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Original Article

Synthesis Characterisation *in silico* Studies and Anti-bacterial Evaluation of Acetaminophen Chalcone Derivatives

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ABSTRACT

Background: Design synthesis of Acetaminophen-chalcone conjugates and finding the *in silico* studies and characterisation of compounds. Determination of biological activity mainly emphasise on Antibacterial activity. Chalcones are a group of plant-derived polyphenolic compounds that possess a wide variety of biological activities. It acts as precursor of several biosynthetic pathways in the production of flavonoids and isoflavonoids. Chalcones shows activity mainly because of an enone pharmacophore in their structures. **Materials and Methods:** Five chalcone derivatives were synthesised by using Claisen Schmidt reaction of appropriate Ketones or substituted Ketones with Benzaldehydes or Substituted Benzaldehydes in presence of Catalyst and Ethanol. The structure of synthesised analogs was determined by its Physico-chemical properties like Solubility, Melting point and thin layer chromatography followed by Spectral analysis IR, ^{1}H NMR. This study provides an overview of an Antibacterial activity of Acetaminophen-Chalcone derivatives. *In silico* studies were performed on all compounds using Molinspiration, Protox, Swiss ADME softwares. This study aimed to investigate the potential antibacterial activity of Acetaminophen-Chalcone compounds using agar diffusion method. **Results:** In this present work we designed five different Chalcone derivatives AS1, AV2, AA3, APC4 and APN5. *In silico* studies were analysed. Antibacterial activities of derived chalcones were screened for *Escheria coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis.* **Conclusion:** APN5 drug shows broad spectrum of antibacterial activity against all the four bacterial strain and in all the respective concentrations.

Keywords: Acetaminophen-chalcones, Paracetamol derivatives, *in silico* studies, Anti-bacterial activity.

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INTRODUCTION

The present work is prompt disclosure of new experimental in many fields of drug design, discovery and elucidation of biological active compounds. Which focus relationship in chemical and biological evaluation on anti-bacterial activity. *In silico* studies will welcome the increase of your understanding of drug molecules. Acetaminophen also commonly known as Tylenol is the most commonly taken analgesic world-wide and is recommended as first line therapy in pain conditions by WHO (World Health Organisation). Acetaminophen drug is commonly used non-narcotic analgesic-antipyretic agents and is a component of many over-the-counter proprietary preparations. USFDA has been approved this drug in 1951. It is available in varied forms like syrups, tablets and effervescent tablets, injections, suppositories and other forms.[1]

Chalcones are the natural pigments found in nature which form the central core for a variety of significant biological compound. They are the aromatic ketones and enones. They are important constituents of many natural sources.[2-4] They are widely distributed in plants and are thought to serve as a starting point for a number of biosynthetic pathways that result in the synthesis of isoflavonoids and flavonoids. Chalcones shows activity mainly because of an enone pharmacophore in their structures.[5,6] The chalcone core is made up of two aromatic rings joined by a three-carbon aliphatic chain.[7] These ketones are α,β-unsaturated and comprise of two aromatic rings with distinct substituents. The electrophonic three carbon α,β -unsaturated carbonyl system, which has a ketoethylenic group in its structure and a planar structure, connects these two aromatic rings.[8,9]

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Chalcones derivatives exhibit wide range of pharmacological actions such as antibacterial, anti-inflammatory, analgesic, anticholinergic, antiplatelet, antiulcer, antioxidant, antimalarial, anticancer, antiviral, anti-leishmanial, anti-diabetic, immunomodulatory, aldose reductase inhibition and as non-purine xanthine oxidase inhibitors.[10] Chalcones are widely used because of their simple structure, open chain model, ease of construction, the feat and promising biological applications. Chalcones have potential research class of compounds with important uses against various diseases.[11,12]

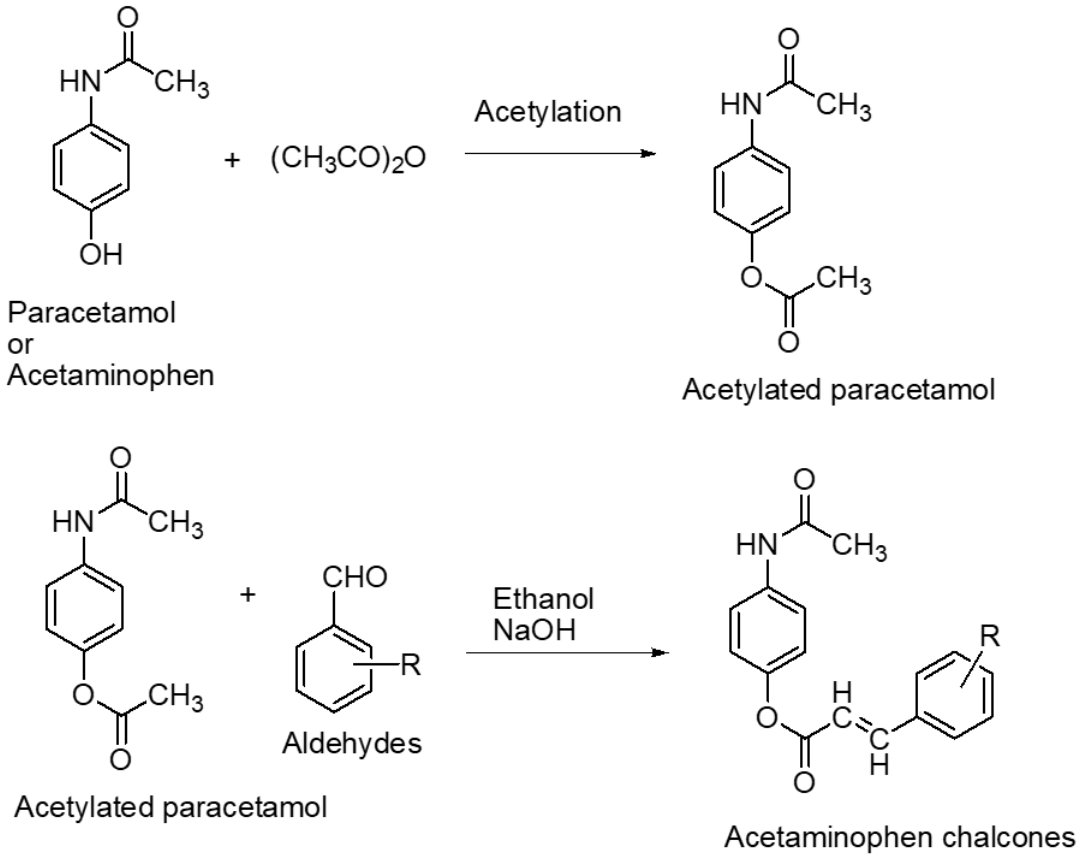
Drug resistance is the reduction in effectiveness of a drug in curing a disease or reducing a patient's symptom.[13] Antibacterial resistances happen when the bacteria develop the ability to defeat the drugs, which are designed to kill them. Due to severe side effects, toxicities and bacterial resistance lead to complications in treatment; so many researchers are working to find a new antibacterial agent that has potency against drug resistant strains.[14,15]

MATERIALS AND METHODS

The paracetamol used are Analytical grade procured from institution. All chemicals used are synthetic grade; different aldehydes were used mentioned in Table 1. All the compound structures were given in Table 2. We determined the melting points of synthesised organic derivative compounds using the open capillary tube method. One of the physical parameters, the melting point, indicates the purity of the organic derivative in the form of pure crystals with sharp and definite melting points. The melting points of the derivatives were performed using melting point apparatus. Solubility: The solubility of the synthesised derivatives was tested using different solvents. The solubility characters were listed. TLC solvent is chloroform: ethyl acetate ratio 9:1. IR spectra were recorded FTIR spectrophotometer and ^{1}H NMR spectra taken from Bruker 600 MHz instrument. Thin-layer chromatography is a chromatography technique used to separate and identify the components in a mixture based on their different physical and chemical properties. TLC is a quick, easy and inexpensive method that requires small amounts of sample.[16-18]

Synthetic Scheme

Scheme 1



Scheme 1: synthetic route of chalcone derivatives.

Synthetic procedure

Acetylation of paracetamol

Place 10 g of paracetamol, 14 mL of acetic anhydride and 2 mL of conc. H_2SO_4 in a 250 mL conical flask. Heat the mixture in the conical flask for 30 min in a water bath. Cool the mixture in ice water and immediately filter off the precipitated product. The filter paper was dried with its content in well-ventilated cabinet for 24 hr.

Preparation of chalcones

A mixture of 0.01 M Acetylated paracetamol and 0.01 M Aldehyde in 10-20 mL of ethanol in a 250 mL conical flask. The mixture was stirred in an ice bath for 1 hr. 10% NaOH was added upon 3 hr of continuous stirring and left overnight. The reaction mixture was cooled and the solid thus obtained was filtered, washed with Dil. HCl. The product was dried and collected.[19,20]

Antibacterial activity by Agar well diffusion method

In our current study, the antibacterial activity was carried out by agar well diffusion method. Here response of organisms to the synthesized compounds was measured and compared with the response of the standard reference drug gentamycin. The four microrganisms used *Staphylococcus aureus* (Gram+ve) and *Bacilus subtilis* (Gram+ve), *Escheria coli* (Gram-ve) and *Pseudomonas aeruginosa* (Gram-ve). Well diffusion method is generally used to evaluate antimicrobial (antibacterial) action of plants or microbial extracts. In this technique, petridish contains muller hinton agar which has been inoculated with respective bacterial inoculum. Agar wells of diameter 6 to 8 mm were made by using sterile cork borer. And a suitable volume and desired concentration of the antibacterial agent were added using sterilized dropping pipettes into the wells and plates were left for 1 hr for preincubation. The plates were incubated at 37°C or 24 hr. These antibacterial agents diffuse from the well which leads to inhibition of bacterial growth in the vicinity of the punctured well. The area up to which the antibacterial agent shows its effect is referred as zone of inhibition, which was measured, calculated and compared with the standard. The media used in this method is Muller Hinton Agar.[21,22]

The *Staphylococcus aureus, Bacilus subtilis* (gram+ve), *Escheria coli* and *Pseudomonas aeruginosa* gram-ve organisms were isolated from pure culture which were left overnight on a suitable nutrient media in a different petridish. Then using a sterile loop or a cotton swab these morphologically similar colonies were picked and suspended in a saline solution and mixed to an even turbidity. The density of the inoculum suspension should be equal to a 0.5 McFarland standard.

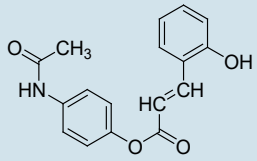
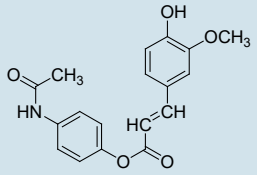
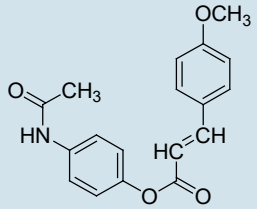
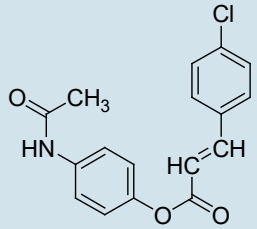
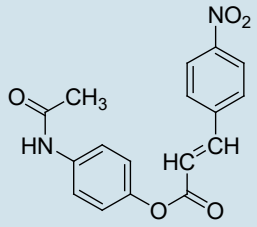
The respective strains swab of these colonies is then streaked across a Mueller-Hinton agar plate to form a bacterial lawn. To obtain uniform growth, the agar plates are streaked with the swab, incubated at 35-37°C for 4-6 hr. McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions. Photometric device is used to measure the density of the suspension. If density is too low, add more bacteria and if it is high add a saline solution.

Each drug compound was dissolved in distilled water to get a required stock solution and then from these stock solution different concentrations such as 250 µg, 125 µg, 62.5 µg, 31.250 µg and 15.625 µg were made. The standard drug used was

Table 1: **List of aldehydes and codes.**

Compound code	Aldehydes
AS-1	Salicylaldehyde
AV-2	Vanillin
AA-3	Anisaldehyde
APC-4	p-Chlorobenzaldehyde
APN-5	p-Nitrobenzaldehyde

Table 2: **Structures of synthesized compounds.**

Codes	Structures
AS1	
AV2	
AA3	
APC4	
APN5	

gentamycin. The zone of inhibition for gentamycin was 16 mm for *E. coli*, 17 mm for *S. aureus* and 22 mm for *Bacillus subtilis* (12 mm above is considered as sensitive).

In silico studies of Acetaminophen-Chalcone derivatives

By using Molinspiration, Protox, Swiss ADME, Chemspider, Chemdraw softwares we have performed *in silico* studies of synthesized derivatives. Smiles format were shown in Table 3. Molinspiration is a privately owned company focused on research development and application oriented modern cheminformatics techniques, especially in connection with the web or internet. Molinspiration tools are independent platform free and may run on any personel computer. Molinspiration was used to obtain parameter such as MiLogP, TPSA and drug likeness scores.[23,24]

PROTOX is an online tool that provides toxic parameters, including LD_{50} values, immunogenicity, mutagenicity, hepatotoxicity and carcinogenicity. This web server tool predicts how toxic small molecules will be. The LD_{50} values for toxic doses are commonly expressed as mg/kg body weight. The term "median lethal dose" (LD_{50}) refers to the dose at which 50% of test subjects die up on exposure to the chemical.[25]

Swiss ADME is drug development software includes pharmacokinetic parameters such as (ADME) involves absorption, distribution, metabolism and excretion, increasingly in the drug discovery process, a free web tools to find pharmacokinetics and drug likeliness and medicinal chemistry of small molecules. Efficient input and easy to predict key parameters collection of molecules for drug discovery and ensured interpretation.[26]

Spectral data of compounds

(E)-4-acetamidophenyl 3-(2-hydroxyphenyl) acrylate: AS1

FTIR (cm^{-1}): 1651 (C=O, amide), 3323 (N-H, amide), 1750 (C=O, Carbonyl), 1505 (Aromatic ring) 2880 (C-H, Alkane), 3035 (CH=, Alkene), 3560 (OH, alcohol): ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.02 (3H,s, methyl), 5.0 (1H,s,OH), 6.32 (1H,d, CH= alkene), 6.68-7.0 (4H,m, Aromatic), 7.05-7.61 (4H, m, Aromatic), 7.91 (1H,d, CH= alkene), 8.0 (1H,s, NH sec. amine).

(E)-4-acetamidophenyl 3-(4-hydroxy-3-methoxyphenyl) acrylate: AV2

FTIR (cm^{-1}): 1651 (C=O, amide), 3321 (N-H, amide), 1714 (C=O, Carbonyl), 1505 (Aromatic ring) 2881 (C-H, Alkane), 3092 (CH=, Alkene), 2800 (OCH_3, methoxy), 3510 (OH, alcohol): ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.02 (3H,s, methyl), 3.73 (3H,s, OCH_3), 5.0 (1H,s,OH), 6.39 (1H,d, CH= alkene), 6.57-6.69 (3H,m, Aromatic), 7.05-7.61 (4H, m, Aromatic), 7.64 (1H,d, CH= alkene), 8.0 (1H,s, NH sec. amine).

(E)-4-acetamidophenyl 3-(4-methoxyphenyl) acrylate: AA3

FTIR(cm^{-1}): 1675 (C=O, amide), 1597 (N-H, amide), 1746 (C=O, Carbonyl), 1509 (Aromatic ring) 2938 (C-H, Alkane), 3027 (CH=, Alkene), 2839 (OCH_3, methoxy),: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.02 (3H,s, methyl), 3.73 (3H,s, OCH_3), 6.39 (1H,d, CH= alkene), 6.72-7.19 (4H,m, Aromatic), 7.05-7.61 (4H, m, Aromatic), 7.64 (1H,d, CH= alkene), 8.0 (1H,s, NH sec. amine).

(E)-4-acetamidophenyl 3-(4-chlorophenyl) acrylate: APC4

FTIR(cm^{-1}): 1640 (C=O, amide), 1592 (N-H, amide), 1681 (C=O, Carbonyl), 1515 (Aromatic ring) 2962 (C-H, Alkane), 3094 (CH=, Alkene), 761 (Cl, chloride),: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.02 (3H,s, methyl), 6.39 (1H,d, CH= alkene), 7.02-7.24 (4H,m, Aromatic), 7.05-7.61 (4H, m, Aromatic), 7.64 (1H,d, CH= alkene), 8.0 (1H,s, NH sec. amine).

Table 3: Smiles format of synthesized compound

Compound codes	Smiles format
AS1	CC(NC1=CC=C(OC(/C=C/C2=C(O)C=CC=C2)=O)C=C1)=O
AV2	CC(NC1=CC=C(OC(/C=C/C2=CC(OC)=C(O)C=C2)=O)C=C1)=O
AA3	CC(NC1=CC=C(OC(/C=C/C2=CC=C(OC)C=C2)=O)C=C1)=O
APC4	O=C(C)NC1=CC=C(OC(/C=C/C2=CC=C(Cl)C=C2)=O)C=C1
APN5	O=C(C)NC1=CC=C(OC(/C=C/C2=CC=C([N+]([O-])=O)C=C2)=O)C=C1

Table 4: Solubility studies of synthesized compounds.

Codes	Chloroform	Ethanol	Water	Ethyl acetoacetate	DMSO
AS1	Insoluble	Soluble	Insoluble	Soluble	Soluble
AV2	Soluble	Soluble	Soluble	Soluble	Soluble
AA3	Soluble	Soluble	Insoluble	Soluble	Soluble
APC4	Soluble	Soluble	Insoluble	Insoluble	Soluble
APN5	Soluble	Soluble	Soluble	Soluble	Soluble

(E)-4-acetamidophenyl 3-(4-nitrophenyl) acrylate: APN5

FTIR(cm^{-1}): 3325 (N-H, amide), 1688 (C=O, Carbonyl), 1600 (Aromatic ring) 2900 (C-H, Alkane), 3080 (CH=, Alkene), 1520 (NO_2, Nitro), ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.02 (3H,s, methyl), 6.68 (1H,d, CH= alkene), 7.05-7.61 (3H,m, Aromatic), 7.56-8.14 (4H, m, Aromatic), 7.78 (1H,d, CH= alkene), 8.0 (1H,s, NH sec. amine).

RESULTS

All the synthesised compounds given were derived from scheme 1. Solubility studies were given in Table 4. Physico-chemical parameters were noted in Table 5. By measuring inhibition zones at different drug concentrations, the study aims to elucidate the drugs' spectrum of activity and concentration-dependent efficacy in Table 6.

Table 5: Physico chemical parameters of synthesized compounds.

Codes	Melting point	Molecular formula	Molecular Weight	Colour	State	R_f	% yield
AS1	152°C	$C_{17}H_{15}O_4N$	297	Burnt umber	Solid	0.38	49.5
AV2	142°C	$C_{18}H_{18}O_5N$	328	Light peach	Solid	0.45	34.9
AA3	130°C	$C_{18}H_{17}O_4N$	311	Brown	Solid	0.23	22.9
APC4	204°C	$C_{17}H_{14}O_3N_1Cl$	315	Light brown	Solid	0.27	15.1
APN5	205°C	$C_{17}H_{14}O_5N_2$	326	Light orange	Solid	0.35	8.28

Table 6: Anti-bacterial activity of compounds.

(R=Resistant)					
Drug Code	**Drug Concentration**				
	250µg	**125µg**	**62.5µg**	**31.250µg**	**15.625µg**
***Bacillus subtilis* (Gram+ve)**					
AS1	18mm	16mm	16mm	15mm	15mm
AV2	16mm	14mm	14mm	16mm	16mm
AA3	12mm	12mm	R	R	R
APC4	16mm	14mm	14mm	14mm	14mm
APN5	20mm	20mm	14mm	12mm	R
***Staphylococcus aureus* (Gram+ve)**					
AS1	32mm	24mm	20mm	R	R
AV2	R	R	R	R	R
AA3	R	R	R	R	R
APC4	R	R	R	R	R
APN5	12mm	R	R	R	R
***Escherichia coli* (Gram-ve)**					
AS1	15mm	12mm	R	R	R
AV2	R	R	R	R	R
AA3	R	R	R	R	R
APC4	R	R	R	R	R
APN5	25mm	21mm	20mm	16mm	14mm
***Pseudomonas aeruginosa* (Gram-ve)**					
AS1	14mm	12mm	R	R	R
AV2	R	R	R	R	R
AA3	14mm	12mm	R	R	R
APC4	R	R	R	R	R
APN5	26mm	22mm	20mm	16mm	12mm

Bacillus subtilis strain bacteria tested AS1, AV2 and APC4 shows a good inhibition across all concentrations and the MIC was found to be at 15.625µg showing zone of inhibition of 15 mm, 16 mm and 14 mm respectively shown in Figures 1-3. This bacterium is sensitive to AA3 drug at only two concentrations 250 µg and 125 µg exhibiting zone of inhibition at 12mm respectively. And it is resistant to other concentration. APN5 Shows a good zone of inhibition at 250 µg, 125 µg, 62.5 µg and 31.250 µg concentration.

Staphylococcus aureus strain bacteria exhibits potent inhibition at higher concentration of AS1 but loses effectiveness at lower concentrations. This strain is sensitive to APN5 at only 250µg concentration showing 12 mm of zone of inhibition and is resistant to all other concentration of drug including MIC.

Escherichia coli strain shows effective sensitivity to APN5 drug and MIC at 15.625 µg of 14 mm zone of inhibition shown in Figure 4. AS1 displays moderate inhibition, with effectiveness being resistant at lower concentration of drug. It shows resistance across all the concentration of AV2, AA3, APC4 compounds.

Pseudomonas aeruginosa strain is maximum sensitive to APN5 drug at all the concentration and shows MIC at 15.625 µg of 12 mm shown in Figure 5. AS1 and AA3 show moderate sensitivity at 250 and 125 µg concentrations. This bacteria show resistance across all the concentration of AV2, APC4 drugs.

Molinspiration results describe that compounds show the log p-value indicating partition coefficient. All compounds show TPSA less than 150A^o indicating good drug permeability in the plasma membrane. Percentage absorption (%ABS) should be in the range of 79.96-89.88% signifying good absorption in the intestine shown in Table 7.

Formula for calculation of percentage absorption: % ABS=109-0.345×TPSA

Bioactivity scores calculation for organic compounds says, 'the probability is that if bioactivity scores are greater than 0', then it is active. If it is between -5 and 0 then moderately active, If less than -5 then inactive. All Compounds 1-5 are moderately active as GPCR ligands, Ion channel modulator, Kinase inhibitor, nuclear receptor ligand, Protease inhibitor, Enzyme inhibitor shown in Table 8.

Protox result analysis for all compounds, the dose predicted LD_{50} is 2000 mg/kg. All compounds come under predicted toxicity is class 4. From the toxicity model report Table 9, all compounds toxicity are inactive towards cytotoxicity, carcinogenicity, mutagenicity, all the compounds showed toxicity active towards hepatotoxicity, immunogenicity. It is *in silico* toxicity prediction very useful for toxicologist, medicinal chemist, computational chemist and regulatory agencies. It saves the time, need of animal testing and cost associated with it.

From the Swiss ADME search, we conclude that all the compounds follow Lipinski rule, all compounds show TPSA less than 150A^o indicating good drug permeability in the plasma membrane, all the compounds shows bioavailability score. All the compounds shows high GI absorption AS1, AA, APC4 shows BBB permeant, all the compounds shows no to P-gp substrate, AS1does not inhibit all the inhibitor, AV2 does not inhibit all the inhibitor except CYP2C9 inhibitor, AA3 inhibits to all the inhibitor except CYP2D6, CYP3A4, APC4 inhibits to all the inhibitor except CYP2D6, APN5 inhibits to all the inhibitor except CYP2D6, CYP3A4 were reported in Table 10.

DISCUSSION

The starting material for synthesis is paracetamol, it is treated with acetic anhydride and few drops of sulphuric acid on slight heating gives a reaction mixture, further on cooling gives Acetylated paracetamol. An equimolar mixture of Acetylated paracetamol and different aromatic aldehydes were treated with 10% NaOH and stirred for 3 hr, solid precipitates, filtered and dried gives Acetaminophen-Chalcone derivatives. TLC were confirmed by using silica gel sheets which are pre-coated Aluminium sheets and melting points were analysed and by using different solvents solubility tested. An FTIR spectrum has been recorded in instrument and interpreted the functional groups. Proton NMR is analysed the number of protons, solvent used is deuterated chloroform, using 600 MHz instrument. An alkene has been formed at 3094 cm^{-1} range reveals that chalcones are formed. Proton NMR 6.39 and 7.34 alkenes have single protons and a chemical shift was recorded.[27,28] This study examines the effectiveness of these synthesized derivatives against four distinct bacterial strains: '*Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*' using Agar well diffusion method. *Bacillus subtilis* strain bacteria tested AS1, AV2

Table 7: Calculation of molecular properties using Molinspiration.

Codes	Mi LogP	TPSA	MW	n atoms	N OH	N OHNH	N violations	n rot	Volume	%ABS
AS1	2.61	75.63	297.31	22	5	2	0	5	266.80	82.91
AV2	2.19	84.86	327.34	24	6	2	0	6	292.35	79.72
AA3	2.91	64.64	311.34	23	5	1	0	6	284.33	86.69
APC4	3.53	55.40	315.76	22	4	1	0	5	272.32	89.88
APN5	4.47	84.16	324.34	24	6	1	0	6	290.87	79.96

Table 8: Bioactivity scores prediction using Molinspiration.

Codes	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear Receptor ligand	Protease Inhibitor	Enzyme Inhibitor
AS1	-0.26	-0.29	-0.24	-0.11	-0.25	-0.14
AV2	-0.23	-0.29	-0.21	-0.13	-0.26	-0.14
AA3	-0.27	-0.32	-0.24	-0.20	-0.26	-0.19
APC4	-0.27	-0.30	-0.28	-0.21	-0.31	-0.21
APN5	-0.28	-0.29	-0.39	-0.21	-0.27	-0.11

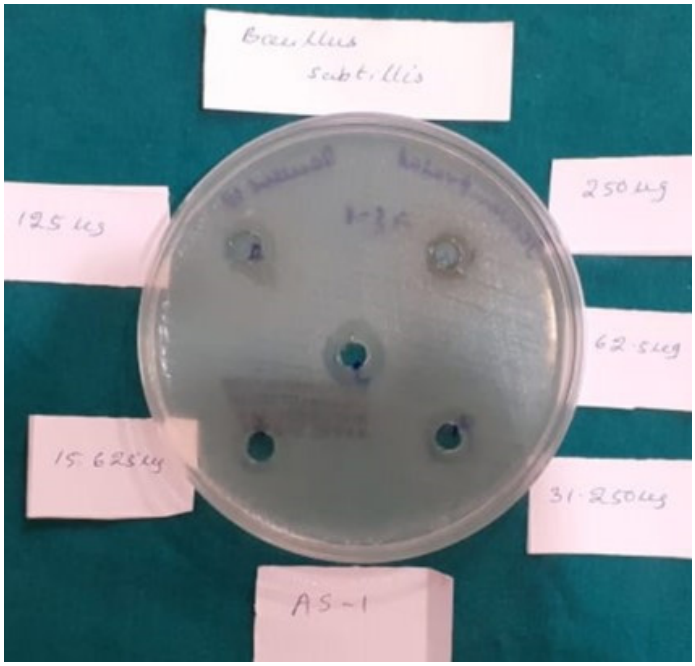


Figure 1: *Bacillus subtilis* is sensitive to AS1 at 15.625 µg concentration.

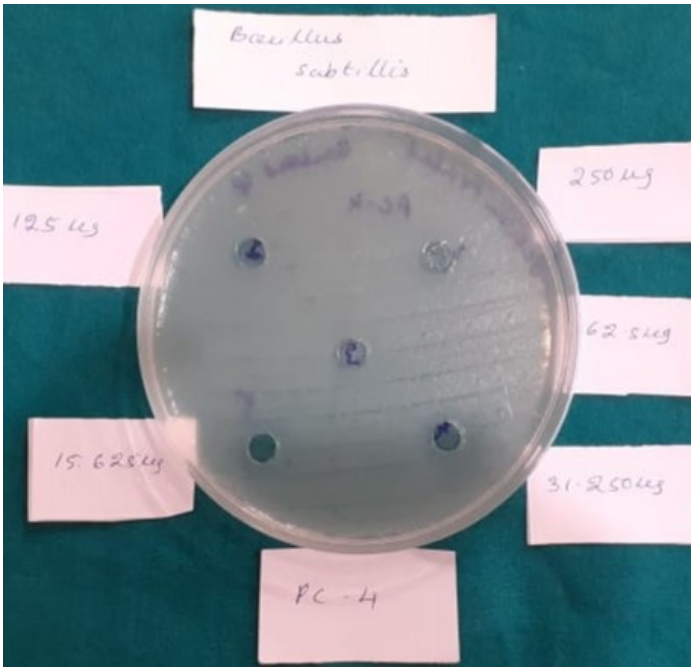


Figure 3: *Bacillus subtilis* is sensitive to APC4 at 15.625 µg concentration

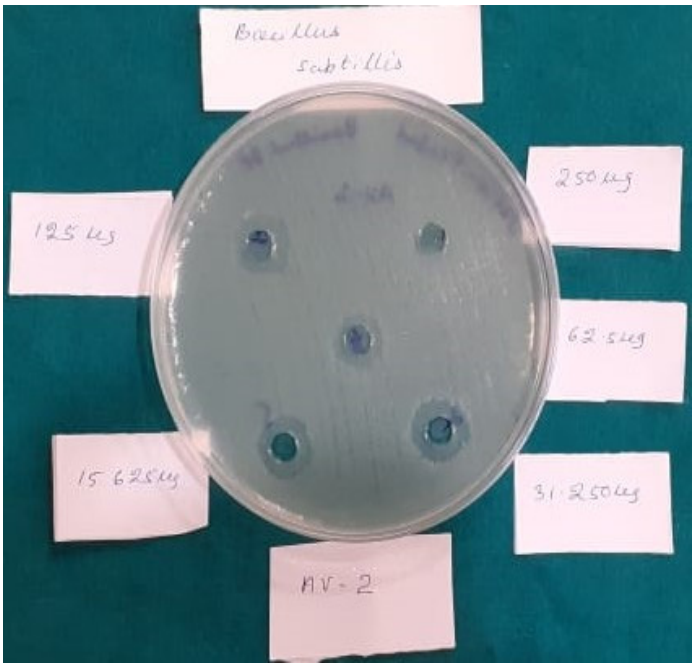


Figure 2: *Bacillus subtilis* is sensitive to AV2 at 15.625 µg concentrations.

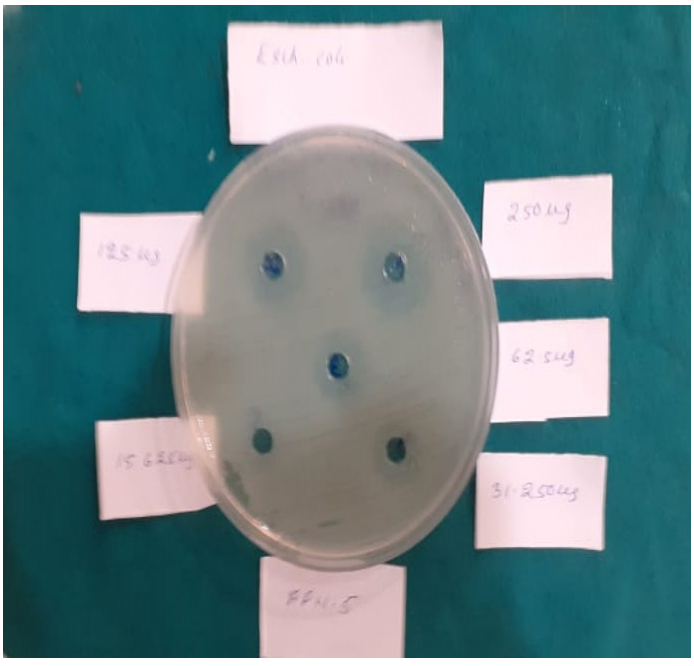


Figure 4: *Escherichia coli* are sensitive to APN5 at 15.625 µg concentrations.

Table 9: Toxicity model report.

Target	AS1	AV2	AA3	APC4	APN5
Hepatotoxicity	Active	Active	Active	Active	Active
Carcinogenicity	Inactive	Inactive	Inactive	Inactive	Inactive
Immunogenicity	Active	Active	Active	Active	Active
Mutagenicity	Inactive	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive

Table 10: Swiss ADME results.

Pharmacokinetics	AS1	AV2	AA3	APC4	APN5
Lipinski	Yes	Yes	Yes	Yes	Yes
TPSA (A^2)	75.63	84.86	64.63	55.40	101.22
Consensus Log $P_{O/W}$	2.52	2.58	2.90	3.46	2.18
Bioavailability score	0.55	0.55	0.55	0.55	0.55
GI absorption	High	High	High	High	High
BBB permeant	Yes	No	Yes	Yes	No
P- gp substrate	No	No	No	no	No
CYP1A2 inhibitor	No	No	Yes	yes	Yes
CYP2C19 inhibitor	No	No	Yes	yes	Yes
CYP2C9 inhibitor	No	Yes	Yes	yes	Yes
CYP2D6 inhibitor	No	No	No	no	No
CYP3A4 inhibitor	No	No	No	yes	No
Log K_p (cm/s)[a]	-6.28	-6.47	-6.13	-5.70	-6.33

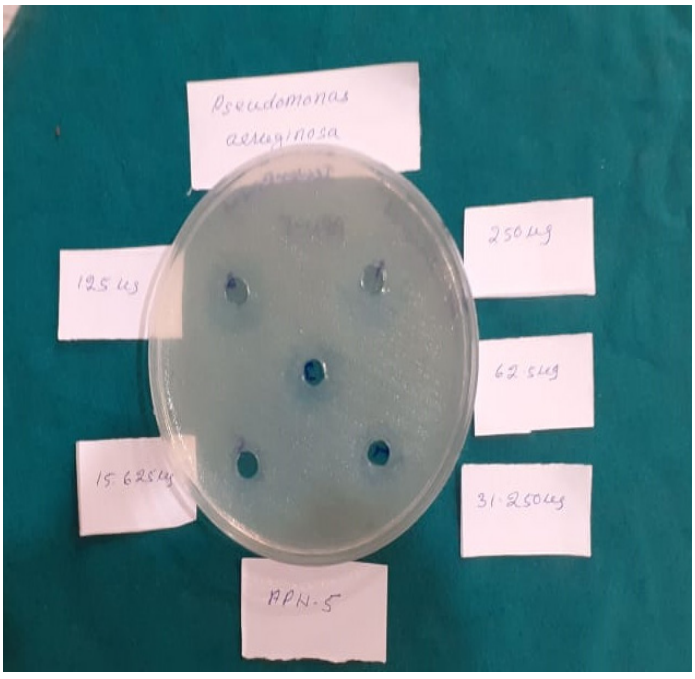


Figure 5: *Pseudomonas aeruginosa* is sensitive to APN5 at 15.625 µg concentrations.

and APC4 shown a good zone of inhibition at all concentrations. *E. coli* strain bacteria tested APN5 shown effective at all concentrations. *Pseudomonas aeruginosa* strain bacteria tested APN5 shown sensitivity at all concentrations. All compounds Molinspiration properties were predicted and calculated the percentage absorption in the gut at range of 79.72-89.88. From the molinspiration results, all synthesized compounds satisfy the Lipinski rule of five properties, stating that compounds were under the limits. Bioactive scores were predicted all compounds which are in negative sign are moderately active at GPCR ligands, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, enzyme inhibitor.[29] From the Protox report we conclude that the predicted toxicity of synthesized compounds is free from toxicity carcinogenicity, mutagenicity, cytotoxicity. All compounds found active toxicity of Hepatotoxicity, Immunogenicity.[30] All the compounds smiles format were uploaded in Swiss ADME showed good GI absorption is high and bioavailability score is 0.55, permeability to BBB (AS1, AA3, APC4) and some enzyme inhibitor parameters have been noted. From this *in silico* studies we predict the possibilities of the therapeutic potential of new drug molecules more effectively.

CONCLUSION

In the present study, a series of new five 4-acetamidophenyl 3-(4-aryl acrylates) derivatives were synthesized and screened for antibacterial activity and evaluation was carried out by using a standard drug. *In silico* studies results reveal that the entire compound showed characteristic properties using all these softwares such as Molinspiration, Protox, Swiss ADME, respectively. The compound APN5 shows better antibacterial activity against *E.coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis*. Further studies are required to perform *in vitro, in vivo* and molecular dynamic studies to know the stability of the drug.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier transforms infrared spectroscopy; **^{1}H-NMR:** Proton Nuclear Magnetic resonance**; MIC:** Minimum inhibitory concentration; **MBC:** Minimum bacterial concentrations; **TLC:** Thin layer chromatography, **δ:** Chemical shift.

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