J. Pharm. Appl. Chem., **1**, No. 1, 27-35 (2015)

Journal of Pharmaceutical and Applied Chemistry
An International Journal

http://dx.doi.org/10.12785/jpac/010104

Synthesis, Antimicrobial Evaluation and Docking Study of Novel Heterocyclic Compounds Bearing a Biologically Active Sulfonamide Moiety

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Received: 18 Mar. 2015, Revised: 6 Apr. 2015, Accepted: 19 Apr. 2015.

Abstract: In the present work, we aimed to synthesize and evaluate *in vitro* antibacterial and antifungal activities of new heterocyclic compounds bearing sulfadiazine moiety. Among the synthesized compounds, arylidine (**3f**) displayed significant antibacterial activity against *S.pneumoniae* (IC$_{\mathbf{50}}$, 22.46µg/mL, comparable to ampicillin IC$_{50}$ value, 22.76 µg/mL),whereas, 2-pyridone (**4f**)showed the highest antifungal activity against *G.candidum* (IC$_{\mathbf{50}}$,8.63µg/mL, comparable to amphotericin B,IC$_{50}$, 11.63 µg/mL).Its antibacterial activity against *S.pneumoniae* was (IC$_{\mathbf{50}}$,13.84 µg/mL, comparable to ampicillin, IC$_{50}$, 22.76µg/mL) and *E.coli* (IC$_{\mathbf{50}}$,29.89 µg/mL, comparable to gentamycin ,IC$_{50}$,29.42µg/mL) respectively.On the other hand 2-imino chromene (**5a**) displayed significant antibacterial activities against *S.pneumoniae* (IC$_{\mathbf{50}}$,19.84µg/mL, comparable to ampicillin , IC$_{50}$, 22.76 µg/mL) and exhibited antifungal activity against *G.candidum* (IC$_{\mathbf{50}}$,12.63 µg/mL, comparable to amphotericin B, IC$_{50}$, 11.63 µg/mL) respectively.In general,all of the synthesized compounds exhibited better antimicrobial activities than sulfadiazine. Molecular docking studies indicated that the newly synthesized compounds could occupy both *p*-aminobenzoic acid (PABA) and pterin binding pockets of the dihydropteroate synthase (DHPS), suggesting that the target compounds could act by the inhibition of microbial DHPS enzyme.These derivatives contain sulfonamide moiety as well as heterocyclic moiety that increase the lipophilic characters of the synthesized compounds hence enhance its absorption**.**

Keywords: *Antimicrobial agents, Molecular docking, Sulfonamide and Pyridone.*

1 Introduction

Cyanoacetanilides are important and versatile reagents, which have been especially used for the synthesis of polyfunctionalized heterocycles[1-3].Heterocyclic compounds bearing sulfonamide moiety possess diverse biological activities including antibacterial[4], carbonic anhydrase inhibitor[5],insulin release inducer[6], antiviral[7],antifungal[8], anticancer[9],and anti-inflammatory activities[10]. Sulfonamides, which act as competitive inhibitors of PABA substrate for the DHPS enzyme active site, inhibit the biosynthesis of dihydrofolic acid [11]. DHPS facilitates the biosynthesis of the folate intermediate 7,8-dihydropteroic acid from PABA and dihydropterin-6-hydroxymethyl pyrophosphate (DHPP).As DHPS participates in the de novo synthesis of folate cofactors by catalyzing the formation of 7,8-dihydropteroate from condensation of *p*-aminobenzoic acid with 6-hydroxymethyl-7,8-dihydropteroate pyrophosphate[12], inhibition of this enzyme by sulfonamides prevents bacterial growth and cell division[13].Considering sulfadiazine derivatives that act as PABA competitive inhibitor[14], our target was to substitute its primary amino group by antimicrobial pharmacophores in order to occupy both the PABA and pterin binding pockets for the DHPS enzyme, to improve their antimicrobial activity. Pyridone and chromene derivatives are important scaffolds in pharmacologically active compounds and exhibit promising antimicrobial activities[15,16]. Based on those facts, new pyridone and chromene derivatives tagged with sulfadiazine moiety, were synthesized (Figure1)

The new compounds were evaluated *in vitro* for their antibacterial activity against *S.pneumoniae* and *B. subtilis* as examples of Gram-positive bacteria, *E. coli* and *P. aeruginosa* as examples of Gram-negative bacteria, while the antifungal activity was evaluated against *A. niger* and *G. candidumm*. Molecular docking studies were used to rationalize the collected biological results**.**

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2 Results and Discussion

2.1 Chemistry

Cyanoacetamide derivatives are of great importance, as they contribute to build polyfunctional molecules [17, 18] with diverse pharmacological activities [4-10]. Based on that, our aim was to synthesize arylidene, 2-pyridone and chromene derivatives starting from sulfadiazine. The reaction of aryl amines with ethylcyanoacetate in *m*-xylene is well known to constitute one of the most widely used synthetic methods [19].Reaction of 4-amino-*N*-(pyrimidin-2yl)benzenesulfonamide (**1**) with ethyl 2-cyanoacetate in *m*-xylene afforded 2-cyano-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)acetamide(**2**)**.**Thus, the Knoevengel condensation of the cyanoacetanilide (**2**) with aromatic Aldehydes furnished the corresponding arylidene derivatives(**3a-g**)[20]**.**The chemical structure of (**3a**) was established by IR spectrum that revealed absorption bands at 1686, 2212, 3347 and 3431cm^{-1} corresponding to carbonyl, nitrile and two NH groups, respectively. Its ^{1}H-NMR spectrum has signals at δ 7.03 due to CH of pyrimidine, 8.29, 10.73 and 11.70 due to CH and two NH protons, 8.49 due to two CH of pyrmidine, in addition to two aromatic protons appear at δ 7.59–8.15.

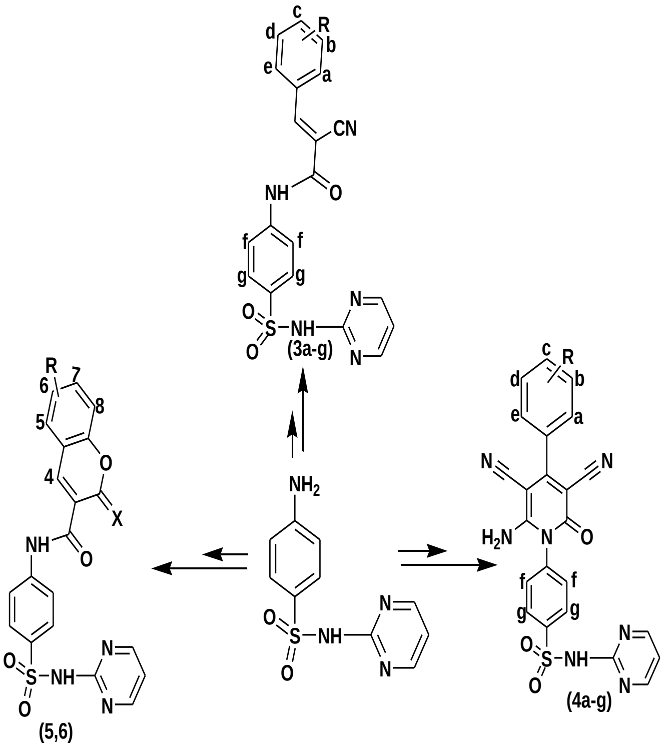
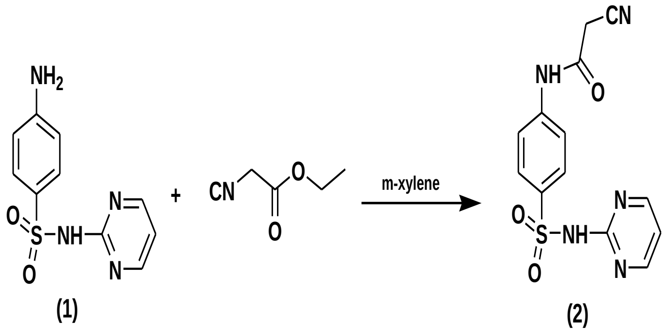


Figure (1): Modification of sulfadiazine moiety.



Scheme (1): Synthesis of cyanoacetanilide (**2**).

Pyridin-2(1*H*)-one derivatives (**4a-g**) were obtained through the reaction of the arylidene derivatives (**3a-g)** and malononitrile in dioxan using triethylamine (TEA) as a catalyst[20]. One-pot reactions of the cyanoacetanilide (**2**), malononitrile and the same aldehydes (1:1:1 molar ratio) under reflux condition in the presence of TEA, afforded the same 2-pyridone derivatives (**4a-g**) [20] in high yield. The IR spectrum of compound (**4d**), taken as a typical example of this series, revealed absorption bands at 1679, 2216,3215 and 3317cm^{-1}, corresponding to carbonyl, nitrile, NH and broad NH_2 groups , respectively. Its ^{1}H-NMR spectrum displayed signals at δ 3.83 due to $-OCH_3$ protons, 7.09 due to CH of pyrimidine and 8.52 due to two CH of pyrimidine. Its mass spectrum displayed a molecular ion peak at m/z 499.

While cyclocondensation of cyanoacetanilide (**2**) with salicylaldehyde derivatives in ethanol, in the presence of ammonium acetate, afforded 2-iminochromene (**5a**, **b**) in high yields. On the other hand, allowing (**2**) to react with salicylaldehyde derivatives in the presence of AcOH/AcONa, afforded chromenones (**6a**,**b**) in good yields[21,22]. The IR spectrum of the product (**5a**) revealed the disappearance of cyano absorption band and showed absorption bands at 1683, 3084, 3329 and 3434 cm^{-1} corresponding to carbonyl and three NH groups, respectively. Its ^{1}H-NMR spectrum displayed signals at δ7.03 due to CH of pyrimidine, 8.55 due to ArH_4, in addition to three NH exchangeable protons at δ 6.76, 9.27, and 13.14.Its mass spectrum established a molecular ion peak at m/z 421.

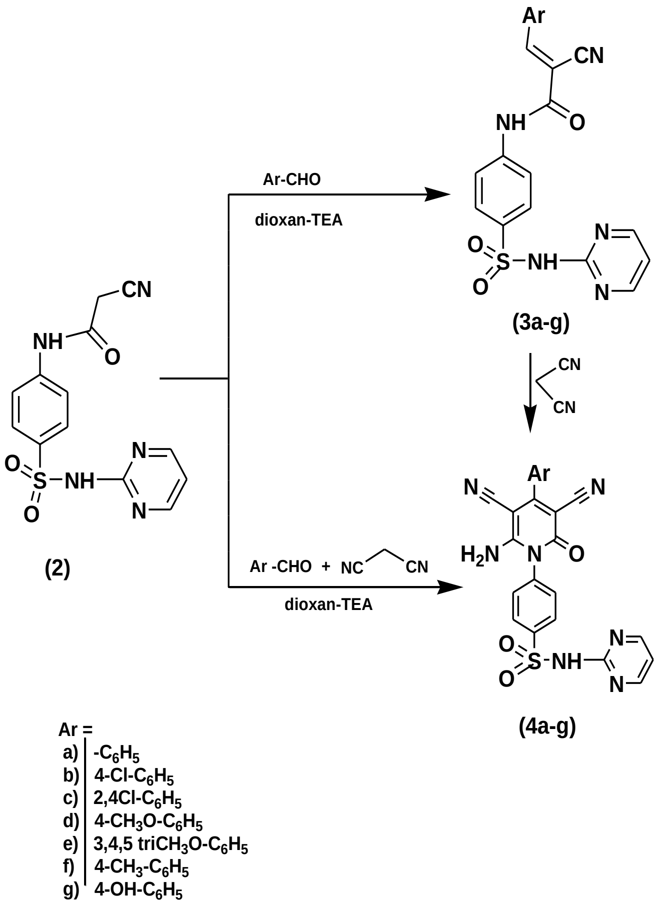
The IR spectrum of the product (**6a**) revealed the disappearance of cyano absorption band and established absorption bands at 1668,1700, 3213 and 3254 cm^{-1} corresponding to CO, COO and two NH groups, respectively. The ^{1}H-NMR spectrum of (**6a**) showed 7.03 due to NH of pyrimidine, 8.89 due to ArH_4 and two NH exchangeable proton at δ 10.90, 11.73.

2.2 Antimicrobial evaluation

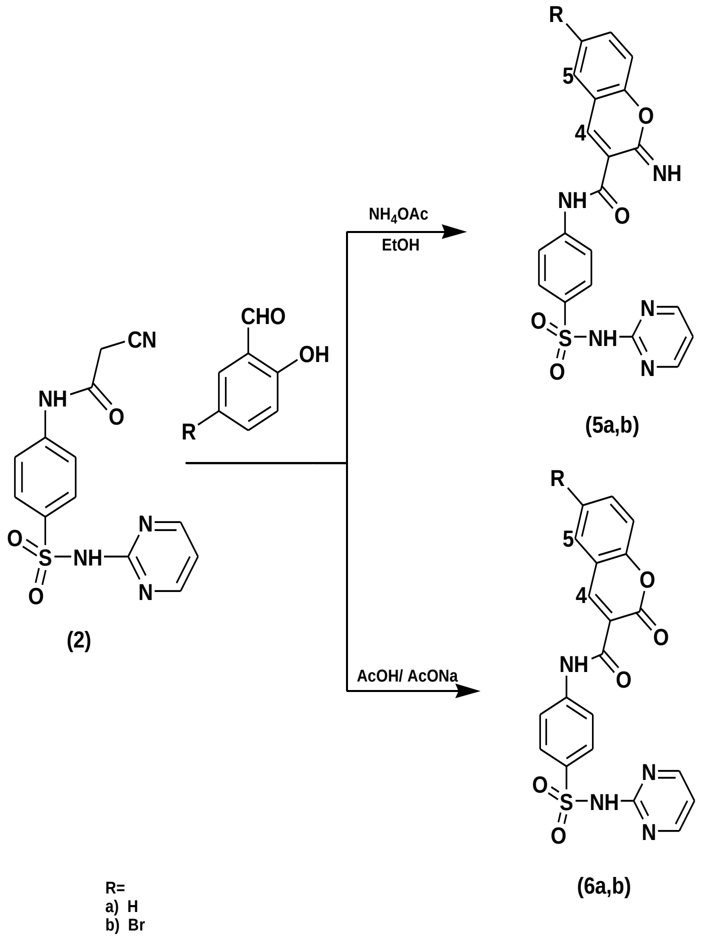
The antimicrobial screening, Minimal inhibitory concentrations(MIC) and Inhibitory concentration 50(IC_{50})of the tested compounds were carried out at the

Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. The newly synthesized target compounds were evaluated for their *in vitro* antimicrobial activities against the human pathogens *Streptococcus pneumoniae* (RCMB-010010), *Bacillis subtilis* (RCMB 010067) as examples of Gram-positive bacteria and *Escherichia coli*(EC) (RCMB 010052) and *Pseudomonas aeruginosa* (RCMB-010043) (PA) as examples of Gram-negative bacteria. They were also evaluated for their antifungal activities against *Aspergillus niger* (RCMB 002007), and *Geotricum candidum* (RCMB 05096).

Micro-titer plate technique was used for the preliminary screening of the antibacterial and antifungal activities. Ampicillin, gentamycin, amphotericin B and sulfadiazine were used as reference drugs. The results were recorded for each tested compound as %inhibition ± SD and summarized in (Table1). (MIC) and (IC_{50} values) were determined for compounds showed significant % inhibition and the results were summarized in (Table 2).



Scheme (2)**:** Synthesis of arylidenes (**3a-g)** and pyridines (**4a-g**).



Scheme (**3**)**:** Synthesis of 2-imino-2*H*-chromene (**5a**,**b**) and 2-oxo-2*H*-chromene(**6a**,**b**).

Most of the newly target compounds appear to have moderate to good activity against all tested microorganisms except *p.aeruginosa*. The most active compound was 2-pyridone (**4f**) as it showed two folds the activity of sulfadiazine against Gram (+ve) bacteria, three folds the activity of sulfadiazine against *E. coli* and five folds the activity of sulfadiazine against fungi (Table1)**.** The pyridone (**4f**) displayed significant antibacterial and antifungal activities. It was much more active than ampicillin against *S.pneumoniae* (IC_{50}µg/mL,13.84µg/mL against 22.76µg/mL), more active than amphotericin B against *G.candidum* (IC_{50} µg/mL, 8.63µg/mL against 11.63µg/mL) and equipotent to gentamicin against *E.coli* (IC_{50} µg/mL, 29.89µg/mL against 29.42µg/mL).

Table (1): Mean of inhibitory % ±standard deviation produced on arrange of clinically pathogenic fungi.

Compds	Gram (+ve) bacteria		Gram (-ve) bacteria		Fungi	
	S.pneumoniae	B. subtilis	E. coli	P.aeruginosa	A.niger	G.candidum
2	11.64±1.2	16.52±0.58	10.61±0.44	NA[a]	12.63±0.58	14.21±1.2
3a	26.85±1.2	43.28±0.58	13.65±0.44	NA	19.32±0.58	23.52±1.2
3b	44.62±0.63	57.95±0.44	32.15±0.58	NA	28.32±0.72	36.25±1.2
3C	23.45±1.2	43.28±0.58	14.32±0.44	NA	14..32±0.58	17.85±1.2
3d	72.14±0.63	79.34±0.58	57.62±0.25	NA	57.26±0.58	67.42±0.72
3e	64.38±0.63	72.62±0.63	51.32±0.58	NA	45.23±0.45	56.34±0.63
3f	86.23±1.2	85.43±0.58	68.34±0.63	NA	64.63±0.63	76.32±0.72
3g	57.62±0.63	63.21±0.63	40.35±0.58	NA	33.65±1.2	46.32±0.72
4a	20.63±0.63	28.14±0.58	19.25±0.58	NA	26.43±0.58	20.63±0.63
4b	32.4±0.63	46.34±0.25	4.62±0.72	NA	13.44±0.72	18.3±0.58
4c	12.63±0.63	14.52±0.25	15.63±0.37	NA	13.44±0.72	15.63±0.58
4d	36.32±0.63	47.32±0.72	15.32±-.63	NA	11.32±0.63	26.32±0.63
4e	18.6±0.63	28.14±0.58	23.64±0.63	NA	16.43±0.63	20.63±0.63
4f	92.35±0.63	97.31±0.44	76.32±0.58	NA	89.32±0.63	96.32±0.37
4g	36.52±0.37	42.62±0.63	19.63±0.42	NA	18.32±0.72	22.76±0.37
5a	88.63±0.72	89.52±0.25	69.32±0.72	NA	83.44±0.72	92.63±0.58
5b	18.42±0.63	18.62±0.44	11.45±0.63	NA	16.42±0.72	18.42±0.63
6a	39.62±0.63	50.31±0.63	19.63±0.58	NA	16.35±0.72	19.65±0.63
6b	20.85±1.2	27.62±0.58	10.63±0.44	NA	13.25±0.58	18.63±1.2
Sulfadiazine	40.52±1.2	53.62±0.58	22.32±0.44	NA	16.35±0.58	20.68±1.2
Gentamicin	NT	NT	75.42±0.58	72.21±0.58	NT	NT
Ampicillin	86.23±0.58	99.62±0.63	NT[b]	NT	NT	NT
Amphotericin B	NT	NT	NT	NT	90.31±0.58	95.21±0.44

[a] NA, No obsereved activity.

[b] NT, Not tested.

On the other hand, chromene derivatives show moderate activity against Gram (+ve) bacteria, *E.coli* and fungi, it was observed that 2-imino chromene (**5a**) demonstrated two folds the activity of sulfadiazine against Gram (+ve) bacteria, three folds the activity of sulfadiazine against *E. coli* and five folds the activity of sulfadiazine against fungi (Table1)**.** 2-Imino chromene (**5a**) was much more active than ampicillin against *S. pneumonia*e (IC_{50}μg/mL,19.84μg/mL against 22.76μg/mL),and equipotent to amphotericin B against *G. candidum* (IC_{50} μg/mL, 12.63μg/mL against 11.63μg/mL).

Some of the arylidine derivatives (**3a-g**) displayed significant antibacterial activities. It was observed that (**3d**,**3e** and **3f**) were the most potent among their series. They possess two folds the activity of sulfadiazine against Gram-positive bacteria and three folds the activity of sulfadiazine against E.*Coli* and fungi. Interestingly, the arylidine (**3f**) was equipotent to ampicillin against *S.pneumoniae* (IC_{50}μg/mL, 22.46μg/mL against 22.76μg/mL).

3 Molecular modeling

Docking simulations were performed using the crystal structure of DHPS from *B. anthracis* (BaDHPS, PDB code: 3TYE)[23,24] bound to the covalent adduct of STZ-DHPP. In general, MOE docking showed that all the studied compounds can interact with BaDHPS in a manner similar to that observed for the STZ-DHPP covalent adduct in the solved crystal structure.Docking study for the arylidine, pyridone and chromene derivatives showed that most of these compound can occupy both *p*-aminobenzoic acid (**Ser221**) and pterin binding pocket (**Asp101**, **Asn120**and **lys220**) with low energy score.

In the pyridone derivatives, the most active compound (**4f**) made a potential hydrogen bond between nitrogen of cyano and the backbone OH group of **Ser221**.Additionally, the oxygen group of sulfonamide moiety forms a hydrogen bond with **Arg254** and arene-cation interaction between benzene sulfonamide and the side chain of **Lys220** with energy score **-10.92**(Figure2).

Table (2): Minimal inhibitory concentrations MIC, μg/mL(IC_{50},μg/mL between brackets) of some new synthesized compounds.

Compds	S.pneumoniae	B. subtilis	E. coli	A.niger	G. candidum
3d	7.81(37.24)	3.9(32.15)	15.63(64.32)	15.63(63.42)	7.81(46.32)
3e	15.63(52.14)	7.81(36.24)	31.25(76.32)	31.25(82.31)	15.63(67.81)
3f	1.95(22.46)	1.95(21.32)	7.81(43.25)	15.63(52.34)	3.9(31.25)
4f	1.95(13.84)	0.98(6.32)	3.9(29.89)	1.95(19.84)	0.98(8.63)
5a	1.95(19.84)	1.95(18.68)	7.81(46.32)	3.9(23.84)	1.95(12.63)
Sulfadiazine	62.5(>125)	31.25(69.24)	125(>125)	125(>125)	125(>125)
Amphotericin B	[a]NT	NT	NT	1.95(22.76)	0.98(4.35)
Gentamicin	NT	NT	7.81(29.42)	NT	NT
Ampicillin	1.95(22.76)	0.98(4.35)	NT	NT	NT

[a] NT, Not tested.

In chromene derivatives, the most active one was (**5a**) formed a possible hydrogen bond between nitrogen of pyrimidine and the backbone NH of **Lys 220**. Moreover, the oxygen group of sulfonamide moiety formed a possible hydrogen bond with NH backbone of **Arg254**, on the other hand arene-cation interaction was established with **Lys220**, **Arg 234** and **Arg 68** with energy score **-10.66**(Figure2).

In the arylidine derivatives, for instance,compound (**3e**) the benzene-sulfonamide moiety occupies virtually the same position observed in the STZ-DHPP-BaDHPS complex; thereby establishing the characteristic hydrogen bond between its sulfonamide oxygen and the backbone NH group of **Ser221** ,additionally two hydrogen bond between oxygen of arylidine and methoxy and the backbone NH group of **Lys220** and **ASn120** respectively with energy score **-9.93**(Figure2).

4 Structure activity relationship (SAR)

It was observed that most of the synthesized *N*-substituted sulfadiazine derivatives have superior antimicrobial activities over sulfadiazine itself. In pyridone derivatives (**4a-g**) the presence of the electron withdrawing chlorine atom results in a moderate antimicrobial activity. When the electronegativity increased (i.e.2,4-dichloro) resulted in further decrease antimicrobial activity. On the other hand, the presence of the electron donating group (-CH3) significantly increased the antimicrobial activity.

In the case of chromene derivatives (Scheme2), 2-imino-2*H*-chromene was found to be more potent than 2-oxo-2*H*-chromene as antimicrobial; substitution of chromene with electronegative group (Br) resulted in decrease of the antimicrobial activity.

Regarding the arylidines series (**3a-g**) (Scheme1)**,** the presence of the electron withdrawing chlorine atom in the acrylamide results in a moderate antimicrobial activity, increasing the electronegativity (i.e.2, 4-dichloro) results in further decrease antimicrobial activity. Presence of the electron donating atom in the acrylamide increases antimicrobial activity of these compounds.

5 Experimental

5.1 Chemistry

Melting points were measured in open capillary tubes using Stuart apparatus and are uncorrected. Elemental microanalysis was carried out at reginal centre for mycology and biotechnology, Al-Azhar University, Cairo, Egypt. The infrared (IR) spectra were recorded using potassium bromide disc technique on a FT IR spectrophotometer at Main Defence Chemical laboratory, Cairo University, Ain Shams University and Al-Azhar University. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were performed on abrucker-300 NMR spectrophotometer 300 MHz at Faculty of (Science, Pharmacy) Cairo University and Main Defence Chemical laboratory. DMSO-d$_6$ was used as a solvent, and the chemical shifts were measured in ppm, relative to TMS as an internal standard. Mass spectra were recorded on a DI-50 unit of Shimadzu GC/ MS-QP 5050A spectrometer at the Regional Centre for Mycology and Biotechnology, Al-Azhar University, and a Hewlett Packard 5988 Spectrometer at Micro analytical Unit, Cairo University. All reactions were monitored by TLC using precoated Aluminium sheets silica gel Merck 60 F254 and were visualized by UV lamp.

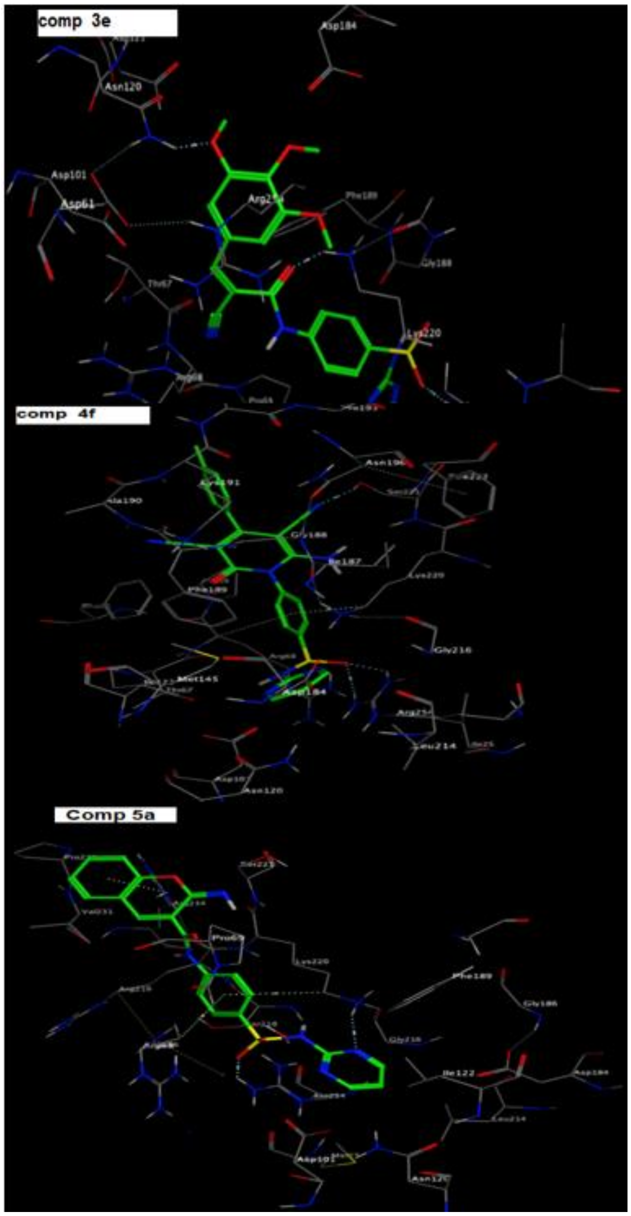


Figure (2)**:** 3D representation of docking of compounds **3e**, **4f** and **5a** in to the active site of 3TYE. Compound **3e** shows three hydrogen bonds interaction with **Ser221**, **Lys220** and **Asn120**.Compound **4f** shows two hydrogen bond interaction with **Ser221** and **Arg254**, in addition to arene-cation interaction between benzene sulfonamide and the side chain of **Lys220**. Compound **5a** shows two hydrogen bond interaction with **Lys220** and **Arg254** and and arene-cation interaction with **Lys220**, **Arg 234** and **Arg 68**. O atoms are colored red, N atoms colored blue and C atoms colored green.

*2-Cyano-N-(4-(N-(pyrimidin-2-yl)sulfamoyl) phenyl) acetamide (**2**)*

Equimolar amounts of sulfadiazine and ethylcyanoacetate, was heated to reflux, in *m*-xylene for 1h, followed by concentration and cooling of mixture. The obtained product was filtered off and recrystallized from acetic acid to afford the titled compound as **Yellow powder** in 64 %yield ,mp180ºC; IR(KBr): υ_{max}./cm^{-1} 3429, 3329 (2NH),2205(CN),1701(C=O);^{1}H-NMR(DMSO-d_6): δ3.93 (s,2H,CH_2),7.02(t,1H,CH-pyrimidine) , 7.69 (dd,2H, *J*=9Hz, $ArH_{\mathbf{g}}$) , 7.93(dd,2H, *J*=9Hz ,$ArH_{\mathbf{f}}$), 8.48 (d,2H,2CH-pyrimidine),10.64(s,1H,NH,D_2O-exchangeable) ,11.68 (s,1H,NH, D_2O-exchangeable).Anal.Calcd for $C_{13}H_{11}N_5$ O_3S(317) C,49.21; H,3.49;N,22.07,found C, 49.48 ; H, 3.53 ; N, 22.19.

*3-Aryl-2-cyano-N-(4-(N-(pyrimidin-2-yl)sulfamoyl) phenyl) acrylamide derivatives (**3a-g**).*

General procedure

To a solution of cyanoacetanilide (**2**) (1mmol) and appropriate aromatic aldehydes (1mmol) in dioxan (20mL),was added few drops of TEA and the reaction mixture was refluxed for 8-10 hours. The solid product so formed was filtered off , washed with EtOH and then recrystallized from DMF to give (**3a**-**g**).

3-Phenyl-2-cyano-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl) phenyl) acrylamide (**3a**).**Yellowish brown powder** in 30% yield, mp 270ºC;IR (KBr) :υ_{max}./cm^{-1} 3431, 3347(2NH) , 2212 (CN),1686 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.03 (t, 1H,CH -pyrimidine), 7.59-8.15 (m,9H,ArH) , 8.29 (s,1H, Olefinic H) , 8.49 (d,2H,2CH- pyrimidine), 10.73 (s,1H,NH, D_2O-exchangeable) ,11.70 (s,1H,NH, D_2o-exchangeable).Anal. Calcd for $C_{20}H_{15}N_5O_3S$ (405) C,59.25 ;H, 3.73 ;N,17.27, found C,59.36 ;H,3.78;N,17.42.

3-(4-Chlorophenyl)-2-cyano-*N*-(4-(*N*-(pyrimidin-2- yl) sulfamoyl)phenyl)acrylamide(**3b**).**Brown powder** in 51% yield, mp 290ºC; IR (KBr):υ_{max}./cm^{-1} 3302,3190 (2NH), 2223 (CN) ,1681(C=O);^{1}HNMR(DMSO-d_6):δ 7.04 (t,1H,CH-pyrimidine), 7.67 (dd,2H, *J*= 9Hz,$ArH_{\mathbf{b+d}}$),7.82 (dd,2H, *J*= 9 H z ,$ArH_{\mathbf{a+e}}$), 7.97-8.00 (m,4H , $ArH_{\mathbf{f+g}}$), 8.29 (s, 1H, Olefinic H) ,8.48 (d,2H,2CH -pyrimidine), 10.75 (s,1H, NH, D_2O-exchangeable),11.72(s,1H,NH,D_2O-exchangeable) ; Anal. Calcd for $C_{20}H_{14}ClN_5O_3S$ (439) C,54.61; H,3.21; N,15.92,found C, 54.74; H, 3.20; N,16.09.

3-(2,4-Dichlorophenyl)-2-cyano-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)acrylamide(**3c**).**Brown powder** in 30% yield, mp 260ºC;IR (KBr):υ_{max}./cm^{-1} 3428,3332(2NH), 2203 (CN),1640(C=O); ^{1}HNMR(DMSO-d_6):δ6.39 (s,1H, NH,D_2O-exchangeable) ,7.08(t,1H,CH-pyrimidine),7.25-7.89 (m,7H, ArH) ,8.40(s,1H,Olefinic H) ,8.50 (d,2H,2CH-pyrimidine),10.71(s,1H,NH, D_2O exchangeable). MS m/z: 473 (M^+); Anal. Calcd for $C_{20}H_{13}C_{l2}N_5O_3S$(473) C,50.64;H,2.76;N,14.77 ,found C,50.81;H,2.79;N,14.89.

3-(4-Methoxyphenyl)-2-cyano-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)acrylamide(**3d**). **Yellowish brown powder** in 50%yield,mp 285ºC; IR (KBr):υ_{max}./cm^{-1} 3409, 3338 (2NH) , 2213 (CN), 1687(C=O); ^{1}H-NMR(DMSO-d_6):δ 3.87(s,3H,OCH_3), 7.02(t,1H, CH pyrimidine),7.15(dd,2H, *J*=9Hz,$ArH_{\mathbf{b+d}}$),7.82(dd,2H,*J*=9Hz, $ArH_{\mathbf{a+e}}$),7.95-8.02 (m,4H,$ArH_{\mathbf{f+g}}$) , 8.22(s,1H,Olefinic H), 8.50 (d,2H,2CH-pyrimidine), 10.60(s,1H, NH, D_2O-exchangeable) ,11.72(s,1H, NH, D_2O-exchangeable). MS m/z:436 (M^++1) .Anal. Calcd for $C_{21}H_{17}N_5O_4S$(435) C,57.92; H,3.93;N,16.08,found C, 58.03; H, 3.97 ; N, 16.2.

3-(3,4,5-Trimethoxyphenyl)-2-cyano-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)acrylamide(**3e**). **Brown powder** in 50% yield, mp 280 ºC; IR (KBr):υ_{max}. /cm^{-1} 3404, 3327(2NH), 2208(CN), 1694(C=O); ^{1}H-NMR (DMSO-d_6): δ 3.78 (s,3H,OCH_3), 3.83(s,6H, 2OCH_3), 7.02(t,1H,CH-pyrimidine),7.40(s,2H,$ArH_{\mathbf{a+e}}$) ,7.82(dd,2H, *J*=9Hz,$ArH_{\mathbf{g}}$),7.96 (dd,2H, *J*=9Hz,$ArH_{\mathbf{f}}$), 8.23(s,1H,Olefinic H), 8.49(d,2H,2CH-pyrimidine),10.68(s,1H,NH,D_2O-exchangeable),11.72(s, 1H,NH,D_2o-exchangeable). MS m/z: 494 (M^+-1). Anal .Calcd for $C_{23}H_{21}N_5O_6S$ (495) C,55.75; H,4.27;N,14.13,found C, 55.89; H, 4.36; N, 14.20.

3-(p-Tolyl)-2-cyano-*N*-(4-(*N*-(pyrimidin-2yl)sulf amoyl)phenyl) acrylamide(**3f**).**Brown powder** in 46% yield, mp 260ºC; IR (KBr):υ_{max}./cm^{-1} 3337, 3109 (2NH) ,2215(CN), 1688 (C=O); ^{1}H-NMR (DMSO-d_6):δ2.40(s,3H,CH_3),7.04(t,1H,CH-pyrimidine),7.40-7.99(m,8H,ArH),8.24(s,1H,Olefinic H),8.49(d,2H,2CH-pyrimidine),10.68(s,1H,NH,D_2O-exchangeable),11.78 (s,1H,NH,D_2O-exchangeable). MS m/z: 419 (M^+). Anal. Calcd for $C_{21}H_{17}N_5O_3S$(419) C,60.13 ;H,4.09 ;N,16.70, found C, 60.40; H, 4.18; N, 16.89.

3-(4-Hydroxyphenyl)-2-cyano-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl) phenyl) acrylamide(**3g**).**Dark brown powder** in 53% yield, mp 270ºC;IR (KBr):υ_{max}. /cm^{-1} 3375(OH) ,3338 , 3111 (2NH), 2214(CN) ,1684(C=O); ^{1}H-NMR (DMSO-d_6):δ 6.67(s,1H,OH,D_2O-exchangeable),6.94-7.01(m,3H,CH–pyrimidine+ $ArH_{\mathbf{b+d}}$) ,7.80-7.96(m,6H,ArH),8.15(s,1H,Olefinic H),8.46 (d,2H,2CH-pyrimidine),10.53(s,2H,2NH,D_2O exchangeable).MS m/z:422(M^++1). Anal.Calcd for $C_{20}H_{15}N_5$ O_4S(421) C,57.00;H, 3.59;N,16.62.,found C, 57.19; H, 3.64; N, 16.76.

Synthesis of 4-(6-amino-4-aryl-3,5-dicyano-2-oxopyridin-1(2*H*-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide derivatives(**4a**-**g**).

General procedure

Method A: a mixture of arylidine (**3a-g**) (10 mmol) and malononitrile (0.66 g, 10 mmol) in dioxan (30 mL)containing TEA (0.5 mL) was heated under reflux for 6 h. the precipitate was filtered off, washed with ethanol and then recrystallized from DMF to give (**4a-g**).

Method B: one pot reaction of (**2**)(10 mmol), aldehyde derivatives (10 mmol), and malononitrile(0.66 g, 10 mmol) in dioxan (30 mL) containing few drops of TEA was heated under reflux for 12 h. the precipitate was filtered off, washed with ethanol and then recrystallized from DMF to give (**4a-g**).

4-(6-Amino-4-phenyl-3,5-dicyano-2-oxopyridin-1 (2*H*-yl)-*N*-(pyrimidin-2yl)benzenesulfonamide(**4a**).**Dark brown powder** in 30% yield, mp>300ºC ;IR (KBr):υ_{max}./cm^{-1} 3431, 3339(NH2), 3211(NH),2214 (CN),1626 (C=O); ^{1}HNMR(DMSO-d_6): 7.07 (t, 1H, CH-pyrimidine) ,7.53-8.19 (m,9H, ArH),8.54 (d,2H, 2CH-pyrimidine).

4-(6-Amino-4-(4-chlorophenyl)-3,5-dicyano-2- oxopyridin-1(2*H*-yl)-*N*-(pyrimidin-2y) benzene sulfonamide (**4b**).**Dark brown powder** in 35% yield,mp >300ºC; IR (KBr):υ_{max}./cm^{-1} 3441,3339 (NH_2), 3198 (NH), 2214 (CN),1637(C=O); ^{1}H-NMR (DMSO-d_6): δ 7.09 (t,1H, CH-pyrimidine),7.54-8.20 (m,8H,ArH) , 8.55(d,2H,2CH-pyrimidine). MS m/z : 502(M^+-1) .Anal. Calcd for $C_{23}H_{14}ClN_7O_3S$ (503) C,54.82;H, 2.80;N,19.46,found C, 55.01; H, 2.78 ; N,19.60.

4-(6-Amino-4-(2,4-dichlorophenyl)-3,5-dicyano-2-oxopyridin-1(2*H*-yl)-*N*-(pyrimidin-2 yl)benzenesulfonamide (**4c**).**Brown powder** in 30% yield, mp>300ºC; IR (KBr):υ_{max}. /cm^{-1} 3330,3192(NH_2) ,3100(NH), 2207(CN),1620(C=O); ^{1}H-NMR(DMSO-d_6): δ 7.45-7.96 (m,8H,8ArH) ,8.49(d,2H,2CH-pyrimidine). MS m/z: 539(M^++2). Anal. Calcd for $C_{23}H_{13}Cl_2$ N_7O_3S (537) C, 51.31; H, 2.43; N, 18.21, found C, 51.52; H, 2.41; N, 18.45.

4-(6-Amino-4-(4-methoxyphenyl)-3,5-dicyano-2-oxopyridin-1(2*H*-yl)-*N*-(pyrimidin-2-yl) benzene sulfona mide(**4d**).**Brown powder** in 20% yield, mp>300ºC; IR (KBr):υ_{max}./cm^{-1} 3317 (br,NH2),3215 (NH),2216 (CN) ,1679(C=O); ^{1}H-NMR(DMSO-d_6) : δ 3.85 (s,1H,OCH_3),7.09 (t,1H,CH-pyrimidine) , 7.11(dd,2H, *J*=9Hz ,$ArH_{\mathbf{b+d}}$), 7.48 (dd,2H, *J*=9Hz, ArH $_{\mathbf{a+e}}$) ,7.56 (dd,2H,*J*=9Hz,$ArH_{\mathbf{g}}$), 8.14 (dd,2H, *J*=9Hz,$ArH_{\mathbf{f}}$), 8.52 (d,2H,2CH-pyrimidine).MS m/z: 499(M^+). Anal.Calcd for C_{24} $H_{17}N_7O_4S$(499) C,57.71; H,3.43; N,19.63 ,found C, 57.89; H, 3.45;N, 19.75.

4-(6-Amino-4-(3,4,5-trimethoxyphenyl)-3,5-dicyano-2-oxopyridin-1(2*H*-yl)-*N*-(pyrimidin-2-yl)benzene sulfona mede(**4e**).**Brown powder** in 20% yield, mp > 300 ºC;IR (KBr):υ_{max}./cm^{-1} 3439 (br,NH_2), 3182 (NH),2211(CN), 1660(C=O); ^{1}H-NMR (DMSO-d_6): δ 3.75 (s,3H, OCH_3) , 3.82(s,6H, 2OCH_3),6.86 (s,2H,$ArH_{\mathbf{a+e}}$), 7.09 (t,1H,CH-pyrimidine), 7.56(dd,2H, *J*=9Hz , $ArH_{\mathbf{g}}$) ,8.17(dd,2H,*J*=9Hz, $ArH_{\mathbf{f}}$),8.55(d,2H,2CH-pyrimidine) .MS m/z: 559 (M^+).Anal. Calcd for $C_{26}H_{21}N_7O_6S$ (559) C, 55.81; H, 3.78; N, 17.52, found C, 55.96; H, 3.86; N, 17.70.

4-(6-Amino-4-(p-tolyl)-3,5-dicyano-2-oxopyridin-1(2*H*-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide(**4f**). **Dark brown powder** in 25% yield, mp>300ºC; IR(KBr): υ_{max}./cm^{-1} 3312(br,NH_2), 3208 (NH) ,2216(CN),1674(C=O); ^{1}H-NMR (DMSO-d_6): δ 2.40 (s,3H, CH_3),6.57(t,1H,CH-pyrimidine), 7.35- 7.42 (m,6H,6ArH), 8.03 (d,2H,$ArH_{\mathbf{f}}$), 8.23 (d,2H, 2CH-pyrimidine).MS m/z :483(M^+) .Anal. Calcd for $C_{24}H_{17}N_7O_3S$ (483) C ,59.62;H,3.54;N,20.28,found C, 59.75; H, 3.58; N, 20.42.

4-(6-Amino-4-(4-hydroxyphenyl)-3,5-dicyano-2-oxopyridin-1(2*H*-yl)-*N*-(pyrimidin-2-yl)benzene sulfonamide (**4g**).**Brown powder** in 32% yield, mp>300ºC; IR(KBr): υ_{max}./cm^{-1} 3429(OH),3342, 3222(NH2), 3104 (NH),2207 (CN),1622 (C=O) ;^{1}HNMR(DMSO-d_6): δ3.93 (s,1H,OH,D_2O- exchangeable),7.09 (t, 1H, CH-pyrimidine) ,7.68-8.49 (m,8H, ArH), 9.09 (d,2H, 2CH-pyrimidine). MS m/z: 484(M^+-1). Anal. Calcd for $C_{23}H_{15}N_7$ O_4S (485) C, 56.90; H, 3.11; N, 20.20, found C, 57.07; H, 3.18; N, 20.36.

Synthesis of 2-imino-*N*-(4-(*N*-(pyrimidin-2-yl) sulfamoyl)phenyl)-2*H*-chromene-3-carboxamide derivatives(**5a-b**)

General procedure:

A mixture of compound (**2**) (0.01 mol), salicylaldehyde or 5-bromo-2-hydroxybenzaldehyde (0.01 mol) and anhydrous ammonium acetate (0.02 mol) in ethanol (20 mL) was refluxed for 4 h. the precipitate was filtered off, washed with ethanol and then recrystallized from DMF to give (**5a-b**).

2-Imino-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)-2*H*-chromene-3-carboxamide(**5a**).**Brown powder** in51%yield,mp >300ºC;IR(KBr):υ_{max}./cm^{-1} 3434, 3329 ,3084(3NH), 1683 (C=O) ,1643(C=N) ;^{1}H-NMR (DMSO-d_6): δ 7.03 (t,1H, CH-pyrimidine),7.27-8.07 (m,8H, ArH), 8.49(d,2H,2CH-pyrimidine), 8.55 (s,1H,$ArH_{\mathbf{4}}$) , 6.67,9.27,13.14 (3s,3H, 3NH,D_2O-exchangeable).MS m/z:421(M^+) .Anal. Calcd for $C_{20}H_{15}$ N_5O_4S(421) C,57.00 ;H,3.59 ;N,16.62,found C, 57.23; H, 3.66; N, 16.89.

6-Bromo-2-imino-*N*-(4-(*N*-(pyrimidin-2-yl) sulfamoyl)phenyl)-2*H*-chromene-3-carboxamide(**5b**).**Dark brown powder** in 45% yield, mp>300ºC;IR(KBr):υ_{max}./cm^{-1} 3437,3318, 3092(3NH), 1694(C=O) ,1641(C=N) ;^{1}H-NMR (DMSO-d_6): δ 6.67 (s,1 H, NH, D_2O-exchangeable), 7.02 (t,1H,CH- pyrimidine), 7.19-7.39 (m,2H, $ArH_{\mathbf{7+8}}$), 7.70-8.00 (m,4H,ArH), 8.08 (s,1H, $ArH_{\mathbf{5}}$), 8.38(s,1H, $ArH_{\mathbf{4}}$), 8.48 (d,2H, 2CH-pyrimidine), 9. 40, 13.04(2s, 2H, 2NH, D_2O-exchangeable).MS m/z: 500(M^++1) .Anal. Calcd for $C_{20}H_{14}BrN_5O_4S$ (499) C, 48.01; H, 2.82; N, 14.00, found C, 48.17; H, 2.86; N, 14.21.

Synthesis of 2-oxo-*N*-(4-(*N*-(pyrimidin-2-yl) sulfamoyl) phenyl)-2*H*-chromene-3-carboxamide derivatives (**6a-b**).

General procedure:

A mixture of compound (**2**) (0.01 mol), salicylaldehyde or 5-bromo-2-hydroxybenzaldehyde (0.01 mol) and fused sodium acetate (0.02 mol) in acetic acid (20 mL) was refluxed for 7h. the precipitate obtained was filtered off, washed with ethanol and then recrystallized from DMF to give (**6a**-**b**).

2-Oxo-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl) phenyl)2*H*-chromene-3-carboxamide(**6a**). **Brown powder** in 41%yield,mp>300ºC;IR(KBr):υ_{max}./cm^{-1}3254,3213(2NH), 1700(COO),1668(CO);^{1}H-NMR(DMSO-d_6): δ 7.03 (t,1H,CH-pyrimidine), 7.45-8.01(m,8H,ArH), 8.50 (d,2H,2CH-pyrimidine),8.89(s,1H,ArH$_4$),10.90, 11.73(2s, 2H, 2NH,D_2O -exchangeable). MS m/z: 421(M$^+$-1).Anal. Calcd for $C_{20}H_{14}N_4O_5S$(422)C,56.87;H,3.34;N,13.26,found C,56.87; H, 3.87; N, 13.31.

6-Bromo-2-oxo-*N*-(4-(*N*-(pyrimidin-2-yl) sulfamoyl) phenyl)-2*H*-chromene-3-carboxamide (**6b**). **Black powder** in 35% yield, mp>300ºC;IR(KBr):υ_{max}./cm^{-1} 3255,3215 (2NH), 1699(COO),1667(CO);^{1}H-NMR(DMSO-d_6):δ7.02-8.89 (m,11H, 11ArH).MS m/z 500(M$^+$): .Anal. Calcd for $C_{20}H_{13}BrN_4O_5S$(500)C,47.92;H,2.61;N,11.18, found C,48.18;H,2.59;N,11.34.

6 Antimicrobial evaluation

Microorganism's strains and preparation of inoculum:*A.niger* (RCMB 002007), *G.candidum* (RCMB 05097),*S.pneumoniae* (RCMB 010010), *B.subtilis* (RCMB 010067), *P.aeruginosa* (RCMB 010043), *E. coli* (RCMB 010052) Strains were used in this study. The microbial suspension equivalent to the turbidity of 0.5 McFarland (108 CFU/ml) standard was prepared from a fresh subculture of tested bacteria in a Mueller Hinton broth (MHB) and tested with fungi in a Sabouraud dextrose broth (SDB) then this suspension was diluted to 106 CFU/ml using MHB for bacteria and Sabouraud dextrose Broth (SDB) for testd fungi. The adjusted microbial inoculum (100 μl) was added to each well of a sterile 96-well flat-bottomed micro titer plate containing the tested concentration of tested samples (100 μl/well). As a result, the last inoculum concentration of 5×105 CFU/ml was obtained in each well. Three wells containing a microbial suspension with no sample using DMSO employed for dissolving the tested compound (growth control) and two wells containing only media (background control) were included in this plate. Optical densities were measured after 24 hours at 37°C for bacteria and after 48 hours at 28°C for fungi using a multi-detection microplate reader at The Regional Center for Mycology and Biotechnology (Sun Rise–Tecan, USA) at 600 nm. Ampicillin, Gentamicin and Amphotericin B were used as standards for Gram-positive bacteria, Gram-negative bacteria and fungi, respectively.

The percentage of microbial inhibitory was calculated using the Microsoft Excel®. Microbial viability % was calculated according to the following equation: Percentage of viability = [1-(ODt/ODc)] × 100%, where ODt is the mean optical density of wells treated with the tested compound and ODc is the mean optical density of untreated cells, while Inhibitory % = (100 – viability) %. For the determination of MIC of tested samples microdilution test was performed in 96-well plates. Two-fold dilutions of each compound were prepared in the test wells, the final drug concentrations being (125–0.004) μg/mL, control wells were prepared with culture medium only and microbial suspension only. The plates were sealed and incubated for 24 hours at 37°C for bacteria and for 48 hours at 28°C for fungi, after each incubation time MIC was detected as the lowest sample concentration that prevented microbial growth. Each MIC was determined three times. The test compounds were also compared using the IC_{50} value, i.e., the concentration of the compound leading to 50% microbial death that was estimated from graphical plots.

7 Conclusion

New series of pyridone and chromene derivatives tagged with sulfadiazine moiety were synthesized and evaluated for their *in vitro* antimicrobial activities. All the newly synthesized compounds were more potent than sulfadiazine. Moreover, some of the targets exhibited better antimicrobial activities than the reference drugs ampicillin, gentamycin and amphotericin B. Docking simulations showed that the studied compounds can be accommodated in the PABA pocket of DHPS thereby inhibiting the enzyme in the same manner reported for sulfa drugs. Additionally, the synergistic effect of combining sulfonamide and biologically active heterocyclic rings in one molecule could explain the targets' observed good results.Compounds (**3f, 4f** and **5a**) were the most active antimicrobial agents in this study.

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