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Design, Synthesis and Evaluation of New Thiazolidine-2, 4-diones as Potent Inhibitors of DNA gyrase B

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Abstract: A new series of thiazolidine-2, 4-diones **9a-j** was designed and efficiently synthesized *via* convenient route and evaluated for their antimicrobial activity. The antimicrobial evaluation was done by agar-cup dilution method using ESKAP (*E.coli* ATCC 25922, *S.aureus* ATCC 29213, *K.pneumoniae* BAA 1705, *A.baumannii* BAA 1605 & *P.aeruginosa* ATCC 27853) microbial strain model. The screening result compared with standard drug levofloxacin showed that the compound **9i** was strongly active against *S.aureus* with MIC (µg/ml) value 0.25. The compounds **9d, 9e** and **9f** were also found to be highly potent against *S.aureus* with MIC (µg/ml) values 2.0, 2.0 and 1.0 respectively. The structures were confirmed with the help of spectral techniques (IR, ^{1}H-NMR, ^{13}C-NMR, and Mass). In *silico* molecular structure designing was accomplished on docking the molecules at the active sites of *S.aureus* GyrB ATPase domain (PDB: 3U2D) co-crystallized with 08B ligand. The docking study revealed that the designed compounds exhibited satisfactory interactions with key residues present at the active site. The results obtained from the docking studies and biological evaluations were highly correlated.

Keywords: Thiazolidine-2,4-dione, Anti-infective, Molecular docking, Antimicrobial

1. Introduction

Nowadays, the increasing rate of infectious diseases through different modes is a serious problem which significantly affects on the health and wealth sectors of the country. Over the past decades, it continues to dominate human population despite huge technological advancements in the health sector[1]. The drug resistance is another major problem which is tremendously affecting on the world economy. According to World Bank Group report, rising drug resistance is a looming threat to our prosperity and sustained economic development in all parts of the world[2]. Recently, the appearance of coronavirus in the Wuhan city of China and pandemics thereafter has threatened the entire population across the world[2]. The germs like bacteria, viruses, fungi and parasites are

responsible for causing infections. At present, the outbreaks of dengue, malaria and zika viral infections are going on across the country. The population explosion and industrial civilization are the key factors responsible for growing rate of infectious diseases. Multidrug resistance *Staphylococcus aureus* and vancomycin resistant *enterococci* are among the most drug resistant bacterial strains. The treatment of microbial infections is one of the most difficult tasks the medicinal community facing today.

Thiazolidin-2,4-dione is an attractive structure in the medicinal chemistry since it has been synthesized and studied the therapeutic potential against different microbial strains[3]. Recently, thiazolidin-2, 4-dione derivatives were hybridized with other bioactive moieties to study the different mechanism of action against the cancer cell lines, the study has showed tremendous potential of some thiazolidine-2, 4-dione derivatives against the numerous cancer cell lines. Angiogenesis is significant process in tumor development, therefore blocking angiogenesis is one of the most important strategies to treat malignancies[4-7]. TZDs have showed very good potential to improve insulin resistance by controlling hyperglycemia which is responsible for metabolic complications in Type 2 diabetes[8-10]. The recent studies conducted on thiazolididine-2,4-dione derivatives possess a wide range of biological activities including antimicrobial[11], anti-inflammatory[12], antihyperglycemic[13], protein tyrosine phosphatase 1B inhibitory[14], antitubercular[15], antioxidant[16], anticancer[17]& antimycobacterial[18].

DNA gyrase is a class of enzymes (also called bacterial *topoisomerase II*) plays crucial role in maintaining nucleoid structure. It catalyzes negative supercoiling of plasmid and chromosomal DNA in bacteria. In the supercoiled state, DNA adopts a specific conformation that allows the large chromosome to function well in the highly constrained space of a bacterial cell to regulate replication and transcription process. Therefore, DNA gyrase is the target of many antibiotics such as nalidixic acid, norfloxacin, ciprofloxacin, novobiocin, metronidazole and clofazimine (**Figure1**). However, due to high toxicity, the clinical use of a few numbers of antibiotics has been restricted and others have limited applications due to multidrug resistance. Nowadays, a simple synthetic strategy and big therapeutic potential of thiazolidine-2, 4-diones have attracted the attention of researchers to study the inhibition and disruption action of DNA topological transitions in the cell structure[19]. The drug designing plays crucial role in medicinal chemistry as it gives fruitful information regarding the pharmacokinetic properties of drugs including adsorption, distribution, metabolism, excretion and toxicity (ADMET) following Lipinski's rule of five. The drugs showing better pharmacokinetic properties are synthesized and screened for their activity study.

In continuation of our efforts in the field of drug designing and synthesis, a novel series of thiazolidine-2, 4-dione derivatives has been designed, synthesized and evaluated for antimicrobial properties and carried out molecular docking study on the active sites of S. *aureus* GyrB ATPase domain (PDB: 3U2D) to observe better efficacy property and binding

affinity of synthesized compounds with ATP binding pockets of DNA gyrase enzyme.

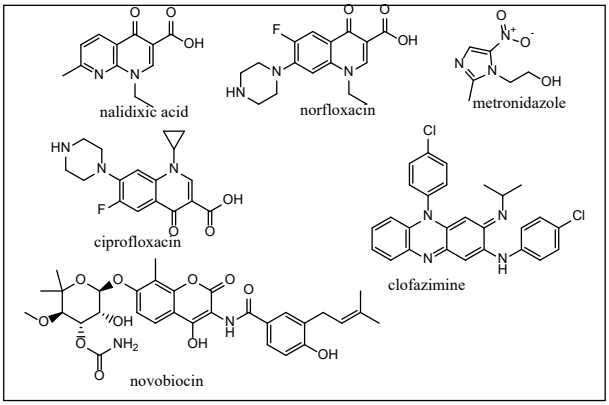


Figure 1: FDA approved antibiotics

2. Computational studies

In *silico* prediction of physicochemical properties:

Physicochemical properties of synthesized compounds (**9a-j)** were studied using QikProp module of Schrodinger software. The latest version of ChemDraw software was used to draw the chemical structures of synthesized compounds. All the structures were saved in the form of mol files and converted to 3D structure using LigPrep module of Schrodinger software. The first ranked structure of each drug was used to predict physicochemical properties. Pharmaceutically relevant important properties such as molecular weight (g/mol), no. of hydrogen bond acceptor, no. of hydrogen bond donors, no. of rotatable bonds, total polar surface area, predicted octanol/water partition coefficient, oral rat acute toxicity, hepatoxicity and no. of Lipinski violation was studied for each drug in the series [20].

2.1: Molecular Docking Study

In the present study, all molecular docking experiments were performed by using GLIDE v3.8 (Schrodinger, LLC, New York) software. The crystal structure of DNA gyrase enzyme was imported from RCSB protein data bank (PDB code: 3U2D) and preprocessed by using protein preparation wizard in Maestro module v10.3 (Schrodinger, LLC, New York) [21]. The necessary steps like generate and refinement have been processed for addition of hydrogen atoms and disulfide bonds at the missing sites on protein molecule. The protein structure was changed to a single unit with the removal of water molecules and other unwanted subunits. After the optimization process, receptor grid generation was proceeded to the previously attached ligand site. The grid is associated with different sets of fields for determining shape and properties of the receptor. The ligand binds with protein residues by using specific force field (OPLS3), generate different poses. The best docked poses have been ranked on the basis of energy function combining empirical and force-field terms [22].

3. Results and Discussion

Synthesis of novel thiazolidine-2, 4-diones (**9a-j**) from substituted aromatic aldehyde (**8**) and N-substituted thiazolidine-2,4-diones (**4**) was carried out using efficient, unique and versatile synthetic route as detailed in **Scheme 3**. Thiazolidine-2, 4-dione (**3**) was prepared from chloroacetic acid (1) and thiourea (2) *via* an efficient, ecofriendly, highly economical and well developed synthetic route as depicted in **Scheme 1**. All literature methods reported till date have utilized excess amounts of strong acid (especially conc. HCL) and required

longer time period (more than 10h) for the preparation of thiazolidine-2, 4-dione. The reported methods also suffer through many drawbacks in respect to yield, reaction conditions, purification and isolation.

The present research work reports herein the development strategy for the preparation of thiazolidine-2,4-dione from chloroacetic acid and thiourea under refluxing conditions on oil bath at 80-90 ^{0}C temperature in acetic acid for 8h. Acetic acid acts as catalyst and there is no need of any solvent to accomplish the reaction. In the end of reaction, the colourless crystals of thiazolidine-2,4-dione were obtained in excellent yield (94%). Purification of the product was done by simply washing the product with cold water. The purified compound was dried and its molecular structure was confirmed by using IR and ^{1}HNMR spectroscopic techniques and melting point. The compound has showed a sharp melting point 125-127 ^{0}C with IR frequency values for N-H stretching band at 3142 cm^{-1}, C=O stretching at 1692 cm^{-1} and CS bending at 890 cm^{-1} and ^{1}H NMR values for the peaks of –NH, & -CH_2 are δ11.97 and δ4.14 respectively. In the next step, N-substituted intermediate compounds of thiazolidine-2,4-dione were prepared from thiazolidine-2,4-dione and alkyl halides under refluxing conditions in acetone in the presence of anhydrous K_2CO_3. The prepared intermediate compounds were purified by recrystallization technique.

Synthesis of an intermediate compound

(**8**) was efficiently achieved from vanillin (**5**). The reaction of vanillin in the presence of bromine and acetic acid gives 3-bromo-4-hydroxy-5-methoxybenzaldehyde (**6**) under cold reaction conditions (below 5 ^{0}C temperature) for 1 to 2h.

The condensation reaction of this compound with ethyl chloroacetate under refluxing reaction conditions in the presence of anhydrous potassium carbonate in acetone afforded ethyl 2-(2-bromo-4-formyl-6-methoxyphenoxy) acetate (7) in excellent yield (87%). The structure of synthesized compound was confirmed by melting point and spectroscopic methods. The appearance of C=O stretching vibration bands at 1720 cm^{-1}and 1685 cm^{-1} confirms the presence of ester and aldehyde functional groups in the synthesized intermediate compound. The ^{1}HNMR peaks at δ9.92ppm, δ5.03ppm and δ3.85ppm are due to the presence of aldehyde, methylene and methoxy groups in the structure. The compound, ethyl 2-(4-formyl-2-methoxy-6-(piperazin-1-yl)phenoxy)acetate (**8**) was prepared under mild reaction conditions in ethylene glycol from the condensation reaction of piperazine with compound (**7**) in the presence of $AlCl_3$ by refluxing reaction mixture for 6h at 110-120 ^{o}C temperature. The synthesized compound has showed peaks in the ^{1}HNMR spectrum at δ1.07(s) for –NH, δ1.21 (t) for –CH_3, δ3.85 (s) for –OCH_3, δ5.02(s) for –CH_2-O, δ6.80-6.70(s) for Ar-H and δ9.92(s) for –CHO groups. Synthesis of target compounds (9a-j) was carried out *via* Knoevenagel condensation reaction from ethyl 2-(4-formyl-2-methoxy-6-(piperazin-1-yl)phenoxy) acetate and thiazolidine-2,4-diones (4a-j) under refluxing reaction conditions in piperidine for 6h (**Scheme 3**).

In the beginning, we have attempted Knoevenagel condensation reaction of 8 with thiazolidine-2,4-dione (4a) in the presence of anhydrous potassium carbonate as catalyst in ethanol medium.

The condensation reaction could not gave satisfactory yield of 5-benzylidenethiazolidine-2,4-dione under refluxing conditions. Further, the same reaction was performed in piperidine under refluxing reaction conditions gave excellent yield (87%) of ethyl (Z)-2-(4-((2,4-dioxothiazolidin-5-ylidene) methyl)-2-methoxy-6-(piperazin-1-yl) phenoxy)acetate in 6h. The structure of synthesized heterocyclic compound was established based on spectral studies (FT-IR, ^{1}H-NMR, ^{13}CMR and Mass). The ^{1}H-NMR spectra shows two signals in the region of δ 6.64-6.32ppm supported the presence of aromatic ring in the structure. The appearance of singlet (s) at δ 8.10-8.02ppm clearly indicated the presence of CH=C group. The compound has showed peak at δ3.84ppm indicated the presence of --OCH_3 group. The presence of –CO-NH-CO-group in **4a** was confirmed by ^{1}H-NMR spectral technique which has showed singlet at δ11.57ppm. The appearance of additional peaks (singlet, quartet and triplet) for the compound at δ 5.07ppm, δ 4.34ppm and at δ 1.20ppm has revealed the presence of –OCH_2COOEt group in the structure.

Table 1: Physical constant, reaction time and % yield of thiazolidine-2,4-dione (9a-j) derivatives

Entry	R	reaction time in (h)	% yield	m.p. (^{0}C)
9a	H	6.0	77	180-182
9b	$CH_2CH{=}CH_2$	5.0	76	187-189
9c	$CH_2CH_2CH_3$	6.0	80	156-158
9d	Me	6.0	85	146-148
9e	C_3H_3	6.5	70	192-194
9f	$CH{=}CH_2$	6.0	74	178-180
9g	$C_6H_5CH_2$	7.0	70	145-148
9h	CH_2COOEt	6.0	74	190-192
9i	CH_2COOH	6.5	70	187-189
9j	C_6H_{13}	7.0	78	174-176

Table 2: Minimum inhibitory concentration (ug/mL) of thiazolidine-2,4-diones against microbial strain and cytotoxicity

	MIC (ug/mL)					
entry	E. coli ATCC 25922	S. aureus ATCC 29213	K. pneumoniae BAA 1705	A. baumannii BAA 1605	P. aeruginosa ATCC 27853	Cytotoxicity (CC50, µg/ml)
9a	>64	16	>64	>64	>32	nd
9b	>64	32	>64	>64	>64	nd
9c	>32	16	>64	>64	>64	nd
9d	>64	2	>64	>64	>64	18
9e	>64	2	>64	>64	>64	10
9f	>64	1	>64	>32	>64	16
9g	>32	8	>64	>64	>64	nd
9h	>64	8	>64	>64	>64	nd
9i	>32	0.25	>64	>16	>64	22
9j	>64	8	>64	>64	>64	nd

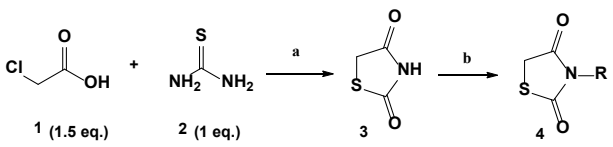
1) *Escherichia coli* ATCC 25922, (2) *S. aureus* ATCC 29213, (3) *Klebsiella pneumoniae* BAA 1705, (4) *Acinetobacter baumannii* BAA 1605 (4) *Pseudomonas aeruginosa* ATCC 27853, nd= Not detected

4. Cytotoxicity Assay:

Cytotoxicity assay was performed against Vero cells using the MTT assay [23]. For all tested compounds, ~10^3 cells/well were added in 96-well plate and incubated at 37^{0}C in the atmosphere of CO_2 (5%). After incubation for 24h, compound was added in the range from 100 to 12.5 µg/mL concentration and further incubated for 72h. A solution

of MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was added in each well and incubated further for 4h at 37^0C, , residual medium was discarded, 0.1 mL DMSO was added into it to soluble the formazan crystals, and colorimetric measurements were carried out (OD) at 540nm filter. Doxorubicin was used as positive control and experimented was repeated twice for accuracy. The CC_{50} was calculated based on the optical density values. CC50 is the lowest concentration of compound that leads to 50% reduction in cell viability

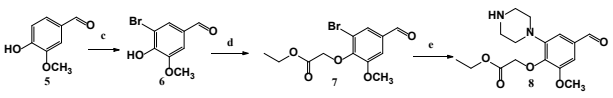
Scheme 1: Synthesis of N-substituted thiazolidine-2,4-diones (4a-j)



Reagents and reaction conditions:

a) AcOH, Reflux, 8h (85%; b) R-X, K_2CO_3, Acetone, Reflux 5-6h (80-94%)

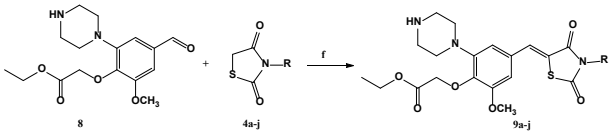
Scheme 2: Synthesis of substituted vanillin derivative (**8**)



Reagents and reaction conditions:

c) Br_2/AcOH; d) Ethylchloroacetate, K_2CO_3, Acetone, Reflux, 6-7h; e) Piperazine, $AlCl_3$, Ethylene glycol, Reflux, 110-120^0C, 5-6h (75%)

Scheme 3: Synthesis of thiazolidine-2,4-dione (**9a-g**) derivatives



Reagents and reaction conditions: f) Piperidine, Reflux 5-6h (85%)

Experimental

All the chemicals of analytical grade were purchased from the supplier and were used for the synthesis without purification. Solvents required for the chemical reactions were distilled out and then used. The melting point determination was performed by using calibrated thermometer and repeated thrice for accuracy. The progress of reaction was monitored by TLC (Thin layer chromatography) silica gel sheets for every synthetic step. KBr pellets were used for recording infrared (IR, KBr, cm-1) on Bruker 12060280 (Software: OPUS 7.2.139.1294) spectrophotometer. Bruker Avance III 400 NMR spectrometer was used to determine ^{1}H spectra in appropriate deuterated solvents and using tetramethylsilane as internal standard and are expressed in parts per million (δ, ppm) downfield from internal standard. Mass spectra were recorded in WATERS-Q-Tof Premier-HAB213 and ESI-MS tech.

Procedure for the synthesis of compound (3):

To the round bottom flask (250mL), a mixture of chloroacetic acid (0.015mol) and thiourea (0.01mol) was heated in acetic acid (5-7mL) under stirring

conditions at the temperature 80-90^{0}C. The progress of reaction was monitored by TLC. A white solid obtained at the end of reaction was poured in to ice cooled water (100mL). The contents of the beaker were further stirred for 5min. The separated solid crystals were filtered off, washed with cold water and dried to give 85% yield. Product formation was confirmed by IR, and ^{1}HNMR spectroscopy techniques and melting point.

Procedure for the preparation of compounds (4a-j):

To the solution of thiazolidine-2,4-dione (3) (0.01mol) in acetone (10mL), alkyl halides (0.01 mol) were added. To the contents of flask the catalytic amount of anhydrous potassium carbonate (0.04mol) was added and the reaction mixture was refluxed on water bath till the completion of reaction (5-7h). On cooling, the contents of flask were poured into ice cooled water and on stirring prepared compound separated out in the form of solid particles. The crude product thus obtained was filtered off, dried and crystallized for ethyl alcohol.

Procedure for the preparation of compound (6):

To the solution of vanillin (0.01mol) in acetic acid (5mL), the known amount of bromine (0.01mol) was added drop wise from the separating funnel maintaining reaction temperature 0-5^{0}C for 1/2 h. The reaction mixture was stirred further for 1 and 1/2h at room temperature. The solid product separates out when the contents of the flask were stirred into ice cooled water for 10 min. The product was filtered off, dried and recrystallized from ethyl alcohol.

Procedure for the synthesis of compound (7):

To the solution of compound (6) (0.01mol) in acetone (7mL) a solution of ethyl chloroacetate (0.01mol) was added drop wise under stirring at room temperature for 1/2h. To the above mixture, a catalytic amount of anhydrous potassium carbonate (0.04mol) was added and the reaction mixture was heated on water bath for 6-7h under refluxing conditions. The progress of reaction was monitored by Thin Layer Chromatography technique. After the completion of reaction, the contents of the flask were cooled to room temperature and poured into ice cooled water. The stirred contents gave solid product which was filtered off, dried and recrystallized from ethyl alcohol.

Procedure for the synthesis of compound (8):

To the solution of compound (7) (0.01mol) in ethylene glycol (10mL), the piperazine (0.015mol) and a catalytic amount of $AlCl_3$ (0.015mol) was added and the reaction mixture was refluxed on oil bath for 5-6h under stirring at the temperature 110-120^{0}C. The progress of reaction was monitored by Thin Layer Chromatography. After the completion of reaction, the contents of the flask were cooled to room temperature and poured into ice cooled water. The stirred contents gave solid product which was filtered off, washed by cold ether, dried and recrystallized from ethyl alcohol. The yield and melting point were recorded.

Red solid; Yield 84%; mp 240-242 ^{o}C; ^{1}H-NMR (400MHz, DMSO-d_6): δ1.07(s)

for –NH, δ1.21 (t) for –CH_3, δ3.85 (s) for –OCH_3, δ5.02(s) for –CH_2-O, δ6.80-6.70(s) for Ar-H and δ9.92(s) for –CHO

General procedure for the synthesis of compounds (9a-j):

In a round bottom flask (250mL) the mixture of piperidine (5mL), thiazolidinone-2,4-diones (4) (0.01mol) and compound (8) (0.01mol) was placed. The contents of the flask were refluxed on water bath for 5-6h. The progress of reaction was monitored by TLC. After the completion of reaction, the contents of the flask was cooled to room temperature and poured into ice. The solid product thus obtained after stirring was filtered off, dried and recrystallized from alcohol.
(Z)-ethyl 2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxy-6-(piperazin-1-yl)phenoxy) acetate (9a). IRυ_{max}/cm^{-1}: 3362 (N-H str., thiazolidine ring), 1718 (C=O str., thiazolidine ring); ^{1}H-NMR (400MHz, DMSO-d_6):11.38 (s, 1H), 7.58 (s, 1H), 7.26 (s, 1H), 7.23 (s, 1H), 4.79 (s, 2H), 4.68 (q, 2H), 3.85 (t, 4H), 3.84 (s, 3H), 2.52 (t, 4H), 1.23 (t, 3H); ^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1, 169.7, 152.5, 144.7, 137.0, 132.3, 129.7, 116.5, 116.4, 113.4, 69.0, 56.2, 56.2, 56.2, 40.0, 39.7, 39.5, 21.0; EIMS m/z: 421.23$[M+1]^+$
Ethyl(Z)-2-(4-((3-allyl-2,4-dioxothiazolidin-5-ylidene) methyl)-2-methoxy-6-(piperazin-1-yl)phenoxy) acetate (9b).

IRυ_{max}/cm^{-1}: 3336 (N-H str., thiazolidine ring), 1708 (C=O str., thiazolidine ring);^{1}H-NMR (400MHz, DMSO-d_6):7.54 (s, 1H), 7.28 (s, 1H), 7.23 (s, 1H), 5.26.-5.07 (m,5H), 4.79 (s, 2H), 4.68 (q, 2H), 3.91 (t, 4H), 3.84 (s, 3H), 2.51 (t, 4H), 1.21 (t, 3H); ^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1, 169.7, 152.5, 144.7, 137.0, 132.3, 132.0, 129.7, 116.5, 116.4, 113.9, 113.4, 69.0, 56.2, 56.2, 56.2, 42.3, 40.0, 39.7, 39.5, 21.0; EIMS m/z: 461.53$[M+1]^+$
(Z)-ethyl-2-(4-((2,4-dioxo-3-propylthiazolidin-5-ylidene)methyl)-2-methoxy-6-(piperazin-1-yl) phenoxy) acetate (9c). IRυ_{max}/cm^{-1}: 3372 (N-H str., thiazolidine ring), 1734 (C=O str., thiazolidine ring); ^{1}H-NMR (400MHz, DMSO-d_6):7.64 (s, 1H), 7.27 (s, 1H), 7.21 (s, 1H), 4.74 (s, 2H), 4.68 (q, 2H), 4.10 (t, 2H), 3.89 (t, 4H), 3.83 (s, 3H), 2.48 (t, 3H), 1.54 (m, 2H), 1.22 (t, 3H), 1.1 (t, 3H) ; ^{13}C-NMR (400MHz, DMSO-d_6): δ180.1, 175.1, 169.7, 152.5, 144.7, 137.0, 132.3, 132.0, 129.7, 116.5, 116.4, 113.9, 113.4, 69.0, 56.2, 56.2, 56.2,46.0, 42.3, 42.3, 21.0, 18.6; EIMS m/z: 463.18$[M+1]^+$
(Z)-ethyl 2-(2-methoxy-4-((3-methyl-2,4-dioxothiazolidin-5-ylidene)methyl)-6-(piperazin-1-yl)phenoxy) acetate (9d). IRυ_{max}/cm^{-1}: 3342 (N-H str., thiazolidine ring), 1728 (C=O str., thiazolidine ring); ^{1}H-NMR (400MHz, DMSO-d_6): 7.61 (s, 1H), 7.28 (s, 1H), 7.25 (s, 1H), 4.74 (s, 2H), 4.68 (q, 2H), 3.89 (t, 4H), 3.84 (s, 3H), 3.25 (s, 3H), 2.48 (t, 4H), 1.23 (t, 3H); ^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1, 169.7, 152.5, 144.7, 137.0, 132.3, 129.7, 116.5, 116.4, 113.4, 69.0, 56.2, 56.2, 56.2, 40.0, 39.7, 39.5, 31.8, 21.0; EIMS m/z: 435.15$[M+1]^+$
(Z)-ethyl-2-(4-((2,4-dioxo-3-(prop-2-yn-1-yl)thiazolidin-5-ylidene)methyl)-2-methoxy-6-(piperazin-1-yl) phenoxy) acetate (9e). IRυ_{max}/cm^{-1}: 3382 (N-H str., thiazolidine ring), 1733 (C=O str., thiazolidine ring); ^{1}H-NMR (400MHz, DMSO-d_6):7.64 (s, 1H), 7.29 (s, 1H), 7.23 (s, 1H), 4.76 (s, 2H), 4.68 (q, 2H), 4.22 (s, 2H), 3.85 (t, 4H), 3.82 (s, 3H), 2.48 (t, 4H), 3.08 (s, 1H), 1.23 (t, 3H) ;^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1,

169.7, 152.5, 144.7, 137.0, 132.3, 129.7, 116.5, 116.4, 113.4, 78.0, 73.7, 69.0, 56.2, 56.2, 56.2, 40.0, 39.7, 39.5, 32.0, 21.0; EIMS m/z: 459.15 $[M+1]^+$

(Z)-ethyl-2-(4-((2,4-dioxo-3-vinylthiazolidin-5-ylidene)methyl)-2-methoxy-6-(piperazin-1-yl)phenoxy) acetate (9f). IRυ_{max}/cm^{-1}: 3342 (N-H str., thiazolidine ring), 1695 (C=O str., thiazolidine ring); ^{1}H-NMR (400MHz, DMSO-d_6): 7.58 (s, 1H), 7.26 (s, 1H), 7.23 (s, 1H), 4.79 (s, 2H), 4.68 (q, 2H), 3.85 (t, 4H), 3.83 (s, 3H), 2.48 (t, 4H), 1.23 (t, 3H); ^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1, 169.7, 152.5, 144.7, 137.0, 132.3, 129.7, 123.9, 116.5, 116.4, 113.4, 105.8 69.0, 56.2, 56.2, 56.2, 40.0, 39.7, 39.5, 21.0; EIMS m/z: 447.23$[M+1]^+$

(Z)-ethyl-2-(4-((3-benzyl-2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxy-6-(piperazin-1-yl)phenoxy) acetate (9g). IRυ_{max}/cm^{-1}: 3364 (N-H str., thiazolidine ring), 1707 (C=O str., thiazolidine ring); ^{1}H-NMR (400MHz, DMSO-d_6): 7.64 (s, 1H), 7.26 (s, 1H), 7.35-7.24 (m, 5H), 7.23 (s, 1H), 4.82 (s, 2H), 4.79 (s, 2H), 4.68 (q, 2H), 3.86 (t, 4H), 3.84 (s, 3H), 2.49 (t, 4H), 1.23 (t, 3H); ^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1, 169.7, 152.5, 144.7, 137.0, 136.5, 132.3, 129.7, 128.5, 126.7, 126.5, 126.3, 116.5, 116.4, 113.4, 69.0, 56.2, 56.3, 56.2, 47.2, 40.0, 39.7, 39.5, 21.0; EIMS m/z: 511.18$[M+1]^+$

Ethyl(Z)-2-(5-(4-(2-ethoxy-2-oxoethoxy)-3-methoxy-5-(piperazin-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl) acetate (9h). IRυ_{max}/cm^{-1}: 3396 (N-H str., thiazolidine ring), 1724 (C=O str., thiazolidine ring); ^{1}H-NMR (400MHz, DMSO-d_6): 7.57 (s, 1H), 7.28 (s, 1H), 7.23 (s, 1H), 4.79 (s, 2H), 4.63 (s, 2H), 4.58 (q, 2H), 4.14 (q, 2H), 3.98 (t, 4H), 3.83 (s, 3H), 2.51 (t, 4H), 1.23 (t, 6H); ^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1, 169.7, 167.3, 152.5, 144.7, 137.0, 132.3, 129.7, 116.5, 116.4, 113.4, 69.0, 61.0, 56.2, 56.3, 56.2, 45.3, 40.0, 39.7, 39.5, 21.0, 18.1; EIMS m/z: 507.20$[M+1]^+$

(Z)-2-(5-(4-(2-ethoxy-2-oxoethoxy)-3-methoxy-5-(piperazin-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl) acetic acid (9i). IRυ_{max}/cm^{-1}: 3388 (N-H str., thiazolidine ring), 1745 (C=O str., thiazolidine ring); ^{1}H-NMR (400MHz, DMSO-d_6): 11.57 (s, 1H), 7.56 (s, 1H), 7.28 (s, 1H), 7.23 (s, 1H), 4.79 (s, 2H), 4.75 (s, 2H), 4.68 (q, 2H), 3.91 (t, 4H), 3.84 (s, 3H), 2.52 (t, 4H), 1.20 (t, 3H); ^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1, 169.7, 167.3, 152.5, 144.7, 137.0, 132.3, 129.7, 116.5, 116.4, 113.4, 69.0, 56.2, 56.2, 56.2, 47.9, 40.0, 39.7, 39.5, 21.0; EIMS m/z: 479.10$[M+1]^+$

Ethyl (Z)-2-(4-((3-(2-chloroacetyl)-2, 4-dioxothiazolidin-5-ylidene)methyl)-2-methoxy-6-(piperazin-1-yl) phenoxy) acetate (9j).

IRυ_{max}/cm^{-1}: 3355 (N-H str., thiazolidine ring), 1727 (C=O str., thiazolidine ring);^{1}H-NMR (400MHz, DMSO-d_6): 7.61 (s, 1H), 7.36 (s, 1H), 7.22 (s, 1H), 4.81 (s, 2H), 4.68 (q, 2H), 4.24 (s, 2H), 3.84 (t, 4H), 3.82 (s, 3H), 2.58 (t, 4H), 1.23 (t, 3H); ^{13}C-NMR (400MHz, DMSO-d_6): δ180.0, 175.1, 169.7, 163.7, 152.5, 144.7, 137.0, 132.3, 129.7, 116.5, 116.4, 113.4, 69.0, 56.2, 56.2, 56.2, 40.0, 39.9, 39.7, 39.5, 21.0; EIMS m/z: 497.10$[M+1]^+$

5. Chemoinformatics analysis

SwissADME and AdmetSAR web tool were used to study chemoinformatics of all synthesized compounds [24-25]. The compounds were evaluated for their

physicochemical, pharmacokinetics, drug likeness, and medicinal chemistry friendliness properties. ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness properties were recorded to support drug discovery (Table 3). The Lipinski rule of five was predicted using experimental and computational approaches [26]. According to Lipinski rule of five, the poor absorption or permeation of drug is possible when it has high molecular weight, more than 5 H-bond donors, 10 H-bond acceptors and calculated Log P is greater than 5. Six compounds abide Lipinski rule of five with zero violation and 04 compounds 9g, 9h, 9i and 9j violates one rule only.

[a]MW=molecular weight (g/mol); nON=no. of hydrogen bond acceptor; nOHNH=no. of hydrogen bond donors; Nrot=no. of rotatable bonds; SASA=Surface active surface area; QPlogPo/w=predicted octanol/water partition coefficient; PHOA=percent human oral absorption; QPlogBB=predicted brain/blood partition coefficient; nVio. = no. of Lipinski violation.

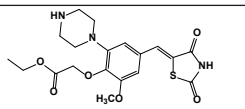
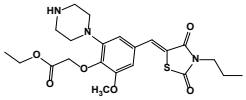
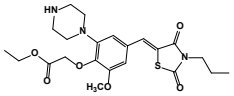
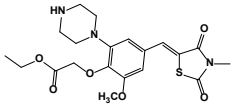
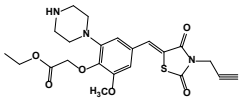
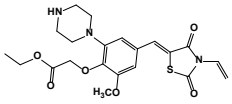
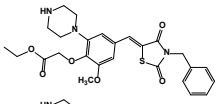
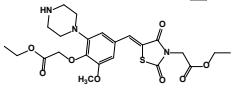
Table 3: **ADME parameters of benzylidenethiazolidine-2,4-diones (9a-j) and ATPase inhibitor of S. aureus (Levofloxacin)[a].**

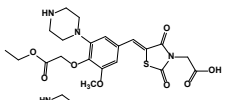
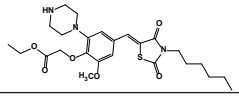
Entry	Compound	MW	nON	nOHNH	Nrot	SASA	QPlogPo/w	PHOA	QPlogBB	nVio.
1	9a	421.47	9.000	2.000	7	668.004	1.369	59.927	-1.421	0
2	9b	461.532	9.000	1.000	9	794.571	3.081	78.336	-1.298	0
3	9c	463.548	9.000	1.000	9	780.541	2.816	75.416	-1.366	0
4	9d	435.494	9.000	1.000	7	711.204	2.006	67.630	-1.301	0
5	9e	459.516	9.000	1.500	9	763.675	2.666	73.242	-1.416	0
6	9f	447.505	9.000	1.000	8	768.941	2.714	75.403	-1.265	0
7	9g	511.592	9.000	1.000	9	872.451	4.348	75.029	-1.227	1
8	9h	507.557	11.000	1.000	10	880.596	2.704	42.526	-2.056	2
9	9i	479.504	11.000	2.000	9	758.237	-0.207	17.887	-2.301	1
10	9j	505.628	9.000	1.000	12	880.988	4.084	70.266	-1.644	1
11	Levofloxacin	361.372	7.250	0.000	1	600.970	-0.378	48.896	-0.455	0

Table 4: **Docking Score, XP Gscore (kcal/mol) and Bonding interactions of approved antibiotics with key amino acids for ATPase inhibition**

Entry	Drug	Docking score	XP Gscore	Interactions with key residues
1	Nalidixic acid	-4.401	-4.446	H-bond Residues: ASN 54 and SER 55; Hydrophobic Residues: GLU 58, ASP 81, GLY 83, GLY 85, THR 173 etc.
2	Norfloxacin	-5.490	-5.589	H-bond Residues: SER 129; Salt Bridge: GLU 58 Hydrophobic Residues: ASP 81, ARG 84, ARG 144, GLY 85, THR 173, ASN 54, SER 55 etc.
3	Ciprofloxacin	-4.816	-4.916	H-bond Residues: ASN 54 and GLU 58; Hydrophobic Residues: ASP 81, GLY 83, GLY 85, THR 173 etc.
4	Novobiocin	-4.443	-4.460	H-bond Residues: ARG 84, ARG 144, GLU 58, ASP 81; Hydrophobic Residues: GLY 85, THR 173, ASN 54, SER 55 etc.
5	Metronidazole	-3.229	-3.229	H-bond Residues: SER55, ASP81; Hydrophobic Residues: GLU 58, , GLY 83, GLY 85, THR 173 etc.
6	Clofazimine	-4.107	-4.109	pi-pi interaction Residues: ARG 84; Hydrophobic Residues: SER 55 & ASP 81 & GLU 58, , GLY 83, GLY 85, THR 173 etc.
7	Levofloxacin	-4.188	-4.331	H-bond Residues: ASN 54; Hydrophobic Residues: SER 55, ASP 81, GLU 58, , GLY 83, GLY 85, THR 173 etc.

Table 5: **Docking Score, XP Gscore (kcal/mol) and interactions of compound (9a-j) with key amino acids for ATPase inhibition**

Entry No.	Compound	Docking score	XP Gscore	Interactions with key residues
1		-5.275	-5.319	H-bond Residues: ARG 144; Hydrophobic Residues: ASP 57, GLU 58, SER 55, ASP 81, ARG 84, GLY 85, GLY 83, THR 173, ASN 54, SER 129 etc.
2		-4.869	-4.875	H-bond Residues: ARG 144, ASN 54; Hydrophobic Residues: ASP 57, GLU 58, SER 55, ASP 81, ARG 84, GLY 85, GLY 83, THR 173, ASN 54, etc
3		-5.024	-5.030	H-bond Residues: ASN 54; GLU 58, ASP 81; Hydrophobic Residues: ASP 57, ARG 144, SER 55, ARG 84, GLY 85, GLY 83, THR 173 , SER 129 etc
4		-5.910	-5.917	H-bond Residues: ASN 54; Salt Bridge: ASP 81, GLU 58; Hydrophobic Residues: ASP 57, ARG 144, SER 55, ARG 84, GLY 85, GLY 83, THR 173,THR 80 etc
5		-6.146	-6.153	H-bond Residues: ASN 54, ARG 144 Hydrophobic Residues: ASP 57, SER 55, ARG 84, GLY 85, GLY 83, THR 173, ASP 81, GLU 58, THR 80 etc.
6		-5.836	-5.843	H-bond Residues: ASN 54, ARG 144; Hydrophobic Residues: ASP 57, SER 55, ARG 84, GLY 85, GLY 83, THR 173, ASP 81, GLU 58, THR 80 etc.
7		-4.295	-4.302	H-bond Residues: ASP 57, ARG 144; Hydrophobic Residues: SER 55, ASN 54, GLY 85, GLY 83, THR 173, ASP 81, GLU 58, SER 129 etc. Pi-pi stacking: ARG 84
8		-5.714	-5.721	H-bond Residues: ASN 54, ARG 144; Hydrophobic Residues: ASP 57, SER 55, ARG 84, GLY 85, GLY 83, THR 173, ASP 81, GLU 58, THR 80 etc.

9		-6.553	-6.559	H-bond Residues: ASP81, ARG84; Hydrophobic Residues: SER 55, ASN 54, GLY 85, GLY 83, THR 173, GLU 58, SER 129 etc.
10		-4.997	-5.003	H-bond Residues: ASN 54, ARG 144; Hydrophobic Residues: ASP 57, SER 55, ARG 84, GLY 85, GLY 83, THR 173, ASP 81, GLU 58, THR 80 etc.

5. Virtual Screening of Antibiotics and New Thiazolidine-2,4-dione-Based Drugs against ATPase of S. aureus

At the outset, molecular docking of the approved antibiotics was carried out with protein 3U2D using the Glide module of the Schrodinger suite. On the basis of docking score and Glide XP Gscore (kcal/mol) the binding affinity of ligands was measured to rank the poses. As per the docking results, FDA approved antibiotic, norfloxacin was realized as the best inhibitor of ATPase of S. aureus with docking score -5.490 and XP Gscore -5.589 kcal/mol respectively (**Table 4,** entry 2). The ciprofloxacin has secured second rank as ATPase inhibitor of S. aureus, with a docking score of -4.816 and XP Gscore of -4.916 (**Table 4**, entry 3).

The docking results of our designed compounds (**Table 5**, Entry 1-10) were compared with the docking results of approved antibiotics (**Table 4**, Entry 1-7) to find out the most suitable drug for protein inhibition. Among the designed drugs, 9i (Table 5, Entry 9) was found to be top scoring with a docking score of -6.553 and XP Gscore of -6.559 respectively. It forms hydrogen bonding with the residues ASP 81 and ARG 84 and hydrophobic interactions with SER 55, ASN 54, GLY 85, GLY 83, THR 173, GLU 58, SER 129 etc.

The second ranked drug, 9e has docking score -6.146 and XP Gscore of -6.153 respectively. This drug forms H-bonding interactions with the residues ASN 54, ARG 144 and hydrophobic interactions with the residues ASP 57, SER 55, ARG 84, GLY 85, GLY 83, THR 173, ASP 81, GLU 58, THR 80 etc.

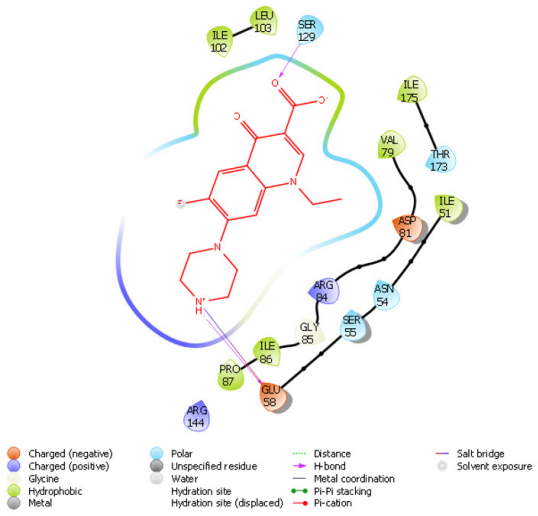
The third ranked drug, 9d shows docking score -5.910 and XP Gscore of -5.917 (Kcl/mol) respectively. This drug has showed H-bonding interaction with the residue ASN 54 and salt bridges with ASP 81 and GLU 58, and hydrophobic interactions with the residues ASP 57, ARG 144, SER 55, ARG 84, GLY 85, GLY 83, THR 173, THR 80 etc. Therefore, norfloxacin, the top ranked drug was considered to build a new library of thiazolidine-2, 4-diones as it was found to be effectively intervening with the functioning of DNA gyrase of *Staphylococcus aureus*.

The ciprofloxacin and norfloxacin, both contains piperazine ring in their chemical structure. Many important drugs were found to contain piperazine scaffold in their structure. Taking into consideration the importance of piperazine, a library of drugs was designed and subjected to molecular docking study against protein 3U2D. Of the screened more than 1500 analogs, 10 analogs were exhibited notable docking score.

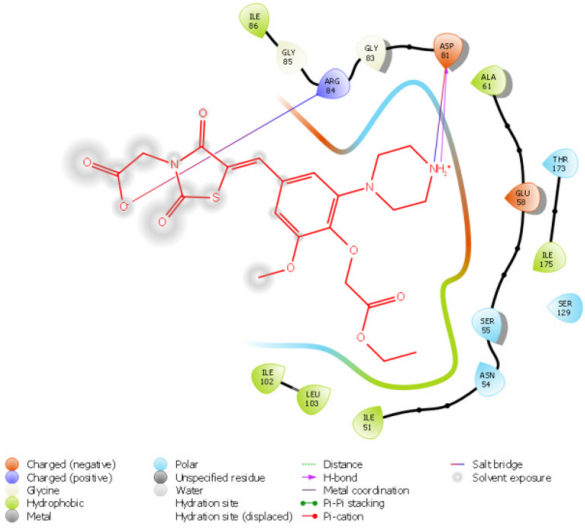
To study the drug-likeness properties, 10 top-ranked analogs along with two antibiotics (ciprofloxacin and norfloxacin) were screened for their ADMET profiles and the results are depicted in Table 3.

Remarkably, six drugs (Table 3, entry 1-6) strictly abide to the Lipinski's "rule of five" and theoretically met the drug-likeness properties. Compound 9i (Table 3, entry 9) also followed the rule with a slight deviation in number of H-bond acceptor (i.e., 11). However, compound 9i exhibited good octanol/water partition coefficient and human oral absorption. On the basis of favorable H-bonding, salt bridge and hydrophobic interactions, compound **9i** was selected for further studies. Compound 9i forms H-bond interactions with ASP81, ARG84; and hydrophobic interactions with SER 55, ASN 54, GLY 85, GLY 83, THR 173, GLU 58, SER 129 etc with the key residues of protein.

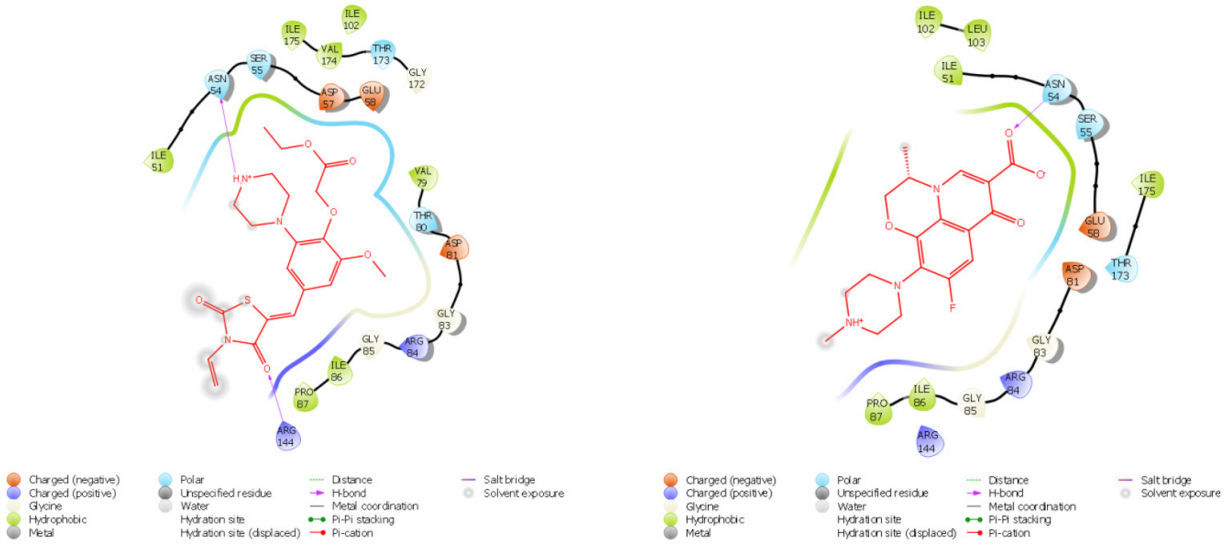
Structure activity relationship (SAR) study was performed to indicate the role of scaffolds present in thiazolidine-2,4-dione compounds. The lead compound **9i** contains two main functionalities, i.e. benzylidene and thiazolidine-2,4-dione. A benzylidene scaffold contains $–OCH_3$, $-OCH_2COOEt$ groups and a piperazine ring. The role of every group of benzylidene functionality was studied to find out the suitable one. When methoxy group was replaced by phenolic –OH group the binding affinity of ligand decreases. The condensation of ester group to phenolic –OH group enhanced the binding affinity of ligand. The acid group forms salt bridge with ARG 84 the key residues of protein. The piperazine ring forms H-bond with ASP 81. The piperazine ring in the structures of approved drugs and in the drugs reported in present paper was found to be very effective against enzyme. In order to find out a more suitable N-substituted group, the role of different groups was tested. A compound 9i incorporating N-substitued acid group showed improved activity against S.aureus.



(a)



(b)



(c) (d)

Figure 2: (a) Norfloxacin-3U2D docked complex, showing interactions with key residues at the active site of (PDB ID: 3U2D); (b) Compound 9i-3U2D docked complex, showing molecular interactions with key residues at the active site of (PDB ID: 3U2D); (c) Compound 9e-3U2D docked complex, showing molecular interactions with key residues at the active site of (PDB ID: 3U2D); (d) Levofloxacin-3U2D bound at the active site of (PDB ID: 3U2D)

6. Conclusion

A series of thiazolidine-2, 4-diones **9a-j** was synthesized and evaluated for their antimicrobial activity. The antimicrobial evaluation against *E.coli* ATCC 25922, *S.aureus* ATCC 29213, *K.pneumoniae* BAA 1705, *A.baumannii* BAA 1605 & *P.aeruginosa* ATCC 27853 microbial strains was carried out to find most potent drugs. The screening result compared with standard drug levofloxacin showed that compound **9i** was strongly active against *S.aureus* with MIC (µg/ml) value 0.25. Molecular docking studies provided theoretical knowledge of structure design based on molecular interactions formed by ligand with selected protein. The results obtained by experimental study were in good agreements with the results of virtual docking. The chemoinformatics study reveals the ADMET profile of all designed compounds. In a series, the first six compounds completely abide Lipinski's rule of five and last four compounds showed little violation. The cytotoxicity values are below 20 as showed by MTT assay clearly indicating that synthesized compounds are suitable to use as potent antimicrobial agents.

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