

Design and Synthesis of Novel Chromeno Pyrimidine analogs of potential Antimicrobial properties

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Abstract

*The aim of the study is to design and synthesize the new bioactive heterocyclic compounds. Pyrimidine is an important class of heterocyclic compound that are known to possess important pharmacological and antimicrobial properties. 2H-chromenes are also known to possess antimicrobial properties. Chromeno pyrimidines are compounds having both pyrimidines and 2H-chromenes in their structures. Hence, the synthesis of novel chromeno pyrimidine containing chloro, fluoro, trifluoromethyl groups have been carried out by multi-step organic synthesis, in order to study their interesting antimicrobial properties. The synthesized compounds were characterized by ¹H NMR, ¹³C NMR, ¹⁹F NMR, FT-IR, GC-MS and elemental analysis. They were screened for their antibacterial activities against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*), Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and anti-fungal activities against *Aspergillus niger* and *Candida albicans* by cup plate method. The fluoro and trifluoro methyl derivatives showed the highest anti-microbial activity compared to the others. The trifluoro derivative (6c₂) exhibited excellent results with the highest inhibition zone of 26mm in 100 µg/ mL against *Bacillus subtilis* and 23mm in 100 µg/ mL against *Pseudomonas aeruginosa*.*

Keywords: Chromeno pyrimidines, antibacterial, antifungal, synthesis, novel drugs.

1. Introduction

It is apparent that the world today in spite of various developments in pharmaceutical research is facing a shortage of new drugs that can bring effective results & control the diseases caused by bacteria and fungi. Growing consumption of various antibiotics for the treatment of microbial infections led to the appearance of multi-drug resistant (MDR) microbial pathogens. Although there are several drugs available for the treatment of microbial infections, 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], antiviral [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 structurally diverse synthetic derivatives [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.

2. 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. ^1H NMR spectra were recorded on Bruker spectrometer (500 MHz) using DMSO- d_6 / CDCl_3 as a solvent. ^{13}C NMR spectra were recorded on Bruker spectrometer (125 MHz) in DMSO- d_6 / CDCl_3 . ^{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, I 2 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 $^\circ\text{C}$ 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 $^\circ\text{C}$); IR ($\nu_{\text{max}}\text{cm}^{-1}$): 3620, 3314, 1700, 1636 1565, 1460; ^1H -NMR (CDCl_3 , 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 (CDCl_3 , 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 ($\text{C}_{13}\text{H}_{10}\text{ClF}_3\text{O}_4 + \text{Na}$) $^+$: 345.0118; found: 345.0135

(3b): Off-yellow solid; M.P (162-165 $^\circ\text{C}$); IR ($\nu_{\text{max}}\text{cm}^{-1}$): 3601, 3000, 2960, 1735, 1620, 1429; ^1H -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, CDCl_3) δ 164.99, 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 $\text{C}_{14}\text{H}_{10}\text{F}_6\text{O}_4$ 356.03 g/ mole is C, 47.71; H, 2.20; F, 32.45; O, 17.84 % HRMS: calcd. For m/z ($\text{C}_{14}\text{H}_{10}\text{F}_6\text{O}_4 + \text{Na}$) $^+$: 356.22; found: 356.05

(3c): Off-white solid, M.P (154-156 $^\circ\text{C}$); IR ($\nu_{\text{max}}\text{cm}^{-1}$): 3640, 3324, 1790, 1676 1565, 1461; ^1H -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); ^{13}C NMR (125 MHz, CDCl_3 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 $\text{C}_{13}\text{H}_{10}\text{F}_4\text{O}_4$ are C, 50.71; H, 3.20; Cl, 8.02; F, 24.45; O, 20.84 %; HRMS: calcd. For m/z ($\text{C}_{13}\text{H}_{10}\text{F}_4\text{O}_4 + \text{Na}$) $^+$: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, monitored 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(154-158 $^{\circ}$ c); IR ($\nu_{\text{max}}\text{cm}^{-1}$) 3629, 3425, 3122, 3111, 1875,1720, 1625, 1592; ^1H -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 $\text{C}_{11}\text{H}_6\text{ClF}_3\text{O}_4$ 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 ($\text{C}_{11}\text{H}_6\text{ClF}_3\text{O}_4 + \text{Na}$) $^+$: 317.611; found: 317.572.

(4b): White solid, M.P (166-168 $^{\circ}$ c); IR ($\nu_{\text{max}}\text{cm}^{-1}$):3631, 3394, 3196, 3022, 2028, 1874, 1725, 1625, 1562, 1492; ^1H -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 $\text{C}_{12}\text{H}_6\text{F}_6\text{O}_4$ is C, 47.71; H, 2.20; F, 32.45; O, 17.84 %; HRMS calcd for m/z $\text{C}_{12}\text{H}_6\text{F}_6\text{O}_4 + \text{Na}$ $^+$: 328.17; found: 328.02

(4c): White solid; M.P(161-162 $^{\circ}$ c); IR ($\nu_{\text{max}}\text{cm}^{-1}$): 3621, 3314, 3196, 3012, 2028, 1864, 1715, 1625, 1552, 1492; ^1H -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, CDCl_3) 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 $\text{C}_{11}\text{H}_6\text{F}_4\text{O}_4$ is C, 47.71; H, 2.20; Cl, 8.02; F, 27.45; N, O, 23.84 %; HRMS: calcd. For m/z ($\text{C}_{11}\text{H}_6\text{F}_4\text{O}_4 + \text{Na}$) $^+$:278.27; found: 278.03.

3. 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 refluxed 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.

(6a₁):Light yellow solid ; M.P(167-169 $^{\circ}$ c); IR ($\nu_{\text{max}}\text{cm}^{-1}$) : 3819 ,3615, 3340, 3310, 2899, 2105, 1971, 1912, 1815, 1616, 1547, ^1H -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, CDCl_3) 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 $\text{C}_{15}\text{H}_{11}\text{ClF}_3\text{N}_3\text{O}_3$ is C, 48.21; H, 2.97; Cl, 9.49; F, 15.25; O, 12.84; N, 11.24 % HRMS: calcd. For m/z ($\text{C}_{15}\text{H}_{11}\text{ClF}_3\text{N}_3\text{O}_3 + \text{Na}$) $^+$:373.01; found: 373.04

(6a₂):Yellow solid ; M.P (176-178 $^{\circ}$ c); IR ($\nu_{\text{max}}\text{cm}^{-1}$): 3634, 3214, 3005, 2654, 2052, 1992, 1699, 1369; ^1H -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 $C_{15}H_8ClF_4N_3O_3$ C, 48.99; H, 2.57; Cl, 9.05; F, 19.45; O, 12.84; N, 10.24 %; HRMS: calcd. For m/z ($C_{15}H_8ClF_4N_3O_3 + Na$)⁺:389.71; found: 389.03

(6a₃): Off-white solid ; M.P(177-179⁰c); IR ($\nu_{max}cm^{-1}$): 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, ¹⁹F NMR δ : -87.02; Elemental analysis for $C_{16}H_8ClF_6N_3O_3$ is C, 43.71; H, 1.20; Cl, 8.06; F, 25.45; O, 10.84; N, 9.01 %; HRMS: calcd. For m/z ($C_{16}H_8ClF_6N_3O_3 + Na$)⁺:439.71; found: 439.03

(6b₁): yellow solid; M.P(192-194⁰c); IR ($\nu_{max}cm^{-1}$): 3899, 3715, 3350, 3312, 2899, 2105, 1961, 1942, ¹H 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); ¹³C NMR (125 MHz, CDCl₃) δ 164.59, 158.35, 157.25, 155.76, 131.99, 129.84, 125.81, 118.64; ¹⁹F NMR δ : -87.02, -62.80; Elemental analysis for $C_{16}H_{11}F_6N_3O_3$ is C, 47.71; H, 2.20; F, 27.45; N, 10.33; O, 11.84 % HRMS: calcd. For m/z ($C_{16}H_{11}F_6N_3O_3 + Na$)⁺:402.27; found: 407.07

(6b₂): Yellow solid ; M.P (197-198⁰c); IR ($\nu_{max}cm^{-1}$): 3632, 3225, 3059, 2647, 2546, 2512, 1978, 1831, 1748; ¹H-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); ¹³C 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; ¹⁹F NMR δ : -95.45, -87.02, -62.80. Elemental analysis for $C_{16}H_{10}F_7N_3O_3$ is C, 45.71; H, 2.20; F, 37.45; N, 9.33; O, 11.84 %; HRMS: calcd. For m/z ($C_{16}H_{10}F_7N_3O_3 + Na$)⁺:425.27; found: 425.07

(6b₃): Yellow solid; M.P (197-199⁰c); IR ($\nu_{max}cm^{-1}$): 3600, 3310, 3100, 2610, 2122, 1926, 1788, 1734, 1656, 1554; ¹H-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); ¹³C 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; ¹⁹F NMR δ : -87.02, -62.80; Elemental analysis for $C_{16}H_{10}ClF_6N_3O_3$ 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 ($\nu_{max}cm^{-1}$): 3501, 3340. 3127, 3100, 2904, 2764, 2648, 2179, 1982, 1953, 1788, 1751; ¹H-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 $C_{15}H_9F_4N_3O_3$ is C, 50.71; H, 2.20; F, 21.45; N, 11.33; O, 13.84 %. HRMS: calcd. For m/z ($C_{15}H_9F_4N_3O_3 + Na$)⁺: 355.27; found: 355.03 ¹⁹F NMR δ : -87.02, -134.13.

(6c₂): Yellow solid; M.P(175-177 °C); IR ($\nu_{max}cm^{-1}$): 3546, 3177, 3165, 3085, 2782, 2489, 2323; ¹H-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, ¹⁹F NMR δ : -87.02, -134.13; Elemental analysis for $C_{16}H_{10}F_7N_3O_3$ is C, 45.71; H, 2.20; F, 31.45; N, 9.33; O, 11.84 %

4. 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 grown for 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.

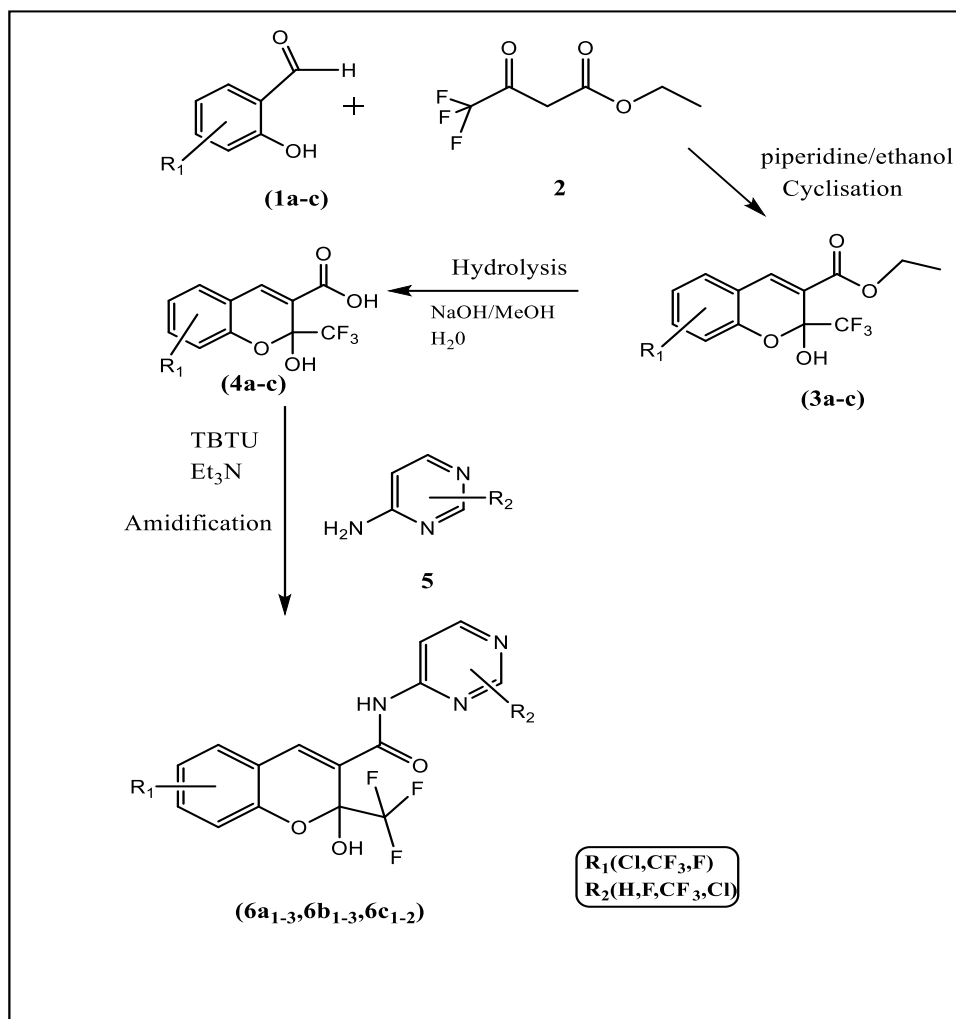
4.2. General procedure for testing the antifungal property

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 labeled 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 °C for 24 h. The diameters of the zones of growth inhibition were measured (mm) and compared to the standard.

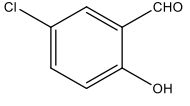
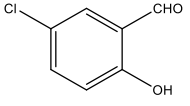
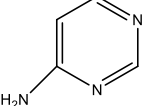
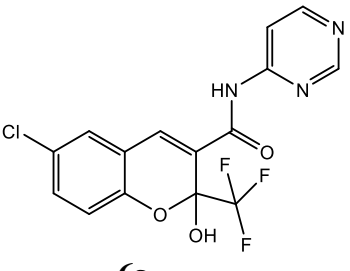
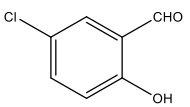
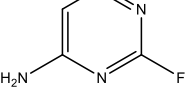
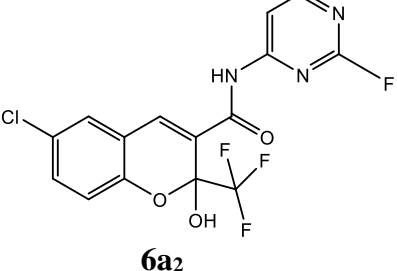
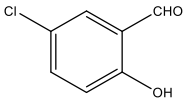
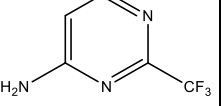
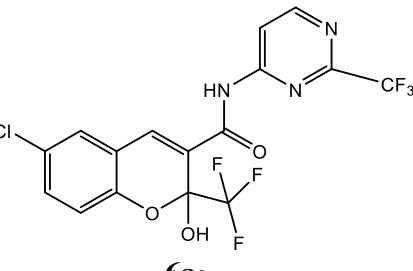
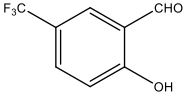
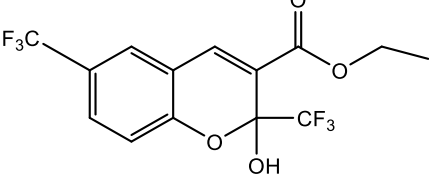
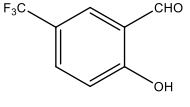
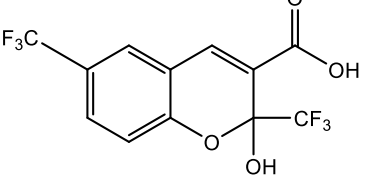
5. Results and Discussion

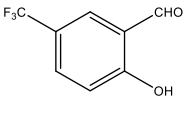
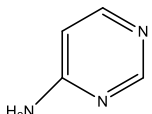
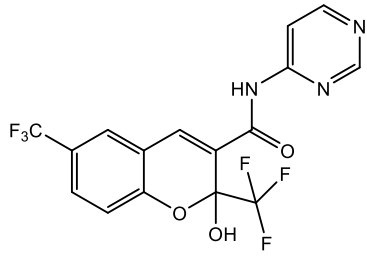
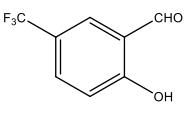
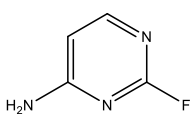
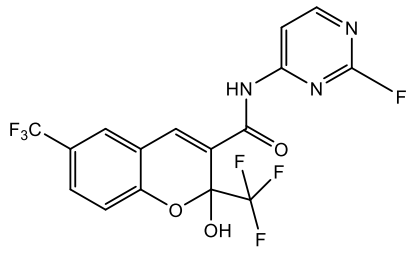
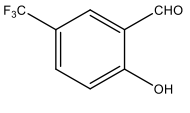
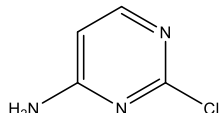
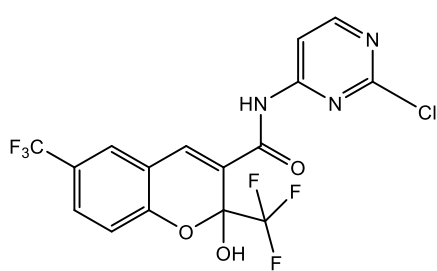
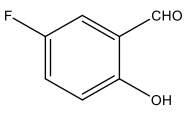
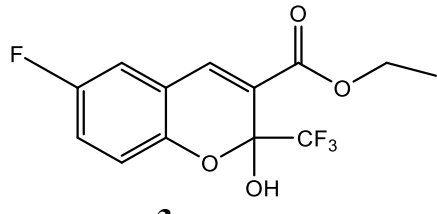
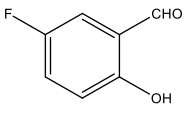
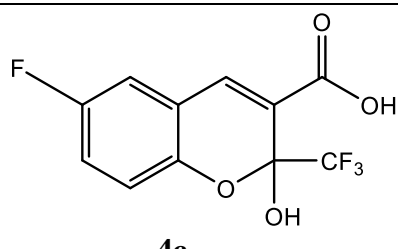
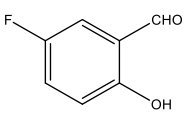
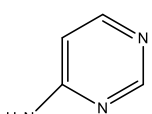
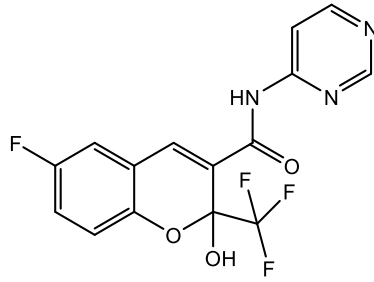
5.1. Synthesis and Characterization

As shown in scheme 1, chromene esters (**3a-c**) possessing the hydroxyl and trifluoro 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 ¹H NMR spectra gave triplet at δ 1.27 ppm and multiplet at δ 4.72 – 3.93 ppm shows the presence of 3H and 2H next to the ester group. The ¹⁹F NMR spectra of **3a** showed at signal δ -87.02 ppm showed the presence one type of fluorine atoms while ¹⁹F spectra of **4a** showed two signals at δ - 87.02, -62.80 ppm showing the presence of fluorine atoms in two different environments. After the initial reaction which required 4h of reflux, the esters were subjected to hydrolysis 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 (**6a₁₋₃**, **6b₁₋₃**, **6c₁₋₂**). Completion of all the reactions was monitored by a simple TLC analysis. The IR spectra of chromeno pyrimidines (**6a₁₋₃**, **6b₁₋₃**, **6c₁₋₂**) showed absorption bands corresponding to N-H stretching in the range of 3350-3210 cm⁻¹. The ¹³C-NMR spectra of all the synthesized compounds showed the quartets of CF₃ and C-2 atom with their corresponding coupling constants $1J_{C,F} = 289-291$ Hz and $2J_{C,F} = 33.6-36.4$ 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. | | - | | 68 |
| 2. | | - | | 74 |

| | | | | |
|----|---|---|---|----|
| |  | | | |
| 3. |  |  |  6a1 | 86 |
| 4. |  |  |  6a2 | 64 |
| 5. |  |  |  6a3 | 61 |
| 6. |  | - |  3b | 88 |
| 7. |  | - |  4b | 67 |

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

| | | | | |
|-----|--|--|--|----|
| 14. | | | | 71 |
|-----|--|--|--|----|

The ^1H NMR spectra of (**6a₁**) 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 (**6a₃**) gave signal at only two signals at δ 8.28 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 (**6a₁₋₂**, **6b₁₋₃**, **6c₁₋₂**) 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 (**6a₁**, **6a₂**, **6c₁**) 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 (**6a₃**, **6b₁**, **6b₂**, **6b₃**, **6c₂**) showed the best activity against all the bacterial strains. The activities of (**6a₃**, **6b₁**, **6b₂**, **6b₃**, **6c₂**) 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 the concentration of test / standard compound(s) | | | | | | | |
|---------------------|--|-----------------------------|----------------------------|-----------------------------|----------------------------|-----------------------------|----------------------------|-----------------------------|
| | <i>S. aureus</i> | | <i>B. subtilis</i> | | <i>E. coli</i> | | <i>P. aeruginosa</i> | |
| Concentration | 50 $\mu\text{g}/\text{mL}$ | 100 $\mu\text{g}/\text{mL}$ | 50 $\mu\text{g}/\text{mL}$ | 100 $\mu\text{g}/\text{mL}$ | 50 $\mu\text{g}/\text{mL}$ | 100 $\mu\text{g}/\text{mL}$ | 50 $\mu\text{g}/\text{mL}$ | 100 $\mu\text{g}/\text{mL}$ |
| 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 (**6a**₁₋₂, **6b**₁₋₃, **6c**₁₋₂) 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 (**6b**₂, **6c**₂) 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) | | | |
|-----------------|--|------------|--------------------|------------|
| | <i>A. niger</i> | | <i>C. albicans</i> | |
| Concentration | 50 µg/ mL | 100 µg/ mL | 50 µg/ mL | 100 µg/ mL |
| 3a | 7 | 11 | 10 | 19 |
| 4a | 7 | 13 | 11 | 17 |
| 6a ₁ | 10 | 15 | 9 | 17 |
| 6a ₂ | 11 | 16 | 11 | 19 |
| 6a ₃ | 11 | 14 | 10 | 16 |
| 3b | 14 | 16 | 10 | 21 |
| 4b | 17 | 19 | 11 | 22 |
| 6b ₁ | 14 | 20 | 16 | 24 |
| 6b ₂ | 16 | 28 | 17 | 30 |
| 6b ₃ | 17 | 26 | 20 | 28 |
| 3c | 12 | 15 | 15 | 22 |
| 4c | 10 | 19 | 13 | 20 |
| 6c ₁ | 14 | 23 | 16 | 27 |
| 6c ₂ | 17 | 30 | 19 | 32 |
| Griseofulvin | 20 | 35 | 21 | 36 |

6. Conclusion

Novel chromeno pyrimidines were synthesized using a simple multi-step strategy 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.

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