FABAD J. Pharm. Sci., 45, 1, 9-18, 2020

RESEARCH ARTICLE

Synthesis, Molecular Docking and Antibacterial Activity of Some Novel Pyridin-2-yl-Carbamodithioates

Rakesh KUMAR PAUL[*°], Mohammed Afzal AZAM[**], Srikanth JUPUDI[***]

Synthesis, Molecular Docking and Antibacterial Activity of some Novel Pyridin-2-yl-carbamodithioates

SUMMARY

In the present study a series of pyridin-2-yl-carbamodithioates (4a-g) were synthesized and characterized by the spectral data. Compounds (4a-g) were synthesized by heating together a mixture of triethyl ammonium N-(2-pyridyl)-dithiocarbamate (1) and the corresponding N'-(chloroacetyl)arylhydrazide/2-chloro-N'(arylsulfonyl) acetohydrazide (3a-g) in refluxing absolute ethanol. Synthesized compounds were evaluated for their in vitro activity against selected Gram-positive and Gram-negative bacterial strains by disk diffusion and two-fold serial dilution methods. All other tested compounds, except 4b exhibited significant activity against the tested strain of Gram-negative bacteria P. aeruginosa 2200 with MIC values in the range of 31.25-62.50 µg/ml. Most of the tested compounds did not show activity against the tested Gram-positive bacteria Staphylococcus aureus NCIM 5022. Compound 4a and 4c exhibited significant activity against Gram-negative bacteria E. coli with MIC value of 15.62 µg/ml. Results are compared with the standard drug ciprofloxacin. The extra-precision molecular docking and binding free energy calculation by MM-GBSA approach was performed in the catalytic pocket of E. coli MurD enzyme. The van der Waals energy term was observed to be the driving force for binding of compounds 4a-g to E. coli MurD enzyme. The outcome of this study shows that pyridin-2-yl-carbamodithioate scaffold can be utilized for the design of potent antibacterial agents.

Key Words: *Carbamodithioates, antibacterial activity, minimum inhibitory concentration, zone of inhibition, molecular docking, MurD enzyme.*

Yeni Bazı Piridin-2-il-karbamoditiyoatların Sentezi, Moleküler Kenetlenmesi ve Antibakteriyel Aktivitesi

ÖZ

Bu çalışmada, piridin-2-il-karbamoditiyoat bileşikleri (4a-4g) sentezlenmiş ve yapıları spektral verilerle aydınlatılmıştır. (4a-g) bileşikleri trietil amonyum N-(2-piridil)-ditiyokarbamat (1) ve ilgili N'-(kloroasetil)arilhidrazid / 2-kloro-N'(arilsülfonil)asetohidrazid (3a-g) türevlerinin karışım halinde absolü etanol içerisinde geri çeviren dik soğutucu altında ısıtılması ile elde edilmiştir. Sentezlenen bileşikler seçilmiş Gram-pozitif ve Gram-negatif bakteri suşlarına karşı disk difüzyon ve iki-kat seri seyreltme yöntemleri kullanılarak in vitro koşullarda test edilmiştir. 4b türevi hariç test edilen bileşiklerin hepsi Gram-negatif bakteri olan P. aeruginosa 2200'ye karşı 31.25-62.50 µg/ml aralığındaki MIC değerleri ile kayda değer aktivite göstermiştir. Bileşiklerin çoğu Gram-pozitif bakteri olan Staphylococcus aureus NCIM 5022'ye karşı etki göstermemiştir. 4a ve 4c bileşikleri Gram-negatif bakteri olan E. Coli'ye karşı 15.62 µg/ml MIC değeri ile anlamlı bir etkiye sahiptir. Test sonuçları standart ilaç olarak siprofloksasin ile kıyaslanmıştır. E. coli MurD enziminin katalitik cebinde moleküler kenetleme ve MM-GBSA ile serbest bağlanma enerjisi hesaplaması yaklaşımı uygulanmıştır. 4a-g Bileşiklerinin E. coli MurD enzimine bağlanmasındaki temel rol oynayan faktörün Van der Waals enerjileri olduğu gözlenmiştir. Yapılan bu çalışma, piridin-2-il-karbamoditiyoat yapısının potent antibakteriyel bileşiklerin tasarlanmasında değerlendirilebileceğini göstermektedir.

Anahtar Kelimeler: *Karbamoditiyoatlar, antibakteriyel aktivite, minimum inhibitor konsantrasyon, inhibisyon alanı, moleküler doking kenetleme, MurD enzimi.*

Received: 15.06.2019
Revised: 12.09.2019
Accepted: 09.10.2019

[*] ORCID: 0000-0002-2269-3181, Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Udhagamandalam- 643001, Tamil Nadu, India
[**] ORCID: 0000-0001-9770-9371, Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Udhagamandalam- 643001, Tamil Nadu, India
[***] ORCID: 0000-0001-8005-1905, Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Udhagamandalam- 643001, Tamil Nadu, India

[°] Corresponding Author; Rakesh Kumar Paul
A Constituent College of JSS Academy of Higher Education and Research, Mysuru
Tel: 8668033011
E-mail: rakeshpaul12324@gmail.com

INTRODUCTION

The alarming rise of resistant bacteria is one of the most urgent threats to the human health worldwide. Infections due to resistant bacterial strains are difficult to treat and imposes huge economic burden on societies worldwide. The discovery of new antibacterial agents acting *via* novel mechanism of action requires urgent attention. Peptidoglycan (PG), an essential component of the bacterial cell is crucial for maintaining the cell shape and osmotic stability (Salton, 1994). It consists of glycan strands of alternating *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) residues cross-linked by short peptides. The biosynthesis of peptidoglycan involves four sets of reactions which are catalyzed by enzymes.

Commonly used antibiotics used in clinic like β-lactams and glycopeptides inhibit the later steps of peptidoglycan biosynthesis. The earlier cytoplasmic steps for the biosynthesis of peptidoglycan precursor are not exploited well. The formation of *N*-acetylglucosamine-*N*-acetylmuramyl pentapeptide, a monomeric building block takes place in the cytoplasm during first stage. In this biosynthetic pathway the adenosine triphosphate (ATP) dependent Mur ligases (MurC-F) plays important by successive addition of L-alanine (L-Ala), D-glutamate (D-Glu), meso-2,6-diaminopimelate (m-DAP) in Gram-negative bacteria or L-lysine (L-Lys) in Gram-positive bacteria and D-alanine–D-alanine dipeptide into UDP-N-acetylmuramic acid (Vollmer et al. 2008, Lugtenberg et al., 1972a, Lugtenberg et al., 1972b, Lugtenberg et al., 1973). The catalytic pocket of all Mur ligases comprises three distinct domains: an N-terminal domain, a central domain and C-terminal domain (Mol et al., 2003, Spraggon et al., 2004, Smith, 2006).

The ligases MurC, MurD, MurE and MurF exhibited high structural and sequence similarity in ATP binding site (Bouhss et al., 1999), while they did not exhibit structural and sequence similarity to any ATP-utilizing human enzyme (Skedelj et al., 2011).

MurD ligase, second in the series catalyzes the addition of D-Glutamic acid (D-Glu) to the cytoplasmic intermediate uridine-5′-diphosphate-N-acetylmueamoyl-L-alanine (UMA) (Bertrand et al., 1999, Walsh et al., 1999, Van Heijenoort, 2001). Because of its high specificity for the D-amino acid substrate and its absence in human, MurD is considered as an attractive target for the antibacterial agents (VanHeijenoort, 2001, Anishetty et al., 2005). Fosfomycin is only one potent drug which is acting on the MurA ligase (Kahan et al., 1974, Hendlin et al., 1969). Carbamodithioate moiety attached to other heterocyclic systems exhibited promising antibacterial activity (Gvozdjakova et al., 1979, Vuksanovic et al., 2013; Vuksanovic et al., 2016). Benzohydrazide and benzenesulfonohydrazide derivatives are also claimed to possess antibacterial activity (Kiran et al., 2017; Haripriya et al., 2017; Wisterowicz, et al., 2012). In view of the above facts, an attempt have been undertaken to club together carbamodithioate moiety with either benzohydrazide or benzenesulfonohydrazide scaffold in one molecular frame to improve the antibacterial activity. Herein, we describe the synthesis and antibacterial screening result of pyridin-2-yl-carbamodithioates **4a-g.** For these compounds, we also performed extra-precision molecular docking and binding free energy calculation by MM-GBSA approach to investigate the binding affinity of these compounds in the catalytic pocket binding residues of *Escherichia coli* MurD enzyme (pdb.2Y10).

MATERIALS AND METHOD

Chemistry

The essential chemicals were of reagent grade and purified when necessary. Melting points were determined with open glass capillaries. The progress of reaction was monitored regularly by using thin layer chromatography (TLC) on silica gel plates (Merck 60 F_{254}, Germany). The infrared spectra were obtained on FT-IR Spectrum 2 (Perkin Elmer) spectrophotometer and the graph was plotted using %Transmission and centimetre inverse (cm^{-1}). The proton NMR spectra were recorded by Bruker AV-III 400 spectrometer (400 MHZ) using DMSO-d_6 as solvent. Chemical shift values were reported as δ ppm using the solvent as the internal standard. The liquid chromatography electrospray ionization mass spectrometry (LC/ESI/MS) (sample dissolved on MeOH or MeCN) was performed using a Shimadzu 2010A (Japan) instrument by positive ionization mode operating at 70 ev.

Synthesis of triethyl ammonium N-(2-pyridyl)-dithiocarbamate (**1**) (Edward, 1956)

1 g (1 mol) 2-aminopyridine, 7.6 ml (1.1 mol) triethylamine and 7.8 ml (1.1 mol) carbon disulphide were dispersed in n-hexane and the mixture was kept aside for several days at room temperature for completion of the reaction. During this period a triethyl ammonium N-(2-pyridyl)-dithiocarbamate separated and solidified as crystalline solid. The separated solid was filtered, washed with n-hexane and dried. M.P.: 83-84 °C [Lit. mp. 84-85 °C (Edward, 1956)].

Synthesis of *N*'-(chloroacetyl)arylhydrazide/2-chloro-*N*'(arylsulfonyl)acetohydrazide (3a-g)

To a stirred solution of appropriate hydrazide (1 mol) in ethylacetate (20 ml), a solution of chloroacetyl chloride (1 mol) in ethylacetate (20 ml) was added dropwise and the resulting mixture was stirred for one hour at room temperature and then further refluxed for 1 hour. After completion of reaction, excess of solvent was evaporated under vacuum and the solid thus separated (**3a-g**) was filtered, washed with n-hexane and used without further purification.

Synthesis of 2-(2-substituted benzoylhydrazinyl)-2-oxoethyl pyridin-2-ylcarbamodithi- oate/2-oxo-2-[2-(substituted phenylsulfonyl)hydrazinyl] ethyl pyridin-2-ylcarbamodi- thioate (4a-g)

To a stirred hot solution of triethylamine salt of N-(2-pyridyl)-dithiocarbamic acid (**1**) (1 mol) in absolute ethanol (4 ml), appropriate pyridin-2-ylcarbamodithioates (**4a-g**) (1 mol) was added gradually. The reaction mixture was refluxed on a water bath. After completion of reaction excess solvent was evaporated under vacuum and the resultant residue was stirred with excess ice-cold water. The solid thus separated was filtered, washed with ice-cold water and recrystallized from aqueous ethanol (Table 1).

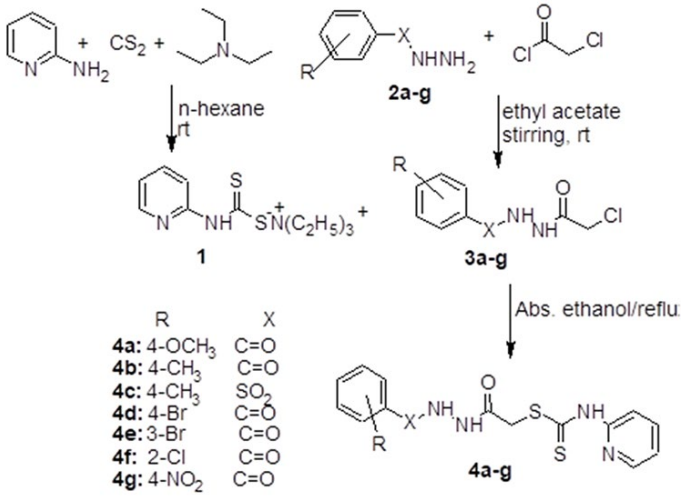


Figure 1. Synthetic route for the title compounds

2-{2-[(4-methoxyphenyl)carbonyl]hydrazinyl}-2-oxoethyl pyridin-2-ylcarbamodithioate (4a)

IR (cm^{-1}): 3289, 3223 (NH), 3019 (ArH), 2865 (CH_3), 1689, 1642 (C=O), 1608 (C=N), 1589 (Ar C=C), 1039 (C-O-C), 1015 (C=S), 841 (p-substituted benzene). ^1H NMR (DMSO-d_6): δ_{ppm} 9.23 (s, 1H, NH), 9.14 (s, 1H, NH), 8.97 (s, 1H, NH), 8.21-6.84 (m, 8H, ArH), 3.82 (s, 3H, OCH_3), 3.52 (s, 2H, CH_2). LC-MS (ESI): m/z calculated for $C_{16}H_{16}N_4O_3S_2$: 376. Found: m/z 376 (M^+), 241, 183, 165, 135, 107, 93, 77.

2-{2-[(4-methylphenyl)carbonyl]hydrazinyl}-2-oxoethyl pyridin-2-ylcarbamodithioate (4b)

IR (cm^{-1}): 3292, 3278 (NH), 3025 (ArH), 2825 (CH_2), 1667, 1631 (C=O), 1609 (C=N), 1499 (Ar C=C), 1015 (C=S), 857 (p-substituted benzene). ^1H NMR (DMSO-d_6): δ_{ppm} 9.43 (s, 1H, NH), 9.35 (s, 1H, NH), 8.89 (s, 1H, NH), 8.43-6.87 (m, 8H, ArH), 3.52 (s, 2H, CH_2), 2.11 (s, 3H, CH_3). LC-MS (ESI): m/z calculated for $C_{16}H_{16}N_4O_2S_2$: 360. Found: m/z 362 (M^+ + 2H), 241, 211, 183, 119, 92, 78.

2-{2-[(4-methylphenyl)sulfonyl]hydrazinyl}-2-oxoethylpyridin-2-ylcarbamodithioate (4c)

IR (cm^{-1}): 3193 (NH), 3032 (ArH), 2928 (CH_2), 1660, 1643 (C=O), 1615 (C=N), 1596 (Ar C=C), 1046 (SO_2), 1030 (C=S), 839 (p-substituted benzene). ^1H NMR (DMSO-d_6): δ_{ppm} 9.21 (s, 1H, NH), 9.15 (s, 1H, NH), 8.97 (s, 1H, NH), 8.23-6.94 (m, 8H, ArH), 2.96 (s, 2H, CH_2), 1.97 (s, 3H, CH_3). LC-MS (ESI): m/z calculated for $C_{15}H_{16}N_4O_3S_3$: 396. Found: m/z 396 (M^+), 211, 185, 169, 140, 96, 92, 78, 76, 65

2-{2-[(4-bromophenyl)carbonyl]hydrazinyl}-2-oxoethyl pyridin-2-ylcarbamodithioate (4d)

IR (cm^{-1}): 3365, 3285 (NH), 3019 (ArH), 2920 (CH_2), 1645, 1623 (C=O), 1605 (C=N), 1595 (Ar C=C), 1012 (C=S), 836 (p-substituted benzene), 659

(C-Br). ^{1}H NMR (DMSO-d_6): δ_{ppm} 10.56 (s, 1H, NH), 10.14 (s, 1H, NH), 9.89 (s, 1H, NH), 7.80-7.67 (m, 8H, ArH), 3.56 (s, 2H, CH_2). LC-MS (ESI): m/z calculated for $C_{15}H_{13}BrN_4O_2S_2$: 425. Found: m/z 475 (M^+ + CH_3OH + H_2O), 241, 211, 184, 169, 155, 93, 76.

2-{2-[(3-bromophenyl)carbonyl]hydrazinyl}-2-oxoethyl pyridin-2-ylcarbamodithioate (4e)

IR (cm^{-1}): 3360, 3206 (NH), 3019 (ArH), 2930 (CH_2), 1670, 1655 (C=O), 1621 (C=N), 1600 (Ar C=C), 1035 (C=S), 624 (C-Br). ^{1}H NMR (DMSO-d_6): δ_{ppm} 9.82 (s, 1H, NH), 10.25 (s, 1H, NH), 10.14 (s, 1H, NH), 10.01 (s, 1H, NH), 8.11-6.98 (m, 8H, ArH), 3.47 (s, 2H, CH_2). LC-MS (ESI): m/z calculated for $C_{15}H_{13}BrN_4O_2S_2$: 425. Found: m/z 443 (M^+ + H_2O), 241, 226, 216, 184, 169, 155, 93, 78.

2-{2-[(2-chlorophenyl)carbonyl]hydrazinyl}-2-oxoethyl pyridin-2-ylcarbamodithioate (4f)

IR (cm^{-1}): 3278, 3213 (NH), 3080 (ArH), 2932 (CH_2), 1645, 1637 (C=O), 1614 (C=N), 1594 (Ar C=C), 1029 (C=S), 779 (C-Cl), 756 (o-substituted benzene). ^{1}H NMR (DMSO-d_6): δ_{ppm} 8.96 (s, 1H, NH), 8.91 (s, 1H, NH), 8.72 (s, 1H, NH), 8.31-6.87 (m, 8H, ArH), 3.23 (s, 2H, CH_2). LC-MS (ESI): m/z calculated for $C_{15}H_{13}ClN_4O_2S_2$: 381. Found: m/z 383 (M^+ + 2), 211, 184, 170, 140, 93, 76, 60.

2-{2-[(4-nitrophenyl)carbonyl]hydrazinyl}-2-oxoethyl pyridin-2-ylcarbamodithioate (4g)

IR (cm^{-1}): 3359, 3231 (NH), 3031 (ArH), 1653, 1652 (C=O), 1620 (C=N), 1598 (Ar C=C), 1529 (NO_2), 1020 (C=S), 845 (p-substituted benzene). ^{1}H NMR (DMSO-d_6): δ_{ppm} 8.97 (s, 1H, NH), 8.87 (s, 1H, NH), 8.77 (s, 1H, NH), 8.12-7.94 (m, 8H, ArH), 3.41 (s, 2H, CH_2). LC-MS (ESI): m/z calculated for $C_{15}H_{13}N_5O_4S_2$: 391. Found: m/z 393 (M^+ + 2), 242, 184, 166, 150, 122, 93, 78.

Table 1. Physical characterization data for synthesized compounds **4a-g.**

Comp.	Molecular Formula	Molecular weight	M.p. (°C)	Yield (%)	Solvent crystallization
4a	$C_{16}H_{16}N_4O_3S_2$	376.45	228-232	66	ethanol
4b	$C_{16}H_{16}N_4O_2S_2$	360.45	168-172	70	ethanol
4c	$C_{15}H_{16}N_4O_3S_3$	396.50	192-196	80	ethanol
4d	$C_{15}H_{13}BrN_4O_2S_2$	425.32	217-220	75	methanol
4e	$C_{15}H_{13}BrN_4O_2S_2$	425.32	194-198	68	methanol
4f	$C_{15}H_{13}ClN_4O_2S_2$	380.87	198-200	72	methanol
4g	$C_{15}H_{13}N_5O_4S_2$	391.42	206-208	60	ethanol

***In vitro* antibacterial screening**

Determination of zone of inhibition

Standard strains of *Escherichia coli* NCIM 2065, *Staphylococcus aureus* NCIM 5022, *Pseudomonas aeruginosa* NCIM 2200 were procured from The Microbial Type Culture Collection and Gene Bank (MTCC) housed at Institute of Microbial Technology [IMTECH], Chandigarh, India. The antibacterial activities of the test compounds **4a-g** were determined by the agar disk diffusion method. Sterile agar plates were seeded with selected bacterial strains (10^8 CFU) and allowed to stay at 37 °C for 3 hours. Ciprofloxacin was used as a control drug at a concentration of 10 µg/ml in sterile dimethyl sulfoxide. Zone of inhibition of bacterial growth around the disk was taken as average in mm (three in dependent evaluations) (Table 2) was calculated.

Determination of minimum inhibitory concentration (MIC)

The MIC values of test compounds **4a-g** (**Table 3**) were determined by the two-fold serial dilution technique as per the guidelines of Clinical and Laboratory Standards Institute, with some modifications (Barry, 1999). The sets of seven dilutions (3.90, 7.81, 15.62, 31.25, 62.5 and 125 µg/ml) of test compounds and standard drug ciprofloxacin were prepared in sterile dimethyl sulphoxide (DMSO). Sterile DMSO was used as a negative control while ciprofloxacin was used as positive control in sterile DMSO. MIC values were determined using the Mueller Hinton medium (Hi-media). The final inoculum size was 10^5 CFU/ml for the antibacterial assay. Tubes were incubation for 24 h at 37±1 °C. Tubes with no visible growth of microorganism were recorded to represent the MIC (Table 3) and results are compared with the standard drug.

Table 2. Determination of zone of inhibition of the synthesized compounds **4a-g**.

Comp.	Zone of inhibition (mm)* (Mean±SD)														
	***S. aureus* (µg/ml)**					***E. coli* (µg/ml)**					***P. aeruginosa* (µg/ml)**				
	150	75	50	25	10	150	75	50	25	10	150	75	50	25	10
4a	-	-	-	-	-	8	-	-	-	-	7	11	7	-	-
4b	15	10	8	-	-	13	11	-	-	-	-	-	-	-	-
4c	-	-	-	-	-	9	9	-	-	-	36	31	17	10	-
4d	10	8	-	-	-	10	8	-	-	-	24	17	15	9	-
4e	-	-	-	-	-	20	8	-	-	-	17	12	10	9	-
4f	-	-	-	-	-	10	9	-	-	-	32	25	18	12	-
4g	13	10	9	7	-	9	8	6	-	-	21	19	16	10	-
Ciprofloxacin	-	-	-	-	25	-	-	-	-	19	-	-	-	-	26

*Average of three independent determinations. Ciprofloxacin tested concentration 10 µg/ml.

S. aureus: Staphylococcus aureus NCIM 5022; *E. coli: Escherichia coli* NCIM 2065; *P. aeruginosa: Pseudomonas aeruginosa* NCIM 2200.

Table 3. Determination of minimum inhibitory concentration of the synthesized compounds **4a-g**.

Comp.	Minimum Inhibition Concentration (µg/ml)		
	S. aureus	*E. coli*	*P. aeruginosa*
4a	>250	15.62	31.25
4b	62.50	31.25	>250
4c	>250	15.62	31.25
4d	125	62.50	31.25
4e	>250	31.25	62.50
4f	>250	>250	31.25
4g	125	62.50	31.25
Ciprofloxacin	31.25	15.62	31.25

S. aureus: Staphylococcus aureus NCIM 5022; *E. coli: Escherichia coli* NCIM 2065; *P. aeruginosa: Pseudomonas aeruginosa* NCIM 2200.

Docking study

The chemical structures of synthesized molecules **4a-g** were sketched using the builder panel of Schrodinger suite 2018-1 and optimized with Ligprep module. Energy minimization was performed with OPLS3 force field (Harder et al., 2016) till root mean square deviation (RMSD) converged to 0.01Å. The structures thus obtained were then used for the modelling studies. The 3D-structure of *E. coli* MurD enzyme (pdb.2Y1O resolution: 1.49 Å) was retrieved from the protein data bank and prepared using Protein Preparation Wizard tool (Sastry et al., 2013). Bond orders were assigned, the missing side chains were added and break up in the protein structure was repaired using prime (Jacobson et al., 2004) module. The protein structure was minimized with OPLS3 force field. The crystallographic water molecules with less than three hydrogen bonds were deleted. Further, restrained minimization was performed until RMSD of heavy atoms converged to 0.30 Å. The active site was defined with a 10 Å radius around the co-crystal ligand and a grid box was generated at the centroid of the active site. The low energy conformations of prepared ligands were docked into the catalytic pocket in 'extra-precision' (XP) mode (Friesner et al., 2006) without applying any constraints.

RESULTS AND DISCUSSION

Chemistry

Compounds **4a-g** was synthesized according to **Scheme 1**. The intermediate triethyl ammonium N-(2-pyridyl)-dithiocarbamate (**1**) was synthesized by the reaction of 2-aminopyridine, carbon disulphide and triethylamine in diethyl ether at room temperature. Other intermediates *N'*-(chloroacetyl) arylhydrazides/2-chloro-*N'*-(arylsulfonyl)acetohydra-zides **3a-g** were synthesized by stirring a mixture of appropriate hydrazides **2a-g** with chloroacetyl chloride in ethyl acetate. *N'*-(Chloroacetyl)arylhydrazides/2-chloro-*N'*-(arylsulfonyl) acetohydrazides (**3a-g**) were further refluxed with intermediate triethyl ammonium N-(2-pyridyl) dithiocarbamate (**1**) in absolute ethanol to yield title compounds **4a-g**.

In the infrared (IR) spectra of compounds **4a-g**, -NH, >C=O and >C=N absorption bands were observed in the range at 3198-3365, 1623-1689- and 1605-1621. In the ^{1}H NMR spectrum of compound **4d** three singlet signals appeared at δ 10.56, 10.14 and 9.89 ppm were assigned to three NH protons. Two

multiplets observed in the range of 7.80-7.67 ppm was due to the eight aromatic protons whereas signal observed at 3.56 ppm was ascribed to two protons of CH_2 group. In the mass spectrum of compound **4d** the band observed at m/z 475 is due to M^++CH_3OH+H_2O which is consistent with its molecular formula $C_{15}H_{13}BrN_4O_2S_2$. In the ^1H NMR spectrum of another compound **4e** three singlet signals appeared at δ 10.25, 10.14 and 10.01 ppm were assigned to three NH protons. Three multiplets detected in the range δ 8.21-6.87 ppm was due to the eight aromatic protons whereas signal observed at δ 3.47 ppm was ascribed to two protons of the CH_2 group. In the mass spectrum of compound **4e** the band observed at m/z 443 is due to M^++H_2O which is consistent with its molecular formula ($C_{15}H_{13}BrN_4O_2S_2$).

Antibacterial Activity

Zone of inhibition (ZI) of the synthesized compounds **4a-g** (**Table 2**) were performed using agar disk diffusion method against *E. coli* NCIM 2065, *S. aureus* NCIM 5022, *P. aeruginosa* NCIM 2200. Compound **4c-g** exhibited promising antibacterial activity against Gram-negative bacteria *P. aeruginosa* 2200 at a concentration of 25 µg/ml (zone of inhibition 9-12 mm), while compound **4b** was found to be inactive against the same strain even at the highest tested concentration of 150 µg/ml. Compounds **4b-g** were found to be moderately active against another tested Gram-negative bacteria *E. coli* NCIM 2065 as evident by the zone of inhibition (8-13 mm) at a concentration of 75 µg/ml. Compounds **4b**, **4d** and **4g** which have shown activity against tested Gram-positive bacteria *S. aureus* NCIM 5022 and all other tested compounds were found to be inactive. It is evident from the above results that tested compounds are more active against the tested Gram-negative bacteria compared to the tested Gram-positive bacteria.

Determination of minimum inhibitory concentration of synthesized compounds **4a-g** (Table 3) was carried out by two-fold micro dilution method. Compounds **4a**, **4c**, **4d**, **4f** and **4g** exhibited significant inhibitory activity (MIC, 31.25 µg/ml in all cases) against *P. aeruginosa* 2200 compared to the standard drug ciprofloxacin (MIC, 31.25 µg/ml). Against *S. aureus* NCIM 5022 only one compound **4b** exhibited activity (MIC, 62.50 µg/ml), while other compounds were observed to be either less or inactive (MIC, 125 µg/ml to >250 µg/ml) compared to the standard drug ciprofloxacin (MIC, 31.25 µg/ml). Compounds **4a** and **4c** were observed to be most active against *E. coli* NCIM 2065 with MIC value of 15.62 µg/ml (in both cases). Compounds **4b**, **4d**, **4e** and **4g** exhibited comparatively less inhibitory activity against this bacterial strain with MIC values in the range 31.25-62.50 µg/ml, when compared to the standard drug ciprofloxacin (MIC, 15.62 µg/ml). Compound **4f** was found to be inactive against this bacterial strain.

Molecular docking

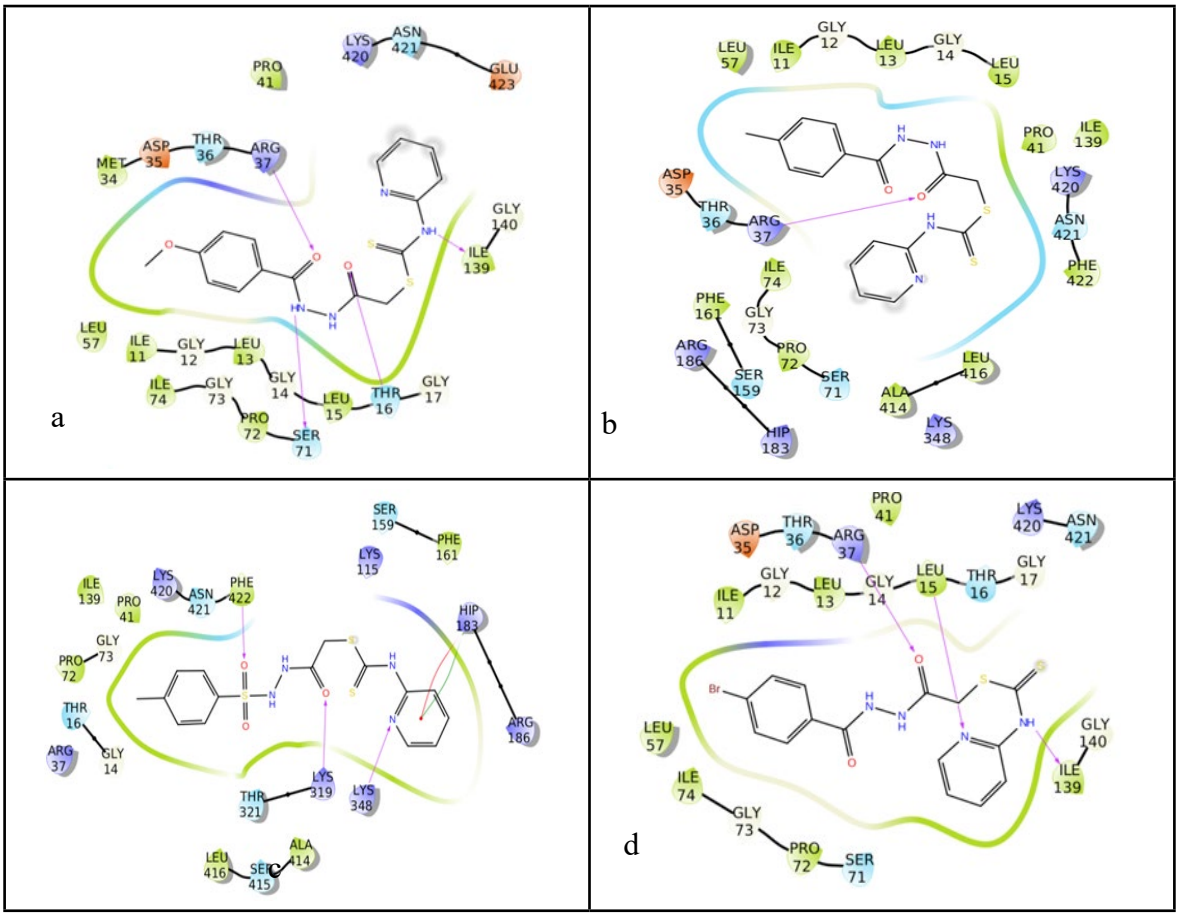
In is evident from Figure **1a-g** that compounds occupied all three domains of the catalytic pocket of *E. coli* MurD enzyme. Most of active compounds showed hydrogen bonding interactions with Arg37, Ser71, Lys329 and Lys348 residues. The observed Glide scores was in the range of -4.29 to -5.34 kcal/mol. Compound **4d** exhibited high binding affinity and occupied N-terminal and central domains of the catalytic pocket. This compound showed three hydrogen bonding interactions, one each with Leu125, Arg37 and Ile139. Precisely, carbonyl oxygen of –NHNHCO- fragment accepted a hydrogen bond from Arg37 whereas –NH- of –SCONH- fragment accepted a hydrogen bond from Leu139. In addition, nitrogen of pyridine ring accepted a hydrogen bond from Leu15. In case of compound **4f** with lowest Glide score (-4.29 kcal/mol) three hydrogen bonding interactions were observed. The carbonyl oxygen of –NHNHCO- fragment accepted a hydrogen bond from Arg37 while NH of SCONH fragment formed a hydrogen bond with Ile139. Pyridine nitrogen of this compound also establishes a hydrogen bond with Leu15. Compound **4c** occupied central and C-terminal domains of the catalytic pocket and established three hydrogen bonding interactions with Lys319, Lys348 and Phe422. Pyridine ring in compound **4c** also established π-cation and π-π stacking interactions one each with the imidazole ring of His183. However, these lucrative hydrophobic interactions was not observed in other compounds. This is in correlation with the highest binding free energy (ΔG_{Bind} -75.71 kcal/mol) of this compound.

The binding free energy (ΔG_{Bind}) was calculated for complexes **4a-g**/2Y1O by MM-GBSA approach using the VSGB 2.0 energy model (Li et al., 2011) and OPLS3 force field. It is evident from Table 4 that binding free energy is in the range of -42.66 to -75.71 kcal/mol. The higher negative values of ΔG_{Bind} in compounds **4c** and **4d** (-75.71 and -72.56 kcal/mol, respectively) indicated that these two compounds have favourable binding interactions and hence stability within the catalytic pocket. In most of the ligands van der Waals and Coulomb energy terms favours the ligand binding. Further, the high negative value indicates that van der Waals energy term is the driving force for binding of inhibitors to *E. coli* MurD enzyme. Hydrogen bond energy term is also favourable binding, while covalent energy term moderately disfavours the ligand binding.

Table 4. Molecular docking and binding free energy (MM/GBSA) calculation (kcal/mol) of compounds **4a-g** in the catalytic pocket of *E. coli* MurD enzyme (pdb.2Y10).

Comp.	[a]G_{score}	[b]G_{energy}	[c]G_{emodel}	[d]XP_{HBond}	[e]ΔG_{Bind}	[f]ΔG_{Cov}	[g]ΔG_{vdW}	[h]Δ_{Coul}	[i]$\Delta G_{H\text{-}bond}$
4a	-5.05	-43.29	-58.43	-1.55	-57.84	20.29	-56.63	-30.09	-4.86
4b	-4.69	-41.51	-55.82	-0.67	-42.66	5.49	-48.79	-41.93	-3.64
4c	-5.21	-47.24	-62.83	-1.4	-75.71	28.3	-73.66	-7.23	-7.46
4d	-5.34	-46.65	-65.05	-0.90	-72.56	19.31	-49.9	-54.3	-8.72
4e	-4.30	-43.62	-61.31	-0.96	-46.92	7.54	-49.19	-38.86	-6.03
4f	-4.29	-45.68	-62.4	-0.80	-45.29	44.05	-38.9	-52.42	-7.09
4g	-4.42	-41.05	-59.42	-0.87	-55.51	11.51	-65.28	7.35	-1.46

[a]G_{score}: glide score; [b]G_{energy}: glide energy; [c]G_{emodel}: glide model energy; dXPHBond: extra-precision hydrogen bond reward; [e]ΔG_{bind}: binding free energy; [f]Δ_{cov}: covalent energy; [g]Δ_{vdW}: van der Waals energy; [h]Δ_{Coul}: Coulomb energy; [i]ΔH-bond: hydrogen bond energy contribution.



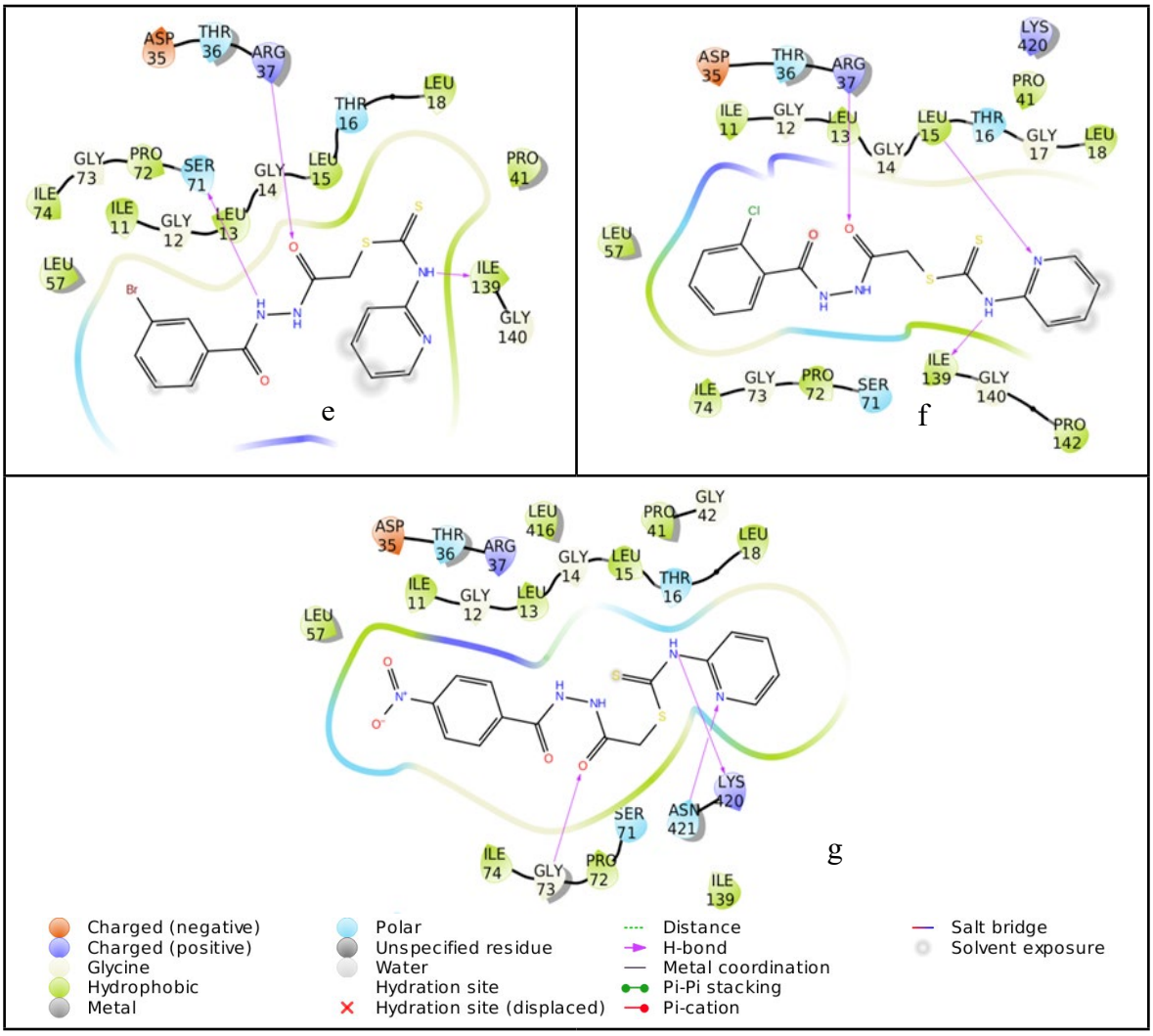


Figure 1. Plot represents 2D-ligand interaction diagram of compounds (a) **4a** (b) **4b** (c) **4c** (d) **4d** (e) **4e** (f) **4f** (g) **4g** in the catalytic pocket of MurD *E. coli* (pdb.2Y10).

CONCLUSION

In the present investigation compounds **4a-g** were synthesized and characterized by spectral data. Compounds were evaluated for their antibacterial activity against Gram-positive bacteria *S. aureus* NCIM 5022 and Gram-negative bacteria *E. coli* NCIM 2065 and *P. aeruginosa* NCIM 2200 by disc diffusion and two-fold serial dilution methods. Except compound **4b**, all other tested compounds exhibited significant activity against the tested strain of Gram-negative bacteria *P. aeruginosa* 2200 with MIC values in the range 31.25-62.50 µg/ml. Most of the tested compounds did not show activity against Gram-positive bacteria *S. aureus* NCIM 5022. Compound **4a** and **4c** revealed significant antibacterial activity against Gram-negative bacteria *E. coli* with MIC value of 15.62 µg/ml, which is comparable to the standard drug ciprofloxacin. Extra-precision docking results of compounds **4a-g** showed favourable hydrogen bonding interactions with residues Leu15, Arg37, Ile139, Lys319 and Lys348 within the catalytic pocket. Binding free energy calculation showed that van der Waals and Coulomb energy terms are favourable for the ligand binding to *E. coli* MurD enzyme.

ACKNOWLEDGEMENTS

The authors are thankful to the JSS Academy of Higher Education and Research, Mysuru for providing support.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

Anishetty, S., Pulimi, M. & Pennathur, G. (2005). Potential drug targets in *Mycobacterium tuberculosis* through metabolic pathway analysis. *Computational Biology and Chemistry*, *29*, 368-378.

Barry, A.L.J. (1999). The Antimicrobial Susceptibility Test, Principle and Practices. Lea and Febiger. 4, 180.

Bertrand, J.A., Auger, G., Martin, L., Fanchon, E., Blanot, D, LeBeller. D., van Hiijenoort J. & Dideberg, O. (1999). Determination of the MurD mechanism through crystallographic analysis of enzyme complexes. *Journal of Molecular Biology*, *289*, 579-590.

Bouhss, A., Dementin, S., Parquet, C., Mengin-Lecreulx, D., Bertrand, J.A., Le Beller, D., Dideberg, O., van Heijenoort, J., Blanot, D. (1999). Role of the ortholog and paralog amino acid invariants in the active site of the UDP-MurNAc-l-alanine:D-glutamate ligase (MurD). *Biochemistry*, *38*, 12240-12247.

Friesner, R.A., Murphy, R.B., Repasky, M.P., Frye, L.L., Greenwood, J.R., Halgren, T.A., Sanschagrin, P.C. & Mainz, D.T. (2006). Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *Journal of Medicinal Chemistry*, 49, 6177-6196.

Gvozdjaková, A., Odlerova Z. & Gogh, T. (1979). Synthesis and tuberculostatic properties of S-alkyl 2-(α-naphthothiazolyl)dithiocarbamates. *Chemical Papers*, *33*, 129-132.

Harder, E., Damm, W. & Maple, J. (2016). OPLS3: a force field providing broad coverage of drug-like small molecules and proteins. *Journal of Chemical Theory and Computation*, *12*, 281-296.

Haripriya, V., Lakxminarayana, E., Chary & MT, (2016). Synthesis and antibacterial activity of some new quinaxaline-bezohydrazides. *Indian journal of Chemistry*, *55B*, 207-212.

Hendlin, D., Stapley, E.O., Jackson, M., Wallick, H., Miller, A.K., Wolf & F.J.(1969). Phosphonomycin, a new antibiotic produced by strains of streptomyces. *Science*, *166*, 122-123.

Jacobson, M.P., Pincus, D.L., Rapp, C.S., Day, T.J., Honig, B., Shaw, D.E., Friesner & R.A. (2004). A Hierarchical approach to all-atom protein loop prediction. *Proteins: Structure Function and Bioinformatics*, *55*, 351-367.

Kahan, F.M., Kahan, J.S., Cassidy, P.J., Kropp & H. (1974). The mechanism of action of fosfomycin (phosphonomycin). *Annals of the New York Academy of Sciences*, *235*, 364-386.

Kamani, K.A., Patel & K.D (2016) Synthesis and pharmacology evolution of new coumarin clubbed carbamodithioate moiety using biocatalyst. *Chemical Science Transactions*, *5*, 886-893.

Kiran, K., Ashok, D., Rao, B.A., Sarasija, M., Rao & A.S (2017). Synthesis, characterisation, and antibacterial activity of some novel vanillin related hydrazone derivatives bearing 1,2,3-triazole ring. *Russian Journal of General Chemistry*, *87*, 1288-1294.

Knott, E.B. (1956). Heterocyclyl-rhodanines and -2-thiohydantoins. *Journal of the Chemical Society*, *0*, 1644-1649.

Li, J., Abel, R., Zhu, K., Cao, Y., Zhao, S., Friesner & R.A. (2011). The VSGB 2.0 Model: A next generation energy model for high resolution protein structure modelling. *Proteins, 79*, 2794-2812.

Lugtenberg, E.J., v Schijndel-van & Dam. A. (1972a). Temperature-sensitive mutants of *Escherichia coli* K-12 with low activities of the L-alanine adding enzyme and the D-alanyl-D-alanine adding enzyme. *Journal of Bacteriology*, *110*, 35-40.

Lugtenberg, E.J., v Schijndel-van & Dam. A. (1972b). Temperature-sensitive mutants of *Escherichia coli* K-12with low activity of the diaminopimelic acid adding enzyme. *Journal of Bacteriology*, *110*, 41–46.

Lugtenberg, E.J., v Schijndel-van & Dam. A. (1973). Temperature-sensitive mutant of *Escherichia coli* K-12 with an impaired D-alanine: D-alanine ligase. *Journal of Bacteriology*, *113*, 96–104.

Mol, C.D., Brooun, A., Dougan, D.R., Hilgers, M.T., Tari, L.W., Wijnands & R.A.(2003). Crystal structures of active fully assembled substrate- and product-bound complexes of UDP-N-acetylmuramicacid: L-alanine ligase (MurC) from *Haemophilus influenzae*. *Journal of Bacteriology*, *185*, 4152-4162.

Salton, M.R.J. (1994). The bacterial cell envelope. A historical perspective. In: Ghuysen JM, Hakenbeck R (ed) Bacterial Cell Wall ,1st edn. Amsterdam, *Elsevier Science B.V*, *1*.

Sastry, G.M., Adzhigirey, M., Day, T., Annabhimoju, R., Sherman & W. (2013). Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *Journal of Computer-Aided Molecular Design*, *27*, 221-234.

Skedelj, V., Tomasic, T., Masic, L.P., Zega & A. (2011) ATP-binding site of bacterial enzymes as a target for antibacterial drug design. *Journal of Medicinal Chemistry*, *54*, 915-929.

Smith, C.A. (2006). Structure, function and dynamics in the mur family of bacterial cell wall ligases. *Journal of Molecular Biology*, *362*, 640-655.

Spraggon, G., Schwarzenbacher, R., Kreusch, A., Lee, C.C., Abdubek, P., Ambing & E. (2004). Crystal structure of an Udp-n-acetylmuramate-alanine ligase MurC (TM0231) from Thermotogamaritima at 2.3 A resolution. *Proteins*, *55*,1078-1081.

VanHeijenoort, J (2001). Recent advances in the formation ofthe bacterial peptidoglycan monomer unit. *Natural Product Reports*, *18*, 503-519.

Vollmer, W., Blanot, D., de Pedro & M.A (2008). Peptidoglycan structure and architecture. *FEMS Microbiology Reviews*, *32*, 149-67.

Vuksanovic, V., Leka, Z., Terzic & N. (2013). Antibacterial effect of synthesized dithiocarbamate K-DAAP. *Fresenius Environmental Bulletin*, *22*, 3803-3807.

Walsh, A.W., Falk, P.J., Thanassi, J., Discotto, L., Pucci, M.J., Ho & H.T. (1999). Comparison of the D-glutamate adding enzymes from selected gram-positive and gram-negative bacteria. *Journal of Bacteriology*, *181*, 5395–5401.

Wisterowicz, K., Gobis, K., Henryk Foks, H., Augustynowicz-Kopec & E. (2012). The synthesis and tuberculostatic activity of benzenesulfonohydrazide derivatives. *Heteroatom Chemistry*, *23*, 99-104.