Synthesis and Antibacterial Evaluation of Novel 1,5-Benzodiazepine–Coumarin Hybrids

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Abstract

A novel series of 1,5-benzodiazepine–coumarin hybrids 3(a–d) was efficiently synthesized via a straightforward three-step pathway using molecular hybridization strategies. The reaction sequence commenced with the preparation of 3-acetylcoumarin (1), followed by enamination with N,N-dimethylformamide dimethyl acetal (DMF-DMA) in the presence of catalytic acetic acid to afford enaminone intermediate 2. Subsequent cyclocondensation of intermediate 2 with various substituted o-phenylenediamines in ethanol using triethylamine (NEt3) under reflux conditions yielded the targeted novel hybrids 3(a–d) in high yields (72%–78%). The chemical structures of all prepared compounds were unambiguously established through 1H and 13C NMR spectroscopic analyses. The in vitro antibacterial evaluation against a representative panel of Gram-positive (S. aureus, B. cereus, L. innocua) and Gram-negative (E. coli, P. aeruginosa) bacterial strains revealed variable to excellent growth-inhibitory activities. Among the tested derivatives, compound 3d (X = NO2) demonstrated exceptional broad-spectrum antibacterial potency, exhibiting the highest inhibition zones (18.49–25.41 mm) and the lowest minimum inhibitory concentration (MIC) values (12.5–25 µg. mL-1). The structure–activity relationship (SAR) analysis underscored the decisive role of electron-withdrawing groups (-NO2 > -Cl) at the aromatic ring in enhancing antimicrobial efficacy compared to electron-donating (-CH3) or unsubstituted (-H) derivatives. Overall, these findings highlight 1,5-benzodiazepine–coumarin hybrids as promising lead structures for the development of new antibacterial agents.

Keywords: Coumarin; 1,5-Benzodiazepine; Enaminone; Molecular hybridization; Antibacterial activity; Structure–activity relationship (SAR).

1. Introduction

Heterocyclic architectures represent one of the most versatile and widely investigated classes of organic molecules, a prominent status owed to their ubiquitous distribution in natural products and their fundamental roles in biological processes [1]. Among these frameworks, nitrogen-containing heterocycles occupy a pivotal position in medicinal chemistry, prized for their rich structural diversity, tuneable reactivity, and broad spectrum of pharmacological profiles [2]. Driven by their proven therapeutic value and crucial role in rational drug design, the past few decades have witnessed significant advancements in the efficient synthesis and structural functionalization of these privileged cores.

Benzodiazepines and their fused-ring derivatives constitute a cornerstone class among fused bicyclic N-heterocycles [3]. The clinical significance of this framework was inaugurated with the discovery of chlordiazepoxide (Librium) by Leo Sternbach in 1955, followed by its commercial introduction in 1960 and the subsequent launch of diazepam (Valium) in 1963, which rapidly became one of the most widely prescribed psychotropic drugs globally [4,5]. Beyond their classical applications as central nervous system agents-functioning as potent anxiolytics [6], anticonvulsants [5,7], sedatives, hypnotics, antidepressants [3,5], and neuroleptics [8]-structural modifications of the benzodiazepine nucleus have substantially broadened their therapeutic horizon. Modern medicinal chemistry efforts have successfully demonstrated their anti-inflammatory [9], cytotoxic, and antiproliferative properties [10], alongside their efficacy as non-nucleoside inhibitors of HIV-1 reverse transcriptase [11], selective modulators of γ-aminobutyric acid $GABA_A$ receptors [12], conformationally constrained dipeptidomimetic inhibitors of caspase-1 [13], and cholecystokinin-A (CCK-A) receptor antagonists [14]. Furthermore, select benzodiazepine derivatives find specialized industrial utility outside pharmacology, serving as colorants and dyes for acrylic fibers in photochemical applications [15]. While 1,4-benzodiazepines are heavily documented, their 1,5-regioisomers have garnered escalating

attention due to their unique chemical reactivity and expanding biological scope, including emerging roles in anticancer, antiviral, and cardiovascular therapies [16–18]. Beyond their intrinsic biological potency, these bicyclic frameworks function as indispensable synthetic synthons for constructing complex polycyclic architectures [19]. The reactive sites on the 1,5-benzodiazepine ring allow for efficient annulation, affording various fused heterocyclic systems such as triazolo-, oxadiazolo-, oxazino-, and furanobenzodiazepines [20,21]. Mechanistically, the classical synthesis of 1,5-benzodiazepines relies on the double nucleophilic cyclocondensation of o-phenylenediamines with diverse three-carbon building blocks, including α,β-unsaturated carbonyl compounds, β-haloketones, or substituted ketones [22,23], as well as condensation reactions involving dehydroacetic acid (DHA) derivatives [24]. To overcome limitations associated with traditional protocols such as prolonged reaction times, harsh conditions, side reactions, and unsatisfactory yields, a wide array of catalytic promoters has been explored. Early methodologies utilized Bronsted/Lewis acids and solid supports (SiO_2, $MgO/POCl_3$, Al_2O_3/P_2O_5) [25,26], which later evolved into rare-earth Lewis acids like $Yb(OTf)_3$ or Sc(OTf)3 under thermal or microwave activation [27]. Modern efforts continuously advocate for greener and milder synthetic protocols employing catalysts such as ceric ammonium nitrate (CAN), organic acids, or $AgNO_3$ [28,29]. On the other hand, coumarin motifs represent another fundamental class of naturally occurring heterocycles endowed with broad antibacterial, antioxidant, and antiproliferative activities. Consequently, the strategy of molecular hybridization-combining the bioactive coumarin core with the 1,5-benzodiazepine framework-presents a compelling approach to access novel molecular entities with amplified biological potential. As part of our ongoing research directed toward the design of functionally diverse heterocycles, we report herein a straightforward and efficient route for the synthesis of novel 1,5-benzodiazepine–coumarinhybrids **3(a–d)**. The synthetic sequence proceeds via the preparation of 3-acetylcoumarin (1), its enamination with N,N-dimethylformamide dimethyl acetal (DMF-DMA) to yield enaminone intermediate 2, and its subsequent cyclocondensation with substituted o-phenylenediamines catalyzed by triethylamine under reflux conditions (Scheme 1).

2. Material and methods

2.1. General Information and Instrumentation

Nuclear magnetic resonance (^1H, ^{13}C, DEPT) spectra were recorded on a Bruker AQS-AVANCE spectrometer operating at 300MHz at 25°C. Deuterated chloroform $CDCl_3$ and dimethyl sulfoxide DMSO-d_6 were utilized as solvents. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS,δ = 0.00 ppm) as an internal standard, or referenced to the residual solvent signals ($CDCl_3$: δ_H = 7,25, δ_C = 77.00 ; DMSO-d_6: δ_H = 2.52, δ_C =39.52). Coupling constants (J) are given in Hertz (Hz). The splitting patterns are designated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), td (triple doublets), q (quadruplet), and m (multiplet or complex signal).

Synthesis of 3-acetyl-2H-chromen-2-one (1)
In a 100 mL round-bottom flask, 2-hydroxybenzaldehyde (3.0 g, 27.0 mmol, 1.0 eq.) was dissolved in 30 mL of ethanol. To this solution, methyl 3-oxobutanoate (or ethyl 3-oxobutanoate) (3.10 g, 27.0 mmol, 1.0 eq.) and 6 drops of piperidine were added. The reaction mixture was stirred at room temperature for 3 hours. The progress of the reaction was monitored by Thin-Layer Chromatography (TLC). Upon completion, the mixture was poured into 100 mL of ice-cold water. The resulting precipitate was collected by filtration, air-dried, and recrystallized from ethanol to yield compound **1** as a solid (80% yield , m.p.111- 118°C)

^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.50 (s, 1H, H-4), 7.67 – 7.60 (m, 2H, H-5, H-7), 7.39 –7.30 (m, 2H, H-6, H-8), 2.72 (s, 3H, CH_3 acetyl). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 195.58 (C=O ketone), 159.20 (C-2, C=O lactone), 155.12 (C-8a, C-O), 147.55 (C-4, CH olefinic), 134.35 (C-7, CH Ar), 130.28 (C-5, CH Ar), 125.16(C-6, CH Ar), 124.80 (C-3), 118.45 (C-4a), 116.73 (C-8, CH Ar), 30.65 (CH_3 acetyl).

General Procedure for the Synthesis of Enaminone (2)
A mixture of 3-acetyl-2H-chromen-2-one 1 (1.88g, 10.0 mmol, 1.0 eq.) and N,N-dimethylformamide dimethyl acetal (1.19 g, 10.0 mmol, 1.0 eq.) was dissolved in 40mL of dichloromethane (CH_2Cl_2) in the presence of a catalytic amount of acetic acid (CH_3COOH). The reaction mixture was heated under reflux for 24h. Upon completion of the reaction (monitored by TLC), the solvent was evaporated under reduced pressure, and the resulting oily residue was precipitated and recrystallized from ethanol to yield the pure enaminone derivative 2.

Compound 2 3-(3-(dimethylamino)acryloyl)-2H-chromen-2-one (75% yield , m.p. 142-144°C) . ^1H NMR (300 MHz, $CDCl_3$) : δ (ppm) 8.42 (s, 1H, H-4 coumarin), 7.85 (d, J = 12.3 Hz, 1H, CH=CH-N), 7.62 – 7.50 (m, 2H, H-5, H-7 coumarin), 7.32 – 7.24 (m, 2H, H-6, H-8 coumarin), 6.30 (d, J = 12.3 Hz, 1H, CO-CH=CH), 3.18 (s, 3H, N-CH_3), 2.99 (s, 3H, N-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 185.40 (C=O enaminone), 160.20 (C-2, C=O lactone), 154.80 (CH=CH-N), 154.10 (C-8a, C-O), 145.30 (C-4, CH olefinic), 132.80 (C-7, CH Ar), 129.20 (C-5, CH Ar), 124.60 (C-3), 126.50 (C-6, CH Ar), 119.20 (C-4a), 116.40 (C-8, CH Ar), 97.80 (CO-CH=CH), 45.20 (N-CH_3), 37.40 (N-CH_3).

Synthesis of Benzodiazepines (3a–3d)
To an ethanolic solution (30 mL) containing compound 2 (2.43 g, 10 mmol, 1.0 eq.), the appropriate substituted aromatic diamine (10 mmol, 1.0 eq.) was added in the presence of a catalytic amount of triethylamine (NEt3). The reaction mixture was heated under reflux with magnetic stirring for 6 hours. After cooling to room temperature, the resulting solid was

collected by vacuum filtration, washed with cold ethanol, and recrystallized from ethanol to yield the corresponding benzodiazepine derivatives 3a–3d.

Compound 3a: 3-(1H-1,5-benzodiazepin-2-yl)-2H-chromen-2-one (Yield: 72%; m.p. 184–186 °C). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 10.15 (br s, 1H, NH), 8.58 (s, 1H, H-4 coumarin), 7.80 (d, J=6.2 Hz, 1H, CH=N diazepine), 7.65 – 7.52 (m, 2H, H-5, H-7 coumarin), 7.35 – 7.26 (m, 2H, H-6, H-8 coumarin), 7.15 – 6.95 (m, 4H, Ar-H diazepine), 5.75 (d, J=6.2Hz, 1H, CH-C=N diazepine).^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 160.10 (C-2, C=O lactone), 154.60 (C-8a, C-O), 152.40 (C-4, C=N imine diazepine), 146.20 (C-4 coumarin, CH olefinic), 140.20 (C-9a, C-NH diazepine), 139.10 (C-5a, C=N diazepine), 137.50 (C-2, C-C bridge diazepine), 133.10 (C-7 coumarin, CH Ar), 129.50 (C-5 coumarin, CH Ar), 128.60 (C-7 diazepine, CH Ar), 127.40 (C-8 diazepine, CH Ar), 124.80 (C-6 coumarin, CH Ar), 124.10 (C-3 coumarin), 122.30 (C-6 diazepine, CH Ar), 119.50 (C-9 diazepine, CH Ar), 118.80 (C-4a coumarin), 116.50 (C-8 coumarin, CH Ar), 98.40 (C-3, CH enamine diazepine).

Compound 3b: 3-(7-methyl-1H-1,5-benzodiazepin-2-yl)-2H-chromen-2-one (Yield: 78%; m.p. 198–200 °C). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 10.08 (br s, 1H, NH), 8.55 (s, 1H, H-4 coumarin), 7.78 (d, J=6.2Hz, 1H, CH=N diazepine), 7.63 – 7.51 (m, 2H, H-5, H-7 coumarin), 7.34 –7.25 (m, 2H, H-6, H-8 coumarin), 7.02 (s, 1H, H-6' Ar diazepine), 6.90 (d, J=8.1 Hz, 1H, Ar-H), 6.82 (d, J=8.1Hz, 1H, Ar-H), 5.70 (d, J=6.2 Hz, 1H, CH-C=N diazepine), 2.32 (s, 3H, Ar-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 161.40 (C-2, C=O lactone), 154.20 (C-8a, C-O), 152.10 (C-4, C=N imine diazepine), 145.00 (C-4 coumarin, CH olefinic), 141.50 (C-9a, C-NH diazepine), 141.20 (C-7, C-CH3 diazepine), 140.00 (C-5a, C=N diazepine), 138.00 (C-2, C-C bridge diazepine), 132.10 (C-7 coumarin, CH Ar), 129.00 (C-5 coumarin, CH Ar), 127.90 (C-8 diazepine, CH Ar), 126.80 (C-6 diazepine, CH Ar), 124.50 (C-6 coumarin, CH Ar), 124.00 (C-3 coumarin), 121.50 (C-9 diazepine, CH Ar), 119.03 (C-4a coumarin), 116.30 (C-8 coumarin, CH Ar), 98.10 (C-3, CH enamine diazepine), 21.10 (Ar-CH_3).

Compound 3c: 3-(7-chloro-1H-1,5-benzodiazepin-2-yl)-2H-chromen-2-one (Yield:75%; m.p. 210–212 °C). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 10.22 (br s, 1H, NH), 8.60 (s, 1H, H-4 coumarin), 7.82 (d, J=6.2 Hz, 1H, CH=N diazepine), 7.66 – 7.54 (m, 2H, H5, H-7 coumarin), 7.37 – 7.27 (m, 2H, H-6, H-8 coumarin), 7.18 (d, J=2.2 Hz, 1H, H-6' Ar diazepine), 7.08 (dd, J=8.5,2.2 Hz, 1H, Ar-H), 6.92 (d, J=8.5Hz, 1H, Ar-H), 5.78 (d, J=6.2 Hz, 1H, CH-C=N diazepine). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 159.95 (C-2, C=O lactone), 154.70 (C-8a, C-O), 152.80 (C-4, C=N imine diazepine), 146.50 (C-4 coumarin, CH olefinic), 142.10 (C-9a, C-NH diazepine), 141.00 (C-5a, C=N diazepine), 137.80 (C-2, C-C bridge diazepine), 133.30 (C-7 coumarin, CH Ar), 129.80 (C-7, C-Cl diazepine), 129.60 (C-5 coumarin, CH Ar), 127.20 (C-8 diazepine, CH Ar), 126.50 (C-6 diazepine, CH Ar), 124.90 (C-6 coumarin, CH Ar), 123.80 (C-3 coumarin), 121.10 (C-9 diazepine, CH Ar), 118.90 (C-4a coumarin), 116.60 (C-8 coumarin, CH Ar), 98.80 (C-3, CH enamine diazepine).

Compound 3d: 3-(7-nitro-1H-1,5-benzodiazepin-2-yl)-2H-chromen-2-one (Yield: 83%; m.p. 242–244 °C). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 10.45 (br s, 1H, NH), 8.68 (s, 1H, H-4 coumarin), 8.05 (d, J = 2.4 Hz, 1H, H-6' Ar diazepine), 7.95 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 7.88 (d, J = 6.2 Hz, 1H, CH=N diazepine), 7.68 – 7.56 (m, 2H, H-5, H-7 coumarin), 7.39 – 7.29 (m, 2H, H-6, H-8 coumarin), 7.01 (d, J = 8.8 Hz, 1H, Ar-H), 5.85 (d, J = 6.2 Hz, 1H, CH-C=N diazepine).^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 159.80 (C-2, C=O lactone), 154.85 (C-8a, C-O), 153.20 (C-4, C=N imine diazepine), 148.50 (C-7, C-NO2 diazepine), 147.10 (C-4 coumarin, CH olefinic), 145.20 (C-9a, C-NH diazepine), 143.10 (C-5a, C=N diazepine), 138.50 (C-2, C-C bridge diazepine), 133.50 (C-7 coumarin, CH Ar), 129.80 (C-5 coumarin, CH Ar), 125.10 (C-6 coumarin, CH Ar), 124.30 (C-8 diazepine, CH Ar), 123.50 (C-3 coumarin), 122.80 (C-6 diazepine, CH Ar), 119.50 (C-9 diazepine, CH Ar), 119.00 (C-4a coumarin), 116.70 (C-8 coumarin, CH Ar), 99.50 (C-3, CH enamine diazepine).

2.2. Determination of antibacterial activity

2.2.1. Bacterial Strains and Culture Conditions

The bacterial strains used as test organisms in this study were categorized into Gram-positive and Gram-negative bacteria. The Gram-positive strains included *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria innocua*. The Gram-negative strains comprised *Escherichia coli* and *Pseudomonas aeruginosa*.

All bacterial strains were cultured at 37 °C using standard nutrient agar/broth and Mueller-Hinton media. Prior to experimental use, working cultures from solid media were sub-cultivated into liquid nutrient broth and incubated at 37°C for 18 h to prepare fresh, exponentially growing microbial suspensions used as active inocula for all subsequent biological assays.

2.2. 2. Antibacterial Activity Assay

The antibacterial activity of the synthesized compounds 3**(a–d)** was quantitatively evaluated using the standard disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [30,31]. The microbial suspensions prepared from the 18 h liquid cultures were standardized to a turbidity equivalent to a 0.5 McFarland standard (1.5×10^{8} CFU/mL) using a densitometer, and then uniformly spread over the Mueller-Hinton agar medium surface to form a homogenous bacterial lawn. The test compounds 3(a–d) were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions of 10 mg/mL. Sterile laboratory filter paper discs (6 mm in diameter, Whatman No.1) were impregnated with 10 μL of each compound solution, yielding a definitive final concentration of 100 μg.disc^{-1}. The prepared sample discs, alongside Chloramphenicol (CHL) as a positive standard antibiotic control and pure DMSO as a negative control, were carefully placed

on the inoculated agar surface. Following incubation at 37 °C for 24 h, the antibacterial efficacy was determined by measuring the diameter of the zones of inhibition (IZ, in mm) around each disc. All experimental assays were performed in triplicate (n=3), and results were expressed as Mean ± Standard Deviation (SD).

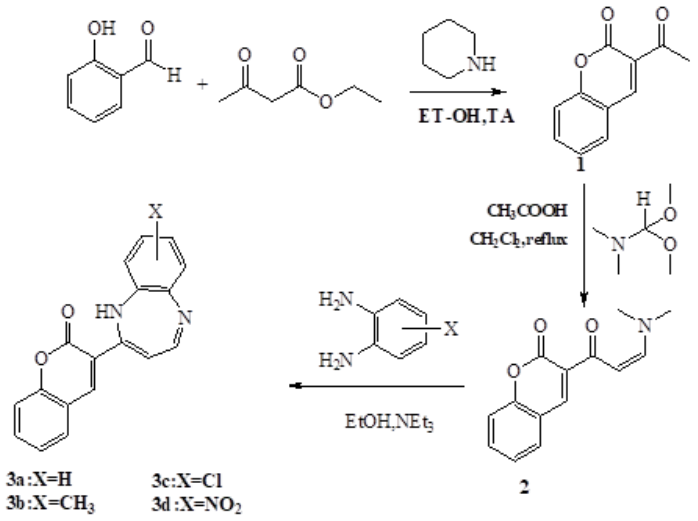
2.2. 3. Determination of Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) of the synthesized compounds 3(a–d) was evaluated using the standard broth microdilution method in 96-well microtiter plates as recommended by the CLSI guidelines [31].Serial two-fold dilutions of the target compounds were prepared in Mueller-Hinton broth to yield a final concentration range of 100,50,25,12.5,6.25, and 3.12 µg/mL. Each well was inoculated with the standardized bacterial suspensions (adjusted to a 0.5 McFarland standard using a densitometer) of both Gram-positive and Gram-negative strains. The microtiter plates were incubated at 37 °C for 24 h. Growth and sterility controls containing medium with DMSO and bacterial inocula without test compounds were assessed simultaneously under the same experimental conditions to confirm bacterial viability and medium sterility. The MIC value was defined and recorded as the lowest concentration of the compound that completely prevented visible bacterial growth.

3.Results and discussion

3.1. Chemistry

The target coumarin-benzodiazepine hybrid derivatives 3(a–d) were synthesized via a three-step sequence starting from 2-hydroxybenzaldehyde, as outlined in **Scheme 1**. 3-Acetyl-2H-chromen-2-one **1** was synthesized via Knoevenagel condensation of 2-hydroxybenzaldehyde with ethyl acetoacetate in the presence of piperidine as a base catalyst in ethanol at room temperature. The intermediate **1** was subsequently reacted with N,N-dimethylamide dimethyl acetal derivatives specifically N,N-dimethylformamide dimethyl acetal (DMF-DMA) in dichloromethane under reflux using acetic acid (CH_3COOH) as a catalyst to yield the corresponding enaminone intermediate **2**. Finally, the cyclocondensation of enaminone **2** with various substituted o-phenylenediamines in ethanol, catalyzed by triethylamine (NEt_3) under reflux conditions, afforded the novel benzodiazepine derivatives **3(a–d)** (where **3a: X= H**; **3b: X = CH_3**; **3c: X= Cl**; and **3d: X = NO_2**). A plausible reaction mechanism for the formation of enaminone intermediate **2** and its subsequent conversion into benzodiazepine hybrids **3(a–d)** is outlined in **Scheme 2**.



Scheme 1. Stepwise synthesis of novel benzodiazepine-coumarin hybrids 3(a–d) starting from 3-acetylcoumarin (1).

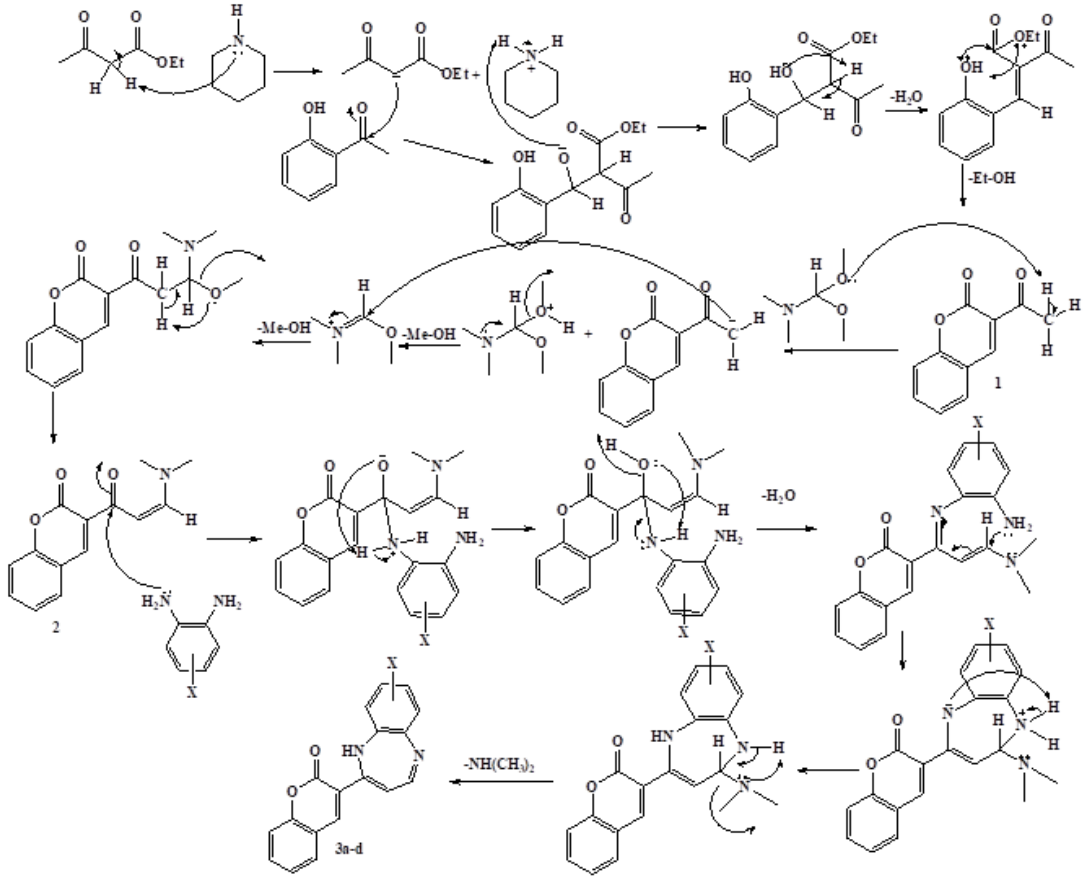
3.2. Structural determination by spectroscopic methods

The molecular structures of precursor **1**, enaminone intermediate **2**, and the target benzodiazepine–coumarin hybrids 3a–d were unambiguously established through ^{1}H NMR and ^{13}C NMR spectroscopic analyses. The initial precursor, 3-acetyl-2H-chromen-2-one (**1**), was synthesized in 80% yield (m.p. 111–118 °C). Its ^{1}H NMR spectrum displayed a characteristic singlet at δ = 8.50 ppm attributed to the olefinic H-4 proton of the coumarin core, along with a sharp three-proton singlet at δ = 2.72 ppm corresponding to the acetyl methyl group (-$COCH_3$). The aromatic protons resonated as multiplets in the range of δ = 7.30-7.67 ppm. In the ^{13}C NMR spectrum, diagnostic signals appeared at δ = 195.58ppm for the acetyl carbonyl (C=O ketone), δ = 159.20ppm for the lactone carbonyl (C-2), δ = 147.55 ppm (C-4), and δ = 30.65 ppm for the acetyl methyl carbon.

Condensation of **1** with N,N-dimethylformamide dimethyl acetal yielded the key enaminone **2** in 75% yield (m.p. 142–144 °C). The ^{1}H NMR spectrum confirmed enaminone formation through two distinct singlets at δ = 3.18 and 2.99 ppm, assigned to the magnetically non-equivalent N-methyl protons (N-CH_3). The E-stereochemistry across the double bond was evidenced by two coupled doublets at δ = 7.85ppm (CH=CH-N) and δ = 6.30 ppm (CO-CH=CH), exhibiting a vicinal coupling constant of J = 12.3Hz. The H-4 singlet resonated at δ = 8.42 ppm. The ^{13}C NMR spectrum revealed the enaminone carbonyl carbon at δ = 185.40ppm, the lactone carbonyl at δ = 160.20 ppm, and the olefinic carbons (CH=CH-N and CO-CH=CH) at δ = 154.80

and 97.80 ppm, respectively. The N-methyl carbons appeared at δ = 45.20 and 37.40 ppm. The targeted hybrids **3a–d** were obtained in good to high yields (72%–83%). In the ^{1}H NMR spectra, all derivatives exhibited a broad singlet in the range of δ = 10.08–10.45 ppm, assigned to the diazepine -NH- proton. The seven-membered ring system was corroborated by a pair of coupled doublets at δ = 7.78–7.88 ppm (CH=N) and δ = 5.70–5.85 ppm (CH-C=N), with a coupling constant of J=6.2 Hz. The coumarin H-4 singlet appeared consistently between δ = 8.55 and 8.68 ppm . Electronic influence from the substituent X was clearly observed: derivative **3b** (X = CH_3) displayed a methyl singlet at δ = 2.32 ppm, whereas electron-withdrawing substituents shifted the -NH- proton downfield to δ = 10.22 ppm for **3c** (X = Cl) and δ = 10.45 ppm for **3d** (X = NO_2).

The ^{13}C NMR spectra of **3a–d** provided final confirmation of cyclocondensation. The lactone carbonyl (C-2) appeared at δ = 159.80-161.40 ppm, while the imine carbon (C-4) and the ring-junction carbon (C-9a) resonated at δ = 152.10-153.20 ppm and δ = 140.20-145.20 ppm, respectively. The C-3 enamine carbon appeared characteristically at δ = 98.10-99.50 ppm, and the methyl carbon of **3b** resonated at δ = 21.10 ppm, fully in agreement with the proposed structure.



Scheme 2. Proposed mechanism for the synthesis of novel benzodiazepine-coumarin hybrids 3(a–d).

3.3. Antibacterial Activity of Synthesized Compounds 3(a–d)

The in vitro antibacterial potential of the newly synthesized target compounds **3(a–d)** was evaluated against a representative panel of microbial pathogens, comprising Gram-positive strains (*Staphylococcus aureus*, *Bacillus cereus*, and *Listeria innocua*) and Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*).

The preliminary screening was conducted using the standard paper-disk diffusion method at a fixed concentration of 100 µg.disc^{-1} (Table 1).

Table 1: Antibacterial activity (Inhibition Zone in mm) of synthesized compounds 3(a–d) at 100 µg.disc^{-1}

Compound	X	Inhibition Zone IZ in mm				
		Gram-positive(+)			*Gram-negative(-)*	
		S. aureus	***B. cereus***	***L. innocua***	***E. coli***	***P.aeruginosa***
3a	**H**	**(11.33 ± 0.17)**	**(13.50 ± 0.15)**	—	**(10.11 ± 0.12)**	—
3b	**CH_3**	**(14.35 ± 0.12)**	**(17.25 ± 0. 06)**	**(10.26 ± 0.10)**	**(13.25 ± 0.08)**	**(09.16 ± 0.08)**

3c	**Cl**	**(18.55 ± 0.08)**	**(21.56 ± 0.06)**	**(15.05 ± 0.14)**	**(19.03 ± 0.06)**	**(16.31 ± 0.17)**
3d	**NO_2**	**(21.08± 0.14)**	**(25.41 ± 0.32)**	**(18.49± 0.17)**	**(23.19 ± 0.19)**	**(19.39 ± 0.36)**
CHL		**(34.60 ± 0.44)**	**(38.37 ± 0.50)**	**(34.80 ± 0.40)**	**(38.60 ± 0.46)**	**(25.73 ± 0.50)**

Note: CHL: Chloramphenicol. Data are expressed as Mean ± Standard Deviation (SD) of three independent replicates (n=3). The symbol (—) indicates no zone of inhibition observed under the tested experimental conditions (disc concentration: 100 $\mu g.disc^{-1}$).

The antibacterial efficacy was quantitatively determined by measuring the inhibition zone diameters (IZ, in mm) and compared against Chloramphenicol (CHL) as a reference standard antibiotic. Furthermore, the minimum inhibitory concentration (MIC, in $\mu g.mL^{-1}$) for each compound was determined using the standard broth microdilution assay to evaluate their quantitative potency (Table 2).

Table 2: Minimum inhibitory concentrations (MIC in $\mu g.mL^{-1}$) for compounds 3(a–d)

Compound	**X**	***Minimum inhibitory concentrations (µg/ml)***				
		Gram-positive(+)			***Gram-negative(-)***	
		S. aureus	***B. cereus***	***L. innocua***	***E. coli***	***P.aeruginosa***
3a	**H**	**100**	**100**	—	**100**	—
3b	**CH_3**	**100**	**50**	**100**	**100**	**100**
3c	**Cl**	**50**	**25**	**50**	**50**	**50**
3d	**NO_2**	**25**	**12.5**	**25**	**25**	**25**

Note: Values represent the minimum inhibitory concentration (µg/mL) determined by the broth microdilution method. The symbol (—) indicates no activity detected at the highest tested concentration (100 µg/mL).

3.3.1. Primary Antibacterial Screening (Disc Diffusion Assay)

As summarized in **Table 1**, the synthesized compounds **3(a–d)** exhibited variable degrees of antibacterial activity against the tested bacterial strains depending on the nature of the substituent substituent (X) attached to the aromatic backbone.
In general, all synthesized derivatives demonstrated moderate-to-excellent antibacterial potency, with inhibition zone diameters ranging between 9.16 mm and 25.41mm. Among the tested Gram-positive bacteria, *Bacillus cereus* displayed the highest susceptibility to all synthesized compounds (13.50 mm ≤ IZ ≤ 25.41 mm), whereas *Listeria innocua* was the most resistant strain.

The primary screening revealed significant insights into the influence of substituents (X) on antibacterial efficacy:

- Effect of Electron-Withdrawing Groups (-NO_2 and -Cl): Compound 3d (X = NO_2) exhibited the most superior antibacterial activity among the entire series against all tested bacterial strains. It displayed prominent inhibition zones against *B. cereus* (IZ = 25.41 ± 0.32 mm), *E. coli* (IZ = 23.19 ± 0.19mm), *S. aureus* (IZ= 21.08 ± 0.14 mm), *P. aeruginosa* (IZ = 19.39 ± 0.36 mm), and *L. innocua* (IZ = 18.49 ± 0.17 mm). Similarly, compound 3c containing a chloro radical (X = Cl) demonstrated substantial activity across all bacterial strains, recording inhibition zones ranging from 15.05 mm to 21.56 mm.
- Effect of Electron-Donating Group (-CH_3) and Unsubstituted Derivative (-H): Compound 3b (X= CH_3) showed moderate broad-spectrum activity (9.16 mm ≤ IZ ≤ 17.25mm). Conversely, the unsubstituted derivative 3a (X = H) exhibited the lowest antibacterial efficacy (10.11 mm ≤ IZ ≤ 13.50 mm) and was entirely inactive against both *L. innocua* and *P. aeruginosa* (no zone of inhibition observed).
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3.3.2. Minimum Inhibitory Concentration (MIC) Determination

To further quantify the antibacterial strength, the MIC values of compounds **3(a–d)** were determined and recorded in **Table 2**. The MIC findings were highly consistent with the disk diffusion outcomes.

- Compound **3d** (X = NO_2) confirmed its position as the most potent candidate in this study, displaying the lowest MIC value of 12.5 $\mu g.mL^{-1}$ against B. cereus, and 25 $\mu g.mL^{-1}$ against *S. aureus*, *L. innocua*, *E. coli*, and *P. aeruginosa.*
- Compound **3c** (X = Cl) exhibited strong growth-inhibitory effects with an MIC of 25 $\mu g.mL^{-1}$ against *B. cereus* and 50 $\mu g.mL^{-1}$ against the remaining four bacterial strains.
- Compounds **3b** (X = CH_3) and **3a** (X= H) displayed higher MIC values (50 $\mu g.mL^{-1}$ to 100 $\mu g.mL^{-1}$), demonstrating considerably weaker antibacterial efficiency.

3.3.3. Structure–Activity Relationship (SAR) Discussion

A clear Structure–Activity Relationship (SAR) can be established based on the nature of the substituent (X) located on the core framework:

Activity Order: 3d ($-NO_2$) > 3c (-Cl) > 3b ($-CH_3$) > 3a (-H)

The incorporation of strong electron-withdrawing and highly polar groups, particularly the nitro ($-NO_2$) and chloro (-Cl) radicals, significantly enhances the antimicrobial potency compared to electron-donating methyl ($-CH_3$) or hydrogen (-H) substituents. The strong electron-withdrawing nature of the nitro group likely facilitates better cell membrane penetration, lipophilicity, and binding affinity toward crucial microbial target enzymes, thereby leading to enhanced bacterial growth inhibition.

4. Conclusion

In summary, we have successfully developed a convenient and efficient synthetic protocol for accessing a novel class of 1,5-benzodiazepine–coumarin hybrids 3(a–d) in good to high yields under mild reflux conditions. The targeted molecular frameworks were constructed via the strategic cyclocondensation of enaminone intermediate 2 with diverse o-phenylenediamines catalyzed by triethylamine. Comprehensive spectroscopic characterization (^{1}H NMR and ^{13}C NMR) unambiguously confirmed the proposed structures and established key stereochemical features, including the trans-configuration of enaminone 2 and the intramolecular hydrogen bonding within the seven-membered diazepine ring. Biological evaluation demonstrated that the synthesized hybrids possess significant antibacterial potential against both Gram-positive and Gram-negative bacterial pathogens. A clear structure–activity relationship (SAR) was delineated, revealing that the nature of the substituent on the fused aromatic nucleus strongly dictates antimicrobial strength. Notably, the introduction of strong electron-withdrawing groups ($-NO_2$ and -Cl) markedly amplified antibacterial efficiency, with the nitro-bearing derivative 3d emerging as the most potent candidate across all tested strains (MIC = 12.5–25 $\mu g.mL^{-1}$). The combination of synthetic accessibility, distinct structural versatility, and pronounced bioactivity establishes these novel hybrid scaffolds as valuable leads for further medicinal chemistry optimizations and prospective drug development against microbial pathogens.

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