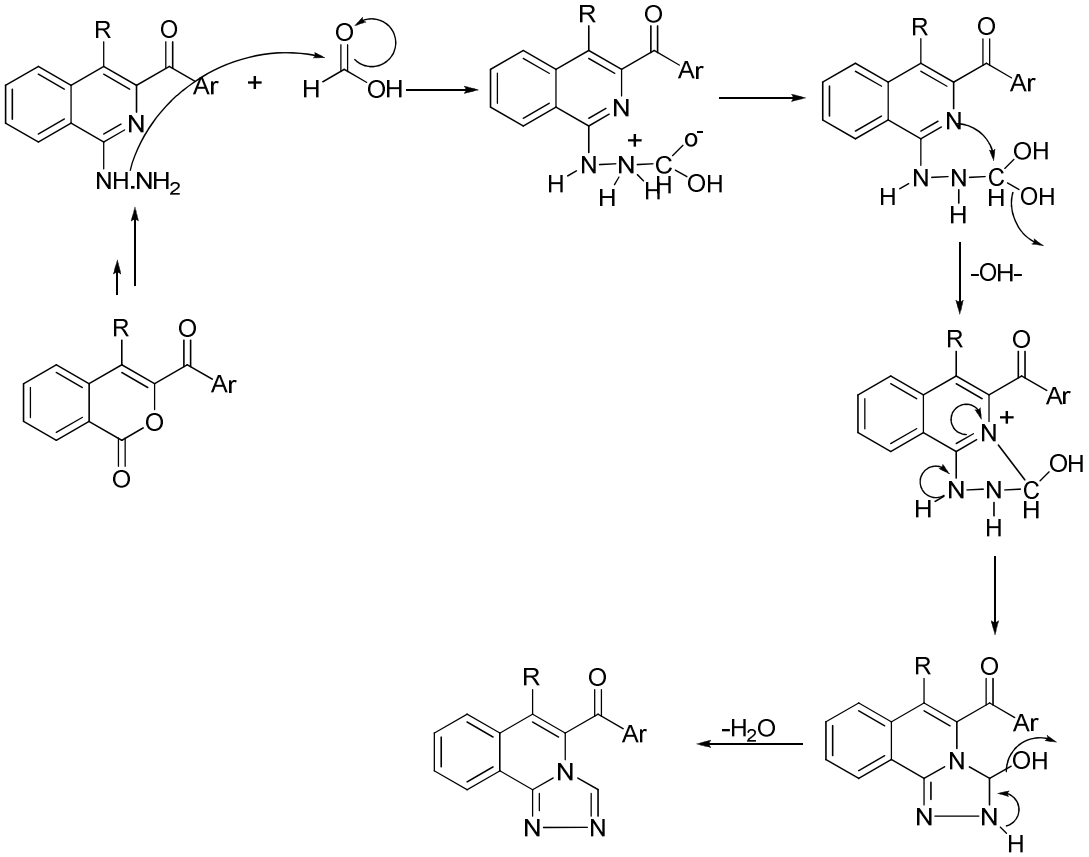

Figure 6. Mechanism; Triazole isoqunoline

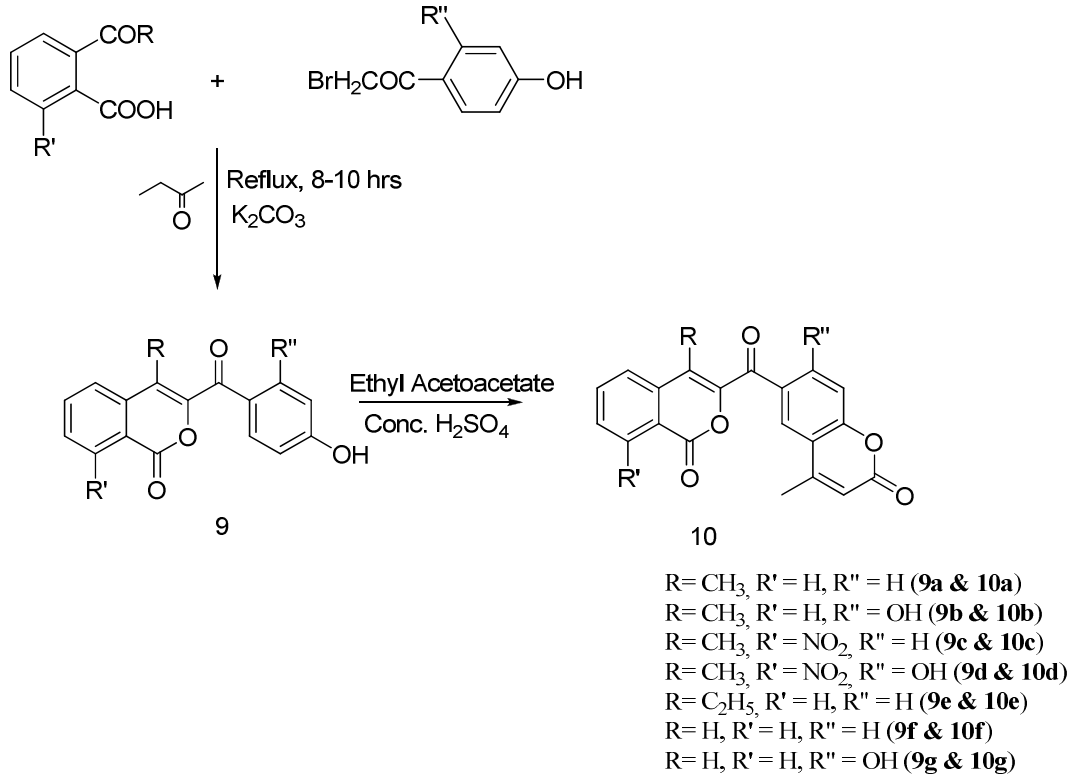

Figure 7. Synthesis of coumarinoyl isocoumarins

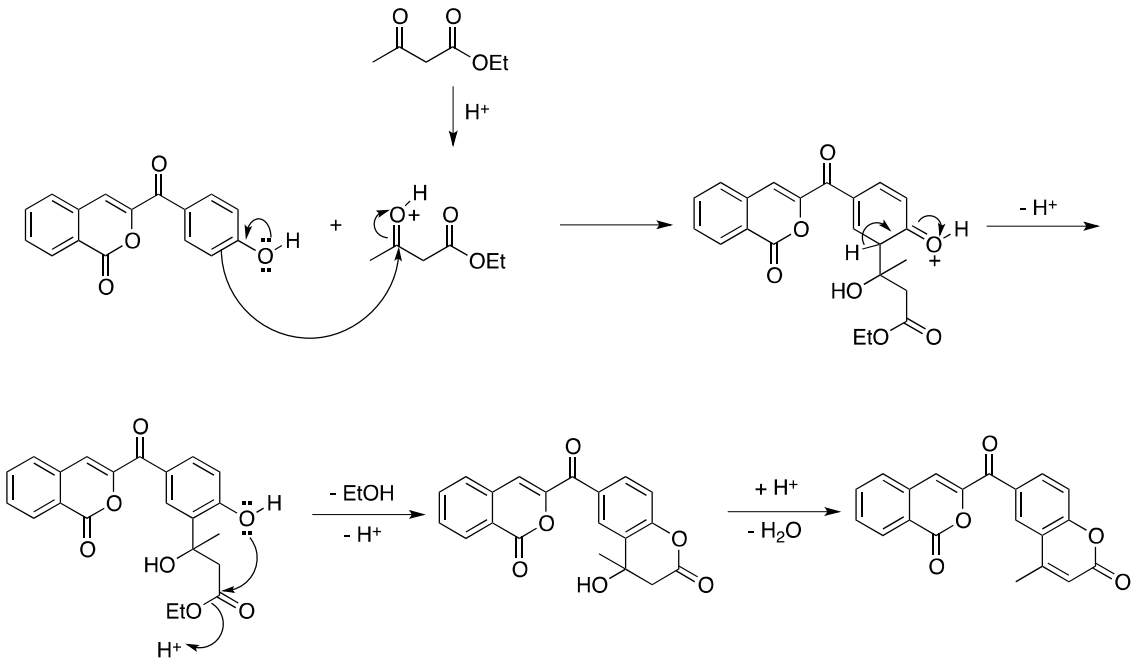

Figure 8. Mechanism; 3-(4'-methyl coumarin-6'-carbonyl) isocoumarin

3. Conclusion

A new series of triazole / tetrazole isoquinolines and coumarinoyl isocoumarin derivatives have been synthesized and evaluated for their antimicrobial activity against gram positive, gram negative bacteria and different fungi namely *Fusarium pallidoroseum, Colletotrichum capsici.* Synthesized compounds were also evaluated for their analgesic activity and tested compounds showed better results when compared with standard drug.

4. Experimental

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 plates using UV/Iodine as visualizing agent and Merck's silica gel (60-120 mesh) was used for column chromatographic purification. Infrared spectra were recorded on FTIR Perkin Elmer spectrophotometer using potassium bromide optics. ^{1}H NMR spectra were recorded on a Bruker spectrometer (400 MHz) using TMS as internal standard and chemical shifts are given in ppm. Mass spectrums were obtained using Thermo Scientific Corporation, DSQ II Mass Spectrometer. Elemental analyses were carried out on Perkin-Elmer C, H, N, S analyzer (Model-2400).

4.1.General procedure for Synthesis of 4-substituted -3- dibenzofuryl isocoumarin (3): o-propionyl benzoic acid (**1b**) (1.91g, 0.01 mmol), 2-bromo acetyl dibenzofuran (**2**) (3.1g. 0.01mmol) and anhy. K_2CO_3, (3.1g, 0.022 mmol) was refluxed for 10-12 hrs in ethyl methyl ketone. Solvent was then removed, 50 mL water added and extracted with 100 mL ethyl acetate. Solvent layer was first washed with sat. sod. bicarbonate solution and then with water, it was finally dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using pet. ether-ethyl acetate (95:5) as eluent to yield compounds 3a-b.

4.2.1. 4-Methyl -3- dibenzofuryl isocoumarin (3a): M.P: 55^{0}C; 80.26% yield; Ir: 1735 cm^{-1} (γ lactone), 1600 cm^{-1} (C=O), 1266 cm^{-1} (C-O); Anal. Calcd $C_{23}H_{14}O_4$ (354.0g): C, 77.96; H, 3.95; Found: C, 77.58; H, 4.10; ^{1}H NMR δ 2.11 (s, 3H, CH_3), 7.34-8.01 (m, 10H, aromatic protons), 8.23 (d, 1H, C_8-H, J = 3.2 Hz); ms: m/z: 355 (M^+ + 1), 340, 311, 264, 174, 160 and 146.

4.2.2. 4-Ethyl -3- dibenzofuryl isocoumarin (3b): M.P 86^{0}C; 80.00% yield; Anal. Calcd $C_{24}H_{16}O_4$ (368.0g): C, 78.26; H, 4.34. Found: C, 78.43; H, 4.71; ^{1}H NMR δ 1.25 (t, 3H, CH_3, J = 7.4 Hz), 2.70 (q, 2H, CH_2, J = 7.2 Hz), 6.88-7.92 (m, 10H, aromatic protons), 8.16 (s, 1H, C_8-H); ms: m/z: 368 (M^+), 312, 278, 250, 186 and 146.

4.3. General procedure for Synthesis of Substituted Isoquinolones (4): A solution of isocoumarin (**3**) (0.1g, 0.00027 mmol) in ethanol (3 mL) was refluxed with liquor ammonia (4mL) for 2.5 hrs. The reaction mixture was left overnight at room temperature. The resultant solid was filtered, washed with water and it was recrystallised from ethanol to give 4a-b.

4.3.1. 3-(Dibenzofuran– 3-carbonyl)-4-methyl isoquinolones (4a): M.P 80^{0}C; 90.15% yield; Ir: 3386 cm^{-1} (N-H), 1680 cm^{-1} (C=O); Anal. Calcd $C_{23}H_{15}O_3N$ (353.0 g): C, 78.18; H, 4.24; N, 3.96; Found: C, 78.02; H, 4.58; N, 4.03.

4.3.2. 3-(Dibenzofuran– 3-carbonyl)-4-ethyl-2H-isoquinoline-1-one (4b): M.P 75^{0}C; 91.00% yield; Ir: 32276 cm^{-1} (N-H), 1695 cm^{-1} (C=O); Anal. Calcd $C_{24}H_{17}O_3N$ (367.0 g): C, 78.47; H, 4.63; N, 3.81; Found: C, 78.50; H, 4.81; N, 3.99.

4.4. General procedure for Synthesis of Substituted 1-Chloroisoquinolines (5): Isoqinoline (**4**) (64 mg, 0.00017 mmol), PCl_5 (42 mg) and $POCl_3$ (0.2 mL) were mixed and the mixture refluxed for 4hrs at 130 ^{0}C. Excess of $POCl_3$ was removed by distillation under reduced pressure. The residue was

decomposed with ice water containing excess of sodium bicarbonate. The product was filtered, washed with water and recrystallised from diethylether-hexane to give 5a-b.

4.4.1. 3-(Dibenzofuran– 3-carbonyl)-1-chloro-4-methyl isoquinoline (5a): M.P 92^{0}C; 87.36% yield; Ir: 1688 cm^{-1} (C=O); Anal. Calcd $C_{23}H_{14}O_2NCl$ (371.5 g): C, 74.29; H, 3.76; N, 3.76; Found: C, 74.49; H, 3.54; N, 3.72.

4.4.2.3-(Dibenzofuran– 3-carbonyl)-1-chloro-4-ethyl isoquinoline (5b): M.P 75^{0}C; 87.33% yield; Ir: 1683 cm^{-1} (C=O); Anal. Calcd $C_{24}H_{16}O_2NCl$ (385.5 g): C, 74.70; H, 4.15; N, 3.63; Found: C, 74.46; H, 4.39; N, 3.91.

4.5.General procedure for Synthesis of Substituted 1-Hydrazinoisoquinolines (6): A mixture of (**5**) (47 mg, 0.00012 mmol), 80% aq. Hydrazine hydrate (0.5mL) and ethanol (1mL) was refluxed at 100^{0}c for 1 hr. The alcohol was distilled off and the residue treated with excess of ammonia, it was filtered and washed with water, then was recrystallised from ethanol to give 6a-b.

4.5.1. 3-(Dibenzofuran– 3-carbonyl)-1-hydrazino -4-methyl isoquinoline (6a): M.P 90^{0}C; 74.00% yield; Ir: 1578 cm^{-1} (N-H); Anal. Calcd $C_{23}H_{17}O_2N_3$ (367.0 g): C, 75.20; H, 4.63; N, 11.44. Found: C, 75.52; H, 4.60; N, 11.61.

4.5.2. 3-(Dibenzofuran– 3-carbonyl)-1-hydrazino -4-ethyl isoquinoline (6b): M.P 95^{0}C. 74.23% yield; Ir: 1614 cm^{-1} (N-H); Anal. Calcd $C_{24}H_{19}O_2N_3$ (381.0 g): C, 75.59; H, 4.98; N, 11.02. Found: C, 75.70; H, 5.21; N, 11.27.

4.6. General procedure for Synthesis of Tetrazolo-(5, 1-a)-isoquinolines (7): To a solution of (**6**) (50 mg) (0.00013 mmol) in 50% aq. HCl (1mL) which was cooled to 0^{0}C, an aq. solution of sodium nitrite (29mg) in 1.0 mL water was added dropwise while maintaining the temperature below 5^{0}C. After the addition was over, the reaction mixture was heated for 1 hr, and then allowed to cool, the solid product was filtered, washed with water, dried and recrystallised from ethanol to give 7a-b.

4.6.1. 3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – Tetrazolo – (5, 1 – a) isoquinoline (7a): M.P 84^{0}C; 60.00% yield; Ir: 1692 cm^{-1} (C=O), 1645 cm^{-1} (C=N), 1567 cm^{-1} (C=C), 1497 cm^{-1} (C-N); Anal. Calcd $C_{23}H_{14}O_2N_4$ (378.0 g): C, 73.00; H, 3.70; N, 14.81; Found: C, 73.30; H, 3.49; N, 15.01; ^{1}H NMR δ 2.20 (s, 3H, CH_3), 7.25-7.99 (m, 10H, aromatic protons), 8.1 (d, 1H, $C_{1'}$-H); ^{13}C NMR ($CDCl_3$) δ 187.0 (C=O, ketone), 150.6 (C=N), 12.7 (CH_3), 123.0 (C), 130.3 (CH), 128.9 (C), 127.1 (CH), 127.1 (C), 136.0 (C), 128.3 (C), 151.8 (=C-), 129.3 (C), 124.1 (CH), 121.8(CH), 131.0 (C), 155.7 (C), 112.5 (CH), 145.1 (C), 107.0 (C), 122.0 (CH), 123.1 (CH), 124.5 (CH), 112 (CH); ms: m/z: 378 (M^+), 363, 316, 289, 212, 184, 169 and 168.

4.6.2. 3 - (Dibenzofuran– 3-carbonyl) – 4 - ethyl – Tetrazolo – (5, 1 – a) isoquinoline (7b): M.P 75^{0}C; 60.63% yield; Anal. Calcd $C_{24}H_{16}O_2N_4$ (392.0 g): C, 73.46; H, 4.08; N, 14.28; Found: C, 73.72; H, 4.29; N, 14.34; ^{1}H NMR δ 1.52 (t, 3H, CH_3, *J* = 7.1 Hz), 2.31 (q, 2H, CH_2, *J* = 7.2 Hz), 7.24-8.00 (m, 10H, aromatic protons), 8.05 (d, 1H, $C_{1'}$-H); ^{13}C NMR ($CDCl_3$) δ 187.0 (C=O, ketone), 150.6 (C=N), 17.5 (CH_3), 20.5 (CH_2), 123.0 (C), 130.3 (CH), 128.9 (C), 127.1 (CH), 127.1 (C), 136.0 (C), 128.3 (C), 151.8 (=C-), 129.3 (C), 124.1 (CH), 121.8(CH), 131.0 (C), 155.7 (C), 112.5 (CH), 145.0 (C), 107.0 (C), 122.0 (CH), 123.1 (CH), 124.5 (CH), 112.0 (CH); ms: m/z: 393 (M^+ + 1), 363, 330, 302, 224, 210, 198 and 183.

4.7. General procedure for Synthesis of 1,2,4-Triazolo-(3, 4-a)-isoquinolines (8): A mixture of (**6**) (50 mg, 0.00013 mmol) and formic acid (0.1 mL) was heated at 130-135^{0}C for 3 hr, and after completion of reaction, mixture was poured into ice cold water. The unreacted formic acid was neutralized with sodium bicarbonate solution. The solid product formed was filtered, washed with water and was carefully recrystallized from ethanol to give 8a-b.

4.7.1. 3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – 1,2,4 -Triazolo – (3, 4 – a) isoquinoline (8a): M.P 78^{0}C; 55.86%; Ir: 1675 cm^{-1} (C=O), 1620 cm^{-1} (C=N), 1559 cm^{-1} (C=C), 1494 cm^{-1} (C-N); Anal. Calcd $C_{24}H_{15}O_2N_3$ (377.0 g): C, 76.39; H, 3.97; N, 11.14; Found: C, 76.01; H, 4.25; N, 11.38; ^{1}H NMR δ 2.16 (s, 3H, CH_3), 7.25-7.99 (m, 10H, aromatic protons), 8.00 (d, 1H, $C_{1'}$-H), 8.07 (d, 1H, N-CH=N); ^{13}C NMR ($CDCl_3$) δ 187.0 (C=O, ketone), 151.0 (C=N), 148.0 (C=N), 12.5 (CH_3), 123.5 (C), 130.0 (CH), 128.0 (C), 127.0 (CH), 127.0 (C), 136.2 (C), 128.0 (C), 152.0 (=C-), 129.3 (C), 124.0 (CH), 122.0 (CH), 132.0 (C), 155.6 (C), 113.0 (CH), 145.2 (C), 106.9 (C), 121.6 (CH), 123.0 (CH), 123.9 (CH), 111.0 (CH); ms: m/z: 377 (M^+), 327, 287, 210, 183 and 169.
4.7.2. 3 - (Dibenzofuran– 3-carbonyl) – 4 - ethyl – 1,2,4 -Triazolo – (3, 4 – a) isoquinoline (8b): M.P 92^{0}C; 55.00% yield; Anal. Calcd $C_{25}H_{17}O_2N_3$ (391.0 g): C, 76.72; H, 4.34; N, 10.74; Found: C, 76.58; H, 4.50; N, 10.92; ^{1}H NMR δ 1.30 (t, 3H, CH_3, *J* = 7.2 Hz), 2.70 (q, 2H, CH_2, *J* = 7.3 Hz), 7.25-8.0 (m, 10H, aromatic protons), 8.06 (d, 2H, $C_{1'}$-H and N-CH=N); ^{13}C NMR ($CDCl_3$) δ 187.0 (C=O, ketone), 151.0 (C=N), 148.0 (C=N), 17.0 (CH_3), 20.1 (CH_2), 123.5 (C), 130.0 (CH), 128.0 (C), 127.0 (CH), 127.0 (C), 136.2 (C), 128.0 (C), 152.0 (=C-), 129.3 (C), 124.0 (CH), 122.0 (CH), 132.0 (C), 155.6 (C), 113.0 (CH), 145.2 (C), 106.9 (C), 121.6 (CH), 123.0 (CH), 123.9 (CH), 111.0 (CH); ms: m/z: 391(M^+), 341, 301, 223, 210, 196 and 169.

4.8. General procedure for the synthesis of (coumarin-3-carbonyl) isocoumarin (10): A mixture of isocoumarin **9** (100 mg, 0.00033 mmol) and ethyl acetoacetate (0.043mL, 0.00033mmol) was added slowly to conc. sulphuric acid (2mL) cooled in ice within 15 mins. The reaction mixture was left overnight. After completion of reaction, the mixture was poured into ice and solid product obtained was filtered and purified by column chromatography using pet. ether – ethyl acetate (80:20) as eluent to give 10a-g.

4.8.1. 4-Methyl-3-(4'-methyl coumarin-6'-carbonyl) isocoumarin (10a): M.P 192^{0}C; 49.37% yield; Ir: 1604.26 cm^{-1}(-C=O), 1706.24 (lactonic ketone); Anal. Calcd $C_{21}H_{14}O_5$ (346.0 g): C, 72.83; H, 4.04; Found: C, 73.19; H, 4.26; ^{1}H NMR δ 2.31 (s, 3H, C_4' –H), 2.55 (s, 3H, C_4-H), 6.85-8.19 (m, 7H, aromatic protons), 8.28 (d, 1H, C_8-H); ^{13}C NMR ($CDCl_3$) δ 163 (C=O, isocoumarin lactone), 161 (C=O, coumarin lactone), 187.0 (C=O, ketone), 14.4 (CH_3), 24.1 (CH_3), 125.0 (C), 137.0 (C), 126.0 (CH), 133.0 (CH), 128.0 (CH), 130.0 (CH), 128.5 (C), 134.3 (C), 133.0 (C), 128.0 (CH), 128.5 (C), 157.0 (C), 122.0 (CH), 129.0 (C), 109.3 (CH), 156.0 (C); ms: m/z: 346 (M^+), 187, 159 and 145.

4.8.2. 4-Methyl-3-(4'-methyl-7'-hydroxy coumarin-6'-carbonyl) isocoumarin (10b): M.P 158^{0}C; 38.09% yield; Anal. Calcd $C_{21}H_{14}O_6$ (362.0 g): C, 69.61; H, 3.86; Found: C, 69.48; H, 4.13; ^{1}H NMR δ 2.25 (s, 3H, C_4' –H), 2.65 (s, 3H, C_4-H), 6.42 (s, 1H, OH), 6.86-8.10 (m, 6H, aromatic protons), 8.25 (d, 1H, C_8-H, *J* = 3.0 Hz); ^{13}C NMR ($CDCl_3$) δ 163.0 (C=O, isocoumarin lactone), 161.0 (C=O, coumarin lactone), 187.0 (C=O, ketone), 14.4 (CH_3), 24.1 (CH_3), 125.0 (C), 137.0 (C), 126.0 (CH), 133.0 (CH), 128.0 (CH), 130.0 (CH), 128.5 (C), 134.3 (C), 133.0 (C), 128.0 (CH), 128.5 (C), 157.0 (C), 122.0 (CH), 129.0 (C), 109.3 (CH), 156.0 (C); ms: m/z: 362 (M^+), 287, 259, 203, 187 and 146.

4.8.3. 4-Methyl-3-(4'-methyl coumarin-6'-carbonyl)-8-nitro isocoumarin (10c): M.P 128^{0}C; 40.28% yield; Ir: 1557 cm^{-1} (-C=O), 1720 cm^{-1} (lactonic ketone), 1344 cm^{-1}(-NO_2); Anal. Calcd $C_{21}H_{13}O_7N$ (391.0 g): C, 64.45; H, 3.32; N, 3.58; Found: C, 64.19; H, 3.68; N, 3.82; ^{1}H NMR δ 2.03 (s, 3H, C_4' –H), 2.56 (s, 3H, C_4-H), 6.59-8.11 (m, 6H, aromatic protons), 8.43 (d, 1H, C_7-H, *J* = 2.2 Hz); ^{13}C NMR ($CDCl_3$) δ 163.0 (C=O, isocoumarin lactone), 162.0 (C=O, coumarin lactone), 187.0 (C=O, ketone), 14.8 (CH_3), 24.7 (CH_3), 126.0 (C), 137.3 (C), 125.0 (C), 150.0 (C-NO_2), 123.0 (CH), 134.6 (CH), 132.0 (CH), 135.0 (C), 136.0 (C), 126.5 (CH), 127.0 (CH), 122.7 (CH), 152.0 (C), 134.0 (C), 155.0 (C), 109.0 (CH); ms: m/z: 391(M^+), 207 and 160.

4.8.4. 4-Methyl-3-(4'-methyl-7'-hydroxy coumarin-6'-carbonyl)-8-nitro isocoumarin (10d): M.P 134^{0}C; 40.00% yield; Anal. Calcd $C_{21}H_{13}O_8N$ (407.0 g): C, 61.91; H, 3.19; N, 3.43; Found: C, 62.35; H, 3.30; N, 3.72; ^{1}H NMR δ 1.99 (s, 3H, C_4' –H), 2.30 (s, 3H, C_4-H),5.34 (s, 1H, OH), 6.59-8.24 (m, 5H, aromatic protons), 8.41 (d, 1H, C_7-H); ^{13}C NMR ($CDCl_3$) δ 163.0 (C=O, isocoumarin lactone), 162.0 (C=O, coumarin lactone), 187.0 (C=O, ketone), 14.4 (CH_3), 24.5 (CH_3), 125.2 (C), 137.4 (C), 124.0 (C), 150.4 (C-NO_2), 122.8 (CH), 134.3 (CH), 132.1 (CH), 135.0 (C), 124.0 (C), 127.5 (CH),

120.0 (CH), 151.0 (C - OH), 138.0 (C), 134.6 (C), 155.0 (C), 109.4 (CH); ms: m/z: 407 (M^+), 377, 361, 331, 209, 187, 159, 145 and 77.

4.8.5. 4-Ethyl-3-(4'-methyl coumarin-6'-carbonyl) isocoumarin (10e): M.P 180^{0}C; 57.83% yield; Ir: 1610.09 cm^{-1}(-C=O), 1714.28 (lactonic ketone); Anal. Calcd $C_{22}H_{16}O_5$ (360.0 g): C, 73.33; H, 4.44; Found: C, 73.30; H, 3.71; ^{1}H NMR δ 1.36 (t, 3H, CH_3, *J* = 7.0 Hz), 2.35 (s, 3H, C_4' –H), 2.95 (q, 2H, CH_2), 6.80-8.10 (m, 7H, aromatic protons), 8.21 (d, 1H, C_8-H); ^{13}C NMR ($CDCl_3$) δ 163.0 (C=O, isocoumarin lactone), 162.0 (C=O, coumarin lactone), 187.0 (C=O, ketone), 11.0 (CH_3), 24.5 (CH_3), 22.0 (CH_2), 130.0 (C), 136.8 (C), 129.0 (C), 131.0 (CH), 127.2 (CH), 134.0 (CH), 126.5 (CH), 134.3 (C), 133.5 (C), 128.8 (CH), 121.5 (CH), 127.9 (CH), 156.5 (C), 127.5 (C), 155.0 (C), 109.5 (CH); ms: m/z: 361 (M^+ + 1), 354, 331, 316, 201, 174 and 118.

4.8.6. 3-(4'-methyl coumarin-6'-carbonyl) isocoumarin (10f): M.P 190^{0}C; 50.71% yield; Anal. Calcd $C_{20}H_{12}O_5$ (332.0 g): C, 72.28; H, 3.61; Found: C, 72.40; H, 3.89; ^{1}H NMR δ 2.32 (s, 3H, C_4' –H), 7.44 (s, 1H, C_4-H), 6.57-8.16 (m, 7H, aromatic protons), 8.42 (dd, 1H, C_8-H, *J* = 1.8 Hz, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 163.0 (C=O, isocoumarin lactone), 161.0 (C=O, coumarin lactone), 187.0 (C=O, ketone), 24.1 (CH_3), 116.0 (CH), 142.0 (C), 137.0 (C), 126.0 (CH), 133.0 (CH), 128.0 (CH), 130.0 (CH), 128.5 (C), 134.3 (C), 133.0 (C), 128.0 (CH), 128.5 (C), 157.0 (C), 122.0 (CH), 129.0 (C), 109.3 (CH), 156 .0 (C); ms: m/z: 330 (M^+ - 2), 187, 146 and 77.

4.8.7. 3-(4'-methyl-7'-hydroxy coumarin-6'-carbonyl) isocoumarin (10g): M.P 230^{0}C; 47.83% yield; Anal. Calcd $C_{20}H_{12}O_6$ (348.0 g): C, 70.58; H, 3.52. Found: C, 70.72; H, 3.88; ^{1}H NMR δ 2.12 (s, 3H, C_4' –H), 5.10 (s, 1H, OH), 7.45 (s, 1H, C_4-H), 6.53-7.99 (m, 6H, aromatic protons), 8.41 (d, 1H, C_8-H, *J* = 3.7 Hz); ^{13}C NMR ($CDCl_3$) δ 163.0 (C=O, isocoumarin lactone), 161.0 (C=O, coumarin lactone), 187.0 (C=O, ketone), 24.1 (CH_3), 116.3 (CH), 141.8 (C), 137.0 (C), 129.0 (C), 130.0 (CH), 127.6 (CH), 134.0 (CH), 126.0 (CH), 121.0 (C), 159.0 (C - OH), 110.0 (CH), 128.5 (CH), 158.0 (C), 121.0 (C), 156.0 (C), 109.8 (CH); ms: m/z: 348 (M^+), 333, 173 and 146.

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