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Design, Synthesis, and Antimicrobial Evaluation of Some New Thiopyrimidin-Benzenesulfonamide Compounds

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Abstract: Bacterial infection poses a serious threat to human life due to its rapidly growing resistance to antibacterial drugs, which is a significant public health issue. This study was focused on the design and synthesis of a new series of 25 analogues bearing 5-cyano-6-oxo-4-substituted phenyl-1,6-dihydropyrimidine scaffold hybridized with different substituted benzene-sulfonamides through thioacetamide linker **M1-25** via the treatment of the key intermediates 2-mercapto-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitriles **1a-c** with different 2-chloro-*N*-substituted benzene-sulfonamide derivatives **4a-e** overnight at room temperature. The spectroscopic data of all the new compounds (IR, ^{1}H-NMR, ^{13}C-NMR, and Mass) was studied in detail to confirm their molecular structures. The antimicrobial activity of the new molecules was studied against various Gram-positive, Gram-negative, and fungal strains. All the tested compounds showed promising broad spectrum antimicrobial efficacy, especially against *K. pneumoniae* and *P. aeruginosa,* with ZOI values ranging from 15 to 30 mm. Furthermore, the most promising compounds M**6**, M**19**,M **20**, and M**25** were subjected to minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. In addition, the anti-virulence activity of the compounds was also examined using multiple biofilm assays. The new compounds revealed promising suppressing activity of microbial biofilm formation in the examined *K. pneumoniae* and *P. aeruginosa* microbial isolates. Additional *in silico* ADMET studies were conducted to represent their oral bioavailability, drug-likeness characteristics, and human toxicity risks. The obtained data suggest the newly prepared pyrimidine-benzene-sulfonamide derivatives may serve as model compounds amenable for further optimization and development of new antimicrobial and antisepsis candidates.

Keywords: Thiopyrimidine; benzene-sulfonamides; antimicrobial activity; bactericidal activity; anti-virulence activity; *in silico* ADMET

1. Introduction

Global public health is seriously threatened by the persistent spread of microbial diseases that are resistant to conventional therapies. According to estimates, antibiotic-resistant pathogenic bacterial infections claim the lives of 700,000 people worldwide each year. In the absence of novel approaches to prevention or treatment, it is projected that 10 million people will pass away from these infectious diseases annually by 2050 [1–3].

Multi-drug-resistant (MDR) bacteria have emerged as a result of illness exposure in hospitals, overconsumption, and inappropriate antibiotic use [4]. World Health Organization recently declared a global health day to be dedicated to "Combat drug resistance: no action today means no cure tomorrow," which encouraged increased research efforts [4]. Antibiotic-resistant bacterial infections are known to be caused by bacteria living in biofilms rather than by free bacteria [4]. Three factors are thought to be responsible for the resistance of biofilm-forming bacteria to traditional antimicrobials: (1) the antimicrobial's inability to pass through the biofilm; (2) the emergence of complex drug resistance characteristics; and (3) the deactivation or modification of antimicrobial enzymes by the biofilm [3]. Given the increased prevalence of life-threatening diseases, the goal is to create pharmaceutical regimens with improved antibacterial properties that offer more consistency and efficiency against infections by resistant bacterial pathogens [5,6].

The earliest class of synthetic antibacterial medications are sulfonamides **(Figure 1)**. Since the 1930s, they have been used in pharmaceutical therapeutics and have proven to be effective against a variety of pathogens and clinical infections [7].

Drugs classified as classical sulfonamides inhibit dihydropteroate synthase (DHPS). They compete with its natural substrate, PABA, thereby blocking folate biosynthesis and subsequently leading to defective thymidine biosynthesis. It has also been documented that sulfonamides interfere with the development of peptidoglycan in a variety of pathogens by blocking specific enzymes (MurB, MurD, and MurE) involved in its biosynthesis. Moreover, sulfonamides have the ability to suppress serine/threonine kinase (Stk1/PknB), resulting in increased sensitivity of MRSA to sublethal concentrations of β-lactams, thus reversing acquired resistance [7,8]. Another mode of action of sulfonamides is the inhibition of carbonic anhydrases (CAs) that participate in pH regulation and CO_2 and bicarbonate-dependent biosynthetic pathways by catalyzing the interconversion between these two small molecules [9]. **Figure 1** represents various FDA-approved sulfonamide antibiotics.

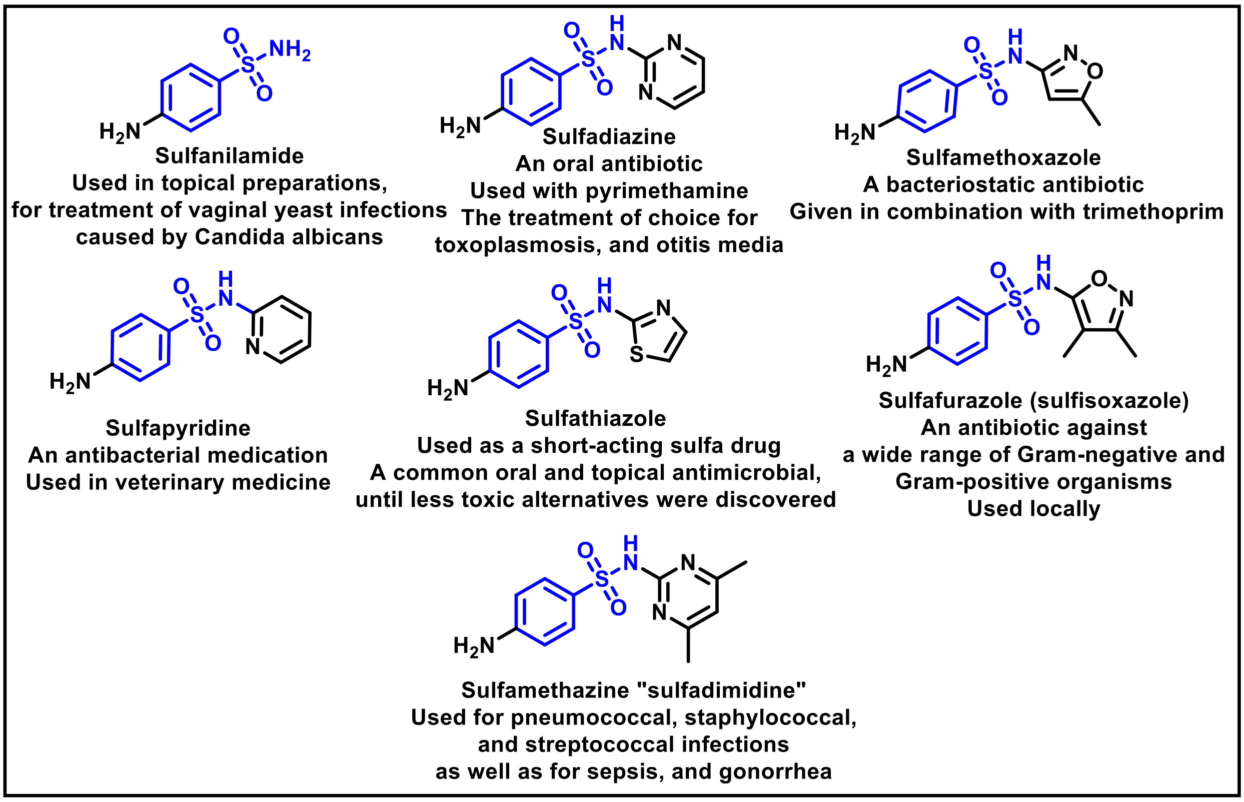


Figure 1. Examples of various FDA-approved sulfonamide antibiotics.

It is well recognized that several heterocyclic scaffolds serve as essential structural components in the majority of the world's most widely prescribed medicinal pharmaceuticals. Among the heterocyclic substances with biological significance are derivatives of pyrimidines, and pyrimidine – carbonitriles [10–14]. The pyrimidine moiety is a crucial component of many physiologically active substances that occur naturally and is involved in both chemical and biological activities [15]. A pyrimidine-based nucleotide that function as a prosthetic scaffold for several enzymes, is involved in a variety of redox reactions in living beings [16].

Researchers have focused on a broad range of pyrimidine-carbonitrile ligands for many years, and these compounds have demonstrated significant roles as physiologically active drugs with antibacterial, antiviral, anticancer, and other properties [17–22].

Carbonitrile has significant diverse biological activities such as anti-allergic, antibacterial, antifungal, anti-HIV, anticonvulsant, anti-inflammatory, and β-lactamase inhibition [23–26]. It is characterized by various properties such as rigidity, stability in *in vivo* environments, hydrogen bonding ability, and modest dipole character [26]. **Figure 2** represents different examples of FDA-approved pyrimidine-based antimicrobial drugs.

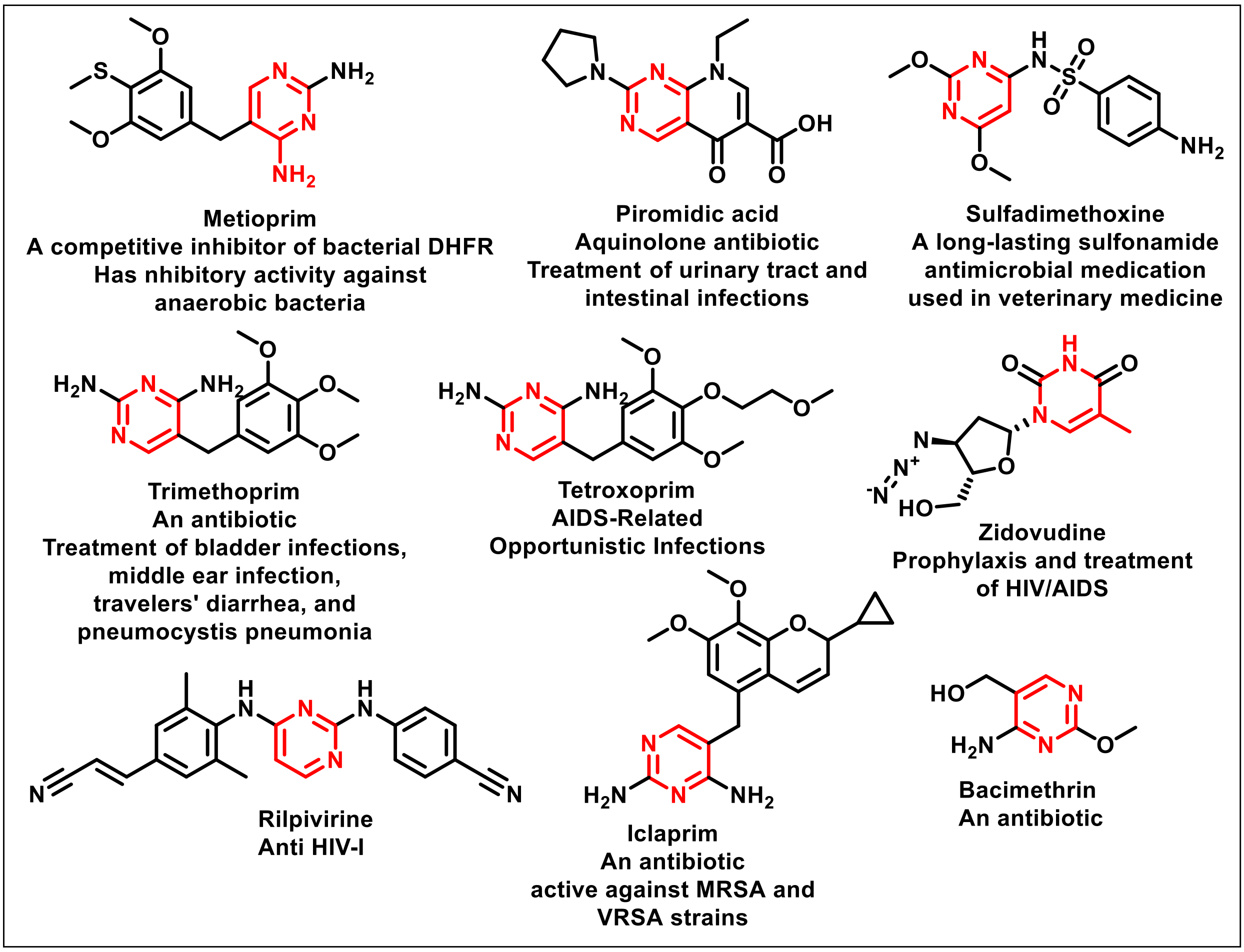


Figure 2. Different examples of FDA-approved pyrimidine-based antimicrobial drugs.

In search of more new potent multi-targeted sulfonamide or sulfonyl drugs to overcome the microbial resistance and reduce the adverse effects, many medicinal chemistry scholars focused on combinations of various substituted benzenesulfonamide ring with other heterocyclic molecules to develop novel formulations with greater effectiveness as well as less toxicity [27].

Also, it has been also reported that the thioacetamide molecule produces antifungal activity, in addition to its ability to interact with SH- and NH_2-containing enzymes and proteins to reveal the antimicrobial activity [28,29].

Taking into account the aforementioned considerations, we designed and created a novel series of substituted benzenesulfonamide derivatives conjugated with various substituted 5-cyano pyrimidine nuclei via a thioacetamide linkage with possible antimicrobial (antibacterial and antifungal) effectiveness. This study represents the synthesis of a series of 2-((5-cyano-6-oxo-4-substitutedphenyl-1,6-dihydropyrimidin-2-yl)thio)-*N*-(4-(*N*-substituted sulfamoyl)phenyl) acetamide derivatives M**1–25 (Figure 3)**. The antimicrobial efficacy of the new target compounds was tested against several human pathogenic microbes (bacteria and fungi). Furthermore, the minimum inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) values were determined for the most potent analogues which aided in examining the synergistic effects of tested compounds. In addition, the anti-virulence activity of latter molecules obtained by their prevention of biofilm formation was also assessed. Moreover, *in silico* methodologies to determine their physicochemical parameters were analyzed in the eyes of Lipinski's rule of five. Further pharmacokinetic parameters were calculated using, Swiss ADME program.

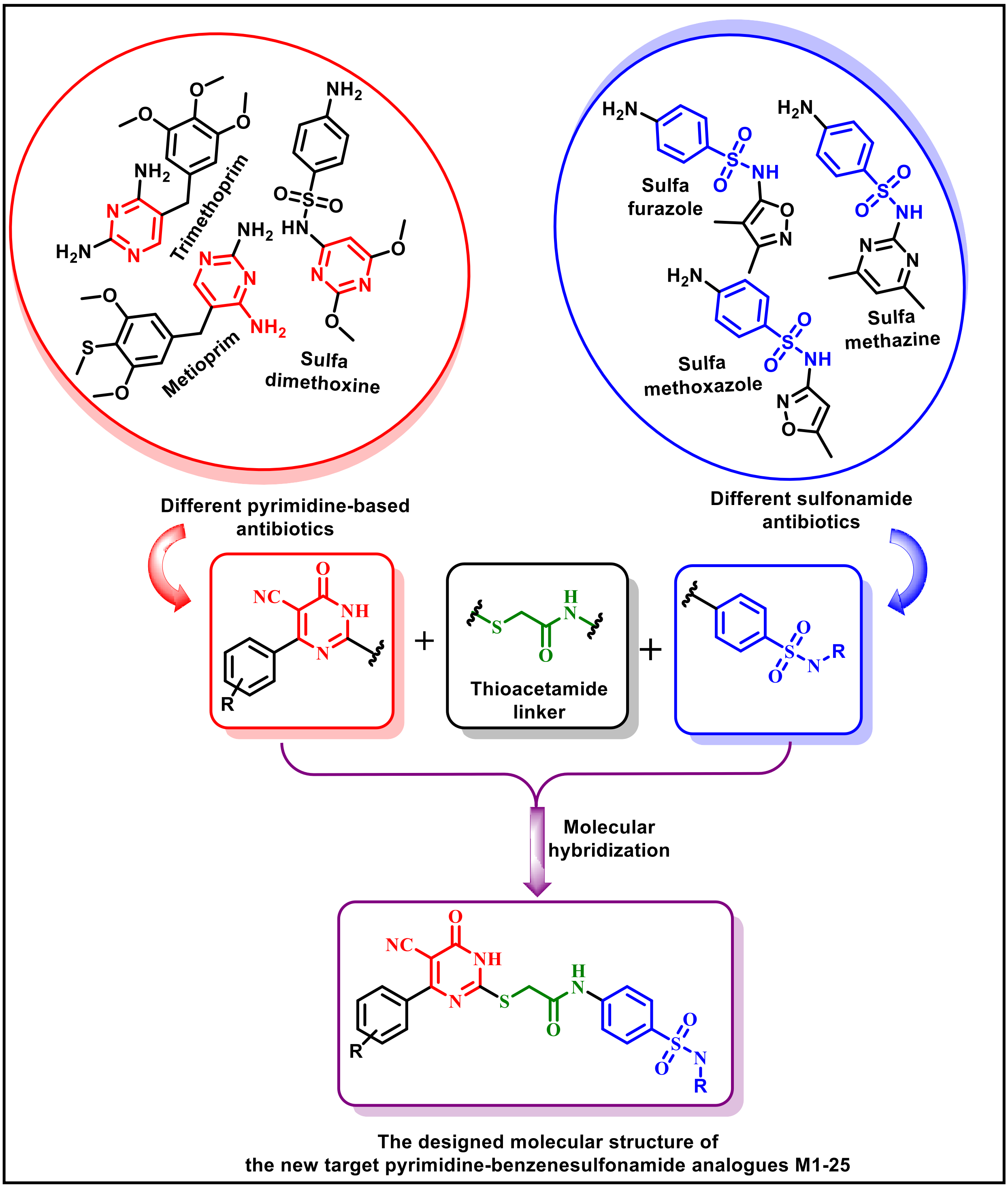


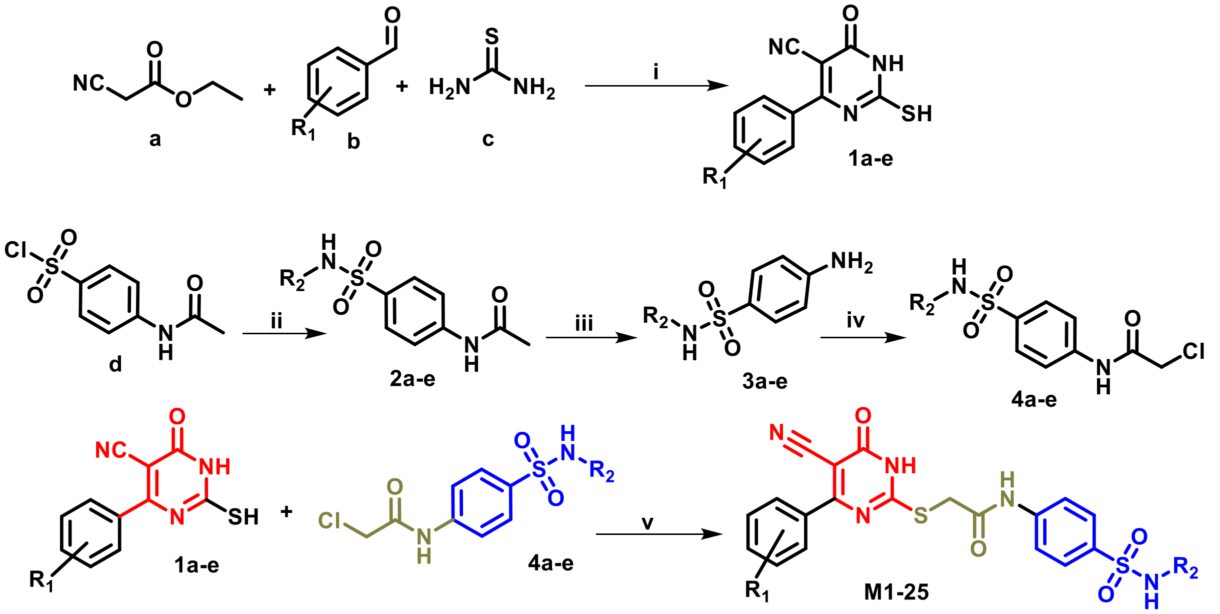
Figure 3. The proposed molecular structures of the new pyrimidine-benzenesulfonamide compounds M**1-25.**

2. Results and Discussion

2.1. Chemistry

The synthetic pathway of the target compounds **M1-25** was illustrated in **Scheme 1**. The 6-substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles (**1a-e**) were obtained through a one-pot reaction of thiourea, ethyl cyanoacetate, and the appropriate aldehyde in the presence of K_2CO_3, providing quantitative yields as previously reported [30]. The 2-chloro-*N*-(4-(*N*-substitutedsulfamoyl)phenyl)acetamide compounds **4a-e** were synthesized via acetylation of various substituted 4-amino-*N*-substituted benzenesulfonamide derivatives **3a-e** with chloroacetyl chloride in anhydrous THF at -10°C under basic conditions [31]. Subsequently, coupling of the key starting 2-thioxo-1,2,3,4-tetrahydropyrimidine compounds **1** with chloroacetamide compounds **4-1-5** in DMF under basic conditions led to the formation of the target 5-cyano-6-oxo-4-phenyl-1,6-dihydropyrimidine-based analogues **M1-25** in excellent yields **(Table 1)**. The structures of the novel compounds were confirmed by their spectral data (^{1}H NMR, ^{13}CNMR, and Mass spectra).

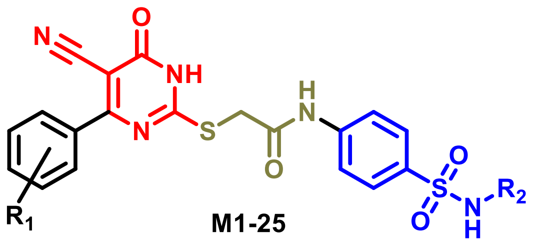
In the ^{1}H NMR spectra of the target pyrimidine-benzenesulfonamide analogs **M1-25**, the amidic NH protons of both the acetamide linker and dihydropyrimidine ring appeared as singlets and very broad singlets downfield in the δ 10.0–13.71 ppm range. The aromatic protons are resolved into four distinct peaks within the range δ 6.80–8.0 ppm. In addition, the 2H of the thioacetamide group appeared as a singlet signal in the region of δ 3.88-4.30 ppm, while the sulfamoyl aliphatic or hetero alicyclic protons appeared in the upfield range at δ 1.03-3.15 ppm (more detail in the Supporting file). Mass spectrometry represented molecular ion peaks of all of the new target benzenesulfonamide derivatives compounds **M1-25** which were in agreement with their expected molecular formulae.



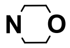
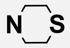
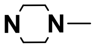
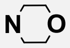
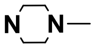
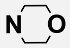
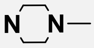
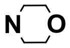
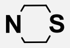
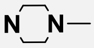
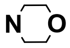
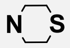
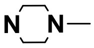
Reagents and conditions:
(i) $KHCO_3$, Ethanol, Reflux for12 hrs ; (ii) NH_2-NH_2.H_2O (99%), Ethanol, Reflux for 4 hrs; (iii) conc. HCL, Ethanol, Reflux for 2 hrs;(iv) Chorloacetyl chloride, THF, K_2CO_3 at, Stirring at ^{0}C; (iv) K_2CO_3, DMF, Stirring at room tempreture.

Scheme 1. Synthesis of the target pyrimidine-benzene-sulfonamide analogs M1-25.

Table 1. Molecular structures of the target compounds **M1-25.**



M1-25

Compounds	R_1	R_2	Compounds	R_1	R_2
M1	H		**M14**	4-Cl	
M2	H		**M15**	4-Cl	
M3	H		**M16**	4-F	
M4	H		**M17**	4-F	
M5	H		**M18**	4-F	
M6	4-Br		**M19**	4-F	
M7	4-Br		**M20**	4-F	
M8	4-Br		**M21**	3-Cl	
M9	4-Br		**M22**	3-Cl	
M10	4-Br		**M23**	3-Cl	
M11	4-Cl		**M24**	3-Cl	
M12	4-Cl		**M25**	3-Cl	
M13	4-Cl				

2.2. Biological Evaluation

2.2.1. Antimicrobial Activity Determination

The newly synthesized sulfonamide compounds M**1-25** were assessed for their antimicrobial characteristics against the bacterial isolates *E. coli* ATCC-25922, *K. pneumoniae, P. aeruginosa* ATCC 27,853 as Gram-negative bacteria, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 35984, and *B. subtilis ATCC 6633* as Gram-positive bacteria, and the fungal strain *C. albicans* ATCC-10231.

These specific strains were selected because of their capacity to form biofilms and in addition to their notable effect on plant and human health. Utilizing the agar-well diffusion process [32], the average diameter of the inhibition zones in millimeters was measured for each tested analogue (3000 μg/mL) against every kind of microbial growth surrounding the discs [32] **(Table 2, Figure 4)**.

The obtained results showed that all the examined analogues are promising antimicrobial candidates against most of tested microbial isolates, producing ZOI ranging from 15 to 30 mm. Interestingly, the bacterial strains *K. pneumoniae* and *P. aeruginosa* bacterial strains exhibited great sensitivity towards all the target compounds M**1–25**. Both *K. pneumoniae* and *P. aeruginosa* are multidrug-resistant pathogens and are associated with serious hospital-acquired infections such as pneumonia and various sepsis syndromes [33–35]. So, these new pyrimidine-benzenesulfonamides can be considered as basic scaffolds for the synthesis of new drugs of high antimicrobial activity that can overcome the virulence and antibiotic resistance of these two organisms.

Moreover, the halophenyl-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl-thio-*N*-substituted sulfonyl phenyl acetamide derivatives M**6**, M**19**, M**20**, and M**25** exhibited the most potent wide-spectrum antimicrobial activity against all the examined isolates, producing ZOIs ranging from 15 to 30 mm. Halogenation may improve permeability and enhance the antimicrobial activity of the sulfonamide pharmacophore. In addition, the halogenated derivatives may also be effective against other multidrug-resistant (MDR) pathovars like *S. aureus* and *S. pneumoniae* [36,37] **(Figure 5)**.

Table 2. The antimicrobial potency of the new molecules **1-25**, expressed as μg/mL.

Microbial organisms	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**	**M15**	**M16**	**M17**	**M18**	**M19**	**M20**	**M21**	**M22**	**M23**	**M24**	**M25**
E. coli ATCC-25922	0	16	18	20	0	20	20	18	25	22	0	20	20	22	19	0	0	0	0	20	0	20	20	24	26
K. pneumoniae	20	22	24	22	22	26	22	22	24	24	20	17	15	22	22	24	24	22	28	26	20	20	19	26	26
P. aeruginosa ATCC 27853	20	22	20	22	24	26	18	18	20	20	22	18	22	22	22	16	20	22	26	30	20	18	20	22	30
S. aureus ATCC 6538	0	0	0	0	17	21	21	0	21	21	15	19	0	15	20	22	16	22	20	26	22	17	18	0	24
S. epidermidis ATCC 35984	0	15	0	0	22	15	21	20	25	17	0	0	0	0	18	20	20	20	22	22	20	20	0	13	20
B. subtilis ATCC 6633	18	22	0	0	22	20	22	17	26	15	18	22	0	13	18	23	25	0	24	15	15	20	0	13	28
C. albicans ATCC-10231	0	0	0	0	17	21	21	0	21	21	15	19	0	15	18	23	16	23	20	26	22	17	18	0	24

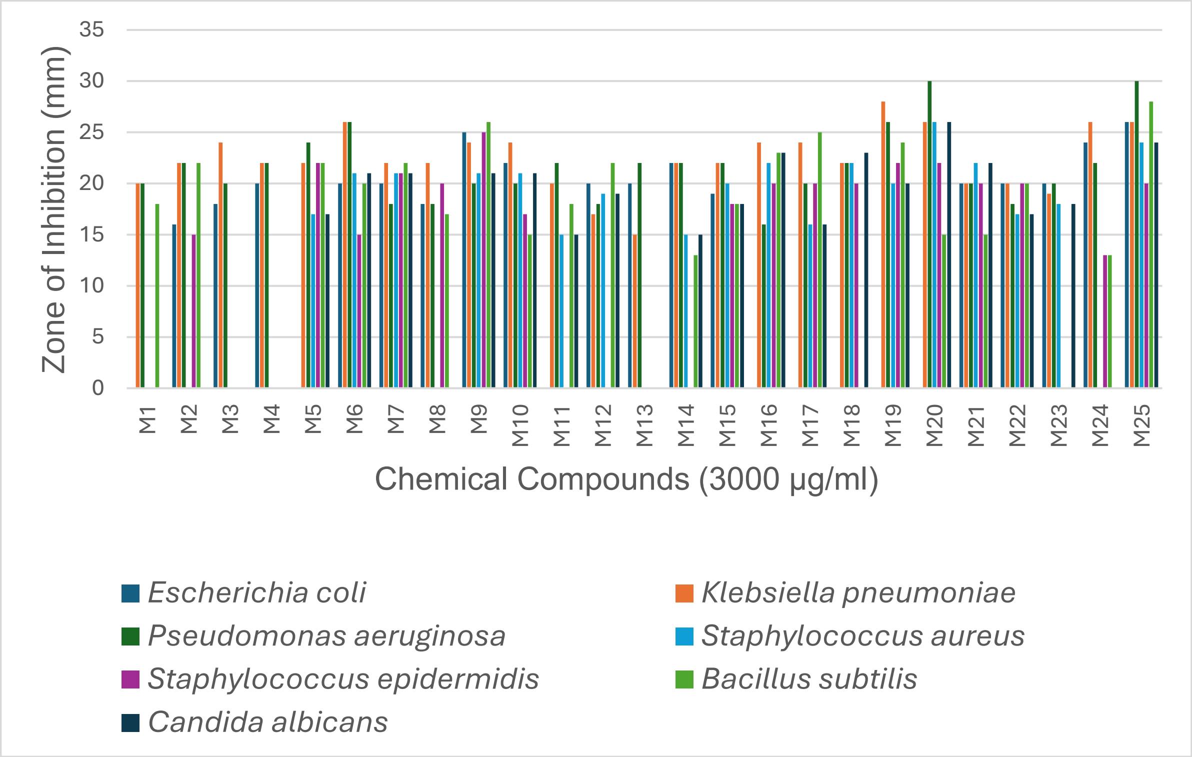
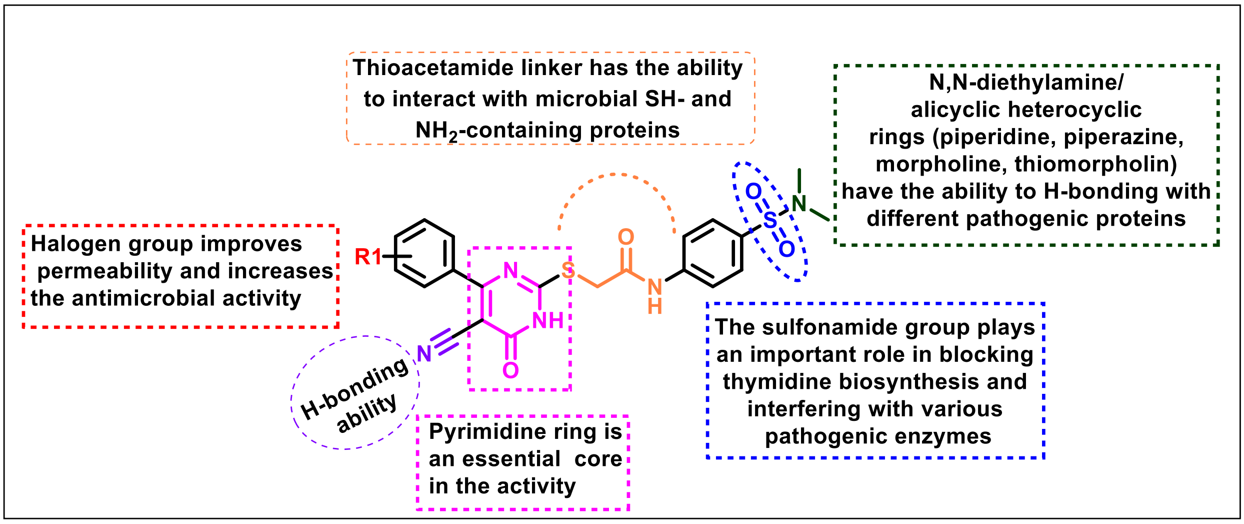


Figure 4. Zone of inhibition (mm) of the new sulfonamide compounds (3000 μg/ml) against various microbial strains.



Thioacetamide linker has the ability to interact with microbial SH- and NH_2-containing proteins

N,N-diethylamine/ alicyclic heterocyclic rings (piperidine, piperazine, morpholine, thiomorpholin) have the ability to H-bonding with different pathogenic proteins

Halogen group improves permeability and increases the antimicrobial activity

H-bonding ability

Pyrimidine ring is an essential core in the activity

The sulfonamide group plays an important role in blocking thymidine biosynthesis and interfering with various pathogenic enzymes

Figure 5. Structure-Activity Relationship of the new compounds in terms of antimicrobial activity.

2.2.2. MIC and MBC of Selected Compounds against More Susceptible Bacteria

Moreover, minimum inhibitory concentration (MIC (µg/m)) as well as the minimum bactericidal concentration (MBC(µg/mL)) assays were performed for the promising compounds M**6**, M**19**, M**20**, and M**25** against *K. pneumonia* and *P. aeruginosa* bacterial strains using the double-sequence dilution method [38,39]. The MIC assay represents the lowest concentration of antimicrobial agent that greatly inhibits microbial growth, while the MBC demonstrates the lowest level of antimicrobial agent leading to microbial death. According to Clinical and Laboratory Standards Institute (CLSI), antibacterial agents are usually evaluated as bactericidal if the MBC is no more than four times the MIC values [40].

The obtained results were summarized in **Table 3**. It was noticed that the MIC values for the tested compounds were 375 µg/mL against both bacterial strains. On the other hand, the MBC values showed variability among the compounds and bacterial strains. For *Klebsiella pneumoniae*, the MBC values for compounds **6** and **19** was 1500 µg/mL, while for compounds 21 and 25, it was 7500 µg/mL. For *Pseudomonas aeruginosa*, the MBC for all four compounds was consistent at 1500 µg/mL. Based on the obtained results, it could be detected that the ratio of MBC/MIC is equal to 4 in the case of compounds M**6** and M**9** against both *K. pneumonia* and *P. aeruginosa* confirming their bactericidal activity. With regard to compounds M**20** and M**25**, their MBC/MIC ratios revealed their bactericidal against *P. aeruginosa* strain and their bacteriostatic impact on *K. pneumoniae* these data implied the newly synthesized sulfonamide derivatives had potentiality to be developed and optimized as bactericidal agents against some resistant strains.

Table 3. MIC and MBC results for the most potent compounds against the respiratory bacterial strains.

Chemical Compounds	Bacterial strains	MIC µg/mL	MBC µg/mL
M6	***Klebsiella pneumoniae***	375 ± 0.00	1500 ± 0.45
	Pseudomonas aeruginosa	375 ± 0.00	1500 ± 0.00
M19	***Klebsiella pneumoniae***	375 ± 0.00	1500 ± 0.29
	Pseudomonas aeruginosa	375 ± 0.00	1500 ± 0.00
M20	***Klebsiella pneumoniae***	375 ± 0.00	7500 ± 0.00
	Pseudomonas aeruginosa	375 ± 0.00	1500 ± 0.00
M25	***Klebsiella pneumoniae***	375 ± 0.00	7500 ± 0.00
	Pseudomonas aeruginosa	375 ± 0.00	1500 ± 0.00

2.2.3. Determination of the Antibiofilm Effect of the Most Promising Compounds Using TCP Method

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A serious public health issue results from infections by microbial biofilms where bacterial biofilms have been demonstrated to be thousand times more resistant to antibacterial drugs compared to those in the planktonic forms [41]. Persistence of the gram-negative airway pathogens such as *K. pneumoniae* and *P. aeruginosa* their survival within the lung is mainly attributed to biofilm through colonization of endotracheal tubes and airways. In addition to the adaptation of these pathogens to the biofilm lifestyle [41–44].

In our study, incubation of *K. pneumonia* and *P. aeruginosa* with the most promising four tested compounds M**6**, M**19**, M**20**, and M**25** at MIC, 2MIC and 4MIC concentrations for 24 h showed moderate to good biofilm formation prevention, with a percentage of inhibition up to 90 % against *K. pneumonia* and 91.8% against *P. aeruginosa* **(Figures 6 and 7)**. The inhibitory ability of these compounds to biofilm formation makes it a promising source of drug leads to control microbial biofilm growth.

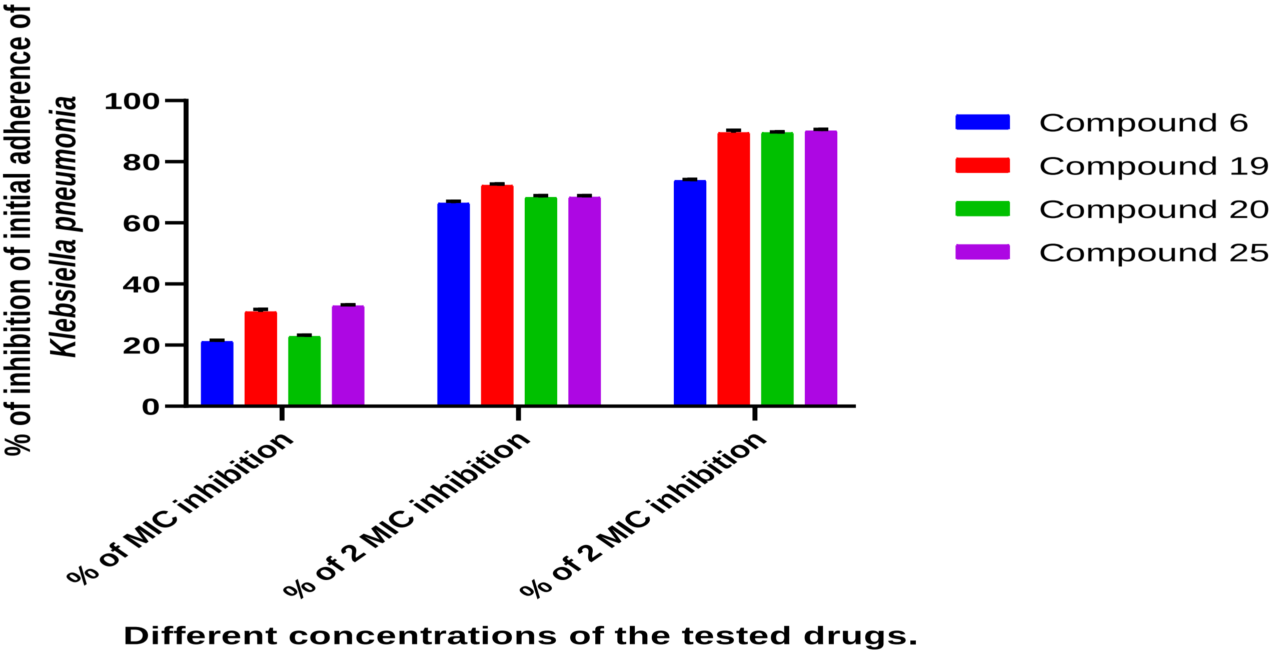


Figure 6. The inhibitory effect of the tested drugs M**6**, M**19**, M**20**, and M**25** on biofilm formation by *K. pneumonia.*

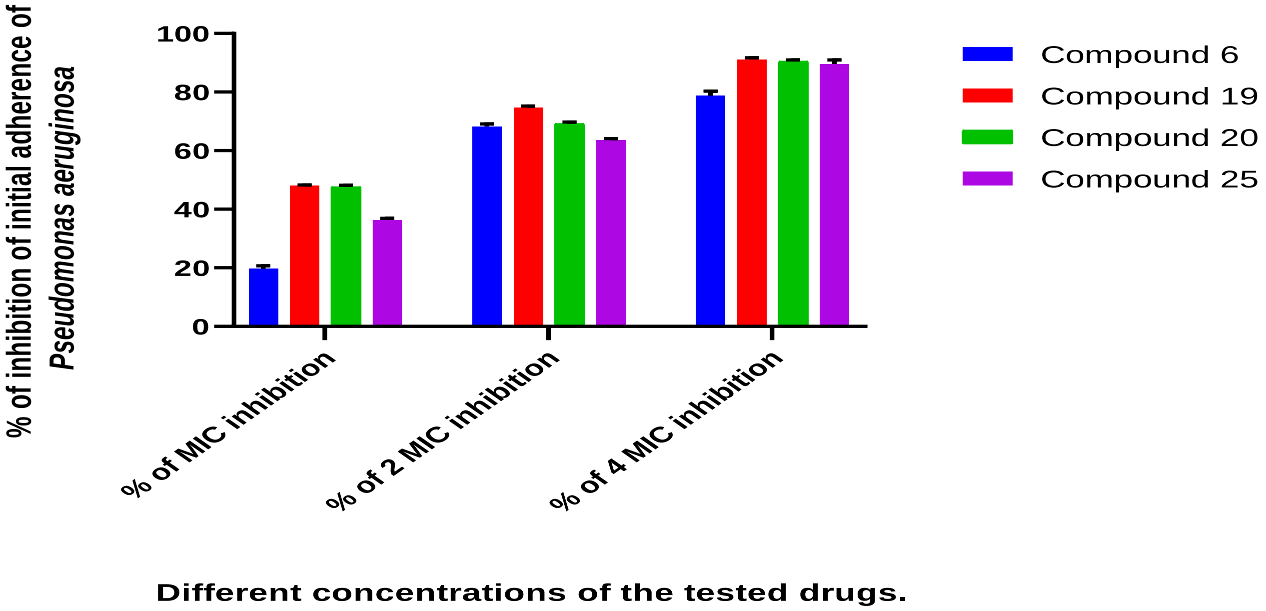


Figure 7. The inhibitory effect of the tested compounds **6**, **19**, **20**, and **25** on biofilm formation by *P. aeruginosa.*

2.3. Physicochemical Properties and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) Studies

The physicochemical properties of the promising candidates M**6**, M**9**, M**20** and M**25** were assessed and illustrated in **Table 4**. It could be seen that all the compounds follow Lipinski's rule. The hits showed that H-bonds acceptor <10, H-bonds donor <5 and logP <5. The compounds showed molecular weight roughly > 500 D, which is considered a violation of the Lipinski's rule. Even though the compounds violate one parameter, the drugs still follow Lipinski's rule. In addition, the recently published data suggested that there is a tendency for orally effective small molecule inhibitors to slightly exceed the 500 D [45]. It could be seen in **Table 3**, most of the molecules showed slightly high total polar surface area (TPSA). In general, these results indicate these promising inhibitors have excellence oral bioavailability and good absorbance.

In order to obtain deep understanding of the pharmacokinetic profile of the hits, the ADMET properties were calculated. The prediction of ADMET is considered an essential study to predict the pharmacokinetic and bioavailability properties of drug-like compounds [46–48]. The ADMET results **(Table 5)** illustrated that, the compounds have a moderate water solubility and good intestinal absorbance. In addition, the hits showed logKp values <-2.5 which indicates the compounds have reasonable skin permeability. The distribution results of the promising hits showed logBB <-1 and logPS <-3 which indicates the inhibitors are poorly distributed to the brain or CNS.

While the metabolism calculations indicate that the inhibitors are metabolized by CYP3A4 enzyme while they could not be substrates or inhibitors for CYP2D6 or CYP1A2 enzymes respectively. The low values of the total clearance of the four molecules reveal that they have good half-lives, and the toxicity study showed no hERG inhibition properties or AMES mutagenicity which suggests the compounds are not mutagenic or tumorigenic.

The bioavailability radars of the compounds **(Figure 8)** showed that the compounds have good pharmacokinetic properties. The pink area of the radars illustrated the lipophilicity, size of the molecules, insolubility, instauration and the flexibility properties. The compounds are almost within the range of conformity. While the polarity properties are slightly increased over the range. In general, the pharmacokinetic properties of the compounds are promising and can be optimized.

In conclusion, compounds M**6**, M**9**, M**20** and M**25** showed satisfactory ADMET properties, the results indicated good absorption, poor penetration to blood brain barrier or the CNS, excellent clearance properties and no toxicity.

Table 4. Physicochemical properties and Lipinski's rule of the most promising compounds.

Compound No.	MW	HBA	HBD	logP (o/w)	TPSA Å^2	Num. rotatable bonds	Lipinski
M6	576.49	6	2	3.04	169.7	8	Yes; 1 violation: MW>500
M9	546.02	7	2	1.72	178.9	6	Yes; 1 violation: MW>500
M20	562.09	6	2	2.35	195	6	Yes; 1 violation: MW>500
M25	562.09	6	2	2.39	195	6	Yes; 1 violation: MW>500

MW = molecular weight, HBA = Hydrogen bond acceptor, HBD = Hydrogen bond donor, log Po/w = Octanol-Water partition coefficient, TPSA=total polar surface area.

Table 5. *In silico* ADMET prediction of compounds M**6**, M**9**, M**20** and **25.**

	Compound M6	Compound M9	Compound M20	Compound M25
Absorption				
Water solubility (log mol/L)	-4.0	-3.8	-3.8	-3.8
Intestinal absorption	82.4	78.7	85.1	85.2
Skin permeability (log Kp)	-2.7	-2.7	-2.7	-2.7
Distribution				
Blood brain permeability(log BB)	-1.46	-1.43	-1.42	-1.42
CNS permeability (log PS)	-2.78	-3.45	-2.73	-2.73
Metabolism				
CYP2D6 substrate	No	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No
Excretion				
Total clearance (log ml/min/Kg)	0.1	0.2	0.1	0.1
Renal OCT2 substrate	NO	No	No	No
Toxicity				
AMES toxicity	No	No	No	No
hERG inhibitor	No	No	No	No
Tumorigenic	No	No	No	No

Irritant	No	No	No	No

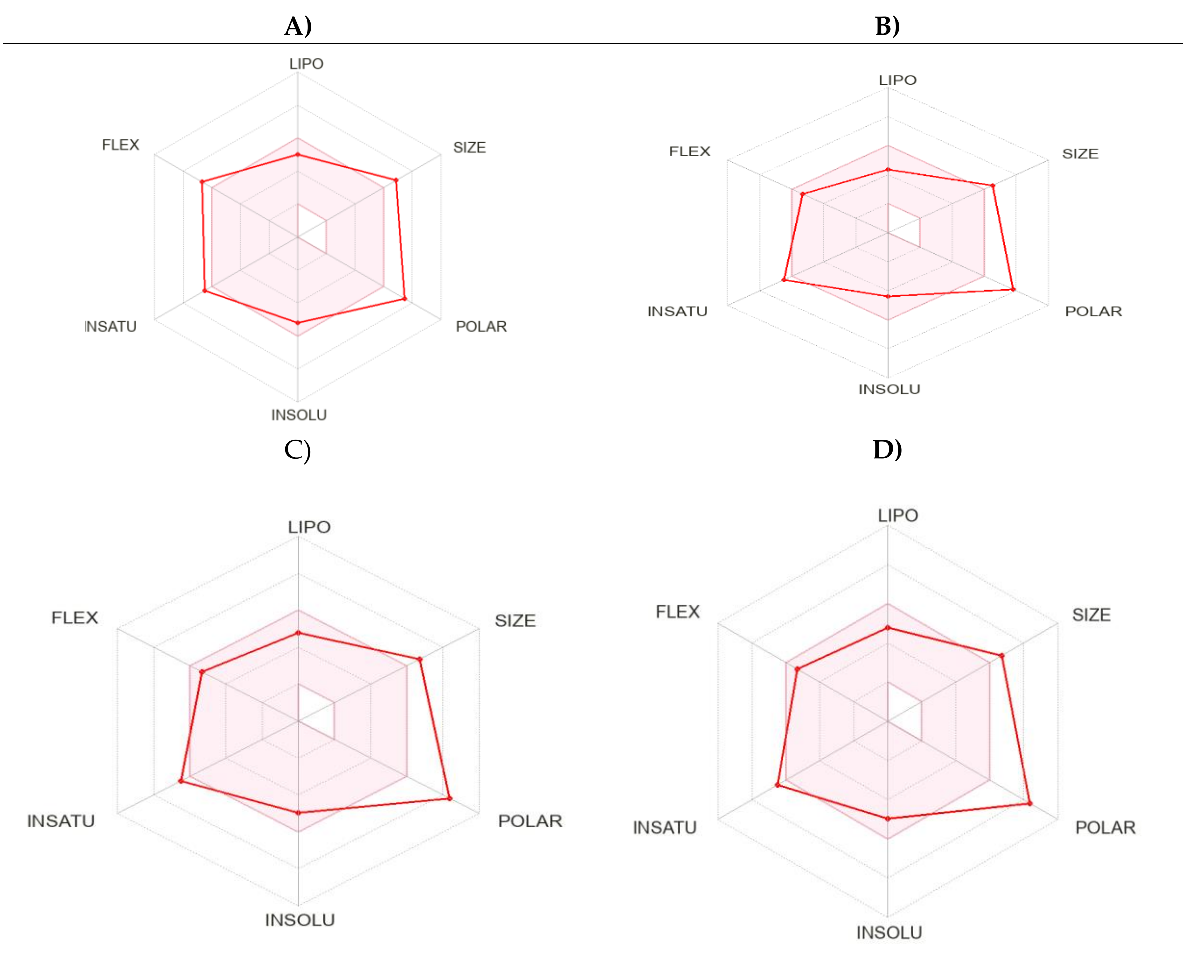


Figure 8. The bioavailability radars of **(A)** compound **6**, **(B)** compound **9**, **(C)** compound **20** and (**D)** compound **25**. The pink areas indicate the optimum properties range. The compounds are almost within the range of conformity, and accepted pharmacokinetics properties.

3. Conclusion

In this study, we have synthesized novel 25 analogues bearing 5-cyano-6-oxo-4-substituted phenyl-1,6-dihydropyrimidine scaffold hybridized with various substituted benzene-sulfonamide derivatives through a thioacetamide linker **M1-25**. These compounds exhibited considerable promise as antimicrobial agents, demonstrating broad-spectrum efficacy against Gram-positive, Gram-negative, and fungal pathogens, with notable effectiveness against the resistant strains *K. pneumoniae* and *P. aeruginosa*. The positive results from minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and biofilm suppression assays underscore their capability to inhibit microbial growth and virulence. Preliminary ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling suggests that these derivatives possess favorable drug-like properties and manageable toxicity. Collectively, these findings underscore the potential of these pyrimidine-benzene-sulfonamide derivatives as promising candidates for further development and optimization in the treatment of microbial infections.

4. Experimental

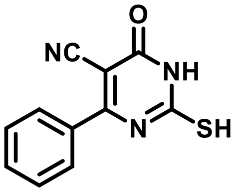
4.1. Chemistry

All reagents and solvents were purchased from commercial suppliers and used without purification unless stated otherwise. The instruments used to determine melting points, spectral data (IR, ^{1}H NMR, ^{13}C NMR, and mass), as well as chemical analyses were included in a detailed description of the in the file of Supporting Information.

4.1.1. General Procedure for Preparation of 6-Substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles [30].

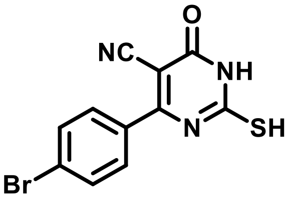
A mixture of thiourea (1.839g, 24 mmol), a suitable aldehyde (24 mmol), ethylcyanoacetate (2.734g, 24 mmol), and K_2CO_3 (4.837g, 24 mmol) was added to a round bottom flask, followed by ethanol (50 mL). The reaction mixture was then heated under reflux for 12 hours and monitored by TLC. The resulting creamy precipitate was filtered, ethanol washed, and vacuum dried. The product was then dissolved in the minimum amount of hot water (100 mL) and acidified with glacial acetic acid to pH 4. The white precipitate was suction filtered and then recrystallized from aqueous DMF.

4.1.1.1. 2-Mercapto-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (1a)



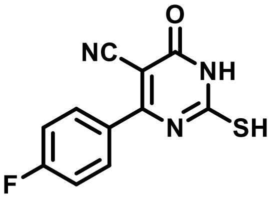
White powder, 81%. mp 255.°C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.58 (dd, J = 8.1, 6.6 Hz, 2H), 7.62 – 7.65 (m, 1H), 7.68 (dd, J = 6.9, 1.7 Hz, 2H), 13.20 (s, 1H), 13.34 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 91.3, 115.2, 128.9, 129.2, 129.8, 132.6, 159.0, 161.4, 176.7.

4.1.1.2. 4-(4-Bromophenyl)-2-mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (1b)



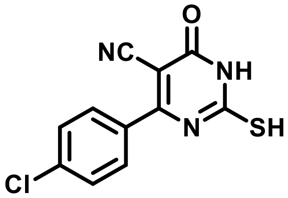
White powder, 85%. mp 284 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.63 (d, J = 8.6 Hz, 2H), 7.80 (d, J = 8.5 Hz, 2H), 13.18 (s, 1H), 13.35 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 91.5, 115.0, 126.3, 129.0, 131.3, 132.0, 158.8, 160.4, 176.6.

4.1.1.3. 4-(4-Fluorophenyl)-2-mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (1c)



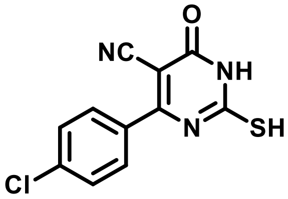
White powder, 84%. mp 270-272 °C, ^{1}H NMR (400 MHz, DMSO-d6) δ 7.44 (dt, 2H), 7.76 (dd, 2H), 13.22 (s, 1H), 13.36 (s, 1H).^{13}CNMR (101 MHz, DMSO) δ 91.4, 115.2, 116.0, 116.3, 126.2, 126.2, 132.1, 132.2, 158.9, 160.5, 163.4, 165.8, 176.6.

4.1.1.4. 4-(4-Chlorophenyl)-2-mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (1d)



White powder, 86%. mp 252.°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 8.7 Hz, 2H), 13.22 (s, 1H), 13.38 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 91.5, 115.1, 128.6, 129.1, 131.2, 137.4, 158.9, 160.4, 176.6.

4.1.1.5. 4-(3-Chlorophenyl)-2-mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (1e)

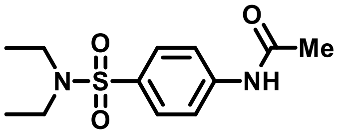


Yellow powder, 80%. mp 229.°C, ^{1}HNMR (400 MHz, DMSO-d6) δ 7.57 – 7.67 (m, 2H), 7.72 (dt, J = 7.7, 1.8 Hz, 1H), 7.78 (t, J = 1.9 Hz, 1H), 13.24 (s, 1H), 13.40 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 91.7, 114.9, 128.0, 129.1, 130.9, 131.7, 132.3, 133.4, 158.8, 160.0, 176.6.

4.1.2. General Procedure for Preparation of N-substituted sulfonyl phenyl acetamides 2

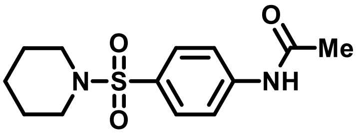
To a solution of 4-acetylaminobenzenesulfonyl chloride (2.34 g, 10 mmol) in methanol, 20 mL of appropriate amine (20 mmol) was added dropwise at room temperature. The reaction mixture was refluxed for 4 hours. After cooling, the solvent was removed under pressure, and water (50 mL) was added. The mixture was stirred at room temperature for 30 minutes. The solid obtained was filtered, washed with cold water, and dried. The compound formed was then recrystallized with 50% ethanol.

4.1.2.1. *N*-(4-(*N*,*N*-diethylsulfamoyl)phenyl)acetamide (2a)



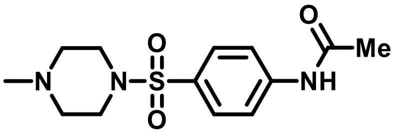
White powder, 2.30g, 85%, ^{1}H NMR (400 MHz, DMSO-d_6)) δ 1.03 (t, J = 7.1 Hz, 6H), 2.09 (s, 3H), 3.13 (q, J = 7.1 Hz, 4H), 7.71 (d, J = 8.9 Hz, 2H), 7.78 (d, J = 8.7 Hz, 2H), 10.30 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 14.5, 24.6, 42.2, 119.2, 128.3, 133.9, 143.4, 169.5.

4.1.2.2. *N*-(4-(piperidin-1-ylsulfonyl)phenyl)acetamide (2b)



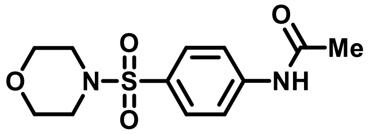
White powder, 2.42g, 86%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (m, 2H), 1.54 (p, J = 5.8 Hz, 4H), 2.10 (s, 3H), 2.86 (t, J = 5.5 Hz, 4H), 7.66 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.8 Hz, 2H), 10.33 (s, 1H). ^{13}CNMR (DMSO-d_6, 101 MHz): δ 23.4, 24.6, 25.1, 47.0, 119.1, 129.1, 129.5, 143.8, 169.5.

4.1.2.3. *N*-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)acetamide (2c)



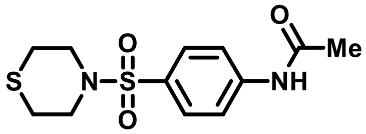
White powder, 2.51g, 84%.^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10 (s, 3H), 2.14 (s, 3H), 2.36 (t, J = 4.9 Hz, 4H), 2.87 (t, J = 5.0 Hz, 4H), 7.66 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 10.36 (s, 1H)^{13}C NMR (101 MHz, DMSO) δ 24.6, 45.7, 46.2, 54.0, 119.1, 128.9, 129.2, 144.0, 169.5.

4.1.2.4. *N*-(4-(morpholinosulfonyl)phenyl)acetamide (2d)



White powder, 2.39g, 84%. ^{1}H NMR (400 MHz, DMSO-d6) δ 2.11 (s, 3H), 2.84 (t, 4H), 3.62 (t, J = 4.7 Hz, 4H), 7.67 (d, J = 8.7 Hz, 2H), 7.84 (d, J = 8.8 Hz, 2H), 10.38 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 24.6, 46.4, 65.7, 119.2, 128.4, 129.4, 144.1, 169.6.

4.1.2.5. *N*-(4-(thiomorpholinosulfonyl)phenyl)acetamide (2e)

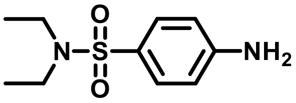


White powder, 2.67 g, 89%.^{1}HNMR (400 MHz, DMSO-d6) δ 2.10 (s, 3H), 2.66 (t, 4H), 3.18 (t, 4H), 7.68 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.9 Hz, 2H), 10.37 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 24.6, 26.9, 48.2, 119.2, 128.9, 130.1, 144.0, 169.6.

4.1.3. General Procedure for Preparation of 4-amino-benzenesulfonamides 3

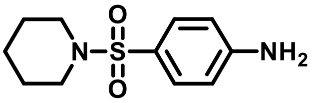
A substituted sulfonyl phenyl acetamide **2** (10 mmol) was added to concentrated HCl (10 mL) and methanol (10 mL) and heated at 80–90°C for two hours. The solution became clear upon heating. After two hours, the solution was concentrated using a rotary evaporator. The resulting solution was then neutralized to pH 7 by the addition of a saturated aqueous solution of Na_2CO_3. The product was washed with cold water and recrystallized with ethanol.

4.1.3.1. 4-Amino-N,N-diethylbenzenesulfonamide (3a)



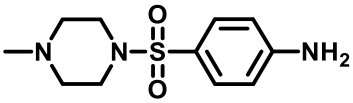
White powder, 2.10g, 92%.^{1}H NMR (400 MHz, DMSO-d6) δ 1.02 (t, J = 7.1 Hz, 6H), 3.07 (q, J = 7.1 Hz, 4H), 5.93 (s, 2H), 6.62 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.7 Hz, 2H). ^{13}CNMR (101 MHz, DMSO) δ 14.5, 42.0, 113.3, 125.1, 129.1, 153.1.

4.1.3.2. 4-(Piperidin-1-ylsulfonyl)aniline (3b)



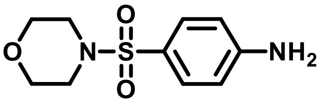
White powder, 2.16g, 90%.^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.18 – 1.45 (m, 2H), 1.53 (p, J = 5.7 Hz, 4H), 2.79 (t, J = 5.4 Hz, 4H), 6.00 (s, 2H), 6.65 (d, J = 8.7 Hz, 2H), 7.34 (d, J = 8.7 Hz, 2H). ^{13}CNMR (101 MHz, DMSO) δ 23.5, 25.2, 47.0, 113.2, 120.7, 129.9, 153.5.

4.1.3.3. 4-((4-Methylpiperazin-1-yl)sulfonyl)aniline (3c)



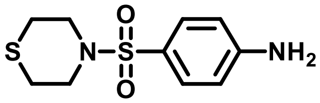
White powder, 2.28 mg, 89%. ^{1}HNMR (400 MHz, DMSO-d6) δ 2.13 (s, 3H), 2.34 (t, J = 4.9 Hz, 4H), 2.80 (t, J = 4.9 Hz, 4H), 6.05 (s, 2H), 6.66 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 8.7 Hz, 2H). ^{13}CNMR (101 MHz, DMSO) δ 45.8, 46.1, 54.0, 113.2, 119.9, 130.0, 153.7.

4.1.3.4. 4-(Morpholinosulfonyl)aniline (3d)



White powder, 2.23g, 92%.^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.78 (t, 4H), 3.61 (t, 4H), 6.09 (s, 2H), 6.67 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.7 Hz, 2H). ^{13}CNMR (101 MHz, DMSO) δ 46.4, 65.8, 113.2, 119.3, 130.2, 153.8.

4.1.3.5. 4-(Thiomorpholinosulfonyl)aniline (3e)

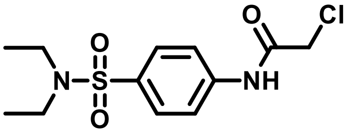


White powder, 2.41g, 93%.^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.65 (t, 4H), 3.11 (t, 4H), 6.06 (s, 2H), 6.66 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.7 Hz, 2H). ^{13}CNMR (101 MHz, DMSO) δ 26.9, 48.2, 113.3, 121.0, 129.7, 153.7.

4.1.4. General Procedure for Preparation of N-substituted sulfonyl phenyl chloro- acetamides [31].

In a flame-dried flask, chloroacetyl chloride (0.631 mg, 5.5 mmol) was added dropwise at 0°C to a solution of sulfonylaniline **3** (1.00 g, 4.3 mmol) in anhydrous THF (30 mL) containing K_2CO_3 (1.18 g, 8.6 mmol). The reaction mixture was stirred for 4 hours and monitored by TLC. After reaction completion, water (60 mL) was added, and the aqueous layer was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to yield compound **4**. The crude product was used for the next step without further purification.

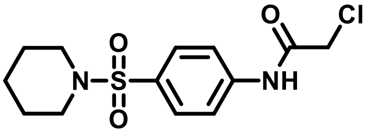
4.1.4.1. 2-Chloro-*N*-(4-(*N*,*N*-diethylsulfamoyl)phenyl)acetamide (4a)



White powder, 88%, ^{1}H NMR (400 MHz, Chloroform-d) δ 1.05 (t, J = 7.2 Hz, 6H), 3.16 (q, J = 7.1 Hz, 4H), 4.15 (s, 2H), 7.68 (d, J = 2.5 Hz, 4H), 8.91 (s, 1H). ^{13}CNMR (101 MHz, CDCl3) δ 14.1, 42.1, 43.1, 120.0, 128.0, 135.6, 141.0, 165.1.

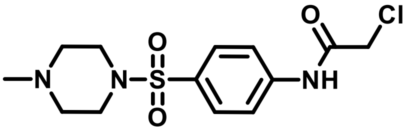
4.1.4.2. 2-Chloro-*N*-(4-(piperidin-1-ylsulfonyl)phenyl)acetamide (4b)

doi:10.20944/preprints202408.1943.v1



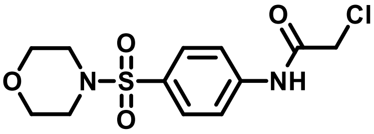
White powder, 91%.¹H NMR (400 MHz, DMSO-d_6δ 1.30 – 1.38 (m, 2H), 1.53 (t, J = 5.6 Hz, 4H), 2.86 (t, J = 5.4 Hz, 4H), 4.31 (s, 2H), 7.70 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 10.70 (s, 1H). ¹³C NMR (101 MHz, DMSO) δ 23.3, 25.1, 44.0, 47.0, 119.6, 129.2, 130.5, 142.9, 165.8.

4.1.4.3. 2-Chloro-*N*-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)acetamide (4c)



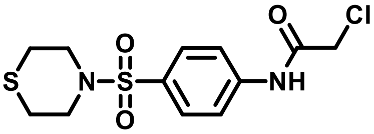
White powder, 94%.¹HNMR (400 MHz, DMSO-d6) δ 2.13 (s, 3H), 2.35 (t, J = 5.0 Hz, 4H), 2.87 (t, J = 4.9 Hz, 4H), 4.32 (s, 2H), 7.71 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 10.79 (s, 1H).¹³CNMR (101 MHz, DMSO) δ 44.0, 45.7, 46.2, 53.9, 119.7, 129.3, 129.8, 143.1, 165.9.

4.1.4.4. 2-Chloro-*N*-(4-(morpholinosulfonyl)phenyl)acetamide (4d)



White powder, 89%, ¹H NMR (400 MHz, Chloroform-d) δ 3.02 (t, 4H), 3.75 (t, 4H), 4.24 (s, 2H), 7.75 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H), 8.48 (s, 1H). ¹³CNMR (101 MHz, CDCl3) δ 42.8, 46.0, 66.1, 119.8, 129.2, 131.1, 141.1, 164.3.

4.1.4.5. 2-Chloro-*N*-(4-(thiomorpholinosulfonyl)phenyl)acetamide (4e)

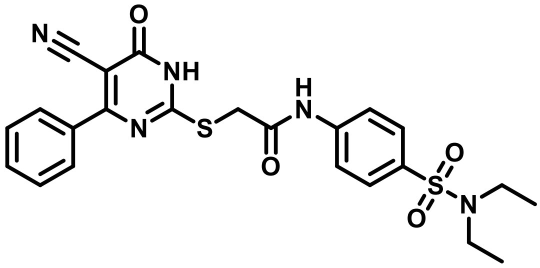


White powder, 85%. ¹H NMR (400 MHz, DMSO-d_6) δ 2.66 (q, J = 4.8, 4.2 Hz, 4H), 3.20 (q, J = 4.8, 4.2 Hz, 4H), 4.32 (d, J = 3.4 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 7.85 (d, J = 8.7 Hz, 2H), 10.73 (s, 1H). ¹³C NMR (101 MHz, DMSO) δ 26.9, 44.0, 48.2, 119.8, 129.0, 131.0, 143.1, 165.9.

4.1.5. General Procedure for Preparation of Final Target Compounds M1-25

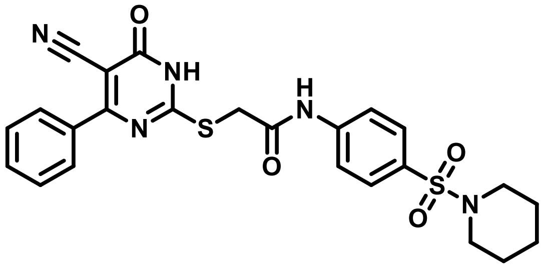
In a dry oven flask, A mixture of compound **1** (1.30 mmole) and K_2CO_3 (1.43 mmole) in 10 mL DMF was stirred for 20 minutes under nitrogen. Then, the appropriate sulfonyl acetamide compound 4 (1.30 mmole) was added to the flask. The reaction mixture was stirred for 20–34 hours under nitrogen at room temperature and monitored by TLC. Upon completion of the reaction, water (60 mL) was added, and the mixture was acidified with glacial acetic acid. Precipitation formed, which was then filtered, washed with cold water, and purified by flash column chromatography.

4.1.5.1. 2-((5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(N,N-diethylsulfamoyl)phenyl)acetamide (M1)



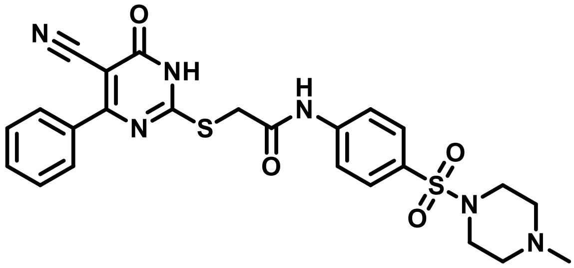
White powder, mp 207-210, 601 mg, 93%.^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03 (t, J = 7.1 Hz, 6H), 3.15 (q, J = 7.1 Hz, 4H), 4.20 (s, 2H), 7.29 (t, J = 7.7 Hz, 2H), 7.50 (t, J = 7.5 Hz, 1H), 7.74 (d, J = 9.1 Hz, 2H), 7.77 (d, J = 9.4 Hz, 2H), 7.82 (d, J = 7.8 Hz, 2H), 10.78 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 14.4, 36.1, 42.1, 93.5, 116.3, 119.3, 128.4, 128.8, 129.2, 132.0, 134.3, 135.5, 143.1, 161.8, 166.3, 166.5, 167.5. MS (ESI) m/z (%): 498.1450 [M+H].

4.1.5.2. 2-((5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(piperidin-1-ylsulfonyl)phenyl)acetamide (M2)



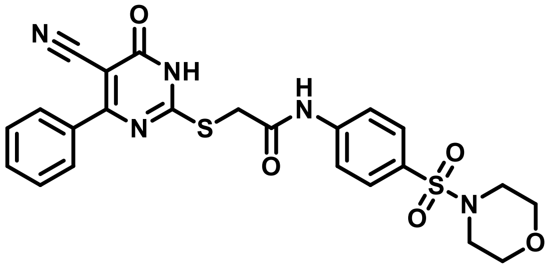
White powder, mp 184-186 629 mg, 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (p, 2H), 1.54 (t, J = 5.8 Hz, 4H), 2.86 (t, J = 5.5 Hz, 4H), 4.22 (s, 2H), 7.29 (t, J = 7.7 Hz, 2H), 7.49 (t, J = 7.5 Hz, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.82 (dd, J = 10.5, 8.1 Hz, 4H), 10.76 (s, 1H), 13.71 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 23.4, 25.1, 36.2, 47.1, 93.7, 116.2, 119.2, 128.8, 129.2, 129.2, 130.0, 132.0, 135.5, 143.4, 161.6, 166.2, 166.5, 167.5. MS (ESI) m/z (%): 509.1640 [M+H].

4.1.5.3. 2-((5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)-N-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)acetamide (M3)



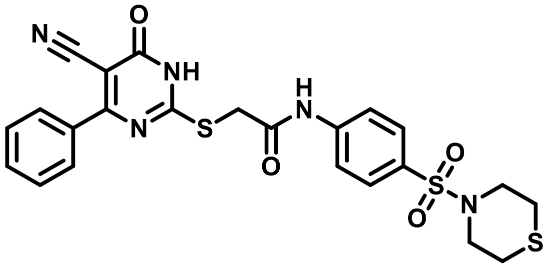
White powder, mp 213-215 ºC, 641 mg, 94%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.67 (s, 3H), 3.16 (s, 8H), 4.10 (s, 2H), 7.37 (t, J = 7.8 Hz, 2H), 7.51 (t, J = 7.4 Hz, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 7.7 Hz, 2H), 7.85 (d, J = 8.4 Hz, 2H), 11.15 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 36.0, 42.9, 43.9, 52.4, 92.1, 117.8, 119.4, 128.5, 128.7, 129.0, 129.5, 131.5, 136.4, 144.1, 167.6, 167.7. MS (ESI) m/z (%): 525.1303 [M+H].

4.1.5.4. 2-((5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(morpholinosulfonyl)phenyl)acetamide (M4)



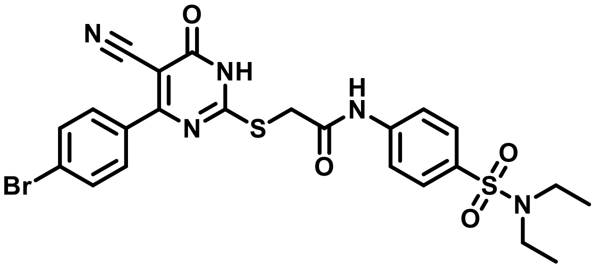
White powder, mp 205-208 °C, 598 mg, 90%^{1}HNMR (400 MHz, DMSO-d6) δ 2.85 (t, J = 4.7, 4.1 Hz, 4H), 3.62 (t, J = 4.7 Hz, 4H), 3.88 (s, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.46 (t, J = 7.3 Hz, 1H), 7.67 (d, J = 8.8 Hz, 2H), 7.76 (d, J = 7.0 Hz, 2H), 7.82 (d, J = 8.8 Hz, 2H), 11.42 (s, 1H).^{13}CNMR (101 MHz, DMSO) δ 35.8, 46.4, 65.8, 90.2, 119.1, 120.0, 128.5, 128.7, 129.4, 130.3, 137.8, 143.9, 167.7, 168.9. MS (ESI) m/z (%): 512.5891 [M+H].

4.1.5.5. 2-((5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(thiomorpholinosulfonyl)phenyl)acetamide (M5)



White powder, mp 216-219 °C, 610, 89%. ^{1}H NMR (400 MHz, DMSO-d6) δ 2.66 (t, J = 4.7 Hz, 4H), 3.19 (t, J = 4.7 Hz, 4H), 3.88 (s, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.46 (t, J = 7.3 Hz, 1H), 7.68 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H), 11.38 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 26.9, 35.8, 48.2, 90.2, 119.2, 120.0, 128.5, 128.7, 129.0, 130.2, 130.3, 137.8, 143.8, 161.5 167.7, 168.9, 171.7. MS (ESI) m/z (%): 528.6730 [M+H].

4.1.5.6. 2-((4-(4-Bromophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(N,N-diethylsulfamoyl)phenyl)acetamide (M6)



White powder, mp 242-245 °C, 696 mg, 93%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03 (t, J = 7.1 Hz, 6H), 3.15 (q, J = 7.1 Hz, 4H), 4.19 (s, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 6.2 Hz, 6H), 10.70 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 14.4, 36.1, 42.2, 93.8, 116.0, 119.3, 125.9, 128.4, 131.1, 131.8, 134.3, 134.6, 142.9, 161.3, 166.4, 166.4, 166. 5. MS (ESI) m/z (%):574.0240 [M-H].

4.1.5.7. 2-((4-(4-Bromophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(piperidin-1-ylsulfonyl)phenyl)acetamide (M7)

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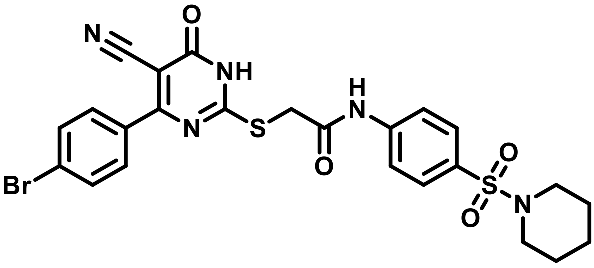
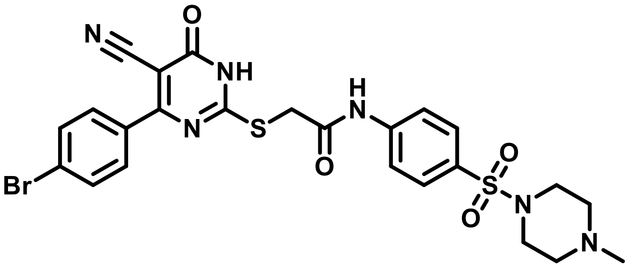


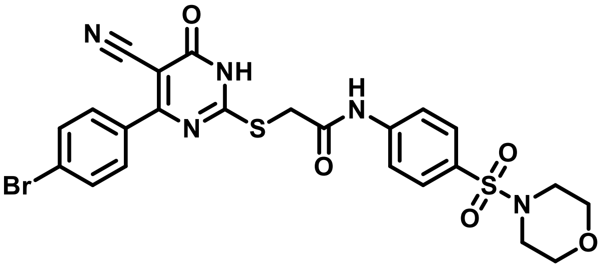
White powder, mp 257-260 ℃, 734 mg, 96%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (p, J = 5.4, 4.7 Hz, 2H), 1.54 (t, J = 5.7 Hz, 4H), 2.86 (t, J = 5.4 Hz, 4H), 4.19 (s, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.75 (d, J = 8.5 Hz, 2H), 7.79 (d, J = 8.7 Hz, 2H), 10.74 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 23.4, 25.1, 36.1, 47.1, 93.8, 116.0, 119.2, 125.9, 129.1, 130.0, 131.2, 131.8, 134.6, 143.2, 161.3, 166.4, 166.5, 166.5. MS (ESI) m/z (%): 588.0470 [M+H].

4.1.5.8. 2-((4-(4-Bromophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)acetamide (M8)



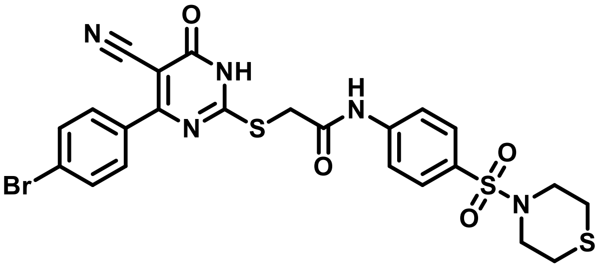
White powder, mp 230-233 ℃, 745mg, 95%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.64 (s, 3H), 3.09 (s, 8H), 3.99 (s, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.72 (d, J = 4.0 Hz, 2H), 7.74 (d, J = 3.8 Hz, 2H), 7.86 (d, J = 8.8 Hz, 2H), 11.21 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 35.9, 43.3, 44.2, 52.6, 91.1, 118.7, 119.4, 124.5, 128.3, 129.5, 130.8, 131.7, 136.3, 144.1, 166.5, 168.2. MS (ESI) m/z (%): 603.0240 [M+H].

4.1.5.9. 2-((4-(4-Bromophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(morpholinosulfonyl)phenyl)acetamide(M9)



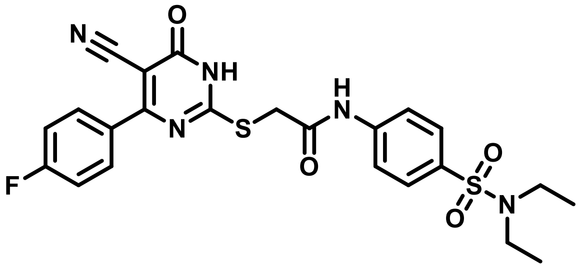
White powder, mp 218-222 ℃, 698mg, 91%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.86 (t, J = 4.7 Hz, 4H), 3.64 (t, J = 4.8 Hz, 4H), 4.21 (s, 2H), 7.48 (d, J = 8.5 Hz, 2H), 7.70 (d, J = 8.6 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 7.82 (d, J = 8.6 Hz, 2H), 10.77 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 36.2, 46.4, 65.8, 93.8, 116.1, 119.3, 125.9, 128.9, 129.5, 131.1, 131.8, 134.7, 143.6, 166.5, 166.6. MS (ESI) m/z (%): 590.0540 [M+H].

4.1.5.10. 2-((4-(4-Bromophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(thiomorpholinosulfonyl)phenyl)acetamide (M10)



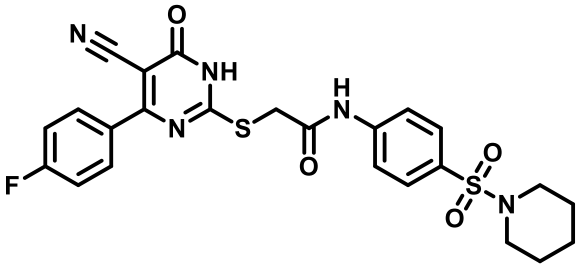
White powder, mp 224-226 ℃, 693mg, 88%, ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.68 (t, J = 4.9 Hz, 4H), 3.20 (t, J = 5.0 Hz, 4H), 4.20 (s, 2H), 7.49 (d, J = 8.5 Hz, 2H), 7.71 (d, J = 8.6 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H), 7.81 (d, J = 8.6 Hz, 2H), 10.77 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 26.9, 48.3, 93.8, 116.0, 119.3, 125.9, 129.0, 130.5, 131.1, 131.8, 134.6, 143.5, 161.3, 166.4, 166.5, 166.6. MS (ESI) m/z (%): 606.0120 [M+H].

4.1.5.11. 2-((4-(4-Fluorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(N,N-diethylsulfamoyl)phenyl)acetamide (M11)



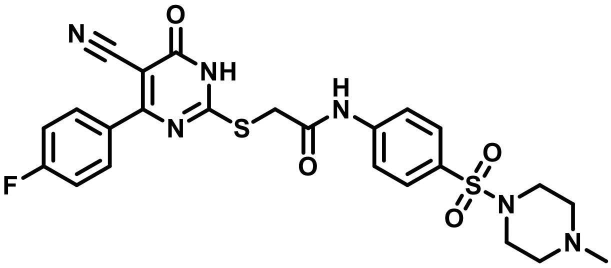
White powder, mp 248-251 ℃, 630mg, 94%, ^{1}H NMR (400 MHz, DMSO-*d*$_6$)δ 1.03 (t, J = 7.1 Hz, 6H), 3.15 (q, J = 7.1 Hz, 4H), 4.21 (s, 2H), 7.13 (t, J = 8.8 Hz, 2H), 7.76 (s, 4H), 7.93 (dd, 2H), 10.72 (s, 1H), 13.96 (s, 1H).^{13}CNMR (101 MHz, DMSO) δ 14.4, 36.1, 42.1, 93.5, 115.7, 115.9, 116.2, 119.3, 128.5, 131.9, 131.9, 134.3, 142.9, 163.1, 165.6, 166.3, 166.5. MS (ESI) m/z (%): 516.6984 [M+H].

4.1.5.12. 2-((4-(4-Fluorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(piperidin-1-ylsulfonyl)phenyl)acetamide (M12)



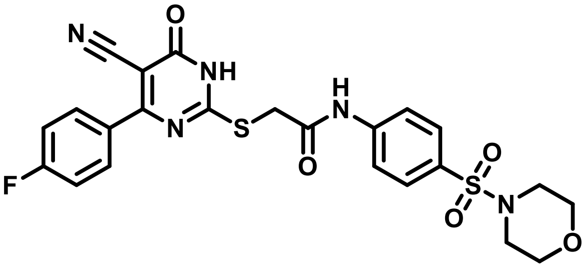
White powder, mp 177-180 ºC, 630mg, 92%, ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.36 (p, 2H), 1.54 (t, J = 5.7 Hz, 4H), 2.86 (t, J = 5.4 Hz, 4H), 4.22 (s, 2H), 7.11 (t, J = 8.8 Hz, 2H), 7.69 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H), 7.93 (dd, J = 8.7, 5.6 Hz, 2H), 10.75 (s, 1H), 13.95 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 23.4, 25.1, 36.2, 47.1, 93.5, 115.7, 115.9, 116.2, 119.2, 129.2, 130.0, 131.9, 132.0, 143.3, 163.1, 165.6, 166.2, 166.3, 166.5. MS (ESI) m/z (%): 528.1124 [M+H].

4.1.5.13. 2-((4-(4-Fluorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)acetamide (M13)



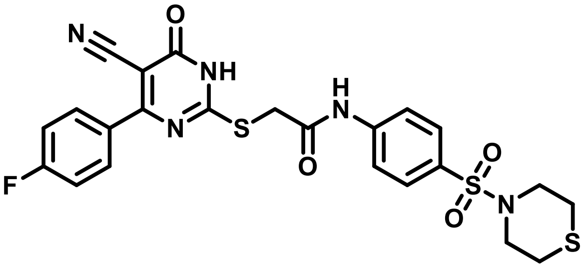
White powder, mp 213-215 °C, 641mg, 91%,^{1}H NMR (400 MHz, DMSO-d6) δ 2.60 (s, 3H), 3.04 (s, 8H), 3.98 (s, 2H), 7.23 (t, J = 8.9 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H), 7.86 (t, 4H), 11.24 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 35.9, 43.5, 44.4, 52.8, 90.9, 115.5, 115.8, 118.9, 119.4, 128.3, 129.5, 131.2, 131.3, 133.5, 133.6, 144.0, 162.5, 165.0, 166.5, 167.8, 168.3, 170.2. MS (ESI) m/z (%): 543.7064 [M+H].

4.1.5.14. 2-((4-(4-Fluorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(morpholinosulfonyl)phenyl)acetamide (M14)



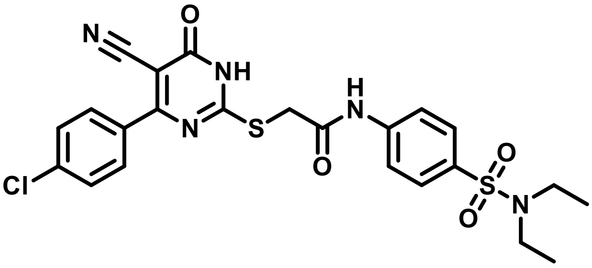
White powder, mp 270-274 °C, 640mg, 93%, ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.85 (t, J = 4.2 Hz, 4H), 3.63 (t, J = 4.7 Hz, 4H), 4.24 (s, 2H), 7.14 (t, J = 8.8 Hz, 2H), 7.70 (d, J = 8.9 Hz, 2H), 7.82 (d, J = 8.9 Hz, 2H), 7.94 (dd, J = 8.7, 5.6 Hz, 2H), 10.77 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 36.2, 46.4, 65.8, 93.6, 115.8, 116.0, 116.1, 119.3, 128.9, 129.5, 131.9, 132.0, 143.6, 161.4, 163.1, 165.6, 166.1, 166.4, 166.6. MS (ESI) m/z (%): 530.7637 [M+H].

4.1.5.15. 2-((4-(4-Fluorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(thiomorpholinosulfonyl)phenyl)acetamide (M15)



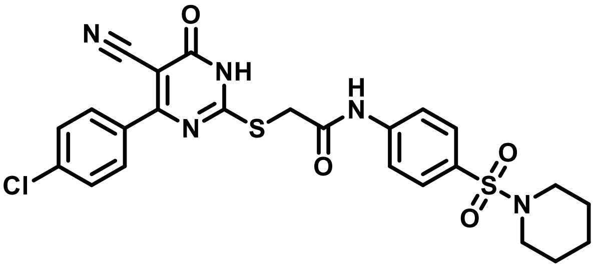
White powder, mp 195-198 °C, 652mg, 92%, ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.65 (t, J = 5.2 Hz, 4H), 3.18 (t, J = 5.0 Hz, 4H), 4.21 (s, 2H), 7.12 (t, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 8.6 Hz, 2H), 7.92 (dd, J = 8.6, 5.4 Hz, 2H), 10.78 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 26.9, 36.1, 48.2, 93.5, 115.8, 116.0, 116.2, 119.4, 129.0, 130.6, 131.8, 131.9, 143.4, 161.5, 163.1, 166.2, 166.4, 166.6. MS (ESI) m/z (%): 546.6345 [M+H].

4.1.5.16. 2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(N,N-diethylsulfamoyl)phenyl)acetamide (M16)



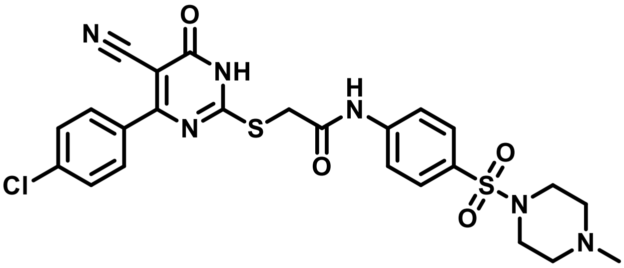
White powder, mp 230-233. ℃, 650mg, 94%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02 (t, J = 7.1 Hz, 7H), 3.14 (q, J = 7.1 Hz, 4H), 4.18 (s, 2H), 7.32 (d, J = 8.5 Hz, 2H), 7.74 (s, 4H), 7.83 (d, J = 8.5 Hz, 2H), 10.72 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 14.4, 36.2, 42.2, 93.8, 116.0, 119.3, 128.5, 128.8, 131.0, 134.3, 134.3, 137.0, 142.9, 161.4, 166.3, 166.4,166.4. MS (ESI) m/z (%): 532.0280 [M+H].

4.1.5.17. 2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(piperidin-1-ylsulfonyl)phenyl)acetamide (M17)



White powder, mp 238-240 ℃, 671mg,95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (p, J = 6.0 Hz, 2H), 1.53 (p, J = 5.7 Hz, 4H), 2.85 (t, J = 5.4 Hz, 4H), 4.19 (s, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.79 (d, J = 8.7 Hz, 2H), 7.83 (d, J = 8.5 Hz, 2H), 10.75 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 23.4, 25.1, 36.1, 47.1, 93.8, 116.0, 119.2, 128.8, 129.2, 130.0, 131.0, 134.3, 137.0, 143.2, 161.4, 166.3, 166.4, 166.5. MS (ESI) m/z (%): 544.0680 [M+H].

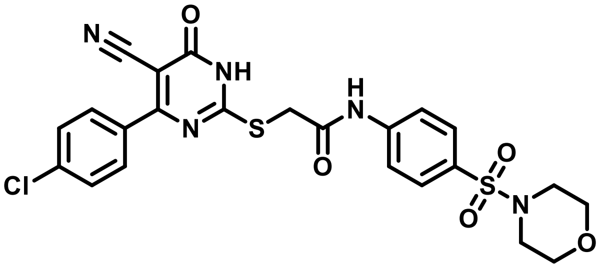
4.1.5.18. 2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)acetamide (M18)



White powder, mp 225-227 ℃, 657mg, 93%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.55 (s, 3H), 2.99 (s, 8H), 3.91 (s, 2H), 7.39 (d, J = 8.6 Hz, 2H), 7.65 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 8.6 Hz, 2H), 7.78 (d, J = 8.7 Hz, 2H), 11.15 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 35.9, 43.4, 44.3, 52.7, 91.0, 118.8, 119.3, 128.3, 128.8, 129.5, 129.8, 130.6, 135.7, 135.9, 144.1, 166.4, 168.3. MS (ESI) m/z (%): 559.0910 [M+H].

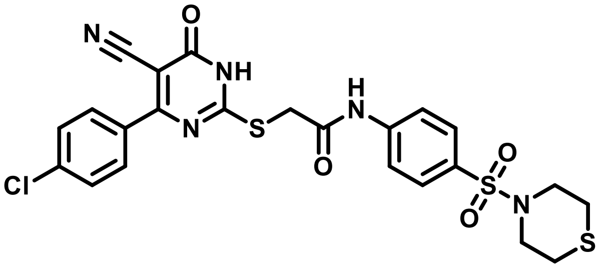
4.1.5.19. 2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(morpholinosulfonyl)phenyl)acetamide (M19)

doi:10.20944/preprints202408.1943.v1



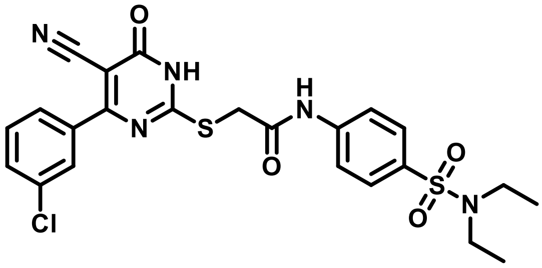
White powder, mp 198-200 ℃, 646 mg, 91%, ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.86 (t, J = 3.8 Hz, 4H), 3.64 (t, J = 4.7 Hz, 4H), 4.21 (s, 2H), 7.36 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.7 Hz, 2H), 10.80 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 36.2, 46.4, 65.8, 93.7, 116.2, 119.3, 128.8, 128.9, 129.5, 131.0, 134.4, 136.9, 143.6, 166.3, 166.6, 166.7. MS (ESI) m/z (%): 546.0620 [M+H].

4.1.5.20. 2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(thiomorpholinosulfonyl)phenyl)acetamide (M20)



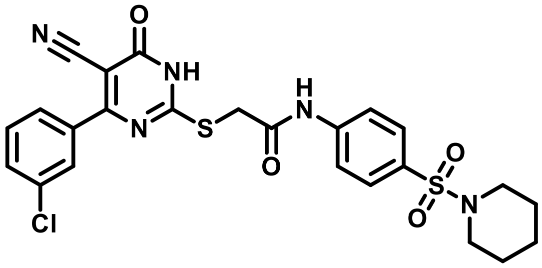
White powder, mp 210-214 ℃, 686 mg, 94%, ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.66 (t, J = 4.7, 4.3 Hz, 4H), 3.19 (t, J = 4.5 Hz, 4H), 4.21 (s, 2H), 7.34 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H), 7.85 (d, J = 8.6 Hz, 2H), 10.76 (s, 1H). ^{13}CNMR (101 MHz, DMSO δ 26.9, 36.2, 48.3, 93.8, 116.0, 119.3, 128.9, 129.0, 130.5, 131.0, 134.3, 137.0, 143.5, 161.4, 166.3, 166.6. MS (ESI) m/z (%): 562.0420 [M+H].

4.1.5.21. 2-((4-(3-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(N,N-diethylsulfamoyl)phenyl)acetamide (M21)



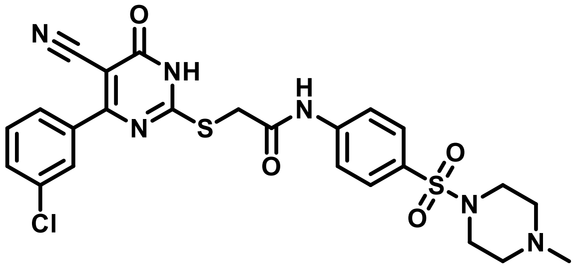
White powder, mp 255-258 ℃, 608 mg, 88%,^{1}H NMR (400 MHz, DMSO-d6) δ 1.02 (t, J = 7.1 Hz, 6H), 3.13 (q, J = 7.1 Hz, 4H), 4.20 (s, 2H), 7.36 (t, J = 7.9 Hz, 1H), 7.55 (d, J = 7.4 Hz, 1H), 7.72 (d, J = 4.7 Hz, 4H), 7.76 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 2.5 Hz, 1H), 10.71 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 14.4, 36.1, 42.2, 94.3, 115.9, 119.5, 127.8, 128.4, 128.6, 130.7, 131.8, 133.8, 134.4, 137.5, 142.8, 161.4, 166.2, 166.3, 166.6. MS (ESI) m/z (%): 532.0350 [M+H].

4.1.5.22. 2-((4-(3-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(piperidin-1-ylsulfonyl)phenyl)acetamide (M22)



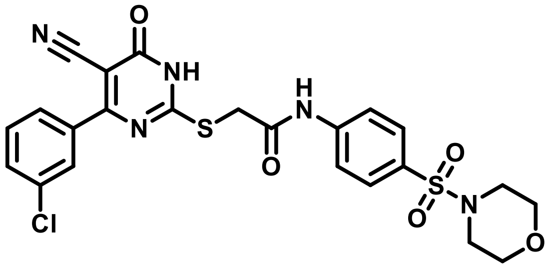
White powder, mp 262-265 °C, 629 mg, 89%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (p, 2H), 1.54 (p, 4H), 2.84 (t, J = 5.0 Hz, 4H), 4.21 (s, 2H), 7.38 (t, J = 8.0 Hz, 1H), 7.55 (d, J = 7.9 Hz, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.78 (dd, 4H), 10.75 (s, 1H).. ^{13}CNMR (101 MHz, DMSO) δ 23.4, 25.1, 36.1, 47.1, 94.3, 116.0, 119.3, 127.8, 128.6, 129.1, 130.0, 130.7, 131.8, 133.7, 137.6, 143.3, 166.2, 166.4, 166.7. MS (ESI) m/z (%): 544.0950 [M+H].

4.1.5.23. 2-((4-(3-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)acetamide (M23)



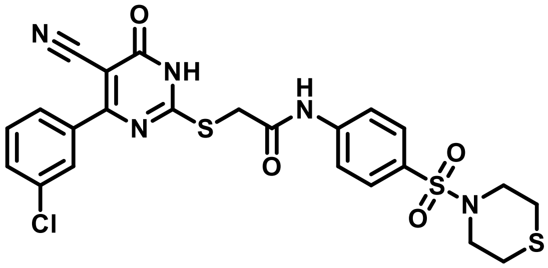
White powder, mp 242-245 °C, 617 mg, 85%,^{1}H NMR (400 MHz, DMSO-d6) δ 2.67 (s, 3H), 3.11 (s, 8H), 4.01 (s, 2H), 7.44 (t, J = 7.9 Hz, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.75 (dd, 4H), 7.87 (d, J = 8.8 Hz, 2H), 11.19 (s, 1H).^{13}C NMR (101 MHz, DMSO) δ 35.9, 43.1, 44.1, 52.5, 91.5, 118.5, 119.4, 127.5, 128.3, 128.4, 129.5, 130.6, 130.7, 133.5, 139.1, 144.1, 166.1, 168.1, 170.3. MS (ESI) m/z (%): 559.0840 [M+H].

4.1.5.24. 2-((4-(3-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(morpholinosulfonyl)phenyl)acetamide (M24)



White powder, mp 261-264 °C, 582 mg, 82%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.76 (t, J = 4.4 Hz, 4H), 3.57 (t, J = 5.9, 3.5 Hz, 4H), 4.16 (s, 2H), 7.29 (t, J = 7.9 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 4.8 Hz, 2H), 7.74 (d, J = 6.6 Hz, 2H), 10.70 (s, 1H), 13.96 (s, 1H).^{13}CNMR (101 MHz, DMSO) δ 36.2, 46.4, 65.8, 94.4, 115.9, 119.4, 127.8, 128.6, 128.8, 129.4, 130.7, 131.8, 133.8, 137.5, 143.6, 161.3, 166.2, 166.4, 166.6. MS (ESI) m/z (%): 546.0240 [M+H].

4.1.5.25. 2-((4-(3-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(thiomorpholinosulfonyl)phenyl)acetamide (M25)



White powder, mp 245-247 ^{o}C, 613 mg, 84%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.67 (t, J = 6.2 Hz, 4H), 3.18 (t, J = 4.1 Hz, 4H), 4.20 (s, 2H), 7.36 (t, J = 7.9 Hz, 1H), 7.57 (dd, J = 8.3, 2.1 Hz, 1H), 7.68 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 4.5 Hz, 2H), 10.83 (s, 1H). ^{13}CNMR (101 MHz, DMSO) δ 26.9, 48.3, MS (ESI) m/z (%):574.0240 [M+H].116.3, 119.4, 127.8, 128.6, 129.0, 130.4, 130.6, 131.7, 133.7, 137.8, 143.5, 166.2, 166.6. MS (ESI) m/z (%): 562.0910 [M+H].

4.2. Biological Evaluation

4.2.1. Materials

This study utilized 25 chemical compounds labeled **M1** to **M25**. All compounds were tested at a concentration of 3000 µg/mL.

4.2.2. Bacterial Strains

The antibacterial activity of the compounds was assessed against a panel of bacterial strains. More details are presented in the file of Supplementary information.

4.2.3. Agar Diffusion-Based Screening of Antimicrobial Activity

The antibacterial screening was performed using the agar diffusion method[49]. More details are presented in the file of Supplementary information.

4.2.4. Measurement of Inhibition Zones

The antibacterial activity was evaluated by measuring the diameter of the inhibition zones in millimeters (mm) around each well. More details are presented in the file of Supplementary information.

4.2.5. Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined by macro dilution method [39,40]. More details are presented in the file of Supplementary information.

4.2.6. Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined as [40] following the MIC assay by subculturing 100 µL from each well that showed no visible growth onto fresh agar plates. The plates were incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of the compound that resulted in a 99.9% reduction in the initial bacterial inoculum. More details are presented in the file of Supplementary information.

4.2.7. Antibiofilm Assay of the Selected Compounds by Tissue Culture Plate Method (TCP):

Inhibition of the initial adherence by *Klebsiella pneumonia* and *Pseudomonas aeruginosa* was by the selected compounds was assessed according to the reported references [41,42]. More details are presented in the file of Supplementary information.

4.3. Physicochemical Properties and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) Studies

The compounds were prepared in sdf format and Data Warrior software was used to calculate the physicochemical properties. While pkCSM platform was used to calculate the ADMET properties using smiles of the compounds as input [45–48].

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability: Data will be made available on request. S.S.A., M.M.A., Y.M.S., H.M.A., A.N.E., M.K.A., S.H.A.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

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