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Synthesis, Characterization and Biological Assessment of Novel Pyrano-Pyrrole Derivatives from Chalcones of 4-Amino 3,5 Dichlorobenzotrifluoride

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Abstract

Chalcones and pyrano-pyrrole derivatives play important roles in pharmaceutical research and innovation. Owing to the significant relevance of chalcones and pyrano-pyrrole derivatives in pharmaceutics, we have attempted to synthesize these biologically active molecules using 4-amino-3,5-dichlorobenzotrifluoride. A series of novel pyrano-pyrrole derivatives was prepared via substituted chalcones using facile synthetic approaches. All synthesized molecules were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry, and were screened for antimicrobial properties.

Keywords: cyclic imide, malononitrile, chalcones, pyrano-pyrrole

1. Introduction

Chalcone is an important class of chemical compounds containing an unsaturated carbonyl group that serves as an essential building block for a variety of key biological molecules. Research on chalcones has received considerable attention in recent years. All compounds with the 1,3-diphenylprop-2-en-1-one core framework, in which two aromatic rings are connected by an α,β-unsaturated carbonyl system, are collectively referred to as chalcones [1]. The development of new drugs and pharmaceutical research both depend heavily on heterocyclic molecules. This field encompasses a vast area of synthetic research, particularly in the pharmaceutical sciences. Cyclic imides prepared from primary amines and cyclic anhydrides have emerged as an important class of organic substrates for agricultural, biological, and chemical applications [2].

Cyclic imides are extensively utilized for advanced derivatization, mainly of chalcones, through substituted aromatic aldehydes.

As noted above, chalcones are highly effective for treating a wide range of illnesses [3]. Additionally, they are used to create some of the most inventive heterocyclic molecules, including pyrano-pyrroles, pyrano-pyrazoles, isoxazoles, flavones, and pyrazolines [4–6]. Recently, renewed global attention has focused on this classic class of heterocyclic compounds because of their significant applications and advantages, specifically in pharmaceutical science and innovative drug design [7]. Several pyrano-pyrrole derivatives have been conveniently prepared by a single-pot multicomponent reaction. A calibrated mixture of aryl aldehyde, malononitrile, and hydroxypyrrole was refluxed in the presence of a catalyst to afford the desired product [8]. Recently, benzopyran derivatives have attracted considerable attention due to their therapeutic significance as anticoagulants, diuretics, and spasmolytics [9–12].

Benzopyrans are also recognized for their remarkable medicinal value against serious diseases [13], including anti-anaphylactic, antitumor, and anticancer activities [14]. According to the literature, benzopyrans have been found in numerous biologically active natural compounds [15]. Additionally, they are utilized as pigments [16], photoactive materials [17], and biodegradable agrochemicals [18].

In contemporary mainstream research, considerable effort is being devoted to developing modern synthetic strategies that complement and explore novel methodologies in heterocyclic chemistry. Researchers are also focusing on significant molecular complexity, higher practical compatibility, and low-cost solvents and reagents. In addition to traditional methods, numerous eco-friendly methodologies have been described in the literature for obtaining complex heterocyclic moieties from convenient precursors under milder reaction conditions. Heterocyclic chemistry plays a significant role in synthesizing highly efficient molecules.

In the current work, 4-amino-3,5-dichlorobenzotrifluoride and succinic anhydride were used to prepare 1-(2,6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidine-2,5-dione (3a). Compound 3a was further derivatized into a novel series of chalcones using substituted aromatic aldehydes in acetic acid. This novel series of chalcones was ultimately utilized to design substituted pyrano-pyrrole derivatives. Many demanding, time-consuming, instrument-intensive, and environmentally unfriendly synthetic approaches have been reported for the synthesis of chalcones and pyrrole-fused derivatives. However, we have developed an effective, safe, and environmentally friendly synthetic method for preparing bis-chalcones and their derivatives. It offers high yield rapidly and at low economic cost. All newly developed molecules were investigated by spectroscopic techniques and evaluated for their antimicrobial properties.

2. Materials and Methods

Melting points were recorded in open glass capillaries and are uncorrected. The chemical structures of the obtained compounds were confirmed by spectral analyses. IR spectra were recorded in KBr pellets on Shimadzu and ATR Bruker alpha FT-IR spectrophotometers. ^{1}H-NMR spectra were recorded at 500 MHz on a Bruker spectrometer. The reaction was monitored by thin-layer chromatography using pre-coated silica gel aluminium plates with a mixture of diethyl ether and ethyl acetate (6:4).

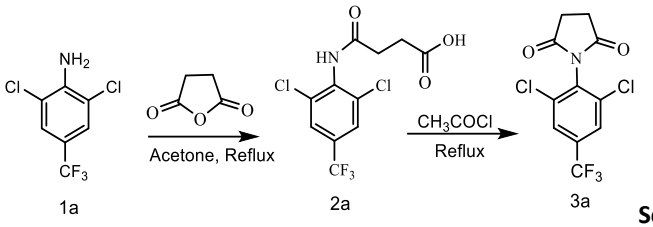
2.1 EXPERIMENTAL SECTION

2.1.1 General Procedure for the Synthesis of N-phenyl Succinimide:

Synthesis of 1-(2,6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidine-2,5-dione (3a)

Succinic anhydride (0.01 mol) was dissolved in benzene (10 mL), and 2,6-dichloro-4-(trifluoromethyl) aniline (0.01 mol) was added with vigorous stirring. 4-((2,6-Dichloro-4-(trifluoromethyl) phenyl) amino)-4-oxobutanoic acid (2a) was formed. This amic acid was cyclized using fresh acetyl chloride under reflux to give

product 3a. The product was recrystallized from ethanol (Scheme 1).



Scheme 1: 1-(2,6-Dichloro-4-(trifluoromethyl) phenyl) pyrrolidine-2,5-dione (3a)

1)1-(2, 6-dichloro-4-trifluoromethyl-phenyl)-pyrolidine -2, 5-Dione (3a):
Molecular formula: $C_{11}H_6Cl_2F_3NO_2$.
Physical appearance: off-white powder.
Nature: amorphous. Yield: 88%.
Melting point: 164–166 °C.
Molecular weight: 312 g/mol.

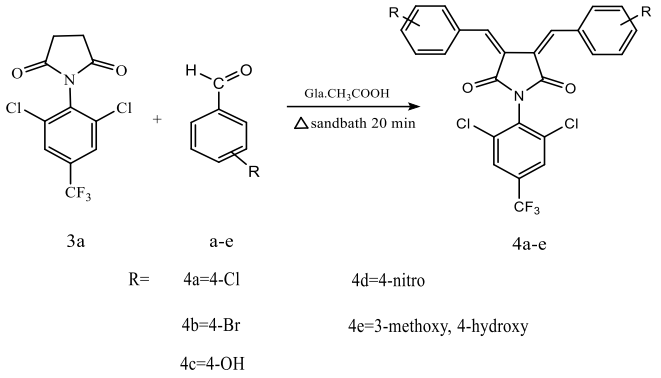
Elemental analysis calcd: C, 38.34; H, 1.94; N, 4.49. Found: C, 38.24; H, 1.83; N, 4.34.

FT-IR (KBr) cm^{-1}: 3000 (C–H stretching, CH_2), 2936 (C–H stretching, Ar–H), 1710 (C=O), 1537 and 1490 (Ar C=C), 1220 (C–N stretching), 1010 (C–F stretching), 826 (C–Cl stretching).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.50 (t, 4H), 7.94 (s, 2H, Ar-H).

2.2.2 General Method for the Synthesis of Chalcones Using Cyclic Imide 3a and Substituted Aromatic Aldehydes:

Synthesis of (3Z,4Z)-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-3,4-bis(substituted-benzylidene) pyrrolidine-2,5-diones (4a–e)
Cyclic imide 3a (0.01 mol) and substituted aromatic aldehyde (a–e) (0.02 mol) were dissolved in glacial acetic acid (10 mL). The reaction mixture was concentrated on a sand bath over a low flame. The progress of the reaction was monitored by TLC using n-hexane:ethyl acetate (8:2) as developing solvent. The colored solid product (4a–e) was obtained and recrystallized from aqueous ethanol.



Scheme-2: Synthesis of bis-chalcones using cyclic imide and derivatives of aromatic aldehyde

1) (3Z,4Z)-3,4-bis(4-chlorobenzylidene)-1-(2,6-dichloro 4(trifluoromethyl) phenyl) pyrrolidine-2,5-dione (4a):
Chemical Formula: $C_{25}H_{12}Cl_4F_3NO_2$
Physical Appearance: Off white solid
Nature of compound: Amorphous
Percentage Yield (%): 91
Melting Point (°C):162
Molecular Weight (g/mol): 554

FT-IR (KBr) cm^{-1}: 3051, 2926 and 2852 (C=C-H stretching), 1529, 1492 and 1425 (Aromatic C=C), 1591 (C=C), 1689 (N-C=O) 1321 (N-C stretching), 821 (C-Cl stretching), 1014 (C-F stretching)

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 8.08 (s, 2H, Ar-H, *J* = 2.3 Hz), 7.92 (m, 4H, Ar-H, *J*=7.5 Hz), 7.58-7.60 (m, 4H, 2Ar-H, *J*=7.5 Hz and =C-H)

2) (3Z, 4Z)-3, 4-bis (4-bromobenzylidene)-1-(2, 6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidine-2, 5-Dione (4b):
Chemical Formula: $C_{25}H_{12}Br_2Cl_2F_3NO_2$
Physical Appearance: Off white solid
Nature of compound: Amorphous
Percentage Yield (%): 90
Melting Point (°C): 192
Molecular Weight (g/mol): 646

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 8.01 (s, 2H, Ar-H, *J* = 2.3 Hz), 7.85-7.95 (m, 4, Ar-H, *J*=7.5 Hz), 7.70-7.75 (m, 4H, *J*=7.5 Hz, 2Ar-H) 7.56 (s, 2H, =C-H)

3) (3Z, 4Z)-3, 4-bis (4-hydroxybenzylidene)-1-(2, 6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidine-2, 5-Dione (4c):
Chemical Formula: $C_{25}H_{14}Cl_2F_3NO_4$
Physical Appearance: Off white solid
Nature of compound: Amorphous
Percentage Yield (%): 92
Melting Point (°C):120
Molecular Weight (g/mol): 520

FT-IR (KBr) cm^{-1}: 3020, 2924 and 2583 (C=C-H stretching), 1591, 1535 and 1440 (Ar-C=C, ethylene) 1678 (N-C=O), 1323 (C-N stretching), 550-850 (C-Cl stretching), 1286 (C-O Stretching) 1016 (C-F stretching), 3184 (Ar-O-H stretching), 1390 (O-H bending).

^{1}H-NMR (500 MHz, DMSO-d$_6$, δ ppm): 8.00 (s, 2H, Ar-H, *J* = 2.3 Hz), 7.75-7.77 (m 4H, Ar-H, *J* = 7.5 Hz), 6.93 (m, 4H, Ar-H, *J* = 7.5 Hz), 7.54 (s, 2H, =C-H), 9.79 (s, 1H, Ar-O-H).

4) (3Z,4Z)-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-3,4-bis (4-hydroxy-3-methoxy benzylidene) pyrrolidine - 2,5-dione (4d):
Chemical Formula: $C_{27}H_{18}Cl_2F_3NO_6$
Physical Appearance: Brown coloured Solid
Nature of compound: Amorphous
Percentage Yield (%): 94
Melting Point (°C): 92
Molecular Weight (g/mol): 580

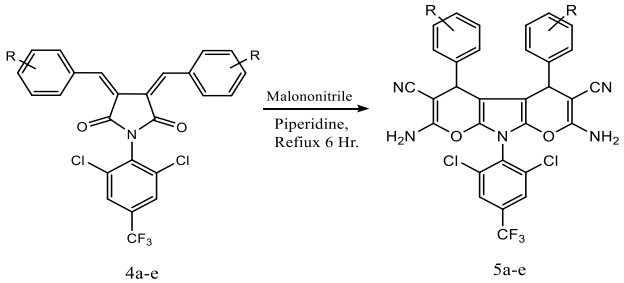
^{1}H-NMR (500 MHz, DMSO-d$_6$, δ ppm): 8.008 (s, 2H, Ar-H, *J* = 2.3 Hz), 7.41-7.43 (m, 4H, Ar-H, *J* = 7.5 Hz), 6.94 (d, 2H, 2Ar-H, *J* = 7.5 Hz), 7.39 (s, 2H, =CH), 9.77 (s, 1H, Ar-O-H), 3.85 (s, 3H OCH_3)

5) (3Z, 4Z)-3, 4-bis (4-nitrobenzylidene)-1-(2, 6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidine-2, 5-Dione (4e):
Chemical Formula: $C_{25}H_{12}Cl_2F_3N_3O_6$
Physical Appearance: Pale yellow solid
Nature of compound: Amorphous
Percentage Yield (%): 89
Melting Point (°C):142
Molecular Weight (g/mol): 578

2.2.3 General methods for the synthesis of Pyrano-pyrrole derivatives using Chalcones:

Synthesis of Pyrano-pyrrole derivatives of (3Z,4Z)-3,4-bis (benzylidene)-1-(2,6-dichloro 4 (trifluoromethyl) phenyl) pyrrolidine-2,5-dione (4a-e) and malononitrile:

Synthesis of 2,7-diamino-9-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4,5-diphenyl-5,9-dihydro-4H-dipyrano [2,3-b:3',2'-d] pyrrole-3,6-dicarbonitrile (5a-e):
Substituted chalcone (4a-e) (1 mmol) was dissolved in 60% ethanol and then malononitrile (2 mmol) was transferred slowly at room temperature. The reaction mixture was refluxed at 95°C for 6 hr. with constant stirring. Reaction was simultaneously observed by TLC using silica-coated metal plates with appropriate solvent system (n-hexane: ethyl acetate 8:2). The reaction mixture was transferred to crushed ice, to furnish solid precipitate. Precipitate was obtained by filtration, wiped via distilled water and allowed to air dry. Finally, the product was recrystallized from methanol to afford desired compounds (5a-e).



4a-e 5a-e

R= 5a=4-Cl 5d=4-nitro

5b=4-Br 5e=3-methoxy, 4-hydroxy

5c=4-OH

Scheme 3: Synthesis of pyrano-pyrrole derivatives using chalcones.

1) 2,7-diamino-4,5-bis(4-chlorophenyl)-9-(2,6-dichloro-4-(trifluoromethyl)phenyl)-5,9-dihydro-4H-dipyrano[2,3-b:3',2'-d]pyrrole-3,6-dicarbonitrile (5a):
Chemical Formula: $C_{31}H_{16}Cl_4F_3N_5O_2$
Physical Appearance: Off white solid
Nature of compound: Crystalline
Percentage Yield (%): 65
Melting Point (°C): 170-172
Molecular Weight (g/mol): 689

FT-IR (KBr) cm^{-1}: 3183 (N-H stretching) 3088, 3021 (C=C-H stretching), 2227 (C-N stretching nitrile), 1668 (N-H bending), 1578 and 1528 (Ar-C=C), 1321 (C-N), 1153 (C-F stretching), 885 and 818 (C-Cl stretching), 695 (N-H Wagging)

2) 2,7-diamino-4,5-bis(4-bromophenyl)-9-(2,6-dichloro-4-(trifluoromethyl) phenyl)-5,9-dihydro-4H-dipyrano [2,3-b:3',2'-d] pyrrole-3,6-dicarbonitrile:
Chemical Formula: $C_{31}H_{16}Br_2Cl_2F_3N_5O_2$
Physical Appearance: White solid
Nature of compound: Crystalline
Percentage Yield (%): 72
Melting Point (^oC):148-150
Molecular Weight (g/mol): 778

3)2,7-diamino-9-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4,5-bis(4-hydroxyphenyl)-5,9-dihydro-4H-dipyrano [2,3-b:3',2'-d] pyrrole-3,6-dicarbonitrile (5c):
Chemical Formula: $C_{31}H_{18}C_{l2}F_3N_5O_4$
Physical Appearance: Yellow solid
Nature of compound: Crystalline
Percentage Yield (%): 70
Melting Point (^oC): 168-170
Molecular Weight (g/mol): 652

FT-IR (KBr) cm^{-1}: 3345 (Ar-O-H stretching broad), 3132 (N-H stretching), 3032 (C=C-H stretching), 2227 (C-N stretching Nitrile), 1673 (N-H bending), 1561, and 1438 (Ar-C=C), 1371 (C-N stretching), 1299 (C-O Stretching), 1114 (C-F stretching), 935 and 834 (C-Cl stretching)

^{1}H-NMR (500 MHz, DMSO-d$_6$, δ ppm): 8.36 (s, 2H, N-H), 7.99 (s, 2H, Ar-H, *J* = 2.3 Hz), 7.78 (dd, 4H, Ar-H, *J* = 7.5 Hz), 6.90 (dd, 4H, Ar-H, *J*= 7.5 Hz), 9.88 (s, 2H, O-H), 4.45 (s, 2H).
^{13}C-NMR (125 MHz, DMSO-d$_6$, δ ppm): 114-129 (d, 8C, Ar-C), 164 (s, 2C, N-C-O), 161 (s, 2C, Ar-C), 147 (s, 3C, Ar-C), 134 (s, 2C, Ar-C), 126-129 (s, 5C, Ar-C), 116 (s, 2C, CN), 74 (s, 2C, Ar-C), 32 (s, 2C), 123 (s, 2C, N-C-), 121 (s, 1C, F-C).

HRMS (ESI): Cal: 652 (exact mass) **Obs:** m/z: 651 [M+H]

4) 2,7-diamino-9-(2,6-dichloro-4-(trifluoromethyl) phenyl) -4,5-bis(4-hydroxy-3-methoxyphenyl) -5,9-dihydro-4H-dipyrano[2,3-b:3',2'-d] pyrrole-3,6-dicarbonitrile (5d):
Chemical Formula: $C_{33}H_{22}Cl_2F_3N_5O_6$
Physical Appearance: Yellow solid
Nature of compound: Crystalline
Percentage Yield (%): 74
Melting Point (^oC):122-124
Molecular Weight (g/mol): 712

FT-IR (KBr) cm^{-1}: 3406 (Ar-O-H stretching), 3271 (N-H stretching), 3021 (C=C-H stretching), 2841 (O-CH_3 stretching), 2221 (C-N stretching Nitrile), 1662 (N-H bending), 1567, 1511, and 1461 (Ar-C=C), 1382 (C-N stretching), 1299 (C-O Stretching), 1153 (C-F stretching), 895 and 957 (C-Cl stretching), 728 (N-H wagging).

^{1}H-NMR (500 MHz, DMSO-d$_6$, δ ppm): 8.30 (s, 2H, N-H), 8.00 (s, 2H, Ar-H, *J* = 2.3 Hz), 7.68 (dd, 2H, Ar-H, *J* = 7.5 Hz), 7.55 (dd, 2H, Ar-H, *J*= 7.5 Hz), 6.90 (s, 2H, Ar-H, *J* = 7.5 Hz), 9.77 (s, 2H, O-H), 4.45 (s, 2H), 3.88 (s, 6H).

5) 2,7-diamino-9-(2,6-dichloro-4-(trifluoromethyl) phenyl) -4,5-bis (4-nitrophenyl)- 5,9-dihydro-4H-dipyrano [2,3-b:3',2'-d] pyrrole-3,6-dicarbonitrile (5e):
Chemical Formula: $C_{31}H_{16}Cl_2F_3N_7O_6$
Physical Appearance: Off-white solid
Nature of compound: Crystalline
Percentage Yield (%): 72
Melting Point (^oC):158-160
Molecular Weight (g/mol): 710

FT-IR (KBr) cm^{-1}: 3177 (N-H stretching), 3021 (C=C-H stretching), 2220 (C-N stretching Nitrile), 1673 (N-H bending), 1578 (N-O asymmetric stretching), 1522 (Ar-C=C), 1388 (N-O symmetric stretching), 1321 (C-N stretching), 1248 (C-O Stretching), 1120 (C-F stretching), 974 and 885 (C-Cl stretching), 684 (N-H wagging).

^{1}H-NMR (500 MHz, DMSO-d$_6$, δ ppm): 8.61 (s, 2H, N-H), 8.44 (dd, 4H, Ar-H, *J* = 7.5 Hz), 8.10 (dd, 4H, Ar-H, *J* = 7.5 Hz), 7.99 (s, 2H, Ar-H, *J* = 2.3 Hz), 4.88 (s, 2H).

^{13}C-NMR (125 MHz, DMSO-d$_6$, δ ppm): 125-131 (d, 10C, Ar-C), 159 (s, 2C, N-C-O), 154 (s, 2C, Ar-C), 149 (s,

3C, Ar-C), 134 (s, 2C, Ar-C), 138 (s, 2C, Ar-C), 124-129 (s, 3C, Ar-C), 114 (s, 2C, CN), 86 (s, 2C, Ar-C), 29 (d, 2C), 120 (s, 1C, F-C)

2.3 BIOLOGICAL STUDY

2.3.1 Antimicrobial Activity

A new series of compounds (5a–e) was evaluated for antimicrobial activity against two bacterial strains, Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli*, and two fungal strains, *Aspergillus niger* and *Candida albicans*, by the disc diffusion method in vitro. Circular paper discs (6 mm) containing a fixed concentration of test sample solution, along with discs saturated with controls, were placed on inoculated solid agar plates. The plates were incubated at the optimum growth temperature, and the results were monitored by observing the zone of inhibition (Table 1).

2.3.2. Antimicrobial Assay

A stock solution (1000 µg/mL) of each test sample was prepared in DMSO. The test was performed by the disc diffusion method using an appropriate volume of test samples. Hi-media antibiotic discs of chloramphenicol and amphotericin-B (100 µg/disc) were used as standard references for antibacterial and antifungal activity, respectively.

Nutrient agar (Hi-media) was used for bacteria, with the following composition (g L^{-1}): sodium chloride 5.0; beef extract 10.0; peptone 10.0 (pH 7.2). Potato dextrose agar (Hi-media) was used for fungi and yeast, with the following composition (g L^{-1}): potato infusion 200; dextrose 20; agar 15; final pH 5.6 ± 0.2 at 25 °C.

Microbiological media were prepared according to the manufacturer's guidelines. Using sterile forceps, paper discs (6 mm diameter) containing a set volume of test sample solution were placed on the surface of inoculated agar plates and pressed down to ensure contact between the disc and the agar surface. In the same manner, paper discs saturated with controls (DMSO and reference standards) were placed on agar plates and incubated at the optimum temperature for 24 h (antibacterial investigation) and 3–7 days (antifungal investigation). The zones of inhibition were measured with a Vernier caliper in millimeters and are tabulated in Table 1.

3. Results and Discussion

A series of bis-chalcones (4a–e) was developed from cyclic imide 3a (Scheme 1) and various substituted aromatic aldehydes using a convenient and environmentally friendly synthetic route (Scheme 2). Compounds 4a–e were further treated with malononitrile to furnish a novel series of pyrano-pyrrole derivatives (5a–e) (Scheme 3). The newly synthesized compounds 3a, 4a–e, and 5a–e were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and HRMS. Because compounds 5a–e were anticipated to be biologically effective entities, they were also investigated for antimicrobial activity against bacterial and fungal strains in vitro.

3.1. Biological Activity

All compounds were tested for antimicrobial properties against bacterial and fungal strains. In the series, most compounds were found to be antibacterial agents

Table 1.0: Antibacterial activity of 5a-e series

Sr. No.	Sample Code	E-Coli	B. Subtills	A. Niger	C. Albicans
1	5a	-	-	12.22±0.02	-
2	5b	7.24±0.06	-	10..34±0.09	7.22±0.20
3	5c	-	6.96 ± 0.12	16.14±0.14	8.68±0.06
4	5d	-	8.32±0.13	7.45±0.09	-
5	5e	-	7.64 ± 0.18	-	8.96±0.08
Standard	Chloramphenicol	20.57± 0.17	19.95±0.28	NA	14.29±0.07
Standard	Amphotericin-B	NA	NA	21.84 ±0.17	14.21±0.12

against both bacterial strains. Compounds 5c, 5d, and 5e were effective against *Bacillus subtilis*, while compound 5b showed a mild antibacterial effect against *Escherichia coli*. These compounds were also investigated for antifungal properties against two fungal strains. Compounds 5c and 5e exhibited good antifungal activity against *Aspergillus niger* and *Candida albicans*, respectively. Compounds 5b and 5c were moderately effective against both fungal strains (Table 1).

4. Conclusion

A novel series of pyrano-pyrroles (5a–e) was successfully synthesized under mild conditions from chalcones 4a–e and malononitrile. Several methods were reviewed, and the most convenient and non-hazardous synthetic strategy was established for preparing pyrano-pyrrole derivatives with ease and minimal work-up difficulty. This approach also provides remarkable yields within a short time.

Aromatic rings bearing electron-donating groups in the initial moieties provided significantly higher yields, whereas rings with electron-withdrawing substituents gave moderate yields. The structures of all synthesized compounds were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. Compounds 5a–e were assayed for antimicrobial activity. Most compounds were good to moderately effective against bacterial and fungal strains, and compound 5e was a significant antifungal agent against *C. albicans*.

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