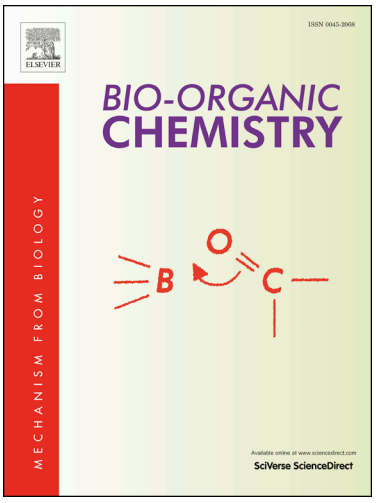

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Aryl fluorosulfate analogues as potent antimicrobial agents: SAR, cytotoxicity and docking studies

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Abstract

A series of aryl fluorosulfate analogues (**1-37**) were synthesized and tested for *in vitro* antibacterial and antifungal studies, and validated by docking studies. The compounds **9, 12, 14, 19, 25, 26, 35, 36** and **37** exhibited superior antibacterial potency against tested bacterial strains, while compounds **2, 4, 5, 15, 35, 36** and **37** were found to have better antifungal activity against tested fungal strains, compared to standard antibiotic gentamicin and ketoconazole respectively. Among all the synthesized **37** analogs, compounds **25**, **26**, **35**, **36** and **37** displayed excellent anti-biofilm property against *Staphylococcus aureus*. The structure-activity relationship (SAR) revealed that the antimicrobial activity depends upon the presence of $–OSO_2F$ group and slender effect of different substituent's on the phenyl rings.

The electron donating (OCH_3) groups in analogs increase the antibacterial activity, and interestingly the electron withdrawing (Cl, NO_2, F and Br) groups increase the antifungal activity (except compound **35, 36** and **37**). The mechanism of potent compounds showed membrane damage on bacteria confirmed by SEM. Compounds **35**, **36** and **37** exhibited highest glide g-scores in molecular docking studies and validated the biocidal property.

Key words: Aryl-fluorosulfates; antimicrobial; docking studies; cytotoxicity.

1. Introduction:

Aryl fluorosulfates were described more than four decades ago, but the chemistry of these compounds is quite unexplored [1]. Recently, new interest in the synthesis of aryl fluorosulfates from the reaction of a phenol with sulfuryl fluoride in the presence of a base was described by Sharpless and co-workers through a SuFEx click chemistry process [2,3]. Sulfuryl fluoride (SO_2F_2, bp = −55.4 °C) is produced by Dow Agro Sciences and is most commonly employed as an insecticide for the control of dry wood termites by whole-structure fumigation. The sulfuryl fluoride has low toxicity and is relatively inexpensive [4]. Recently, the fluorosulfates based probes have become vital tools in chemical biology and molecular pharmacology [5,6]. Aryl fluorosulfates probes have also been found to possess substantial efficacy in chemical and medicinal chemistry [2]. Nowadays bacterial infections, especially today with the emergence of multidrug-resistant bacteria caused by the misuse of antibiotics, are becoming a serious problem. Unfortunately, the traditional drugs used in clinics are exhibiting less effectiveness in the treatment of infections [7]. Therefore, it is of great significance to develop new types of antimicrobial agents, especially those with new drug targets or with the ability to overcome drug resistance [8]. However, the chemical biology toolkit still needs urgent attention to significantly improve and expand the palette of useful synthetic transformations that can be harnessed to understand biology and drug discovery. We inspired by the special features of aryl fluorosulfates analogues and our

ongoing research program [9-11]. Herein, we report the synthesis of a series of aryl fluorosulfates (**1-37**) and their utilization as antimicrobial agents in the field of drug discovery through exploiting the relative chemical stability of fluorosulfates and carrying out a number of unique fluorosulfates-sparing functional group modifications for their antibacterial and antifungal activities studies. In addition, in this work, we also carried out molecular docking studies of those synthetic compounds in order to correlate their structural motif with their antibacterial and antifungal activities. The experimental results and docking studies suggest these compounds will create powerful and valuable chemical tools for chemical biology research and drug discovery.

2. Results and Discussion

2.1. Chemistry

Aryl fluorosulfates were synthesized **(Fig. 1)** directly from the phenols and SO_2F_2 following the procedure described by Sharpless and co-workers [2]. All the derivatives were obtained in good to excellent yield. The formation of the $–OSO_2F$ was confirmed by the presence of fluorine (F) peak in ^{19}F NMR and the absence of -OH peak in ^{1}H NMR spectra. All the chemical structures were confirmed by ^{1}H, ^{13}C, ^{19}F NMR and mass spectral analysis (Supporting information).

2.2. Biology

2.2.1. Antibacterial activity

Aryl fluorosulfates (**1-37**) were synthesized and screened for their *in vitro* antibacterial activity against two Gram-positive strains *Staphylococcus aureus* and *Bacillus subtilis* as well as two Gram-negative strains *Escherichia coli* and *Klebsiella pneumonia* by using the agar well diffusion method [12], as well as micro dilution method [13]. The results of antibacterial screening were summarized in **Table 1** and **Table 2**. All assays were performed in triplicate and the results were expressed as the mean of the diameter of

inhibition zone in millimeter (mm). Gentamicin was used as the standard drug for the antibacterial screening. Recently, K. B. Sharpless and his research group was reported arylfluorosulfates containing analogs showed potential anticancer agents [14]. We inspired the biological importance of arylfluorosulfates groups, we first time reported to arylfluorosulfates is good antimicrobial agents. In the present investigations *S. aureus* used as a model organism and anti-biofilm activity study was carried out for all **37** compounds. Among **37** analogs the best was **25** (0.84±0.18), **26** (0.82±0.20), **35** (0.76±0.10), **36** (0.58±0.31), **37** (0.51±0.23) and they exhibited as classic compounds for the control of diseases. Among these classic compounds analog **36** and **37** are highly potent in nature (**Fig. 2**). To support the antimicrobial activity, the MIC of compound **37** treated with *S. aureus* over night and cell morphology was observed in SEM showing cell rupture, variation in the structure can be visualized (**Fig. 3)**. This indicates that, all potent nature of the compounds are acting on cell membrane and involved in the destabilization of cell and leads to death of the bacteria was hypothesized in the present investigation.

The antimicrobial activities those synthetic aryl fluorosulfates were evaluated accordingly. The results revealed that most of the compounds have shown moderate to excellent activity against the four tested bacterial strains. Compounds **9**, **12**, **14**, **19**, **25**, **29**, **35**, **36** and **37** showed superior antibacterial activities compared to the reference drug gentamicin. These activities may be associated with the presence of both electrons donating group (OCH_3) and sulfonylfluoride ($-OSO_2F$) groups in the analogs. Compounds **36** and **37** exhibited excellent antibacterial activities against all of the tested bacterial strains. It may be explained by the presence of two methoxy groups and two $–OSO_2F$ groups in the molecules. Compound **36** was more active than compound **37**, in which the presence of double bond may slightly reduce the antibacterial activity. Interestingly, compound **35** with no electron donating groups but more $-OSO_2F$ groups presented in the molecule, displayed good

antibacterial activity against all the tested bacterial pathogens, this analog may be preliminarily prove that the antibacterial activity depends on -OSO_2F group. Compound **29** showed good antibacterial activities against the Gram-negative bacterial strains *Escherichia coli* and *Klebsiella pneumonia*, which may be caused by the antibacterial activity of indole moiety. Compounds **24** and **25** exhibited excellent antibacterial activity against all the tested bacterial strains. It may be assumed that antibacterial activity increases along with the increasing of -OSO_2F group number on the benzene ring. Both the compounds **24** and **25** strongly confirmed that the bacterial activities have a significant relationship with the presence of the –OSO_2F groups. Compounds **9**, **12** and **14** showed good antibacterial activity. It may be explained by the presence of methoxy group on the benzene ring. Compound **14** showed good antibacterial activities compared to compounds **9** and **12**. The substituent of *ortho*, *meta* and *para* positions of methoxy groups on the phenyl rings also led to the slight difference in the antibacterial activity. Compound **20** showed good antibacterial activities against the Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* due to the presence of another phenyl ring. Compound **24** showed superior antibacterial activity against the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumonia* and moderate activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis.* The rest of the compounds **2-6**, **8**, **10**, **13** and **15-18** showed least antibacterial activity, which may be explained that electron withdrawing groups (Cl, F, Br and NO_2) reduced the antibacterial activity. Compounds **32-34** showed nil antibacterial activity against all the tested bacterial strains, in which the presence of iodine (I) and CF_3 groups on the five membered rings may reduce the antibacterial activity.

2.2.2. Antifungal activity

All the synthesized aryl fluorosulfates (**1-37**) were screened for their *in vitro* antifungal activity against four fungal strains, namely, *Aspergillus niger*, *Fusarium*

moniliforme, *Fusarium oxysporum* and *Candida albicans* by using agar well diffusion method [15] as well as micro dilution method [16]. The results of antifungal screening were summarized in **Table 2** and **Table 3**. All assays were performed in triplicate and the results were presented as the mean of the diameter of inhibition zone in millimeter (mm). Ketoconazole was used as the standard drug for the antifungal activity screening.

The results revealed that compounds **2**, **4**, **5**, **15**, **35**, **36** and **37** showed good to excellent antifungal activities against all the tested fungal strains. Compounds **36** and **37** were shown excellent antifungal activities, which may be explained in the presence of the two methoxy and two $–OSO_2F$ groups on the phenyl ring. Compound **35** displayed good antifungal activities due to the presence of two $–OSO_2F$ groups on the two phenyl rings. Compounds **2**, **4**, **5** and **15** exhibited good antifungal activities against all the tested fungal pathogens. It may be related to the presence of electron withdrawing (Cl, NO_2 and Br) groups on the phenyl ring. Compound **3** showed good antifungal activities against the *Fusarium moniliforme* and *Fusarium oxysporum* fungal strains. Compound **17** showed good antifungal activity against the *Fusarium moniliforme* and *Fusarium oxysporum* fungal strains due to the presence of $–CF_3$ groups on the phenyl ring which may increase the antifungal activity. The compounds (**9**, **12** and **14**) displayed less antifungal activities against the tested fungal pathogens. It is speculated that the presence of electron donating (OCH_3) groups on the phenyl ring may reduce the antifungal activity. The remaining compounds showed very less or poor activity against the tested all pathogens which suggest that other functional groups (CN, CH_3, NH_2 and I) don't influence the antifungal activity.

Based on their promising antimicrobial activity, these synthetic compounds were further tested for their minimum inhibitory concentration (MIC). The results showed that, compounds **35, 36** and **37** exhibited excellent MBC and MFC activity against all the bacterial and fungal strains (MIC values were below the standards). Compounds **9** exhibited good

MBC activity against the bacterial pathogens of *Staphylococcus aureus* and *Bacillus subtilis*. Compounds **14** and **25** displayed good MBC activity against all the tested bacterial pathogens. Compound **29** showed good MBC activity against the Gram-positive strains *Staphylococcus aureus* and *Bacillus subtilis* as well as Gram-negative strains *Escherichia coli* and *Klebsiella pneumonia* respectively. Compounds **2**, **4**, **5** and **15** showed good MFC activity against all the fungal pathogens (MIC values were below the standards). On the basis of these promising results, we are convinced that aryl fluorosulfates will be widely used in the near future for the development of antimicrobial drugs.

2.2.3. Docking studies

The MurB enzyme catalysis repeating disaccharide and pentapeptide units of the bacterial peptidoglycan layer are connected by a lactyl ether bridge biosynthesized from phosphoenolpyruvate. It is well known that structure and mechanism of the MurB enzyme will permit unusual enol ether reduction reaction. Hence disruption at the active side of MurB, causes inhibition of peptidoglycan. The bacterial Hfq is a protein, which plays an important role in the regulation of genes in cooperation with sRNAs. Hfq has two or more sites to bind RNA(s) including U-rich and/or the poly (A) tail of mRNA. Whereas, the AmpC β-lactamase provide a multi-resistance to the β-lactam antibiotics. These are keys check point where inhibitors designed to enzymes could work as potent antibacterial compound and may facilitate as candidate of next-generation antimicrobial agents.

The mevalonate 5-diphosphate decarboxylase produces an isopentenyl diphosphate a building block for polyisoprenoid synthesis from Mevalonate pathway. Isopentenyl diphosphate is a critical pathway for growth of the human bacterial pathogen *Enterococcus faecalis*. The mevalonate 5-diphosphate decarboxylase suggested as a therapeutic target for the treatment of drug-resistant bacterial infections. Whereas α-1,2-Mannosyltransferase is an member of glycosyltransferase family, which actively take part in biosynthesis of cell wall

glycoproteins of *Saccharomyces cerevisiae*. The α-1,2-Mannosyltransferase is known to implicated in virulence of *Candida albicans*. The Sec3p-N interacts with Rho1p and membrane containing PIP_2 in the process of exocytosis. Hence, attempt was focused for potent antimicrobial compound which act on key point of virulent pathogenic bacteria and fungi which hampers its growth.

Molecular docking depicts the structural geometry, drawn against the interaction of the protein with ligand binding mode by evaluating the energy scores of different bound poses of the ligand within functional site of the protein template with a scoring function. The best scoring reflects the promising targets for drug candidates, whose actions depend upon the inhibition or regulation of the target protein functions [17]. Among the 37 ligands (**Scheme Fig. 1**), **M-36** was found to be more potent than the standards used in the *in vitro* methodologies. The docking studies results were represented against potent compound M-36 **(Fig. 4 and 5)**. Antibacterial potency of **M-36** was also found potent against MurB. The structure of ligand **M-36** is depicted as structure analog of curcumin [18] which is known to possess antimicrobial as well as antioxidant activities, not surprisingly, **M-36** in our docking result also showed tremendous bioactivity. It is worthy to note, a **M-36** formed hydrogen bond with the hydroxyl group of hydrophilic amino acids in MurB (1MBT) and AmpC β-lactamase (Supporting information **Table S1**). Hence, it can be reasonably speculated that compound **M-36** inhibited the bacterial peptidoglycan bio-synthesis by restricting the vital MurB enzyme from carrying out its function [19]. MurB, a critical enzyme involved in bacterial cell wall synthesis, we have established one plausible target for the synthesized molecules **M-36**. Bacterial Hfq is a protein that plays an important role in the regulation of genes in cooperation with sRNAs. An RNA chaperone Hfq acts as a central player in post-transcriptional gene regulation in several Gram-negative bacteria [20]. Ligand **M-37** was

found to be slightly more potent than **M-36** because **M-37** obstruct the RNA binds in the post-transcriptional process of protein synthesis (Supporting information **Table S2**).

2.2.4. Molecular Dynamics

The molecular dynamic simulations of MurB complex with compound **36** (**Fig 6**) simulated their interaction under explicit solvent conditions. The OPLS 2005 potential energy analysis of the docked complex showed a gradual decrease and appeared to remain stable after 5 ns to MD simulations. In MD simulation, there was change in the positioning of compound **36** in relation to the protein indicating conformational change of the ligand. This clearly deduces the compound **36** closely interact with MurB active and causes conformational changes as the compound binds to the protein. The VMD package revealed that, MurB structure formed number of salt bridges involving residues. These salt bridges were formed intra as well as inter bond with MurB polypeptide. Thus, from the present study it is concluded that MurB from *Escherichia coli* docked with Compound **36** is a stable complex when subjected to MD simulation in presence of explicit solvent.

The antifungal property of **M-36** showed good antifungal activity against those chosen protein templates (**Table 5**). 1FI4 is responsible for sterol/isoprenoid biosynthesis, 3A58 where Rho- and phosphoinositide-dependent localization is present and 1S4N is involved in the biosynthesis of yeast cell walls glycoproteins [21-24], Docking studies showed that the amino acid residues of enzyme 1FI4 played an active role in the interaction with ligand **M-36** (**Table 5**). Ligand **M-36** was found to be more potent with the glide XP score of -5.74 kcal/mol (Supporting information **Table S1 and S2**) showing strong interaction with Lys123 via the hydrogen bond and salt-bridge, whereas the hydrogen bond between M-36 and Val40 was formed *via* a catalytical water molecule. Thereby we can assume that **M-36** might have interference Rho- and phosphoinositide-dependent localization. On the other hand, in case the of 1FI4, **M-37** was better than **M-36** with the glide XP score of -6.84 kal/mol and forming a

hydrogen bond with Thr75. Ligand, **M-36** had glide XP score of -6.21kcal/mol in case of 1S4N, forming a hydrogen bond with Val282. Our antifungal docking studies demonstrated that **M-36** is connected more effectively with above mentioned targets than the known antifungal compounds.

QikProp, the prediction program was used to calculate pharmacokinetic ADME (absorption, distribution, metabolism and excretion) properties consisting of principal descriptors and physiochemical properties. Qikprop modules predict the range of molecular properties for the newly synthesized compounds to compare them with 95% of those known drugs. All the ligands obey the Lipinski's rules: QPpolrz, QPlogPC16, QPlogPoct, QPlogPw, QPlogPo/w, QPlogS, CIQPlogS, QPlogHERG, QPPCaco, QPlogBB, QPPMDCK, QPlogKp, QPlogKhsa, Human Oral Absorption, Percent Human Oral Absorption, SAfluorine, SA amide O, number of Nitrogen and Oxygen and Lipinski Rule of Five. Based on the above results, it may be concluded that **M-36** possesses the potentiality to be utilized as a better antibacterial and antifungal drug candidate (Supporting information **Table S3**).

2.2.5. Cytotoxicity assay

The cytotoxicity evaluation of the synthesized compounds were carried out using MDA-MB-231 is a triple negative breast cancer cell line. In order to ascertain the likely safety of compounds for their potential use [25,26] a standard cell-based toxicity analysis was performed and showed different performance of synthesized compounds response to the MDA-MB-231 cells in the present investigations. The compounds **9**, **12**, **36** and **37** are less cytotoxic and compounds **4**, **5**, **13**, **23**, **24**, **29** and **35** are moderate and compounds **6**, **16**, **22, 32** and **33** showed high toxicity to the cells. But rest of the compounds is negligible in the effect against MDA-MB-231 cells. The greater effect of compounds **9** (36±0.13), **12** (48±0.12), **36** (38±0.36) and **37** (40±0.28) µg/mL has IC_{50} value compared to the standard doxorubicin having IC_{50} value of 21 µg/mL. This significant effect represents the

permeability of the synthesized compounds to the cell. It also confirms the effect of compound can pass through the membrane to cause the death of the cell and consistence with the reported literatures [26].

3. Conclusion

In the present investigation, a series of simple aryl fluorosulfates were synthesized in good yields and tested for their preliminary *in vitro* antibacterial and antifungal activities. It was that found compounds (**9**, **12**, **14**, **19**, **25**, **26**, **35**, **36** and **37)** displayed good antibacterial activity while compounds (**5**, **9**, **10**, **11**, **12**, **28** and **30)** demonstrated good antifungal activities compared to that of the standard antibiotics gentamicin and ketaconazole. Among **37** analogs, the compounds **36** and **37** are highly potent in nature. Further, SAR study showed that the presence of -OSO_2F groups, electrons donating (OCH_3) and withdrawing (Cl, NO_2, F and Br) groups on the phenyl ring played an important role in the antimicrobial activities. Molecular docking studies were performed for all the synthesized compounds, among them compounds **35**, **36** and **37** showed the highest glide g-scores. On the basis of these promising results, we convinced that aryl fluorosulfates will be widely used in the near future for antimicrobial drugs.

4. General Experimental details

All reactions were carried out under an air atmosphere. Unless otherwise specified, NMR spectra were recorded in $CDCl_3$ on a 500 or 400 MHz (for ^1H), 471 or 376 MHz (for ^{19}F), or 126 MHz (for ^{13}C) spectrometer. All chemical shifts are reported in ppm relative to TMS (^1H NMR, δ ppm) as an internal standard. All chemicals and reagents were purchased from Energy Chemicals, China and used without further purification. The coupling constants are reported in Hertz (Hz). The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High resolution mass spectroscopic analysis was performed on a Bruker MicroTOF QII mass spectrometer in

positive mode. Progress of the reaction was monitored by TLC using silica gel coated on glass plates the compounds on the TLC plates were detected under UV light.

4.1. Procedure for the synthesis of aryl fluorosulfates **(1-37)**

In a 50 mL three-necked flask equipped with a stirring bar, phenol (2 mmol, 1.0 equiv) and Et_3N (1.2 equiv) were dissolved in 10 mL CH_2Cl_2. SO_2F_2 was introduced by bubbling through the solution. The reaction was monitored by TLC. After 4-12 h at room temperature before concentrating under vacuum. The crude product was purified by silica gel chromatography by gradient elution with 5−20% EtOAc / Petroleum ether to give pure product (**1-37**).

Note: All the aryl fluorosulfates are identical to those reported regarding the ^{1}H, ^{19}F and ^{13}C NMR to those reported in the previous papers [26-29]. Syntheses and characterization of the unprecedented new compounds are reported as below.

Full characterization of the new compounds

4.2. 3-(Trifluoromethyl)phenyl sulfofluoridate **(13)**

Petroleum ether / ethyl acetate = 20 : 1 (v / v) as eluent for column chromatography. white solid, 84% Yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, *J* = 7.8 Hz, 1H), 7.66 (t, *J* = 8.1 Hz, 1H), 7.63 (s, 1H), 7.57 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.8, 133.2 (q, *J* = 34.6 Hz), 131.3, 125.7 (q, *J* = 3.6 Hz), 124.5, 122.8 (q, *J* = 273.4 Hz), 118.5 (q, *J* = 4.6 Hz); ^{19}F NMR (471 MHz, CDCl3) δ: 38.4 (1F), -62.9 (3F); HRMS m/z [M+]: Calcd for $C_7H_4F_4O_3S$: 243.9817; Found: 243.9816

4.3. 4-(Tert-butyl)phenyl sulfofluoridate **(18)**

Petroleum ether / ethyl acetate = 20 : 1 (v / v) as eluent for column chromatography. Colorless oil, 89% Yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 8.6 Hz, 2H), 1.35 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.0, 147.9, 127.3, 120.2, 34.7,

31.2; ^{19}F NMR (471 MHz, $CDCl_3$) δ: 37.1; HRMS m/z [M+]: Calcd for $C_8H_{13}FO_3S$: 232.0569; Found: 232.0562

4.4. 3,4-Dimethylphenyl sulfofluoridate **(19)**

Petroleum ether / ethyl acetate = 20 : 1 (v / v) as eluent for column chromatography. Colorless oil, 81% Yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.4 Hz, 1H), 7.13 (s, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 2.32 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 148.2, 139.3, 137.5, 131.1, 121.5, 117.8, 19.9, 19.3; ^{19}F NMR (471 MHz, $CDCl_3$) δ: 37.1; HRMS m/z [M+]: Calcd for $C_8H_9FO_3S$: 204.0256; Found: 204.0261

4.5. 4-(Benzyloxy)phenyl sulfofluoridate **(21)**

Petroleum ether / ethyl acetate = 20 : 1 (v / v) as eluent for column chromatography. White solid, 85% Yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.38 (m, 4H), 7.35 (t, *J* = 6.6 Hz, 1H), 7.25 (d, *J* = 8.6 Hz, 2H), 7.01 (d, *J* = 9.1 Hz, 2H); 5.07 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.5, 143.8, 136.2, 128.8, 128.3, 127.5, 122.0, 116.1, 70.6; ^{19}F NMR (471 MHz, CDCl3) δ: 36.5; HRMS m/z [M+]: Calcd for $C_{13}H_{11}FO_4S$: 282.0362; Found: 282.0370

4.6. 4'-Cyano-[1,1'-biphenyl]-4-yl sulfofluoridate **(22)**

Petroleum ether / ethyl acetate = 10 : 1 (v / v) as eluent for column chromatography. White solid, 85% Yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.4 Hz, 2H), 7.78 (t, *J* = 8.5 Hz, 4H), 7.47 (d, *J* = 8.4 Hz, 2H); ^{19}F NMR (471 MHz, $CDCl_3$) δ 38.1; ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.2, 143.6, 140.0, 132.8, 129.3, 127.9, 121.7, 118.5, 112.0; HRMS m/z [M+]: Calcd for $C_{13}H_8FNO_3S$: 277.0209; Found: 277.0215

4.7. Benzene-1,3,5-triyl trisulfofluoridate **(25)**

Petroleum ether / ethyl acetate = 10 : 1 (v / v) as eluent for column chromatography. White solid, 86% Yield. ^{1}H NMR (500 MHz, $CDCl_3$) δH: 7.53 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 150.2, 115.6; ^{19}F NMR (471 MHz, $CDCl_3$) δ: 40.1; HRMS m/z [M+]: Calcd for $C_6H_3F_3O_9S_3$: 371.8891; Found: 371.8899

4.8. 2-Iodocyclopenta-1,4-dien-1-yl sulfofluoridate **(32)**

Petroleum ether / ethyl acetate = 20 : 1 (v / v) as eluent for column chromatography. Colorless oil, 84% Yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.14 (t, *J* = 7.5 Hz, 1H); ^{19}F NMR (471 MHz, $CDCl_3$) δ: 42.2; ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.4, 140.8, 130.2, 130.0, 121.7, 88.4; HRMS m/z [M+]: Calcd for $C_5H_4FIO_3S$: 301.8910; Found: 301.8915

4.9. (Z)-4-(7-(4-((fluorosulfonyl)oxy)-3-methoxyphenyl)-3-hydroxy-5-oxohept-3-en-1-yl)-2-methoxyphenyl sulfurofluoridate **(36)**

Petroleum ether / ethyl acetate = 5 : 1 (v / v) as eluent for column chromatography. Off white solid, 85% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.3 (s, 0.7H), 7.19 (d, *J* = 8.1 Hz, 2H), 6.86 (d, *J* = 1.5 Hz, 2H), 6.78 (d, *J* = 8.8 Hz, 2H), 5.39 (s, 0.7H), 3.87 (s, 6H), 3.55 (s, 0.3H), 2.92 (t, *J* = 7.6 Hz, 3.3H), 2.88-2.81 (m, 1.6H), 2.58 (*J* = 7.5 Hz, 3.3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.5, 192.4, 151.0, 142.8, 137.4, 122.2, 120.5, 113.7, 113.6, 99.9, 56.1, 44.8, 39.6, 31.2, 29.1; ^{19}F NMR (471 MHz, $CDCl_3$) δ: 39.5; HRMS m/z [M+]: Calcd for $C_{21}H_{22}F_2O_{10}S_2$: 536.0622; Found: 536.0630

4.10. 4-((1E,3Z,6E)-7-(4-((fluorosulfonyl)oxy)-3-methoxyphenyl)-3-hydroxy-5-oxohepta-1,3,6-trien-1-yl)-2-methoxyphenyl sulfurofluoridate **(37)**

Petroleum ether / ethyl acetate = 5 : 1 (v / v) as eluent for column chromatography. Off white solid, 83% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.7 (s, 1H), 7.62 (d, *J* = 15.9 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.21-7.19 (m, 4H), 6.61 (d, *J* = 15.9 Hz, 2H), 5.89 (s, 1H), 3.98 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.8, 151.5, 139.7, 139.0, 136.6, 125.9, 122.9, 120.6, 112.5, 56.3; ^{19}F NMR (471 MHz, $CDCl_3$) δ: 40.3; HRMS m/z [M+]: Calcd for $C_{21}H_{18}F_2O_{10}S_2$: 532.0309; Found: 532.0315

5. Biology

5.1. Antibacterial activity

In vitro antibacterial activity was evaluated against human pathogens of both gram positive organisms namely *S. aureus* and *B. substilis* as well as gram negative organisms namely *E. coli* and *K. pneumoniae* by agar well diffusion method and micro dilution method with slight modifications [12].

5.1.1. Agar Well Diffusion Method [3]

The microorganisms were inoculated into the sterilized nutrient broth and maintained at 37 °C for 24 hours. On the day of testing, bacteria were subcultured separately in 100 mL of sterilized nutrient broth. Inoculated subcultured broths were kept at room temperature for the growth of inoculums. Using the sterile cork borer, wells (6 mm) were made into each petriplate. The compounds were dissolved in DMSO of 5 mg/mL and from this 5, 10, 15 and 20 µL (25, 50, 75, 100 µg/well) were added into the wells by sterile pipettes. The antibiotic standard gentamicin for antibacterial activity (as positive control) was tested against the pathogens. The samples were dissolved in DMSO and showed no inhibition act as negative control. The plates were incubated at 37 °C for 24 h for bacteria growth. After appropriate incubation the inhibition zone diameter of each well was measured. Duplicates were maintained and the average values were calculated for eventual assessing of antimicrobial activity.

5.1.2. Microdilution Method [30]

All the microorganisms were grown in Muller-Hinton broth. After cultivation for 16–18 h at 37 °C, bacteria were harvested and their density was determined by measuring OD at A_{600}. MIC of the compounds was determined by agar dilution method. Suspension of each microorganism was prepared to contain approximately (1×10^4- 2×10^4 CFU/mL) and applied to the plates with serially diluted compounds to be tested (dissolved in DMSO) and the reference drug gentamicin incubated at 37 °C overnight. Minimum inhibitory concentration was considered to be the lowest concentration that completely inhibited the

growth of microorganisms on the plates. Diameter of Zone of inhibition (mm) was measured after 24 h and MIC values were determined.

5.2. Antifungal activity

In vitro antifungal activity was evaluated against human pathogens of *A. niger*, *F. moniliforme* and *F. oxysporum* by agar well diffusion method well as microdilution method with slight modifications [15].

5.2.1. Agar well diffusion method [31]

The microorganisms were inoculated into the sterilized nutrient broth and maintained at 37 °C for 24 hours. On the day of testing, bacteria were subcultured separately into 100 mL of sterilized nutrient broth. Inoculated subcultured broths were kept at room temperature for the growth of inoculums. Using the sterile cork borer, wells (6 mm) were made into each petriplate. The compounds were dissolved in DMSO of 5 mg/mL and from this 5, 10, 15 and 20 µL (25, 50, 75, 100 µg/well) were added into the wells by using sterile pipettes. Simultaneously the antifungal standards ketoconazole for antifungal activity (as positive control) were tested against the pathogens. The samples were dissolved in DMSO and showed no inhibition act as negative control. The plates were incubated at 28 °C for 48 h for fungi growth. After appropriate incubation the diameter of zone of inhibition zone diameter of each well was measured. Duplicates were maintained and the average values were calculated for eventual antimicrobial activity.

5.2.2. Microdilution Method [15]

Sabouraud agar was used for the preparation of plates. Suspension of each microorganism was prepared to contain 10^5 CFU/mL. The agar plates were inoculated with fungal strains and serially diluted test compounds and reference drug dissolved in DMSO. The plates were incubated at 25 °C for 48–72 h. Minimum inhibitory concentration was considered to be the lowest concentration that completely inhibited the growth of

microorganisms on the plates. Diameter of inhibition zone (mm) was measured after 48 h and MIC values were determined.

Action of compounds on *Shigella flexneri* biofilm growth

Inoculum preparation

The *S. flexneri* was grown in nutrient agar at 35 ℃ for 18-20 h and cells were harvested by centrifuging at 5000 rpm for 8 min at 4 ℃. Wash the cells thrice in sterile saline solution and re-suspend pelleted cells in saline solution. Cell density adjusted to an optical density at 600 nm (OD600) of 0.1 using UV-visible spectrometer and viable counts of approximately 6 log cfu/mL.

5.3. Anti-biofilm activity of compounds

The qualitative and quantitative test was performed for determination of biofilm production using microtiter plate method (MtP) [32]. The experiment was set according to reported method (dos Santos Rodrigues et al., 2017) with slight modifications, 20 µl aliquots of cell suspension was inoculated into each one of six well polystyrene microtiter plate containing 180 µl of nutrient agar supplemented with glucose (10 g/100 mL). MtP was covered and incubated at static condition of 37 °C for 18 h to favors greater adherence *S. flexneri*. After, each well was washed thrice with saline solution; cells were fixed with 150 µl of methanol for 20 min and dry the MtP at room temperature. The cells were stained with crystal violet (0.5%) for 15 min then discard the contents and wash thrice with 200 µl of saline solution. Dry the MtP, using 150 µl of 95% ethanol then dye bound to the cells was eluted for 30 min and absorbance at 490 nm was determined using microplate spectrophotometer.

5.4. Docking Studies

The co-ordinates of 1F1H (Mevalonate 5-diphosphate decarboxylase), 3A58 (Sec3p - Rho1p complex), 1S4N (α1,2-Mannosyltransferase), 3HSB (YmaH (Hfq), 1MBT (MurB),

1KE4 (AmpC β-lactamase) from *Bacillus substilis* were obtained from the Brookhaven Protein Data Bank9 Ligand were drawn using Maestro 2D sketcher and energy minimize was computed by OPLS 2005. Proteins were prepared by retrieving into Maestro platform (Schrödinger, Inc.). Protein structure was corrected, by using Prime software module of Schrödinger to correct the missing loops and in the protein. Water molecules from YmaH were removed beyond 5 Å from the hetero atom respectively. Water molecules which are thought to be important in aiding the interaction between the receptor were optimized during protein pepwizard. Automated, necessary bonds, bond orders, hybridization, explicit hydrogens and charges were assigned. OPLS 2005 force field was applied to the protein to restrained minimization and RMSD of 0.30 Å was set to converge heavy atoms during the pre-processing of protein before starting docking. Using Extra-precision (XP) docking and scoring each compound were docking into the receptor grid of radii 20Å × 20Å × 20Å and the docking calculation were judge based on the Docking score and Glide score. Molecular visualization was done under Maestro workspace [33].

5.5. Molecular Dynamics

Molecular dynamics (MD) simulations were performed to validate the stability of ligand at the active site using Desmond program inbuilt with OPLS 2005 module. The MurB-Compound 36 complex was positioned in an orthorhombic cell soaked with a pre-equilibrated box of explicit solvent including 0.15M NaCl salt with single-point charge (SPC) water model in a cubic box, with geometrical dimension of 10 Å×10 Å× 10 Å. All overlapping solvent molecules were removed and an appropriate number of counter ions were added to maintain charge neutrality. The energy minimization for both SPC water models and the protein before subjecting the system to MD simulation performed using default relaxation protocol with an interface for 10 ns. The average structure of the simulated complex that was

generated was used for computing the distances of interacting residues as well as salt bridge formation.

5.6. Cytotoxicity assay

Cell culture

The Human triple negative breast cancer cells (MDA-MB- 231) was purchased from the National Center for Cell Sciences (NCCS), Pune, India. They were maintained in DMEM under standard cell culture conditions at 37 ℃ and 5% CO_2 in a humidified environment.

5.7. MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of the compounds as previously described [34]. The optical density was measured at 620 nm in an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

$$\% \text{ of Viability} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \times 100$$

Statistical analysis

All studies were implemented in triplicate. All data are expressed as the mean ± standard deviation (SD) and P values of less than 5% were considered statistically significant.

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Competing financial interests: The authors declare no competing financial interests.

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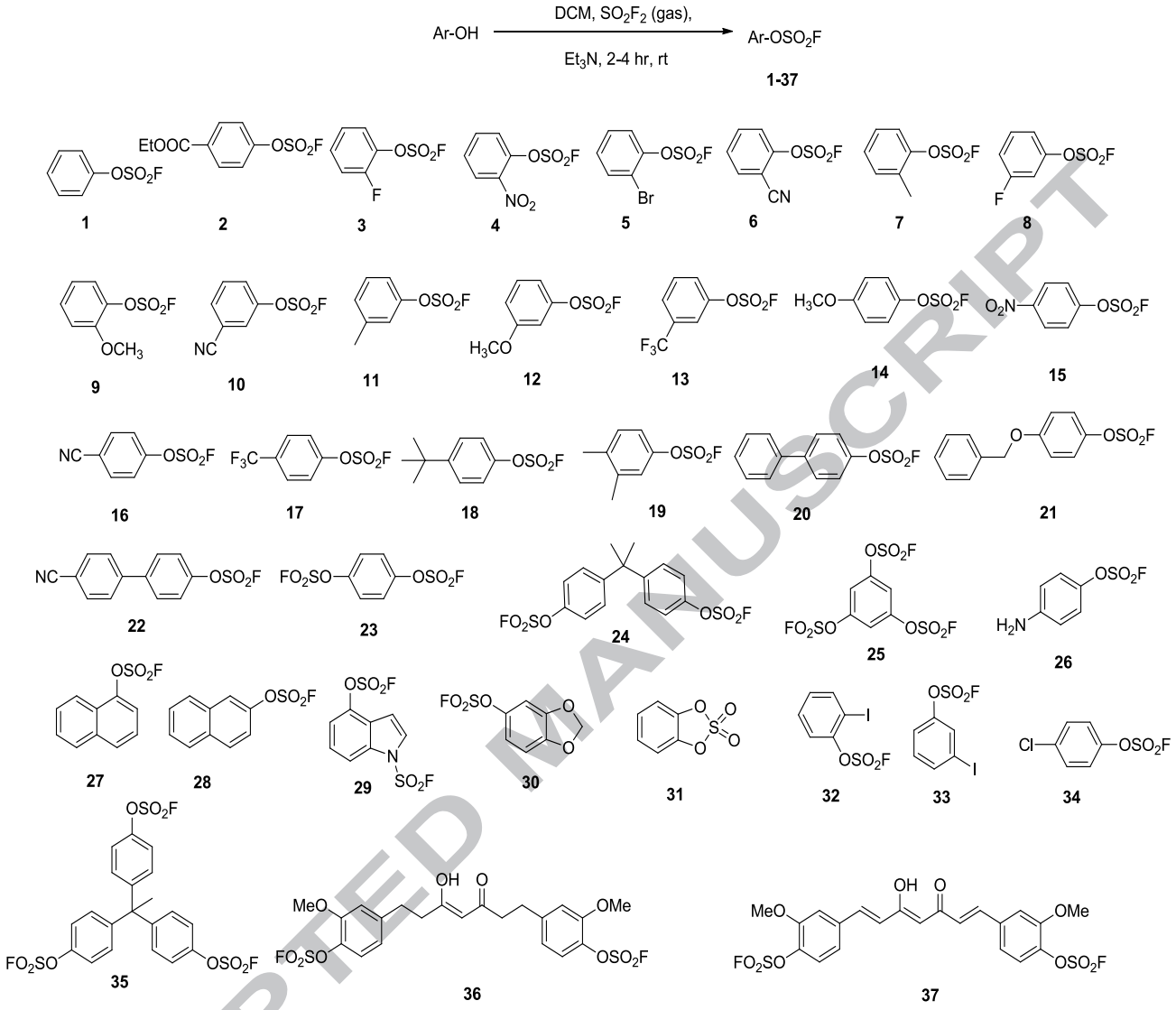

Figure 1: Scheme representation of synthesis of compounds (**1-37**).

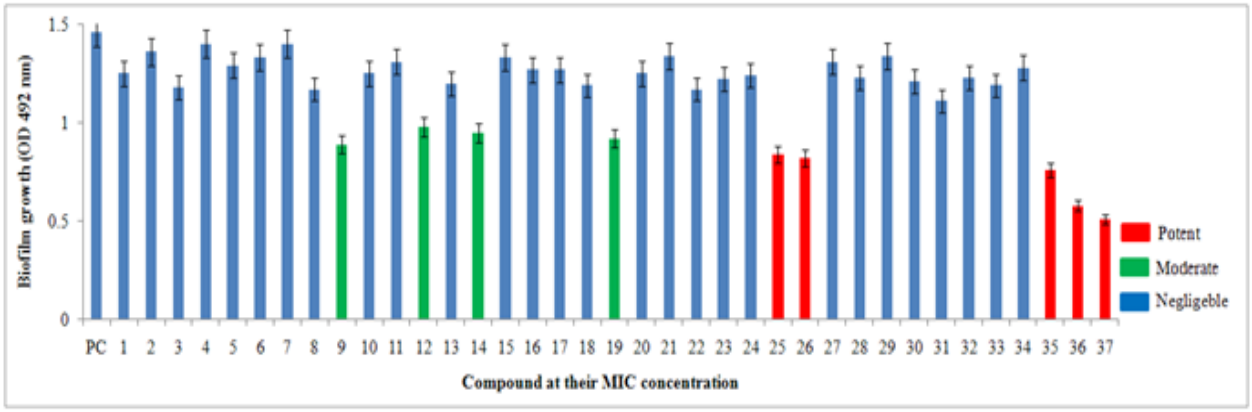

Figure 2: The antibiofilm activity of aryl fluorosulfate analogs. The all 37 synthetic analogs were test for anti-biofilm activity agasint *S. aureus* in 96 well palte using crustal violet method. The obtained results showed tat, the compounds **25**, **26**, **35**, **36**, and **37** are highly potent in nature and compounds **9**, **12**, **14**, and **19** are moderate in nature compared to the others had negligeble activity agaisnt *S. aureus* biofilm growth was depicted.

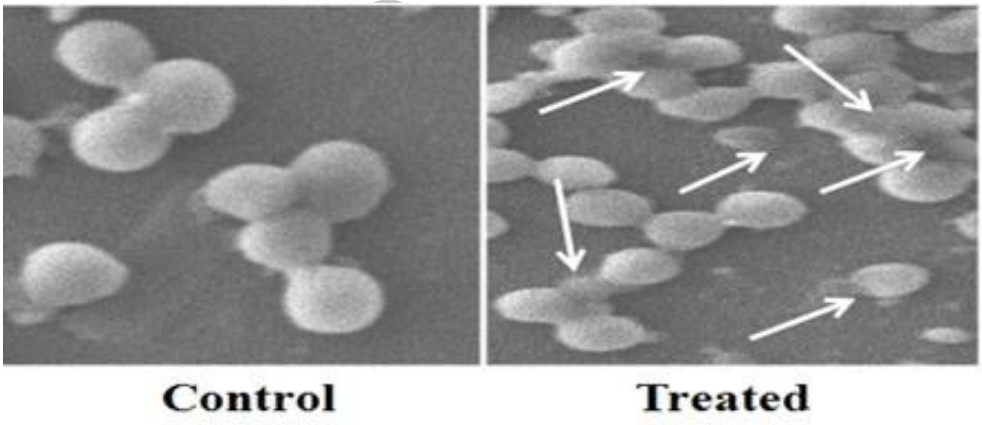

Figure 3: The SEM image of *S. aureus*. The *S. aureus* was treated with MIC concentration of compound **37** overnight and processed sample was observed in SEM. The results showed that, the highly potent compound **37** act on cell membrane of *S. aureus* leading to structural variations and death of the organism was indicated in arrow in the depicted figure.

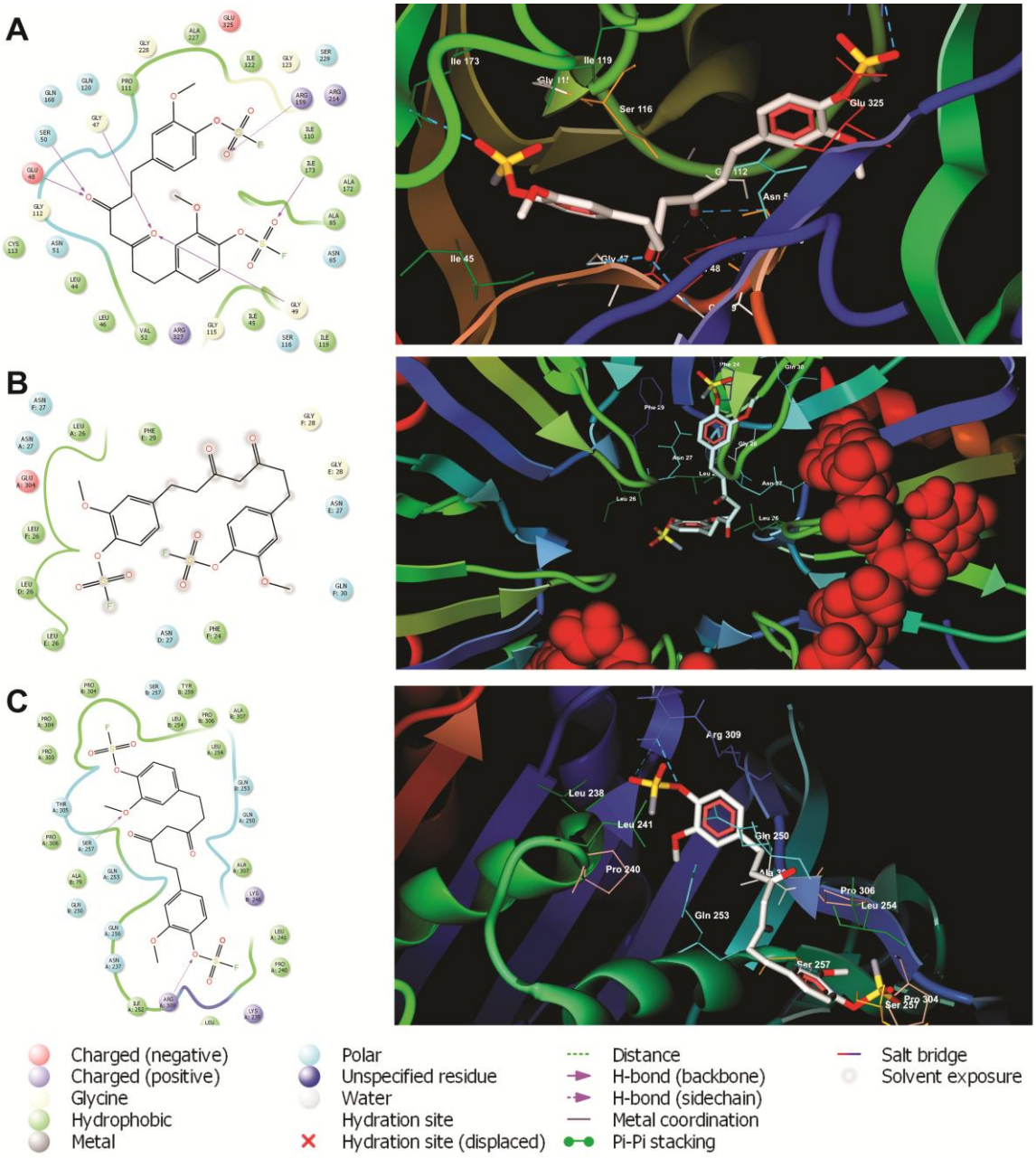

Figure 4: (A) Molecular interaction of MurB (PDB ID: 1MBT), (B) YmaH (Hfq) (PDB ID: 3HSB) and (C) AmpC β-lactamase (PDB ID: 1KE4) with compound 36, showing the hydrogen bond with the backbone amino groups on left side and secondary structure respective protein depicting the best docked pose for compound **36**. RNA is represented in CPK colored in red.

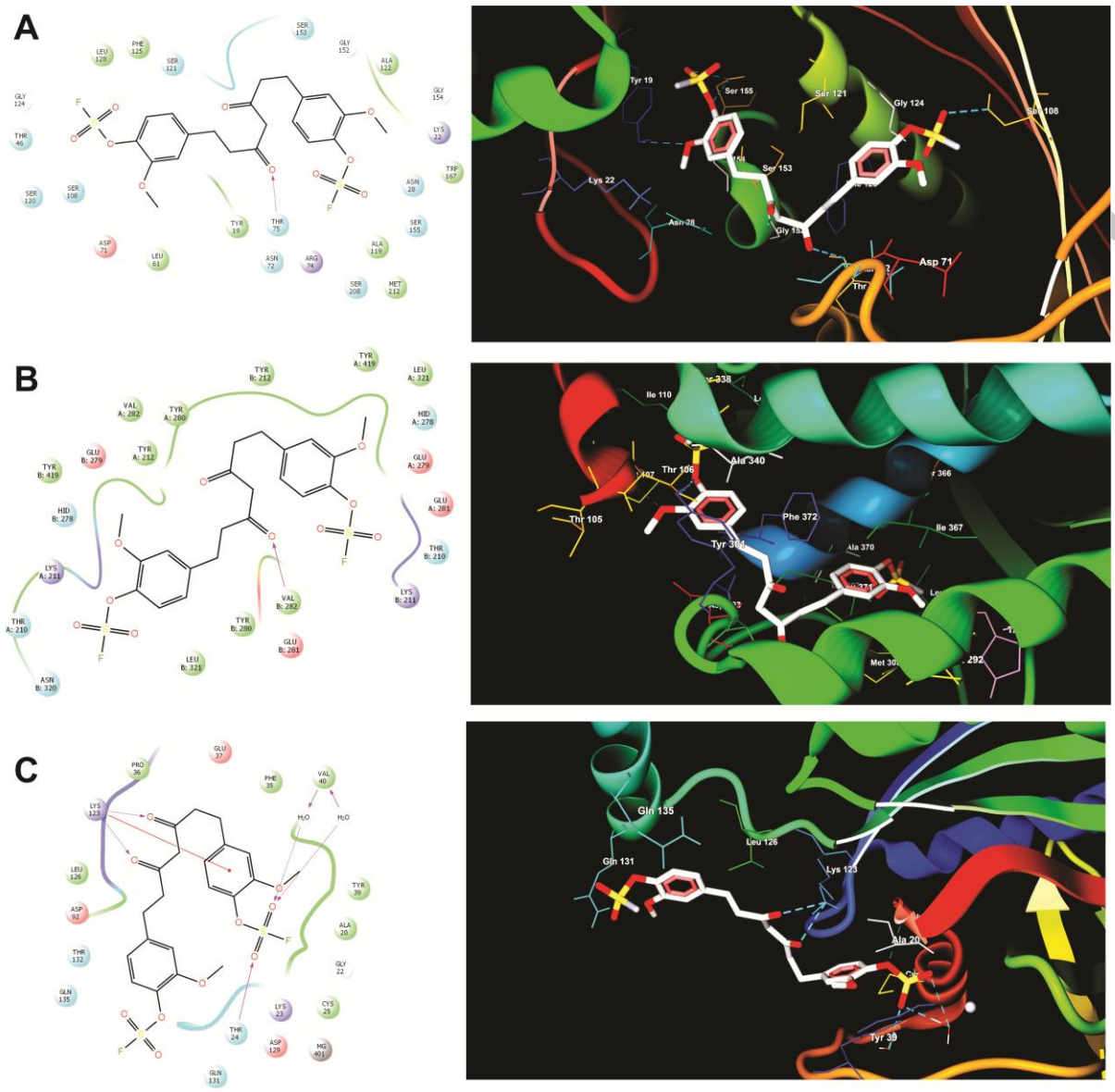

Figure 5: (A) Molecular interaction of Mevalonate 5-diphosphate decarboxylase (PDB ID: 1FI4), (B) α1,2-Mannosyltransferase (PDB ID: 1S4N) and (C) Sec3p - Rho1p complex (PDB ID: 3A58) with compound 36, showing the hydrogen bond with the backbone amino groups on left side and secondary structure respective protein depicting the best docked pose for compound **36** at the catalytical site.

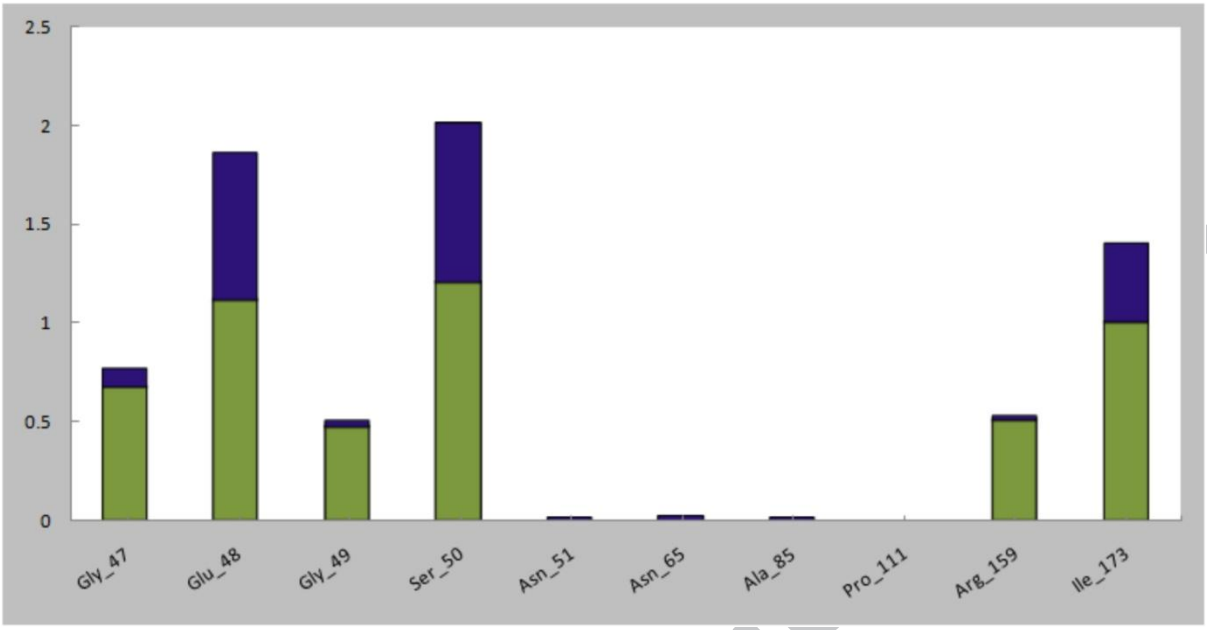

Figure 6: Simulative interactive diagram showing MurB docked with compound 36 simulated in water environment using Desmond application of Schrondinger software. The complex was subjected for 10ns MD simulations. Green color represent backbone donor whereas blue color represent side-chain acceptor.

Table 1: Antibacterial activity of synthesized compounds (**1-37**)

Entry	Zone of Inhibition (mm)[a]															
	Gram-positive bacteria								Gram-negative bacteria							
	Staphylococcus aureus				*Bacillus substilis*				*Escherichia coli*				*Klebsiella pneumonia*			
	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/Ml	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL
01	07±1	11±0	15±2	18±0	06±1	11±2	16±1	19±1	10±1	13±1	17±2	20±1	07±0	11±0	16±0	19±1
02	04±1	09±1	14±1	16±2	05±1	09±1	12±1	16±0	-	-	-	-	-	-	-	-
03	07±1	10±1	13±1	16±0	06±1	09±2	12±1	16±1	05±1	08±1	11±1	13±0	-	-	-	-
04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
05	-	-	-	-	-	-	-	-	07±1	11±1	15±2	19±1	09±1	13±1	15±1	17±1
06	-	-	-	-	-	-	-	-	04±1	06±1	10±4	15±1	07±1	10±2	14±2	17±1
07	05±1	09±2	12±1	16±0	06±1	10±1	12±1	14±2	04±1	06±1	10±2	14±0	-	-	-	-
08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
09	15±1	22±1	27±2	31±0	15±1	23±2	28±2	31±1	13±1	19±2	26±2	31±1	15±1	21±2	27±3	31±1
10	05±1	09±1	11±2	15±2	07±2	10±1	15±1	19±1	-	-	-	-	04±1	10±1	14±1	17±1
11	07±1	10±1	13±1	16±0	06±0	10±1	13±1	17±0	-	-	-	-	-	-	-	-
12	17±1	24±1	28±2	30±1	16±1	21±2	27±1	31±0	15±1	23±1	27±2	31±2	17±1	22±1	28±2	32±1
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	18±1	24±2	30±1	34±1	17±2	25±1	31±2	34±1	16±1	22±2	30±1	33±2	19±1	24±2	30±1	34±1
15	-	-	-	-	-	-	-	-	05±1	09±1	11±0	14±1	06±1	10±2	14±1	17±0
16	10±1	14±2	17±0	21±1	09±1	12±1	17±1	21±0	06±1	10±1	14±1	18±0	10±1	12±0	17±0	21±1
17	07±1	10±2	14±1	16±1	-	-	-	-	-	-	-	-	06±1	09±1	13±1	16±1
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	15±1	20±	24±1	30±1	20±1	24±1	30±1	34±2	07±1	10±1	12±1	14±0	08±1	11±2	14±0	16±1
20	14±1	19±1	22±1	26±1	13±1	17±1	23±2	27±1	13±1	17±2	21±1	26±1	07±1	11±2	15±0	17±1
21	09±1	14±2	17±1	20±1	18±1	11±2	15±2	19±1	-	-	-	-	-	-	10±1	13±1
22	12±1	15±2	20±2	26±1	13±1	19±2	22±4	27±1	10±2	19±2	24±1	29±1	10±1	14±2	19±1	23±1
23	10±2	16±1	21±1	23±1	11±1	16±2	21±1	24±1	10±1	15±1	19±2	23±1	09±1	14±2	18±1	21±1
24	12±1	15±2	19±1	27±1	09±1	13±1	17±1	26±0	08±1	13±2	18±1	25±1	11±0	16±1	20±1	28±1
25	20±1	27±2	33±1	38±1	19±1	26±1	32±1	36±2	19±1	25±1	29±1	33±2	19±1	26±2	31±1	38±1
26	09±1	12±1	19±1	26±1	06±1	14±1	21±2	24±2	07±1	10±0	12±1	16±1	08±2	10±2	14±1	19±2
27	-	-	-	-	07±1	11±2	15±1	17±2	08±1	12±1	15±1	19±1	08±1	12±1	14±2	17±2
28	08±1	12±1	15±2	18±1	07±1	12±2	16±1	18±1	-	-	-	-	-	07±0	10±1	15±1
29	15±1	22±1	27±2	30±2	14±1	17±1	21±2	25±1	16±2	24±2	30±1	35±2	16±2	19±1	25±1	30±1
30	10±1	15±1	21±1	24±0	08±1	14±2	20±1	23±1	07±1	14±1	19±1	21±1	06±1	09±1	14±1	18±1
31	07±2	11±2	15±1	18±2	06±2	12±1	16±2	19±1	07±1	10±2	11±1	15±0	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

33	-	-	-	-	-	-	-	-	05±1	09±2	11±2	14±0	07±1	10±2	12±1	14±2
34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	20±1	27±1	35±1	41±2	21±2	28±2	37±1	43±2	19±1	28±3	36±1	41±2	18±2	27±1	37±2	44±3
36	22±1	31±1	37±1	42±2	20±1	29±2	39±2	44±1	19±1	27±2	36±1	41±1	20±1	30±1	39±2	44±2
37	18±1	26±1	34±1	40±2	18±2	27±2	36±2	41±1	17±1	25±2	34±1	39±2	19±1	28±1	37±1	43±1
Std	**19±1**	**25±2**	**31±1**	**34±2**	**18±1**	**24±2**	**30±1**	**33±1**	**21±1**	**27±2**	**31±1**	**34±2**	**19±1**	**25±**	**31±1**	**34±2**
Control DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.
Std: Gentamicin**, NA:** No activity**, (±)** Standard deviation

Table 2: Minimum inhibitory concentration (MIC) of the synthesized compounds

Entry	MIC (µg/mL) Values[a]							
	Antibacterial				Antifungal			
	S. aureus	***B. substilis***	***E. Coli***	***K. pneumonia***	***A. niger***	***F. moniliforme***	***F. oxysporum***	***C. albicans***
2	-	-	-	-	24±2	24±0	23±3	22±4
4	-	-	-	-	22±4	24±0	25±0	22±3
5	-	-	--	-	22±3	22±0	24±4	21±4
9	22±2	25±4	26±3	28±0	-	-	-	-
12	25±2	27±4	24±4	24±0	-	-	-	-
14	25±7	24±4	24±4	24±3	-	-	-	-
15	-	-	-	-	26±3	24±4	22±3	28±4
24	20±4	21±2	20±3	21±3	-	-	-	-
25	22±4	23±4	26±3	28±0	-	-	-	-
29	28±4	28±4	24±2	26±4	-	-	-	-
35	19±4	20±4	19±2	18±0	24±3	25±0	23±0	24±0
36	18±4	17±4	18±2	18±0	25±3	22±3	21±3	20±2
37	18±2	17±4	16±4	20±3	22±3	21±4	22±4	20±4
Std (B)	**27±4**	**27±3**	**26±3**	**28±3**	-	-	-	-
Std (F)	-	-	-	-	**30±4**	**28±4**	**28±3**	**27±0**

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

Std (B): Gentamicin for antibacterial; **Std (F)**: Ketoconazole for antifungal

Table 3: Antifungal activity of the synthesized compounds

Entry	Zone of Inhibition (mm)[a]															
	Aspergillus niger				*Fusarium moniliforme*				*Fusarium oxysporum*				*Candida albicans*			
	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL
01	06±1	11±0	16±1	20±1	05±1	09±1	12±1	15±1	06±1	10±1	13±1	16±1	06±1	10±1	14±2	17±1
02	12±1	19±2	26±1	31±1	14±1	21±0	28±1	32±2	10±2	19±1	25±1	30±1	08±1	12±1	16±1	22±1
03	04±1	09±2	12±1	16±1	16±1	25±2	31±1	36±1	17±1	23±2	30±1	34±1	05±1	10±1	14±2	19±1
04	15±1	23±1	30±1	34±1	07±1	26±1	33±1	36±1	14±2	22±1	29±1	32±1	15±1	23±1	30±1	33±1
05	15±1	20±1	28±1	33±1	16±1	22±3	29±1	34±2	19±1	26±1	32±2	36±2	19±1	29±2	33±1	38±2
06	04±1	09±1	13±1	17±1	06±1	11±1	16±1	19±1	04±1	09±1	13±1	17±1	04±1	10±1	14±1	19±2
07	-	-	-	-	06±1	10±1	13±1	16±2	07±1	13±1	17±1	19±2	-	-	-	-
08	-	-	-	-	-	-	-	-	-		-	-	04±1	09±1	11±1	14±2
09	07±1	12±1	16±1	18±2	06±1	10±2	15±1	19±1	04±1	09±1	13±1	18±1	03±0	09±1	14±1	17±1
10	04±1	09±1	11±1	13±1	06±1	09±1	13±1	16±1	05±1	07±2	10±1	16±1	09±1	11±2	16±1	20±1
11	05±1	10±2	16±1	19±1	06±1	09±2	11±2	15±1	-	-	-	-	-	-	-	-
12	04±1	10±2	14±1	16±2	04±1	09±2	14±1	16±2	05±1	07±1	13±1	17±1	06±1	07±2	12±1	15±1
13	-	-	-	-	-	-	-	-	04±1	10±2	14±2	20±1	06±1	08±1	13±1	19±1
14	07±1	10±2	12±1	15±1	05±1	09±1	13±1	16±1	06±1	10±1	13±1	16±2	06±1	10±1	13±2	16±1
15	15±1	23±1	30±2	34±2	14±1	22±3	30±1	34±2	18±1	26±1	31±2	36±2	17±1	27±1	31±1	34±1
16	08±1	12±2	16±1	20±1	07±1	13±1	16±1	20±1	-	-	-	10±1	06±1	11±0	15±1	19±2
17	-	-	-	-	13±1	19±1	28±1	32±2	16±1	22±2	29±2	31±1	07±1	11±2	16±0	19±1
18	-	-	-	-	-	-	-	-	06±1	10±1	15±1	19±2	07±1	13±1	17±2	21±1
19	-	-	-	-	-	-	--	-	07±1	10±1	15±1	16±2	06±1	09±2	13±1	16±0
20	06±1	09±2	12±1	16±1	05±1	09±2	13±1	16±1	07±1	11±2	15±1	18±1	-	-	-	-
21	05±1	09±1	15±1	16±2	06±1	10±2	14±1	18±2	06±1	11±2	16±0	19±1	-	-	05±1	10±2
22	08±1	13±1	17±1	21±0	07±1	11±2	15±1	19±0	-	-	-	-	-	-	-	-
23	08±1	13±2	19±1	22±1	07±2	11±2	16±1	20±0	06±1	11±1	16±1	19±0	06±1	12±1	16±1	19±1
24	10±1	14±2	18±1	20±1	08±1	13±1	17±2	21±0	08±1	12±1	16±1	22±1	07±1	12±2	16±1	20±1
25	06±1	11±2	16±1	19±2	07±2	12±1	17±1	21±1	6±1	11±2	17±1	19±1	10±1	16±1	20±1	25±1
26	08±1	12±1	16±1	20±3	09±1	15±2	19±1	23±1	08±1	10±2	17±1	22±1	08±1	13±1	18±1	22±1
27	05±1	09±1	13±2	16±2	-	-	-	-	-	-	-	-	08±1	13±1	16±1	20±1
28	07±1	12±1	17±1	21±1	-	-	-	-	-	-	-	-	08±1	13±2	19±1	23±1
29	10±1	14±1	19±1	23±0	09±1	13±2	16±1	19±1	08±1	13±1	17±1	20±1	08±1	12±1	16±1	18±1
30	07±1	10±2	14±2	17±1	06±1	10±1	16±1	17±2	04±1	09±2	13±1	16±1	06±1	11±1	16±1	20±0
31	03±0	09±2	13±1	18±1	-	-	-	-	-	-	-	-	08±1	13±1	17±1	21±2
32	04±1	09±1	12±1	15±1	-	-	-	-	-	-	-	-	06±1	10±1	13±1	18±1

33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	04±1	10±1	14±1	16±1	-	-	-	-
35	16±1	22±1	29±1	32±1	19±1	26±2	31±1	34±2	17±2	25±1	31±1	34±1	17±1	22±3	29±1	33±2
36	18±1	27±2	34±1	40±2	20±1	28±1	37±1	40±1	19±1	28±2	35±2	40±2	17±1	25±1	32±1	38±2
37	17±1	25±1	30±1	37±3	16±1	25±1	34±1	36±2	16±1	24±1	30±2	33±2	15±1	22±1	27±1	33±2
Std	18±1	24±1	29±2	33±1	19±1	25±1	29±2	33±1	17±1	24±1	29±1	33±1	18±1	26±2	30±2	34±2
Control DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

Std: Ketoconazole, **NA:** No activity, **(±)** Standard deviation

Table 4: Evaluation of cytotoxicity for synthesized compounds **(1-37)**

Entry	MDA-MB- 231[a] IC_{50} µg/mL
1	-
2	-
3	-
4	62±0.24
5	57±0.65
6	75±0.01
7	61±0.12
8	42±0.21
9	36±0.13
10	56±0.41
11	48±0.11
12	40±0.12
13	55±0.20
14	34±0.10
15	29±0.31
16	70±0.33
17	64±0.21
18	50±0.14
19	52±0.42
20	56±0.10
21	58±0.31
22	77±0.21
23	48±0.31
24	49±0.21
25	47±0.15
26	72±0.10
27	61±0.31
28	62±0.22
29	44±0.16
30	59±0.14
31	65±0.22
32	71±0.31
33	74±0.27
34	61±0.29
35	52±0.27
36	38±0.36
37	40±0.28
Doxorubicin	**21±0.27**

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

Table 5. Predicted amino acid residues map in protein templates, determining molecular interactions with ligand **M-36.**

PDB-ID	**Protein molecule**	**Amino acid residues in H-bond**	**Amino acids residues in antifungal proteins with ligand within distance of 3.5Å with ligand**
1FI4	Mevalonate 5-diphosphate decarboxylase	Thr75	Arg74, Lys22, Asn28, Trp167, Met212, Ala119, Ser155, Trp167, Ala122, Gly152, Ser153, Ser121, Phe125, Ser108, Asp71, Leu61, Tyr19
3A58	Sec3p - Rho1p complex	Lys123, Val40, Thr24	Pro36, Glu37, Phe35, Tyr39, Ala20, Gly22, Cys25, Lys23, Asp129, Gln131, Gln135, The132, Asp92, Leu126
1S4N	α1,2-Mannosyltransferase	Val282	Tyr212, Tyr419, Leu321, His278, Glu279, Glu281, Thr210, Lys211, Val282, Tyr280, Glu281, Leu321, Asn320, Val281, Glu279, Tyr419
3HSB	YmaH (Hfq)	ND	Asn27, Leu26, Asn27, Glu304, Phe24, Gln27, Gly28
1MBT	MurB	Gly47, Ser50, Glu48, Gly49, Ile173, Arg159	Pro11, Gln120, Gln168, Asn51, Cys113, Leu46, Val52, Arg327, Gly115, Ile45, Ser116, Ile119, Asn65,Ala172, Ala85, Ser229, Gly123, Ile122, Glu325
1KE4	AmpC β-lactamase	Arg309, Ser257	Ser257, Tyr259, Leu254, Ala307, Leu254, Gln253, Ala307, Lys246, Leu241, Pro240, Lys238, Arg309, Ile252, Asn237, Gln256, Gln253, Ala79, Pro306, Thr305

Aryl fluorosulfate analogues as potent antimicrobial agents: SAR, cytotoxicity and docking studies

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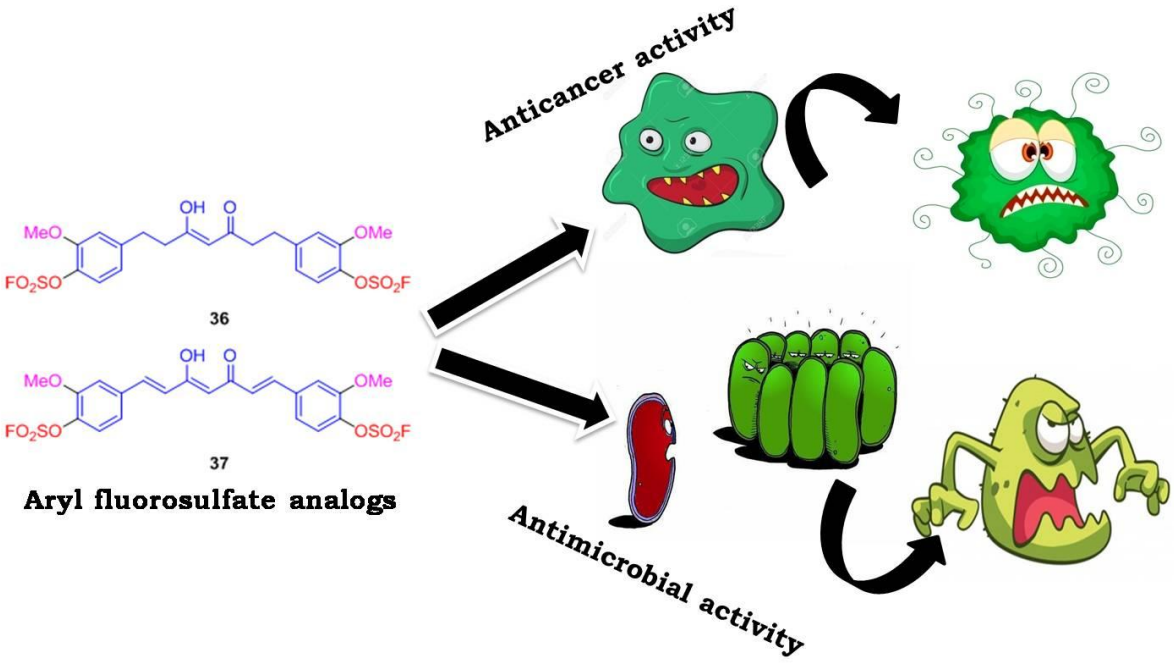

Research Highlights

1. Aryl fluorosulfates were synthesized directly from the phenols and SO_2F_2 in a simple method and all compounds were tested as antimicrobial, cytotoxicity and docking studies.
2. The compounds **9, 12, 14, 19, 25, 26, 35, 36** and **37** exhibited superior antibacterial activity.
3. Compounds **2, 4, 5, 15, 35, 36** and **37** were found to have better antifungal activity.
4. The structure-activity relationship (SAR) revealed that the antimicrobial activity depends upon the presence of $–OSO_2F$ group and slender effect of different substituent's on the phenyl rings. The electron donating (OCH_3) groups in analogs increase the antibacterial activity, and interestingly the electron withdrawing (Cl, NO_2, F and Br) groups increase the antifungal activity (except compound **35, 36** and **37**).