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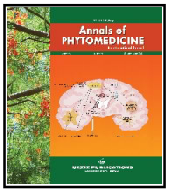

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Synthesis and docking study of aryl acetamide derivatives as an antimicrobial agents

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Abstract

The present study focused on the synthesis of an acetamide-bearing agent (aryl chloroacetamide) via condensation between substituted amines and chloroacetylchloride. The structural elucidation of synthesised compounds was performed by UV, IR, proton NMR, and mass spectral methods. *In silico* predictions of physiochemical and ADMET properties were also performed and found to be true as the synthesised compounds are orally bioavailable. The antibacterial and antifungal investigations were done by adapting the diffusion technique. The compound F_3, followed by F_5, displayed good growth inhibitory action against the tested bacterial and fungal strains at a concentration of 800 µg/100 µl. The activity may be due to the presence of diaryl-substituted nitrogen in the compound. In addition, a molecular docking study of the synthesised derivatives showed that the compound F_7 had a high binding affinity when compared with the standard ligand. From this study, we conclude that the aryl chloroacetamide compounds possess antibacterial and antifungal activities, which are further supported by their *in silico* study results and could be considered a promising analogue for the treatment of microorganism-associated disorders and inflammation.

1. Introduction

Careless use of antimicrobials has resulted in an alarming increase in microbial resistance. This highlights the need to create new chemical entities from synthetic or natural sources with distinctive modes of action in order to alleviate microbial disease. Growing worldwide public health concerns include the growth and spread of multidrug-resistant (MDR) strains of *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and non-fermentative gram-negative bacilli such as *Pseudomonas aeruginosa* (Hawkey *et al*., 2018). Additionally, the lack of effective antifungal medications, the rise of antifungal resistance, and the emergence of MDR fungal diseases pose a serious threat to the public's health. When the immune system of the colonised host is compromised, MDR bacterial and fungal pathogens can produce catastrophic infections that frequently do not respond to conventional or even last-resort therapies. Hence, the creation of brand-new, highly effective antimicrobial substances that simultaneously target a number of sites in bacterial or fungal pathogens might aid in the battle against antibiotic resistance (Zvarych *et al*., 2021).

There has been much interest focused on halogenated acid derivatives due to their ability to eradicate or inhibit the growth of bacteria, fungi, parasites, and viruses. N-aryl-2-chloroacetamides function as herbicides, antibacterials, antifungals, and disinfectants. Herbicides containing chloroacetamide groups can be exemplified by 2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide and 2-chloro-N-(2,6-dimethylphenyl)-N-[(3-methoxythiophen-2-yl)methyl] acetamide has been used in the control of weeds and grass in crops (Ahmad, 2020; Dehghani *et al*., 2013). The structural characteristics of chloroacetamides make them a desirable synthetic platform for the synthesis of various heterocyclic systems of aziridines (Concellon *et al*., 2010), N-lactams (Guthrie *et al*., 2009), imidazolidine (O'Reilly *et al*., 2009), piperazine (Paczal *et al*., 2006), and macrocyclic ligands (Busato *et al*., 2003) through the nucleophilic replacement of chlorine, reactive N-H group, methylene, and ketonic groups. Additionally, some chloroacetamide reagents have been used in solid-state chemistry as well as biomarkers and polymer modification. Acetamide-bearing molecules possess pharmacological properties such as antimicrobial (Sharma *et al*., 2019), anticancer (El-Abd *et al*., 2022; Yadav *et al*., 2017), herbicide (El-Zemity *et al*., 2021), antibacterial (Murtaza *et al*., 2019), antifungal (Tang *et al*., 2019; Katke *et al*., 2011), and antioxidant (Abdel Latif *et al*., 2020). Further, chloroacetamides have a mild electrophilic property, which makes them react with cysteine and histidine aminoacids in cross-linking a variety of biomolecules with proteins (Olszewska *et al*., 2016).

The current work is focused on the synthesis of aryl chloroacetamide derivatives of biologically significant moieties and their testing to assess their potential against bacteria and fungi strains. A molecular docking study was also performed against the enzymes DNA gyrase, Mur B, fungal NMT, and DHFR; those enzymes are responsible for their suppression by preventing bacterial and fungal reproduction.

2. Materials and Methods

All the reagents and solvents used for this study were obtained from chemical suppliers and used without further purification of the

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reagents. Digital melting point equipment was utilised for the determination of the melting points of the synthesised compounds using the open capillary method and presented uncorrected. IR spectra of synthesised compounds were captured using the KBr pellet technique using a Schimadzu Fourier Transform spectrophotometer in the frequency range of 400 cm^{-1} to 4000 cm^{-1}. Proton NMR spectra for compounds were obtained in deuterated chloroform solvent using a Bruker Advance 400 MHz Spectrometer. Chemical shifts were measured in parts per million, and trimethylsilane served as an internal standard. A JEOL GC-Mate spectrometer was used to take mass spectra of synthesised compounds.

2.1 Synthetic procedure

0.02 moles of the corresponding substituted amine were dissolved in 10 ml of dichloromethane, followed by 0.02 moles of chloroacetyl chloride in 10 ml of dichloromethane, which were added dropwise at an interval of 1 min for 25 min to the amine solution. The solution was stirred using a magnetic stirrer for 3 h in an ice bath at 0-5ºC, followed by stirring for 3 h at room temperature. The completion of the reaction was monitored by TLC using hexane and ethyl acetate in a ratio of 3:7 as a mobile phase and silica gel as a stationary phase. The visualisation of spots was done by iodine vapour and/or UV chambers. The mixture was poured into a beaker containing ice-cold water. The compound was dried and recrystallized using the corresponding solvent to get a pure product.

2.2 Molecular and ADMET properties determination

The online tool, molinspiration cheminformatics, determined the drug-like properties of the compounds. The ADMET property predictions were done by the web-based tool Pre ADMET.

2.3 Assessment of antimicrobial activity

2.3.1 Minimum inhibitory concentration (MIC)

Bacterial strains tested: *Bacillus subtilis*, *Pseudomonas vulgaris*, *Escherichia coli,* and *Klebseilla pneumoniae*.

Fungal strains tested: *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger,* and *Aspergillus fumigatus.*

Serial dilutions of the chloroacetamide compounds in the range of concentrations 800, 400, 200, 100, 50, and 25 µg/100 µl were added to the corresponding microbial growth medium in separate test tubes. These test tubes were then inoculated with the bacterial and fungal strains separately to be tested. The test tubes were then allowed to incubate overnight, and then the turbidity of the medium was observed (Wiegand *et al*., 2008).

2.3.2 Antibacterial and antifungal activity

The diffusion method was adapted for the determination of activity. Nutrient agar medium for bacterial growth and Sabouraud dextrose agar medium for fungal growth were used. Based on the MIC value, the synthesised samples were prepared at concentrations of 100, 200, 400, and 800 µg/100 µl, respectively, in DMF. Growth medium and petri plates were sterilised by autoclaving at 121°C for about 30 min at 15 lb pressure. Under aseptic conditions in the laminar airflow chamber, about 20 ml of the medium was dispensed into each petri plate to yield a uniform depth of 4 mm. After solidification of the media, 18 h cultures of bacterial strains in nutrient agar medium and fungal strains in Sabouraud dextrose agar medium were swabbed on the surface of the plates, respectively. The well was prepared by using a cork borer, followed by the loading of 100 µl of each synthesised sample at their respective concentrations into the distinct well. DMF was employed as the negative control, and the concentration of 10 µg/ml each of gentamycin for antibacterial activity and fluconazole for antifungal activity, respectively, was used as the positive control. The sample-loaded plates were then kept at 37°C for 24 h to observe the zone of inhibition for antibacterial activity and 48 h for antifungal activity (Saravana Kumari *et al*., 2020).

2.4 Molecular docking study

The drug discovery software molecular operating environment (MOE) was used for analysing the interaction of synthesised acetamide derivatives with diverse microbial proteins. Target proteins chosen for both antibacterial and antifungal activity separately were DNA gyrase (PDB code: 1KZN), Mur B (PDB code: 2Q85), N-myristoyl transferase (PDB code: 4CAW), and Candida albicans DHFR (PDB code: 4HOE).

2.4.1 Preparation of protein

The structures of proteins such as DNA gyrase, Mur B, N-myristoyl transferase, and candida DHFR were downloaded from the protein databank. DNA gyrase and Mur B belong to the classification of a bacterial protein from the organism *E. coli*; N-myristoyl transferase belongs to the classification of a fungal protein from the organism *Aspergillus fumigatus;* and DHFR belongs to the classification of a fungal protein from the organism *Candida albicans*. The PDB file was imported into MOE software to prepare the protein. All the bound water molecules and heteroatoms were removed from the complex using the sequence (SEQ) window, a default option in the MOE programme. Both polar and non-polar hydrogens were added, and the 3D structure was corrected and used for docking simulation.

2.4.2 Preparation of ligand

The chemical structures of chloroacetamide derivatives were drawn using ACD Chemsketch software. The SMILES structure of the compounds was incorporated into the builder option of the MOE programme. After energy minimization and generation of the 3D structure, the potential energy and partial charge were corrected and used for the docking study.

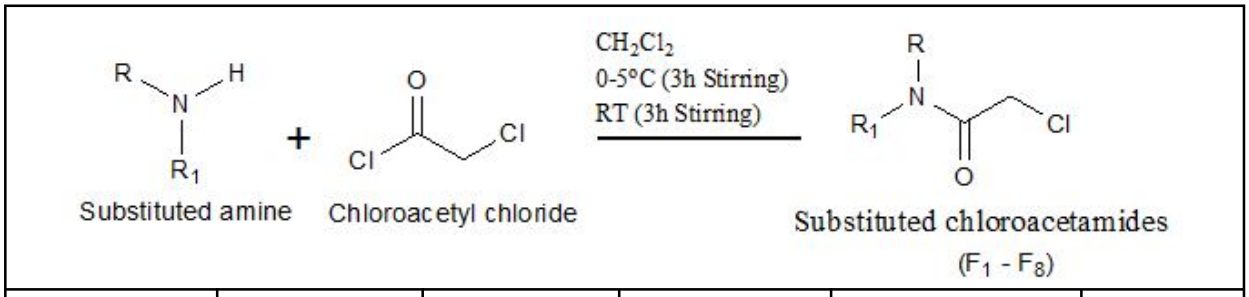
2.4.3 Docking process

The optimised target ligands were docked with the enzymes DNA gyrase (1KZN), Mur B (2Q85), N-myristoyl transferase (4CAW), and DHFR (4HOE). For docking simulations, the placement was set as a triangular matcher, the number of retaining was set at 10, and the refinement was set as a forcefield on the MOE suite to generate 10 poses of each target ligand confirmation. The most appropriate docked ligand target structure was selected on the basis of higher S-score values. The S-score is the value calculated by the built-in scoring functions of MOE on the basis of ligand binding affinity with the receptor protein after docking. In addition, the ligand-receptor interaction, followed by the 3D ligand protein pose, was viewed for interpretation.

3. Results

3.1 Chemistry

Aryl chloroacetamide derivatives were synthesised by condensation reactions between substituted amines and chloroacetyl chloride in the presence of dichloromethane (Scheme 1). The target compounds were obtained in good yield.



Compounds	R	R^1	Compounds	R	R^1
F_1	2-pyridyl	H	F_5	phenyl	methyl
F_2	benzyl	H	F_6	2-chlorophenyl	H
F_3	phenyl	phenyl	F_7	salicyl	H
F_4	octadecyl	H	F_8	4-nitrophenyl	H

Scheme 1: Synthesis of chloroacetamide.

Thin-layer chromatography was done to check the purity of the synthesised compounds using a silica gel G-coated plate as the stationary phase and hexane and ethyl acetate as the mobile phases (7:3). Iodine vapour as a visualising agent and a UV chamber for spot identification The melting points of the compounds were determined using the open capillary method and presented uncorrected. These synthesised chloroacetamides were confirmed by spectral methods (Manjuparkavi *et al*., 2022). Physical data yield and spectral data were summarised below.

2-chloro-*N*-(pyridin-2-yl) acetamide (F_1)

Pink powder, yield: 30%, m.p (C): 77, λmax (nm): 505, IR (γcm^{-1}): 3110.97 (amide N-H str), 3060.62 (Ar C-H str), 2995.25 (methylene C-H Str), 1581.52 (pyr C=N Str), 1681.81 (amide C=O Str), 1541.52 (Ar C=C Str), 775.33 (C-Cl Str). ^{1}H NMR (γ ppm): 4.1 (2H, s), 6.8-8.3 (4H, s), 7.8 (1H, s); MS (m/z): 169.7 ($C_7H_7ClN_2O^+$), 135.1 ($C_7H_7N_2O^+$), 122.3 ($C_6H_6N_2O^+$).

N-benzyl-2-chloroacetamide (F_2)

White powder, yield: 42, m.p (C): 84, λmax (nm): 389, IR (γcm^{-1}): 3278.78 (amide N-H Str), 3006.82 (Ar C-H Str), 2937.38 (methylene C-H Str), 1649.02 (amide C=O Str), 1427.23 (Ar C=C Str), 696.25 (C-Cl Str), ^{1}H NMR (γppm): 4.3 (2H, s), 4.5 (2H, d), 6.9-7.2 (5H, m), 8.1 (1H, t); MS (m/z): 183.8 ($C_9H_{10}ClNO^+$), 148.3 ($C_9H_{10}NO^+$), 106.7 ($C_7H_9N^+$), 78.4 ($C_6H_6^+$).

2-chloro-N, N- diphenyl acetamide (F_3)

Light blue powder, yield: 54, m.p (C): 103, λmax (nm): 477, IR (γcm^{-1}) 3004.69 (Ar C-H Str), 2945.10 (methylene C-H Str), 1679.88 (amide C=O Str), 1492.80 (Ar C=C Str), 750.26 (C-Cl Str), ^{1}H NMR (γppm): 4.4 (2H, s), 7.2-7.7 (10H, m); MS (m/z): 244.6 ($C_{14}H_{12}ClNO^+$), 169.2 ($C_{12}H_{11}N^+$), 77.6 ($C_6H_6^+$).

2-chloro-N-octadecylacetamide (F_4)

White powder; Yield: 88; m.p (C): 114; λmax (nm): 362; IR (γcm^{-1}): 3290.33 (Amide N-H Str), 2954.74 (methyl C-H Str), 2916.17, 2848.67 (methylene C-H Str), 1647.10 (amide C=O Str), 773.40 (C-Cl Str); ^{1}H NMR (γppm): 0.9 (3H, t), 1.24-1.29 (CH_2, m), 7.5 (1H, d); MS (m/z): 345.54 ($C_{20}H_{40}ClNO^+$).

2-chloro-N-methyl-N-phenylacetamide (F_5)

Brown powder; Yield: 47%; m.p (C): 110; λmax (nm): 396; IR (γcm^{-1}): 3045.39 (Ar C-H Str), 2883.38 (methyl C-H Str), 2759.95 (methylene C-H Str), 1674.10 (amide C=O Str), 1494.73 (Ar C=C Str), 705.90 (C-Cl Str); ^{1}H NMR (γppm): 2.6 (3H, s), 4.2 (2H, s), 7.0-7.3 (5H, m); MS (m/z): 183.0 ($C_9H_{10}ClNO^+$), 135.3 ($C_8H_9NO^+$), 106.2 ($C_7H_8N^+$), 92.7 ($C_6H_7N^+$), 78.4 ($C_6H_6^+$).

2-chloro-N-(2-chlorophenyl) acetamide (F_6)

White powder; Yield: 63%; m.p (C): 85; λmax (nm): 462; IR (γcm^{-1}): 3269.12 (Amide N-H Str), 3043.46 (Ar C-H Str), 2999.10 (methylene C-H Str), 1672.17 (amide C=O Str), 1539.09 (Ar C=C Str), 757.97 (C-Cl Str); ^{1}H NMR (γppm): 4.5 (2H, s), 6.7-7.7 (4H, m), 8.2 (1H, d); MS (m/z): 203.6 ($C_8H_7Cl_2NO^+$), 168.3 ($C_8H_7ClNO^+$), 155.2 ($C_7H_6ClNO^+$), 93.4 ($C_6H_7N^+$), 77.6 ($C_6H_6^+$).

4-(2-chloroacetamido)-2-hydroxybenzoic acid (F_7)

Grey powder; Yield: 45%; m.p (C): 290; λmax (nm): 489; IR (γcm^{-1}): 3259.47 (phenolic O-H Str), 3218.97 (amide N-H Str), 3097.47 (Ar C-H Str), 2993.32 (methylene C-H Str), 2611.44-2528.50 (carboxylic O-H Str), 1668.31 (amide C=O Str), 1569.95 (carboxylic C=O Str), 1454.23 (Ar C=C Str), 763.76 (C-Cl Str); ^{1}H NMR (γppm): 4.1 (2H, s), 5.4 (1H, s), 7.4-7.9 (3H, d), 8. 3 (1H, d), 10.1 (1H, s); MS (m/z): 229.8 ($C_9H_8ClNO_4^+$), 180.7 ($C_8H_7NO_4^+$), 152.1 ($C_7H_6NO3^+$), 78.4 ($C_6H_6^+$).

2-chloro-N-(4-nitrophenyl) acetamide (F_8)

Light green powder; yield: 65%; m.p (C): 178; λmax (nm): 507; IR (γcm^{-1}): 3226.35 (amide N-H Str) 3103.25 (Ar C-H Str), 2827.45 (methylene C-H Str), 1685.67 (amide C=O Str), 1571.88, 1562.23 (Ar C=C Str), 1506.23 (Ar-NO_2 Str), 773.40 (C-Cl Str); ^{1}H NMR (γppm): 4.2 (2H, s), 7.7-8.3 (4H, m), 7.9 (1H, d); MS (m/z): 213.7 ($C_8H_7ClN_2O_3^+$), 138.2 ($C_6H_6N_2O_2^+$), 92.6 ($C_6H_7N^+$).

3.2 Molecular property determination

Molinspiration's open-access web tool programme is based on the Lipinski rule of five, which predicts drug-like properties of compounds. The drug-like properties of the synthesised compounds are tabulated in Table 1.

Table 1: Molecular properties of synthesized compounds

Compounds	Log P	TPSA	nat	nON	nOHNH	nVio	nrotb	Volume
F_1	0.82	41.99	11	3	1	0	2	141.61
F_2	1.42	29.10	12	2	1	0	3	162.57
F_3	3.66	20.31	17	2	0	0	3	217.56
F_4	8.43	29.10	23	2	1	1	18	376.55
F_5	1.97	20.31	12	2	0	0	2	162.71
F_6	2.35	29.10	12	2	1	0	2	159.30
F_7	1.63	86.62	15	5	3	0	3	180.79
F_8	1.68	74.92	14	5	1	0	3	169.10

TPSA-Total Polar Surface Area, nat - Number of atoms, nON-Hydrogen bond acceptor, nOHNH - Hydrogen bond donor, nVio - Number of violations, nrotb-Number of rotatable bonds.

3.3 ADMET prediction

The pharmacokinetic properties of the synthesised chloroacetamide compounds were predicted using the online web tool PreADMET (Table 2).

Table 2: ADMET properties of synthesized chloroacetamides

Compounds	BBB	$CaCO_2$	CYP inh /subs	%HIA	Pgp-inh	PPB	AMES Test	Carcino-genicity	hERG-inh (risk)
F_1	0.50	21.14	Non	94.59	Non	8.09	Mutagen	Positive	Low
F_2	0.88	1.55	Non	97.15	Non	45.44	Mutagen	Positive	Medium
F_3	1.77	28.53	Non	100	Inh	89.80	Mutagen	Positive	Low
F_4	16.7	43.40	Non	97.54	Inh	100	Non	Negative	Low
F_4	16.7	43.40	Non	97.54	Inh	100	Non	Negative	Low
F_5	1.45	27.25	Non	100	Non	36.16	Mutagen	Positive	Medium
F_6	0.66	20.62	Non	91.75	Inh	60.56	Mutagen	Positive	Low
F_7	0.44	21.09	Non	86.72	Non	35.16	Mutagen	Positive	Low
F_8	0.66	20.62	Non	91.75	Inh	60.56	Mutagen	Positive	Low

BBB: Blood brain barrier, Pgp: pearmeability glycoprotein, $CaCO_2$: cell permeability, CYP inh/subs: Cytochrome inhibition/substrate, PPB: plasma protein binding, HIA:Human intestinal absorption, hERG: human ether-a-go-go related gene potassium channel inhibition.

3.4 *In vitro* antimicrobial activity

3.4.1 Minimum inhibitory concentration (MIC)

The synthesised chloroacetamide derivatives did not show any visible growth at the lowest concentration of 100 µg/100 µl.

3.4.2 Antibacterial and antifungal activity

The synthesised chloroacetamide derivatives were prepared and tested in concentrations of 100, 200, 400, and 800 µg/100 µl respectively, in DMF on bacterial and fungal strains for their growth inhibitory activity. The growth inhibitions measured in terms of zone of inhibition in mm diameter are shown in Tables 3 and 4, respectively.

Table 3: Growth inhibitory activity of chloroacetamides on bacterial strains

Compounds	Conc.	Zone of inhibition in mm diameter			
	(µg/100 µl)	*B. subtilis*	*E. coli*	*P. vulgaris*	*K. pneumoniae*
F_1	100	18.00 ± 0.58	16.70 ± 0.33	22.00 ± 1.73	18.00 ± 1.15
	200	21.30 ± 0.67	19.60 ± 1.67	23.33 ± 4.37	24.66 ± 0.66
	400	24.00 ± 0.58	24.30 ± 0.88	24.00 ± 3.05	25.66 ± 1.45
	800	31.00 ± 0.58	25.00 ± 0.58	30.00 ± 2.88	27.00 ± 0.58
F_2	100	17.30 ± 1.45	24.70 ± 2.19	-	-
	200	19.67 ± 1.2	28.30 ± 4.41	19.00 ± 0.58	16.00 ± 0.58
	400	21.00 ± 0.58	28.00 ± 1.15	22.33 ± 1.20	22.33 ± 0.88
	800	28.00 ± 1.53	34.30 ± 4.26	23.33 ± 0.88	28.00 ± 1.15
F_3	100	29.00 ± 0.58	32.67 ± 1.33	17.00 ± 0.58	28.00 ± 1.15
	200	29.00 ± 0.58	33.00 ± 1.15	18.00 ± 1.15	33.66 ± 1.85
	400	29.00 ± 0.58	35.00 ± 3.21	19.00 ± 0.58	33.66 ± 1.85
	800	30.30 ± 0.88	36.67 ± 3.33	19.33 ± 1.20	34.00 ± 0.58
F_4	100	16.00 ± 0.58	24.00 ± 3.05	17.66 ± 1.45	-
	200	17.00 ± 0.58	25.00 ± 2.5	20.00 ± 2.88	-
	400	17.60 ± 1.20	27.70 ± 4.26	24.33 ± 3.48	-
	800	18.00 ± 1.15	28.00 ± 2.65	24.66 ± 0.88	-
F_5	100	17.30 ± 0.88	20.30 ± 0.33	28.33 ± 1.45	18.00 ± 1.15
	200	18.66 ± 1.20	24.00 ± 1.52	29.33 ± 2.33	21.66 ± 2.33
	400	20.00 ± 1.15	25.00 ± 3.28	34.33 ± 2.60	27.33 ± 0.08
	800	20.30 ± 0.88	27.00 ± 0.58	34.66 ± 0.33	32.66 ± 0.88
F_6	100	19.60 ± 0.88	-	-	22.00 ± 0.58
	200	23.00 ± 0.58	-	-	23.00 ± 1.15
	400	25.00 ± 0.58	17.30 ± 0.88	-	23.66 ± 1.45
	800	27.33 ± 0.88	22.30 ± 1.20	-	26.66 ± 0.88
F_7	100	-	-	22.00 ± 0.58	16.00 ± 0.58
	200	-	-	23.00 ± 0.58	17.00 ± 0.58
	400	13.33 ± 0.33	17.30 ± 1.76	24.00 ± 0.58	-
	800	15.00 ± 0.58	22.60 ± 1.45	25.33 ± 0.33	-
F_8	100	13.33 ± 0.33	22.00 ± 0.58	19.00 ± 0.58	16.00 ± 0.58
	200	15.33 ± 0.88	23.60 ± 0.88	23.33 ± 1.76	18.00 ± 0.58
	400	15.66 ± 0.88	24.60 ± 0.88	26.33 ± 0.88	19.00 ± 0.58
	800	18.33 ± 0.33	25.60 ± 0.88	29.33 ± 0.88	20.33 ± 0.88
Standard (Gentamycin)	10 µg/ml	30.33 ± 1.20	28.66 ± 0.88	29.33 ± 0.88	28.00 ± 0.57

Values are presented as n=3, Mean ± SEM.

Table 4: Growth inhibition on fungal strains by chloroacetamides

Compounds	Conc. (µg/100 µl)	Zone of inhibition in mm diameter			
		C. albicans	*A. flavus*	*A. fumigatus*	*A. niger*
F_1	100	14.33 ± 0.33	17.33 ± 0.33	13.00 ± 0.33	-
	200	15.33 ± 0.88	21.33 ± 0.33	16.33 ± 0.88	-
	400	16.33 ± 0.88	21.33 ± 0.88	19.00 ± 1.00	-
	800	22.00 ± 0.58	22.66 ± 0.88	21.66 ± 0.88	-
F_2	100	-	18.66 ± 0.33	-	-
	200	-	20.33 ± 0.33	-	-
	400	-	20.33 ± 0.33	-	-
	800	-	21.66 ± 0.33	-	-
F_3	100	28.33 ± 1.45	22.33 ± 1.76	24.00 ± 1.00	29.66 ± 0.33
	200	29.66 ± 0.33	25.66 ± 1.76	26.66 ± 0.88	31.66 ± 0.88
	400	30.33 ± 2.40	29.00 ± 1.00	29.00 ± 0.58	34.00 ± 0.58
	800	32.00 ± 1.15	30.00 ± 0.58	30.00 ± 0.58	37.00 ± 1.73
F_4	100	-	-	-	-
	200	-	-	-	-
	400	-	-	-	-
	800	-	-	-	-
F_5	100	-	17.66 ± 0.88	24.66 ± 1.20	15.00 ± 1.15
	200	19.33 ± 1.45	24.33 ± 0.33	25.33 ± 1.20	-
	400	19.66 ± 0.88	25.00 ± 1.15	27.66 ± 1.45	-
	800	29.00 ± 2.02	29.66± 2.02	28.66 ± 0.88	24.66 ± 1.76
F_6	100	-	14.66 ±1.76	-	-
	200	-	16.00 ± 1.15	-	-
	400	-	20.00 ± 1.15	-	-
	800	-	23.00 ± 1.15	-	-
F_7	100	-	-	-	-
	200	-	-	-	-
	400	-	19.33 ± 0.66	-	-
	800	-	23.33 ± 0.66	-	-
F_8	100	-	19.33 ± 0.88	-	-
	200	-	26.00 ± 0.66	-	-
	400	-	-	-	-
	800	-	-	-	-
Standard (Fluconazole)	10 µg/ml	17.33 ± 1.20	21.33 ± 1.20	21.00 ± 0.57	24.33 ± 0.88

Values are presented as n=3, Mean ± SEM.

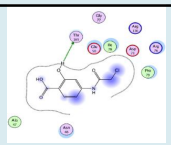
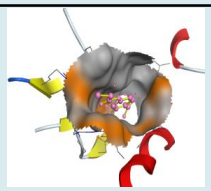
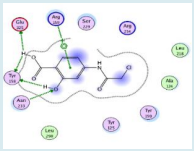
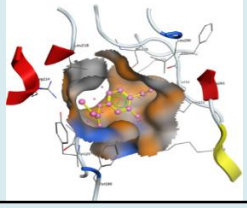
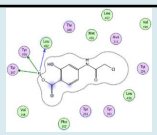
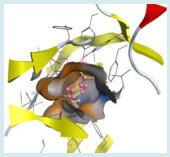
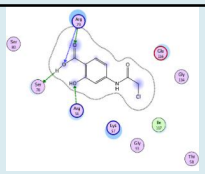
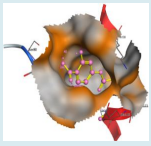
3.5 Molecular docking studies

Molecular docking has become a valuable tool in drug design and discovery processes to predict the mode of interaction of ligand molecules with macromolecular targets. An optimised conformation of the ligand-receptor complex with less binding energy was considered. The results of the binding affinity of synthesised chloroacetamides are shown in Table 5. Among the synthesised compounds, the compound F_7 bearing salicyl substituted nitrogen showed the highest binding affinity in all the targeted enzymes tested and had scores of -10.2 (DNA gyrase), -9.6 (Mur B), -14.2 (N-Myristoyl transferase), and -11.6 (DHFR), respectively. The protein ligand interactions are shown in Table 6.

Table 5: Binding affinity of synthesized chloroacetamides

Compounds	Binding affinity (kcal/mol)			
	DNA gyrase subunitB (1KZN)	Mur B (2Q85)	N-Myristoyl transferase (4CAW)	*C. albican* DHFR (4HOE)
F_1	-7.4	-7.0	-7.0	-7.6
F_2	-8.4	-7.6	-7.7	-7.8
F_3	-7.7	-8.9	-8.2	-9.9
F_4	-9.8	**-10.3**	-11.9	-10.0
F_5	-	-	-	-
F_6	-7.7	-7.3	-7.3	-8.2
F_7	**-10.2**	-9.6	**-14.2**	**-11.6**
F_8	-8.1	-7.7	-8.9	-8.8
Ciprofloxacin	-9.6	-	-	-
Fosfomicin	-	-8.0	-	-
[Cyclohexylethyl]-[[[[4-[2-methyl-1-imidazolyl-butyl] phenyl] acetyl]-seryl]-lysinyl]-amine	-	-	-15.3	-
Sulphadiazine	-	-	-	-10.3

Table 6: Interaction of compound F_7 with target protein

Target protein	2D interaction	3D interaction	Interactions
1KZN			Side chain acceptor (Thr 165); Greasy centre (Ala 47, Ile 78, Pro 79); Acidic centre (Glu 50, Asp 73);Basic centre (Arg 136, Arg 76)
(2Q85)			Side chain acceptor (Glu 325, Tyr 158); Greasy centre (Leu 218, Ala 124, Leu 290); Acidic centre (Glu 325); Basic centre(Arg 159, Arg 214); Arene-cation (Arg 159);
NMT(4CAW)			Side chain acceptor (Tyr 159, Tyr 147); Backbone donor (Leu 492); Greasy centre (Leu 492, Leu 457, Leu 436, Met 491, Val 490, Val 148, Phe 157)
C. albican DHFR (4HOE)			Side chain acceptor (Ser 781); Side chain donor (Arg 56, Arg 79); Backbone acceptor (Arg 79); Greasy centre (Ile 117); Acidic centre (Glu 116); Basic centre (Arg 79, Arg 56, Lys 57);

4. Discussion

Drug-like properties are of great importance for drug substances because they influence oral bioavailability, metabolism, excretion, toxicity, and biological activity. If, a molecule complies with the Lipinski rule, *i.e*., molecular weight ≤500 Da, lipophilicity score ≤5, hydrogen bond donors ≤5, hydrogen bond acceptors ≤10, and as per the Ghose and Veber rule, number of atoms between 20 and 70, molar refractivity between 40 and 130, then it is considered a drug-like molecule. From the above results, it was found that there was no violation of these properties, which indicated that the chloroacetamide derivatives agrees with these rules. Hence, all the compounds exhibited drug-like properties.

In addition to structural properties, potency, and selectivity, the pharmacokinetic properties (absorption, distribution, metabolism, excretion, and toxicity) of the molecule are crucial for the development of a successful drug molecule. From the above results, most of the synthesised compounds were found to have good absorption in the human intestine, medium intestinal permeability, and were non-substrate for Pgp, thus being predicted to be orally bioavailable. Synthesised compounds have low penetration into the CNS, so they are CNS inactive. Inhibition of hERG indicates a low risk of cardiovascular side effects. The compounds were also found to display non-carcinogenicity in rats.

The lowest concentration of the antimicrobial substance needed to kill the microbes is termed the MIC. This can be explained by the broth tubes that appeared turbid, indicative of bacterial growth and fungal growth, respectively, while tubes that remain clear indicate no growth of microorganisms (Amsa *et al*., 2022; Punit *et al*., 2021). Synthetic chloroacetamides showed no growth at 100 µg/100 µl. This was considered a MIC value. From the results obtained from the antibacterial study, all the compounds were found to be highly active against all the tested organisms at a higher concentration of 800 µg/100 µl. The compound F_3 was found to have the most promising growth inhibitory action against the gram-negative organisms *E. coli* and *K. pneumoniae* with 36 ± 3.33 mm and 34 ± 0.58 mm, respectively, and F_5 with 34 ± 0.33 mm inhibition on *P.vulgaris*. F_1 was shown to be the most active agent against the gram-positive organism *B. subtilis* with 31 ± 0.58 mm.

Based on the results of the antifungal study, it was found that the compound F_3 had the most effective agent against all the tested fungal strains at 800 µg/100 µl. Diaryl substituted chloroacetamide (F_3) was found to be highly active, followed by compound F_5, with a single aryl ring, and methyl substituted chloroacetamide, which was shown to be most active compared to standard.

Bacterial DNA gyrase (type II topoisomerase) has the function of catalysing the introduction of ATP-dependent negative supercoils into double stranded closed circular DNA and influencing the topology state of DNA. DNA gyrase has received a lot of attention as the intracellular target of antibacterial and anticancer agents (Bates and Maxwell, 2005). Peptidoglycan is a major component of the bacterial cell wall and has been a key target in antibacterial development due to its significant role in preserving the osmotic equilibrium between cell and environment. The MurA enzyme moves the enolpyruvate group from phosphoenolpyruvate to UDP-N-acetylglucosamine to make enolpyruvyl-UDP-N-acetylglucosamine, which is a part of peptidoglycan. Following this step, MurB completes the formation of UDP-N-acetyl muramic acid (UDP-MurNAc) using NADPH. UDP-MurNAc can serve as an attachment for the peptide portion. Resulting pentapeptides facilitate the cross-linking that provides rigidity to the cell wall (Park, 1987; Bugg and Walsh, 1992). NMT plays a crucial role in the transfer of myristate from myristoyl CoA to N-terminal glycine residues present in a variety of eucaryotic cellular and some viral proteins. NMT has been mentioned as a target since it participates in signalling networks and is necessary for the development of human pathogens, including *Candida* species. Significant differences exist between humans' and candida's NMT, which is helpful for the rational design of fungal growth inhibitors (Gordon *et al*., 1991; Thinon *et al*., 2014). The transformation of dihydrofolate into tetrahydrofolate in the folate biosynthesis pathway is catalysed by the enzyme DHFR. Tetrahydrofolate is necessary for many metabolic processes, including the formation of purines, pyrimidines, and DNA. Cell death occurs as a result of the suppression of DHFR, which ultimately prevents the synthesis of DNA. Folate biosynthesis is considered a drug target for the production of antifungal agents. Inhibition of DHFR offers an efficient target for the development of inhibitors with potent antifungal action and sufficient selectivity towards DHFR (Paulsen *et al*., 2009). According to structural biology research, the active sites differ between human and candida species and could be used to create selective inhibitors (Liu *et al*., 2018).

Molecular docking studies of synthesised compounds with different microbial target proteins, including bacteria and fungi, showed that the compound F_7 had the highest binding score. Antibacterial and antifungal agents have diverse mechanisms of action, viz., they may inhibit cell wall synthesis, depolarize the cell membrane, inhibit protein synthesis, inhibit nucleic acid synthesis, and inhibit metabolic pathways in bacteria and fungi. Based on this available mechanism of action, our study has hypothesised the selection of proteins of interest, such as those needed for cell wall synthesis and nucleic acid synthesis. We aimed to go with these two mechanisms of action. Among the synthesised compounds, F_3 showed the best antibacterial and antifungal activity against the tested microbes at 800 µg/100 µl. However, the *in silico* docking result showed the compound F_7 had a better and slightly improved binding affinity score in the range of -9 to -14 kcal/mol when compared to other compounds. This uneven antimicrobial and *in silico* result may be due to the selection of the protein or to the presence of hydrogen bond donor and acceptor groups like -NH, -OH, C=O, *etc*., which are present in the structure of the compound F_7. Moreover, the compound F_3 fails to have this group as it contains only tertiary nitrogen in its structure. This may be the reason for uneven results with target compounds.

5. Conclusion

The synthesis of derivatives bearing acetamide is carried out by the condensation of substituted amines and chloroacetyl chloride and is characterised structurally by means of spectral methods. Molecular property evaluation of compounds revealed that the acetamides fulfil the requirements of the Lipinski rule of five and are predicted to be orally bioavailable. The ADMET properties of synthesised acetamides were predicted to be well absorbed in the human intestine, inactive in the CNS, low risk of CVS side effects, and non-carcinogenic in rats. The lowest concentration of the synthesised compounds to inhibit the growth of bacteria and fungi was determined and found to be 100 µg/100 µl. The antibacterial and antifungal activities of synthesised

chloroacetamides were determined. The compound (F_3) with diaryl substituted nitrogen showed promising growth inhibition against all the tested bacterial and fungal strains at 800 µg/100 µl, and followed by compound F_5 with a single aryl ring and methyl substituted nitrogen, which was shown to be most active compared to standard. Molecular docking studies of synthesised compounds showed that the compound F_7 had the highest binding score. This result may be due to the presence of a higher number of hydrogen bond donors and acceptors in F_7. The study concluded that the acetamide derivatives may act as the lead and require optimisation.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article

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