Research Article

The facile and efficient ultrasound-assisted synthesis, molecular docking and antimicrobial evaluation of 1, 3-dithiane derivatives.

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ABSTRACT

A new, simple and efficient method for the synthesis of novel 1, 3-dithiane derivatives under ultrasonic conditions *via* a one-pot three-component reaction of activated methylene group malononitrile with carbon disulfide in the presence of arylidene malononitriles was reported. The effects of $LiOH.H_2O$ as a base under ultrasonic conditions at different concentrations have been investigated and the reaction provided products with good yields at 40-50ºC temperature. Explorations of synthesized 1,3-dithiane substituted **(**ketene dithioacetals) compounds for the antimicrobial study were found to be effective towards *S. aureus* (ACPDM-5 and ACPDM-13) with a zone of inhibition at 26mm and 22mm, which is compared to that of standard ciprofloxacin (26mm). This made our study to explore the inhibition mechanism with the help of molecular docking studies with possible binding energies (-6.4 to -8.9 kJ/mol) by pyrx 0.8 software to represent a good prediction of interactions between the ligand and protein (2XCT). Based on the *in-vitro* and *in-silico* studies a series of ketene dithioacetals compounds may be helpful for further studying SAR, ADMET and designing antimicrobials that are more potent.

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INTRODUCTION

In, heterocyclic chemistry, organosulfur compounds which contain sulfur in their cyclic structure is well known for their pharmacological activities (1). Sulfur containing drugs such as sulfonamides, thioethers, sulfones, penicillins and cephalosporins moieties which are well studied both on synthesis and on application during the past decades but, there is very little research carried out on synthesis and pharmacological evaluation of dithianes.

Dithiane is a six-membered cyclohexane derivative in which two methylene positions are being replaced by two sulfur atoms which exists in three forms i.e., 1,2 –dithiane, 1,3–dithiane, and 1,4-dithiane. 1,3-dithiane or 1,3-dithiacyclohexane, is one of the constituents isolated from garlic and other allium species. 1,3-dithiane-incorporated compounds have been reported to possess pesticidal, insecticidal, and human 5α-reductase inhibitory activity. Recently, a number of dithiane-incorporated pregnane derivatives were synthesized and evaluated for their *in-vitro* antifungal and antibacterial activity (2).

1,3-dithiane substituted with olefin in 2nd position forms compounds ketene dithioacetals, which are useful and convenient reagents for the synthesis of a variety of heterocyclic compounds (3-8). These molecules facilitate a nucleophilic character by the olefinic linkage present with the help of electron releasing alkylthio groups (9-11). Functional groups at the α-carbon such as cyano, oxo, nitro, sulfonyl, phosphonoyl, trifluoromethyl, bromo, iodo, chloro-/bromo-ethenyl, ethynyl and silyl etc. along with alkylthio groups were made interest for organic synthesis. The most suitable method for synthesis of functionalized ketene dithioacetals involves the reaction of an active methylene compound with carbon disulfide in the presence of suitable bases like lithium dialkyl amide (12), sodium hydride (13), potassium *t*-butoxide (14), KF/Alumina (15) and trimethylamine (16-17) subsequent alkylation with an alkylating agent. Therefore, with the aim to prepare new α-functionalized ketene dithioacetals from the reaction of α-cyano ketene dithioacetals by malononitrile and carbon disulfide, with electrophiles such as arylidene-malononitriles derivatives in one component reaction with the help of $LiOH.H_2O$ (lithium hydroxide monohydrate) to afford synthesis of 2-(6-amino-5-cyano-4-aryl-4*H*-1,3-dithiin-2-ylidene)-malononitrile as novel compounds.

Development of eco-friendly, cheaper, simpler, and more efficient methodologies for synthesis of widely used organic

compounds from readily available reagents is one of the major challenges for chemists in organic synthesis (18). Compared to conventional techniques, ultrasound assisted methods are widely used technique in organic synthesis as the procedure is more convenient, useful in the synthesis of a wide range of organic, inorganic and nanostructured materials with high yields, shorter reaction time and milder conditions and finally cost effective (19). Applying a high power ultrasonic wave into liquid reaction mixtures is known to cause a variety of chemical transformations (19). In this study we have, therefore, decided to apply ultrasonic technique to synthesize novel1, 3-dithiane substituted (ketene dithioacetal) derivatives.

MATERIALS AND METHODS

General

All the chemicals were purchased from Sigma Aldrich Ltd., (Germany) and Merck with the quality of analytical grade and were used without any further purification. The melting points of synthesized compounds were determined by the open capillary tube method and the results are uncorrected. The progress of the reaction was monitored by thin layer chromatography (TLC) plates, which were precoated with aluminum silica gel 60F 254 procured from Merck (Germany) using solvent system were n-hexane: Ethyl acetate (1.5:0.5) by UV absorption for visualization. The synthesized compounds were characterized by IR, ^{1}H-NMRspectroscopy.FTIR spectra were recorded in KBr on Bruker Alpha. ^{1}H-NMR spectra were recorded on Bruker Avance-III 400 MHz instrument with $CDCl_3$ as solvent and chemical shift values are reported relative to tetramethylsilane (TMS) as an internal standard.

All the reactions were carried out using an ultrasonic cleaner (with a frequency of 50 Hz and a nominal power 170 W) by locating the reaction flask in the maximum energy area in the cleaner; where the surface of reactants (reaction vessel) is slightly lower than the level of the water (20).

General procedure for the preparation of compounds (ACPDM-1 to ACPDM-15)

A mixture of active methylene compound malononitrile **1** (1.0 mmol), carbon disulfide **2** (3.0 mmol), Arylidene malononitrile **3** (1 mmol) and lithium hydroxide monohydrate ($LiOH.H_2O$) (0.1 mmol) were added to a reaction flask containing 10 ml of ethanol at room temperature. The mixture was then irradiated in the water bath of an ultrasonic cleaner at the room temperature for the period as indicated in Table 2, during

ultrasonication the reaction was maintained at 40-50°C. After completion of the reaction, (determined by TLC) the reaction mixture was poured in cold water and precipitate obtained was collected. The crude product was recrystallized from ethanol to give pure compounds ACPDM-1 to ACPDM-15with 84 to 98 % yields.

2-(6-amino-5-cyano-4-phenyl-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-1)

Cream crystals; Mp: 42-44ºC IR υ/cm^{-1} (KBr):3367, 3217, 3032, 2223, 1658, 1589, 1257, 616. ^{1}H NMR (400 MHz, Chloroform-*d*): δ = 1.25 (s, 1H, CH), 7.527-7.658 (m, 3H, Ar), 7.779 (s, 2H, NH_2), 7.897-7.926 (m, 2H, Ar-H).

2-(6-amino-5-cyano-4-(4-(dimethylamino) phenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-2)

Brown crystals; Mp: 56-58ºC IR υ/cm^{-1}(KBr): 3554, 3350, 3077, 2201, 1608, 1556, 1252, 723. ^{1}H NMR (400 MHz, Chloroform-*d*): δ = 3.139 (s, 7H, CH, and CH_3), 6.678-6.701 (d, *J* = 9.2 Hz, 2H, Ar), 7.464 (s, 2H, NH_2), 7.803-7.82 (d, *J* = 7.6 Hz, 2H, Ar).

2-(6-amino-5-cyano-4-(3-nitrophenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-3)

Dark yellow crystals; Mp: 80-82ºC IR υ/cm^{-1}(KBr): 3367, 3299, 3040, 2229, 1659, 1596, 1524, 1215, 620. ^{1}H NMR (400 MHz, Chloroform): δ = 1.263(s, 1H, CH), 7.771-7.811 (t, *J* = 8.0 Hz, 1H, Ar), 7.882(s, 2H, NH_2), 8.311-8.334 (m, 1H, Ar), 8.458-8.487(m, 1H, Ar), 8.651-8.660(t, *J* = 2.0 Hz, 1H, Ar).

2-(6-amino-5-cyano-4-(3, 4-dimethoxyphenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-4)

Brown crystals; Mp: 70-72ºC IR υ/cm^{-1}(KBr): 3469, 3376, 3010, 2221, 1644, 1564, 1271, 1252, 625. ^{1}H NMR (400 MHz, Chloroform): δ = 3.982 (s, 7H, CH, OCH_3), 6.946-6.967 (d, *J* = 8.4 Hz, 1H, Ar), 7.367--7.394 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H, Ar), 7.636 (s, 2H, NH_2), 7.675-7.680 (d, *J* = 2.0 Hz, 1H, Ar)

2-(6-amino-4-(2-chlorophenyl)-5-cyano-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-5)

Light Brown crystals; Mp: 75-77ºC IR υ/cm^{-1}(KBr): 3465, 3356, 3023, 2227, 1636, 1582, 862, 619. ^{1}H NMR (400 MHz, Chloroform): δ = 1.263 (s, 1H, CH), 7.429-7.483 (m, 1H, Ar), 7.544-7558 (m, 2H, Ar), 8.171-8.192(d, *J* = 7.2 Hz, 1H, Ar), 8.270 (s, 2H, NH_2).

2-(6-amino-4-(3-chlorophenyl)-5-cyano-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-6)

Cream crystals; Mp: 98-100ºC IR υ/cm^{-1}(KBr): 3465, 3356, 3030, 2227, 1657, 1587, 837, 620. ^{1}H NMR (400 MHz, Chloroform): δ = 1.255 (s, 1H, CH),

7.476-7.517 (m, 1H, Ar), 7.588-7.616 (m, 1H, Ar), 7.720 (s, 2H, NH_2), 7.827-7.846 (m, 2H, Ar).

2-(6-amino-5-cyano-4-(p-tolyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-7)

Yellow crystals; Mp: 126-128ºC IR υ/cm^{-1}(KBr): 3452, 3365, 3034, 2221, 1655, 1625, 625. ^{1}H NMR (400 MHz, Chloroform): δ = 2.458 (s, 4H, CH, and CH_3), 7.328-7.348 (d, *J* = 8.0 Hz, 2H, Ar), 7.718 (s, 2H, NH_2), 7.802-7.822 (d, *J* = 8.0 Hz, 2H, Ar).

2-(6-amino-5-cyano-4-(4-fluorophenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-8)

Brown crystals; Mp: 180-182ºC IR υ/cm^{-1}(KBr): 3498, 3378, 3037, 2229, 1660, 1593, 1239, 616. ^{1}H NMR (400 MHz, Chloroform): δ = 1.255 (s, 1H, CH), 7.209-7.259 (m, 2H, Ar), 7.736 (s, 2H, NH_2), 7.934-7.984 (dd, *J* = 4.8 Hz, *J* = 3.2 Hz, 2H, Ar).

2-(6-amino-5-cyano-4-(3-hydroxyphenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-9)

Brown crystals; Mp: 150-152ºC IR υ/cm^{-1}(KBr): 3428, 3367, 2240, 1649, 1571, 615. ^{1}H NMR (400 MHz, Chloroform): δ = 1.255 (s, 1H, CH), 5.489 (s, 1H, OH), 7.102-7.131 (m, 1H, Ar), 7.362-7.463 (m, 3H, Ar), 7.713 (s, 2H, NH_2).

2-(6-amino-5-cyano-4-(3, 4, 5-trimethoxyphenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-10)

Dark yellow crystals; Mp: 140-142ºC IR υ/cm^{-1}(KBr): 3563, 3364, 3018, 2219, 1642, 1567, 1256, 631. ^{1}H NMR (400 MHz, Chloroform): δ = 3.911 (s, 7H, CH and OCH_3), 3.983 (s, 3H, OCH_3), 7.190 (s, 2H, Ar), 7.647 (s, 2H, NH_2).

2-(6-amino-4-(10-chloroanthracen-9-yl)-5-cyano-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-11)

Brown crystals; Mp: 148-150ºC IR υ/cm^{-1}(KBr): 3385, 3255, 3049, 2233, 1612, 1593, 628. ^{1}H NMR (400 MHz, Chloroform): δ = 4.031 (s, 1H, CH), 7.690 (s, 2H, NH_2), 7.976-7.981 (m, 4H, Ar), 8.070-8.075 (m, 4H, Ar).

2-(6-amino-5-cyano-4-(2-nitrophenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-12)

Dark brown crystals; Mp: 98-100ºC IR υ/cm^{-1}(KBr): 3367, 3299, 3040, 2229, 1659, 1596, 1524, 1215, 620. ^{1}H NMR (400 MHz, Chloroform): δ = 1.254 (s, 1H, CH), 7.786-7.900 (m, 3H, Ar), 8.343-8.363 (d, *J* = 8.0 Hz, 1H, Ar), 8.445 (s, 2H, NH_2).

2-(6-amino-5-cyano-4-(2-fluorophenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-13)

Cream crystals; Mp: 88-90ºC IR υ/cm^{-1}(KBr): 3498, 3378, 3037, 2229, 1660,

1593, 1160, 616. ^{1}H NMR (400 MHz, Chloroform): δ = 1.225 (s, 1H, CH), 7.225-7.253 (m, 1H, Ar), 7.318-7.357 (m, 1H, Ar), 7.611-7.669 (m, 1H, Ar) 8.097 (s, 2H, NH_2), 8.262-8.303 (m, 1H, Ar)

2-(6-amino-5-cyano-4-(4-hydroxyphenyl)-4H-1, 3-dithiin-2-ylidene) malononitrile (ACPDM-14)

Yellow crystals; Mp: 66-68ºC IR υ/cm^{-1}(KBr): 3428, 3361, 2240, 1649, 1571, 615. ^{1}H NMR (400 MHz, Chloroform): δ = 1.255 (s, 1H, CH), 5.880 (s, 1H, OH), 6.949-6.971 (m, 2H, Ar), 7.644 (s, 2H, NH_2), 7.865-7.886 ((m, 2H, Ar).

2-(6-amino-5-cyano-4-(thiophen-2-yl)-4H-1, 3-dithiin-2-ylidene) malononitrile-(ACPDM-15)

Cream crystals; Mp: 88-90ºC IR υ/cm^{-1}(KBr): 34989, 3378, 3070, 2229, 1660, and 1593. 615. ^{1}H NMR (400 MHz, Chloroform): δ = 1.254 (s, 1H, CH), 7.270-7.283 (m, 1H, Ar), 7.805-7.819 (m, 1H, Ar), 7.869 (s, 2H, NH_2), 7.875-7.889 (m, 1H, Ar)

BIOLOGICAL EVALUATION

Antimicrobial activity

Four bacterial strains of Gram-positive (*Bacillus subtilis* ATCC 6633, and *Staphylococcus aureus* ATCC 29213), Gram-negative (*Escherichia coli* ATCC 25922) and as a representative fungus (Candida albicans ATCC 10231) were used for *in vitro* antimicrobial activity. The antimicrobial potential of the newly synthesized 1, 3-dithiane derivatives (ketene dithioacetals) were investigated towards the tested microorganisms and expressed as the diameter of the inhibition zones according to the agar plate diffusion method (21). 100 µL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/ mL for bacteria or 10^5 cells/mL for fungi. One mL of each sample (at 1 mg/mL) was added to each well (10 mm diameter holes cut in the agar gel). The plates were incubated for 24 h at 37 °C (for bacteria and yeast) and for 72 h at 27 °C (for filamentous fungi), each test was determined in triplicate. After incubation, the microorganism's growth was observed. Ciprofloxacin was used as standard antibacterial drugs while Fluconazole was used as standard antifungal drug. The resulting zone of inhibition were measured in millimeters and used as criterion for the antimicrobial activity. Solvent controls (DMSO) were included in each experiment as negative control. DMSO was used for dissolving the test compounds and showed no inhibition zones, confirming that it has no influence on growth of the tested microorganisms.

COMPUTATIONAL STUDIES

Computational Tools

To understand the molecular mechanism involved in antibacterial activities, we carried out the docking studies. At first, the ligand structures of newly synthesized 2-(6-amino-5-cyano-4-aryl 4*H*-1,3-dithiin-2-ylidene) malononitrile (ACPDM-1-ACPDM-15) was generated using Chem Draw 12.0 software. The structures were saved as a .cdx format then energy minimization was carried out and saved as .mol2 (ligand) file format. Simultaneously, the SMILE file format of all compounds were obtained from Chem Draw 12.0 in order to use them to carryout docking studies. The docking studies of all compounds were carried out with Pyrx 0.8 software (22).

Molecular docking studies

The crystal structure of DNA gyrase in complex with ciprofloxacin (PDB ID: 2XCT) (23) with resolution of 3.35A$^{\circ}$ was accessed from protein data bank (http://www.rcsb.org). For preparation of protein, a default procedure was followed by removing water molecules, polar hydrogen's were added and kollman united atom charges were assigned. Protien energy was minimized with spdbv and saved as .pdb format. For the preparation of ligands, software such as Chemdraw 12.0 in which 2D structure was converted into 3D structure and energy minimization was carried by MM2 for each ligand. Molecular Docking was carried out by virtual screening tool by pyrx 0.8. The grid centre was set at X=14.53, Y= 36.95, Z= 93.94. The grid size was set to 64.51, 84.97, 109.22 xyz points with grid spacing of 0.5A^{o} by keeping all parameters in defaults for docking. The visualization was done using discovery studio visualizer 2020 for receptor-ligand interactions.

RESULTS AND DISCUSSION

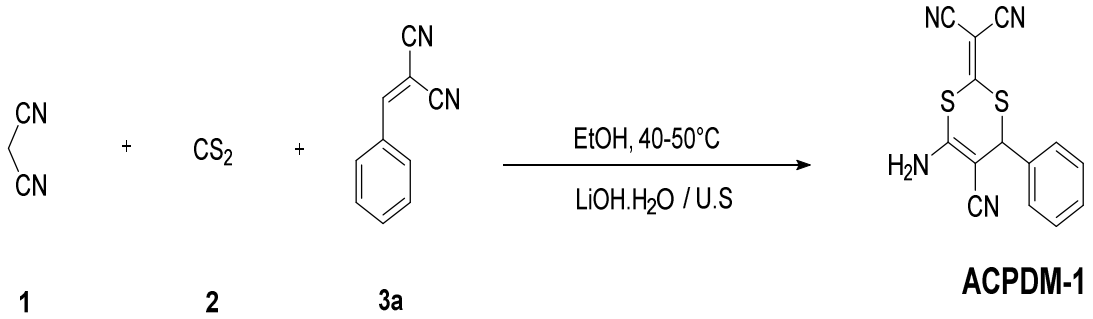
Reaction conditions and optimization

The optimization of reaction conditions, including the concentration of base, the reaction temperature and the equivalents of the starting materials was investigated under ultrasonication conditions. Previously several bases like NaOH, K_2CO_3, and trimethylamine were used in conventional methods for the reaction. Attempt has been made to use hydroxide like $LiOH.H_2O$, which has significant covalent character due to small size of the Li^+ ion. Water is used in knoevenegal condensation reaction as in ethanol/water medium as solvent by elimination of water molecule by with drawl of α-acidic proton from active methylene where adjacent two electron withdrawing groups are present to form the benzylidene derivatives. Initially the reactions were carried by refluxing and stirring in presence of lithium hydroxide

monohydrate at room temperature, but we have found that the reaction was not progressive and the yields were less than 50 % even after refluxing for 1 to 3 hrs.

Making the point of view in abstraction a model reaction was carried out by activated methylene compound like malononitrile **1** (1mmol) was reacted with carbon disulphide **2** (1mmol) for 0.5h (30 min) under ultrasonication and followed with addition of benzylidene malononitrile derivatives **3** (1mmol) in ethanol (Table 1) which was progressed under room temperature as a basic experiment (Table 1, Entry 1). Then the reaction was progressed by increasing the temperature to 40-50°C, only a 30 % amount of product was observed (Table 1, Entry 2). This indicates that temperature effects the reaction so reflux was carried out but only 45 % amount was observed (Table 1, Entry 3). To observe the effects of concentration of the starting materials we therefore increased the concentration of carbon disulphide to 2mmol (Table 1, Entries 4-5) and further up to 3.5 mmol and progressed the reactions at room temperature and 40-50°C under normal reflux and ultrasonication conditions. We found a great increase in the yields up to 95% (Table 1, Entries 4-7). Increasing the concentration beyond 3mmol did not improve the yield more than 93% (Table 1, Entry 8). So by taking into account of 1:3:1 ratio a series of experiments were performed when malononitrile **1**of 1mmol was reacted with carbon disulphide **2** of 3mmol and arylidene malononitrile **3** of 1mmol in ethanol at 40-50°C to obtain optimum results of optimized compound 2-(6-amino-5-cyano-4-aryl-4*H*-1,3-dithiin-2-ylidene) malononitrile under ultrasonication conditions.

Table 1. Optimization of 2-(6-amino-5-cyano-4-phenyl-4*H*-1, 3-dithiin-2-ylidene) malononitrile



1 **2** **3a** **ACPDM-1**

Entry	Solvent	Base[a]	Temp. (°C)[b]	mmol of	Yield (%)[c]	Ultrasonication time (in min)
1	Ethanol	$LiOH.H_2O$	r.t.	1:1:1	N.R	0
2	Ethanol	$LiOH.H_2O$	40-50°C	1:2:1	30	0
3	Ethanol	$LiOH.H_2O$	reflux	1:3:1	45	0
4	Ethanol	$LiOH.H_2O$	r.t.	1:2:1	68	30
5	Ethanol	$LiOH.H_2O$	40-50°C	1:2:1	72	30
6	Ethanol	$LiOH.H_2O$	r.t.	1:3:1	84	30
7	Ethanol	$LiOH.H_2O$	40-50°C	1:3:1	95	30
8	Ethanol	$LiOH.H_2O$	40-50°C	1:3.5:1	93	30

[a]Amount of base was 0.1mmol

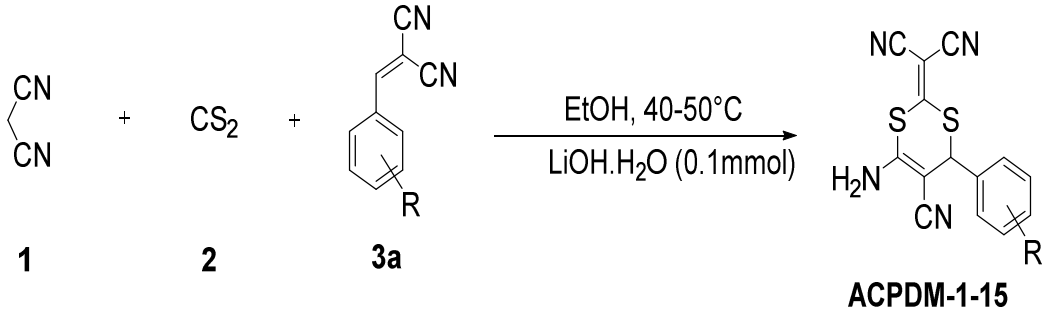
[b]Reaction time for non-ultrsonic conditions was 6h

[c]Yield is given for isolated product

U.S in the reaction indicates Ultrasonication

To the best of our knowledge, all the synthesized compounds are novel depicted in Table 2, were characterized by Infrared spectroscopy, ^{1}H-NMR analysis. For instance, the ^{1}H NMR spectrum methine proton was observed as singlet at δ = 1.25 ppm, a D_2O-exchangeable signal at δ =7.77 ppm which is attributed to the NH_2 group, and a multiplet at δ = 7.52-7.65 ppm and 7.89-7.92 ppm for the aromatic protons of the phenyl ring were also observed.

Table 2. Synthesis of 1, 3-dithiane derivatives (ACPDM-1-ACPDM-15).



Product	R	Ultrsonic Time (h)	Yield (%)
ACPDM-1	H	0.5	95
ACPDM-2	4-$(CH_3)_2NC_6H_4$-	0.5	85
ACPDM-3	3-NO_2 C_6H_4-	0.5	91
ACPDM-4	3,4-di$OCH_3C_6H_3$-	0.5	90
ACPDM-5	2-ClC_6H_4-	0.5	87
ACPDM-6	3-ClC_6H_4-	0.5	84
ACPDM-7	4-$CH_3C_6H_4$-	0.5	89
ACPDM-8	4-FC_6H_4-	0.5	87
ACPDM-9	3-OHC_6H_4-	0.5	91
ACPDM-10	3,4,5-triOCH_3 C_6H_2-	0.5	90
ACPDM-11	10- Cl- 9-anthraldehyde	0.5	95
ACPDM-12	2-$NO_2C_6H_4$	0.5	95
ACPDM-13	2-F C_6H_4	0.5	98
ACPDM-14	4-OHC_6H_4	0.5	94
ACPDM-15	2-thiophene-carboxaldehyde	0.5	92

Biological Evaluation

Antimicrobial Evaluation

The newly synthesized 1, 3-dithiane derivativesACPDM-1-ACPDM-15were tested for their antimicrobial activity and their resulting inhibition zones were measured in mm diameter, in **Table 3**. Among the tested compounds, both ACPDM-5 and ACPDM-13 were found to be most active for bacterial strains and ACPDM-13 were found to be active for fungal strains. Among the different substituents attached to the ketene dithioacetals which exerted potential antimicrobial activity against gram positive and gram negative bacteria are the electron withdrawing groups to an aromatic ring like fluoro, chloro and nitro groups attached in ortho positions compared to that of an electron donating groups.

Table 3. *In-vitro* mean diameter of inhibition zone (mm) for the synthesized1, 3-dithiane derivatives against pathogenic bacteria and fungi.

Compounds	*B. Subtilis*	*S. Aureus*	*E. Coli*	*C. Albicans*
ACPDM-1	**9**	**12**	**11**	**na**
ACPDM-2	**14**	**14**	**na**	**17**
ACPDM-3	**12**	**13**	**13**	**11**
ACPDM-4	**8**	**11**	**18**	**18**
ACPDM-5	**24**	**26**	**22**	**12**
ACPDM-6	**na**	**na**	**15**	**9**
ACPDM-7	**10**	**12**	**na**	**16**
ACPDM-8	**13**	**17**	**11**	**na**
ACPDM-9	**13**	**na**	**8**	**14**
ACPDM-10	**15**	**14**	**na**	**10**
ACPDM-11	**18**	**18**	**18**	**15**
ACPDM-12	**19**	**15**	**18**	**11**
ACPDM-13	**22**	**22**	**19**	**22**
ACPDM-14	**11**	**14**	**9**	**15**
ACPDM-15	**19**	**12**	**14**	**19**
Ciprofloxacin	**26**	**26**	**24**	**-**
Fluconazole	**-**	**-**	**-**	**25**

na: Not active / no activity

Computational Results

Molecular docking studies

Considering the outcome of antimicrobial activity, it was thought worthy to execute computational method like molecular docking studies by substantiating *the in-vivo* results with *in-silico* studies. The enzyme DNA Gyrase is selected for the present study which is one of the topoisomerases II classes are involved in winding and unwinding of DNA during the process of replication and transcription. Gyrase enzyme is present in both prokaryotic and eukaryotic cell but the enzymes are not entirely similar in structure or sequence, which affects the topological state of DNA, and have different affinities for different molecules hence it is considered as an important intracellular target for antibacterial agents as a representative model for other DNA topoisomerases [22]. Further investigation was made based on fact to study for binding mode, docking score energy and

the predictable type of interactions between the new chemical entities and binding site of the target protein of DNA Gyrase enzyme. Among the synthesized compounds ACPDM-5, ACPDM-11, ACPDM-12 and ACPDM-13 exhibited good DNA gyrase affinity along with standard ciprofloxacin. They established good poses that are represented in form of hydrogen bonds, binding affinities and amino acids depicted in **Table 4**. All the docked molecules were subjected to 2D and 3D protein-ligand interaction analysis. **Figure 1** represents the further extrapolation of binding conformation of the docked molecules.

Table 4. Molecular Docking Study of novel 1, 3-dithiane derivatives in the active site of 2XCT.

LIGANDS	BINDING AFFINITY	NO. OF HYDROGEN BONDS	AMINO ACIDS
ACPDM-1	-6.8	3	GLN B:1095, SER B: 1112, MET B: 1113
ACPDM-2	-7	5	ARG B:1092, SER B: 1112 (2), ASP B 1116, ALA B: 1118
ACPDM-3	-6.9	8	GLY B: 436, SER B: 438, ALA B: 439, ASP B: 508, ASP B: 510 (2), GLY B: 1082, SER B: 1084
ACPDM-4	-6.4	6	ARG B:1092 (2), GLN B:1095, SER B: 1112 (2), MET B: 1113
ACPDM-5	-7.5	7	ARG B:1092 (2), GLN B:1095, SER B: 1112, MET B: 1113, GLY B: 1115, GLN B: 1267
ACPDM-6	-6.9	3	GLN B:1095, SER B: 1112 (2)
ACPDM-7	-6.8	3	ASN B: 1269, ARG B: 1272 (2)
ACPDM-8	-7.1	3	GLN B:1095, SER B: 1112, MET B: 1113
ACPDM-9	-7.1	4	ARG B: 517, GLN B: 541 (2), THR B: 544, ARG B: 1012
ACPDM-10	-6.4	5	THR B: 1059, LEU B: 1136, ILE B: 1139, ASN B: 1140, LYS B: 1141
ACPDM-11	-8.9	4	ASN B: 1054, LEU B: 1136, ASN B: 1140, LYS B: 1141
ACPDM-12	-7.6	7	ARG B:1092 (2), GLN B: 1095, SER 1112 (2), MET B: 1113, GLN B: 1267
ACPDM-13	-7.4	7	ARG B:1092 (2), GLN B: 1095 (2), SER 1112 (2), MET B: 1113
ACPDM-14	-6.9	6	THR B: 507, ALA B: 540, GLN B: 541, GLU B: 1017, GLU B: 1020, SER B: 1021
ACPDM-15	-6.5	4	ARG B:1092, SER B: 1112 ASP B: 1116, ALA B: 1118
CIPROFLOXACIN	7.4	3	GLN B: 1095 (2), ALA B: 1118

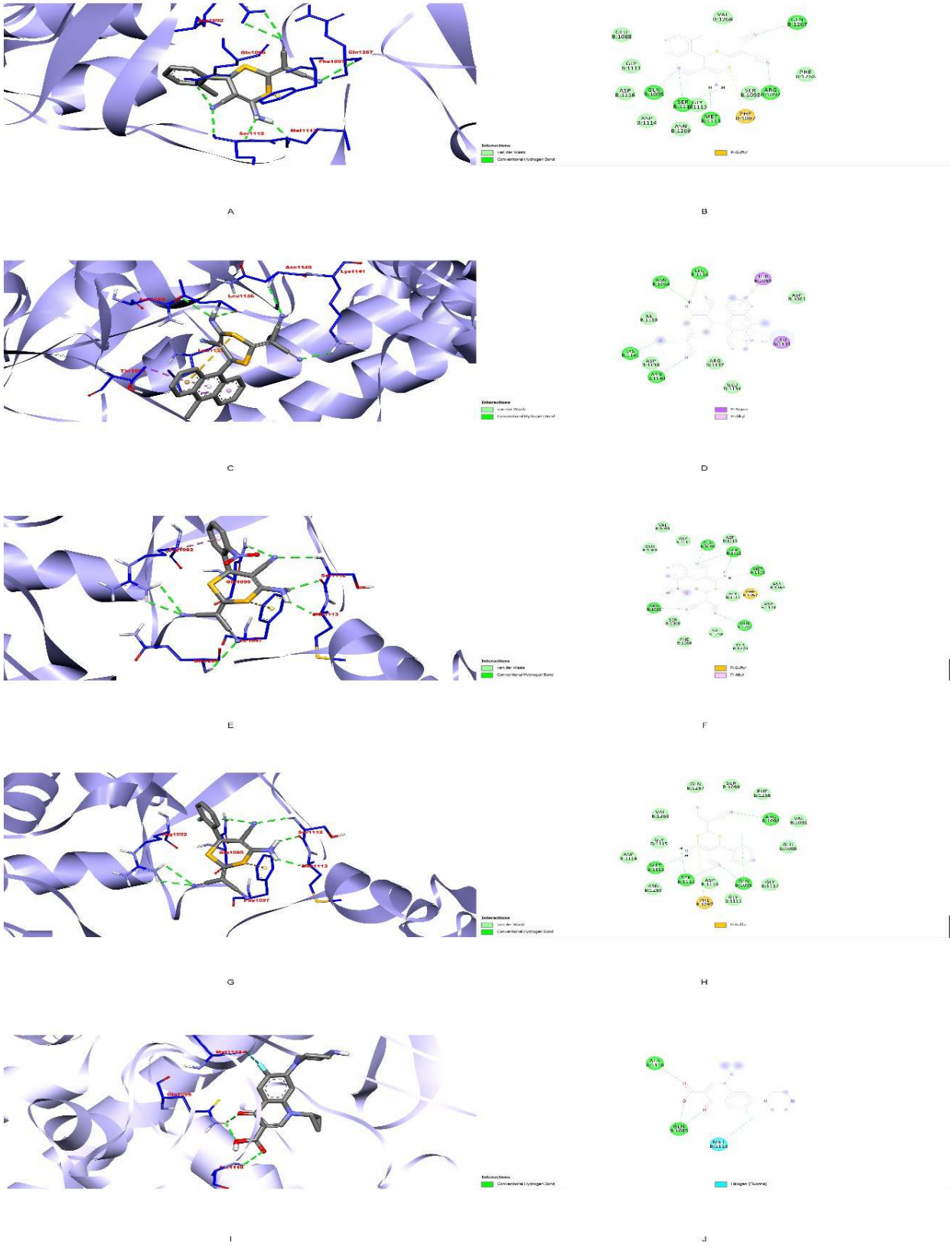


Figure 1: Docking poses of 1,3-dithiane derivativesACPDM-5 (A & B), ACPDM-11 (C & D), ACPDM-12 (E & F), ACPDM-13 (G & H), and Ciprofloxacin (I & J)

CONCLUSION

A novel, green synthetic and one-pot three component reaction approach was developed to synthesize 15 (ACPDM-1 toACPDM-15) new1,3-dithiane derivatives (ketene dithioacetals) by reacting active methylene compounds like carbon disulfide, and arylidene malononitriles in the presence of lithium hydroxide monohydrate as a base under ultrasonic conditions. Among the synthesized compounds, compounds ACPDM-5 and ACPDM-13 were found to be effective against gram positive *S. aureus*. Molecular docking simulations were carried out for receptor-ligand interactions which revealed that the compound ACPDM-5 and ACPDM-13 have good binding modes and affinity towards DNA gyrase binding site comparable to that of ciprofloxacin. Further, for the best outfit of these compounds in-silico studies like 3D-QSAR and ADMET studies could be performed to design highly potent 1, 3-dithiane derivatives.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

No animals were used.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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