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Synthesis of novel ketene dithioacetals via one pot reaction: Molecular modelling *in-silico* Admet studies and antimicrobial activity

Mohammad Arif Pasha[a], Sumanta Mondal[a*] and Naresh Panigrahi[a]

[a]*Department of Pharmaceutical Chemistry, GITAM Institute of Pharmacy, Rushikonda, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, 530045, India*

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A B S T R A C T

A simple and efficient method for the synthesis of fifteen novel ketene dithioacetals (2-(6-amino5-cyano-4-aryl-4*H*-1,3-dithiin-2-ylidene) malononitrile) *via* a one-pot three-component reaction of activated methylene group malononitrile with carbon disulfide in the presence of arylidene malononitriles were reported. The effects of LiOH.H_2O as a base at different concentrations have been investigated and can provide products in good yields at 40-50ºC temperature (54-89%). All the synthesized ketene dithioacetals compounds (MCB1-MCB15) were checked for favorable pharmacokinetic parameters along with toxicities which are based on drug-likeness explained by Lipinski's rule of five by Med chem designer software correlated with that of pkCSM online tool. Explorations of synthesized ketene dithioacetals compounds for the antimicrobial study were found to be effective towards *Staphylococcus aureus* (MCB5 and MCB13) 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). Further evaluation of druggability and ADMET predictions compounds MCB5 and MCB13 were found to be effective. Based on the *in-vitro* and *in-silico* studies a series of ketene dithioacetals compounds may be helpful for further studying SAR and designing more potent antimicrobials.

1. Introduction

Development of organic molecules from one pot reaction has made considerable attention because no necessity for purification of organic intermediates and usage of solvents has been slashed. Environment friendly methodologies or processes are developing day to day for widely used organic compounds that are readily obtainable reagents which is one of the major challenges for chemists in organic synthesis. Attention has been made for organic synthesis which is green, mild and simple procedures. In this context, lithium hydroxide monohydrate (LiOH.H_2O) is used as a green basic catalyst for the organic synthesis.

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, penicillin's and cephalosporin's moieties which are well studied both on synthesis

* Corresponding author.
E-mail address: mondalresearch@gmail.com (S. Mondal)

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and application during the past decades but, little study was carried out so far for 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 molecule facilitate a nucleophilic character by the olefinic linkage present with the help of electron releasing alkylthio groups.[9-10] 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.[11] 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 triethylamine[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 $LiOH.H_2O$ to afford synthesis of 2-(6-amino-5-cyano-4-aryl-4*H*-1,3-dithiin-2-ylidene) malononitrile as novel compounds.

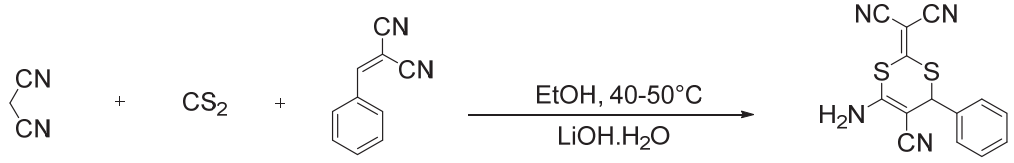
2. Results and Discussion

2.1.Reaction conditions and mechanism

The optimization of reaction conditions, including the concentration of base, the reaction temperature and the equivalents of the starting materials was investigated. Previously reactions were carried out by several bases like NaOH, K_2CO_3, and triethylamine[16-17]. So, an attempt has been made to use hydroxide like $LiOH.H_2O$ which has significant covalent character due to the small size of the Li^+ ion. $LiOH.H_2O$ is used in knoevenegal condensation reaction as in ethanol/water medium as solvent by elimination of water molecule by withdrawal of α-acidic proton from active methylene where adjacent two electron withdrawing groups are present to form the benzylidene derivatives.[18-19] 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 and followed with addition of benzylidene malononitrile derivatives **3** (1mmol) in ethanol(**Table 1**, Entries 1-8) which was progressed under room temperature as a basic experiment, we found that the reaction was not accelerated (**Table 1**, Entry 1). Then the reaction was progressed by increasing the temperature to 40-50°C trace amount of product was observed (**Table 1**, Entry 2). This indicates that temperature affects the reaction so reflux was carried out but only trace amount was observed (**Table 1**, Entry 3). This made us to increase the concentration of starting material in larger amounts by increasing the concentration of carbon disulphide to 2mmol (**Table 1**, Entries 4-5) and further to 3mmol (**Table 1**, Entries 6-7) and progressed the reactions under room temperature and in other by maintaining the temperature at 40-50°C. We found that the reaction was accelerated by an increase in carbon disulphide concentration along with maintaining the temperature at 40-50°C 30% yield at 2mmol and 85% yield was obtained at 3mmol. Increasing the concentration beyond 3mmol did not improve the yield more than 85% (**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.

To the best of our knowledge, all the synthesized compounds 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 = 1.256 ppm, a D_2O-exchangeable signal at δ =7.779 ppm which is attributed to the NH_2 group, and a multiplet at δ = 7.527-7.658 ppm and 7.897-7.926 ppm for the aromatic protons of the phenyl ring were also observed.

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

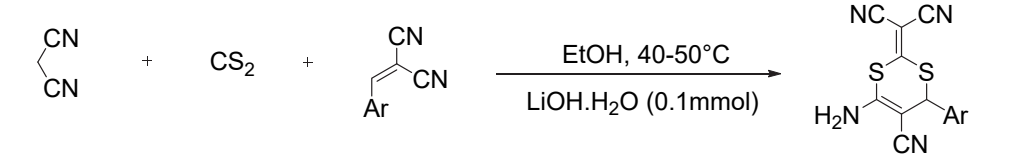


1 **2** **3** **4 (MCB1-MCB15)**

Entry	Base[a]	Temp. (°C)[b]	mmol of	Yield (%)[c]
1	$LiOH.H_2O$	r.t.	1:1:1	N.R
2	$LiOH.H_2O$	40-50°C	1:1:1	Trace
3	$LiOH.H_2O$	reflux	1:1:1	Trace
4	$LiOH.H_2O$	r.t.	1:2:1	N.R
5	$LiOH.H_2O$	40-50°C	1:2:1	30
6	$LiOH.H_2O$	r.t.	1:3:1	35
7	$LiOH.H_2O$	40-50°C	1:3:1	85
8	$LiOH.H_2O$	40-50°C	1:3.5:1	84

[a]Amount of base was 0.1mmol, [b]Reaction time was 6h, [c]Yield is given for isolated product, N.R=No Reaction, r.t= room temperature.

Table 2. Synthesis of ketene dithioacetals 4 (MCB1-MCB15) in solvent ethanol



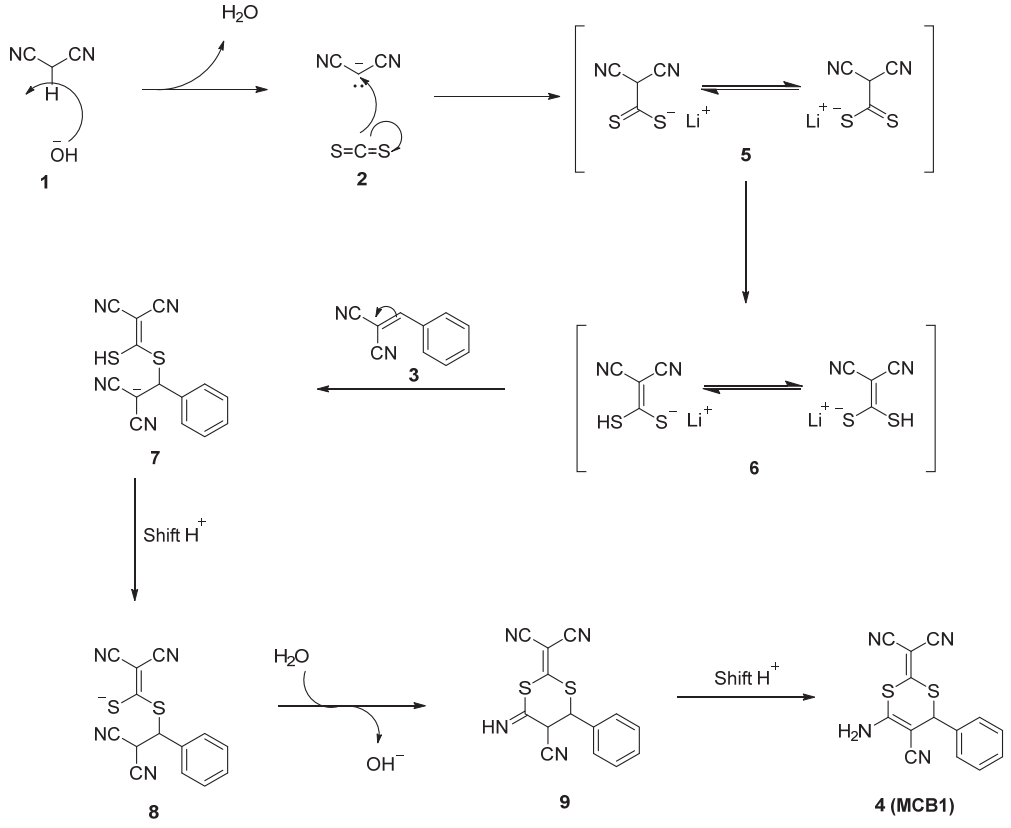
1 **2** **3** **4 (MCB1-MCB15)**

Product	Ar	Time (h)	Yield (%)[a]
MCB1	C_6H_5-	4	66
MCB2	4-$(CH_3)_2NC_6H_4$-	3	66
MCB3	3-NO_2 C_6H_4-	3	57
MCB4	3,4-di$OCH_3C_6H_3$-	7	54
MCB5	2-ClC_6H_4-	3	71
MCB6	3-ClC_6H_4-	3	58
MCB7	4-$CH_3C_6H_4$-	3	60
MCB8	4-FC_6H_4-	7	60
MCB9	3-OHC_6H_4-	2	89
MCB10	3,4,5-triOCH_3 C_6H_2-	11.25	88
MCB11	10- Cl- 9-anthraldehyde	11	66
MCB12	2-$NO_2C_6H_4$-	7.30	64
MCB13	2-F C_6H_4-	6.35	62
MCB14	4-OHC_6H_4-	5.30	64
MCB15	2-thiophene carboxaldehyde	6.20	61

[a]Isolated yield.

A proposed mechanism for the formation of 2-(6-amino-5-cyano-4-phenyl-4*H*-1,3-dithiin-2-ylidene)malononitrile **4 (MCB1)** is illustrated in **scheme 1**. The chemical reaction involves with active methylene compound like malononitrile **1** with carbon disulfide **2** in presence of strong base like

$LiOH.H_2O$ gave the intermediates lithium 2,2-dicyanoethanedithioate **5** and lithium 2,2-dicyano-1-mercaptoethenethiolate **6.** Nucleophilic attack of intermediates **5** and **6** by sulfur anion on the electron deficient double bond of benzylidene malononitrile **3** could lead to formation of intermediate **7** via michael addition reaction. Then, hydrogen transfer takes place intramolecularly which produces intermediate **8,** which further undergoes nucleophilic attack by second sulfur anion with the cyanide carbon to form a cyclic structure affording the intermediate **9**. The final steps involve a 1,3 hydrogen-shift which leads to formation of final product **4 (MCB1)**.



Scheme 1. The proposed mechanism for the synthesis of 2-(6-amino-5-cyano-4-phenyl-4*H*-1,3-dithiin-2-ylidene) malononitrile

2.2.Biological Evaluation

2.2.1. *Antimicrobial Evaluation*

The fifteen newly synthesized ketene dithioacetals derivatives MCB1-MCB15 were tested for their antimicrobial activity against two Gram-positive bacterial strains *Bacillus Subtilis* and *Staphylococcus aureus* and Gram-negative bacterial strain *Escherichia Coli* along with their fungal strain *Candida Albicans* with their resulting inhibition zones were measured in mm diameter, in **Table 3**. Among the tested compounds, MCB5 and MCB13 were found to be most active against Gram-positive bacterial strains *Bacillus Subtilis* (24 and 22 mm respectively) and *Staphylococcus aureus* (26 and 22 mm respectively) and MCB13 were found to be active for fungal strain *Candida Albicans* (22mm respectively). Among the different substituent's 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 synthesized ketene dithioacetals compounds against pathogenic bacteria and fungi.

Compounds	*B. Subtilis*	*S. Aureus*	*E. Coli*	*C. Albicans*
MCB1	9	12	11	na
MCB2	14	14	na	17
MCB3	12	13	13	11
MCB4	8	11	18	18
MCB5	24	26	22	12
MCB6	Na	na	15	9
MCB7	10	12	na	16
MCB8	13	17	11	na
MCB9	13	na	8	14
MCB10	15	14	na	10
MCB11	18	18	18	15
MCB12	19	15	18	11
MCB13	22	22	19	22
MCB14	11	14	9	15
MCB15	19	12	14	19
Ciprofloxacin	26	26	24	-
Fluconazole	-	-	-	25

2.3. Computational Results

2.3.1. Molecular docking studies

Considering the outcome of antimicrobial activity, it was thought worthy to execute computational methods 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 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.[20] 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 protien of DNA Gyrase enzyme. The fifteen synthesized compounds showed good binding energy towards the target protien 2XCT ranging from -6.4 to -8.9 kcal/mol, among them MCB5 (-7.5 kcal/mol), MCB11 (-8.9 kcal/mol), MCB12 (-7.6 kcal/mol), and MCB13 (-7.4 kcal/mol), exhibited good DNA gyrase binding affinity along with standard ciprofloxacin. Moreover, the changes in binding energies coincided well with the experimental data of antimicrobial studies found that MCB5 and MCB13 found to be effective. With the observations from the biological data and the molecular docking results, might suggest that the antibacterial activities of these compounds are seemingly derived from the interaction between the compounds and the enzyme DNA Gyrase. However, there was not always a correlation among the *in vitro* and *in-silico* study outcome. Although compounds MCB11 and MCB12 showed good binding affinity for 2XCT with value -8.9 kcal/mol and -7.6 kcal/mol they showed low antimicrobial activity against all the microorganisms. The compounds exhibit good poses that are represented in the 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. **Fig. 1** represents the further extrapolation of binding conformation of the docked molecules.

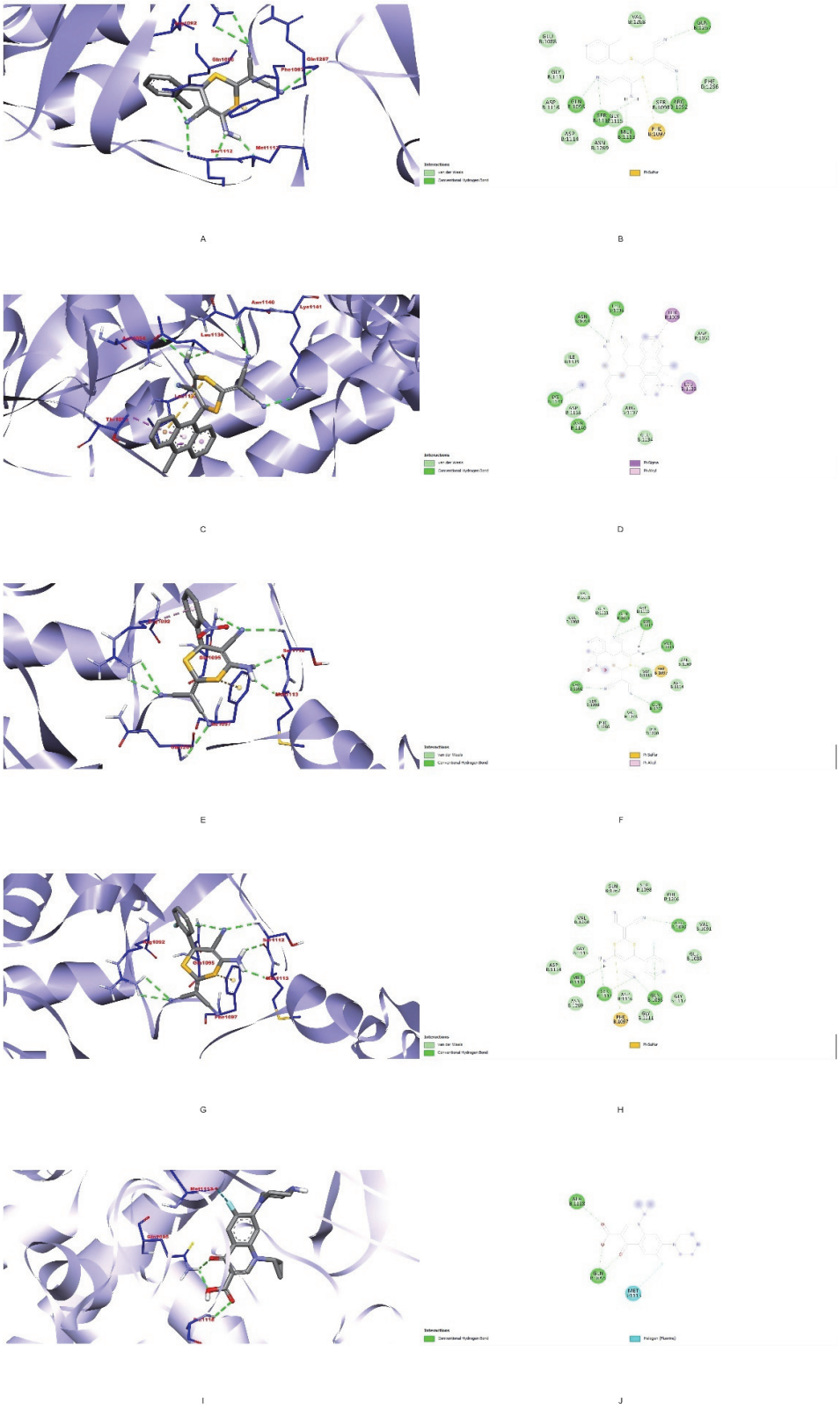


Fig. 1. Docking poses of ketene dithioacetals MCB5 (A & B), MCB11 (C & D), MCB12 (E & F), MCB13 (G & H), and Ciprofloxacin (I & J)

Table 4. Molecular Docking Study of 2XCT for synthesized ketene dithioacetals compounds

LIGANDS	BINDING AFFINITY Kcal/mol	No. OF HYDROGEN BONDS	AMINO ACIDS
MCB1	-6.8	3	GLN B:1095, SER B: 1112, MET B: 1113
MCB2	-7	5	ARG B:1092, SER B: 1112 (2), ASP B 1116, ALA B: 1118
MCB3	-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
MCB4	-6.4	6	ARG B:1092 (2), GLN B:1095, SER B: 1112 (2), MET B: 1113
MCB5	-7.5	7	ARG B:1092 (2), GLN B:1095, SER B: 1112, MET B: 1113, GLY B: 1115, GLN B: 1267
MCB6	-6.9	3	GLN B:1095, SER B: 1112 (2)
MCB7	-6.8	3	ASN B: 1269, ARG B: 1272 (2)
MCB8	-7.1	3	GLN B:1095, SER B: 1112, MET B: 1113
MCB9	-7.1	4	ARG B: 517, GLN B: 541 (2), THR B: 544, ARG B: 1012
MCB10	-6.4	5	THR B: 1059, LEU B: 1136, ILE B: 1139, ASN B: 1140, LYS B: 1141
MCB11	-8.9	4	ASN B: 1054, LEU B: 1136, ASN B: 1140, LYS B: 1141
MCB12	-7.6	7	ARG B:1092 (2), GLN B: 1095, SER 1112 (2), MET B: 1113, GLN B: 1267
MCB13	-7.4	7	ARG B:1092 (2), GLN B: 1095 (2), SER 1112 (2), MET B: 1113
MCB14	-6.9	6	THR B: 507, ALA B: 540, GLN B: 541, GLU B: 1017, GLU B: 1020, SER B: 1021
MCB15	-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

2.3.2. Druggability Validations through Lipinski's Filter

To upsurge efficacy in relation to the biological activity and fastidiousness while settling the conformity of the drug-like physicochemical properties termed by Lipinski's rule which is a characteristic approach in drug discovery process.[21] Lipinski's rule of five (Ro5) support as a widespread and considerable rule of thumb in the consideration of drug-likeness and also in the determination of the molecule's potential as an orally active drug or as a candidate drug with respect to its pharmacological or biological activity. Lipinski's rule covers some of the important molecular properties of a drug's pharmacokinetics like ADME, but still it fails to anticipate if a compound is pharmacologically active. A conformity of the drug candidate's is purely based on Rule of 5 that offers more promise to the candidate drug for ultimate success during clinical trials and therefore the higher chance of reaching the market.[22] As depicted in **Table 5**, the predicted Lipinski's filters almost positive for all proposed 2-(6-amino-5-cyano-4-aryl-4*H*-1,3-dithiin-2-ylidene) malononitrile compounds. None of the synthesized compounds (MCB1-MCB15) showed negative values. These most potent compounds were found to have substituted with electron withdrawing groups like chlorine, fluorine and nitro present in the ortho position of the aromatic ring.

2.3.3. ADMET and Pharmacokinetics Predictions

For accelerating opportunities in the discovery of new targets and to find out lead compounds with predicted biological activity depends on *in-silico* drug likeness prediction along with further ADME/Tox tools. For effective metabolism and action, a good drug candidate is absorbed in specified time and well distributed throughout the organism/system. **Table 5** depicts the drug likeness properties of the synthesized new ketene dithioacetals as test compounds predicted using Med Chem designer. From **Table 5**, it can be seen that the partition coefficient (S+log *P*) value ranges from -0.817 to 5.017 and TPSA (Topological Polar Surface Area), ranges from 97.39 to 143.21, indicates the surface

belonging to polar atoms in the compound where increased TPSA is associated with diminished membrane permeability and compounds with higher TPSA were better substrates for p-glycoprotein which is responsible for drug efflux from cell.

Table 5. Druggability and Lipinski's filter prediction results of MCB1-MCB15

Compounds	MlogP	S+logP	S+logD	RuleOf5	RuleOf5_Code	MWt	M_NO	T_PSA	HBDH
MCB1	0.321	2.708	2.706	0	None	296.374	4	97.39	2
MCB2	0.306	2.817	2.815	0	None	339.443	5	100.63	2
MCB3	-0.182	-0.817	-0.82	0	None	342.379	7	137.7	3
MCB4	-0.197	2.98	2.979	0	None	356.427	6	115.85	2
MCB5	0.845	3.247	3.246	0	None	330.819	4	97.39	2
MCB6	0.845	3.187	3.186	0	None	330.819	4	97.39	2
MCB7	0.577	3.02	3.019	0	None	310.401	4	97.39	2
MCB8	0.718	3.082	3.081	0	None	314.364	4	97.39	2
MCB9	-0.199	2.081	2.059	0	None	312.373	5	117.62	3
MCB10	-0.445	2.963	2.962	0	None	386.453	7	125.08	2
MCB11	2.328	5.017	5.016	0	None	430.94	4	97.39	2
MCB12	0.368	2.614	2.611	0	None	341.371	7	143.21	2
MCB13	0.718	3.097	3.096	0	None	314.364	4	97.39	2
MCB14	-0.199	2.211	2.198	0	None	312.373	5	117.62	3
MCB15	-0.137	2.445	2.443	0	None	302.4	4	97.39	2
Ciprofloxacin	0.588	-0.806	-0.826	0	None	331.349	6	74.57	2

Notes: MlogP. Moriguchi's estimation of logP. S+logP. LogP calculated using Simulations Plus' highly accurate internal model. S+logD. LogD at user-specified pH (default 7.4), based on S+logP. Rule Of Five. Lipinski's Rule of Five: a score representing the number of probable problems a structure might have with passive oral absorption. MWt=molecular weight; M_NO. A total number of Nitrogen and Oxygen atoms. T_PSA. The topological polar surface area in square angstroms. HBDH. A number of Hydrogen bond donor protons. The presence of a code means that the corresponding Lipinski rule was violated.

In order to ensure the druggability potential of the synthesized ketene dithioacetals compounds (MCB1-MCB15), ADMET properties along with the other pharmacokinetic properties were predicted using pkCSM online tool. The established outcome was substituted and found to have correlated well with ADMET values that were predicted by using Med chem designer software. Studies predicted that all the synthesized ketene dithioacetals compounds will be well absorbed and intestinal absorption was found to be 84.073 to 100.

Skin permeability is a vital property for enhancing drug effectiveness in the development of the transdermal drug delivery systems. A drug barely penetrates the skin if log Kp is more than -2.5 cm/h.[23] From **Table 6** it can be seen that the skin permeability (Kp) of synthesized ketene dithioacetals derivatives ranges from -2.559 to -3.233 cm/h (< -2.5). Therefore, it can be predicted that all the synthesized derivatives have good skin penetration.

The Caco-2 (human epithelial colorectal adenocarcinoma cells) monolayer of cells is widely used as an *in vitro* model of the human intestinal mucosa to predict the intestinal permeability for the absorption of orally administered drugs and investigate drug efflux by measuring the log of the evident permeability coefficient (log Papp; log cm/s). A compound is considered to have a high Caco-2 permeability if it has Papp > 8 x 10^{-6} cm/s. High Caco-2 permeability is translated into predicted log Papp values > 0.90cm/s.[23] From **Table 6** it can be seen that the Caco-2 permeability value (log Papp) of the synthesized ketene dithioacetals compounds ranges from -0.365 to 0.923 cm/s, in which MCB1, MCB5, and MCB13 have high Caco-2 permeability and other predicted that the compounds have a moderate to low Caco-2 permeability.

The volume of distribution (VD) is a parameter representing the calculated value of an individual drug that will be circulated at an equal level of blood plasma. This model is established from the estimation of the steady-state volume of distribution (VDss) which is represented as log L/kg. According to Pires *et al*[23] VDss >2.81 L/kg (log VDss > 0.45) is categorized as high whereas VDss

<0.71 L/kg (log VDss < -0.15) is categorized as low. From **Table 6** it can be seen that VDss values of the synthesized ketene dithioacetals ranges from 0.612 to 0.078, and one compound has a VDss value of < -0.15 i.e. MCB10, so it can be predicted that all the derivatives can be distributed evenly providing an equal level of blood plasma.

The ability of a drug to cross the Blood-Brain Barrier (BBB) is an important parameter to consider for reducing side effects and toxicities or to improve the efficacy of drugs whose pharmacological activity is within the brain. The BBB permeability is calculated *in vivo* as log BB, the logarithmic ratio of the brain-to-plasma drug concentration. Compounds are able to pass through the BBB promptly when log BB is higher than 0.3, but compounds with log BB smaller than -1 barely reach the brain.[23] From **Table 6** it can be seen that the ranges of log BB value of synthesized ketene dithioacetals compounds range from -0.86 to -1.016, which means greater than -1, so it can be predicted that all the derivatives are able to penetrate the blood-brain barrier moderately (except MCB10).

The central nervous system permeability of blood-surface area product (log PS) is a direct measurement that can be obtained from *in situ* brain perfusions with the drug directly injected into the carotid artery. This lacks the systemic distribution effects which may demolish brain penetration. Compounds with a log PS > -2 are considered to penetrate, while those with log PS < -3 are considered as unable to penetrate the central nervous system.[23] From **Table 6** it can be seen that the log PS value of the synthesized ketene dithioacetals derivatives ranges from -1.293 to -2.999 which penetrate the central nervous system easily.

Cytochrome P450 is an important detoxification enzyme that oxidizes xenobiotics to facilitate their excretion from the body. The cytochrome P450's is responsible for the metabolism of many drugs. Inhibitors of the P450's can dramatically alter the pharmacokinetics of these drugs, so it is significant to evaluate whether a given compound is likely to be a cytochrome P450 substrate. The two main isoforms responsible for drug metabolism are cytochrome P2D6 (CYP2D6) and Cytochrome P3A4 (CYP3A4).[23] From **Table 6** it can be seen that almost all synthesized ketene dithioacetals compounds do not affect or inhibit the CYP2D6 and CYP3A4 enzymes (except MCB11).

Drug clearance occurs primarily as a combination of hepatic clearance and renal clearance which is measured by the proportionality constant. To achieve steady-state concentrations depends on bioavailability and administered at the appropriate dosing rate. The higher the CLtot value of the drug, the faster the clearance process.[23] From **Table 6** it can be seen that the CLtot value of synthesized ketene dithioacetals ranges from 0.177 to 0.62 ml/min/kg which predicts the rate of excretion of drugs.

Ames toxicity test is to determine the toxicity of the compound. The AMES test is used to predict the mutagenic potential of a compound using bacteria. A positive test indicates that the compound is mutagenic and therefore may act as a carcinogen.[23] From **Table 6** it can be seen that except MCB2 and MCB4 all other synthesized ketene dithioacetals compounds are predicted to have mutagenic effects.

Hepatotoxicity is the most concerning safety aspect in the drug development process. The toxicity of the compounds shows at least one pathological or physiological hepatic event was considered hepatotoxic and highly related to liver disruption.[23] From **Table 6** it can be seen that all the ketene dithioacetals derivatives are not hepatotoxic.

The probable toxicity of prospective synthesized ketene dithioacetals compounds has to be assessed. The acute toxicity and relative toxicity of different compounds can be determined from the lethal dosage value. To complement the toxicity prediction of synthesized ketene dithioacetals compounds there is an acute oral toxicity test for rodents (LD50) and an acute toxicity classification of compounds based on the Globally Harmonized System (GHS) using the pkCSM online tool.[23] From **Table 6** it can be seen that the prediction for the LD50 values of the synthesized ketene dithioacetals compounds ranges from 2.027 kg/mol to 3.164 kg/mol.

Acute toxicity class of compounds ranges with in category 1 (LD50= ≤ 5mg/kg), category 2 (LD50 = > 5 ≤ 50 mg/kg), category 3 (LD50 = > 50 ≤ 300 mg/kg), category 4 (LD50 = >300 ≤ 2000 mg/kg), category 5 (LD50 = > 2000 ≤ 5000 mg/kg), category 6 (LD50 = > 5000 mg/kg), which states that all the ketene dithioacetals derivatives belong to category 5 indicates that all are relatively low acute toxic compounds. (https://unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev05/English/ST-SG-AC10-30-Rev5e.pdf).

Table 6. ADMET Properties of the synthesized 2-(6-amino-5-cyano-4-aryl-4*H*-1,3-dithiin-2-ylidene) malononitrile compounds by pkCSM online tool

	Absorbtion			Distribution			Metabolism		Excretion		Toxicity		
Compounds	Intestinal Absorbtion (%)	Skin permeability (log Kp)	Caco-2 permeability (log Papp in 10^{-6} cm/s)	VDss (log L/kg)	BBB Permeability (log BB)	CNS Permeability (log PS)	CYP2D6 Inhibitor (Yes/No)	CYP3A4 Inhibitor (Yes/No)	Total Clearance (log ml/min/kg)	AMES (Yes/No)	Hepatotoxicity (Yes/No)	LD50 (mol/kg)	Class
MCB1	90.117	-2.549	0.923	0.28	-0.356	-1.877	No	No	0.304	Yes	No	2.879	5
MCB2	87.674	-2.743	0.795	0.28	-0.518	-1.967	No	No	0.275	No	No	2.856	5
MCB3	86.947	-2.688	-0.365	0.247	-0.86	-2.124	No	No	0.328	Yes	No	2.405	5
MCB4	85.069	-3.188	0.829	-0.1	-0.796	-2.169	No	No	0.476	No	No	2.982	5
MCB5	91.293	-2.578	0.911	0.253	-0.524	-1.763	No	No	0.237	Yes	No	3.082	5
MCB6	91.293	-2.605	0.842	0.278	-0.524	-1.758	No	No	0.177	Yes	No	3.037	5
MCB7	90.575	-2.583	0.771	0.302	-0.366	-1.802	No	No	0.245	Yes	No	2.786	5
MCB8	90.588	-2.847	0.836	0.078	0.574	-1.909	No	No	0.155	Yes	No	2.721	5
MCB9	84.073	-3.211	0.377	0.105	-0.437	-2.039	No	No	0.192	Yes	No	2.731	5
MCB10	79.438	-3.221	0.812	-0.266	-1.016	-2.956	No	No	0.62	No	No	2.972	5
MCB11	100	-2.719	0.539	0.612	-0.623	-1.293	No	Yes	0.347	Yes	No	2.027	5
MCB12	86.903	-2.675	-0.414	0.346	-0.874	-2.139	No	No	0.388	Yes	No	2.538	5
MCB13	90.531	-2.675	0.923	0.095	-0.557	-1.901	No	No	0.276	Yes	No	2.939	5
MCB14	84.091	-3.233	0.431	0.057	-0.472	-2.043	No	No	0.187	Yes	No	2.681	5
MCB15	90.463	-2.598	0.893	0.137	-0.526	-1.917	No	No	0.243	Yes	No	3.164	5
Ciprofloxacin	96.466	-2.734	0.492	-0.17	-0.587	-2.999	No	No	0.633	No	Yes	2.891	5

3. Conclusion

Based on a Michael addition reaction novel fifteen new ketene dithioacetals derivatives were designed and synthesized by the reaction of active methylene compounds, carbon disulfide, and arylidene malononitrile in the presence of lithium hydroxide monohydrate as a base by the one-pot reaction. All the synthesized compounds were screened against three bacterial strains and one fungal species. Among the synthesized compounds, MCB5 and MCB13 compounds were found to be effective against *S. aureus* with a zone of inhibition at 26mm and 22mm which is compared to that of standard ciprofloxacin (26mm). Further, molecular docking simulations were carried out with protein 2XCT for receptor-ligand interactions which revealed that the compound MCB5 and MCB13 have binding mode and an affinity to DNA gyrase binding site comparable to that of ciprofloxacin. Further, *in-silico* studies were carried out for druggability by Lipinski's rule in which none of the molecules fails. ADMET studies reveal that all compounds come under category 5 which states low acute toxic compounds.

Herein, further studies can be made to optimize and develop new antimicrobial compounds, and modifications in the structures can be made to identify more biologically potent compounds.

4. Experimental

4.1.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. Spectral data of the compounds were routinely checked by IR, ^{1}H-NMR spectroscopies. 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.

4.2.General procedure for the preparation of compounds (MCB1-MCB15)

A mixture of active methylene compound like malononitrile **1** (1.0 mmol), carbon disulfide **2** (3.0 mmol), and lithium hydroxide monohydrate (0.1 mmol) was stirred in 10 ml of ethanol at room temperature for 0.5 h. Arylidene malononitrile **3** (1 mmol) was added and the reaction was stirred by maintaining 40-50°C for 3-11.25 h. After completion of the reaction, determined by TLC, the reaction mixture was poured in cold water and precipitate was collected. The crude product was recrystallized from ethanol to give pure compounds **4** (MCB1-MCB15) with 54 to 89% yields.

4.3 Physical and Spectral Data

4.3.1. 2-(6-amino-5-cyano-4-phenyl-4H-1,3-dithiin-2-ylidene)malononitrile (MCB1)

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). ESI-MS *m/z*; 297.05 $[M+H]^+$ calculated, 296.38.

4.3.2. 2-(6-amino-5-cyano-4-(4-(dimethylamino)phenyl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB2)

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). ESI-MS *m/z*; 340.15 $[M+H]^+$ calculated, 339.45.

4.3.3. 2-(6-amino-5-cyano-4-(3-nitrophenyl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB3)

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). ESI-MS *m/z*; 342.44 $[M+H]^+$ calculated, 341.37.

4.3.4. 2-(6-amino-5-cyano-4-(3,4-dimethoxyphenyl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB4)

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). ESI-MS *m/z*; 357.49 $[M+H]^+$ calculated, 356.43.

4.3.5. 2-(6-amino-4-(2-chlorophenyl)-5-cyano-4H-1,3-dithiin-2-ylidene)malononitrile (MCB5)
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). ESI-MS *m/z*; 331.92 $[M+H]^+$ calculated, 330.82.

4.3.6. 2-(6-amino-4-(3-chlorophenyl)-5-cyano-4H-1,3-dithiin-2-ylidene)malononitrile (MCB6)
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). ESI-MS *m/z*; 331.93 $[M+H]^+$ calculated, 330.82.

4.3.7. 2-(6-amino-5-cyano-4-(p-tolyl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB7)
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). ESI-MS *m/z*; 311.39 $[M+H]^+$ calculated, 310.40.

4.3.8. 2-(6-amino-5-cyano-4-(4-fluorophenyl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB8)
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). ESI-MS *m/z*; 315.45 $[M+H]^+$ calculated, 314.37.

4.3.9. 2-(6-amino-5-cyano-4-(3-hydroxyphenyl)-4H-1,3-dithiin-2-ylidene) malononitrile (MCB9)
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). ESI-MS *m/z*; 313.29 $[M+H]^+$ calculated, 312.38.

4.3.10. 2-(6-amino-5-cyano-4-(3,4,5-trimethoxyphenyl)-4H-1,3-dithiin-2-ylidene) malononitrile (MCB10)
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). ESI-MS *m/z*; 387.40 $[M+H]^+$ calculated, 386.46.

4.3.11. 2-(6-amino-4-(10-chloroanthracen-9-yl)-5-cyano-4H-1,3-dithiin-2-ylidene) malononitrile (MCB11)
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). ESI-MS *m/z*; 431.88 $[M+H]^+$ calculated, 430.94.

4.3.12. 2-(6-amino-5-cyano-4-(2-nitrophenyl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB12)
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). ESI-MS *m/z*; 342.42 $[M+H]^+$ calculated, 341.37.

4.3.13. 2-(6-amino-5-cyano-4-(2-fluorophenyl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB13)
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). ESI-MS *m/z*; 315.28 $[M+H]^+$ calculated, 314.37.

4.3.14. 2-(6-amino-5-cyano-4-(4-hydroxyphenyl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB14)
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). ESI-MS *m/z*; 313.39 $[M+H]^+$ calculated, 312.39.

4.3.15. 2-(6-amino-5-cyano-4-(thiophen-2-yl)-4H-1,3-dithiin-2-ylidene)malononitrile (MCB15)
Cream crystals; Mp: 88-90ºC IR υ/cm^{-1}(KBr): 34989, 3378, 3070, 2229, 1660, 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). ESI-MS *m/z*; 303.44 $[M+H]^+$ calculated, 302.41.

4.4. Biological Evaluation

4.4.1. Antimicrobial activity

Four bacterial strains of Gram-positive (*Bacillus Subtilis* ATCC 6633, and *Staphylococcus aureus* ATCC 29213), Gram-negative (*Escherichia coli* ATCC 25922) and representative fungi (*Candida albicans* ATCC 10231) were used in our *in-vitro* antimicrobial activity. The antimicrobial potential of the newly synthesized ketene dithioacetals was investigated towards the tested microorganisms and expressed as the diameter of the inhibition zones according to the agar plate diffusion method.[24] 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.

4.5. Computational studies

4.5.1. Computational Tools

To execute all *in-silico* studies, at first, the ligand structures of 2-(6-amino-5-cyano-4-aryl 4*H*-1,3-dithiin-2-ylidene) malononitrile (MCB1-MCB15) was generated using Chem Draw 12.0 software. The structures were saved as a .cdx format then changed into .pdb (ligand) file format. Simultaneously, the SMILE file format of all compounds was obtained from Chem Draw 12.0 in order to use them to obtain the druggability data. The druggability of all compounds was predicted by docking the synthesized ligand with Pyrx 0.8 software[25], Spdbv[26], MedChem Designer^{TM} software version 5.5 (http://www.simulationsplus.com/software/medchem-designer) [Simulations Plus Inc. Lancaster, CA] was used which is based on the software's built-in algorithm, and pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction/).

4.5.1.1.Molecular docking studies

4.5.1.1.1. Accession of ligand and protein

The crystal structure of DNA gyrase in complex with ciprofloxacin (PDB ID: 2XCT) [27] with resolution of 3.35A$^{\circ}$ was accessed from protein data bank (http://www.rcsb.org). For preparation of

protein, water molecules were deleted, 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 0.5Aº by keeping all parameters in defaults for docking. The visualization was done using discovery studio visualizer 2020 for receptor-ligand interactions.

4.5.1.2.Pharmacokinetic Properties and toxicity prediction studies.

Drug-likeness prediction was carried out based on the Lipinski rule of five with consistent properties and satisfies the rule containing good absorption when taken orally as active pharmacological agent. It is applied to evaluate *in vivo* capabilities of the synthesized molecules. Any newly synthesized molecule with more than 5H-bond donors(-OH, NH), more than 10H-bond acceptors (N, O), molecular weight greater than 500 and the log *P* greater than 5 unsatisfies the rule of five. Med chem. Designer^{TM} and pkCSM prediction tool was used for drug-likeness and toxicity prediction of the synthesized ligands. Med chem. Designer^{TM} programme estimates the physicochemical properties like rule of five, octonal/water partition coefficient (S+LogP), water/octanol partition coefficient at specicific pH 7.4 (S+logD), Topological polar surface area (TPSA), molecular weight, count of nitrogen and oxygens (M_NO), etc. Meanwhile, pkCSM predictor provides information regarding absorption parameters like Human Intestinal Absorption (HIA), Oral bioavailability, Caco-2 permeability, distribution parameters like Plasma Protein Binding (PPB), Blood Brain Barrier (BBB), metabolism parameters like Cytochrome P450 (CYP450) substrate, inhibitor, excretion parameters like half time (t1/2), renal clearance and toxicity parameters like organ toxicity, genomic toxicities for drug candidates.

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