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Original Research Article

Synthesis and Comprehensive Evaluation of Antimicrobial and Antioxidant Activities of 2-(benzylthio)-4-hydroxy-6-(methylthio) pyrimidine-5-carbonitrile and Its Derivatives

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Abstract: The brief study of 2-(benzylthio)-4-hydroxy-6-(methylthio) pyrimidine-5-carbonitrile and its derivatives, describes the methodical synthesis and characterization of novel pyrimidine derivatives. Equimolar amounts of S-benzylthiuronium chloride and ethyl 2-cyano-3,3-bis(methylthio)acrylate were precisely reacted in the first synthesis. This important step was carried out in the commonly used solvent N,N'-dimethylformamide (DMF) with the help of a catalytic quantity of anhydrous potassium carbonate, which serves as a base to boost the cyclization reaction. The newly created substituted pyrimidine intermediate then functioned as a flexible foundation for additional functionalization. Reactions using a wide variety of nucleophiles that contain N and O were used to accomplish this. The replacement of thiomethyl group at sixth position gives substituted pyrimidine derivatives, which includes different phenols, substituted aromatic amines, hetaryl amines, and active methylene compounds. The structural integrity and authenticity of each synthesized compound were confirmed by using physicochemical data and spectroscopic methods, like infrared (IR), mass spectrometry, and (^{1}H NMR) spectroscopy. The biological strength of these newly prepared compounds were explored by evaluating their in vitro antioxidant activity using the 96-well microplate method and their antibacterial activities using the Agar Well plate method.

Keywords: Pyrimidine, DMF, Potassium carbonate, antimicrobial evaluation, antioxidant evaluation etc.

INTRODUCTION

In bio-organic and medicinal chemistry, electron-rich nitrogen-containing heterocyclic molecules like pyrimidines has plays significant role. because Due to pyrimidine bases in pyrimidines, such as thymine, cytosine, and uracil, are main components of nucleic acids and important building blocks of RNA and DNA, the pyrimidine nucleus is an important component in biological systems [1]. The pyrimidine moiety possessed compounds show a wide variety of biological and medicinal properties[2-3]. Pyrimidine substituted derivatives shows wide-ranging therapeutic applications[4], including antimicrobial[5-7], antibacterial[8,9], antifungal[10], antiviral[11], antimalarial[12], antitubercular[13], antileishmanial[14], antioxidant[15,16], anti-inflammatory[17,18], antihistaminic[19], antiallergic[20], analgesic[21], antipyretic[22], anticancer[23], anti-HIV activities[24] and antidepressants[25].

The development of new heterocyclic compounds

with enhanced therapeutic applications and low toxicity has been encouraged by the expansion of these health problems. The development of resistant to antibiotics in modern-day medicine is the main problem in the growth of bacterial strains, especially infections caused by Escherichia coli and Staphylococcus aureus [26]. The development of novel antibacterial and chemotherapeutic drugs the pyrimidine derivatives have become alternative options.

In the continuing efforts toward the synthesis of biologically related heterocyclic ring systems, has been more focused on the advance synthesis of novel substituted pyrimidine derivatives having multiple bioactive fractions within the same molecular basis. So according to literature serve, In this present work, we synthesized novel 2-(benzylthio)-4-hydroxy-6-(methylthio)pyrimidine-5-carbonitrile and its derivatives. The produced pyrimidine nucleus was synthesized by simple condensation of S-benzylthiuronium chloride with ethyl 2-cyano-3,3-bis(methylthio)acrylate. The formed product intermediate was again refluxes with various substituted nucleophiles, such as substituted aryl amines, substituted phenols, heteryl amines, and active methylene compounds, to gain diverse 6-substituted pyrimidine derivatives. These newly synthesized molecules shows the multiple pharmacologically active structural motifs and were evaluated for their antimicrobial activity and antioxidant activity in comparison with standard reference drugs, with the purpose of identifying capable of compounds having enhanced functional therapeutic potential.

RESULTS AND DISCUSSION

a) Experimental:

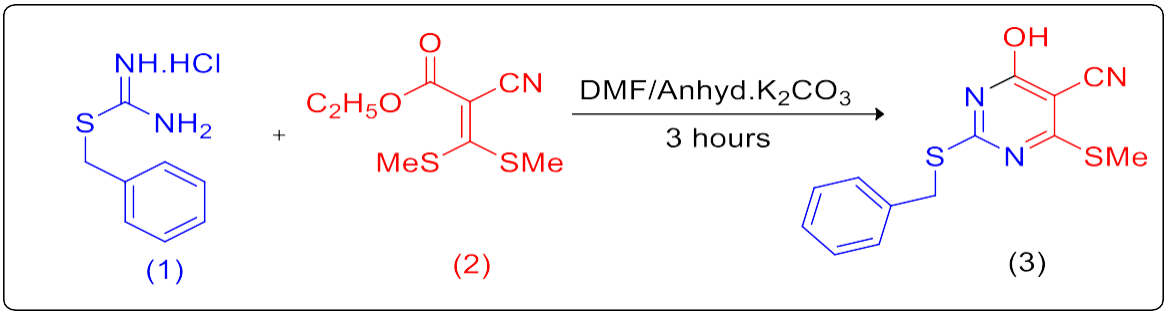
All materials were acquired from SD Fine, Spectrochem, and Loba Chem. An Electrothermal IA 9000 SERIES digital melting point device was used to determine the uncorrected melting points of the produced compounds. Thin-layer chromatography (TLC) was performed on pre-coated sheets of silica gel-C plates with a thickness of 0.25 mm to verify the purity of each product. FT-IR spectra were captured using an infrared spectrophotometer in KBr pallets or Nujol. Using tetramethylsilane (TMS) as an internal standard, ^{1}H-NMR spectra was recorded using a Brukner advance spectrophotometer operating at 400 MHz frequency. Mass spectra were obtained using the EI technique at 70 eV on a FT-VC-7070 H Mass spectrometer.

Synthesis of 2-(benzylthio)- 4-hydroxy-6-(methylthio)pyrimidine-5-carbonitrile (3):

S-benzylthiuronium chloride (1) (0.01 mol) and ethyl 2-cyano-3,3-bis(methylthio) acrylate (2) (0.01 mol) were refluxed for three hours in 20 mL of N, N'-dimethyl formamide, with anhydrous potassium carbonate (10 mg) acting as a catalyst. After cooling to room temperature, ice-cold water was added to the reaction mixture. To obtain pure (3), the separated solid product was filtered, cleaned with water, and recrystallized from ethanol.

Synthesis of 6-substituted derivatives of 2-(benzylthio)-4-hydroxy-6- (methylthio)pyrimidine-5-carbonitrile (4a-c and 7a-c)

In N, N'-dimethyl formamide (15 mL) and a catalytic quantity of anhydrous potassium carbonate (10 mg), a mixture of (3) (0.001 mmol) reacted individually with different aromatic amines, substituted phenols, active methylene, and heteryl amine (0.001 mmol) for three–four hours. The reaction mixture was added to ice-cold water after cooling to room temperature. After filtering and washing with water, the separated solid product was recrystallized from ethanol to yield pure 4a-c and 7a-c.



Scheme 1. Synthesis of 2-(benzylthio)-4-hydroxy-6-(methylthio)pyrimidine-5-carbonitrile (3)

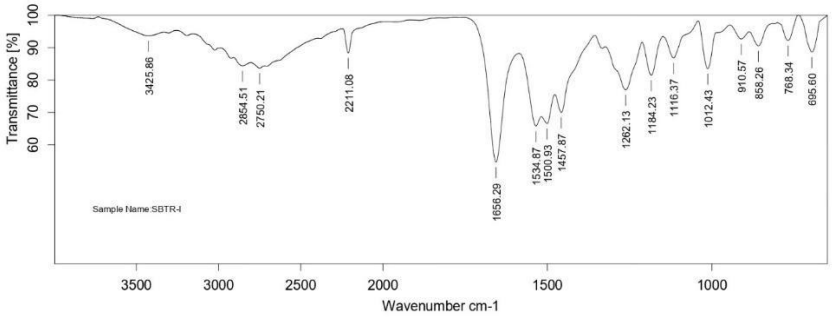


Figure 1. FTIR Spectra of 2-(benzylthio)-4-hydroxy-6-(methylthio)pyrimidine-5-carbonitrile

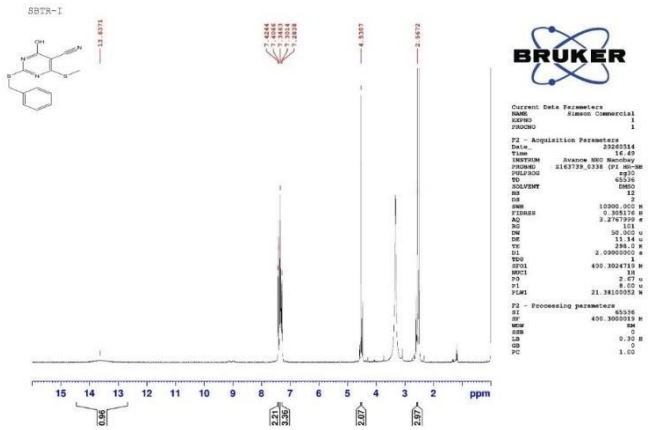
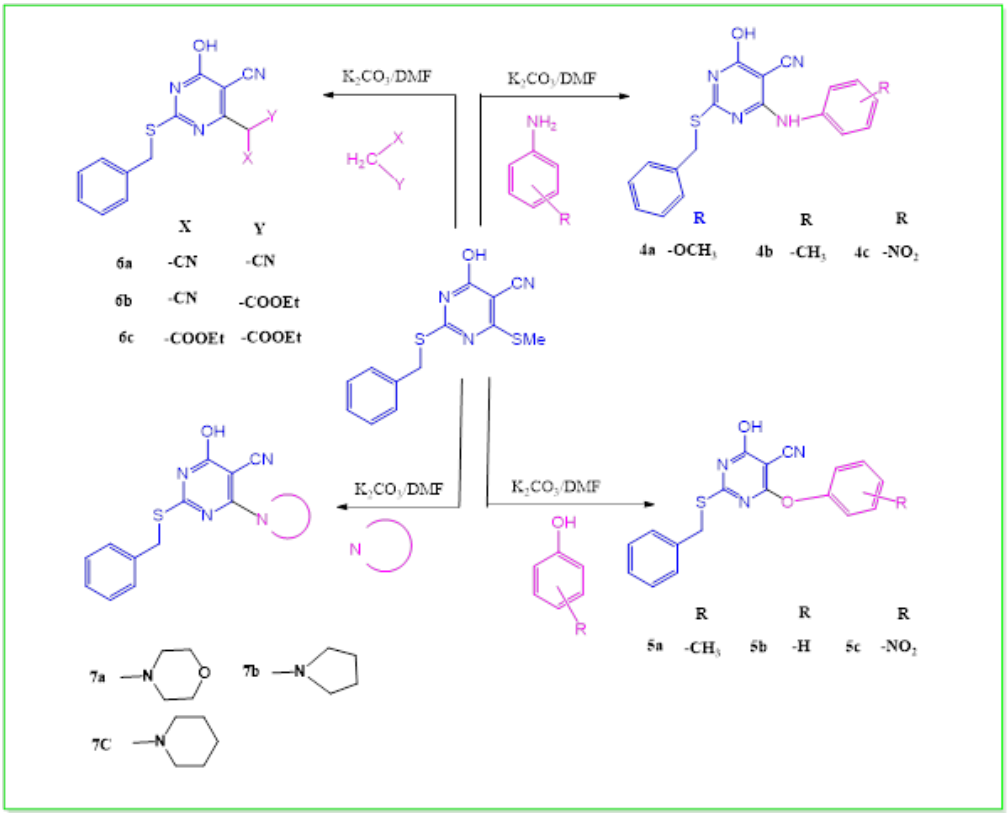


Figure 2. [1]H-NMR Spectra of 2-(benzylthio)-4-hydroxy-6-(methylthio)pyrimidine-5-carbonitrile



Scheme 2. Synthesis of substituted 6-methylthio pyrimidine derivatives

Table: 1 Physico-chemical data of newly synthesized compounds.

Sr. No.	Comp. code	Color	M.F.	M. Wt.	M.P.(^{0}C)	Yield (%)
1	3	Yellow	$C_{13}H_{11}N_3OS_2$	289	190-192	81
2	4a	Pale yellow	$C_{19}H_{16}N_4O_2S$	364	250-252	71
3	4b	Slightly yellow	$C_{19}H_{16}N_4OS$	348	213-315	67
4	4c	Slightly yellow	$C_{18}H_{14}N_4OS$	334	207-209	61
5	5a	Slightly Brown	$C_{19}H_{15}N_3O_2S$	349	244-246	70
6	5b	Brown	$C_{18}H_{13}N_3O_2S$	335	222-225	69
7	5c	Slightly yellow	$C_{18}H_{12}N_4O_4S$	380	253-255	64
8	6a	Brown	$C_{15}H_9N_5OS$	307	260-262	71
9	6b	Slightly Brown	$C_{17}H_{14}N_4O_3S$	354	255-267	68
10	6c	Slightly Brown	$C_{18}H_{19}N_3O_4S$	373	240-242	64
11	7a	Brown	$C_{16}H_{16}N_4O_2S$	328	240-242	68
12	7b	Slightly orange	$C_{16}H_{16}N_4OS$	312	211-213	67
13	7c	Red brown	$C_{17}H_{18}N_4OS$	326	203-205	70

Spectral and Physical Data:

Spectral analysis, including IR, ^{1}H NMR, and mass spectral data, was used to determine the structures of freshly synthesized compounds. The CN stretching frequency band was produced between 2220 and 2250 cm^{-1}, while the carbonyl stretch occurred between 1640 and 1670 cm^{-1}. One of the most important methods for confirming the presence of organic compounds is ^{1}H NMR. The signal at δ 2.56 ppm for compound (3) indicates the presence of an –SCH_3 group. However, in compounds (4a-c and 7a-c), the existence of a peak in the aromatic region (δ 7.24-7.57 ppm), active methylene, and hetaryl amines proves that the substitution occurs at the 6-position, while the absence of a peak at δ 2.60 ppm indicates the removal of the –SCH_3 group. Additionally, the molecular weights of the structures and the molecular ion peaks in the mass spectra were in good agreement.

2-(benzylthio)-4-hydroxy-6-(methylthio) pyrimidine-5-carbonitrile (3):

IR (KBr/cm^{-1}): 2211 (CN), 3425 (NH_2): ^{1}H NMR (400 MHz, DMSO-d6, ppm) δ 2.56 (s, 3H, -CH_3), δ 4.53 (s, 2H, -SCH_2), δ 7.28-7.42 (m, 5H Ar-H), δ 13.63 (s, 1H, - OH) ; ^{13}C NMR (DMSO-d6, ppm): δ = 13.36 (SCH_3), δ = 34.60 (SCH_2), 89.76 (C-CN), 115.47 (CN), 127.88, 127.93, 127.97, 129.09, 129.28, 136.78, (Ar-C), 166.43 (C-SCH_3), 167.05 (-S-C=N), 174.09 (C-NH_2), EI-MS(m/z: RA%):288 (M-1).

2-(benzylthio)-4-hydroxy-6-((4-methoxyphenyl)amino)pyrimidine-5-carbonitrile (4a):

IR (KBr/cm^{-1}): 2213 (CN), 3209, 3414 (NH_2): ^{1}H NMR (400 MHz, DMSO-d6, ppm) δ 3.79 (s, 3H, - OCH_3), δ 4.39 (s, 2H, - SCH_2), δ 7.24-7.57 (m, 9H Ar-H), δ 8.45 (s, 1H, -NH-Ar), δ 11.03 (s, 1H, - OH); Mass: m/z = 364 (M+).

2-(benzylthio)-4-hydroxy-6-phenoxypyrimidine-5-carbonitrile (5b):

IR (KBr/cm^{-1}): 2214 (CN), 3433 (NH_2): ^{1}H NMR (400 MHz, DMSO-d6, ppm) δ 4.55 (s, 2H, - SCH_2), δ 7.27-7.43 (m, 10H Ar-H), δ 13.63 (s, 1H, - OH); Mass: m/z = 335 (M^+).

2-(2-(benzylthio)-5-cyano-6-hydroxypyrimidin-4-yl)malononitrile(6a):

IR (KBr/cm^{-1}) 2212 (CN), 3424 (NH_2): ^{1}HNMR (400 MHz, DMSO-d6, ppm): δ = 4.55 (s, 2H, -SCH_2-Ar) δ 4.73 (s, 1H, CH), 7.28-7.41 (m, 5H), 13.57 (s,1H, -OH): EI-MS (m/z: RA%): 306 (M-1).

2-(benzylthio)-4-hydroxy-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (7b):

IR (KBr/cm^{-1}): 2213 (CN), 3435 (NH_2): ^{1}HNMR (400 MHz, DMSO-d6, ppm): 1.88 (m, 4H, pyrrolidine H), 3.65 (m, 4H, pyrrolidine H), δ = 4.45 (s, 2H, -SCH_2-Ar), 7.29-7.41 (m, 5H), δ 11.06 (s, 1H, - OH); Mass: m/z = 311 (M-1).

c] Antimicrobial activity

The antibacterial activity study demonstrated that the 6-substituted derivatives of 2-(benzylthio)-4-hydroxy-6-(methylthio)pyrimidine-5-carbonitrile (4a-c and 7a-c) exhibited varying degrees of inhibitory effects against Staphylococcal and Escherichia coli infections. The standard antibiotic, streptomycin, showed the highest antibacterial activity, producing zones of inhibition of 35 mm against S. aureus and 32 mm against E. coli, thereby validating the experimental method. Among the tested compounds, the antibacterial activity generally increased with increasing

concentration from 5 mg/ml to 10 mg/ml, indicating a concentration-dependent antimicrobial efficacy. Compounds 4a-c and 7a-c demonstrated comparatively stronger antibacterial activity. In particular, compound -6a exhibited the highest antibacterial activity among all the test samples, showing zones of inhibition of 24 mm against S. aureus and 26 mm against E. coli at conc. of 10 mg/ml. At higher concentration the compounds 7b, 5a, and 5b showed good antibacterial effects, with zone of inhibition ranging from 20–22 mm for both bacterial strains. The results indicate that various tested compounds possess promising broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The activity results observed for the tested compounds shown in table-2 which focuses the presence of potent bioactive antimicrobial moieties.

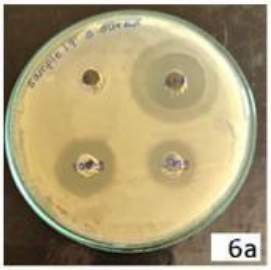


Figure 3. Antimicrobial Study

Table No. 2 Antimicrobial activity of 2-(benzylthio)-4-hydroxy-6-(methylthio) pyrimidine-5-carbonitrile and its 6-substituted derivatives

Sr No.	Compounds	Concertation	Gram positive	Gram negative
			S.aureus	*E.Coli*
1	Standard Streptomycin	1 mg/ml	***35***	***32***
2	4a	5 mg/ml	15	14
		10 mg/ml	20	19
3	4b	5 mg/ml	09	12
		10 mg/ml	13	16
4	5a	5 mg/ml	16	15
		10 mg/ml	20	21
5	5b	5 mg/ml	17	15
		10 mg/ml	22	20
6	6a	5 mg/ml	18	17
		10 mg/ml	24	26
7	7b	5 mg/ml	14	16
		10 mg/ml	20	22

d] Antioxidant activity:

The antioxidant potential of the 6-substituted derivatives of 2-(benzylthio)-4-hydroxy-6-(methylthio)pyrimidine-5-carbonitrile (4a-c and 7a-c) are summarized in table-2 was evaluated using the DPPH (2,2-diphenyl-1- picrylhydrazyl) free radical scavenging assay in a 96-well plate format. DPPH is a stable free radical that exhibits a deep violet color, which decreases upon reduction by antioxidant compounds capable of donating hydrogen atoms or electrons. The degree of discoloration reflects the radical scavenging activity of the test samples. In the present study, Ascorbic Acid, used as the standard antioxidant, showed 91.88% inhibition at a concentration of 1000 µg/mL, confirming the reliability of the assay. for this assay 1000 µg/mL DPPH was used as the control. All the analyzed samples give varying range of antioxidant activity at equal concentration.

In the examined samples, the highest antioxidant activity with (70.97%) inhibition is shown by 6a compound, followed by 4a having inhibition (67.04%). The compounds 4b (63.71%), and 3 (61.71%) indicates the moderate antioxidant activity, whereas 5b (55.78%), and 7b (54.38%) showed relatively lower but still considerable activity. The lowest antioxidant effect was observed in 5a, which has 49.06% inhibition zone. From the result of present work, it can be concluded that 2-(benzylthio)-4-hydroxy-6-(methylthio) pyrimidine-5-carbonitrile and its derivatives are essential to boost the antioxidant activity.

The synthesized selected compounds were initially screened for antioxidant activity using the DPPH radical scavenging test. Proton radical scavenging is an important mechanism of antioxidants. The DPPH radical of odd electron gives a strong absorption maximum at 517 nm and is purple color. When the odd electron of the DPPH radical is paired with hydrogen from free radical-scavenging antioxidants, turn the color of the solution changes from purple to yellow color, to form

reduced DPPH: H. A 1 ml (1 mM) test sample was added to an equal amount of a 0.1 mM solution of DPPH in ethanol. At room temperature for 30 min of incubation, by using the microplate reader the DPPH reduction was measured at 517 nm. The results of DPPH reduction are tabulated in Table 3 in comparison with Ascorbic acid as a standard.

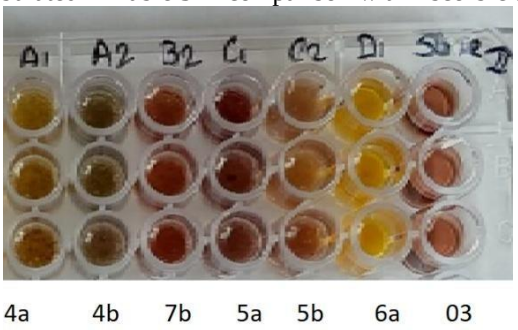


Figure 4. Antioxidant Examination

Table 3. Antioxidant activity of particular compounds:

Sr. No.	Compounds	Antioxidant activity
		DPPH radical scavenging activity (%)
1	3	61.71±0.575
2	4a	67.04±0.495
3	4b	63.71±0.545
4	5a	49.06±0.765
5	5b	55.78±0.664
6	6a	70.97±0.436
7	7b	54.38±0.685
8	Ascorbic acid	91.88±0.122

CONCLUSION

In the present work, we successfully synthesized 2-(benzylthio)-4-hydroxy-6- (methylthio)pyrimidine-5-carbonitrile (3) and its 6-substituted derivatives (4a-c & 7a-c). The antibacterial activity of all the newly synthesized derivatives against S. aureus and E. coli was measured in vitro. And also antioxidant activity examined for the selected compounds. The mostly compounds, such as 3, 4a, 4b, 5a, 5b, 6a, and 5b, revealed good activities.

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