© 2018 IJSRSET | Volume 4 | Issue 1 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section : Engineering and Technology

Synthesis and Antimicrobial Activity of Some Newer Derivative of Pyrimidine

Dr. Deepshikha
M.Sc.(IIT ROORKEE), B.Ed., M.Phill(IIT ROORKEE), Ph.D., Assistant Professor, Department of Chemistry, M. M. College Modinagar, Ghaziabad, Uttar Pradesh, India

ABSTRACT

Some New Bioactive heterocyclic compounds were synthesize among them pyrimidine compounds were found to possess important pharmacological and antimicrobial properties. Pyrimidine and to chromenes have structural and biological activities relationship so halogenated chromeno pyrimidine were synthesis where structure were characterized by NMR, IR, UB,GC-MS and elemental analysis. They were screened for antimicrobial activity. The Fluoro and methyl Heterocyclic showed highest biological activity.

Keywords: Chromene, pyramidines, antibacterial, antifungal, synthesis, novel drugs

I. INTRODUCTION

They are still not completely effective and still possess a certain degree of toxicity and quickly develop resistance due to large scale use [1]. Therefore, there is an immediate need for new antibacterial and antifungal chemical compounds alternatives to the existing ones. Heterocycles containing the chromene moiety and pyrimidine exhibit interesting features that make them an attractive target for microbial pathogens. Chromenes represent an important class of naturally occurring compounds [2, 3]. They have gained industrial recognition due to their contribution towards several biologically active compounds possessing significant pharmacological activities as anti-bacterial [4], antivirus [5], anti-cancer [6] and antimalarial [7] properties. Studies conducted previously had reported that substituted chromenes with the receptors increase the ability of molecule in preventing the disorder and possess different pharmacological activities with lower toxicity [8, 9].

Pyrimidine, a heterocyclic ring has strucuturally diverse synthetic derivaties [10, 11] which are reported to exhibit antimicrobial property against a variety of bacteria, fungi and displayed their potential as a polyfunctional backbone for new antimicrobial agents [12–20]. In general, studies showed some significantly biological properties when there is a combination of two different heterocyclic moieties in a single framework [21]. For instance, pentafluorophenylammonium triflate (PFPAT) showed potential against all the fungal and bacterial strains [22]. Similarly, 1,2,4-triazolo [3,4,-b][1,3,4]thiadiazole-6-yl] selenophenol pyrimidines compounds have showed promising antioxidant, antifungal and antibacterial activity [23]. Although these compounds have been widely studied individually due to their inherent pharmacological properties but only recently, fused structures incorporating both pyrimidine and chromene moieties were synthesized. Several studies have been conducted previously for antimicrobial activities, and the majority had focused on the synthesis of Chromeno-pyrano-pyrimidines, chromeno-pyrimidine-amine and Pyrimidine-thienopyridine derivatives [24–26]. The synthesized compounds have been evaluated for their antimicrobial activities respectively. Synthesis of chromeno pyrimidines has

been of considerable recent interest as they exhibit biological activity in comparison to other heterocycles. Prompted by these observation, we proposed a novel heterocyclic compounds, a new type of hybrid that had both chromene and pyrimidine moieties, i.e., 6-Chloro-2-hydroxy-N- (pyrimidin-4-yl)-2- (trifluoromethyl) chroman-3-carboxamide derivatives with a view to produce promising biologically active compounds. Further an efficient and practical method for the synthesis was introduced, along with antifungal and antimicrobial potential of the newly derived chromeno pyrimidines.

II. EXPERIMENTAL

All the chemicals used in the study were of LR grade and obtained from Sigma Aldrich. Melting points were determined in open capillary tubes sealed at both end using an electrical melting point apparatus. IR spectra were obtained on a Shimadzu-8400 FTIR spectrophotometer and the samples were made into pellets using KBr powder. ^{1}H NMR spectra were recorded on Bruker spectrometer (500 MHz) using DMSO-d6/ CDCl3 as a solvent. ^{13}C NMR spectra were recorded on Bruker spectrometer (125 MHz) in DMSO-d6/ CDCl3.^{19}F NMR spectra were recorded on 376 MHz in $CDCl_3$ solvent. All the reactions were monitored by thin layer chromatography (TLC) on pre coated silica gel paper having mesh size 60 - 254. Spots were visualized using UV light, I2 vapors, or $KMnO_4$

2.1. General method for the synthesis of substituted 2H-Chromene ester derivatives (3a-c)

A mixture of aromatic aldehyde (**1a-c)**) (1.00 g, 6.4 mmol), ethyl-4,4,4-trifluoro acetoacetate (**2**) (1.424 mL, 6.4 mmol), piperidine (63 µL,0.64 mmol) in ethanol (20 mL) was refluxed in an oil bath at 93^{0}C for 4 hours with stirring under inert nitrogen atmosphere. The progress of the reaction was monitored by TLC using 20 % ethyl acetate- hexane solvent system. The yellow solution turned dark brown. The reported compound (27) was purified by column chromatography using hexane.

(3a): Crystalline white solid; M.P(117-119^{0}c); IR (vmaxcm^{-1}): 3620, 3314, 1700, 1636 1565, 1460; ^{1}H-NMR (CDCl3, 500 MHz) δ 7.74 (dd, J = 2.0, 1.0 Hz, 1H), 7.56 – 7.25 (m, 2H), 7.54(s,1H),7.00 (d, J = 7.5 Hz, 1H), 4.72 – 3.93 (m, 2H),, 1.27 (t, J = 5.9 Hz, 3H).^{13}C NMR (CDCl3, 75 MHz) (δ): 166.4, 151.0, 137.9, 133.4, 128.6, 127.6, 118.7, 117.4; ^{19}F NMR (δ): -87.02; HRMS: calc. For m/z (C13H10ClF3O4 + Na) +: 345.0118; found: 345.0135

(3b):Off-yellow solid; M.P(162-165^{0}c); IR (vmaxcm^{-1}): 3601, 3000, 2960, 1735, 1620, 1429; ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.84 (dp, J = 1.7, 0.8 Hz, 1H), 7.81 – 7.44 (m, 2H), 7.54(s,1H),7.08 (d, J = 7.5 Hz, 1H), 4.67 (dq, J = 12.4, 5.9 Hz, 1H), 4.00 (dq, J = 12.4, 5.9 Hz, 1H),1.27 (t, J = 5.9 Hz, 3H). ^{13}C NMR (125 MHz, CDCl3) δ164.99, 155.76, 131.10,129.98, 129.84, 125.81, 118.64, 116.60, 114.80. ^{19}F NMR δ: -87.02, -62.80; Elemental analysis for C14H10F6O4 356.03 g/ mole is C, 47.71; H, 2.20; F, 32.45; O, 17.84 % HRMS: calcd. For m/z (C14H10F6O4 + Na) +:356.22; found: 356.05

(3c):Off-white solid, M.P(154-156^{0}c); IR (vmaxcm^{-1}): 3640, 3324, 1790, 1676 1565, 1461;^{1}H-NMR (400 MHz, Chloroform-d) δ 7.57 – 7.25 (m, 2H), 7.54(s,1H), 7.29 – 6.88 (m, 2H), 4.55 (dq, J = 12.4, 5.9 Hz, 1H), 4.11 (dq, J = 12.4, 5.9 Hz, 1H), 1.27 (t, J = 5.9 Hz, 3H); 13C NMR (125 MHz, CDCl3 NMR Solvents) δ 165.48, 164.07, 155.03, 152.59, 131.16, 130.08, 126.18, 118.36, 117.30, 115.74, 113.48; ^{19}F NMR δ: -87.02-134.13 Elemental analyses for C13H10F4O4 are C, 50.71; H, 3.20; Cl, 8.02; F, 24.45; O, 20.84 %; HRMS: calcd. For m/z (C13H10F4O4 + Na) +:306.27; found: 306.03

2.2. General method for the Synthesis of substituted 2H-chromene-3-carboxylic acid derivatives (4a-c)

The ester compound (3a-c) (3.22 g, 0.01 mmol) was dissolved in 20 ml of methanol and the NaOH (2 g) dissolved in 30 mL water was added slowly to the ester solution. The reaction mixture was refluxed for 4 hours, monitered by TLC (solvent: 25 % ethyl acetate- hexane). After cooling, the concentrated HCl

(5mL) was added to give a white precipitate that was filtered and recrystallized from cold water.

(4a):white solid, M.P(154-1580c); IR (vmaxcm^{-1}) 3629, 3425, 3122, 3111, 1875,1720, 1625, 1592; ^{1}H-NMR (500 MHz, Chloroform-d) (δ): 9.45 (s, 1H), 7.79 (dd, J = 2.0, 1.0 Hz, 1H), 7.72 (d, J = 0.9 Hz, 1H), 7.54(s,1H), 7.45 (dd, J = 7.5, 2.0 Hz, 1H), 7.02 (d, J = 7.4 Hz, 1H); ^{13}C NMR (125 MHz,) (δ): 168.80, 136.17, 130.18, 128.81, 127.29, 124.50, 122.36, 120.21, 118.07, 117.68, 117.27, 99.03, 98.39. ^{19}F NMR δ: -87.02. Elemental analysis for C11H6ClF3O4 294.61 g/ mole is C, 44.85; H, 2.05; Cl, 21.03; F, 19.35; O, 21.7 % HRMS: calcd. For m/z (C11H6ClF3O4 + Na) +: 317.611; found: 317.572.

(4b): White solid, M.P (166-1680c); IR (vmaxcm^{-1}):3631, 3394, 3196, 3022, 2028, 1874, 1725, 1625, 1562, 1492; ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.93 – 7.50 (m, 2H), 7.54(s,1H),7.37 (d, J = 1.0 Hz, 1H), 7.08 (d, J = 7.5 Hz, 1H), ^{13}C NMR (125 MHz,) δ 165.74, 155.76, 131.99, 129.84, 125.81, 118.64, 115.70; ^{19}F NMR δ: -87.02, -62.80; Elemental analysis for C12H6F6O4 is C, 47.71; H, 2.20; F, 32.45; O, 17.84 %; HRMS calcd for m/z C12H6F6O4 + Na +: 328.17; found: 328.02

(4c): White solid; M.P(161-162^{0}c); IR (vmaxcm^{-1}): 3621, 3314, 3196, 3012, 2028, 1864, 1715, 1625, 1552, 1492; ^{1}H-NMR (107 MHz, Chloroform-d) δ 7.53 – 7.23 (m, 2H), 7.54(s,1H), 7.23 – 6.94 (m, 2H); ^{13}C NMR (125 MHz, CDCl3) δ 165.74, 164.45, 155.03, 152.59, 132.53, 131.44, 126.18, 118.36, 117.30, 115.74, 113.48, 112.73; ^{19}F NMR δ: -87.02,-134.13; Elemental analysis for C11H6F4O4 is C, 47.71; H, 2.20; Cl, 8.02; F, 27.45; N; O, 23.84 %; HRMS: calcd. For m/z (C11H6F4O4 + Na) +:278.27; found: 278.03.

III. GENERAL METHOD FOR THE SYNTHESIS OF CHROMENO PYRIMIDINES

The compound (4a-c) (carboxylic acid 0.01mmol) and substituted aromatic amines (5) (amine 0.01mmol) were dissolved in 1mL of dry dichloromethane. N,N,N′,N′-tetramethyluranium O-(benzotriazol-1-yl) tetrafluoroborate (TBTU)(0.02mmol) and triethylamine(0.03mmol) was added. The reaction mixture was refuxed for 18-20 hours, and monitored by TLC using ethyl acetate and hexane solvent. The product was purified by column chromatography using hexane as the solvent.

(6a$_1$): Light yellow solid ; M.P(167-169^{0}c); IR (vmaxcm^{-1}) : 3819 ,3615, 3340, 3310, 2899, 2105, 1971, 1912, 1815, 1616, 1547, ^{1}H-NMR (500 MHz, Chloroform-d) δ 9.37 (s, 7.54(s,1H),1H), 8.83 (dd, J = 1.5, 0.4 Hz, 1H), 8.58 (dd, J = 5.0, 0.4 Hz, 1H), 7.53 (dd, J = 2.0, 1.0 Hz, 1H), 7.37 – 7.04 (m, 2H), 6.91 – 6.54 (m, 2H); ^{13}C NMR (125MHZ, CDCl3) δ 172.54, 171.41(CO), 158. 76, 156.60, 150.91, 128.99, 127.62, 126.80, 125.80, 118.61, 115.39, 109.47, 100.55, ^{19}F NMR δ: -87.02. Elemental analysis for C15H11ClF3N3O3 is C, 48.21; H, 2.97; Cl, 9.49; F, 15.25; O, 12.84; N, 11.24 % HRMS: calcd. For m/z (C15H11ClF3N3O3 + Na) +:373.01; found: 373.04

(6a$_2$): Yellow solid ; M.P (176-178^{0}c); IR (vmaxcm^{-1}): 3634, 3214, 3005, 2654, 2052, 1992, 1699, 1369; ^{1}H-NMR (500 MHz, Chloroform-d) δ 9.47 (s, 1H), 8.28 (d, J = 5.0 Hz, 1H), 7.53 (dd, J = 2.0, 1.0 Hz, 1H), 7.54(s,1H), 7.39 – 7.05 (m, 2H), 7.02 – 6.65 (m, 2H);^{13}C NMR (125 MHz) δ 172.54, 171.41, 168.44, 159.02, 155.53, 154.05, 150.91, 128.99 127.62, 126.80, 125.80, m 118.61, 115.39, 104.32, ^{19}F NMR δ: -87.02, -95.5. Elemental for C15H8ClF4N3O3 C, 48.99; H, 2.57; Cl, 9.05; F, 19.45; O, 12.84; N, 10.24 %; HRMS: calcd. For m/z (C15H8ClF4N3O3 + Na) +:389.71; found: 389.03

(6a$_3$): Off-white solid ; M.P(177-179^{0}c); IR (vmaxcm^{-1}): 3898, 3678, 3399,3577, 2627, 1995, 1778, 1735, 1588, 1404; 1H-NMR (500 MHz, Chloroform-d) δ 9.44 (s, 1H), 8.52 (d, J = 5.0 Hz, 1H), 7.80 (d, J = 5.0 Hz, 1H), 7.53 (dd, J = 2.0, 1.0 Hz, 1H), 7.54(s,1H), 7.40 – 7.05 (m, 2H), 6.79 (d, J = 7.5 Hz, 1H); 13C NMR (125 MHz, CDCl3) δ 172.54, 171.41, 160.89, 159.70, 155.00, 153.84, 150.91, 128.99 – 127.62, 126.80, 125.80, 124.76, 118.61, 115.07, 109.81, ^{19}F NMR δ: -87.02; Elemental analysis for C16H8ClF6N3O3 is C, 43.71;

H, 1.20; Cl, 8.06; F, 25.45; O, 10.84; N, 9.01 %; HRMS: calcd. For m/z (C16H8ClF6N3O3 + Na) +:439.71; found: 439.03

(6b1):yellow solid; M.P(192-194^{0}c); IR (vmaxcm-1): 3899, 3715, 3350, 3312, 2899, 2105, 1961, 1942, ^{1}H NMR (400 MHz, Chloroform-d) δ 9.34 (s, 1H), 8.90 – 8.48 (m, 2H), 7.63 – 7.19 (m, 4H), 6.88 (d, J = 7.3 Hz, 1H), 7.54(s,1H);^{13}C NMR (125 MHz, CDCl3) δ 164.59, 158.35, 157.25, 155.76, 131.99, 129.84, 125.81, 118.64; ^{19}F NMR δ: -87.02, -62.80; Elemental analysis for C16H11F6N3O3 is C, 47.71; H, 2.20; F, 27.45; N, 10.33; O, 11.84 % HRMS: calcd. For m/z (C16H11F6N3O3 + Na) +:402.27; found: 407.07

(6b2): Yellow solid ; M.P (197-1980c); IR (vmaxcm^{-1}): 3632, 3225, 3059, 2647, 2546, 2512, 1978, 1831, 1748; ^{1}H-NMR (500 MHz, Chloroform-d) δ 9.49 (s, 1H), 8.28 (d, J = 5.0 Hz, 1H), 7.64 – 7.20 (m, 3H), 7.54(s,1H),7.04 – 6.75 (m, 2H);^{13}C NMR (125MHz,) δ 168.35, 164.59 , 158.93, 156.35 , 154.73 , 154.02 , 131.99 , 129.84 , 125.81 , 118.64, 116.60 , 114.80 , 105.53; ^{19}F NMR δ: -95.45, -87.02, -62.80. Elemental analysis for C16H10F7N3O3 is C, 45.71; H, 2.20; F, 37.45; N, 9.33; O, 11.84 %; HRMS: calcd. For m/z (C16H11F6N3O3 + Na) +:425.27; found: 425.07

(6b3):Yellow solid; M.P (197-1990c); IR (vmaxcm^{-1}): 3600, 3310, 3100, 2610, 2122, 1926, 1788, 1734, 1656, 1554; ^{1}H-NMR (500 MHz, Chloroform-d. 9.48 (s, 1H), 8.30 (d, J = 5.0 Hz, 1H), 7.64 – 7.20 (m, 3H), 7.54(s,1H), 7.04 – 6.64 (m, 2H);^{13}C NMR (125 MHz, CDCl3) δ 172.54, 171.41, 160.12, 158.83, 156.17, 152.96, 129.02, 126.67, 124.85, 118.62, 115.26, 108.59, 107.23; ^{19}F NMR δ: -87.02, -62.80; Elemental analysis for C16H10ClF6N3O3 is C, 43.71; H, 2.20; Cl, 8.02; F, 25.45; N, 10.33;

(6c1): Off orange solid; M.P (171-1730c); IR (vmaxcm^{-1}): 3501, 3340. 3127, 3100, 2904, 2764, 2648, 2179, 1982, 1953, 1788, 1751; ^{1}H-NMR (500 MHz, Chloroform-d) δ 9.37 (s, 1H), 8.83 (dd, J = 1.5, 0.4 Hz, 1H), 8.58 (dd, J = 5.0, 0.4 Hz, 1H) 7.54(s,1H), 7.38 – 7.04 (m, 2H), 6.95 – 6.54 (m, 3H);13C NMR (125 MHz, CDCl3) δ 165.07 – 164.07, 158.35, 157.25, 155.03, 152.59, 131.99, 126.18, 118.36, 117.30, 115.74, 113.48, 112.73, 109.48, 106.92, 105.16, 104.53, 95.43; Elemental analysis for C15H9F4N3O3 is C, 50.71; H, 2.20; F, 21.45; N, 11.33; O, 13.84 %. HRMS: calcd. For m/z (C15H9F4N3O3 + Na)+: 355.27; found: 355.03 19F NMR δ: -87.02 ,-134.13.

(6c2):Yellow solid; M.P(175-177 °C); IR (vmaxcm^{-1}): 3546, 3177, 3165, 3085, 2782, 2489, 2323; ^{1}H-NMR (400 MHz, Chloroform-d) δ (ppm): 8.92 – 8.56 (m, 2H), 7.71 (s, 1H), 7.54(s,1H),6.80 – 6.33 (m, 3H), 3.90 (t, J = 9.1 Hz, 1H), 3.54 (dd, J = 17.1, 8.9, 0.9 Hz, 1H), 3.07 (dd, J = 17.1, 9.2, 1.0 Hz, 1H); 13C NMR (125 MHz, CDCl3) δ 172.54, 171.41, 164.11, 160.89, 159.70, 154.45, 148.18, 128.62, 125.27 – 124.22, 118.61, 116.80, 116.42 – 115.22, 114.75, 111.97, 111.23, 109.81, 100.55, ^{19}F NMR δ: -87.02, -134.13; Elemental analysis for C16H10F7N3O3 is C, 45.71; H, 2.20; F, 31.45; N, 9.33; O, 11.84 %

IV. SCREENING OF COMPOUNDS FOR ANTIMICROBIAL PROPERTIES

4.1. General procedure for testing the antibacterial property

The antibacterial activities of newly synthesized chromeno pyrimidines along with compounds (**3a-c**) and (**4a-c**) were tested against four selected bacterial strains, Staphylococcus aureus, Bacillus subtilis which are Gram-positive bacteria; and Escherichia Coli, Pseudomonas aeruginosin, that are Gram-negative bacteria by cup-plate method [29]. Second, the Sterilized Nutrient agar medium was sterilized and distributed (100 ml each) into two 250 mL conical flasks. Finally, they were allowed to cool room temperature. The bacterial sub-cultures were grownfor 18-24 h, which were added to each of these media and were shaken systematically. Thus, the bacterial cultures were uniformly distributed in the various media. Equal quantities of the agar medium were added into sterilized Petri dishes and it was ensured that each 45-50 ml of the medium was present in each Petri dish. The medium was allowed

to solidify and a sterile cork borer, consuming a diameter of 6 mm, was used to punch the agar media to prepare the cups.

The compounds used under the study were dissolved in DMF to obtain solutions having the required concentrations (50, 100 µg/ mL). 1 mL of each solution was filled into the cups. Then, the Petri dishes were placed in an inverted position and incubated for 24-48 h at 37°C in an incubator. Growth inhibition zones developed surrounding each cup after which, their diameters were measured (mm) and compared to standard drugs, Streptomycin and Procaine penicillin.

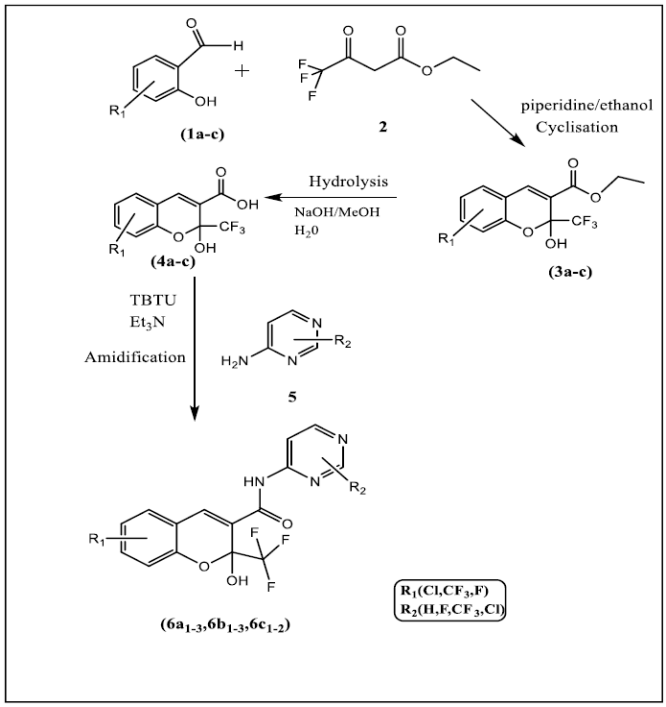
4.2. General procedure for testing the antifungal property

The antifungal activities of newly synthesized compounds along with compounds (**3a-c**) and (**4a-c**) were tested against two fungi Aspergillus niger and Candida albicans at concentrations of 50 µg/ mL and 100 µg/ mL by cup-plate method [28]. Griseofulvin was chosen as the standard.The potato dextrose agar medium was sterilized and then incubated for 72 h. Fungi were subcultured and added uniformly by stirring the media. Petri dishes were cleaned and labelled into which the media were poured. They were then allowed to solidify. The plates were then bored to make cups, four of them in each plate. 0.1 mL of the two test dilutions were added to two cups and the two corresponding test dilutions of the standard to the other two cups. After leaving the plates for 2-3 h to allow diffusion to take place, they were incubated at 37 ^{0}C for 24 h. The diameters of the zones of growth inhibition were measured (mm) and compared to the standard.

V. RESULTS AND DISCUSSION

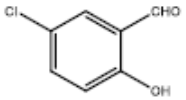
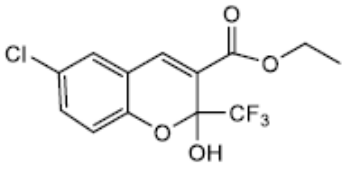
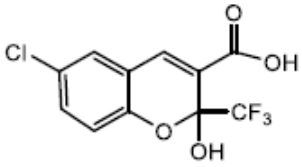
5.1. Synthesis and Characterization

As shown in scheme 1, chromene esters **(3a-c)** possessing the hydroxyl and trifluro groups were initially synthesized as intermediates utilizing piperidine catalysed multi component reaction of aromatic aldehydes(**1a-c**) and ethyl-4,4,4-trifluoro acetoacetate in refluxing ethanol. various aldehydes as shown in Table 1) were utilized for the synthesis of these compounds. The formation of the products was confirmed by comparison of the analytical data in the case of (**3a-c**) with the reported data. The IR spectra of **(3a-c)** showed absorption bands at 3620,3601,3640 cm^{-1} for free OH,1700,1735,1790 cm^{-1} for CO in esters. The ^{1}H NMR spectra gave triplet at δ1.27ppm and multiplet at δ 4.72 – 3.93ppm shows the presence of 3H and 2H next to the ester group. The ^{19}F NMR spectra of **3a** showed at signal δ -87.02ppm showed the presence one type of fluorine atoms while ^{19}F spectra of **4a** showed two signals at δ - 87.02, -62.80ppm showing the presence of fluorine atoms in two different environments. After the initial reaction which required 4h of reflux, the esters were subjected to hydroylsis using NaOH to obtain corresponding chromene carboxylic acids as intermediates(**4a-c**). A sequential addition of substituted pyrimidines, TBTU and triethylamine to the same reaction mixture containing compounds(**4a-c**) afforded the final products amides(**6a$_{1-3}$,6b$_{1-3}$,6c$_{1-2}$**). Completion of all the reactions was monitored by a simple TLC analysis. The IR spectra of chromeno pyrimidines **(6a$_{1-3}$, 6b$_{1-3}$, 6c$_{1-2}$)** showed absorption bands corresponding to N-H stretching in the range of 3350-3210cm^{-1}. The ^{13}C-NMR spectra of all the synthesized compounds showed the quartets of CF3 and C-2 atom with their corresponding coupling constants 1JC,F = 289–291 Hz and 2JC,F = 33.6–36.4 Hz, which appeared at 122.0–123.0 ppm and 95.2–96.6 ppm respectively.

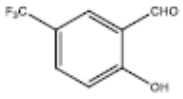
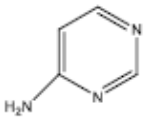
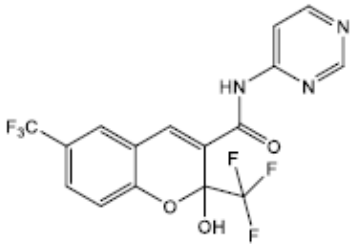
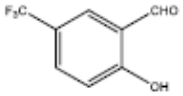
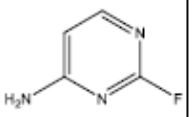
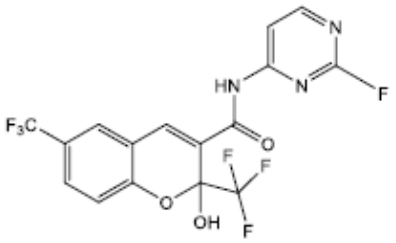
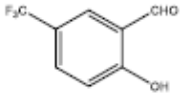
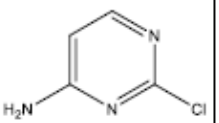
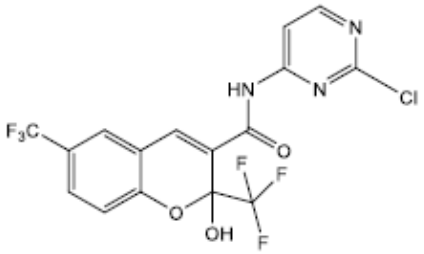
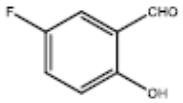
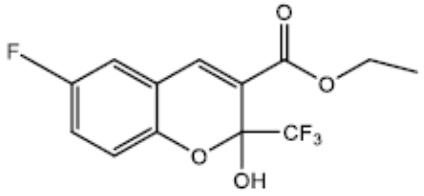
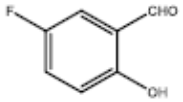
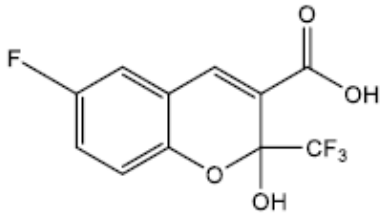
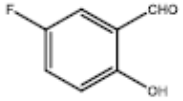
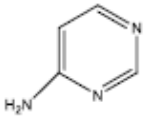
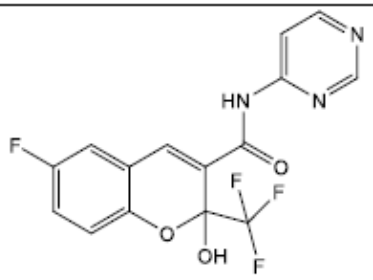


Scheme 1. Route for the synthesis of chromeno pyrimidines

Table 1. Synthesis of Novel Chromeno pyrimidines

Entry Number	Aromatic Aldehyde	Substituted Pyrimidine	Synthesized Compound structure	Yield %
1.		-	 **3a**	68
2.		-	 **4a**	74

d

8.			 **6b1**	48
9.			 **6b2**	64
10.			 **6b3**	51
11.		-	 **3c**	64
12.		-	 **4c**	62
13.			 **6c1**	69

The ^{1}H NMR spectra of (**6a$_1$**) showed signals at δ 8.83,8.58 and 6.67 indicating the presence of three hydrogen atoms in the pyrimidine ring while spectra of (**6a$_3$**) gave signal at only two signals at δ8.28 and 6.90 indicating the substitution of the pyrimidine ring.

5.2. Antibacterial Activity

The results of the anti-bacterial screening are presented in Table 2. From the table, it can be seen that compounds (**3a-c**) and (**4a-c**) exhibited some antibacterial property. However, activities of the chromeno pyrimidines (**6a$_{1-2}$, 6b$_{1-3}$, 6c$_{1-2}$**) were significantly higher as compared to compounds (**3a-c**) and (**4a-c**).

This shows that the introduction of a pyrimidine moiety increased the anti-bacterial properties. The substituents on the aromatic ring and pyrimidine rings had an effect on the anti-bacterial properties. Compounds (**6a$_1$, 6a$_2$, 6c$_1$**) showed moderate anti-bacterial activities against all the four strains. The fluoro substituents showed activities better than the other substituents. This was consistent with the results of a previous study [29]. The 2, 4- difluoro substituent (**6a$_3$, 6b$_1$, 6b$_2$, 6b$_3$, 6c$_2$**) showed the best activity against all the bacterial strains. The activities of (**6a$_3$, 6b$_1$, 6b$_2$, 6b$_3$, 6c$_2$**) were comparable to standard antibiotics, such as streptomycin and procaine penicillin.

Table 2. Screening of the compounds for antibacterial property

Compound	Inhibition zones (mm) against concentration of test/standard compound(s)							
	S. aureus		B. subtilis		E. coli		P. aeruginosa	
Concentration	50 μ g/ mL	100 μ g/ mL	50 μ g/ mL	100 μ g/ mL	50 μ g/ mL	100 μ g/ mL	50 μ g/ mL	100 μ g/ mL
3a	8	11	7	10	7	11	8	10
4a	9	13	8	11	9	12	7	11
6a$_1$	10	14	11	15	13	17	12	15
6a$_2$	12	16	10	17	11	15	10	15
6a$_3$	18	22	19	24	16	21	18	23
3b	13	19	16	20	15	19	14	19
4b	13	19	20	22	19	21	17	19
6b$_1$	15	17	14	17	13	17	12	18
6b$_2$	17	20	18	25	17	23	17	23
6b$_3$	18	22	20	26	18	20	18	24
3c	9	14	8	13	10	11	14	13
4c	10	15	11	14	9	13	11	16
6c$_1$	13	16	11	18	11	18	12	20
6c$_2$	19	23	20	26	17	21	18	23
Streptomycin	-	-	-		21	26	21	26
Procaine penicillin	22	27	23	28	-	-	-	-

5.3. Antifungal activity

The results of the anti-fungal screening are presented in Table 3. The results of the antifungal screening were similar to the antibacterial screening. All the compounds showed anti-fungal activity. The antifungal activities of the chromeno pyrimidines (**6a1-2, 6b1-3, 6c1-2**) were considerably higher than that of compounds (**3a-c**) and (**4a-c**), which again indicated that the incorporation of the pyrimidine ring to chromene moiety had a positive effect on the anti-fungal properties. The effects of the different substituents on the anti-fungal properties were also studied. The chromeno pyrimidines that contained fluoro substituents showed very good anti-fungal properties. Amongst all the chromeno pyrimidines, the difluoro compounds (**6b2, 6c2**) showed the best anti-fungal activity against both the fungal strains.

Table 3. Screening of the compounds for antifungal property

Compound	Inhibition zones (mm) against the concentration of test / standard compound(s)			
	A. niger		C. albicans	
Concentration	50 µg/ mL	100 µg/ mL	50 µg/ mL	100 µg/ mL
3a	7	11	10	19
4a	7	13	11	17
6a1	10	15	9	17
6a2	11	16	11	19
6a3	11	14	10	16
3b	14	16	10	21
4b	17	19	11	22
6b1	14	20	16	24
6b2	16	28	17	30
6b3	17	26	20	28
3c	12	15	15	22
4c	10	19	13	20
6c1	14	23	16	27
6c2	17	30	19	32
Griseofulvin	20	35	21	36

VI. CONCLUSION

Newer chromeno pyrimidines were synthesized in good yields. The synthesized compounds were well characterized and tested for their antibacterial and antifungal activities. The fluoro-substituted compounds showed the greatest activities. These results give an insight into the structure-property relationships, which are tremendously important for the design of further new antimicrobial compounds.

VII. REFERENCES

[1]. R. Kharb, M. Tyagi and A. K. Sharma, "Current status and future scenario of pyrimidine derivatives having antimicrobial potentia," Der Pharma Chem., vol. 6, no. 4, pp. 298–320, 2014.

[2]. R. D. Murray, J. Mendez, and S. A. Brown, The Natural Coumarins: Occurrence, Chemistry and Biochemistry, vol.9 Hoboken, New Jersey, United States: John Wiley & Sons, 1982.

[3]. M. A. Al-Haiza, M. S. Mostafa, and M. Y. El-Kady, "Synthesis and Biological Evaluation of Some New Coumarin Derivatives," Molecules, vol. 8, no. 2, pp. 275–286, Feb. 2003.

[4]. M. A. Bhat, N. Siddiqui, S. A. Khan, and M. I. Mohamed, "Synthesis of triazolothiazolidinone derivatives of coumarin with antimicrobial activity," Acta Pol Pharm, vol. 66, no. 6, pp. 625–632, 2009.

[5]. W. A. El-Sayed, M. M. M. Ramiz, and A. A.-H. Abdel-Rahman, "Anti-Hepatitis B Virus

Activity of New N4 -β-D-Glycoside Pyrazolo[3,4-d]pyrimidine Derivatives," Publ. J. Nat. Res.,vol.21, pp. 323–328, 2009.

[6]. M. S. Mustafa, M. M. El-Abadelah, M. A. Zihlif, R. G. Naffa, and M. S. Mubarak, "Synthesis, and Antitumor Activity of Some N1-(Coumarin-7-yl) Amidrazones and Related Congeners," Molecules, vol. 16, no. 12, pp. 4305–4317, May 2011.

[7]. P. Thompson and L. Webel, Antimalarial agents: chemistry and pharmacology. vol.6,New York: Academic Press, 1972.

[8]. E. McChesney and C. Fitch, "4-Aminoquinolines," in Antimalarial drugs II. Current antimalarials and new drug developments, W. Peters and W. Richards, Eds. Berlin, vol.31,1984, pp. 3–60.

[9]. S. Bhatia and M. Gupta, "1, 3, 4-Oxadiazole as antimicrobial agents: An overview," J. Chem. Pharm. Res., vol. 3, no. 3, pp. 137–147, 2011.

[10]. A. Choudhury, H. Chen, C. N. Nilsen, and K. L. Sorgi, "A chemoselective aniline–chloropyrimidine coupling in a competing electrophilic environment," Tetrahedron Lett., vol. 49, no. 1, pp. 102–105, Jan. 2008.

[11]. M. D. M. H. Bhuiyan, K. M. D. M. Rahman, M. D. K. Hossain, A. Rahim, M. I. Hossain, and M. Abu Naser, "Synthesis and antimicrobial evaluation of some new thienopyrimidine derivatives.," Acta Pharm., vol. 56, no. 4, pp. 441–50, 2006.

[12]. M. R. Prasad, J. Prashanth, K. Shilpa, and D. P. Kishore, "Synthesis and Antibacterial Activity of Some Novel Triazolothienopyrimidines.," ChemInform, vol. 38, no. 40, Oct. 2007.

[13]. H. M. Aly, N. M. Saleh, and H. a. Elhady, "Design and synthesis of some new thiophene, thienopyrimidine and thienothiadiazine derivatives of antipyrine as potential antimicrobial agents," Eur. J. Med. Chem., vol. 46, no. 9, pp. 4566–4572, 2011.

[14]. B. V. Ashalatha, B. Narayana, K. K. V. Raj, and N. Kumari, "Synthesis of some new bioactive 3-amino-2-mercapto-5,6,7,8-tetrahydrof 11benzothknol 2,3-d 1pyrimi¬din-4(3H)-one derivatives," Eur. J. Med. Chem, vol. 42, pp. 719–728, 2007.

[15]. T. George, C. Kaul, R. S. Gmwal, and R. Tahilramani, "Antihypertensive and mono-amine oxidase inhibitory activity of some derivatives of 3-formy1-4-oxo-4H¬pyrido11,2-alpyrimidine," J. Med. Chem, vol. 14, pp. 913–915, 1971.

[16]. O. Bruno, C. Brullo, A. Ranise, S. Schenone, F. Bondavalli, E. Barocelli, V. Ballabeni, M. Chiavarini, M. Tognolini, and M. Impicciatore, "Synthesis and pharmacological evaluation of 2,5-cycloamino-5H-[1]benzopyrano[4,3-d]pyrimidines endowed with in vitro antiplatelet activity," Bioorg Med Chem Lett, vol. 11, no. 11, pp. 1397–1400, 2001.

[17]. R. K. Russell, J. B. Press, R. a. Rampulla, J. J. McNally, R. Falotico, J. a. Keiser, D. a. Bright, and A. Tobia, "Thiophene systems. 9. Thienopyrimidinedione derivatives as potential antihypertensive agents," J. Med. Chem., vol. 31, no. 9, pp. 1786–1793, 1988.

[18]. H. Liu, H.-Q. Wang, and Z.-J. Liu, "Synthesis and herbicidal activity of novel pyrazolo[3,4-d]pyrimidin-4-one derivatives containing aryloxyphenoxypropionate moieties.," Bioorg. Med. Chem. Lett., vol. 17, no. 8, pp. 2203–2209, 2007.

[19]. J.-M. Wang, T. Asami, S. Yoshida, and N. Murofushi, "Biological Evaluation of 5-Substituted Pyrimidine Derivatives as Inhibitors of Brassinosteroid Biosynthesis," Biosci. Biotechnol. Biochem., vol. 65, no. 4, pp. 817–822, Jan. 2001.

[20]. P. Acosta, B. Insuasty, A. Ortiz, R. Abonia, M. Sortino, S. A. Zacchino, and J. Quiroga, "Solvent-free microwave-assisted synthesis of novel pyrazolo[4′,3′:5,6]pyrido[2,3-d]pyrimidines with potential antifungal activity," volume 18.Arab. J. Chem., Mar. 2015.

[21]. M. Mamaghani, F. Shirini, E. Bassereh, and R. Hossein Nia, "1,2-Dimethyl-N-butanesulfonic acid imidazolium hydrogen sulfate as efficient ionic liquid catalyst in the synthesis of indeno fused pyrido[2,3-d]pyrimidines," J. Saudi Chem. Soc., vol. 42, pp. 1–7, Dec. 2014.

[22]. M. Ghashang, S. S. Mansoor, and K. Aswin, "Pentafluorophenylammonium triflate (PFPAT) catalyzed facile construction of substituted chromeno[2,3-d]pyrimidinone derivatives and their antimicrobial activity," J. Adv. Res., vol. 5, no. 2, pp. 209–218, Mar. 2014.

[23]. Y. Kotaiah, K. Nagaraju, N. Harikrishna, C. Venkata Rao, L. Yamini, and M. Vijjulatha, "Synthesis, docking and evaluation of

antioxidant and antimicrobial activities of novel 1,2,4-triazolo[3,4-b][1,3,4]thiadiazol-6-yl)selenopheno[2,3-d]pyrimidines," Eur. J. Med. Chem., vol. 75, pp. 195–202, Mar. 2014.

[24]. J. V. Prasad, N. R. Kumar, K. A. Solomon, K. R. S. S. Nilaventhan, R. C. Lowrence, N. Saisubramanian, and G. Gopikrishna, "One pot synthesis of fused chromeno-pyrano-pyrimidines and evaluation of their antimicrobial activity," Indian J. Chem., vol. 53B, no. 03, pp. 345–351, 2014.

[25]. H. M. Aly and N. M. Saleh, "Utility of a pyrimidine thione derivative in the synthesis of new fused pyrimido[4,5d]pyrimidine, pyrido[2,3-d]pyrimidine and different types of thienopyrimidine derivatives," Int. J. Adv. Res., vol. 2, no. 4, pp. 694–702, 2014.

[26]. R. B. Patil and S. D. Sawant, "Synthesis, Docking Studies and Evaluation of Antimicrobial and in Vitro Antiproliferative Activity of 5h-Chromeno 4,3-D Pyrimidin-2-Amine Derivatives," Int. J. Pharm. Pharm. Sci., vol. 7, no. 2, pp. 304–308, 2015.

[27]. C. Xu, G. Yang, C. Wang, S. Fan, L. Xie, and Y. Gao, "An Efficient Solvent-Free Synthesis of 2-Hydroxy-2-(trifluoromethyl)-2H-chromenes Using Silica-Immobilized L-Proline," Molecules, vol. 18, no. 10, pp. 11964–11977, Sep. 2013.

[28]. G. C. Ramaprasad, B. Kalluraya, B. S. Kumar, and S. Mallaya, "Synthesis, antibacterial and antifungal activities of some novel 1,3,4-oxadiazole analogues," Int. J. Pharm. Pharm. Sci., vol. 4, no. 4, pp. 210–213, 2012.

[29]. V. K. Sahu, A. K. Singh, and D. Yadav, "Review article on 1, 3, 4-Oxadiazole derivaties and it's Pharmacological activities," Int. J. ChemTech Res., vol. 3, no. 3, pp. 1362–1372, 2011.

[30]. S. Sharma, P. K. Sharma, N. Kumar, and R. Dudhe, "Chemistry and pharmacological potentials of Oxadiazoles: A Review," Der Pharma Chem., vol. 2, no. 4, pp. 253–263, 2010.