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Original Article

STEREO SELECTIVE SYNTHESIS OF NOVEL PYRAZOLE AND COUMARIN APPENDED BRIDGED PYRANS AS ANTIMICROBIAL AGENTS

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ABSTRACT

Objectives: The aim of the present study was to synthesize a series of novel bridged pyrans as antimicrobial agents.

Methods: An isomeric mixture of 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, 2(a-f) and 3-(4-ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, 3(a-f) were synthesized by the reaction of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-phenyl-1H-pyrazole-4-carbaldehyde) 1(a-f) and ethyl alcohol in the presence of conc. H_2SO_4. The synthesized compounds were evaluated for their antimicrobial activity.

Results: The structures of the new bridged pyran analogues 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano [3',2':6,7] chromeno [4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, 2(a-f) and 3-(4-ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano [2',3':5,6] chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, 3(a-f) were confirmed by spectral studies and elemental analysis. The compounds 2e and 3e were having-$CONH_2$ substitution and 2f and 3f were having-$CSNH_2$ substitutions in the pyrazole rings showed antibacterial at minimum concentrations against all the tested organisms.

Conclusions: Results of the antimicrobial activity reveal that some of the synthesized compounds act as potential antimicrobial agents against different fungal and bacterial organisms.

Keywords: Antibacterial, Antifungal, Cyclisation, Formyl pyrazoles, Inhibitory.

INTRODUCTION

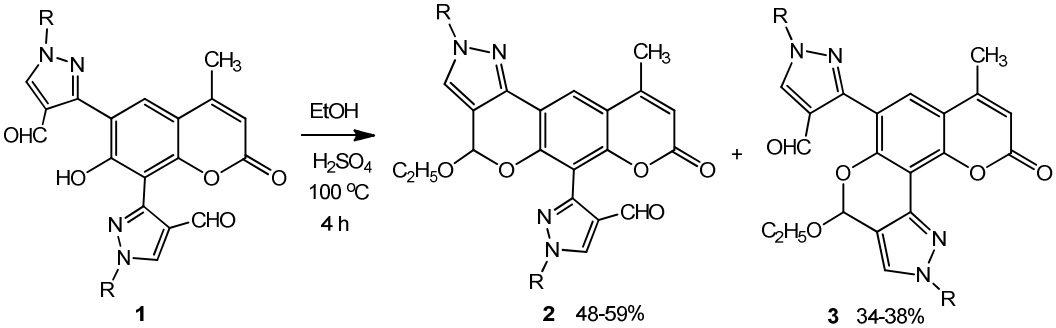
The construction of complex molecular architectures that exhibit greater biological potency in a facile and efficient manner remains an overarching goal for the chemists. In recent years coumarin libraries have attracted great attention because of their synthetic utility as building blocks for the construction of bioactive molecules. Coumarin derivatives are known to a wide range of activities; such as an antioxidant, antimicrobial, anti-HIV, antibiotic, anticancer, muscle relaxant, anti-inflammatory and anticoagulant properties [1]. An efficient synthesis of poly functionalized 4H-pyrans is carried out in one pot synthesis from an aldehyde, malononitrile and an active methylene diketo compound using a heterogeneous Mg/la mixed oxide catalyst [2]. A facile one-pot expeditious synthesis of 2-amino-4H-pyrans and 2-amino-5-oxo-5, 6, 7, 8-tetrahydro-4H-chromenes under solvent-free conditions using magnesium oxide as a catalyst is reported [3].

Pyrazole scaffolds drew a great deal of attention due to its contribution in biological and pharmacological fields [4]. The pyrazole nucleus has pronounced pharmacological applications such as anti-inflammatory [5], antimicrobial [6], antioxidant [7] and analgesic activity [8]. They have a long history of applications in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals.

In search of new antimicrobial agents and in continuation of our work on coumarin analogues we herein report the synthesis of a series of isomeric bridged pyrans tagged to pyrazole and coumarin skeleton and their antimicrobial activities.

Experimental

In a typical procedure, a series of isomeric bridged pyrans tagged to pyrazole and coumarin moiety 2(a-f) and 3(a-f) were synthesized by the condensation reaction of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-aryl-1H-pyrazole-4-carbaldehyde), 1(a-f) in ethyl alcohol (10 ml) in the presence of conc. H_2SO_4 (5 ml) under reflux conditions (Scheme-1).



1 **2** 48-59% **3** 34-38%

a) R = C_6H_5; b) R = 4-$CH_3C_6H_4$; c) R = 4-$OCH_3C_6H_4$;
d) R = 4-FC_6H_4; e) R = $CONH_2$; f) R = $CSNH_2$.

Scheme-1: Synthetic route for the bridged pyrans

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique [9]. The nutrient broth, which contains logarithmic serially two-fold diluted amount of test compound and controls was inoculated with approximately 5 x 10^5 c. f. u of actively dividing bacteria cells. The bacterial cultures were incubated for 24 h at 37 °C and fungi cultures were incubated for 72 h at 37 °C; the growth was monitored visually and spectrophotometrically. The lowest concentration required to arrest the growth of bacteria and fungi was regarded as minimum inhibitory concentration (MIC). The synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Bacillus substilis*, *Staphylococus aureus* and antifungal activity against *Aspergillus niger, Aspergillus flavus, C. albicans*. The antibiotics streptomycin and nystatin were used as standard drugs against bacteria and fungi species respectively. The experiments were carried out in triplicate; the results were taken as a mean of three determinations.

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on a Nujol mull on Shimadzu 8300 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 MHz and 125 MHz spectrophotometer respectively in $CDCl_3$ with TMS as an internal standard. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrophotometer TOF mode. Elemental analysis was performed on a Thermo Finnigan Flash EA 1112 CHN analyzer. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane: ethyl acetate (9:2) as eluent.

Typical procedure for synthesis of 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde, 2a

To a solution of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-phenyl-1H-pyrazole-4-carbaldehyde), 1a (2.58g, 0.005 mol) in ethyl alcohol (10 ml), concentrated sulfuric acid (5 ml) was drop wise and the mixture was refluxed for 4 h at 80 °C. The progress of the reaction was monitored by TLC. After completion, the solvent was removed in vacuo. The resulting mass was extracted in to ether (30 ml), washed successively with NaOH and $NaHCO_3$. The organic phase was dried over anhydrous sodium sulphate. The solvent was evaporated to dryness to get isomeric mixture of 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano [3',2':6,7] chromeno [4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde, 2a and 3-(4-ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano [2',3': 5,6]chromeno[4,3-c]pyrazol -6-yl)-1-phenyl-1H-pyrazole-4-carbal-dehyde, 3a (Scheme-1). The products were purified by column chromatography using hexane and ethyl acetate (9:2 v/v) as eluent. The same procedure was used in all cases.

RESULTS AND DISCUSSION

3-(4-Ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde, 2a

Solid in 59% (3.20g) yield; m. p. 192-194 °C. IR (Nujol, γ cm^{-1}): 1772 (s) (lactone C=O str), 1733 (s) (aldehyde C=O str), 1219 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.051 (t, 3H, CH_3), 2.423 (s, 3H, CH_3), 3.920 (q, 2H, OCH_2), 6.026 (s, 1H, C_9-H), 6.304 (s, 1H, C_4-H), 7.423-7.728 (m, 12H, Ar-H), 8.320 (s, 1H, 5m ring-H), 9.782 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.06 (1C, CH_3), 20.13 (1C, CH_3), 62.33 (1C, OCH_2), 112.59 (1C), 113.64 (1C), 114.87 (1C), 115.30 (1C), 116.02 (1C), 116.86 (1C), 117.40 (1C), 119.72 (4C), 123.22 (1C), 126.16 (2C), 128.13 (1C), 128.90 (4C), 130.17 (1C), 138.46 (2C), 147.80 (1C), 148.63 (1C), 151.12 (1C), 152.94 (1C), 156.10 (1C), 161.18 (1C, C_8), 181.32 (1C, CHO). MS (m/z): 545 (MH^+), 544 (M^+), 516, 488, 280 (100%, base peak), 236. Anal. Calcd. for $C_{32}H_{24}N_4O_5$: C, 70.58; H, 4.44; N, 10.29%; Found: C, 70.49; H, 4.26; N, 10.21%.

3-(4-Ethoxy-2-(4-methylphenyl)-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde, 2b

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis (1-(4-methyl phenyl)-1H-pyrazole-4-carbaldehyde), **1b** (2.72g, 0.005 mol) as solid in 48% (2.75g) yield; m. p. 144-146 °C. IR (Nujol, γ cm^{-1}): 1765 (s) (lactone C=O str), 1726 (s) (aldehyde C=O str), 1213 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.089 (t, 3H, CH_3), 2.314 (s, 6H, CH_3), 2.453 (s, 3H, CH_3), 3.984 (q, 2H, OCH_2), 6.066 (s, 1H, C_9-H), 6.202 (s, 1H, C_4-H), 7.405-7.748 (m, 10H, Ar-H), 8.263 (s, 1H, 5m ring-H), 9.707 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.62 (1C, CH_3), 20.30 (1C, CH_3), 21.40 (2C, CH_3), 62.66 (1C, OCH_2), 112.16 (1C), 113.04 (1C), 114.44 (1C), 115.33 (1C), 116.21 (1C), 119.42 (4C), 123.12 (1C), 128.76 (1C), 129.32 (4C), 130.37 (1C), 134.78 (2C), 135.14 (2C), 147.88 (1C), 148.66 (1C), 150.32 (1C), 151.64 (1C), 152.49 (2C), 156.11 (1C), 161.36 (1C, C_8), 180.66 (1C, CHO). MS (m/z): 573 (MH^+), 572 (M^+), 544, 516, 280 (100%, base peak), 236. Anal. Calcd. for $C_{34}H_{28}N_4O_5$: C, 71.32; H, 4.93; N, 9.78%; Found: C, 71.20; H, 4.78; N, 9.73%.

3-(4-Ethoxy-2-(4-methoxyphenyl)-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde, 2c

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde), **1c** (2.88g, 0.005 mol) as solid in 49% (2.95g) yield; m. p. 178-180 °C. IR (Nujol, γ cm^{-1}): 1769 (s) (lactone C=O str), 1732 (s) (aldehyde C=O str), 1221 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.126 (t, 3H, CH_3), 2.394 (s, 3H, CH_3), 3.813 (s, 6H, OCH_3), 3.955 (q, 2H, OCH_2), 6.148 (s, 1H, C_9-H), 6.322 (s, 1H, C_4-H), 7.361-7.707 (m, 10H, Ar-H), 8.123 (s, 1H, 5m ring-H), 9.869 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.62 (1C, CH_3), 20.30 (1C, CH_3), 55.40 (2C, OCH_3), 62.10 (1C, OCH_2), 111.32 (1C, C_9), 112.04 (4C), 113.20 (1C), 114.12 (4C), 114.93 (1C), 115.32 (1C), 116.00 (1C), 117.56 (1C), 123.22 (1C), 130.30 (1C), 131.18 (1C), 132.78 (2C), 145.06 (1C), 147.33 (2C), 151.44 (1C), 153.56 (1C), 154.86 (1C), 157.08 (2C), 162.10 (1C, C_8), 179.86 (1C, CHO). MS (m/z): 605 (MH^+), 604 (M^+), 576, 548, 280 (100%, base peak), 236. Anal. Calcd. for $C_{34}H_{28}N_4O_7$: C, 67.54; H, 4.67; N, 9.27%; Found: C, 67.40; H, 4.57; N, 9.14%.

3-(4-Ethoxy-2-(4-fluorophenyl)-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde, 2d

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl) bis (1-(4-fluoro phenyl)-1H-pyrazole-4-carbaldehyde), **1d** (2.76g, 0.005 mol) as solid in 54% (3.15g) yield; m. p. 182-185 °C. IR (Nujol, γ cm^{-1}): 1763 (s) (lactone C=O str), 1724 (s) (aldehyde C=O str), 1213 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.105 (t, 3H, CH_3), 2.343 (s, 3H, CH_3), 3.908 (q, 2H, OCH_2), 6.244 (s, 1H, C_9-H), 6.440 (s, 1H, C_4-H), 7.224-7.682 (m, 10H, Ar-H), 8.189 (s, 1H, 5m ring-H), 9.804 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.02 (1C, CH_3), 20.14 (1C, CH_3), 63.00 (1C, OCH_2), 112.02 (1C, C_9), 113.16 (1C), 113.53 (1C), 114.32 (1C), 115.14 (4C), 115.52 (1C), 116.32 (4C), 116.68 (1C), 117.26 (1C), 123.12 (1C), 128.18 (1C), 130.14 (1C), 134.18 (2C), 146.23 (1C), 148.13 (1C), 150.65 (1C), 152.36 (1C), 155.46 (1C), 159.28 (2C), 162.34 (1C, C_8), 180.22 (1C, CHO). MS (m/z): 581 (MH^+), 580 (M^+), 552, 524, 280 (100%, base peak), 236. Anal. Calcd. for $C_{32}H_{22}F_2N_4O_5$: C, 66.20; H, 3.82; N, 9.65%; Found: C, 66.07; H, 3.66; N, 9.49%.

6-(1-Carbamoyl-4-formyl-1H-pyrazol-3-yl)-4-ethoxy-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c] pyrazole-2-carboxamide, 2e

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(4-formyl-1H-pyrazole-1-carboxamide), **1e** (4) (2.25g, 0.005 mol) as solid in 50% (2.39g) yield; m. p. 200-203 °C. IR (Nujol, γ cm^{-1}): 1771 (s) (lactone C=O str), 1730 (s) (aldehyde C=O str), 1217 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.166 (t, 3H, CH_3), 2.404 (s, 3H, CH_3), 3.902 (q, 2H, OCH_2), 6.215 (s, 1H, C_9-H), 6.363 (s, 1H, C_4-H), 7.636 (s, 1H, C_{11}-H), 7.923 (s, 4H, NH_2), 8.26 (s, 1H, 5m ring-H), 8.677 (s, 1H, 5m ring-H), 9.803 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.56 (1C, CH_3), 21.10 (1C, CH_3), 62.24 (1C, OCH_2), 110.72 (1C), 112.46 (1C), 112.82 (1C, C_9), 114.02 (1C), 114.51 (1C), 115.66 (1C), 126.48 (1C), 128.88 (1C), 135.12 (1C), 136.28 (1C), 142.33 (1C), 144.63 (1C), 147.54 (1C), 152.10 (1C), 156.31 (1C), 157.30 (2C, $CONH_2$), 161.14 (1C, C_8), 167.22 (1C, CHO). MS (m/z): 479 (MH^+), 478 (M^+), 450, 422, 280 (100%, base peak), 236. Anal. Calcd. for $C_{22}H_{18}N_6O_7$: C, 55.23; H, 3.79; N, 17.57%; Found: C, 55.20; H, 3.64; N, 17.44%.

6-(1-Carbamoyl-4-formyl-1H-pyrazol-3-yl)-4-ethoxy-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazole-2-carbothioamide, 2f

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(4-formyl-1H-pyrazole-1-carbothioamide), **1f** (4) (2.41g, 0.005 mol) as solid in 56% (2.85g) yield; m. p. 174-178 °C. IR (Nujol, γ cm^{-1}): 1766 (s) (lactone C=O str), 1735 (s) (aldehyde C=O str), 1215 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.101 (t, 3H, CH_3), 2.414 (s, 3H, CH_3), 3.882 (q, 2H, OCH_2), 6.128 (s, 1H, C_9-H), 6.343 (s, 1H, C_4-H), 7.638 (s, 1H, C_{11}-H), 7.864 (s, 1H, 5m ring-H), 8.076 (s, 1H, 5m ring-H), 8.622 (s, 4H, NH_2), 9.844 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.78 (1C, CH_3), 20.86 (1C, CH_3), 62.68 (1C, OCH_2), 111.72 (1C), 112.54 (1C, C_9), 112.96 (1C), 114.88 (1C), 115.24 (1C), 116.12 (1C), 126.72 (1C), 128.74 (1C), 135.27 (1C), 136.7 (1C), 142.21 (1C), 144.51 (1C), 147.33 (1C), 152.32 (1C), 155.44 (1C), 162.36 (1C, C_8), 174.10 (2C, $CSNH_2$), 180.26 (1C, CHO). MS (m/z): 511 (MH^+), 510 (M^+), 482, 454, 280 (100%, base peak), 236. Anal. Calcd. for $C_{22}H_{18}N_6O_5S_2$: C, 51.76; H, 3.55; N, 16.46%; Found: C, 51.82; H, 3.45; N, 16.30%.

3-(4-Ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde, 3a

Solid in 36% (1.95g) yield; m. p. 189-190 °C. IR (Nujol, γ cm^{-1}): 1765 (s) (lactone C=O str), 1728 (s) (aldehyde C=O str), 1214 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.052 (t, 3H, CH_3), 2.422 (s, 3H, CH_3), 3.940 (q, 2H, OCH_2), 6.126 (s, 1H, C_9-H), 6.316 (s, 1H, C_4-H), 7.416-7.745 (m, 12H, Ar-H), 8.319 (s, 1H, 5m ring-H), 9.770 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.22 (1C, CH_3), 20.18 (1C, CH_3), 62.21 (1C, OCH_2), 112.50 (1C), 113.50 (1C), 114.66 (1C), 115.43 (1C), 116.32 (1C), 116.88 (1C), 117.68 (1C), 119.74 (4C), 123.98 (1C), 126.02 (2C), 128.10 (1C), 128.94 (4C), 130.16 (1C), 138.41 (2C), 147.36 (1C), 148.60 (1C), 151.06 (1C), 152.54 (1C), 152.10 (1C, C_8), 156.15 (1C), 185.30 (1C, CHO). MS (m/z): 545 (MH^+), 544 (M^+), 516, 488, 442, 353, 249 (100%, base peak). Anal. Calcd. for $C_{32}H_{24}N_4O_5$: C, 70.58; H, 4.44; N, 10.29%; Found: C, 70.36; H, 4.28; N, 10.11%.

3-(4-Ethoxy-8-methyl-10-oxo-2-(4-methylphenyl))-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde, 3b

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde), **1b** (2.72g, 0.005 mol) as solid in 38% (2.17g) yield; m. p. 147-149 °C. IR (Nujol, γ cm^{-1}): 1772 (s) (lactone C=O str), 1731 (s) (aldehyde C=O str), 1216 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.123 (t, 3H, CH_3), 2.281 (s, 6H, CH_3), 2.487 (s, 3H, CH_3), 3.760 (q, 2H, OCH_2), 6.112 (s, 1H, C_9-H), 6.306 (s, 1H, C_4-H), 7.452-7.790 (m, 10H, Ar-H), 8.436 (s, 1H, 5m ring-H), 9.780 (s, 1H, CHO). MS (m/z): 573 (MH^+), 572 (M^+), 516, 470, 249 (100%, base peak). Anal. Calcd. for $C_{34}H_{28}N_4O_5$: C, 71.32; H, 4.93; N, 9.78%; Found: C, 71.24; H, 4.77; N, 9.56%.

3-(4-Ethoxy-2-(4-methoxyphenyl)-8-methyl-10-oxo-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde, 3c

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis (1-(4-methoxy phenyl)-1H-pyrazole-4-carbaldehyde), **1c** (2.88g, 0.005 mol) as solid in 37% (2.23g) yield; m. p. 172-174 °C. IR (Nujol, γ cm^{-1}): 1770 (s) (lactone C=O str), 1730 (s) (aldehyde C=O str), 1222 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.099 (t, 3H, CH_3), 2.346 (s, 3H, CH_3), 3.855 (s, 6H, OCH_3), 3.980 (q, 2H, OCH_2), 6.133 (s, 1H, C_9-H), 6.338 (s, 1H, C_4-H), 7.380-7.745 (m, 10H, Ar-H), 8.469 (s, 1H, 5m ring-H), 9.789 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.66 (1C, CH_3), 20.38 (1C, CH_3), 55.56 (2C, OCH_3), 62.19 (1C, OCH_2), 112.08 (1C, C_9), 112.22 (4C), 113.26 (1C), 114.56 (4C), 114.98 (1C), 115.74 (1C), 116.08 (1C), 117.88 (1C), 123.20 (1C), 130.34 (1C), 131.28 (1C), 132.26 (2C), 145.38 (1C), 147.40 (2C), 151.40 (1C), 152.10 (1C, C_8), 153.55 (1C), 154.92 (1C), 157.18 (2C), 183.32 (1C, CHO). Anal. Calcd. for $C_{34}H_{28}N_4O_7$: C, 67.54; H, 4.67; N, 9.27%; Found: C, 67.42; H, 4.48; N, 9.36%.

3-(4-Ethoxy-2-(4-fluorophenyl)-8-methyl-10-oxo-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde, 3d

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis (1-(4-fluoro phenyl)-1H-pyrazole-4-carbaldehyde), **1d** (2.76g, 0.005 mol) as solid in 36% (2.08g) yield; m. p. 177-178 °C. IR (Nujol, γ cm^{-1}): 1775 (s) (lactone C=O str), 1734 (s) (aldehyde C=O str), 1224 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.219 (t, 3H, CH_3), 2.338 (s, 3H, CH_3), 3.914 (q, 2H, OCH_2), 6.241 (s, 1H, C_9-H), 6.466 (s, 1H, C_4-H), 7.220-7.686 (m, 10H, Ar-H), 8.185 (s, 1H, 5m ring-H), 9.820 (s, 1H, CHO). Anal. Calcd. for $C_{32}H_{22}F_2N_4O_5$: C, 66.20; H, 3.82; N, 9.65%; Found: C, 66.10; H, 3.64; N, 9.44%.

6-(1-Carbamoyl-4-formyl-1H-pyrazol-3-yl)-4-ethoxy-8-methyl-10-oxo-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazole-2-carboxamide, 3e

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(4-formyl-1H-pyrazole-1-carboxamide), **1e** (2.25g, 0.005 mol) and ethyl alcohol (0.002 mol) as solid in 36% (1.72g) yield; m. p. 205-206 °C. IR (Nujol, γ cm^{-1}): 1762 (s) (lactone C=O str), 1722 (s) (aldehyde C=O str), 1112 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.162 (t, 3H, CH_3), 2.404 (s, 3H, CH_3), 3.923 (q, 2H, OCH_2), 6.210 (s, 1H, C_9-H), 6.362 (s, 1H, C_4-H), 7.646 (s, 1H, C_7-H), 7.923 (s, 4H, NH_2), 8.262 (s, 1H, 5m ring-H), 8.672 (s, 1H, 5m ring-H), 9.834 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.52 (1C, CH_3), 21.48 (1C, CH_3), 62.36 (1C, OCH_2), 110.70 (1C), 112.38 (1C), 112.80 (1C, C_9), 114.00 (1C), 114.50 (1C), 115.42 (1C), 126.43 (1C), 128.81 (1C), 135.10 (1C), 136.24 (1C), 142.30 (1C), 144.23 (1C), 147.30 (1C), 152.56 (1C), 156.26 (1C), 151.30 (2C, $CONH_2$), 152.14 (1C, C_8), 187.20 (1C, CHO). MS (m/z): 479 (MH^+), 478 (M^+), 450, 422, 249 (100%, base peak). Anal. Calcd. for $C_{22}H_{18}N_6O_7$: C, 55.23; H, 3.79; N, 17.57%; Found: C, 55.08; H, 3.60; N, 17.39%.

6-(1-Carbamothioyl-4-formyl-1H-pyrazol-3-yl)-4-ethoxy-8-methyl-10-oxo-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazole-2-carbothioamide, 3f

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(4-formyl-1H-pyrazole-1-carbothioamide), **1f** (4) (2.41g, 0.005 mol) as solid in 38% (1.93g) yield; m. p. 170-171 °C. IR (Nujol, γ cm^{-1}): 1778 (s) (lactone C=O str), 1738 (s) (aldehyde C=O str), 1226 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.118 (t, 3H, CH_3), 2.414 (s, 3H, CH_3), 3.895 (q, 2H, OCH_2), 6.129 (s, 1H, C_9-H), 6.348 (s, 1H, C_4-H), 7.684 (s, 1H, C_{11}-H), 7.846(s, 1H, 5m ring-H), 8.022 (s, 1H, 5m ring-H), 8.560 (s, 4H, NH_2), 9.789 (s, 1H, CHO). Anal. Calcd. for $C_{22}H_{18}N_6O_5S_2$: C, 51.76; H, 3.55; N, 16.46%; Found: C, 51.88; H, 3.33; N, 16.26%.

The structures of the synthesized compounds were provided by IR, ^{1}H NMR, ^{13}C NMR, MS studies and elemental analysis. For instance, in IR spectra, the stretching frequencies of the compounds 2(a-f) and 3(b-e) showed a strong absorption bands in the region 1778-1762 $cm^{-1}$and 1738-1722 cm^{-1} for lactone and aldehydic C=O bonds respectively. A strong and intense absorption band is absorbed in the region 1226-1112 $cm^{-1}$was assigned to C-O bonds.

In ^{1}H NMR spectra, the compounds 2(a-f) and 3(a-f) showed the absorption signals due to aromatic and substituent protons in the expected region. The compounds 2(a-f) showed consistent pattern signals due to C_9-H and C_4-H appeared as singlet in the region δ 6.000-6.100 ppm, and δ 6.200-6.300 ppm. The–CHO proton appeared as singlet in the region δ 9.700-9.900 ppm. While the compounds 3(a-f) showed consistent pattern signals due to C_9-H and C_4-H appeared as singlet in the region δ 6.120-6.150 ppm, and δ 6.300-6.400 ppm. The–CHO proton appeared as singlet in the region δ 9.770-9.980 ppm.

In ^{13}C NMR, all compounds showed the signals due to aromatic and substituent carbons at the expected region. The carbonyl carbons absorbed in the region δ 179.00-185.00 ppm. The synthesized compounds 2(a-f) showed significantly stable molecular ion peaks with a relative abundance ranging up to 18-66% and the base peak at m/z 280; While 3(a-f) showed a base peak at m/z 249. Further, all compounds showed satisfactorily CHN analysis with a deviation of±0.20% from the theoretically calculated values, which strongly favor the formation of the products.

The results of MICs) of the synthesized compounds 2(a-f) and 3(a-f) tested against different bacterium were tabulated in table-1.

Table 1: MIC's of the synthesized compounds 2(a-f) and 3(a-f) tested against bacteria species

Compound	Minimum inhibitory concentrations in µg/ml		
	S. aureus	*B. substilis*	*E. coli*
2a	50	**	200
2b	100	**	100
2c	50	**	150
2d	50	**	200
2e	25	150	50
2f	25	100	50
3a	150	200	150
3b	200	200	150
3c	150	150	200
3d	150	200	150
3e	25	50	25
3f	25	25	50
Streptomycin	25	50	25

Values are expressed as mean of the three determinations (n=3), **No inhibition observed even at a higher concentration of 200 µg/ml

The synthesized compounds exerted moderate to good antibacterial activity against the tested organisms. Compounds 2(a-d) failed to show antibacterial activity against *B. substilis* even at a concentration higher than 200 µg/ml, but showed moderate activity against the species *S. aureus* and *E. coli*. Compounds 3(a-d) showed moderate activities against all the bacterium tested. Compounds 2e and 3e were having-$CONH_2$ substitution and 2f and 3f were having-$CSNH_2$ substitutions in the pyrazole rings showed antibacterial at minimum concentrations against all the tested organisms.

The results of MICs) of the synthesized compounds 2(a-f) and 3(a-f) tested against different fungi species were tabulated in table-2.

Table 2: MIC's of the synthesized compounds 2(a-f) and 3(a-f) tested against fungi species

Compound	Minimum inhibitory concentrations in µg/ml		
	A. niger	*A. flavus*	*C. albicans*
2a	100	200	100
2b	100	150	150
2c	200	150	150
2d	150	200	100
2e	50	50	25
2f	25	50	25
3a	100	150	150
3b	150	150	100
3c	200	150	200
3d	100	20	150
3e	25	50	25
3f	25	50	50
Nystatin	25	50	25

Values are expressed as mean of the three determinations (n=3).

The synthesized compounds showed promising antifungal activity against the tested organisms. Compounds 2(a-d) and 3(a-d) showed moderate activities against all the bacterium tested in comparison with that of the reference standard. Compounds 2e and 3e were having-$CONH_2$ substitution and 2f and 3f were having-$CSNH_2$ substitutions in the pyrazole rings showed antifungal activities at minimum concentrations against all the tested organisms.

CONCLUSION

The synthesized compounds showed promising *in vitro* antifungal activity against the tested organisms. The compounds 2e and 3e were having-$CONH_2$ substitution and 2f and 3f were having-$CSNH_2$ substitutions in the pyrazole rings showed antibacterial and antifungal activity at minimum concentrations against all the tested organisms. Their MIC values indicate that these compounds act as potential antimicrobial agents. The compounds 2(a-d) and 3(a-d) showed antimicrobial activity at a higher concentration comparison with those of standard antibiotics used as reference drugs.

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CONFLICT OF INTERESTS

Declared None

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