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Unveiling The Potential: Coumarin Amide Derivatives Infused With Piperidine As Antimicrobial And Antioxidant Agents For Treating Microbiological Infections

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Abstract
A series of novel coumarin hybrids (compounds 6a–j) was synthesized using piperidine and its acetamide derivatives. The chemical structures of the newly developed products were elucidated through FTIR, ^{1}H NMR, ^{13}C NMR spectroscopy, and mass spectrometry techniques. Evaluation of the synthesized compounds for antibacterial, antifungal, and antioxidant activities was carried out using ampicillin, Nystatin, and Ascorbic acid as reference standards. The compounds exhibited noteworthy activity against the tested microorganisms, ranging from good to moderate. Notably, all compounds displayed substantial activity against Aspergillus Niger NCIM 501. Particularly, compounds 6a, 6f, 6i, and 6j demonstrated potent and broad-spectrum antimicrobial activity. Although the synthetic compounds exhibited lower antioxidant activity compared to the reference standard, compounds 6a and 6e demonstrated comparable activity at higher concentrations.
*Piperidine and its acetamide derivatives were used to create a series of new coumarin hybrids (compounds **6a–j**). Structures of newly synthesized products were depicted using FTIR, ^{1}H NMR,^{13}C NMR spectroscopy, and mass spectrometry techniques. The synthesized compounds were assessed for antibacterial, antifungal, and antioxidant activities by using ampicillin, Nystatin, and Ascorbic acid as the reference standards. All Compounds were found to have good to moderate activity against tested microorganisms. Interestingly all compounds have shown good activity against Aspergillus Niger NCIM 501. Additionally, compounds **6a, 6f, 6i,** and **6j** have demonstrated potent and broad-spectrum activity. Despite having less antioxidant activity than the reference standard when compared to synthetic compounds, at higher concentrations compounds **6a** and **6e** show comparable activity.*

Key Word:*Coumarin, Piperidine, antimicrobial, Antioxidant*

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I. INTRODUCTION

Antimicrobial agents represent a pivotal category of therapeutics since its inception in 1928 in the form of Penicillin that has revolutionized the landscape of infectious disease treatment. These medications effectively combat a broad spectrum of microorganisms, encompassing bacteria, viruses, fungi, and parasites, either by impeding their growth or by outright elimination.The historical significance of antimicrobial agents lies in their ability to curb the spread of infectious diseases, contributing significantly to the reduction of morbidity and mortality globally.The impact of antimicrobial agents cannot be overstated. Prior to their discovery, infectious diseases could rapidly disseminate through populations, leading to epidemics.[1]The World Health Organization (WHO) estimates that infectious illnesses cause 9.2 million deaths worldwide each year.[2]The key infectious diseases that lead to greater death rates include lower respiratory infections, diarrheal illnesses, tuberculosis, HIV/AIDS, and malaria. Emerging infectious illnesses like COVID-19[3-4]and neglected tropical diseases like dengue, the Zika virus,[5] and Ebola[6] are other infections that significantly raise the global burden of disease.[2]

New developments in antimicrobial therapies have been limited by regulatory barriers, higher failure rates, and antimicrobial resistance.[1,7-8-11] The most important class of medications is not being developed enough because of the complexity of bacterial infections and the challenges associated with developing an effective and safe medication. Despite the difficulties caused by antimicrobial resistance, antimicrobial drugs continue to be a vital component of contemporary medicine.

Simultaneously, Antioxidants play a pivotal role in cellular defense mechanisms, serving as crucial protectors against the harmful effects of free radicals. Free radicals, which are highly reactive molecules, can induce oxidative stress and damage various cellular components, including DNA, proteins, and lipids. which in turn develop cancer. Antioxidants are compounds that can shield cells from this damage[13-18]. By neutralizing free radicals, they stop oxidative stress and cellular damage from occurring.

In the realm of medicinal chemistry, the utilization of heterocycles has become commonplace, owing to their expansive range of pharmacological activities, including antibacterial, anticancer, and anti-inflammatory properties and their unique ability to target specific biological receptors[19]. These cyclic compounds, distinguished by the presence of at least one non-carbon element in the ring, such as sulphur, nitrogen, or oxygen, e.g. coumarin, piperidine, benzimidazole, imidazole, pyridine, and pyrimidine offer diverse functionalities and have proven instrumental in the development of therapeutic agents. The incorporation of heterocyclic motifs into medicinal compounds serves to enhance their potency, solubility, selectivity, and pharmacokinetic characteristics.

The focus of the current study lies in the exploration of a hybrid compound derived from coumarin and piperidine, along with its amide derivative. This investigation aims to unravel the impact of these compounds on antimicrobial and antioxidative properties. By delving into the synergistic potential of the coumarin and piperidine hybrid, we seek to understand how this novel structure may offer enhanced therapeutic benefits in terms of combating microbial infections and providing antioxidant-mediated cellular protection. The study not only contributes to the expanding field of heterocyclic-based medicinal chemistry but also holds the promise of unveiling new avenues for the development of potent and multifunctional therapeutic agents.

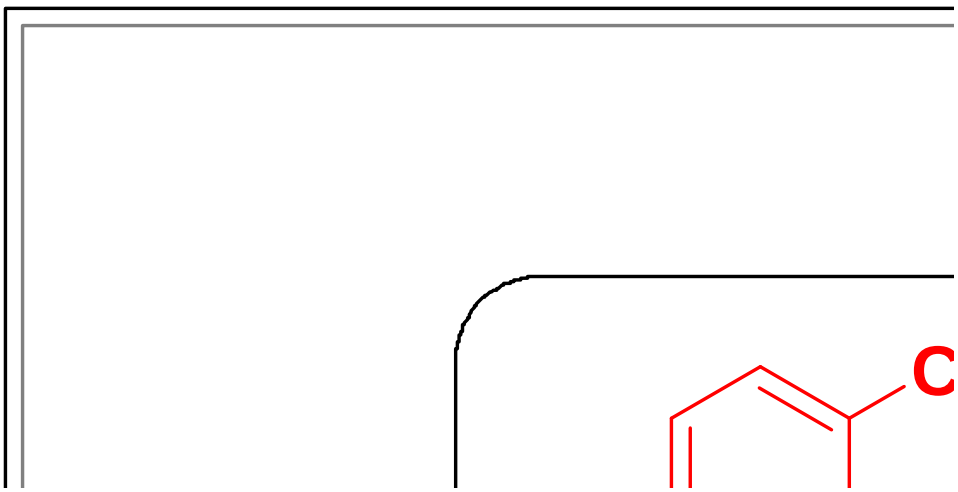

Fig. 1:synthesized hybrid heterocyclic molecules containing multiple pharmacophores.

The vast array of coumarin derivatives, with more than 1300 identified in natural sources, underscores the significance of this class in both organic synthesis and the realm of natural products. Characterized by an oxygen heterocyclic benzopyran system and belonging to the lactone family, coumarin displays a unique structural framework that contributes to its diverse pharmacological properties. Coumarin derivatives have garnered attention across various therapeutic domains, showcasing a multitude of pharmacological activities:antifungal[20-21], antibacterial[22], anti-inflammatory[23], antitumor[24], antiviral, antioxidant[25-26], anticoagulant[27-28], anticancer, and anti-HIV[29] properties.

Piperidine, characterized as a saturated N-heterocycle, holds a distinctive position in medicinal chemistry due to its favorable impact on drug solubility, ultimately enhancing metabolic processes[30-31]. The versatile nature of piperidine-based compounds is evidenced by their wide-ranging pharmacological properties, making them valuable candidates in drug development of antihypertensive, antibacterial, antimalarial, anticonvulsant , anti-inflammatory, antiproliferative, antitubercular, and antioxidant agent.Therefore, the exploration of piperidine moieties in drug design and development is an attractive tool for the creation of novel therapeutic agents.

Both coumarin and piperidine have individually exhibited noteworthy antibacterial characteristics, and the amalgamation of these two components in hybrid molecules presents an intriguing avenue for exploration. The primary objective of the current study is to scrutinize the impact of these newly designed hybrid compounds on their antibacterial qualities. In particular, our focus lies on the synthesis and evaluation of a spectrum of amide derivatives derived from this hybrid structure. This approach allows for a comprehensive investigation into the influence of various functional groups e.g. electron withdrawing group and electron donating group.

The significance of this research lies in the potential discovery of hybrid molecules that not only harness the inherent antibacterial properties of coumarin and piperidine but also leverage the strategic incorporation of amide functionalities. Such insights can pave the way for the development of novel antibacterial agents with enhanced potency, selectivity, and possibly reduced resistance. This study aligns with the broader goal of advancing our understanding of molecular design principles in the pursuit of effective antimicrobial agents, holding implications for the future development of therapeutic strategies in bacterial infections.

II. MATERIAL AND METHODS

All chemicals such as 5-Methoxy Salicylaldehyde, Diethyl malonate, 4-Amino-1-Boc-piperidine, 4N HCl in 1,4-Dioxane, different derivatives of benzoic acid, DIPEA, TEA, and solvents such as Methanol, MDC, DMF, Ethyl acetate, n-Hexane were obtained from Sigma-Aldrich, Avra and used without purification. Melting points were recorded on G-Lab melting point apparatus. Infrared spectra were recorded with the help of Bruker spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained from a Jeol 400 MHz spectrometer. Silica gel (Merck 60, 100-200 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates pre-coated with silica gel (Merck 60 F254, 0.25 mm). Organic solutions were dried over anhydrous Na_2SO_4 as required. The solvents were distilled out on Buchi rotary evaporator. Biological activity evaluation was done at Aster analytics research institute. The antibacterial and antifungal activities were accomplished by using the microbroth dilution method against Ampicillin and Nystatin as a standard. Antioxidant activity was accomplished by using the DPPH method against the Ascorbic acid standard.

Chemistry:

As shown in scheme 2, we discussed the multistep procedure for creating straightforward and innovative heterocyclic hybrid compounds. These compounds are composed of coumarin bound to piperidine amide derivatives.

Scheme 1: i) Piperidine, Acetic acid (Cat.), Diethyl malonate, Ethanol, Reflux ii), NaOH, Ethanol, Reflux, HCl.

According to Scheme 1, 6-methoxy-2-oxo-2H-chromene-3-carboxylic acid **compound 1** was made in two steps, the first of which involved the Knoevenagel condensation of 5-Methoxy Salicyldehyde and diethyl malonate and the subsequent alkaline hydrolysis of Ethyl Carboxlate to produce free carboxylic acid as an assistant for the molecule's construction [32].

Scheme 2: i) HATU/ DIPEA, DMF, 0-5°C to RT, 12 h. ii) 4N HCl in 1, 4-Dioxane, 0-5°C to RT 6 h iii) HATU/ DIPEA, DMF, 0-5°C to RT, 12 h

Tert-butyl 4-(6-methoxy-2-oxo-2H-chromene-3-carboxamido) piperidine-1-carboxylate (**Compound 3**) was synthesized by reacting compound **1** with 4-amino-1-boc-piperidine (**compound 2**).in presence of HATU as coupling agent and DIPEA as base. **Compound 3** is then subjected to an acidic environment (4 N HCl in 1,4-Dioxane) to produce **compound 4**, which is 6-methoxy-2-oxo-N-(piperidine-4-yl)-2H-chromene-3-carboxamide hydrochloride. The last step was utilizing **compound 4** as a building block for synthesis of various amide derivatives **6a-j** using substituted benzoic acid and HATU and DIPEA in DMF.

Chemical Procedure

Preparation of Tert-butyl 4-(6-methoxy-2-oxo-2H-chromene-3-carboxamido) piperidine-1-carboxylate (Compound 3):

To a stirred solution of 6-methoxy-2-oxo-2H-chromene-3-carboxylic acid (1eq) in DMF HATU (1.5 eq) and DIPEA (3.0 eq), was added at 0-5°C followed by addition of 4-amino-1-boc-piperidine (1.2 eq). The reaction mixture was stirred at rt for 16 h. Progress of the reaction was monitored by TLC. After complete consumption of starting material reaction was quenched by adding ice cold water to it and extracts with ethyl acetate (2X 100 mL) and organic layer is dried over sodium sulphate, and evaporated organic layer under vacuum to afford crude material which was purified by column chromatography silica 100-200 mesh using 70 to 80 % ethyl acetate in hexane as an eluent to afford pure final compound. MS (ESI)m/z calcd. For $C_8H_{11}N_3O_2S$: $[M+H]^+$ 213.26, observed mass 213.5**.**

Synthesis of 2-chloro-N-(4-(morpholine-4-carbonyl) thiazol-2-yl) Acetamide (Compound 4): To a stirred solution of step-01 product. (**Compound 3**) (1 eq) 4N HCL in Dioxane is added at 0°C. The reaction mixture was stirred at 25-30°C for 6 h. Progress of the reaction was monitored by TLC. After completion of starting material concentrate reaction mixture completely to dryness to afford off white solid used for next reaction. (68.94% yield). MS (ESI) m/z calcd. for $C_{10}H_{12}ClN_3O_3S$: $[M + H]^+$ 290.73, found 291.0.

General Procedure for the synthesis of Compound 6 a-j:

To a stirred solution of different acid (1.2 eq) in DMF HATU (1.5 eq) and DIPEA (3 eq) was added at 0°C followed by addition of step-02 product (**compound 3**) (1 eq). The reaction mixture was stirred at rt for 16 h. Progress of the reaction was monitored by TLC. After Starting material (**compound 3**) is completely exhausted reaction is quenched by adding ice cold water and extracted with ethyl acetate to afford crude material which was purified by column chromatography silica 100 to 200 mesh using 70 % to 80 % ethyl acetate in hexane as an eluent to afford final solid product.

Compound 6a: off white solid, (79.33% yield); mp = 172-173 °C; IR: νmax/cm $^{-1}$ = 2857, 1693, 1594, 1365, 1166;^{1}H NMR(400 MHz, DMSO) δ 8.84(1H, s), 8.64(1H, d J = 7.8Hz), 7.92(1H, d J= 8.7Hz), 7.43(5H, m), 7.14(1H, d J = 2.1Hz), 7.05(1H, dd J1 = 8.7Hz, J2 = 2.3Hz), 4.36(1H, s), 4.10(1H, m), 3.90(3H,s), 3.60(1H, m), 3.11(3H, m), 1.90 (2H, d J= 48.5Hz), 1.55(2H, dd J1 = 19.8Hz, J2 =10.3Hz); ^{13}C NMR (100 MHz, DMSO) δ 31.0 (2C, s), 46.2 (1C, s), 53.5 (2C, s), 56.2 (1C, s), 100.2 (1C, s),112.0 (2C, s), 113.6 (1C, s), 114.6 (1C, s), 126.6-128.3 (3C, 127.8 (s), 127.8 (s)), 129.3 (2C, s), 131.5 (1C, s), 136.2 (1C, s),

147.8(1C,s), 156.1 (1C, s), 160.6 (1C, s), 160.9 (1C, s), 164.4 (1C, s), 169.0 (1C, s). MS (ESI) m/z calcd. for $C_{23}H_{22}ClN_2O_5$: $[M + H]^+$ 407.44 , found 407.15.

Compound 6b: off white solid, (80.69% yield); mp = 175-173 °C; IR: νmax/cm $^{-1}$= 2963, 1694, 1648, 1595, 1396, 1167; ^{1}H NMR (400 MHz, DMSO): δ 8.84(1H, s), 8.64(1H d J=7.7Hz), 7.92(1H, d J=8.8Hz), 7.52(2H d J=8.5Hz), 7.45(2H d J=8.5Hz), 7.14(1H, d J=2.3Hz), 7.05(1H, dd J1=8.7Hz, J2=2.4Hz), 4.34(1H, s), 4.10(1H, m), 3.90(3H s), 3.51(1H, s), 3.13(2H d J=59.8Hz), 1.87(2H, d J=33.7Hz), 1.55(2H, m);^{13}C NMR (100 MHz, DMSO: : δ 31.0 (1C, s), 31.4 (1C, s), 45.8 (1C, s), 46.1 (2C, s), 56.2 (2C,s) 100.2 (1C, s), 112.0 (1C, s), 113.6 (1C, s), 114.6 (1C, s), 128.4 (2C, s), 128.6 (1C, s), 131.5 (2C, s), 134.0 (1C, s), 134.9 (1C, s), 147.8 (1C, s), 156.1 (1C, s), 160.6 (1C, s), 160.9 (1C, s), 164.4 (1C, s), 167.9 (1C, s); MS (ESI) m/z calcd. for $C_{23}H_{21}ClN_2O_5$: $[M + H]^+$ 441.88, found 441.06

Compound 6c: off white solid , (78.21% yield); mp = 195-196 °C; IR: νmax/cm $^{-1}$ = 2853, 2232, 1686, 1620, 1524, 1164; ^{1}H NMR (400 MHz, DMSO): δ 8.84(1H, s), 8.64(1H,d J= 7.8Hz), 7.92(1H, d J=8.8Hz), 7.38(2H, d J=8.7Hz), 7.14(1H, d J=2.3Hz), 7.05(1H, dd J1=8.7Hz, J2 = 2.4Hz), 6.99(2H, d J= 8.7Hz), 4.27(1H, s), 4.10(1H, m), 3.90(3H, s), 3.80(3H, s), 3.61(1H, td J1=6.6Hz, J2=4.0Hz), 3.14(2H, m), 1.89(2H, s), 1.53(2H, m); ^{13}C NMR (100 MHz, DMSO: δ 31.0 (2C, s), 46.2 (1C, s), 50.1 (2C, s), 56.0 (1C, s), 112.2 (1C, s), 112.5 (1C, s), 112.9 (1C, s), 113.5 (1C, s), 115.5 (1C, s), 127.8-127.9 (3C, 127.8 (s), 127.8 (s)), 128.4 (2C, s), 136.1 (1C, s), 136.9 (1C, s), 147.8(1C,s), 156.1 (1C, s), 160.6 (1C, s), 160.9 (1C, s), 164.4 (1C, s), 169.0 (1C, s).; MS (ESI) m/z calcd. For $C_{24}H_{24}N_2O_6$: $[M + H]^+$ 437.46, found 437.16

Compound 6d: Pale yellow solid , (81.50% yield); mp = 201-202 °C; IR: νmax/cm $^{-1}$ = 2921, 2853, 1692, 1233, 1163; ^{1}H NMR (400 MHz, DMSO): δ 8.85(1H, s), 8.65(1H, d J=7.7Hz), 7.92(1H, d J=8.8Hz), 7.83(2H, d J=8.1Hz), 7.65(2H, d J=8.0Hz), 7.14(1H d J=2.2Hz), 7.05(1H dd J1=8.7Hz, J2=2.4Hz), 4.38(1H d J=12.2Hz), 4.11(1H m), 3.90(3H s), 3.46(1H d J=12.8Hz), 3.21(1H t J=11.7Hz), 3.07(1H m), 1.98(1H d J=10.3Hz), 1.82(1H, d J=10.6Hz), 1.58(2H, d J=10.6Hz) ; ^{13}C NMR (100 MHz, DMSO) δ 30.9 (1C, s), 31.4 (1C, s), 40.2 (1C, s), 45.7 (1C, s), 46.1(1C, s), 56.2 (1C, s), 100.2 (1C, s), 112.1 (1C, s), 113.6 (1C, s), 114.6 (1C, s), 122.5 (1C, s), 125.2 (1C, s), 125.4 (1C, s), 127.4 (1C, s), 129.3 (1C,s), 129.6(1C, s), 131.5 (1C, s), 140.3 (1C, s), 147.8 (1C, s), 156.1 (1C, s), 160.6 (1C, s), 160.9 (1C, s), 164.4 (1C, s), 167.6 (1C, s).; MS (ESI) m/z calcd. for $C_{24}H_{21}F_3N_2O_5$: [M + H]+ 475.44, found 475.04

Compound 6e: off white solid , (79.56% yield); mp = 225-226 °C; IR: νmax/cm $^{-1}$ = 2947, 2850, 1693, 1593, 1355, 1155; ^{1}H NMR (400 MHz, DMSO):δ 8.84(1H s), 8.64(1H d J=7.8Hz), 7.92(1H d J=8.7Hz), 7.46(2H d J=8.4Hz), 7.35(2H d J=8.2Hz), 7.14(1H, d J=2.2Hz), 7.05(1H dd J1= 8.7Hz, J2=2.3Hz), 4.34(1H s), 4.10(1H m), 3.90(3H s), 3.60(1H s), 3.12(2H d J=53.6Hz), 1.90(2H d J=21.3Hz), 1.53(2H dd J1=19.6Hz, J2=10.8Hz), 1.30(9H, s); ^{13}C NMR (100 MHz, DMSO): δ 31.1 (3C, s), 32.1 (2C, s), 34.7 (1C, s), 47.1 (1C, s), 50.1 (2C, s), 56.0 (1C, s), 112.2 (1C, s), 112.5 (1C, s), 112.9 (1C, s), 113.5 (1C, s), 115.5 (1C, s), 125.7 (2C, s), 128.0 (2C, s), 136.1 (1C, s), 136.9 (1C, s), 147.9 (1C, s), 151.9 (1C, s), 156.1 (1C, s), 160.5 (1C, s), 164.3 (1C, s), 169.1 (1C, s) ; MS (ESI) m/z calcd. for $C_{27}H_{30}N_2O_5$: $[M + H]^+$ 463.55, found 463.15.

Compound 6f: off white solid , (84.80% yield); mp = 212-213 °C ; IR: νmax/cm $^{-1}$ = 2853, 1749, 1343, 1161; ^{1}H NMR (400 MHz, DMSO): δ 8.84(1H, s), 8.64(1H, d J=7.7Hz), 7.92(1H, d J= 8.8Hz), 7.31(2H, d J=8.0Hz), 7.25(2H, d J= 7.8Hz), 7.14(1H, d J=1.9Hz), 7.05(1H, dd J1=8.7Hz, J2=2.3Hz), 4.34(1H, s) 4.09(1H, m), 3.90(3H, s), 3.59(1H, s), 3.12(2H, d J=36.1Hz), 2.34(3H s), 1.89(2H d J1=26.4Hz), 1.53(2H dd J1=19.7Hz, J2=10.7Hz) ; ^{13}C NMR (100 MHz, DMSO): δ 20.9 (1C, s), 46.2 (2C, s), 56.2 (1C, s), 78.6 (1C, s), 78.9 (1C, s), 79.2 (1C, s), 100.3 (1C, s), 112.1 (1C, s), 113.7 (1C, s), 114.8 (1C, s), 126.8 (1C, s), 128.9 (2C, s), 131.5 (2C, s), 133.2 (1C, s), 139.0 (1C, s), 147.9 (1C, s), 156.2 (1C, s), 160.7 (1C, s), 160.9 (1C, s), 164.5 (1C, s), 169.2 (1C, s) ; MS (ESI) m/z calcd. for $C_{24}H_{24}N_2O_5$: $[M + H]^+$ 421.47, found 421.12.

Compound 6g: Off white solid , (76.32% yield); mp = 185-186 °C; IR: νmax/cm $^{-1}$ = 2920, 2853, 1621, 1525, 1271; ^{1}H NMR (400 MHz, DMSO): : δ 8.83(1H, s), 8.63(1H, d J=7.7Hz), 7.93(1H, m), 7.39(1H s), 7.24(2H, m), 7.14(1H, d J=2.2Hz), 7.05(1H, dd J1=8.8Hz, J2= 2.3Hz), 4.41(1H, d J=10.7Hz), 4.08(1H, s), 3.90(3H s), 3.26(1H d J=13.1Hz), 3.09(2H, m), 2.89(1H, s), 2.73(1H, s), 2.24(3H, d J=28.3Hz), 1.96(1H d J1=10.4Hz); ^{13}C NMR (100 MHz, DMSO): δ 18.4 (1C, s), 31.0-31.4 (2C, s), 45.2 (1C, s), 46.2 (2C, s), 56.2 (1C, s), 100.2 (1C, s), 112.0 (1C, s), 113.6 (1C, s), 114.5 (1C, s), 125.8 (1C, s), 127.4 (1C, s), 129.8 (1C,s), 131.5 (1C, s), 132.9 (1C, s), 135.4 (1C, s), 136.3 (1C, s), 136.9 (1C, s), 147.8 (1C, s), 156.1 (1C, s), 160.7 (1C, s), 164.4 (1C, s), 167.4 (1C, s) ; MS (ESI) m/z calcd. for $C_{24}H_{23}ClN_2O_5$: $[M + H]^+$455.91, found 455.09.

Compound 6h: Off white solid , (85.52% yield); mp = 235-236 °C ; IR: νmax/cm $^{-1}$ = 2919, 2853, 1628, 1526, 1445, 1166; ^{1}H NMR (400 MHz, DMSO): δ 8.83(1H, sδ 9.24(1H s), 8.81(1H s), 8.55(1H d J=7.7Hz), 7.90(1H d J=8.8Hz), 7.13(1H, m), 7.03(3H m), 6.71(2H dd J1=17.3Hz, J2=8.4Hz), 4.22(1H d J1=12.9Hz), 3.99(1H m), 3.89(3H s), 3.76(1H d J=26.7Hz), 3.59(2H s), 3.16(1H t J=11.5Hz), 2.85(1H m), 1.82(2H m), 1.31(2H m); ^{13}C NMR (100 MHz, DMSO): δ 32.1 (1C, s), 32.7 (1C, s), 38.9 (1C, s), 44.1 (1C, s), 46.2 (2C, s), 56.2 (1C, s), 112.0 (1C, s), 113.6 (1C, s), 114.7 (1C, s), 115.2 (1C, s), 121.4 (1C, s), 125.9 (2C, s), 129.7 (2C, s), 131.4 (1C, s), 147.7 (1C, s), 155.8 (1C, s), 156.1 (1C, s), 160.7 (1C, s), 160.9 (1C, s), 164.4 (1C, s), 169.1 (1C, s) ; MS (ESI) m/z calcd. for $C_{24}H_{24}N_2O_6$: $[M + H]^+$ 437.46, found 437.16.

Compound 6i: Off white solid , (84.25% yield); mp = 267-268 °C; IR: νmax/cm $^{-1}$ = 2922, 2853, 1691, 1628, 1524, 1163; ^{1}H NMR (400 MHz, DMSO): δ 8.82(1H s), 8.58(1H d J= 7.7Hz), 7.91(1H d J=8.8Hz), 7.44(2H m), 7.26(2H m), 7.13(1H d J=2.2Hz), 7.04(1H dd J1 = 8.8Hz, J2=2.3Hz), 4.22(1H d J=13.3Hz), 4.04(1H m), 3.90(4H s), 3.75(2H s), 3.22(1H t J=11.5Hz), 2.87(1H m), 1.87(2H dd J1=12.5Hz, J2=2.7Hz), 1.38(2H dd J1=19.8Hz, J2=9.1Hz); ^{13}C NMR (100 MHz, DMSO): δ 31.0 (1C, s), 31.7 (1C, s), 44.0 (2C, s), 46.1 (2C, s), 56.2 (1C, s), 100.2 (1C, s), 112.0 (1C, s), 113.6 (1C, s), 114.7 (1C, s), 121.3 (1C, s), 128.3 (1C, s), 129.0-130.2 (2C, 129.1 (s), 130.2 (s)), 131.4 (1C, s), 131.90 (1C, s), 138.8 (1C, s), 147.7 (1C, s), 156.1(1C, s), 160.7 (1C,s), 160.9 (1C, s), 164.3 (1C, s), 168.2 (1C, s); MS (ESI) m/z calcd. for $C_{24}H_{23}BrN_2O_5$: $[M + H]^+$ 499.36, found 499.02.

Compound 6j: Off white solid, (73.44% yield); mp = 218-219 °C; IR: νmax/cm $^{-1}$ = 2924, 2854, 1619, 1523, 1154; ^{1}H NMR (400 MHz, DMSO): δ 8.83(1H s), 8.63(1H d J=7.7Hz), 7.92(1H d J=8.8Hz), 7.45(2H d J=8.0Hz), 7.31(1H m), 7.14(1H d J=2.2Hz), 7.05(1H dd J1=8.7Hz, J2=2.4Hz), 4.21(1H d J=13.3Hz), 4.04(4H m), 3.90(3H s), 3.36(1H m), 2.90(1H t J=11.1Hz), 1.99(1H d J=10.3Hz), 1.89(1H d J=10.4Hz), 1.58(1H m), 1.38(1H, m); ^{13}C NMR (100 MHz, DMSO δ 31.2 (1C,s), 31.9 (1C, s), 35.4 (1C, s), 43.8 (1C, s), 46.2 (2C, s), 56.2 (1C, s), 100.2 (2C, s), 112.0 (1C, s), 113.6 (1C, s), 114.9 (1C, s), 127.9 (2C, s), 129.0 (1C, s), 131.5 (1C, s), 133.2 (1C,s), 135.4 (1C, s), 147.7 (1C, s), 156.1 (1C, s), 160.8 (1C, s), 160.9 (1C, s), 164.4 (1C, s), 165.9 (1C, s) ; MS (ESI) m/z calcd. for $C_{24}H_{22}Cl_2N_2O_5$: $[M + H]^+$ 489.35, found 489.01.

In-vitro Antibacterial and antifungal activity by Micro broth dilution method:

A volume of 100 µL of synthesized compounds **(6a-j)** in 10% (v/v) DMSO (usually a stock concentration of 1 mg/mL for synthesized compound) was added into the first row of the plate. 50 µL of nutrient broth and 50 µL of normal saline were added to each well of the plate. Serial dilutions were performed using a multichannel pipette such that each well had a total of l00 µL of the test material in serially descending concentrations. 10 µL of resazurin indicator solution was added to each well. Finally, 0.5 McFarland standard microbial suspension of l0 µL of bacterial and fungal suspension was added to each well to achieve a concentration of 1.5×108CFU/mL (for bacteria) and 0.5-2.5×103 yeast cells or spores/mL (fungi) [63-64]. 2 Each plate had a column with Ampicillin as the positive control and DMSO as the negative control for bacteria and Nystatin as the positive control and DMSO as the negative control in the case of Fungi. The plates were prepared in triplicates and placed in an incubator set at 37 °C for 18-24 h. for bacteria and 25 °C for 48 h. for Fungi. Final concentrations of the compounds in the liquid media ranged from 1000 to 0.0038 µg/mL. Microbial suspensions were added per each well containing broth and various concentrations of the examined compounds. After incubation, the MIC was determined spectrophotometrically as the lowest concentration of the samples showing complete bacterial or fungal growth inhibition. Appropriate DMSO, sterile, and growth controls were carried out. The media with no tested substances were used also as controls. Any color changes from purple to pink or to colorless indicates the growth of microbes. The lowest concentration at which no color change occurred was taken as the MIC value of the synthesized compound [33].

DPPH Assay method for the evaluation of Antioxidant activity

The molecule 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is characterized as a stable free radical by the delocalization of the free electron around the molecule so that the molecule does not combine to form a stable molecule. The stability of the electron also gives deep violet color, characterized by an absorption band in ethanol solution at about 517 nm. When a solution of DPPH is mixed with that of a reacting species that can donate a hydrogen atom, this gives rise to the reduced form with the loss of this violet color [34-35]. The percentage DPPH inhibition activity of the synthesized compound **(6 a-j)** and the ascorbic acid are measured at different concentrations between 25-400 µg/mL and reported.

III. RESULT

In-vitro Antibacterial Activity

Novel acetamide derivatives of hybrid scaffold consisting of coumarin and piperidine, **6a-j** were evaluated for antibacterial activity by using the micro broth dilution method against a set of Gram-positive (Bacillus subtilis NCIM 2063 and Staphylococcus aureus NCIM 2079) and gram-negative (Escherichia coli NCIM 2065; PV: Proteus vulgaris NCIM 2813) bacterial microorganisms and ampicillin used as a reference standard.

Compound	R=	Minimum Inhibitory Concentration (MIC) in µg/mL					
		Gram +ve Bacteria		Gram -ve Bacteria		Fungal Microorganism	
		BS	***SA***	***EC***	***PV***	***AN***	***CA***
6a		187.5	**187.5**	187.5	**187.5**	**250**	250
6b		375	250	375	**250**	**250**	**375**
6c		375	**93.7**	250	375	**187.5**	**93.7**
6d		187.5	375	375	**187.5**	**187.5**	375
6e		250	375	375	375	**250**	**250**
6f		375	**187.5**	375	**187.5**	**250**	250
6g		**93.7**	**187.5**	250	375	**187.5**	375
6h		250	250	375	**187.5**	**250**	250
6i		250	**125**	250	**93.7**	**187.5**	375
6j		375	**93.7**	375	**250**	**187.5**	**93.7**
	Standard	**125**	**187.5**	**125**	**250**	**250**	**187.5**

Table 1: Antibacterial and antifungal activities of compound **6a-j** against *BS:* Bacillus subtilis NCIM 2063; *SA*: Staphylococcus aureus NCIM 2079, *EC*: Escherichia coli NCIM 2065; *PV*: Proteus vulgaris NCIM 2813, *AN*: Aspergillus Niger NCIM 501; and *CA*: Candida albicansNCIM 3471 microorganisms, standard: Ampicillin (For gram Positive and negative bacterial microorganisms); Nystatin (for fungal microorganisms).

In-Vitro Antioxidantactivity

Table 2: Antioxidant activities of compound **6a-j** (Values are mean ± SEM of triplicate determinations)

Conc. µg/mL	***DPPH % Inhibition***										
	Standard	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**	**6j**
25	34.52	23.56	22.41	23.56	23.05	22.04	24.01	23.69	22.59	22.03	24.25
50	63.25	55.45	54.26	53.66	52.14	50.48	53.69	52.61	54.75	53.02	52.45
75	75.45	69.22	68.59	70.12	69.47	68.59	71.02	72.14	70.55	68.95	70.44
100	86.95	73.26	74.25	71.25	72.30	72.56	74.25	73.69	72.14	73.26	74.01
200	93.45	82.55	83.56	82.45	80.56	81.47	82.36	80.25	81.25	82.36	80.25
300	97.44	88.56	85.44	87.55	86.36	87.44	86.57	84.55	87.59	88.56	87.41
400	98.69	**92.55**	91.02	92.36	92.01	**93.55**	91.24	92.36	90.25	91.25	92.33

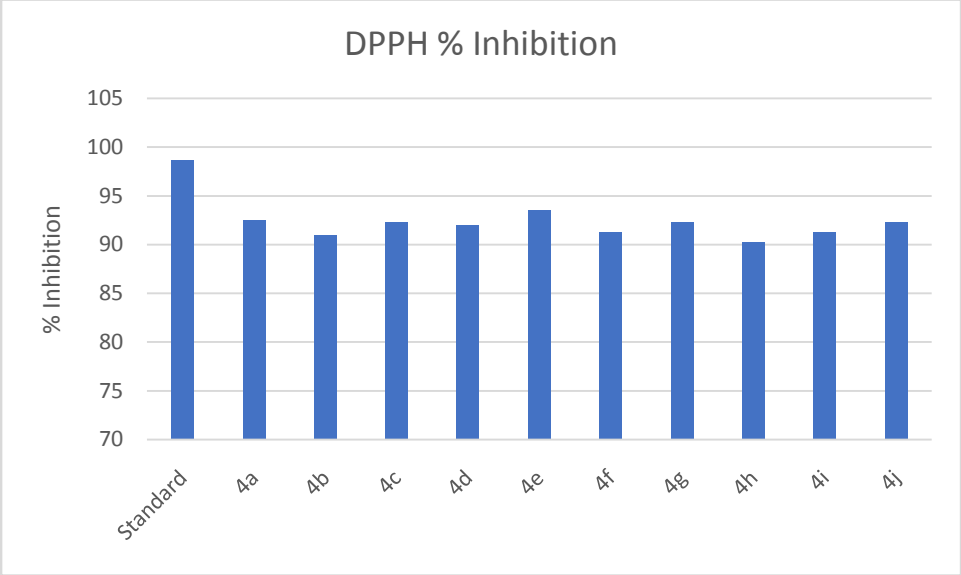

Fig. 2: Effect of Novel hybrids on inhibition of DPPH free radical.

IV. DISCUSSION

In-vitro Antibacterial Activity

According to the results obtained and shown in Table 1, it is observed that all compounds have the ability to suppress the growth of different strains of bacteria to varying degrees. It was noticed that most of the compounds in the series have shown better inhibition of gram-negativeProteus vulgaris NCIM 2813 bacterial microorganisms. Moreover, compounds **6b, 6c, 6i,** and **6j** have shown potent and broad-spectrum activity against all tested microorganisms. It is observed that introduction of electron withdrawing group on phenyl ring (Compound **6b, 6d, 6i, 6j**) increases antibacterial property of compound against gram negative bacteria Proteus vulgaris NCIM 2813. Additionally, if substitution of halogen is at meta (**6i)** and ortho position (**6j)** compound has shown positive impact on antibacterial activity against gram positive Staphylococcus aureus NCIM 2079. The presence of electron donating group ($-CH_3$) at ortho position **(6g)** showed eminent activity against gram positive bacteria Bacillus subtilis NCIM 2063. Based on the comparison of compounds **6a, 6e**, and **6f**, it is possible to anticipate that a decrease in the activity against the tested microorganism occurs when the +inductive effect of substitution at the para position increases. Furthermore, the antibacterial activity against gram negative bacteria Proteus vulgaris NCIM 2813 is positively impacted by the strong electron donating group at para position **(6h).** While the electron donating group at para position **(6c)** is relatively weaker, it has a significant impact on the antibacterial activity against gramme positive bacteria. Staphylococcus aureus NCIM 2079

In-vitro Antifungalactivity

Compound **6a-j**'s in vitro antifungal activity has been determined and presented in Table 1. The growth of Aspergillus niger and Candida albicans has been demonstrated to be inhibited by the provided series of hybrid compounds, albeit to varying degrees. The activity of every compound against Aspergillus Niger NCIM 501 was remarkably significant. Additionally, compounds **6c** and **6j** revealed antifungal activity against the microorganism Candida albicans NCIM 3471. These results suggest that the methoxy group at para position (**6c**) and the dichloro group at ortho position (**6j**) have contributed to the increased antifungal activity of the aforementioned scaffold.

In-Vitro Antioxidantactivity

New derivatives of the piperidine and coumarin hybrid molecule **6a-j** are examined for their in vitro antioxidant activity using the DPPH assay at doses ranging from 25 to 400 g/mL.

Table 2's findings revealed that the compounds' potential to scavenge free radicals was found to be concentration dependent. Over 90% of DPPH free radicals were inhibited by all compounds. Compounds **6a** (92.55%) and **6e** (93.55%) showed the greatest percentage of inhibition among the hybrid compounds examined in this investigation and are equivalent to the activity of the ascorbic acid employed as a reference, as shown in Fig. 2. Trifluoro methane substitution in the para position of the phenyl ring significantly increased the scaffold's antioxidant activity.

V. CONCLUSION

The synthesis, structural elucidation, and biological assessment of 10 new amide derivatives of a hybrid scaffold made of coumarin and piperidine are presented in the current work. All compounds had good yields after being purified through recrystallization or column purification. A study of antimicrobial and antioxidant properties of reported compounds disclosed that all compounds can suppress the growth of different strains of bacteria and fugues to varying degrees. It was noticed that most of the compounds in the series have shown better inhibition of Aspergillus Niger NCIM 501 microorganisms. Introduction of electron withdrawing group on phenyl ring (Compound **6b, 6d, 6i, 6j**) increases antibacterial property of compound against gram negative bacteria Proteus vulgaris NCIM 2813.Substitution of halogen at meta (**6i**) and ortho position (**6j**) compound has shown positive impact on antibacterial activity against gram positive.Additionally, compounds **6c** and **6j** revealed antifungal activity against the microorganism Candida albicans NCIM 3471. The results suggest that the methoxy group at para position (**6c**) and the dichloro group at ortho position (**6j**) have contributed to the increased antifungal activity of the aforementioned scaffold.Moreover, compounds **6b, 6c, 6i**, and **6j** have shown potent and broad-spectrum activity against all tested microorganisms. Furthermore, findings revealed that the compounds' potential to scavenge free radicals was found to be concentration dependent. Over 90% of DPPH free radicals were inhibited by all compounds. All these results can be useful for future efforts to synthesize and evaluate coumarin derivatives. Additionally, it can also be foreseen that these compounds can be further studied for other microorganisms as well as further functional group modification to selected hybrid scaffold may result in discovery of broad-spectrum antimicrobials**.**

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