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Synthesis, Characterization, *In-vitro* and *In-silico* Studies of 5,5-dimethyl-3-(phenylamino)-cyclohex-2-enone

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Abstract: The present study reveals that the greener synthesis, *in-silico*, and *in-vitro* studies of 5,5-dimethyl-3-(phenylamino)-cyclohex-2-enone (DMPC) derivatives. The solvent-free method is one of the most adorable methods for current environmental situations. It is one of the safe and ecological methods to synthesize cyclohex-2-enone compounds in the presence of sodium bisulfate used as a catalyst. It is highly economical. The synthesized cyclohexenone compounds are structure elucidated with the help of IR, ^{1}H and ^{13}C NMR values. All the DMPC (1-5) compounds have a good number of H-bond interactions with notable interaction scores. DMPC-1 has one H-bond interaction with an interaction score -6.7 kJ/mol. DMPC-4 has four H-bond interactions and two hydrophobic interactions with an interaction score -7.5 kJ/mol. DMPC-5 also has -7.5 kJ/mol as an interaction score with three H-bond interactions and one hydrophobic interaction. Only the compound DMPC-4 has a good binding score with 1JIJ. The binding score value is -8.4kJ/mol; this is the only value nearer to the standard drug binding score than other compounds. All the compounds have good interaction with 1UAG bacterial protein than interact with 1JIJ bacterial protein. This interaction study revealed that the DMPC (1-5) compounds follow the cell wall synthesis mechanism when treated with bacterial strains. The catalyst sodium bisulfate is a re-useable one for the synthesis process.

Keywords: cyclohex-2-enone; microwave; auto dock irradiation.

1. Introduction

Present decade, Microwave irradiation methods are mostly followed to reduce the hazards and production of greenhouse gases during organic synthesis [1]. The most important source for heating purposes is the microwave oven [2]. The microwaves are used to synthesize organic compounds with reduced reaction time and improve in the yield, selectiveness process [3]. The organic synthesis is done by microwave, also known as an environmentally friendly process, with cleanliness associated solvent and wastes free process [4]. The many enamines are synthesized by using the microwave irradiated method [5-7].

The inherent structure features of dimethyl substituted cyclohexanone form several reactive centers in its ring; the reactive positions are C-1, C-2, and less extent to C-6, C-3. β-enamines is one of the best class of enamines with high stability [8]. Enamines are mostly stable because of its intermolecular H-bond formation. The enamines form a N-C=C-C=O conjugation, which has high reactivity, enamines used as reactive intermediates in much synthetic organic chemistry to prepare many heterocyclic compounds [9-12]. The β-enamine also has a wide range of applications in various fields [13-15]. β-enamines has a significant

pharmacological activity and exhibit mainly anticonvulsant activity [16-17]. Two methods simply synthesize these biological important and valuable β-enamines; one is the direct conventional method between the amine and 1,3-diketone with water removal by refluxing. The second method is transformation completed using activators such as microwave irradiation and other homogenous, heterogeneous catalysis [18-20]. β-enamines are effective intermediate on the synthesis of pharmaceuticals, amino acids, peptides, and alkaloids [21-22]. β-enamines are effectively synthesized by using n-number of catalyst.

In-silico studies are more economical and efficient methods to predict the pharmacokinetic properties of designed small organic moieties [23]. The combination of metabolics and transcriptomics information is simplified to identify the designed compounds' metabolic pathways by data processing method [24]. The molecular transfusion is one of the best methods to design and developed new compounds with improved affinity and potency compared with previous parent compounds [25]. In order to predict a safer and efficient drug candidate for designed organic molecules, the ADMET profile was raised too early stage estimation parameters [26]. Hence in-silico study helps one or more molecules with quick, predictive models access pharmacokinetics, physicochemical, and other drug-like parameters [27].Now, here shows that the synthesis of β-enamine via a microwave-assisted process using sodium bisulfate silicate is a catalyst. The present article also explains the structural elucidation, docking studies, ADME property, and *in-vitro* bacterial activity of the above-mentioned compounds.

2. Materials and Methods

2.1. Instruments.

The organic inhibitor's skeleton structure was confirmed by using Perkin-Elmer 1650 spectrometer and Bruker AMX 400 NMR spectrometer. The Perkin-Elmer 1650 was used for IR studies with the absorption unit cm^{-1}. Bruker AMX instrument was used to predict and carry out the ^{1}H and ^{13}C NMR values of the DMPC compounds with the unit ppm, and the standard was TMS, $CDCl_3$, as a solvent. The single-mode microwave reactor was used for synthesis purposes under control pressure, temperature, and power. Maspec MSW 9629 spectrometer was used for obtaining the mass spectra value of the inhibitor.

2.2. Procedure for synthesis of DMPC compounds.

The chemical p-nitro aniline (1mmol) was taken in the Erlenmeyer flask 150 ml. The weighed 1mmol of dicarbonyl compound was added in the Erlenmeyer flask. It was introduced in a domestic microwave oven (godrej) and irradiated for 3-10 min (260 W).

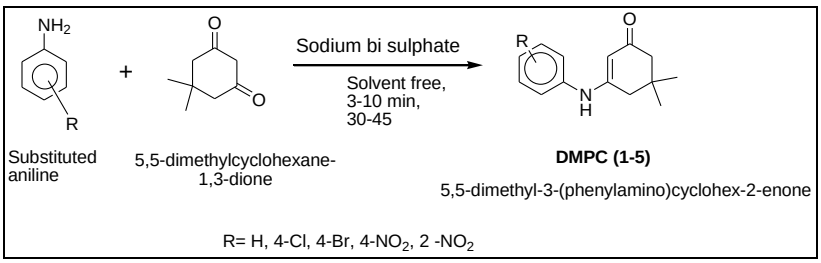


Figure 1. The microwave-assisted pathway of 5,5-dimethyl-3-(phenylamino)cyclohex-2-enones.

The oven was turned off always after 3-minutes periods of heating to avoid evaporation of the reagents. The reaction was monitored by TLC, and after completion, the flask cooled, and the products were removed from the flask either by scratching or with a pipette and weighed for yields determination.

2.3.Molecular docking.

The Auto dock 4.2.5.1 version program was used for the molecular docking studies of synthesized DMPC derivatives. The given literary method was followed to find the docking scores [28].

2.4. ADME.

The DMPC compound structure was subjected to absorption, distribution, metabolism, and excretion (ADME) study using the Swissadme online tool. The tool has the basic information about solubility (S), log P, polar surface area (TPSA), Hydrogen bond acceptor (Hd. Ac.), Hydrogen bond donor (Hd. Dn.), and other some of the basic information. The above parameters help understand the ADME property of any drugs or organic molecules. The compound has a drug property, which means it must obey the rule of five described by Lipinski. The Lipinski rules are: the compound must have Molecular weight ≤ 500, Hydrogen bond acceptor≤ 10, hydrogen bond donor ≤ 5, log p ≤ 5, and molar refractivity≤140. The other most important properties of the compound are that they have polar surface area range between 7to200, S range above -4, the drug score value above 0.5, and drug-likeness score as in positive values for synthesized organic compounds [29,30]. The percentage of absorption calculating by the known formula [31,32],

$$\%Abs = 109-0.345*TPSA$$

2.5. Antimicrobial screening test.

The disk diffusion method is used to carry out the antimicrobial studies of target DMPC compounds. Sterilized inoculums and sterile swabs were used. One negative and one positive strain were used for anti-bacterial screening. Ciprofloxacin was used as standard drugs in the microbial studies. Other steps were adopted from reference [33].

3. Results and Discussion

3.1. Structural confirmation of the organic inhibitor.

The DMPC target compounds were synthesized using Schiff base reaction between substituted aniline and 5,5- dimethyl substituted cyclohexan-1,3-dione via microwave irradiation. The microwave method is one of the best and eco-friendly to synthesize this type of organic inhibitors. It is also a time-saving, economical method. The skeleton structures were confirmed by using elemental analysis and spectral data's which were given below.

DMPC-1 (5,5-dimethyl-3-(phenylamino)cyclohex-2-enone): Yield 70%, M.P 165ºC, Molecular Formula $C_{14}H_{17}NO$; %Calcd. (Found); C%= 78.00 (78.10), H% = 7.93 (7.96), N% = 6.50 (6.51), O%= 7.36 (7.43). M/z (M) +:215.3; FT-IR (KBr, cm^{-1}): 3042.09 cm-1(Aromatic CH stretching), 2889.54cm^{-1} (Aliphatic CH stretching), 1656.67 cm^{-1}(Amide C=O stretching), 1418.05 cm^{-1}(C=N Stretching), 645.15, 735.42, 784.42 cm^{-1}(Aromatic ring stretching); ^{1}H NMR ($CDCl_3$, δ ppm, 400 MHz) 1.02-1.11 (m, 6H of CH_3), 1.88-2.86 (m, 4H of methylene in cyclohexanone), 3.62 (S, 1H of NH), 5.54 (d, 1H of C-2 of cyclohexanone), 6.48-

7.15(Aromatic protons); ^{13}C NMR ($CDCl_3$, δ ppm,100 MHz): 198.93 (C=O), 27.21 (methyl group in cyclohexanone ring), 99.08 (C-2 of cyclohexanone), 162.35 (C-3 of cyclohexanone), 43.08 (C-4 of cyclohexanone), 31.59 (C-5 of cyclohexanone), 52.73 (C-6 of cyclohexanone),122.56-144.08 (Aromatic carbons).

DMPC-2 (5,5-dimethyl-3-(4-chlorophenylamino)cyclohex-2-enone): Yield 72%, M.P 278ºC, Molecular Formula $C_{14}H_{16}NOCl$; %Calcd. (Found); C%= 67.35 (67.33), H% = 6.41 (6.46), N% = 5.61 (5.61) O%= 6.41 (6.41), Cl% = 14.21 (14.20); FT-IR (KBr, cm^{-1}): 3076.23 cm^{-1}(Aromatic CH stretching), 2888.45cm^{-1} (Aliphatic CH stretching), 1652.25 cm^{-1}(Amide C=O stretching), 1412.51 cm^{-1}(C=N Stretching), 654.15, 710.54, 783.91 cm^{-1}(Aromatic ring stretching); ^{1}H NMR ($CDCl_3$, δ ppm, 400 MHz) 1.03-1.25 (m, 6H of CH_3), 2.14-2.84 (m, 4H of methylene in cyclohexanone), 3.62 (S, 1H of NH), 5.68 (d, 1H of C-2 of cyclohexanone), 6.45-7.87(Aromatic protons); ^{13}C NMR ($CDCl_3$, δ ppm,100 MHz): 198.82 (C=O), 28.42 (methyl group in cyclohexanone ring), 97.43 (C-2 of cyclohexanone), 162.14 (C-3 of cyclohexanone), 43.48 (C-4 of cyclohexanone), 32.95 (C-5 of cyclohexanone), 51.25 (C-6 of cyclohexanone),117.71-142.53 (Aromatic carbons).

DMPC-3 (5,5-dimethyl-3-(4-bromophenylamino)cyclohex-2-enone): Yield 71%, M.P 238ºC, Molecular Formula $C_{14}H_{16}NOBr$; %Calcd. (Found); C%= 57.18 (57.16), H% = 5.38 (5.48), N% = 4.78 (4.76) O%= 5.42 (5.44), Br%= 27.14 (27.16). M/z (M) +:350,352; FT-IR (KBr, cm^{-1}): 3066.11 cm^{-1}(Aromatic CH stretching), 2885.51cm^{-1} (Aliphatic CH stretching), 1658.78 cm^{-1}(Amide C=O stretching), 1417.68 cm^{-1}(C=N Stretching), 642.3, 709.8, 763.81 cm^{-1}(Aromatic ring stretching); ^{1}H NMR ($CDCl_3$, δ ppm, 400 MHz) 1.06-1.48 (m, 6H of CH_3), 2.18-2.58 (m, 4H of methylene in cyclohexanone), 3.12 (S, 1H of NH), 5.76 (d, 1H of C-2 of cyclohexanone), 6.97-7.53(Aromatic protons); ^{13}C NMR ($CDCl_3$, δ ppm,100 MHz): 198.52 (C=O), 28.61 (methyl group in cyclohexanone ring), 97.56 (C-2 of cyclohexanone), 162.84 (C-3 of cyclohexanone), 43.38 (C-4 of cyclohexanone), 33.09 (C-5 of cyclohexanone), 50.73 (C-6 of cyclohexanone),124.28-138.93 (Aromatic carbons).

DMPC-4 (5,5-dimethyl-3-(4-nitrophenylamino)cyclohex-2-enone): Yield 78%, M.P 284ºC, Molecular Formula $C_{14}H_{16}N_2O_3$; %Calcd. (Found); C%= 64.42 (64.60), H% =6.28 (6.20), N% = 10.80 (10.76) O%= 18.39 (18.44). M/z (M) +: 260.12; FT-IR (KBr, cm^{-1}): 3065.23 cm^{-1}(Aromatic CH stretching), 2881.74cm^{-1} (Aliphatic CH stretching), 1657.15 cm^{-1}(Amide C=O stretching), 1417.88 cm^{-1}(C=N Stretching), 652.30, 712.57, 788.99 cm^{-1}(Aromatic ring stretching); ^{1}H NMR ($CDCl_3$, δ ppm, 400 MHz) 1.04-1.50 (m, 6H of CH_3), 2.14-2.64 (m, 4H of methylene in cyclohexanone), 3.09 (S, 1H of NH), 5.48 (d, 1H of C-2 of cyclohexanone), 6.72-7.94 (Aromatic protons); ^{13}C NMR ($CDCl_3$, δ ppm,100 MHz): 198.95 (C=O), 29.05 (methyl group in cyclohexanone ring), 98.18 (C-2 of cyclohexanone), 162.23 (C-3 of cyclohexanone), 43.14 (C-4 of cyclohexanone), 32.89 (C-5 of cyclohexanone), 50.83 (C-6 of cyclohexanone), 121.92-146.88 (Aromatic carbons).

DMPC-5 (5,5-dimethyl-3-(2-nitrophenylamino)cyclohex-2-enone): Yield 76%, M.P 283ºC, Molecular Formula $C_{14}H_{16}N_2O_3$; %Calcd. (Found); C%= 64.48 (64.60), H% = 6.28 (6.20), N% = 10.78 (10.76) O%= 18.40 (18.44); FT-IR (KBr, cm^{-1}): 3065.47 cm^{-1}(Aromatic CH stretching), 2882.12 cm^{-1} (Aliphatic CH stretching), 1657.85 cm^{-1}(Amide C=O stretching), 1417.56 cm^{-1}(C=N Stretching), 653.03, 713.61, 785.96 cm^{-1}(Aromatic ring stretching); ^{1}H NMR ($CDCl_3$, δ ppm, 400 MHz) 1.05-1.56 (m, 6H of CH_3), 2.09-2.46 (m, 4H of methylene in cyclohexanone), 3.11 (S, 1H of NH), 5.45 (d, 1H of C-2 of cyclohexanone), 6.74-7.96 (Aromatic protons); ^{13}C NMR ($CDCl_3$, δ ppm,100 MHz): 198.91 (C=O), 29.12 (methyl group in cyclohexanone ring), 98.16 (C-2 of cyclohexanone), 162.24 (C-3 of cyclohexanone), 43.18

(C-4 of cyclohexanone), 32.91 (C-5 of cyclohexanone), 50.85 (C-6 of cyclohexanone),121.98-145.93 (Aromatic carbons).

3.2. Molecular docking studies.

The molecular docking studies of the synthesized compounds are done using the software Auto dock tool. The low value of B.S. (Binding Score) shows excellent activity against bacterial strains. All the DMPC compounds subjected to 1UAG (UDP-N-ACETYLMURAMOYL-L_ALANINE:D-GLUTAMATE LIGASE) MurD Protein, which is involved in the cell wall mechanism derived from *E. coli* (gram-negative) strain and 1JIJ (crystal structure of *S. aureus* complex with SB-239629) protein derived from *S. aureus* (gram-positive) bacterial strain.

3.3. 1UAG.

DMPC compounds showed notable binding scores compared with standard. The compounds DMPC-4,5 showed B.S. -7.5 kJ/mol, the nearer interaction score with standard drug. Other than DMPC-1 has a good B.S. value -6.8kJ/mol. All the compounds have significant hydrogen bond interactions and hydrophobic interaction. The compound DMPC-4 has four hydrogen bond interactions with the amino acid residues TYR A: 180, TYR A: 36, LYS A: 84, and ASP A: 40. The compounds DMPC-3, 5 have three hydrogen bond interactions and one hydrophobic interaction. This docking result exhibit that the DMPC compounds may follow the cell wall mechanism on bacterial strains. The docking results of DMPC compounds with 1UAG bacterial protein given in Table 1.

Table 1. The docking results of DMPC compounds with 1UAG bacterial protein.

Compound	B.S	HBI-bond length	HBI	HPI- bond length	HPI
DMPC-1	-6.8	2.29	SER A: 264	5.11	HIS A:267
DMPC-2	-6.2	2.93, 2.22	GLY A:332, LEU A: 330	4.39, 3.46, 6.01	VAL A:335, LEU A: 339
DMPC-3	-6.4	2.00, 2.10, 2.86	LEU A: 416, SER A: 415	5.38	PHE A:422
DMPC-4	-7.5	2.44,2.69, 2.70,2.47, 2.07	TYR A: 170, TYR A: 36 LYS A:84 ASP A:40	4.48, 4.43, 3.93	HIS A: 50 ALA A: 39
DMPC-5	-7.5	2.84, 2.15, 3.03	TYR A:36, GLN A: 196 GLN A: 174	4.13	LEU A: 70
Ciprofloxacin	-7.7	2.05 2.47 2.65	ASN A: 178 ASN A: 271 GLU A: 327	4.12	ALA A: 328

3.4. 1JIJ.

The compounds docked with 1JIJ showed that all the compounds have fewer B.S. values than the standard drug. Only the compound DMPC-4 has the B.S. value -8.4 kJ/mol and four hydrogen bond interactions and two hydrophobic interactions. Other than the compound, DMPC-5 shows a good number of hydrogen bond interactions than the standard one. The docking result of DMPC compounds with 1JIJ showed that the DMPC compounds did not follow the mechanism of 1JIJ bacterial strain. The compounds literally follow the mechanism of 1JIJ; that's why the compounds have a less binding score with 1JIJ.

3.5. ADME.

All the target compounds DMPC has good ADME prediction values. The bio-availability score of all the compounds is 0.55.

Mostly all the target compounds in-silico ADME values are satisfy the Lipinski rule of five. All the compounds should obey the Lipinski rule with 0 violations. The polar surface area value is high for DMPC-4 and DMPC-5 compounds rather than others in the series. The target compounds have solubility values in the range of -3 to -4. All the compounds have the best absorption % value. All the compounds exhibit acceptable ADME values, which are tabulated in Table 3.

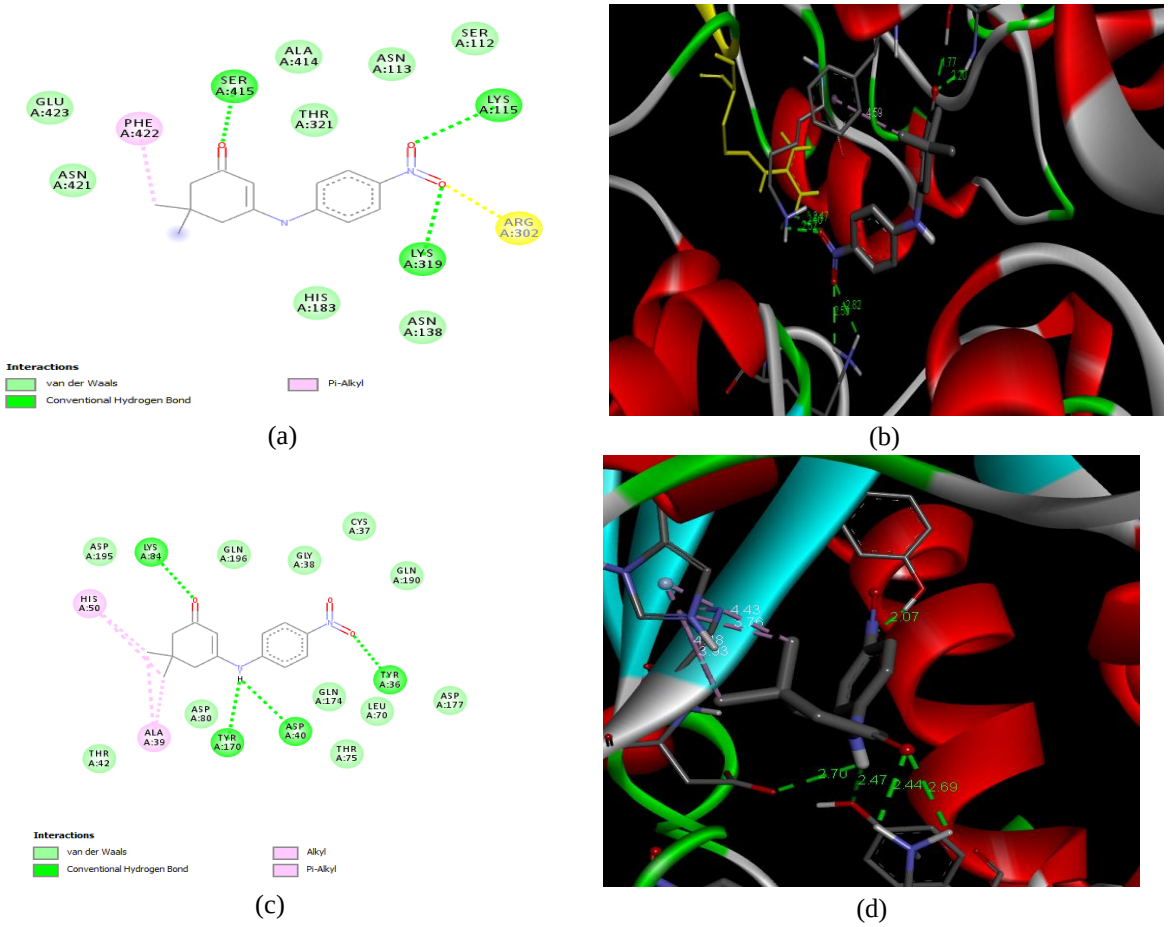


Figure 2. (a) 2D structure of DMPC-4 with 1UAG protein, (b) 3D structure of DMPC-4 with 1UAG protein, (c) 2D structure of DMPC-4 with 1JIJ protein (d) structure of DMPC-4 with 1JIJ protein.

Table 2. The docking result of DMPC compounds with 1JIJ bacterial protein.

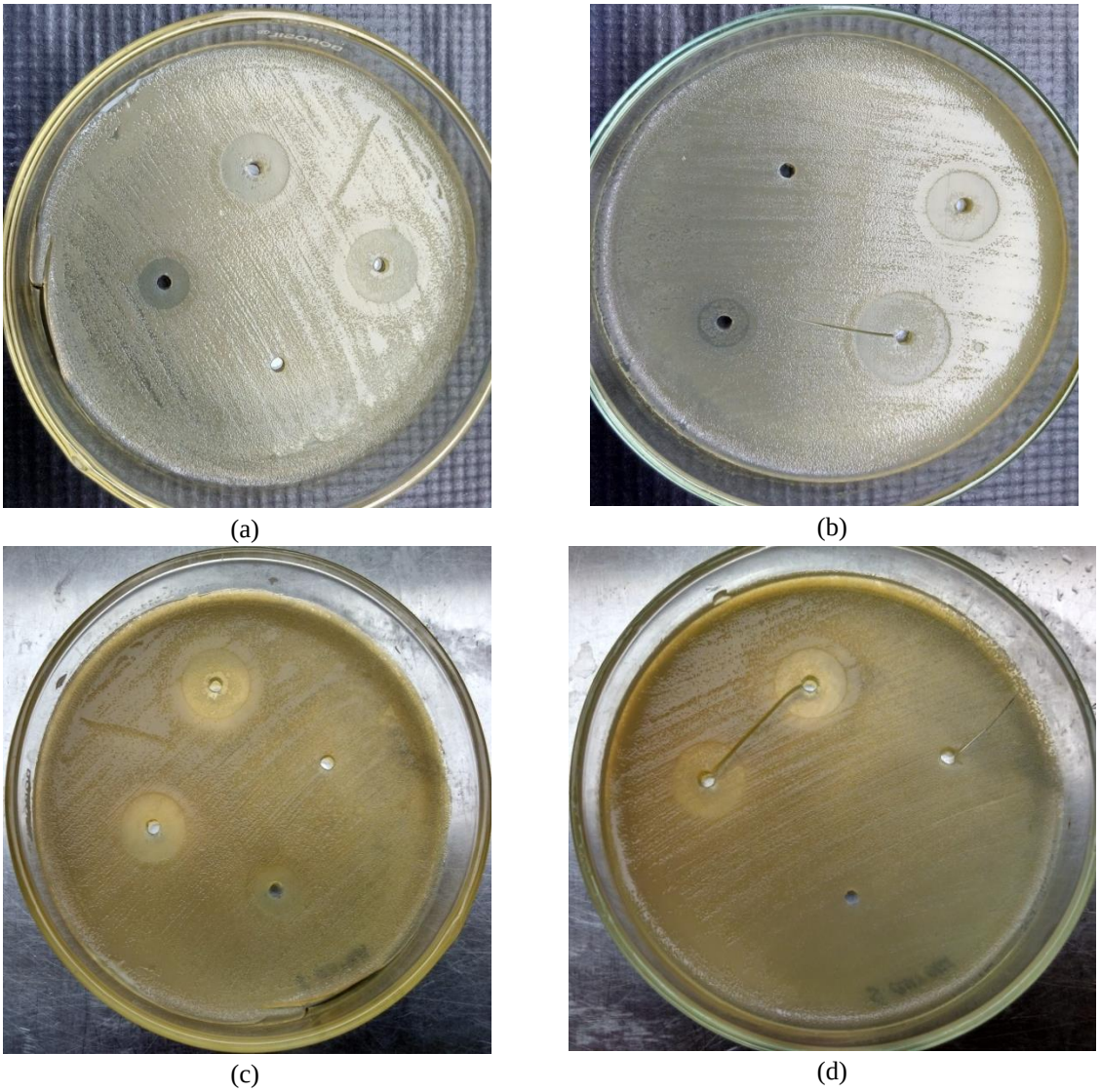
Compound	B.S	HBI-bond length	HBI	HPI- bond length	HPI
DMPC-1	-6.7	3.05	ARG A:12	5.09	PHE A: 273, ARG A: 59
DMPC-2	-7.0	2.32	ASP A: 195	3.65, 4.94, 4.38, 5.05	LEU A: 52, VAL A: 224, PRO A: 53, CYS A: 37
DMPC-3	-6.8	-	-	4.62, 5.16, 5.32	CYS A: 37 TYR A: 36 HIS A: 50
DMPC-4	-8.4	2.07, 2.44, 2.47, 2.69, 2.70	LYS A: 84 TYR A: 170 ASP A: 40 TYR A:36	3.76, 3.93, 4.43, 4.48	HIS A: 50 ALA A: 39
DMPC-5	-7.9	2.15	TYR A: 36	4.13	LEU A: 70

Compound	B.S	HBI-bond length	HBI	HPI- bond length	HPI
		2.84 3.03	GLN A:196 GLN A: 174		
Ciprofloxacin	-9.5	2.10 2.32 2.40	TYR A: 36 THR A: 75	4.56	CYS A: 37

Table 3. ADME prediction values of DMPC compounds.

Compound	Formula	Molecular Weight	H-bond donors	H-bond acceptors	Molecular Refractivity	TPSA	% of Abs	Log S	Log P
DMPC-1	$C_{14}H_{17}NO$	215.29	1	1	66.75	29.1	98.96	-3.27	2.37
DMPC-2	$C_{14}H_{16}ClNO$	249.74	1	1	71.76	29.1	98.96	-3.92	2.9
DMPC-3	$C_{14}H_{16}BrNO$	294.19	1	1	74.45	29.1	98.96	-3.98	3.02
DMPC-4	$C_{14}H_{16}N_2O_3$	260.29	1	3	75.57	74.92	83.15	-4.62	1.2
DMPC-5	$C_{14}H_{16}N_2O_3$	260.29	1	3	75.57	74.92	83.15	-4.05	1.2

Acceptable values: n-Heavy atoms, MW<500, Log P < 5, n-HyA≤ 10, n-HyD ≤ 5, M.Rty < 120, Log S ≤ -5, TPSA (30≤ to ≥ 140), Drug Score ≥ 0.5, %Abs (≤ 80% (best), 30-80 (moderate), ≥ 30% (poor)).



(a) (b) (c) (d)

Figure 3. (a) *in-vitro* studies result (a) bacterial strain of the compound DMPC-4 averse to *E. coli*, (b) bacterial strain of the compound DMPC-5 averse to *E. coli*, (c) bacterial strain of the compound DMPC-4 averse to *S. aureus*, (d) bacterial strain of the compound DMPC-5 averse to *S. aureus.*

3.6. Anti-bacterial study.

The synthesized compounds are screened with bacterial strains at 2.5,5 and 10 µg/mL. For the anti-bacterial study, use the one gram-negative strain *E. coli* and one gram-positive strain *S. aureus* with three different concentrations. Overall, the study showed that the DMPC compounds have a good inhibition zone level when treated with *E. coli* than the treat with *S. aureus*. The DMPC compounds may have the best active against gram-negative bacteria than that the gram-positive bacteria. The DMPC-1,3 compounds have a noticeable zone of inhibition values compared with the standard drug. The zone of inhibition values of DMPC compounds with *E.coli* and *S.aureus* bacterial strains at different concentrations are given in Table 4 and Figure 3.

Table 4. Antimicrobial studies of DMPC1-5

Sample	**Zone of Inibition (diameter in mm)**					
	E.coli			*S.aureus*		
	10µg/mL	5µg/mL	2.5µg/mL	10µg/mL	5µg/mL	2.5µg/mL
DMPC-1	20	18	12	15	13	11
DMPC-2	15	12	10	19	16	12
DMPC-3	18	16	14	14	12	10
DMPC-4	16	14	10	16	14	13
DMPC-5	15	13	12	16	14	12
Ciprofloxacin	28	22	19	38	32	26

3.7. Recover and reuse of catalyst.

The catalyst ($NaHSO_4$-SiO_2) was recovered from the reaction mixture and reused by washed with acetone, filtered, dried, and activated. The catalyst was very efficient to 5 cycles of reaction carried out. So, that up to 5 cycles, the catalyst was collected and reused. The compared values up to five runs the yield are more or less similar rate. There is no wide change in the yield of synthesized compounds DMPC (1-5). The following graph represents the compared yield of compounds during reaction cycles.

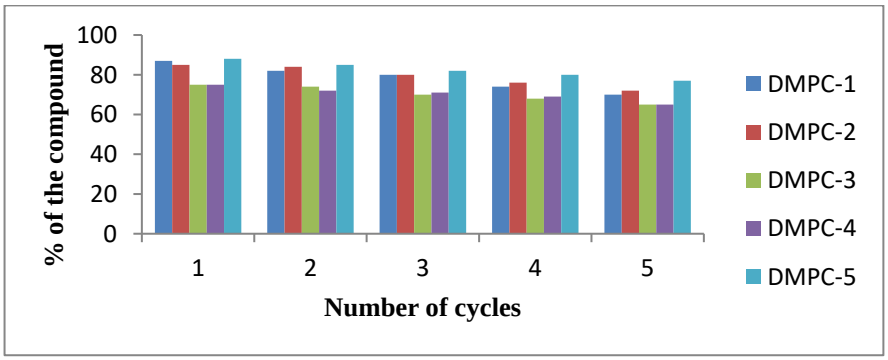


Figure 2. Recovery and reuse of the heterogeneous catalyst $NaHSO_4$-SiO_2.

4. Conclusions

The DMPC 1-5 compounds were successfully synthesized by using re-useable sodium bisulfate as a catalyst with the help of a microwave. The catalyst was reused up to five cycles of every targeted compound's synthesis reactions and indicates that the $NaHSO_4$-SiO_2 has a highly effective catalyst in a dry media synthesis of DMPC compounds. The compounds' skeleton structure is characterized and confirmed from the IR, ^{1}H NMR, and ^{13}C NMR values. The compounds are subjected to docking studies and ADME studies. All the compounds have acceptable values in the ADME studies. From docking studies, all the DMPC compounds may

follow the cell-wall synthesis against bacterial strains. All have good binding interaction with 1UAG than 1JIJ. The in-vitro anti-bacterial studies also showed that the DMPC compound good active against gram-negative *E. coli* than the gram-positive *S. aureus*.

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Conflicts of Interest

The authors declare no conflict of interest.

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