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Gewald reaction for the synthesis of benzo[4,5]-thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives and it's antimicrobial study

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ABSTRACT

A variety of schiff base of 3-amino-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3*H*)-one have been synthesized using novel and yield efficient protocol i.e., Gewald condensation reaction by four step processes. The characterization of synthesized diverse molecules was characterized by various spectroscopic methods such as IR, Mass, and NMR. The hybrids of thieno-pyrimidines were utilized to check their efficiency as anti-microbial activity against various Gram-positive *(S. aureus, S. pyogenes,* MRSA- *multi-resistance S. aureus)* and Gram negative *(E. coli, P. aeruginosa, Klebsiella pneumonia)* bacteria, using five anti-microbial agents as reference standard. It was found that 4a (anti-fungal agent) and 4e (anti-bacterial agent) shown best efficiency and which can lead in the area of medicinal field.

***Keywords*:** Gewald Reaction, Antimicrobial Study, Benzo[4,5]thienopyrimidines

1. INTRODUCTION

In order to start a new drug discovery project and to find biologically active compounds, different options are available [1]. Hits can be obtained via a virtual screening approach or can be copied from scientific or patent literature. Very often, drug discovery projects start with a

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high-throughput screening campaign of commercially available compound libraries against the target of interest. The main interest of the laboratory of medicinal chemistry stands for the synthesis and biological evaluation of aromatic heterocycles. This search revealed that the number of available heterocycles is mainly limited to well-known nitrogen containing compounds such as quinazolines, indoles and benzimidazole [2].

Thiophene, thiazole, and pyrimidine derivatives have been used as therapeutic drugs over years. Among them thiophene containing compounds are well known to exhibit various biological effects [3]. Most efficient protocol for the synthesis of these thiophene derivatives is intramolecular cyclization via nucleophilic displacement, Gewald method, thio-claisen rearrangement, and dehydrophotocyclization [4].

Pyrimidine pharmacophore is an important and integral part of DNA and RNA and play an essential role in several biological processes and also have considerable chemical and pharmacological utility as antibiotics, antibacterial, cardiovascular as well as agrochemical and veterinary product [5]. These derivatives were found to possess a range of diverse activities such as anti-inflammatory and analgesic, antimicrobial, anti-avian influenza virus (H_5N_1), against herpes simplex virus type-1 (HSV-1) and hepatitis-A virus (HAV), serotonin 5-HT6 receptor antagonist, anti-arrhythmic agents, etc. Pyrimidine analogs have been already demonstrated as platelet aggregation inhibitors, antagonists, anti-conceptive and anti-parkinsonism agents. Pyrimidines occupy an outstanding position in organic and medicinal chemistry for their high biological activity. The pyrimidine core is a structural constituent of vital Biomolecules like DNA and of critically important drugs like Fluorouracil, Etravirine, Risperidone, Iclaprim, Avanafil, and Rosuvastatin [6].

Thienopyrimidines comprise a thiophene ring fused with the pyrimidine moiety similar to the imidazole moiety in purines [7]. Thienopyrimidines in general have become an interesting structural element in development of pharmaceutical compounds and have among others been evaluated as cGMP phosphodiesterase inhibitors, anti-viral agents, anti-inflammatory agents, anti-microbial agents, but also as kinase inhibitors and potential anti-cancer agents. Over the last two decades, many thieno pyrimidines have been found to exhibit a variety of pronounced activities. Many of their derivatives have been synthesized as potential anticancer, analgesic, antimicrobial and antiviral agents [8].

Present work was described the studies on Schiff's base of 3-amino-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one from literature survey, we synthesized, purified, characterized and analyzed for microbial efficacy for the series of different kinds of novel 3-(benzylideneamino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin -4(3*H*)-one.

2. MATERIAL AND METHODS

2. 1. Materials

Chemicals and solvents were purchased from the Sigma-Aldrich Chemical Co., Merck chemical, Finar and Spectrochem Ltd. The entire chemicals were used without further purification. Thin-layer chromatography was accomplished on 0.2 mm precoated plates of Silica gel G60 F_{254} (Merck). Visualization was made under UV light (254 and 365 nm). IR spectra were recorded on an "IR Affinity-1S spectrophotometer (Shimadzu)". ^{1}H (400 MHz) and ^{13}C (101.1 MHz) NMR spectra were recorded on a "Bruker AVANCE II spectrometer" in

DMSO-d_6. Chemical shifts are expressed in δ ppm downfield from TMS. Mass spectra were determined by Auto injector system on a "GC-MS (Agilent 7820A-5977B, Santa Clara, CA, USA) mass spectrometer". Solvents were evaporated with a "Roteva rotary evaporator". Melting points were measured in open capillaries and are uncorrected.

2. 2. Methods

Procedure for the synthesis of Ethyl 2-amino-6-methyl-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate (1)

An equimolar mixture of elemental sulphur (6.3 g, 0.19 mol) and morpholine (17.1 g, 0.19 mol) was stirred at 25 °C for 15-20 min. until the sulphur was completely dissolved. To it 4-methylcyclohexanone (22.0 g, 0.19 mol) and ethyl cyanoacetate (22.0 g, 0.19 mol) were added. The reaction mixture was stirred for 10h. After completion of reaction which was monitored by TLC, the reaction mixture was cooled to room temperature and the solid residue was collected, recrystallized from ethanol to afford **Intermediate 1** as white solid (30.0 g).

Yield: 94%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.97-0.99 (d, 3H, *J* = 6.4 Hz, -CH_3 attached to -CH); 1.23-1.27 (t, 3H, *J* = 7.2 Hz, -CH_3 attached to -CH_2); 1.28-1.30 (m, 1H); 1.73-1.76 (m, 2H); 2.01-2.07 (m, 1H); 2.45-2.51 (m, 2H); 2.73-2.78 (m, 1H); 4.11-4.17 (q, 2H, *J* = 5.4 & 12.6 Hz, -OCH_2); 7.21 (s, 2H, -NH_2). Elemental analysis for $C_{12}H_{17}NO_2S$ Calculated: % C, 60.22; % H, 7.16; % N, 5.85; Found: % C, 60.18; % H, 7.13; % N, 5.86

Procedure for the synthesis of Ethyl 2-acetamido-6-methyl-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carboxylate (2)

Intermediate 1 (10.0 g, 0.04 mol) was added to 50 mL of acetic anhydride at room temperature. The reaction mixture was refluxed for 3h. After completion of reaction it was monitored by TLC and it was poured in to ice crushed water. The solid separated was filtered, washed with water and dried under vacuum to give **Intermediate 2** as pale yellow solid (8.0 g).

Yield: 88%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00-1.02 (d, 3H, *J* = 6.4 Hz, -CH_3 attached to -CH); 1.29-1.32 (t, 3H, *J* = 7.0 Hz, -CH_3 attached to -CH_2); 1.31-1.33 (m, 1H); 1.79-1.82 (m, 2H); 2.14-2.19 (m, 1H); 2.21 (s, 3H, -$COCH_3$); 2.56-2.62 (m, 2H); 2.64-2.88 (m, 1H); 4.24-4.30 (q, 2H, *J* = 6.8 & 14.0 Hz, -OCH_2); 10.95 (s, 1H, -NH). Elemental analysis for $C_{14}H_{19}NO_3S$ Calculated: % C, 59.76; % H, 6.81; % N, 4.98; Found: % C, 59.71; % H, 6.78; % N, 5.02.

Procedure for the synthesis of 3-amino-2,7-dimethyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-d]pyrimidin-4(3*H*)-one (3)

Intermediate 2 (8.0 g, 0.03 mol) was dissolved in 40 mL of ethanol at room temperature. To it 40 mL of hydrazine hydrate was added and the reaction mixture was refluxed for 10h. After completion of reaction which was monitored by TLC, The solid separated was filtered, washed with ethanol and dried under vacuum. The crude product was purified by recrystallization in ethanol to give Intermediate 3 as off white solid (4.8 g).

Yield: 87%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03-1.05 (d, 3H, *J* = 6.8 Hz, -CH_3 attached to -CH); 1.34-1.41 (m, 1H); 1.84-1.88 (m, 2H); 2.28-2.35 (m, 1H); 2.53 (s, 3H, -CH_3 attached to -C); 2.69-2.82 (m, 2H); 3.01-3.09 (m, 1H); 5.80 (s, 2H, -N-NH_2). Elemental analysis for $C_{12}H_{15}N_3OS$ Calculated: % C, 57.81; % H, 6.06; % N, 16.85; Found: % C, 57.78; % H, 6.05; % N, 16.87. Mass (m/z): 249 (M^+).

General procedure for the synthesis of 3-((Substitutedbenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-d]pyrimidin-4(3*H*)-one. (4a-4j)

For the preparation of final product, **Step III** product **(3)** (0.1 mol) and various aromatic aldehyde (0.1 mol) were added in methanol containing RBF under acidic condition (Ac-OH; 0.001 mol). It was stirred at room temperature for 2-5 hours. After completion of the reaction (monitored by TLC), reaction mixture was poured into ice cold water, filtered and dried using vacuum filtration apparatus. Purification of final molecules was carried out by column chromatography by using silica gel (60-120 mesh) in ethyl acetate: n-hexane **(8:2)** as a mobile phase. Identification and characterization of derived products were carried out by various spectroscopic techniques *i.e.*, IR, NMR, MS and elemental analysis.

2. 3. Analytical Data

3-((2,4-Dimethoxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]-thieno [2,3-d]pyrimidin-4(3*H*)-one. (4a)

This compound was obtained as off-white solid. Yield: 92%. mp 162-164 °C. IR (cm^{-1}): 3006 (C-H Stretching), 1677 (C=O Stretching), 1664 (C=N Stretching), 1226 (C-N Stretching), 1249 (C-O Alkyl aryl ether stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08-1.10 (d, 3H, *J* = 6.8 Hz, -CH_3 attached to -CH); 1.39-1.48 (m, 1H); 1.90-1.99 (m, 2H); 2.35-2.42 (m, 1H); 2.55 (s, 3H, -CH_3 attached to -C); 2.80-2.90 (m, 2H); 3.19-3.24 (m, 1H); 3.82 (s, 3H, -OCH_3); 3.83 (s, 3H, -OCH_3); 6.89-6.91 (t, 1H, *J* = 8.8 Hz, Ar-H); 7.07-7.10 (dd, 1H, *J* = 3.2 & 9.2 Hz, Ar-H); 7.68-7.69 (d, 1H, *J* = 3.2 Hz, Ar-H); 9.13 (s, 1H, -N=CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.50, 22.50, 25.32, 29.28, 30.57, 33.28, 55.87, 56.13, 76.73, 77.05, 77.37, 110.32, 112.67, 121.12, 121.19, 131.52, 132.41, 152.89, 153.55, 154.32, 155.38, 160.85, 164.50. Elemental analysis for $C_{21}H_{23}N_3O_3S$ Calculated: % C, 63.45; % H, 5.83; % N, 10.57; Found: % C, 63.41; % H, 5.80; % N, 10.55. Mass (m/z): 397 (M^+).

3-((2,3-Dimethoxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]-thieno [2,3-d]pyrimidin-4(3*H*)-one. (4b)

This compound was obtained as off-white solid. Yield: 90%. mp 154-156 °C. IR (cm^{-1}): 3021 (C-H Stretching), 1675 (C=O Stretching), 1660 (C=N Stretching), 1229 (C-N Stretching), 1251 (C-O Alkyl aryl ether stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09-1.11 (d, 3H, *J* = 6.4 Hz, -CH_3 attached to -CH); 1.42-1.47 (m, 1H); 1.91-1.99 (m, 2H); 2.35-2.42 (m, 1H); 2.58 (s, 3H, -CH_3 attached to -C); 2.80-2.89 (m, 2H); 3.17-3.23 (m, 1H); 3.96 (s, 6H, -OCH_3); 6.93-6.95 (d, 1H, *J* = 8.0 Hz, Ar-H); 7.31-7.34 (dd, 1H, *J* = 1.8 & 8.2 Hz, Ar-H); 7.57 (d, 1H, *J* = 2.0 Hz, Ar-H); 8.69 (s, 1H, -N=CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.50, 22.50, 25.32, 29.28, 30.57, 33.28, 55.87, 56.13, 76.73, 77.05, 77.37, 110.32, 112.67, 121.12, 121.19, 131.52, 132.41, 152.89, 153.55, 154.32, 155.38, 160.85, 164.50. Elemental analysis for $C_{21}H_{23}N_3O_3S$ Calculated: % C, 63.45; % H, 5.83; % N, 10.57; Found: C, 63.42; % H, 5.89; % N, 10.55. Mass (m/z): 397 (M^+).

2,7-Dimethyl-3-((2,3,4-trimethoxybenzylidene)amino)-5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-d]pyrimidin-4(3*H*)-one. (4c)

This compound was obtained as off-white solid. Yield: 81%. mp 184-186 °C. IR (cm^{-1}): 3010 (C-H Stretching), 1675 (C=O Stretching), 1669 (C=N Stretching), 1231 (C-N Stretching), 1253 (C-O Alkyl aryl ether stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09-1.11 (d, 3H, *J* = 6.4 Hz, -CH_3 attached to -CH); 1.40-1.50 (m, 1H); 1.91-1.99 (m, 2H); 2.35-2.43 (m, 1H); 2.58

(s, 3H, -CH_3 attached to -C); 2.81-2.90 (m, 2H); 3.17-3.23 (m, 1H); 3.94 (s, 6H, -OCH_3); 3.95 (s, 3H, -OCH_3); 7.14 (s, 2H, Ar-H); 8.76 (s, 1H, -N=CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.37, 155.82, 149.47, 149.31, 141.03, 135.78, 129.28, 124.59, 120.26, 119.99, 107.23, 60.65, 56.79, 35.00, 32.70, 31.22, 23.35. Elemental analysis for $C_{22}H_{25}N_3O_4S$ Calculated: % C, 61.81; % H, 5.89; % N, 9.83; Found: % C, 61.8; % H, 5.85; % N, 9.81. Mass (m/z): 427 (M^+).

3-((2,5-dichlorobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-d]pyrimidin-4(3*H*)-one. (4d)

This compound was obtained as off-white solid (0.33 g). Yield: 86%. mp 194-196 °C. IR (cm^{-1}): 3011 (C-H Stretching), 1681 (C=O Stretching), 1660 (C=N Stretching), 1232 (C-N Stretching), 1245 (C-O Alkyl aryl ether stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09-1.11 (d, 3H, J = 6.8 Hz, -CH_3 attached to -CH); 1.42-1.47 (m, 1H); 1.90-1.99 (m, 2H); 2.35-2.42 (m, 1H); 2.59 (s, 3H, -CH_3 attached to -C); 2.80-2.90 (m, 2H); 3.17-3.23 (m, 1H); 7.35-7.38 (dd, 1H, J = 2.0 & 8.6 Hz, Ar-H); 7.46-7.49 (d, 1H, J = 11.2 Hz, Ar-H); 8.14-8.17 (d, 1H, J = 8.4 Hz, Ar-H); 9.39 (s, 1H, -N=CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.37, 155.82, 149.73, 147.32, 135.78, 134.33, 133.57, 132.55, 131.48, 129.37, 129.28, 128.32, 119.99, 35.00, 32.70, 31.22, 23.35, 21.62, 20.61. Elemental analysis for $C_{19}H_{17}Cl_2N_3OS$ Calculated: % C, 56.16; % H, 4.22; % N, 10.34; Found: % C, 56.12; % H, 4.19; % N, 10.45. Mass (m/z): 406 (M^+).

3-((2-methoxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno-[2,3-d]pyrimidin-4(3*H*)-one. (4e)

This compound was obtained as off-white solid. Yield: 87%. mp 180-182 °C. IR (cm^{-1}): 3000 (C-H Stretching), 1674 (C=O Stretching), 1670 (C=N Stretching), 1225 (C-N Stretching), 1248 (C-O Alkyl aryl ether stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09-1.11 (d, 3H, *J* = 6.4 Hz, -CH_3 attached to -CH); 1.42-1.47 (m, 1H); 1.91-1.98 (m, 2H); 2.35-2.42 (m, 1H); 2.59 (s, 3H, -CH_3 attached to -C); 2.81-2.88 (m, 2H); 3.17-3.23 (m, 1H); 3.87 (s, 3H, -OCH_3); 7.09-7.12 (m, 1H, Ar-H); 7.39-7.41 (m, 2H, Ar-H); 7.48 (Broad, 1H, Ar-H); 8.87 (s, 1H, -N=CH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 21.49, 22.56, 25.32, 29.26, 30.54, 33.27, 55.44, 122.29, 119.16, 121.12, 122.33, 129.94, 131.52, 132.70, 133.90, 153.07, 155.53, 159.92, 160.85, 167.22. Elemental analysis for $C_{20}H_{21}N_3O_2S$ Calculated: % C, 65.37; % H, 5.76; % N, 11.44; Found: % C, 65.32; % H, 5.77; % N, 11.39. Mass (m/z): 367 (M^+).

3-((2-Bromobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno-[2,3-*d*] pyrimidin-4(3*H*)-one. (4f)

This compound was obtained as white solid. Yield: 83%. mp 158-160 °C. IR (cm^{-1}): 3003 (C-H Stretching), 1676 (C=O Stretching), 1661 (C=N Stretching), 1230 (C-N Stretching), 692 (C-Br Alkyl aryl ether stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09-1.11 (d, 3H, *J* = 6.8 Hz, -CH_3 attached to -CH); 1.41-1.48 (m, 1H); 1.91-1.99 (m, 2H); 2.35-2.41 (m, 1H); 2.60 (s, 3H, -CH_3 attached to -C); 2.80-2.89 (m, 2H); 3.16-3.21 (m, 1H); 7.34-7.38 (t, 1H, *J* = 8.0 Hz, Ar-H); 7.65-7.68 (m, 1H, Ar-H); 7.74-7.77 (d, 1H, *J* = 8.0 Hz, Ar-H); 8.07-8.08 (t, 1H, *J* = 2.0 Hz, Ar-H); 8.96 (s, 1H, -N=CH). ^{13}C NMR (100 MHz, $CDCl_3$): 21.48, 22.65, 25.31, 29.25, 30.51, 33.26, 121.09, 123.13, 127.82, 130.43, 131.09, 131.54, 132.90, 134.73, 135.25, 153.14, 155.55, 160.81, 164.94. Elemental analysis for $C_{19}H_{18}BrN_3OS$ Calculated: % C, 54.81; % H, 4.36; % N, 10.09; Found: % C, 54.83; % H, 4.30; % N, 10.03. Mass (m/z): 415 (M^+).

2,7-dimethyl-3-((2-methylbenzylidene)amino)-5,6,7,8-tetrahydrobenzo[4,5]thieno-[2,3-d]pyrimidin-4(3*H*)-one. (4g)

This compound was obtained as white solid. Yield: 89%. mp 184-186 °C. IR (cm^{-1}): 3011 (C-H Stretching), 1680 (C=O Stretching), 1669 (C=N Stretching), 1236 (C-N Stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.07-1.09 (d, 3H, *J* = 6.4 Hz, -CH_3 attached to -CH); 1.52-1.56 (m, 1H); 1.90-1.96 (m, 2H); 2.40-2.49 (m, 1H); 3.01 (s, 3H, -CH_3 attached to -C); 2.80-2.84 (m, 2H); 3.15-3.20 (m, 1H); 3.93 (s, 3H, -CH_3 attached to Aromatic ring); 7.19-7.22 (m, 1H, Ar-H); 7.49-7.55 (m, 2H, Ar-H); 7.69 (Broad, 1H, Ar-H); 8.91 (s, 1H, -N=CH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 21.49, 22.56, 25.32, 29.26, 30.54, 33.27, 55.44, 122.29, 119.16, 121.12, 122.33, 129.94, 131.52, 132.70, 133.90, 153.07, 155.53, 159.92, 160.85, 167.22. Elemental analysis for $C_{20}H_{21}N_3OS$ Calculated: % C, 68.35; % H, 6.02; % N, 11.96; Found: % C, 68.31; % H, 6.05; % N, 11.88. Mass (m/z): 351 (M^+).

3-((3-hydroxy-4-ethoxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo-[4,5] thieno[2,3-d]pyrimidin-4(3*H*)-one. (4h)

This compound was obtained as off white solid. Yield: 93%. mp 192-194 °C. IR (cm^{-1}): 3410 (-OH Stretching), 3008 (C-H Stretching), 1682 (C=O Stretching), 1669 (C=N Stretching), 1236 (C-N Stretching), 1239 (C-O Alkyl aryl ether stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08-1.10 (d, 3H, *J* = 6.8 Hz, -CH_3 attached to -CH); 1.42-1.45 (m, 1H); 1.46-1.50 (t, 3H, *J* = 7.0 Hz, -CH_3 attached to -OCH_2); 1.90-1.97 (m, 2H); 2.05-2.41 (m, 1H); 2.56 (s, 3H, -CH_3 attached to -C); 2.80-2.89 (m, 2H); 3.17-3.22 (m, 1H); 4.16-4.22 (q, 2H, *J* = 7.0 & 13.8 Hz, Ar-H); 6.31 (Broad, 1H, -OH); 6.98-7.00 (d, 1H, *J* = 8.4 Hz, Ar-H); 7.25-7.28 (m, 1H, Ar-H); 7.52-7.53 (d, 1H, *J* = 1.6 Hz, Ar-H); 8.63 (s, 1H, -N=CH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.77, 21.51, 22.48, 25.31, 29.27, 30.55, 33.27, 64.72, 109.6, 114.49, 121.08, 124.74, 125.4, 131.44, 132.57, 146.34, 150.22, 152.87, 155.57, 160.86, 168.02. Elemental analysis for $C_{21}H_{23}N_3O_3S$ Calculated: % C, 63.45; % H, 5.83; % N, 10.57; Found: % C, 63.39; % H, 5.8; % N, 10.51. Mass (m/z): 397 (M^+).

2,7-dimethyl-3-((4-methylbenzylidene)amino)-5,6,7,8-tetrahydrobenzo[4,5]thieno-[2,3-d] pyrimidin-4(3*H*)-one. (4i)

This compound was obtained as white solid. Yield: 89%. mp 184-186 °C. IR (cm^{-1}): 3012 (C-H Stretching), 1680 (C=O Stretching), 1667 (C=N Stretching), 1229 (C-N Stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.06-1.08 (d, 3H, *J* = 6.4 Hz, -CH_3 attached to -CH); 1.44-1.51 (m, 1H); 1.91-1.95 (m, 2H); 2.37-2.41 (m, 1H); 3.05 (s, 3H, -CH_3 attached to -C); 2.71-2.79 (m, 2H); 3.19-3.22 (m, 1H); 3.90 (s, 3H, -CH_3 attached to Aromatic ring); 7.27-7.32 (m, 1H, Ar-H); 7.40-7.44 (m, 2H, Ar-H); 7.60 (Broad, 1H, Ar-H); 8.82 (s, 1H, -N=CH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 21.49, 22.56, 25.32, 29.26, 30.54, 33.27, 55.44, 122.29, 119.16, 121.12, 122.33, 129.94, 131.52, 132.70, 133.90, 153.07, 155.53, 159.92, 160.85, 167.22. Elemental analysis for $C_{20}H_{21}N_3OS$ Calculated: % C, 68.35; % H, 6.02; % N, 11.96; Found: % C, 68.31; % H, 6.05; % N, 11.88. Mass (m/z): 351 (M^+).

3-((4-Chlorobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno-[2,3-*d*] pyrimidin-4(3*H*)-one. (4j)

This compound was obtained as white solid. Yield: 88%. mp 158-160 °C. IR (cm^{-1}): 3009 (C-H Stretching), 1670 (C=O Stretching), 1669 (C=N Stretching), 1230 (C-N Stretching), 842 (C-Cl Stretching). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09-1.11 (d, 3H, *J* = 6.8 Hz, -CH_3 attached

to -CH); 1.41-1.48 (m, 1H); 1.91-1.99 (m, 2H); 2.35-2.41 (m, 1H); 2.60 (s, 3H, -CH_3 attached to -C); 2.80-2.89 (m, 2H); 3.16-3.21 (m, 1H); 7.34-7.38 (t, 1H, *J* = 8.0 Hz, Ar-H); 7.65-7.68 (m, 1H, Ar-H); 7.74-7.77 (d, 1H, *J* = 8.0 Hz, Ar-H); 8.07-8.08 (t, 1H, *J* = 2.0 Hz, Ar-H); 8.96 (s, 1H, -N=CH). ^{13}C NMR (100 MHz, $CDCl_3$): 21.48, 22.65, 25.31, 29.25, 30.51, 33.26, 121.09, 123.13, 127.82, 130.43, 131.09, 131.54, 132.90, 134.73, 135.25, 153.14, 155.55, 160.81, 164.94. Elemental analysis for $C_{19}H_{18}ClN_3OS$ Calculated: % C, 54.81; % H, 4.36; % N, 10.09; Found: % C, 54.83; % H, 4.30; % N, 10.03. Mass (m/z): 371 (M^+).

2. 4. Place of study and screening criteria

Antibacterial evaluation was carried out at the Department of Microbiology, RK University, Rajkot. The primary screen was done of 10 compounds at a various concentration level using duplicate measurements. Out of screened compounds all compounds show moderate to average activity. None of the compounds show higher activity as compared to the standard drugs. Confirmation of the activity was done by dose response assay (MIC) against the affected organism using five various strains *i.e.,* Gram-positive *(S. aureus, S. pyogenes,* MRSA- *multi-resistance S. aureus)* and Gram negative *(E. coli, P. aeruginosa, Klebsiella pneumonia)* bacteria, using ampicillin, ciprofloxacin, chloramphenicol, nystatin, cephazoline and itraconazole as the reference antibacterial agent. Antifungal activity was carried out using selected fungal strain *(C. albicans, A. niger).* Results were expressed in minimum inhibition concentration (MIC). All the newly synthesized compounds were also evaluated against Gram –Ve and Gram +Ve strains at two different concentrations (10 µg/ml and 5 µg/ml) as a zone of inhibition. For zone of inhibition cefazolin and ampicillin were used as a control [9].

2. 5. Protocol for MIC determination

Different pathogenic bacteria like *E.coli*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus aureus* and *Klebsiella pnemoniea* for MIC tests were procured from clinical laboratories and the cultures were maintained by repeated transfers in N-Agar slants. Minimum inhibitory concentration of assayed compound was determined by broth dilution method, under defined test conditions, and inhibits the visible growth of the bacterium being investigated. The susceptibilities of assayed compounds towards bacteria were determined by measurement of MIC and also to evaluate the activity of new antimicrobial agents. For broth dilution, Bacteria (3×108 cells/ml) were inoculated into Nutrient broth medium containing of different concentrations of the compound (2, 4, 6, 8, 10, 20, 40 µg/ml). Growth was assessed after incubation for 24 h and the MIC value was assessed by observation in the absence of visual turbidity. Protocol used for zone of inhibition measurements is described in our previously published paper [9-11].

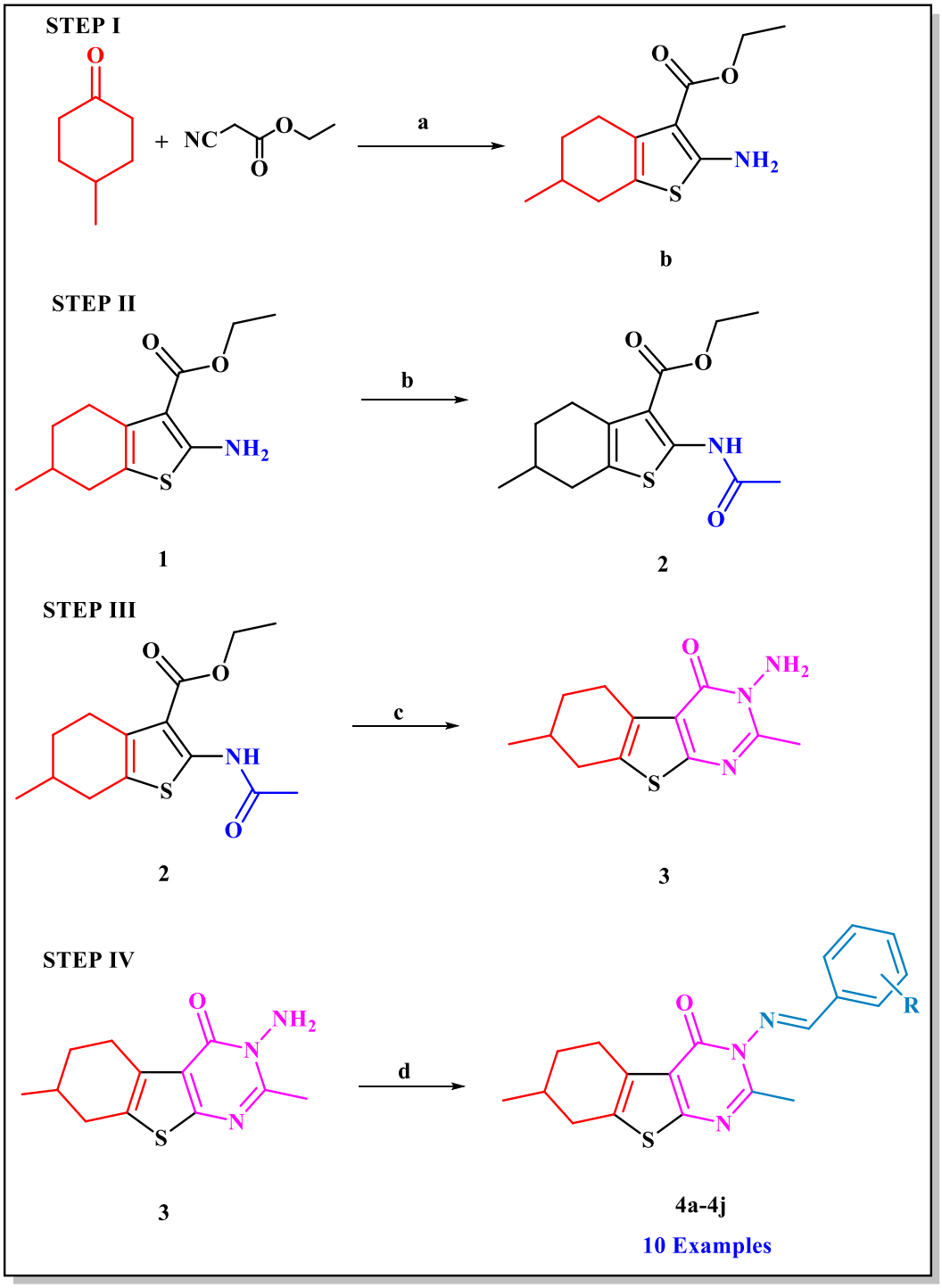
3. RESULTS AND DISCUSSION

3. 1. Chemistry

Intermediate 1 was synthesized by the well-known Gewald reaction between 4-methylcyclohexane, ethylcyanoacetate and sulphur powder. However there are so many methods for the synthesis of Intermediate 1, among them, this method gave very good yield. For effective progress of reaction was carried out by using different weak organic bases like

triethylamine, pyridine, morpholine and piperidine. **Scheme 1.** Shows the reaction scheme for the synthesis of target compounds using 4 steps.

Scheme 1. Reaction and Conditions: (a) Sulfur, Morpholine, RT, 10 Hrs; **(b)** Acetic Anhydride, Reflux, 3 Hrs; **(c)** NH_2NH_2, Ethanol, Reflux, 10 Hrs; **(d)** Various phenyl aldehyde, Acetic Acid, Methanol, RT, 2-5 Hrs.



Reaction Scheme 1: Synthesis of 3-((Substitutedbenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydro benzo[4,5]-thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(4a-4j)**.

R =	**4a** = 2,4-Dimethoxy	**4f** = 2-Bromo
	4b = 2,3-Dimethoxy	**4g** = 2- Methyl
	4c = 2,3,4-Trimethoxy	**4h** = 3-Hydroxy-4-ethoxy
	4d = 2,5-Dichloro	**4i** = 4-Methyl
	4e = 2-Methoxy	**4j** = 4-Chloro

In this short communication, we have tried both conventional heating as well as microwave assisted synthesis for the production of Intermediate 1. By comparing these two routes of synthesis, we came to conclude that the microwave assisted synthesis was taken more time for the completion of reaction and yield was minute in quantity as compared to conventional heating. The acetylation reaction of Intermediate 1 using acetic anhydride in MDC solvent gave analytically pure **Intermediate 2** under reflux conditions. **Intermediate 3** was synthesized by the reaction between Intermediate 2 and hydrazine hydrate in reflux reaction condition in polar protic solvent (Ethanol).

Target compounds **(4a-4j)** were obtained by the reaction between intermediate 3 and different aromatic aldehydes (Schiff's base formation) under acetic acid media (Notice that under concentrated H_2SO_4 media, it degrades the product). The reaction optimization using various aliphatic aldehydes showed lots of other impurities along with the minute percentage of target scaffolds. The equimolar mixture of ethyl acetate and hexane gave good crystals with single spot in TLC (column eluent) and showed neat NMR spectra.

3. 2. Spectroscopic Analysis

A characteristic band in IR spectra of cyclic amide showed strong peak in region ~1650 cm^{-1} and various substituents present in aromatic ring confirms the formation of final adducts. These assignments are in agreement with the predicted scaffold.

DMSO-d_6 was used to perform the ^1H NMR spectra of intermediate steps and $CDCl_3$ was used to carry out final product NMR (^1H and ^{13}C). The final product exhibited specific peak for the arylidene proton at ~8.20 δppm. The aromatic methyl group (~2.7 δppm) and cyclic aliphatic methyl (~1.20 δppm) were clearly distinguished based on their deshielding and shielding effect respectively. The aromatic ring carbons are also in covenant with the theoretical values. The mass spectra of corresponding 3-((Substitutedbenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-*d*]pyrimidin-4(3*H*)-one show their molecular formula weight and found to be in well agreement with the literature.

3. 3. Antimicrobial Evaluation

Activity Assay

From the result of biological evaluation, some of the compounds tested were found to have moderate antibacterial and antifungal activity. Remaining most of the compounds gives higher activity. From the MIC values of compounds (**Table 1)**, it is observed that compounds **4a, 4b,**

4e and **4f** are active against gram +ve bacterial strain. Compounds **4a, 4d, 4e, 4f,** and **4i** are remarkably active against gram –ve bacterial strain. Most of the compounds are active against anti-fungal strain except **4c, 4g** and **4h** due to function group effects. By observing zone of inhibition (**Table 2)**, it is clear that at low concentration compounds are active and high concentration results are variable compared to standard drugs. At low concentration compounds **4f, 4g** and **4j** gives better results against gram +ve bacterial strain. It is interesting to note that most of the compounds are inactive against anti-fungal strain in a zone of inhibition.

Table 1. Antimicrobial and antifungal screening of compounds as a MIC **(4a-4j)**.

	Antibacterial Activity				**Anti-Fungal Activity**	
	Minimum inhibitory concentration µg/ml				**Minimum inhibitory concentration µg/ml**	
	Gram +Ve Bacteria		**Gram -Ve Bacteria**			
	S. aureus	***S. pyogenes***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
Ciprofloxacin	7.8	7.8	15.62	15.62	-	-
Chloramphenicol	7.8	7.8	7.8	7.8	-	-
Nystatin	-	-	-	-	31.25	31.25
Itraconazole	-	-	-	-	15.62	15.62
4a	**9.7**	11.22	**15.64**	**15.60**	**16.62**	**31.60**
4b	**8.00**	**9.01**	31.50	61.50	**16.52**	72.25
4c	16.52	62.65	31.22	31.50	45.67	65.50
4d	15.84	19.58	**15.80**	65.25	**32.10**	62.72
4e	**7.81**	**9.25**	**7.81**	62.50	**32.25**	**32.75**
4f	**8.81**	11.25	**15.80**	**15.75**	**16.30**	**16.25**
4g	31.00	**7.81**	16.80	31.10	68.80	75.25
4h	15.90	51.63	31.10	60.00	65.50	75.25
4i	15.92	15.50	**15.82**	16.25	**32.25**	**32.50**
4j	30.25	62.55	16.00	**7.95**	**31.50**	**32.75**

Table 2. Antimicrobial screening of compounds as a Zone of inhibition **(4a-4j)**.

	E. coli **Gram -Ve**		***Klebsila*** **Gram -Ve**		***Streptococcus*** **Gram +Ve**		***Staphylococcus*** **Gram +Ve**	
	Cefazolin				**Ampicillin**			
Low Con.	9		15		17		15	
High Con.	10		19		20		19	
4a	13	14	10	12	11	12	0	0
4a	15	16	11	12	9	11	0	0
4b	12	15	12	12	7	11	0	0
4b	12	14	11	13	9	10	0	0
4c	14	14	11	11	8	10	0	0
4c	12	13	11	13	9	11	0	0
4d	12	14	11	11	9	12	0	0
4d	14	14	11	14	9	10	0	0
4e	14	14	11	11	8	10	0	0
4e	12	13	11	13	9	11	0	0
4f	**10**	**11**	**16**	**16**	10	10	0	0
4f	12	14	11	12	8	10	0	0
4g	**11**	**10**	**17**	**18**	9	11	0	0
4g	13	14	11	11	7	8	0	0
4h	13	13	12	12	10	10	0	0
4h	13	12	10	13	8	7	0	0
4i	12	13	13	11	9	10	0	0
4i	13	13	11	13	9	9	0	0
4j	**11**	**11**	**15**	**16**	9	11	0	0
4j	13	14	11	11	7	8	0	0

4. CONCLUSIONS

In this part, we have successfully synthesized and purified the small and diverse **3-(benzylideneamino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*) -one derivatives (4a-4j)**. It was identified using various analytical techniques like ^{1}H NMR, ^{13}C NMR, Mass spectrometry and elemental analysis. The final adduct were isolated in four steps of reaction using readily available material and reagents. To follow the green chemistry approach and for the economical viable route of synthesis, we have tried the reaction in various reaction condition and the reaction was optimization using different weak organic base, different solvents, different temperature, etc. It was observed that electron donating and halogens were shown great influence to enhance the anti-microbial activity, and out of ten synthesized compounds seven were shown best agreement with the standard drugs.

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