Journal of Infection and Public Health 13 (2020) 1951–1960

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Original Article

Antimicrobial activity of novel 5-benzylidene-3-(3-phenylallylideneamino)imidazolidine-2,4-dione derivatives causing clinical pathogens: Synthesis and molecular docking studies

Daoud Ali [a], Saud Alarifi [a], Sathish Kumar Chidambaram [b], Surendra Kumar Radhakrishnan [b], Idhayadhulla Akbar [b,*]

[a] Department of Zoology, College of Sciences, King Saud University (KSU), P.O. Box 2455, Riyadh 11451, Saudi Arabia
[b] Research Department of Chemistry, Nehru Memorial College (Affiliated to Bharathidasan University), Puthanampatti - 621007, Tiruchirappalli District, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 3 August 2020
Received in revised form
14 September 2020
Accepted 27 September 2020

Keywords:
Imidazolin-2,4-dione
Antibacterial activity
Antifungal activity
1U1Z
1AI9 kinases molecular docking

ABSTRACT

Background: This work is development of new hydantoin molecules as treatment of potential antibacterial and antifungal activity against clinical pathogens causing infectious disease. Synthesized compounds were evaluated in molecular docking studies, the most effective compound is used to dock against the targets of 1U1Z, and 1AI9 kinases, to evaluate its binding affinity, hoping to rationalize and obtain potent of antibacterial, antifungal agents.

Material and method: The FTIR, ^{1}H & ^{13}C NMR, and mass spectra were used to conform new molecules and their evaluation of antimicrobial activity. Gram-negative bacteria of *Pseudomonas aeruginosa* (ATCC-27853), *Klebsiella pneumoniae* (recultured) and *Escherichia coli* (ATCC-25922), and gram-positive bacteria of *Enterococcus faecalis* (recultured) and *Staphylococcus aureus* (ATCC-25923) were evaluated for all compounds. The *in vitro* antifungal activity was evaluated against *Cryptococcus neoformans* (recultured), *Candida albicans* (recultured), *Aspergillus niger*, *Microsporum audouinii* (recultured) and *Aspergillus fumigatus* (recultured) for all synthesized compounds.

Result: Antibacterial screening, we identified highly active antimicrobial agents for this study for example; gram-negative bacterial screening of **3g** was highly (MIC: 0.25 μg/mL) active in contradiction of *P. aeruginosa*, whereas bacterial screening of **3e** and **3h** were more active (MIC: 2 μg/mL) in contradiction of *K. pneumoniae* and also **3g** was more (MIC: 2 μg/mL) active in contradiction of *E. faecalis* than standard ciprofloxacin. Antifungal activity, the **3b** was more active (MIC: 0.25 μg/mL) against *C. albicance,* **3g** (MIC: 2 μg/mL) and **3h** (MIC: 4 μg/mL) were more potential of *A. funigatus,* and the compound **3c** was highly (MIC: 4 μg/mL) *active* on *M. audouinii* than clotrimazole. Molecular docking studies also supported the new finding of potent antimicrobial agents, the compound **3g**, **3b**, and controls Ciprofloxacin, Clotrimazole were checked again proteins 1U1Z and 1AI9 by Autodock Vina program. The compound **3g** was highest binding affinity (−8.4 kcal/mol) than ciprofloxacin (−8.2 kcal/mol) in 1U1Z protein and the compound **3b** was highest binding affinity (−8.8 kcal/mol) than clotrimazole (−6.8 kcal/mol) in 1AI9 protein respectively.

Conclusion: A novel set of imidazolidine-2,4-dione compounds **3a–h** have synthesized and characterized successfully. The screening of antimicrobial activity shows that all compounds possess antimicrobial activities. In addition, the objective of the study was succeeded with a few of the promising molecules, which are proving to be a potential treatment of bacterial infection candidates.

* Corresponding author.
E-mail addresses: a.idhayadhulla@gmail.com, idhayadhulla@nmc.ac.in (I. Akbar).

https://doi.org/10.1016/j.jiph.2020.09.017

Introduction

The imidazolidin-2,4-diones moiety (hydantoin group) embodies an significant of pharmacophore, which involved bio activities of antifungicidal [1], anti-inflammatory [2], Hypoglycemic [3], Serotonin transporter activity [4], active in *Pseudomonas aeruginosa* [5], antibacterial and antifungal activities [6], antitumor activity, and molecular modeling study [7], and HIV-1 fusion inhibitors [8]. Fig. 1 indicates some natural and synthetic imidazoline-2,4-dione derivatives. This research, focus lead molecules of hydantoin related derivatives have been delivering the microbial pharmaceuticals filed. Based on this selection, a new compound hydantocidin which found in *Streptomyces hygroscopicus* SANK 63584, it is application of non-selective herbicidal [9], and tryptophan type aplysinopsins from marine organisms. Earlier reports, aplysinopsin analogs have various biological activities, particularly inflection of neurotransmissions [10]. Naamines, naamidines, and marine natural products have been novel molecules for phytopathogenic fungi and virus [11], and also nitrofurantoin antibacterial agent was active against various bacterial infection caused. Nitrofurantoin to have good activity against such as *E. coli*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Coagulase negative staphylococci*, *Klebsiella* species, *Citrobacter species, Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus subtilis* species, these organisms used for treatment of infectious disease [12]. However side effects identified that nitrofurantoin such as headaches, loss of appetite, include nausea, liver problems, and or urinary tract prophylaxis may occur [13]. Basically imidazolidin-2-4-dione derivatives are good biological behavior and particularly antibacterial activity, based on above observation, we find new imidazolidin-2-4-dione target molecules for antimicrobial agent with low side effects. Therefore, we have to make novel hydantoin core molecules and evaluation of antimcrobial activity with focusing molecular docking studies.

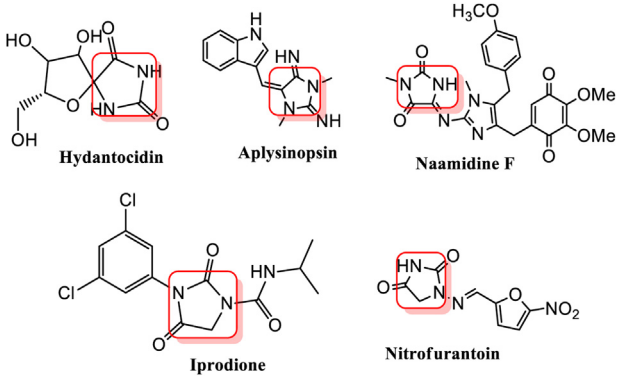


Fig. 1. Biologically active natural imidazolidin-2,4-dione.

Material and methods

Chemistry

FT-IR(KBr, 4000–400 cm^{-1})-Shimadzu 8201PC used to analyze the functional groups present in all compounds. ^{1}H NMR(300 MHz) & ^{13}C NMR(75 MHz)- Bruker spectrometer. Thin layer chromatography-check the purity on silica gel plates. JMS D-300 (70 eV) used to record Mass spectrum (EI). The elemental analyses were checked by all compounds via Vario EL III- model.

Synthesis of (*E*)-2-((*E*)-3-phenylallylidene)hydrazinecarboxamide (1)

A reaction mixture, cinnamaldehyde (0.01 mol, 1.32 g), semicarbazone (0.01 mol, 0.75 g), and ethanol (10 mL) were taken in RB flask with reflux up to 2 h in RT. Evolution of reaction was checked via thin layer chromatography. Purified compound was separated from column chromatography.

Yellow solid; Yield: 76%; M.p. 152–154 °C; mw189; IR(KBr): 3323, 2974, 1655, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ : 10.80 (s, 1H, NH), 7.52–7.40(m, 5H, Ph-ring), 7.25 (d, J = 3.1 Hz, 1H, CH), 7.12(s, 1H), 6.85 (d, J = 3.1 Hz, 1H), 6.10(s, 2H); ^{13}C NMR (75 MHz) δ : 157.1, 137.8, 134.2, 134.1, 133.1, 128.5, 127.5, 127.2, 126.5; EI-MS, m/z (relative intensity %): m/z 190.21 (M^+, 16%); HREIMS: m/z: calcd for $C_{10}H_{11}N_3O$: 189.0831, found 189.0841; Anal. calcd. For $C_{10}H_{11}N_3O$: C, 63.48; H, 5.86; N, 22.21; Found: C, 63.49; H, 5.84; N, 22.19.

3-(3-phenylallylideneamino)imidazolidine-2,4-dione (2)

The compound **1** (0.01 mol, 1.892 g), fused sodium acetate (0.03 mol, 2.46 g), ethylchloroacetate (0.01 mol, 1.22 g), and ethanol (10 mL) were taken RB flask and stirred at RT up to 4 h. The final solid material was filtered and recrystallized from suitable solvent.

Yield: 80%; brown solid; M.p. 167–169 °C; mw 229; IR(KBr): 2981, 1659, 1648 cm^{-1}; ^{1}H NMR (300 MHz) δ 10.78(s, 1H, NH), 7.68–7.38(m, 5H, Ar–H), 7.35(s, 1H, HC=N), 7.20 (s, 1H), 3.82(s, 2H); ^{13}C NMR (75 MHz): δ 164.3, 157.8, 137.1, 135.6, 134.1, 133.1, 128.5, 128.1, 127.0, 126.5, 45.2; MS (m/z): m/z 229.21 (M^+, 23%); HREIMS: m/z: calcd for $C_{12}H_{11}N_3OS$: 229.0121, found 229.0165; Anal. calcd. For $C_{12}H_{11}N_3OS$: C, 62.87; H, 4.84; N, 18.33; found: C, 62.83; H, 4.81; N, 18.30.

(Z)-5-benzylidene-3-((*Z*)-((*E*)-3-phenylallylidene)amino) imidazolidine-2,4-dione(3a)

The compound **2** (0.01 mol, 2.29 g), benzaldehyde (0.01 mol, 1.06 g), and ethanol (10 mL) were taken RB flask and stirred at RT up to 1 h. Purity was check by thin layer. After the completed reaction, filtered of final compound **2** and used suitable solvent for recrystallization of the final product. The remaining compounds (**3b–3i**) where prepared by following by above procedure.

Yellow solid; Yield 89%; M.p. 242–225 °C; m.w.317; IR (KBr): 3032, 2921, 1668, 1633, 1090 cm^{-1}; ^{1}H NMR(300 MHz) δ 10.59(s, 1H, HN), 7.66–7.60 (d, J = 3.7 Hz, 1H,), 7.62–7.32 (m, 10H), 7.20(s, 1H, HC=N), 6.54–6.51(d, J = 3.7 Hz, 1H), 5.28 (s, 1H) ; ^{13}C NMR (75 MHz) δ 158.5, 157.6, 137.3, 136.8, 135.6, 128.1, 127.9, 127.0, 126.5, 123.0, 114.0; MS (m/z): 318.14 (M^+, 16%); HREIMS: m/z: calcd for $C_{19}H_{15}N_3S_2$: 318.0221, found 318.0125; Anal. calcd. For $C_{19}H_{15}N_3S_2$: C, 71.91;H, 4.76;N, 13.24; Found: C, 71.90; H, 4.75; N, 13.21;

5-(4-chlorobenzylidene)-3-((*Z*)-((*E*)-3-phenylallylidene)amino)imidazolidine-2,4-dione (3b)

Yellow solid; Yield: 80%; M.p. 234–238 °C; mw 350; IR (KBr):3038, 2926, 1664, 1631, 1082, 681 cm^{-1}; ^{1}H NMR (300 MHz): δ10.78 (s, 1H, HN), 7.70–7.68 (d, J = 3.7 Hz, 1H), 7.67–7.62 (m, 5H,), 7.61–7.59(d, J = 7.25 Hz, 2H, Ph–Cl), 7.48–7.44(d, J = 7.20 Hz, 2H, Ph–Cl), 7.22(s, 1H, CH), 6.50(s, 1H, CH), 5.27 (s, 1H, CH); ^{13}C NMR (75 MHz): δ 158.4, 157.2, 137.1, 136.6, 135.6, 133.2, 133.0, 128.1, 128.7, 128.6, 127.9, 127.0, 126.6, 123.2, 114.9; MS (m/z): 351.79 (M+H^+, 16%); HREIMS: m/z: calcd for $C_{19}H_{14}ClN_3O_2$: 351.0321, found 351.0155; Anal. calcd. For $C_{19}H_{14}ClN_3O_2$: C, 64.87; H, 4.01; Cl, 10.08; N, 11.94; O, 9.10; Found: C, 71.90; H, 4.75; N, 13.21;

(*Z*)-5-(4-hydroxybenzylidene)-3-((*Z*)-((*E*)-3-phenylallylidene)amino)imidazolidine-2,4-dione(3c)

Yellow solid; Yield: 83%; M.p. 210–213 °C; mw 332; IR (KBr): 3557, 3039, 2929, 1636, 1661, 1082 cm^{-1}; ^{1}H NMR (300 MHz): δ 10.68 (s, 1H), 7.64–7.67 (5H, m), 7.63 (d, J = 3.7 Hz, 1H), 7.55–7.53(d, J = 7.18 Hz, 2H, Ph–OH), 7.22(s, 1H, CH), 6.64–6.62(d, J = 7.20 Hz, 2H, Ph–OH), 6.52(s, 1H), 5.32(s, 1H, –OH), 5.26 (s, 1H, CH); ^{13}C NMR (75 MHz): δ 158.7, 157.8, 155.9, 137.4, 136.2, 135.6, 130.5, 128.1, 127.9, 127.0, 126.2, 125.8, 122.9, 114.3, 114.1; MS (m/z): 333.14 (M+H^+, 22%); HREIMS: m/z:

calcd for $C_{19}H_{15}N_3O_3$: 333.0181, found 333.0215; Anal. calcd. For $C_{19}H_{15}N_3O_3$: C, 68.46; H, 4.54; N, 12.61; O, 14.40; Found: C, 68.45; H, 4.75; N, 13.21;

(*Z*)-5-(4-methoxybenzylidene)-3-((*Z*)-((*E*)-3-phenylallylidene)amino)imidazolidine-2,4-dione(3d)

Yellow solid; Yield: 81%; M.p. 230–233 °C; mw 347.37; IR(KBr): 3039, 2945, 2852, 1635, 1662, 1085 cm^{-1}; ^1H NMR (300 MHz) : δ 10.61 (s,1H, HN), 8.28–8.24(d, 2H, J = 7.02 Hz, Ph–OCH_3), 7.68–7.64(m, 10H), 7.62 (d, J = 3.7 Hz, 1H, CH), 7.24(s, 1H, CH), 6.96–6.94(d, J = 7.05 Hz, 2H, Ph–OCH_3), 6.54(s, 1H, CH), 5.89(s, 1H, –CH), 5.28(s, 1H, CH), 3.83(s, 3H); ^{13}C NMR (75 MHz):δ 158.7, 158.5, 157.2, 137.7, 136.0, 135.2, 128.2, 127.4, 127.2, 126.2, 123.4, 122.9, 116.3, 115.9, 114.1, 55.1; MS (m/z): 348.15 (M^+, 16%); HREIMS: m/z: calcd for $C_{20}H_{17}N_3O_3$: 348.07121, found 348.0745; Anal. calcd. For $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10; Found: C, 70;10; H, 4.94; N, 12.11;

(*Z*)-5-(4-methylbenzylidene)-3-((*Z*)-((*E*)-3-phenylallylidene)amino)imidazolidine-2,4-dione(3e)

Yellow solid; Yield: 86%; M.p. 205–217 °C; mw 331.37; IR (KBr):3039, 2956, 1664, 1630, 1084 cm^{-1}; ^1H NMR (300 MHz)δ: 10.69 (1H, s, HN), 7.64(d, J = 3.7 Hz, 1H, CH), 7.66–7.64 (m, 5H, Ph), 7.58–7.51(d, J = 7.26 Hz, 2H, Ph–CH_3), 6.51(s, 1H, CH), 7.22(s, 1H, CH), 7.16–7.12(d, J = 7.21 Hz, 2H, Ph–CH_3), 5.90(s, 1H, –CH), 5.26(s, 1H, CH), 2.31(s, 3H); ^{13}C NMR (75 MHz): δ 158.7, 157.60, 138.5, 137.1, 136.6, 134.9, 128.0, 127.7, 127.2, 128.7, 126.7, 123.3, 114.2, 21.0; MS (m/z): 332.16 (M+H^+, 35%); HREIMS: m/z: calcd for $C_{20}H_{17}N_3O_2$: 332.0701, found 332.0712; Anal. calcd. For $C_{20}H_{17}N_3O_2$: C, 72.49; H, 5.17; N, 12.68; Found: C, 72.50; H, 5.18; N, 12.66.

(*Z*)-5-(4-(dimethylamino)benzylidene)-3-((*Z*)-((*E*)-3-phenylallylidene)amino)imidazolidine-2,4-dione(3f)

Yellow solid; Yield: 89%; M.p. 211–216 °C; mw 360; IR (KBr): 3039, 2917, 1662, 1635, 1088 cm^{-1}; ^1H NMR (300 MHz): δ 10.58(s, 1H, HN), 7.72 (d, J = 3.7 Hz, 1H), 7.70–7.68 (m, 5H, –Ph), 7.68–7.64(d, J = 7.10 Hz 2 H), 7.24(s, 1H), 6.73–6.70(d, J = 7.08 Hz, 2H, Ph–$N(CH_3)_2$), 6.51(s, 1H), 5.26(s,1H), 3.08(s, 6H) ; ^{13}C NMR (75 MHz):δ 158.7, 157.4, 151.2, 137.6, 136.6, 135.8, 129.5, 128.0, 127.8, 127.0, 126.0, 123.6, 123.1, 114.9, 110.5, 42.1; MS (m/z): 361.10 (M+H^+, 33%); HREIMS: m/z: calcd for $C_{21}H_{20}N_4O_2$: 361.10 01, found 361.10 0252; Anal. calcd. For $C_{21}H_{20}N_4O_2$: C, 69.98; H, 5.59; N, 15.55; Found: C, 69.99; H, 5.60; N, 15.54;

(*Z*)-5-(4-bromobenzylidene)-3-((*Z*)-((*E*)-3-phenylallylidene)amino)imidazolidine-2,4-dione(3g)

Yellow solid; Yield: 80%; M.p. 212–215 °C; mw396; IR (KBr): 3038, 2924, 1668, 1631, 1082, 754 cm^{-1}; ^1H NMR (300 MHz): δ 10.72 (s, 1H, HN), 7.69 (d, J = 3.7 Hz, 1 H), 7.66–7.60 (m, 10H,), 7.62–7.59(d, J = 7.62 Hz, 2H, Ph-Br), 7.59(s, 1H), 7.55–7.52(d, J = 7.60 Hz, 2H, Ph–Br), 6.54 (s, 1H), 5.24(s, 1H) ; ^{13}C NMR (75 MHz): δ 158.2, 157.2, 137.0, 136.2, 135.8, 133.6, 132.0, 129.2, 128.2, 127.8, 127.9, 126.9, 123.2, 122.4, 114.2; MS (m/z): 397.10 (M+H^+, 05%); HREIMS: m/z: calcd for $C_{19}H_{14}BrN_3O_2$: 397.1001, found 397.1052; Anal. calcd. For $C_{19}H_{14}BrN_3O_2$: C, 57.59; H, 3.56; N, 10.60; found: C, 57.58; H, 3.55; N, 10.62;

(*Z*)-5-(4-nitrobenzylidene)-3-((*Z*)-((*E*)-3-phenylallylidene)amino)imidazolidine-2,4-dione(3h)

Yellow solid; Yield: 86%; M.p. 239–241 °C; MW 362; IR (KBr): 3033, 2922, 1665, 1640, 1095, 878 cm^{-1}; ^1H NMR (300 MHz): δ 10.69(s, 1H, HN), 8.22–8.19 (d, 2H, J = 7.52 Hz, Ph-NO_2), 8.12–8.09 (d, J = 7.50 Hz, 2H), 7.69–7.67 (m, 5H), 7.64 (d, 1H, J = 3.7 Hz, CH), 7.18(s, 1H), 6.51(s, 1H), 6.06(s, 1H), 5.20 (s, 1H) ; ^{13}C NMR (75 MHz):δ 158.8 156.9, 148.5, 141.2, 137.3, 137.1, 134.3, 128.7, 128.5, 127.0, 126.9, 126.2, 123.3, 122.2, 116.2, 114.0; MS (m/z): 363.14 (M+H^+, 16%); HREIMS: m/z: calcd for $C_{19}H_{14}N_4O_4$: 363.1412, found 363.1401; Anal. calcd. For $C_{19}H_{14}N_4O_4$: C, 62.98; H, 3.89; N, 15.46; found: C, 62.97; H, 3.88; N, 15.44.

Table 1
Antibacterial screening of test compounds **1**, **2**, and 3**a**–**i** (MIC, μg/mL).

Compound	Gram-negative			Gram-positive	
	E. coli	*P. aeruginosa*	*K. pneumoniae*	*S. aureus*	*E. faecalis*
1	>100	>100	>100	>100	>100
2	64	32	>100	>100	64
3a	16	16	16	64	16
3b	2	64	8	8	16
3c	4	16	16	16	4
3d	64	8	4	8	32
3e	32	2	2	8	8
3f	16	4	8	2	16
3g	8	0.25	4	0.5	2
3h	4	16	2	2	8
3i	2	32	16	4	4
Ciprofloxacin	0.5	0.5	4	0.5	4

(*Z*)-5-(4-aminobenzylidene)-3-((*Z*)-((*E*)-3-phenylallylidene)amino)imidazolidine-2,4-dione(3i)

Yellow solid; Yield: 81%; M.p. 217–219 °C; MW 332.13; IR (KBr) : 3319, 3034, 2934, 1664, 1630, 1084 cm^{-1} ; ^1H NMR (300 MHz): δ 10.63 (s, 1H, HN), 7.66 (d, J = 3.7 Hz, 1H, CH), 7.64–7.60 (m, 5H), 7.54–7.51(d, J = 7.56 Hz, 2H, Ph-NH_2), 7.20(s, 1H), 6.61(s, 1H), 6.33–6.29(d, J = 7.51 Hz, 2H, Ph-NH_2), 6.24(s, 2H), 5.90(s, 1H, –CH), 5.26(s, 1H, CH); ^{13}C NMR (75 MHz): δ 158.9, 157.4, 146.5, 137.0, 136.4, 135.4, 129.6, 128.0, 127.7, 127.2, 126.5, 124.6, 123.1, 115.2, 113.1, 114.0; MS (m/z): 333.14 (M^+, 26%); HREIMS: m/z: calcd for $C_{19}H_{16}N_4O_2$: 333.1212, found 333.1012; Anal. calcd. For $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85; N, 16.86; found: C, 68.67; H, 4.86; N, 16.82.

Biological activity

Antibacterial activity

The activity was tested for all compounds by disc diffusion method [14]. The gram-positive bacteria were used to analysis *E. faecalis, S. aureus*, and gram-negative bacteria were used to analysis *K. pneumoniae, P. aeruginosa*, and *E.coli*. All tested samples were dissolved in DMSO at 100 μg/mL concentration. The inhibition was stored at 37 °C and measurement of inhibition was check after 24-h. Test compounds were compared with ciprofloxacin.

Antifungal activity

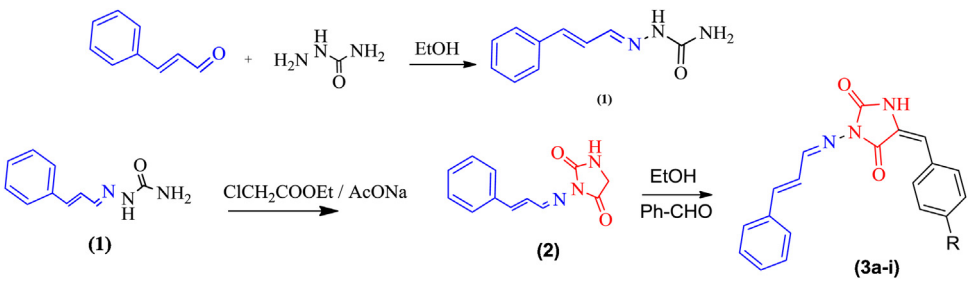
Antifungal activity was tested for all compounds by Agar dilution method [14]. The compounds **1**, **2**, and **3a**–**h** were tested by *C. albicans*, *M. audouinii*. *Cr. neoformans*, *A. fumigatus*, and *A. niger* fungal species. Concentration of the sample was prepared by 100 μg/mL in DMSO. Test compounds were compared with clotrimazole.

Evaluation of the minimum inhibitory concentration (MIC)

The test samples were dissolved separately in DMSO (dimethylsulfoxide) at 64 μg/mL concentration. Various dilutions (64, 32,. . ..0.5 μg/mL) were prepared by twofold dilutions. The microorganism suspensions 106CFU/mL was inoculated on corresponding wells and incubated at 36 °C for 24 h. MIC values are represented in Tables 1 and 2.

Molecular docking studies

Docking was used to inspect the interface, binding mode between compound **3g**, ciprofloxacin, **3b** and clotrimazole with proteins 1U1Z and 1AI9 using Autodock Vina 1.1.2 [15] and input files by AutoDock Tools 1.5.6 package. FabZ ((3R)-hydroxyacyl-



Scheme 1. Synthetic route for synthesis of imidazolidine-2-4-dione derivatives.

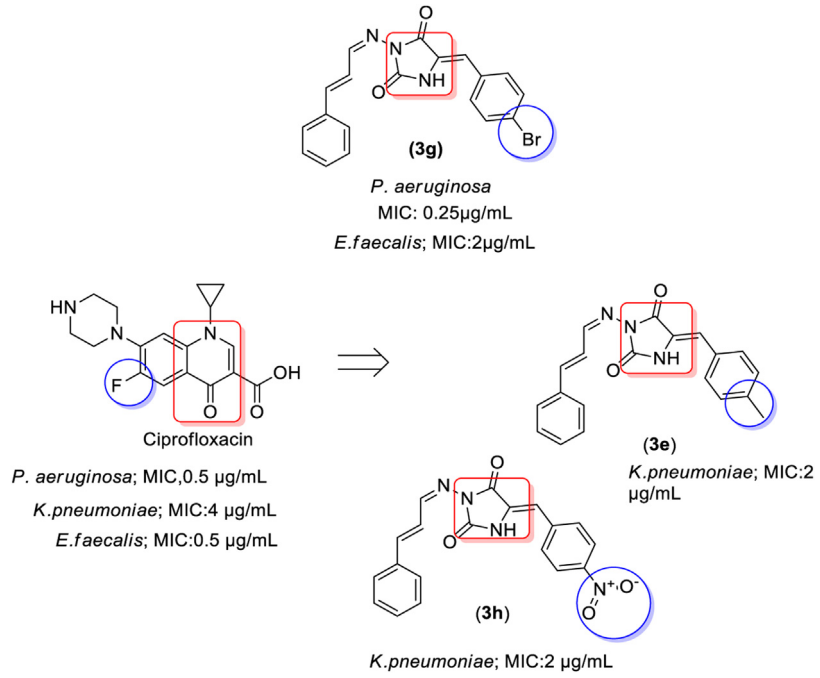


Fig. 2. Structure activity relation of antibacterial activity.

Table 2
Antifungal screening test compounds **1**, **2**, **3a–i** (MIC, μg/mL).

Compd. no.	*A. niger*	*C. albicans*	*A. fumigatus*	*Cr. neoformans*	*M. audouinii*
1	>100	>100	>100	>100	>100
2	32	>100	>100	32	32
3a	16	8	16	16	8
3b	32	0.25	8	8	**8**
3c	16	32	16	4	4
3d	8	16	8	8	8
3e	2	8	16	32	16
3f	8	2	8	16	8
3g	16	1	2	4	16
3h	4	8	4	16	8
3i	2	8	16	32	16
Clotrimazole	1	0.5	8	4	8

acyl carrier protein dehydratase (FabZ) of *P. aeruginosa* (PDB ID: 1U1Z) and Dihydrofolate reductase from *C. albicans* (PDB ID: 1AI9) were downloaded from (http://www.rcsb.org) protein data bank for antimicrobial screening respectively. The 3D structures of the ligands were drawn and energy minimized through ChemDraw Ultra 12.0 and Chem3D Pro softwares. The search grid of 1U1Z protein was identified as center_x: 19.127, center_y: 41.669, and center_z: 126.834 with dimensions size_x: 36, size_y: 32, and size_z: 34 with a space of 1.0 Å. The search grid of 1AI9 protein was identified as center_x: 27.873, center_y: −10.945, and center_z: 12.224 with dimensions size_x: 24, size_y: 24, and size_z: 28 with spacing of 1.0 Å. Therefore, the results were evaluated by discovery studio 2019 database.

ADME and molecular property prediction

The estimation of compound **3b**, **3g**, **ciprofloxacin**, and **clotrimazole** were studied by theoretical approach of ADME and toxicity by using Lipinski's "Rule of Five" [16]. Lipinski's parameters was predicted by web tool Swiss ADME [17]. The evaluation was measured via tPSA (topological polar surface area) [18]. Bioavailability is driven through gastrointestinal absorption [19].

The percentage of calculation: % ABS = 109 − (0.345 × TPSA). The water solubility, CYP2D6, CYP2D9, P-glycoprotein inhibition and phospholipidosis (PLD) induction were also projected.

Results and discussion

Chemistry

The compound **1** was synthesized via aromatic aldehyde react with semicarbazide in ethanol by condensation method. The compound **2** was synthesized from compound **1** reacted to ethyl chloroacetate and sodium acetate in ethanol medium via cyclization method. The **3a–i** were synthesized via the condensation of imidazolidin-2,4-one **2** with benzaldehyde in ethanol medium at 60 °C (Scheme 1). The final products were obtained yield between 80 to 89%.

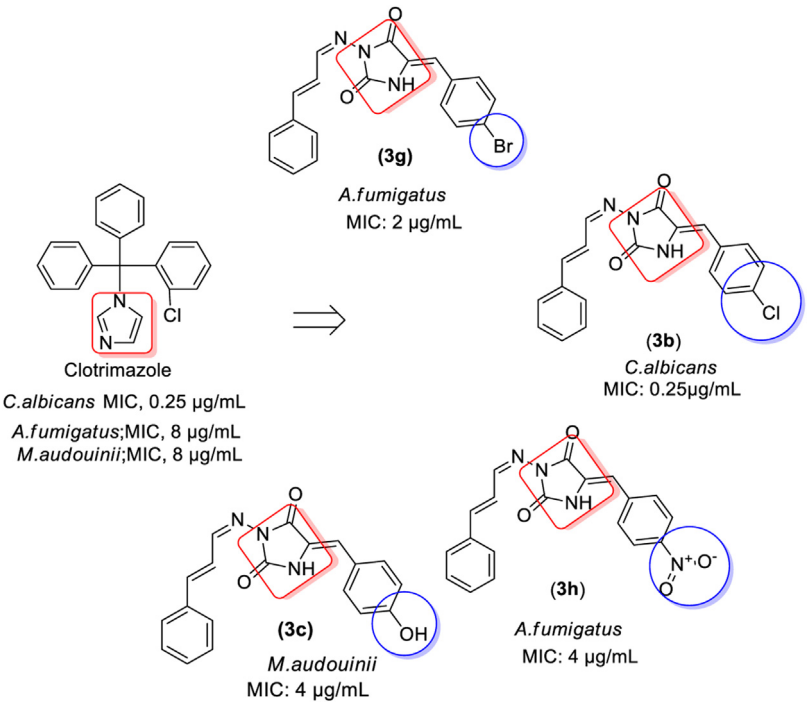


Fig. 3. Structure activity relation of antifungal activity.

Table 3
Molecular docking interaction of compounds **3g**, **ciprofloxacin**, **3b** and **clotrimazole** against proteins **1U1Z** and **1AI9**.

Proteins	Compound no.	Binding affinity (kcal/mol)	No. of H-bonds	H-bonding residues
1U1Z	3 g	−8.4	0	–
	Ciprofloxacin	−8.2	1	Asn47
1AI9	3b	−8.8	2	Ile19, Ala11
	Clotrimazole	−6.8	–	–

The IR absorption bands were measured by compound **1**, which shows that at C═O, HC═N, and NH_2 groups performed the signal at 1645, 1653, and 3323 cm^{-1}. The ^1H NMR of 1 displays the peaks at δ 7.10, 10.80, and 6.10 allocate to HCN, NH, and $-NH_2$ protons. The ^{13}C NMR of compound **1** displays chemical shift at δ 137.8, and 157.1 assign to the C═N, and —CO carbons. Furthermore, molecular ion peak (*m*/*z*) at 190.21 (M^+, 16%), which is projected exact molecular weight of compound **1** via mass spectrometer.

The IR absorption bands of compounds **2**, which shows the IR peak range at 1653, 1648, and 2974 cm^{-1} consistent to HCN, C═O, and NH groups. The proton NMR of **2** shows that proton peaks at δ 7.35, 3.82, and 10.80 constant to the HCN, H_2C-N, and NH protons. The ^{13}C NMR values of δ 137.1, 157.4, and 45.2 were conformed the carbon group C═N, C═O, and CH_2—N presence in compound **2**, respectively. In addition, the confoeation of molecular weight was determined by Mass spectral analysis, which was conformed by molecular ion peak (m/z) at 229.21 match with projected with a molecule weight of compound **2**.

The compounds **3a**–**3i** were confirmed by cyclolized imidazolidine-2,4-dione with further condensation of aromatic aldehydes, the importance of the IR spectral peak at C═N, C═O, NH corresponding to the 1640–1668, 1661–1668, and 2917–2956 cm^{-1} respectively. ^1H NMR spectral obtained important proton peaks at δ 7.18–7.26, 10.58–10.38, and 5.20–5.28 ppm resultant to the protons HCN, NH, and HC═C. The ^{13}C NMR of carbon peaks obtained at δ 137.0–137.7, 122.9–123.6, and 158.2–158.9 resultant to the, C═N, C—N and —CO carbons correspondingly.

Antimicrobial activity screening

Structure activity relationships of active molecules show in Figs. 2 and 3. The compounds **1** and **2** showed low active compared with compounds **3a**–**3i** against both antibacterial and antifungal activitives.

The antibacterial activity result is shows in Table 1, all compounds screened by a bacterial strain for instance *E. coli*, *P. aeruginosa*, and *K. pneumoniae* whereas the compounds were screened for gram positive bacteria strain of *E. faecalis* and *S. aureus*.

The compounds were low active in *E. coli* bacterial species were as Ciprofloxacin have significant active in this species (MIC: 0.5 μg/mL).

Compound **3g** have more active (MIC: 0.25 μg/mL) against *P. aeruginosa and E. faecalis* (MIC: 2 μg/mL) than *with* ciprofloxacin (MIC: 0.5 μg/mL) in *P. aeruginosa* and Ciprofloxacin (MIC: 4 μg/mL) in *E. faecalis* bacterial pathogens. The compound **3g** bearing a 4′-Br halogen group on the phenyl with imidazolidin-2,4-dione showed potential of bacterial activity. Compound **3b** have a 4′-Cl para position phenyl group on the imidazolidin-2,4-dione showed a potential active (MIC: 0.25 μg/mL). For antibacterial pathogens in *P. aeruginosa.*

Compounds **3e** and **3h** are equally more active (MIC: 2 μg/mL) in *K. pneumoniae,* then ciprofloxacin (MIC: 4 μg/mL), however the compound **3e** have methyl group on phenyl with imidazoline equal to the Nitro group presence of **3h** compounds in *K. pneumoniae* species.

The compound **3g** has equipotential (MIC: 0.5 μg/mL) and highly (MIC: 2 μg/mL) active against *S. aureus* and *E. faecalis* compared

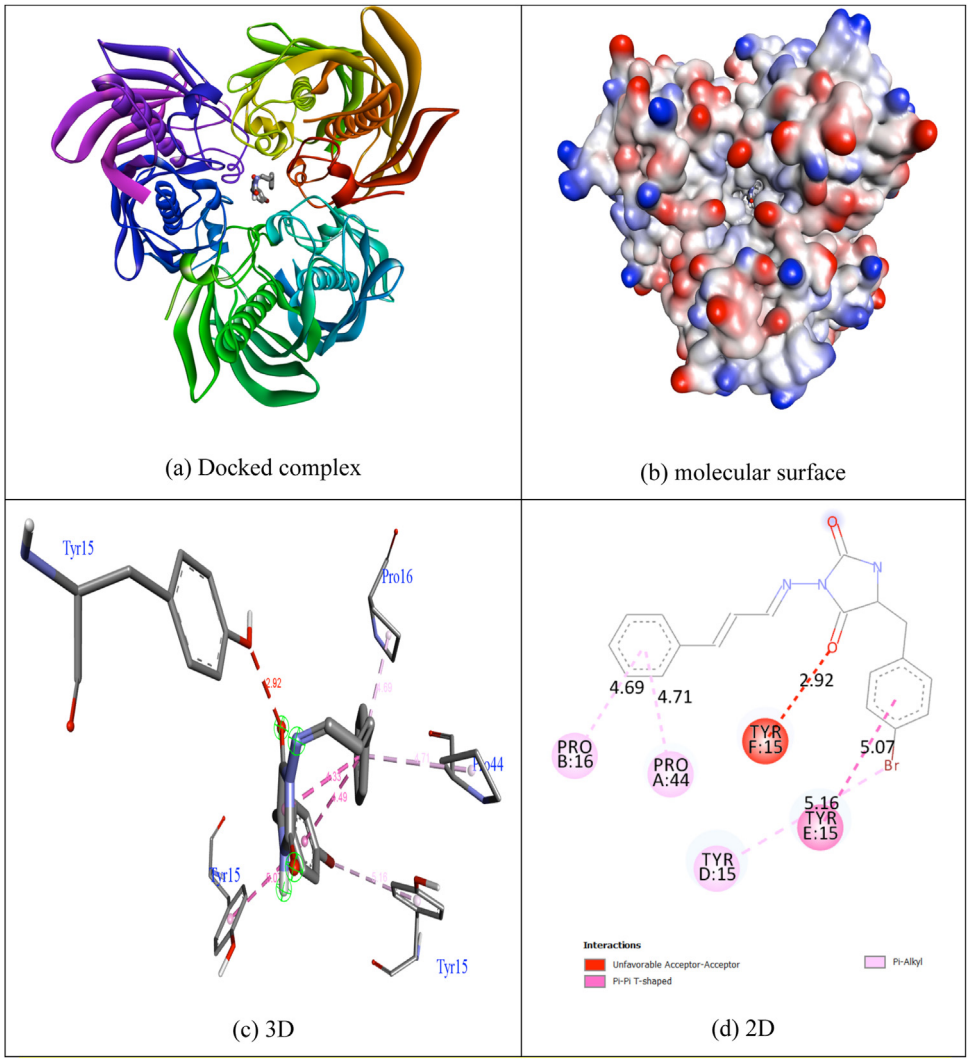


Fig. 4. Molecular docking interaction of compound **3g** within the binding site of **1U1Z** protein.

Table 4
ADME and molecular property of compounds **3b**, **3g**, **ciprofloxacin** and **clotrimazole**.

Comp.	tPSA	%Abs	MW	RoB	HBD	HBA	MR	IlogP (MlogP)	LogS	CYP2D6 inhibitor
Rule	≤140 Å^2	>50	≤500	≤10	≤5	≤10	40–130	<5	>−4	–
3b	61.77	87.68	351.79	4	1	3	106.27	3.21 (3.23)	−4.60	No
3g	61.77	87.68	396.24	4	1	3	108.96	3.21 (3.34)	−4.91	No
Ciprofloxacin	74.57	83.27	331.34	3	2	5	95.25	2.24 (1.28)	−1.32	No
Clotrimazole	17.82	102.85	344.84	4	0	1	101.84	3.07 (4.38)	−5.80	Yes

Abbreviations: tPSA (topological polar surface area); %Abs (absorption); MW (molecular weight); RoB (number of rotatable bonds); HBD (number of hydrogen bond donors); HBA (number of hydrogen bonds acceptors); MR (molar refractivity); IlogP (logarithm of compound partition coefficient between n-octanol); LogS (logarithm of water solubility).

with Ciprofloxacin (MIC: 0.5 μg/mL) due to halogen group presence both standard and compound **3g**, it is plying equal activity in *S. aureus* species.

The antifungal activity result is shows in Table 2, all compounds screened against various fungal species. All compounds are low active in *A. niger*, fungal species compared with clotrimazole(MIC = 1 μg/mL). The compound **3b** has highly active (MIC = 0.25 μg/mL) in *C.albicans* than standard Clotrimazole (MIC = 0.5 μg/mL) due to the compound **3b** baring a 4′-Cl functional group halogen group act as higher performance of clotrimazole derivatives.

The compound **3g** (MIC = 2 μg/mL) and **3h** (MIC = 4 μg/mL) have highly more against *A. fumigatus* than standard Clotrimazole (MIC = 8 μg/mL); The compound **3b** and **3g** have equipotent active (MIC = 4 μg/mL) against *Cr. neoformans* than with standard clotrimazole (MIC = 4 μg/mL). The compound **3c** has more active (MIC = 4 μg/mL) and **3b**, **3d**, **3f**, **3h** are equipotent active compared with Clotrimazole(MIC = 8 μg/mL) against *M. audouinii fungal species.*

Molecular docking

The advance perception into the plausible mechanism of biological activities docking, simulations were performed. The compounds **3g**, **ciprofloxacin** and **3b**, **clotrimazole** were considered for their docking concert with proteins **1U1Z** and **1AI9**

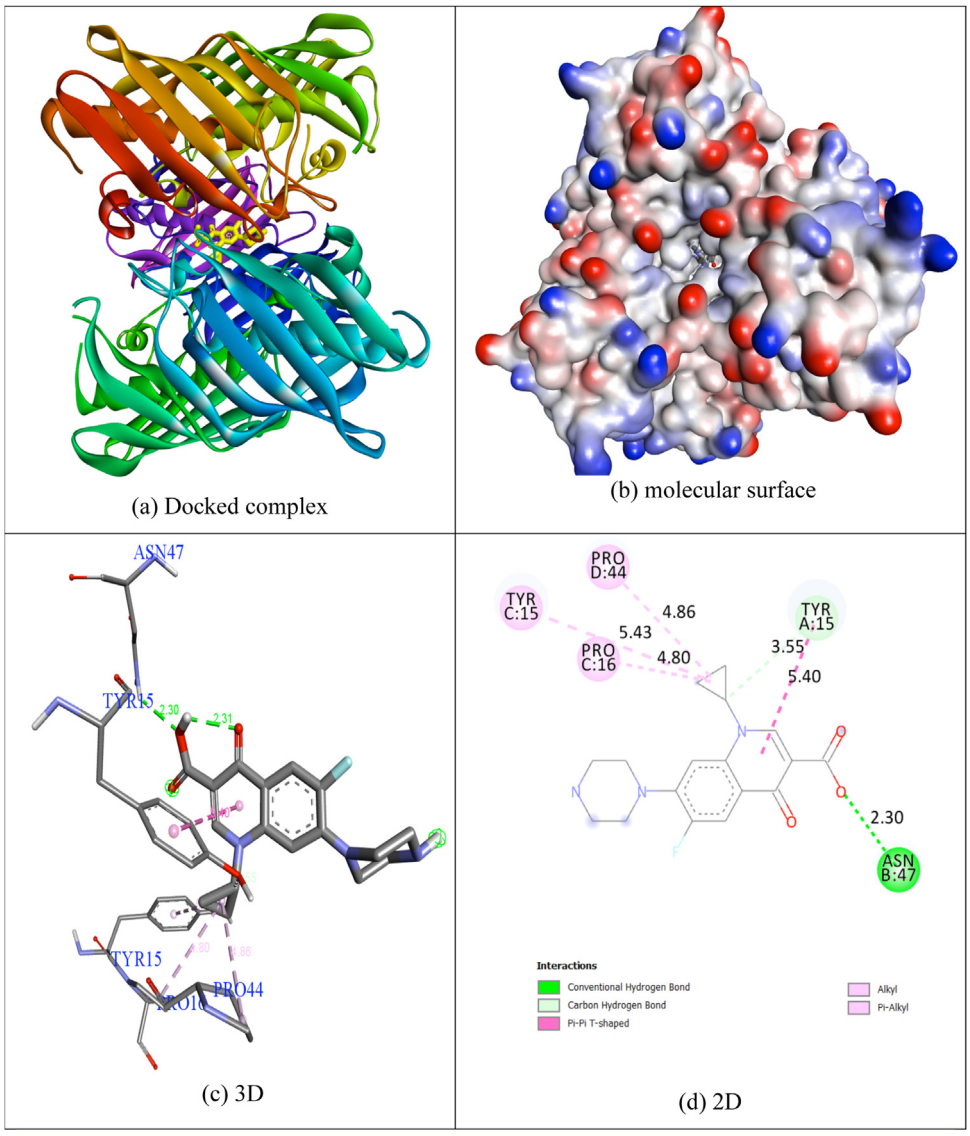


Fig. 5. Molecular docking interaction of control **ciprofloxacin** within the binding site of **1U1Z** protein.

via Autodock Vina program. All of this tested inhibitors shows negative binding energy. The compound **3g** shows remarkable inhibition ability with the binding energy (−8.4 kcal/mol) than control **ciprofloxacin** (−8.2 kcal/mol) in **1U1Z** protein. The compound **3b** shows remarkable inhibition ability with the binding energy (−8.8 kcal/mol) than control **clotrimazole** (−6.8 kcal/mol) in **1AI9**. Hydrogen bonding plays a major role for H-donor and the H-acceptor atoms between the protein-ligand interaction that promising respective compounds were less than 3.5 Å in target proteins **1U1Z** and **1AI9** signifies the strong hydrogen bonding [20]. Compound **3g** could not connect any hydrogen bond contact **1U1Z** receptor. The Tyr15, Pro16 and Pro44 were complex in hydrophobic interactions. The hydrogen bonding and hydrophobic interactions of amino acid residues in **1U1Z** protein with compound **3g** was shown in Fig. 4. The control **ciprofloxacin** was interfaced with one hydrogen bond interaction of the receptor **1U1Z**. The residues Asn47 (bond length: 2.30) was complex in hydrogen bonding interface. The residues of amino acid Tyr15, Pro16 and Pro44 were complex in hydrophobic relations. The hydrogen bonding and hydrophobic interactions of amino acid residues in **1U1Z** protein with control **ciprofloxacin** was shown in Fig. 5. Compound **3b** was formed two hydrogen bond interactions in **1AI9**. The Ile19 (bond length: 1.87) and Ala11 (bond length: 2.47) were complex in hydrogen bonding interfaces. The Val10, Met25, Phe36, Ile112 and Ala115 were involved in hydrophobic connections. The hydrogen bonding and hydrophobic interactions of amino acid residues in **1AI9** protein with compound **3g** was shown in Fig. 6. The clotrimazole couldn't connect any hydrogen bond contact **1AI9** receptor. The Lys57 and Ala115 were complex in hydrophobic interfaces. The hydrogen bonding and hydrophobic connections of amino acid residues in **1AI9** protein with control **clotrimazole** was shown in Fig. 7. The results displayed that the compounds **3g** and **3b** having the remarkable inhibition ability than control compounds **ciprofloxacin** and **clotrimazole** in respective target protein. Docking score is available in Table 3.

ADME and molecular property prediction

Development of bioactive compounds are playing a major role in therapeutic agents [21]. The representation such as hydrogen-bounding capacity, reduced molecular flexibility, intesti-

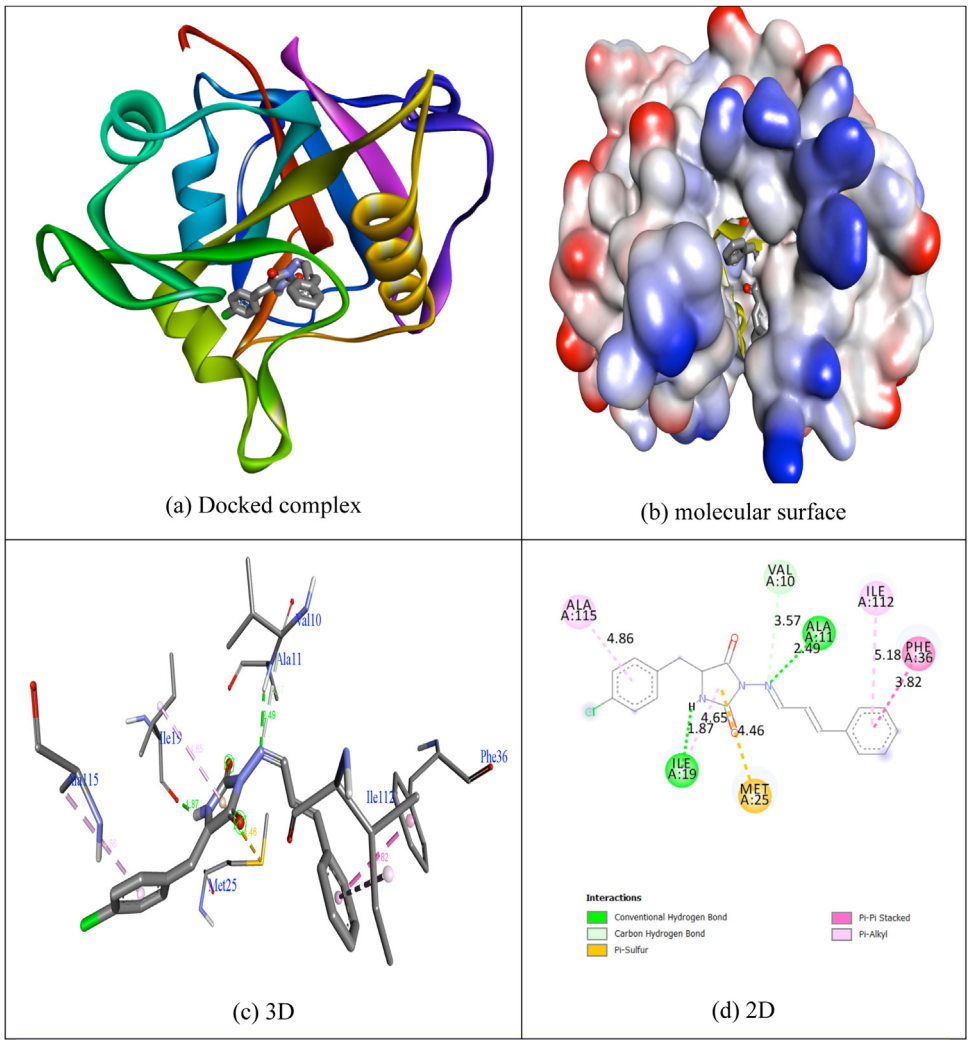


Fig. 6. Molecular docking interaction of compound **3b** within the binding site of **1AI9** protein.

nal absorption, and low polar surface area are the main forecasters of this study [22]. The compounds **3b**, **3g**, **ciprofloxacin** and **clotrimazole** passes Lipinski's "Rule of 5" with 0 violations (Table 4). The molecules were described by the number of rotatable bonds, and also pass the oral bioavailability conditions, displaying low conformational flexibility. The passive molecular transport over membranes, as well as the blood–brain barrier was correlated with the property Topological polar surface area (tPSA) [18]. The tested compounds having tPSA value of <140 $Å^2$ passes criteria. The tested compounds displayed absorption percent of (%Abs = >50), which indicates high bioavailability. The acceptable bioavailability through oral route was (>50%). The compounds **3b**, **3g** and **clotrimazole** displayed moderate water solubility (−logS value of > −4) except **ciprofloxacin** (−logS value of −1.32) shows excellent water solubility. The side effects of liver dysfunction were not anticipated upon the direction **3b**, **3g** and **ciprofloxacin** because it predicted as non-inhibitors of CYP2D6 and that was anticipated in **clotrimazole** due to the property of CYP2D6 inhibitor. The P-glycoprotein (P-gp) is involved in drug metabolism, intestinal absorption, brain diffusion, and its inhibition can extremely modify protection [23]. The drug persuaded is considered by the additional accretion of phospholipids in tissues, and that related to drug prompted toxicity [24]. The tested compounds **3b** and **3g** were not make P-gp and phospholipidosis. Keeping the above results of ADME and toxicity, the compounds **3b** and **3g** shows respectable pharmacokinetic properties, and recognized as drug-like, passing Lipinski's "Rule of 5" with 0 violations.

Conclusion

The novel 5-benzylidene-3-(3-phenylallylideneamino) imidazolidine-2,4-dione molecules were synthesized by cyclization method. Antibacterial screening in gram-negative bacteria, the compound **3g** was more active (MIC: 0.25 μg/mL) in *P. aeruginosa*, and compounds **3e** and **3h** were highly responsive (MIC: 2 μg/mL) in *K. pneumoniae*, **3g** also more inhibition (MIC: 2 μg/mL) in *E. faecalis* than ciprofloxacin. Antifungal activity, the compound compounds **3b** was more response (MIC: 0.25 μg/mL) in *C. albicance* and **3g** (MIC: 2 μg/mL), and **3h** was (MIC: 4 μg/mL) as more potent in *A. funigatus* and the compound **3c** was highly responsive (MIC: 4 μg/mL) than clotrimazole standard in *M. audouinii.* The compound **3g**, **3b**, and controls Ciprofloxacin, Clotrimazole were considered for their docking with proteins 1U1Z and 1AI9 via Autodock Vina database. The novel molecule of **3g** shows respectable binding affinity (−8.4 kcal/mol) than ciprofloxacin binding affinity (−8.2 kcal/mol) in 1U1Z protein and the compound **3b** displays the respectable binding affinity (−8.8 kcal/mol) than clotrimazole (−6.8 kcal/mol) in 1AI9 protein respectively. The results show that the test compounds having the remarkable inhibition ability than respective controls in antibacterial, antifungal. Therefore

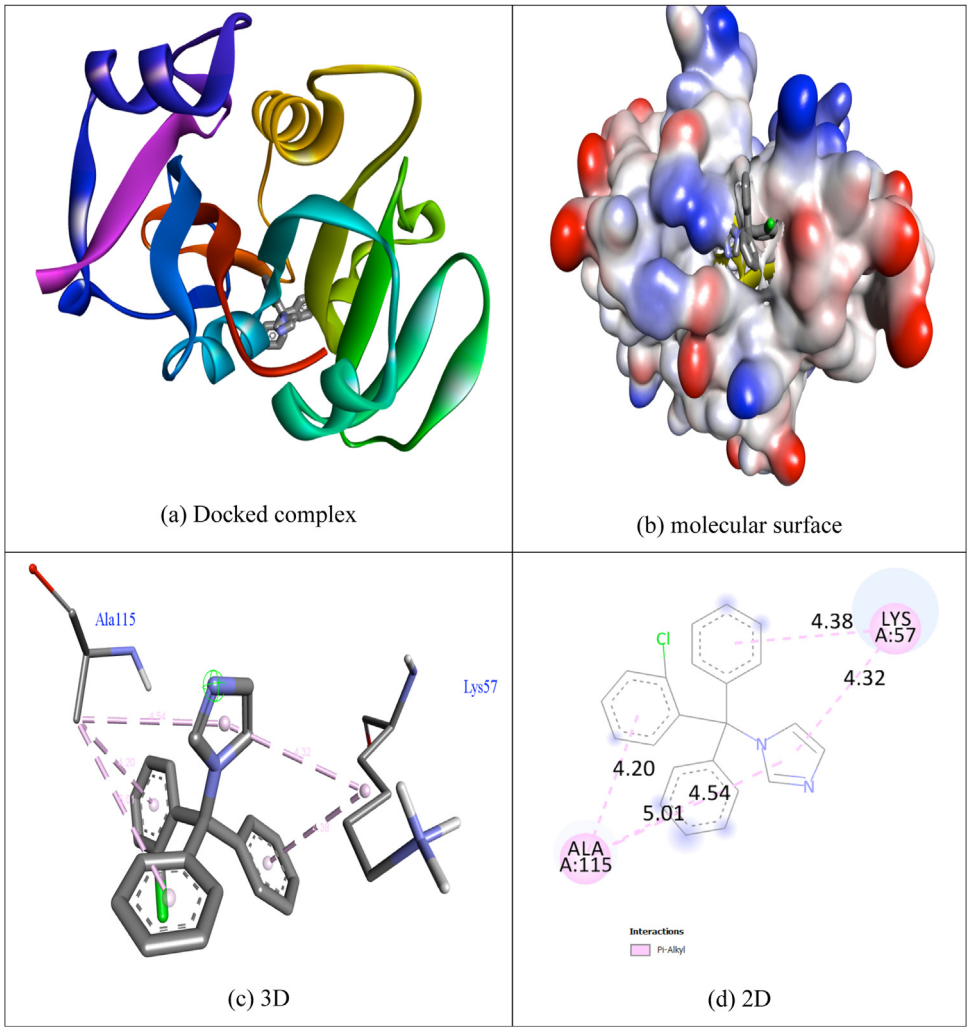


Fig. 7. Molecular docking interaction of control **clotrimazole** within the binding site of **1AI9** protein.

imidazolidine-2,4-dione could novel molecules for antimicrobial agents and which further development in vivo underway.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgement

This work was funded by Researchers Supporting Project number (RSP-2020/ 27), King Saud University, Riyadh, Saudi Arabia.

References

[1] Dolezel J, Hirsova P, Opletalova V, Dohnal J, Marcela V, Kunes J, et al. Rhodanineacetic acid derivatives as potential drugs: preparation, hydrophobic properties and antifungal activity of (5-arylalkylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl)acetic acids. Molecules 2009;14:4197–212, http://dx.doi.org/10.3390/molecules14104197.

[2] Menezes EHC, Góes AJS, Diu MBS, Galdino SL, Pitta IR, Luu-Duc C. Synthesis and structure of substituted benzyl imidazolidinedione and chlorobenzyl thiazolidinedione compounds. Pharmazie 1992;46:457–8.

[3] Hussain A, Kashif MK, Naseer MM, Rana Usman A, Hameed Shahid. Synthesis and in vivo hypoglycemic activity of new imidazolidine-2,4-dione derivatives. Res Chem Intermed 2015;41:7313–26, http://dx.doi.org/10.1007/s11164-014-1814-3.

[4] Zagórska A, Czopek A, Pawłowski M, Dybała Małgorzata, Siwek Agata, Nowak Gabriel. Serotonin transporter activity of imidazolidine-2,4-dione and imidazo[2,1-f]purine-2,4-dione derivatives in aspect of their acid–base properties. Med Chem Res 2012;21:3455–9, http://dx.doi.org/10.1007/s00044-011-9883-y.

[5] Mohamed Basant, Abdel-Samii Zakaria K, et al. Synthesis of imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one derivatives as inhibitors of virulence factors production in *Pseudomonas aeruginosa*. Arch Pharm 2020;353:e1900352, http://dx.doi.org/10.1002/ardp.201900352.

[6] El-Sharief Marwa AMSh, Abbas Samir Y, Zahran Medhat A, Mohamed Yehia A, Ragab Ahmed, Ammar Yousry A. New 1,3-diaryl-5-thioxo-imidazolidin-2,4-dione derivatives: synthesis, reactions and evaluation of antibacterial and antifungal activities. Z Naturforsch B 2016;7:875–81, http://dx.doi.org/10.1515/znb-2016-0054.

[7] El-sayed Selwan M, El-Ashmawy Mahmoud B, Bayoumi Said M, Hassan Ghada S, et al. New imidazole-4-one and imidazolidine-2,4-dione analogs: design, synthesis, antitumor activity, and molecular modeling study. Am J Physiol Biochem Pharmacol 2018;7:24–41, http://dx.doi.org/10.5455/ajpbp.20180427101802.

[8] Ibrahim TS, Bokhtia RM, AL-Mahmoudy AMM, Taher ES, AlAwadh MA, Elagawany M, et al. Design, synthesis and biological evaluation of novel 5-((substituted quinolin-3-yl/1-naphthyl) methylene)-3 substituted imidazolidin-2,4-dione as HIV-1 fusion inhibitors. Bioorg Chem 2020;99:103782, http://dx.doi.org/10.1016/j.bioorg.2020.103782.

[9] Nakajima M, Itoi K, Takamatsu Y, Kinoshita T, Okazaki T, Kawakubo K, et al. Hydantocidin: a new compound with herbicidal activity from *Streptomyces hygroscopicus*. J Antibiot 1991;44:293–300, http://dx.doi.org/10.7164/antibiotics.44.293.

[10] Lewellyn K, Bialonska D, Chaurasiya ND, Tekwani BL, Zjawiony JK. Synthesis and evaluation of aplysinopsin analogs as inhibitors of human monoamine oxidase

A and B. Bioorg Med Chem Lett 2012;22:4926–9, http://dx.doi.org/10.1016/j.bmcl.2012.06.058.
[11] Guo P, Li G, Liu Y, Lu A, Wang Z, Wang Q. Naamines and naamidines as novel agents against a plant virus and phytopathogenic fungi. Mar Drugs 2018;16:311, http://dx.doi.org/10.3390/md16090311.
[12] Gupta K, Scholes D, Stamm WE. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. JAMA 1999;281:736–8, http://dx.doi.org/10.1001/jama.281.8.736.
[13] Lauren LR, Craig SG, Philippe EZ. Risks of long-term use of nitrofurantoin for urinary tract prophylaxis in the older patient. Urolog Sci 2016;27:193–8, http://dx.doi.org/10.1016/j.urols.2016.07.004.
[14] Surendra Kumar R, Moydeen M, Al-Deyab SS, Aseer M, Idhayadhulla A. Synthesis of new morpholine-connected pyrazolidine derivatives and their antimicrobial, antioxidant, and cytotoxic activities. Bioorg Med Chem Lett 2017;27:66–71, http://dx.doi.org/10.1016/j.bmcl.2016.11.032.
[15] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 2010;31:455–61, http://dx.doi.org/10.1002/jcc.21334.
[16] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001;46:3–26, http://dx.doi.org/10.1016/S0169-409X(96)00423-1.
[17] Swiss ADME. Available online: http://www.swissadme.ch [Accessed 20 November 2016].
[18] Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 2000;43:3714–7, http://dx.doi.org/10.1021/jm000942e.
[19] Daina A, Zoete V. A BOILED-egg to predict gastrointestinal absorption and brain penetration of small molecules. Chem Med Chem 2016;11:1117–21, http://dx.doi.org/10.1002/cmdc.201600182.
[20] Taha M, Ismail NH, Khan A, Shah SAA, Anwar A, Halim SA, et al. Synthesis of novel derivatives of oxindole, their urease inhibition and molecular docking studies. Bioorg Med Chem Lett 2015;25:3285–9, http://dx.doi.org/10.1016/j.bmcl.2015.05.069.
[21] Newby D, Freitas AA, Ghafourian T. Decision trees to characterise the roles of permeability and solubility on the prediction of oral absorption. Eur J Med Chem 2015;90:751–65, http://dx.doi.org/10.1016/j.ejmech.2014.12.006.
[22] Azam F, Madi AM, Ali HI. Molecular docking and prediction of pharmacokinetic properties of dual mechanism drugs that block MAO-B and adenosine A2A receptors for the treatment of Parkinson's disease. J Young Pharm 2012;4:184–92, http://dx.doi.org/10.4103/0975-1483.100027.
[23] Fromm MF. P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. Int J Clin Pharmacol Ther 2000;38:69–74, http://dx.doi.org/10.5414/CPP38069.
[24] Nonoyama T, Fukuda R. Drug induced phospholipidosis pathological aspects and its prediction. J Toxicol Pathol 2008;21:9–24, http://dx.doi.org/10.1293/tox.21.9.