In-silico Studies, Synthesis, and Antibacterial Evaluation of Thiophene Linked Isoxazole Derivatives

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this work a series of thiophene linked isoxazole derivatives (LK1-LK8) was synthesized by cyclization of different substituted thiényl chalcones (PL1-PL8). The structures of the synthesized compounds were characterized by IR, ¹H NMR and mass spectral data. These derivatives were evaluated for antibacterial activities. Compounds LK7 showed excellent antibacterial activities amongst the synthesized compounds with MIC value 6.75 µg/ml. Molecular docking of these linked isoxazole derivatives (LK1-LK8) was also performed with crystal structure of staphyrase B 24kDa (PDB code: 5 4URM). All the isoxazole derivatives (LK1-LK8) were docked into same groove of the binding site of native co-crystallized (1R,4aS,5S,6S,8aR)-5-[(5S)-1-(3-O-acetyl-4-O-carbamoyl-6-deoxy-2-O-methyl-alpha-L-talopyranosyl)-4 hydroxy 2-oxo-5-(propan-2-yl)-2,5-dihydro-1H-pyrrol-3-yl]carboxyl-6-methyl-4 1, 2, 3, 4 methylid-ene,4a,5,6,8a-octahydonaphthalen-1-yl][2,6-dideoxy-3-C-[(1S)-1-([3,4-dichloro-5-methyl-1 H-pyrrol-2-yl] carbonyl]amino)ethyl]-beta-D-ribohexopyranoside) ligand for activity explanation and exhibited good ligand interaction and binding affinity were of range -2.04 to -4.34 kcal/mol.

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1. INTRODUCTION

Treatment of bacterial infection remain important and challenging task due to its multiple factors such developing infectious disease, increase in number of multiple drug resistance bacteria and side of the drug presently used in the treatment [1-3]. These factors have enormously increased the importance specially in hospitalised and immune compromised patient. Although large number of antibiotics and chemotherapeutics are present in the market but due development of resistance to these drugs there is always a need of new class of compound to overcome this problem. However, many studies have been carried out to overcome this problem, but desirable result is still a challenge. In regard we have planned to synthesise a new class of compounds having thiophene and Isoxazole ring and evaluate its antimicrobial properties.

Thiophene is five membered heterocyclic rings, its derivatives show multiple activities such as antimicrobial [4], analgesic and anti-inflammatory [5], antihypertensive [6], and antitumor activity [7]. Isoxazole is an azole with an oxygen atom next to the nitrogen. Isoxazole and its derivatives have a broad range of chemotherapeutic action such as antibacterial activity [8], anti-tubercular activity [9], anti-depressant [10], anti-convulsant [11], anti-hyperglycaemic, anti-alzheimers, [12], anti-obesity or hypolipidemic activity, anti-inflammatory [13], antioxidant, and anti-aging activity [14]. Some of the drugs that contain Isoxazole rings are Valdecoxib, Leflunomide, Flucloxacillin, Cloxacillin and Oxacillin. Hence owing to the wide range of activities of these heterocyclic compound it was planned to synthesise derivatives containing these two heterocyclic rings and to compare the affinities of these compounds to the target protein crystal structure of staph gyrase B 24kDa PDB Code 4URM by molecular docking studies and evaluate its antibacterial properties by tube dilution method [15,16,17].

2. MATERIALS AND METHODS

All the reactions have been carried out under the prescribed laboratory conditions. All the synthetic work has been done by procuring the available analytical grade solvents and laboratory grade reagents. For the synthesis of the thiophene linked Isoxazole derivatives the reagents used are substituted benzaldehyde, 2-acetyl thiophene, sodium hydroxide, ethanol, hydroxylamine hydrochloride, sodium acetate, glacial acetic acid which were procured from Sigma-Aldrich.

Purity of the intermediates and final compounds were monitored by thin layer chromatography (TLC) using silica gel G plates. The spots were visualized under UV light and solvent. n-hexane: ethyl acetate (7:3) mixture was used as solvent for running the TLC of these compounds. All IR spectra were recorded in Alpha Bruker using ATR method. 1H NMR spectra were recorded at 400 MHz Bruker Avance II NMR Spectrometer. Mass spectrum was recorded on GC-MS Perkin Elmer Clarus 680 Spectrometer obtained by electro impact ionization method.

2.1 Preparation of Thiaryl Chalcones: PL1-PL8

In a 250 ml conical flask 5mmol of substituted aldehyde and ethanol were added. When all the contents were dissolved 5 mmol substituted 2-acetyl thiophene was added followed by 3-4 drops of aqueous sodium hydroxide. The mixture was kept for overnight stirring. The flask was then kept in the refrigerator for 2 hours. The solid was filtered and was then recrystallized using an ethanol as solvent. [18] The physicochemical properties of these thiaryl chalcones are mentioned in Table 1.

2.2 Preparation of Isoxazole Derivatives: LK1-LK8

The mixture of previously prepared thiaryl chalcone derivatives (0.01mole) along with hydroxylamine hydrochloride (0.01mole) was added to a 250ml conical flask. To this sodium acetate and glacial acetic acid were added along with 50ml ethanol and was stirred. The mixture was then refluxed for 8 to 10 hours. The reaction mixture was cooled and poured into ice-cold water. It is then filtered and dried to get the final product. The product was recrystallized using ethanol as the solvent [19]. The physicochemical properties of these isoxazole derivatives are mentioned in Table 2.

2.3 Minimum Inhibitory Concentration

The broth dilution test is one of the standard methods for determining the level of resistance to an antibiotic. Serial dilutions of the antibiotic are
made in a liquid medium which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration of antibiotic preventing appearance of turbidity is the minimal inhibitory concentration (MIC). After preparation of different concentrations of the test compound in nutrient broth (by using the broth dilution method), we inoculate them with the test organism. The MIC is determined after incubation by choosing the lowest concentration in which no growth occurs. The MIC and the zone of inhibition are inversely correlated. In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. Conversely, the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition [20].

![Chemical structure](image)

**Fig. 1. Scheme for synthesis of thiophene linked isoxazole derivatives (LK1-LK8)**

### Table 1. Physicochemical data of chalcone derivatives (PL1-PL8)

| Compound Code | R | R<sub>1</sub> | R<sub>2</sub> | Molecular formula | Molecular weight | Melting Point (°C) | R<sub>f</sub> value | % Yield |
|---------------|---|-------------|-------------|--------------------|------------------|-------------------|-----------------|---------|
| PL-1          | H | H           | N(CH<sub>3</sub>)<sub>2</sub> | C<sub>15</sub>H<sub>16</sub>NOS | 257              | 130-132           | 0.62            | 72      |
| PL-2          | H | H           | Cl          | C<sub>13</sub>H<sub>9</sub>ClOS | 248              | 136-138           | 0.58            | 74      |
| PL-3          | H | NO<sub>2</sub> | C<sub>13</sub>H<sub>9</sub>N<sub>2</sub>O<sub>3</sub>S | 259              | 144-146           | 0.71            | 76      |
| PL-4          | H | Cl          | N(CH<sub>3</sub>)<sub>2</sub> | C<sub>15</sub>H<sub>14</sub>CINOS | 291              | 150-152           | 0.56            | 68      |
| PL-5          | H | Cl          | Cl          | C<sub>13</sub>H<sub>9</sub>ClO<sub>2</sub>S | 281              | 134-136           | 0.64            | 72      |
| PL-6          | H | NO<sub>2</sub> | Cl          | C<sub>13</sub>H<sub>8</sub>CINO<sub>3</sub>S | 293              | 126-128           | 0.67            | 74      |
| PL-7          | Br| H           | NO<sub>2</sub> | C<sub>13</sub>H<sub>8</sub>BrNO<sub>3</sub>S | 338              | 150-152           | 0.74            | 60      |
| PL-8          | Br| H           | N(CH<sub>3</sub>)<sub>2</sub> | C<sub>15</sub>H<sub>14</sub>BrNOS | 335              | 156-158           | 0.66            | 66      |

### Table 2. Physicochemical data of isoxazole derivatives: (LK1-LK8)

| Compound code | R | R<sub>1</sub> | R<sub>2</sub> | Molecular formula | Molecular weight | Melting Point (°C) | R<sub>f</sub> value | % Yield |
|---------------|---|-------------|-------------|--------------------|------------------|-------------------|-----------------|---------|
| LK-1          | H | H           | N(CH<sub>3</sub>)<sub>2</sub> | C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub>S | 272              | 168-170           | 0.56            | 64      |
| LK-2          | H | H           | Cl          | C<sub>13</sub>H<sub>10</sub>ClNOS | 263              | 154-156           | 0.62            | 70      |
| LK-3          | H | NO<sub>2</sub> | C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>S | 274              | 144-146           | 0.61            | 72      |
| LK-4          | H | Cl          | N(CH<sub>3</sub>)<sub>2</sub> | C<sub>15</sub>H<sub>15</sub>CINOS | 306              | 174-176           | 0.64            | 70      |
| LK-5          | H | Cl          | Cl          | C<sub>13</sub>H<sub>10</sub>ClNOS | 296              | 180-182           | 0.68            | 75      |
| LK-6          | H | NO<sub>2</sub> | Cl          | C<sub>13</sub>H<sub>10</sub>CINO<sub>3</sub>S | 308              | 200-202           | 0.72            | 65      |
| LK-7          | Br| H           | NO<sub>2</sub> | C<sub>13</sub>H<sub>10</sub>BrNO<sub>3</sub>S | 353              | 214-216           | 0.76            | 72      |
| LK-8          | Br| H           | N(CH<sub>3</sub>)<sub>2</sub> | C<sub>15</sub>H<sub>15</sub>BrN<sub>2</sub>O<sub>5</sub>S | 350              | 206-208           | 0.77            | 70      |
2.3.1 Procedure

Double concentration of the nutrient broth was prepared. Distribute each 2.5 ml into 8 test tubes and label them A1 to A8. Distribute 2.5 ml in two test tubes and label them as positive control and negative control. Prepare drug stock solution of 2000 µg/ml by dissolving the drug in water. From this stock solution the following dilutions were prepared; 2.5 ml of the stock solution diluted to 25 ml with water to give 200 µg/ml. Serial dilution of the same was performed to give 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.25 µg/ml respectively. Add 2.5 ml each double concentration nutrient broth to 2.5 ml of the above dilutions so that the concentration further gets halved. i.e., 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, and 3.12 µg/ml respectively. Add 2.5 ml of water to positive control and negative control tube and mix well. Mix all the tubes well close with n cotton plugs and sterilize by autoclaving 15 lbs./sq. in (121° C) for 15 min. Cool the tubes to room temperature and inoculate all the tubes except in the negative control tube. Incubate all the tubes for 48 hrs and observe the turbidity. The MIC of isoxazole derivatives is determined.

3. RESULTS

Spectral Data

Table 3. MIC of isoxazole derivatives: (LK1-LK8) By tube dilution method

| Comp Code | MIC (µg/ml) |
|-----------|-------------|
| LK-1      | 25          |
| LK-2      | 25          |
| LK-3      | 25          |
| LK-4      | 25          |
| LK-5      | 12.5        |
| LK-6      | 12.5        |
| LK-7      | 6.75        |
| LK-8      | 12.5        |
| Ciprofloxacin | 1.5 |

2.4 Molecular Docking Study

Molecular docking study was performed to know the interactions between ligand (synthesized compounds) and receptor. All computational analysis was carried out on Schrodinger 2018-3 suite device Maestro 11.7.012, (ligprep, glide XP docking, grid generation). This software package programmed on DELL Inc.27" workstation machine running on Intel Core i7-7700 CPU@ 3.60 GHz x8, processor with 8GB RAM and 1000 GB hard disk with Linux –X6_64 as the operating system. For docking calculation, the Staphylococcus aureus Gyrase B 24kDa (PDB code: 4URM) was downloaded from protein data bank and refined using protein preparation wizard. Docking score were calculated using maestro (Schrodinger) software. The binding affinity was assessed in terms of binding free energies (S-score, kcal/mol). The docking score, amino acid, and forces responsible for interaction isoxazole derivatives LK1-LK8 with is shown in Tables 4 & 5. The 2-D and 3-D interaction of compound LK5 and LK7 in Figs. 2 & 3.
N, N-dimethyl-4-(3-(thiophen-2-yl)-4,5-dihydroisoxazol-5-yl) aniline (LK-1)

IR (KBr) $v_{\text{max}}$ 1515 (Ar C=C str), 3115 (Ar C-H bending), 2930 (aliphatic C-H str), 1240 (C-O-N str), 720 (C-S str)

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 2.75 (6H, s), 2.86-3.11 (2H, 2.94, dd), 5.59 (1H, dd), 6.65 (2H, ddd), 6.99-7.24 (4H, dd) 7.13 (dd), 7.18 (ddd), 7.41 (1H, dd).

MS: $m/z$ = 273 (M$^+$)

5-(2,4-dichlorophenyl)-3-(thiophen-2-yl)-4,5-dihydroisoxazole (LK-5)

IR (KBr) $v_{\text{max}}$ 1515 (Ar C=C str), 3115 (Ar C-H bending), 2930 (aliphatic C-H str), 1245 (C-N-O str), 720 (C-S str), 615 (C-Cl str).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 2.83-3.07 (2H, 2.90 (dd), 2.99 (dd)), 5.99 (1H, dd), 7.00-7.29 (4H, 7.06 (dd), 7.13 (dd), 7.23 (dd), 7.22 (dd), 7.41 (1H, dd), 7.59 (1H, dd).

MS: $m/z = 351$ (M$^+$)

Table 4. Data of docking score of LK1-LK8

| Compounds | LK-1 | LK-2 | LK-3 | LK-4 | LK-5 | LK-6 | LK-7 | LK-8 |
|-----------|------|------|------|------|------|------|------|------|
| Binding energy kcal/mol | -2.02 | -2.03 | -2.34 | -2.02 | 2.22 | -2.39 | -4.34 | -2.68 |

Fig. 2. Ligand interaction and the binding mode of compound LK5 with 4UR
Table 5. Data of amino acid and bond interaction and of LK1-LK8

| S.No | Compound Code | Hydrophobic interaction | Polar interaction | H-bond Positively charged | Negatively charged |
|------|---------------|-------------------------|-------------------|---------------------------|-------------------|
| 1    | LK-1          | ILE 102, ILE 51, ILE 175, ILE 86 | THR 173, SER 128, ASN 54, SER 55 | ARG 84 | ASP 81, ASP 57, GLU 58 |
| 2    | LK-2          | PRO 87, ILE 86, ILE 175, ILE 102, ILE 51 | THR 173, SER 128, ASN 54, SER 55 | GLY 85 | ARG 84 | ASP 81, GLU 58 |
| 3    | LK-3          | ILE 86, PRO 87, ILE 102, VAL 101, ALA 98 | GLN 91, THR 173, SER 55, ASN 54 | | ASP 81, GLU 58 |
| 4    | LK-4          | ALA 98, VAL 101, ILE 102, PRO 87, ILE 86 | THR 173, SER 55, ASN 54, GLN 91 | | GLU 58, ASP 81 |
| 5    | LK-5          | ILE 102, ILE 51, ILE 175, ILE 86, PRO 87 | SER 55, ASN 54, GLN 91 | GLY 85 | GLY 85 | GLU 58, ASP 81 |
| 6    | LK-6          | ALA 98, VAL 101, ILE 102, PRO 87, ILE 86 | THR 173, SER 55, ASN 54, THR 173 | | GLU 58, ASP 81 |
| 7    | LK-7          | ALA 108 ILE 102, ILE 86, ILE 175, ILE 51, VAL 79 | SER 128, THR 173, ASN 54, SER 55 | | ASP 57, ASP 81 |
| 8    | LK-8          | ILE 175, ILE 51, VAL 79, LEU 103, ILE 102, ILE 86, PRO 87 | SER 128, THR 173, ASN 54, SER 55 | | ASP 81, GLU 58 |

4. DISCUSSION

In this present work different substituted novel thiophene linked isoxazole derivatives were synthesized using two step reactions. In step one thienyl chalcones (PL1-PL8) were synthesized from substituted 2-acetyl thiophene and different substituted aldehyde. The formation of thienyl chalcones was confirmed with IR peak 1647 cm$^{-1}$, shows the presence of enones (=C=C=O). In step two thienyl bonded isoxazole derivatives (LK1-LK8) were synthesized from thienyl chalcones by treating with hydroxylamine hydrochloride and confirmed from spectral analysis. The final compound LK5 showed IR peak at 1240cm$^{-1}$ of C-N-O str of isoxazole and chemical shift value of 2.90 (dd), 2.99 (dd)), 5.99 (1H, dd), 3H of isoxazole ring confirm the formation of compounds in step 2. Compound LK-8 showed the IR peak at 625 cm$^{-1}$ confirm the presence of C-Br bond. The chemical shift value 2.86-3.11 (2H, 2.94, dd) confirm the formation of isoxazole ring in compound LK1 and LK8. The final compounds (LK1-LK8) binding pattern with the crystal structure of staphylococcal nuclease A 24kDa (PDB code: 54URM) was also studied and the binding energy was in the range of -2.04 to -4.34 kcal/mol. Compound LK7 has the best interaction with the receptor having binding energy -4.34 kcal/mol. Compound LK7 interacted with ALA 108, ILE 102, ILE 86, ILE 175, ILE 51, VAL 79, SER 128, THR 173, ASN 54, SER 55, ASP 57 and ASP 81 amino acid of the receptor with hydrophobic, polar interaction negatively charged forces. Compound LK2 and LK5 formed the hydrogen bond with GLY 85 amino acid of receptor staphylococcal nuclease A 24kDa showed the binding energy -2.03 & - 2.22 kcal/mo. All the Compounds LK1-LK8 interaction with receptor amino acid and forces responsible for interaction
Fig. 3. Ligand interaction and the binding mode of compound LK7 with 4URM

are listed in Table 5. The interaction pattern of LK5 & LK7 compounds with the receptor is shown in the Figs. 2 & 3. These compounds were further tested for antibacterial activity against Gram -ve bacteria *E.coli* using serial dilution method and minimum inhibitory concentration of these compounds were measured. Among all the synthesized compounds compound LK7 showed excellent result with MIC 6.75 µg/ml whereas LK5, LK6 and LK8 good result having MIC 12.5 µg/ml whereas standard drug ciprofloxacin having MIC 1.5 µg/ml. Compound LK7 have bromine substitution on thiophene and nitro group on the aryl ring whereas in compound LK5 have chlorine substitution on ortho and para position of aryl ring. Thus, it shows the presence of electron withdrawing such chlorine, nitro and bromine enhances the antibacterial activity of the compounds.

5. CONCLUSION

The study reports the successful synthesis of thiophene linked isoxazole derivatives from cyclisation of thiencyl chalcones with moderate yields and most synthesized compounds have shown significant antibacterial activity. The docking studies shows good interaction with various amino acid of the receptor and the ligand through different bonds. The antibacterial properties of compound having electronegative substitution on the ring have shown excellent results thus favour the antibacterial properties whereas electropositive substituent on the ring shows less activity thus decrease the antibacterial properties. Although, these compounds have diverse biological properties, and their structures allow wide substitutions thus draw more interest to synthesize new antimicrobial compounds.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for
any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yoneyama H, Katsumata R. Antibiotic resistance in bacteria and its future for novel antibiotic development. Bioscience, biotechnology, and biochemistry. 2006;23;70(5):1060-75.
2. Molton JS, Tambyah PA, Ang BS, Ling ML, Fisher DA. The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. Clinical infectious diseases. 2013;56(9):1310-8.
3. Goldberg DE, Siliciano RF, Jacobs Jr WR. Outwitting evolution: fighting drug-resistant TB, malaria, and HIV. Cell. 2012;148(6):1271-83.
4. Bondock S, Fadaly W, Metwally MA. Synthesis and antimicrobial activity of some new thiazole, thipene and pyrazole derivatives containing benzothiazole moiety. European Journal of Medicinal Chemistry. 2010;45(9):3692-701.
5. Molvi KI, Mansuri M, Sudarsanam V, Patel MM, Andrabi SM, Haque N. Synthesis, anti-inflammatory, analgesic, and antioxidant activities of some tetrasubstituted thiophenes. Journal of enzyme inhibition and medicinal chemistry. 2008;23(6):829-38.
6. Shah R, Verma PK. Therapeutic importance of synthetic thiophene. Chemistry Central Journal. 2018;12(1):1-22.
7. Gulpalli KC, Bodige S, Ravula P, Endoori S, Vanaja GR, Babu GS, Chandra JN, Seelam N. Design, synthesis, in silico and in vitro evaluation of thiophene derivatives: A potent tyrosine phosphatase 1B inhibitor and anticancer activity. Bioorganic & medicinal chemistry letters. 2017;27(15):3558-64.
8. Bhatt AH, Parekh HH, Pankh AR. Synthesis of pyrazolones and isoxazoles as potential antimicrobial agents. Heterocyclic Communications. 1998;4(4):361-6.
9. Balaji NV, HariBabu B, Rao VU, Subbaraju GV, Nagasee KP, Kumar MM. Synthesis, screening and docking analysis of hispolon pyrazoles and isoxazoles as potential antitubercular agents. Current topics in medicinal chemistry. 2019;19(9):662-82.
10. Kumar J, Akhtar M, Ranjan C, Chawla G. Design, synthesis and neuropharmacological evaluation of thipene incorporated isoxazole derivatives as antidepressant and antianxiety agents. International Journal of Pharmaceutical Chemistry and Analysis. 2015;14:274-83.
11. Eddington ND, Cox DS, Roberts RR, Butcher RJ, Edafiogho IO, Stables JP, Cooke N, Goodwin AM, Smith CA, Scott KR. Synthesis, and anti convulsant activity of enaminones: 4. Investigations on isoxazole derivatives. European journal of medicinal chemistry. 2002;37(8):635-48.
12. Agrawan N, Mishra P. The synthetic and therapeutic expedition of isoxazole and its analogs. Medicinal Chemistry Research. 2018;27(5):1309-44.
13. Patra A, Batra S, Bhaduri AP, Khanna A, Chander R, Dikshit M. Isoxazole-based derivatives from Baylis–Hillman chemistry: Assessment of preliminary hypolipidemic activity. Bioorganic & medicinal chemistry. 2003;11(10):2269-76.
14. Koufaki M, Fotopoulou T, Kapetanou M, Heropoulos GA, Gonos ES, Chondrogianni N. Microwave-assisted synthesis of 3, 5-disubstituted isoxazoles and evaluation of their anti-ageing activity. European journal of medicinal chemistry. 2014;83:508-15.
15. Kumar KA, Govindaraju M, Kumar GV. Synthesis of isoxazoles via 1, 3-dipolar cycloaddition reactions and their antimicrobial activity. Indian Journal of Heterocyclic Chemistry. 2010;20(2):183-4.
16. Raghavendra KR, Prabhudeva MG, Kumar D, Kumar KA, Jayadevappa HP. Isoxazoles—a biocompatible radical scavenging agents: Citrus juice mediated environmentally benign synthesis and characterization, Asian Journal of Chemistry. 2020;32(12):2997-3001.

17. Lu J, Patel S, Sharma N, Soisson SM, Kishii R, Takei M, Fukuda Y, Lumb KJ, Singh SB. Structures of kibdelomycin bound to Staphylococcus aureus GyrB and ParE showed a novel U-shaped binding mode. ACS chemical biology. 2014;9(9):2023-31.

18. Kabli RA, Khalaf AA, Zimaity MT, Khalil AM, Kaddah AM, and Alrifaie HA. Synthesis of a new series of furyl and thienyl substituted pyrazolines starting with furyl and thienyl chalcones. Journal of the Indian Chemical Society. 1991;68(1):47-51.

19. Voskienė A, Mickevičius V. Cyclization of chalcones to isoxazole and pyrazole derivatives. Chemistry of heterocyclic compounds. 2009;45(12):1485–1488.

20. Andrews JN. Determination of minimum inhibitory concentration. J Antimicrob Chemother. 2001;48:5-16.

21. Tilak K, Renuka N, Kumar V, Kumar A. An accessible approach to the synthesis of thiophene appended pyrazoline carbothioamides as antimicrobial agents, Journal of Chemical and Pharmaceutical Research. 2015;7(3):1845-1849.

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