"An efficient synthesis of designed 4-thiazolidinone fused pyrimidine derivatives as potent antimicrobial agents"

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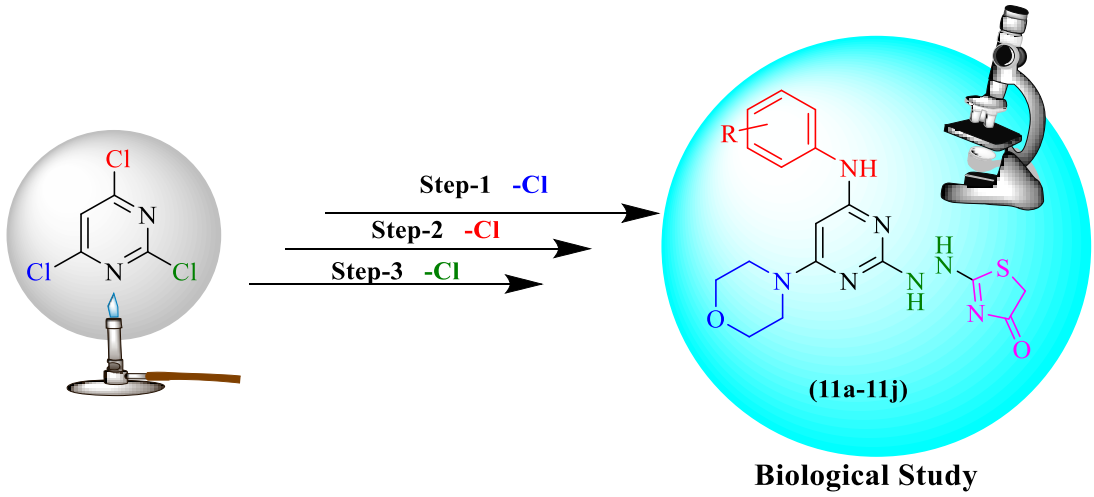
Abstract:

A novel series of hybrid 2-substituted ((pyrimidin-2-yl)hydrazinyl)thiazolidin-4-one derivatives were synthesized by means of aromatic nucleophilic displacement of chlorine atoms of 2,4,6-trichloro pyrimidine. Synthesis of some novel 2-(2-(6-morpholino-4-substituted(phenyl amino)pyrimidin-2-yl)hydrazinyl)thiazol-4(5H)-one derivatives have been carried out by the displacement of chlorine atoms on the basis of functionality concept on varying conditions. The synthesized hydrazinyl thiazolidin-4-one pyrimidine derivatives were evaluated for their expected antimicrobial activity; where, the majority of these compounds showed potent antibacterial and antifungal activities against the tested strains of bacteria and fungi. Afforded title analogs were subsequently characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy. SAR and HOMO-LUMO studies were also carried out for confirming the structure biological activity. Thus, these studies suggested that hydrazinyl pyrimidine derivatives bearing thiazolidinone moiety are interesting scaffolds for the development of novel antimicrobial agents.

Keywords: Pyrimidine, 4-Thiazolidinone, Antimicrobial activity, HOMO-LUMO/FMO

Graphical Abstract:



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Introduction:

In previous years, the population of human affected with several diseases caused by multidrug resistant *gram-positive* and *gram-negative* pathogenic bacteria so it became necessary to develop more efficient heterocyclic compounds [1-4]. As we know that heterocyclic chemistry comprises at least half of all organic chemistry research worldwide and also many natural drugs such as quinine, papavarine, emetine, atropine, procaine, codeine, reserpine and morphine are based on hetero atoms [5,6]. The antibiotics developed not just to treat human infection diseases, but also their property used in agriculture as well as in aquaculture. Since 1940s, Penicillin used in human therapeutics as an antibiotic and various other antibiotics are being continuously used [7]. Over a century, pyrimidine derivatives are important azaheterocycles that have inspired the development of new methodologies for their chemical synthesis [8,9]. At very early period in the history of organic chemistry, pyrimidines "*m*-diazine" were known as the breakdown products of uric acid [10]. The first pyrimidine derivative to be isolated was Alloxan in 1818 by Brugnatelli, oxidizing uric acid with nitric acid [11] also a pyrimidine nucleus is found in structure of many biological structures such as vitamins and in nucleic acids (**Figure-1**). A pyrimidine has many properties, electrophilic aromatic substitution gets more difficult while nucleophilic aromatic substitution gets easier. Additionally the advancement of the pyrimidine derivatives are secured by the replacement of the –Cl group from the commercially available 2,4,6-trichloro pyrimidine and design a new amine substituted pyrimidine derivatives. In the pyrimidine moiety on 2^{nd} position five membered saturated heterocyclic ring substitution leads to anthelmintic, antiparkinsonism, antiviral types of activities. Besides these, 2^{nd} and 4^{th} position of pyrimidine with substitution of amino groups leads to anticancer, antifungal, antibacterial and antiviral types of activities [12]. Inspired by these framework herein we designed some new compounds having saturated five-membered ring thiazolidinone on the 2^{nd} position of pyrimidine and other occupied 4^{th} & 6^{th} positions having amine substitution to get the new methodology for the development of new series of antimicrobial agents contains pyrimidine as a core moiety. Some of the most available marketed drugs containing pyrimidine in its core unit are shown in **Figure-2**.

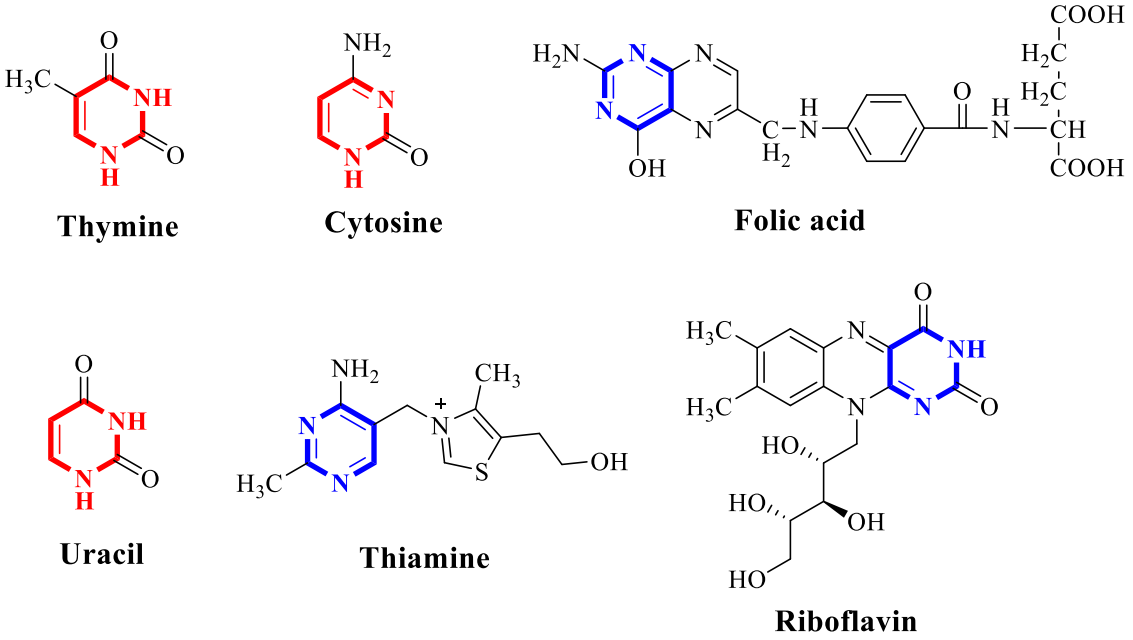


Figure-1: Some Vitamins & Nucleic acids containing pyrimidine nucleus.

On the other hand, numerous thiazolidinones containing various heterocylces have also proven to be of better pharmacological and therapeutically interest. The chemistry of thiazolidinone compounds has received a great interest because of their interesting biological activities such as anticonvulsant [13], hypnotic [14], anti-inflammatory [15], anticancer [16], antioxidant [17], antiproteolytic [18], antitubercular [19], anthelmintic [20], cardiovascular effects [21], antibacterial [22,23], antiviral [24], antifungal [25], insecticidal and herbicidal activities. In recent years, part of our research effort has focused on the synthesis and application of thiazolidinone derivatives containing heterocyclic rings. We have developed an efficient method for the synthesis of thiazolidininyl compounds by the coupling of various nucleophiles [26-28].

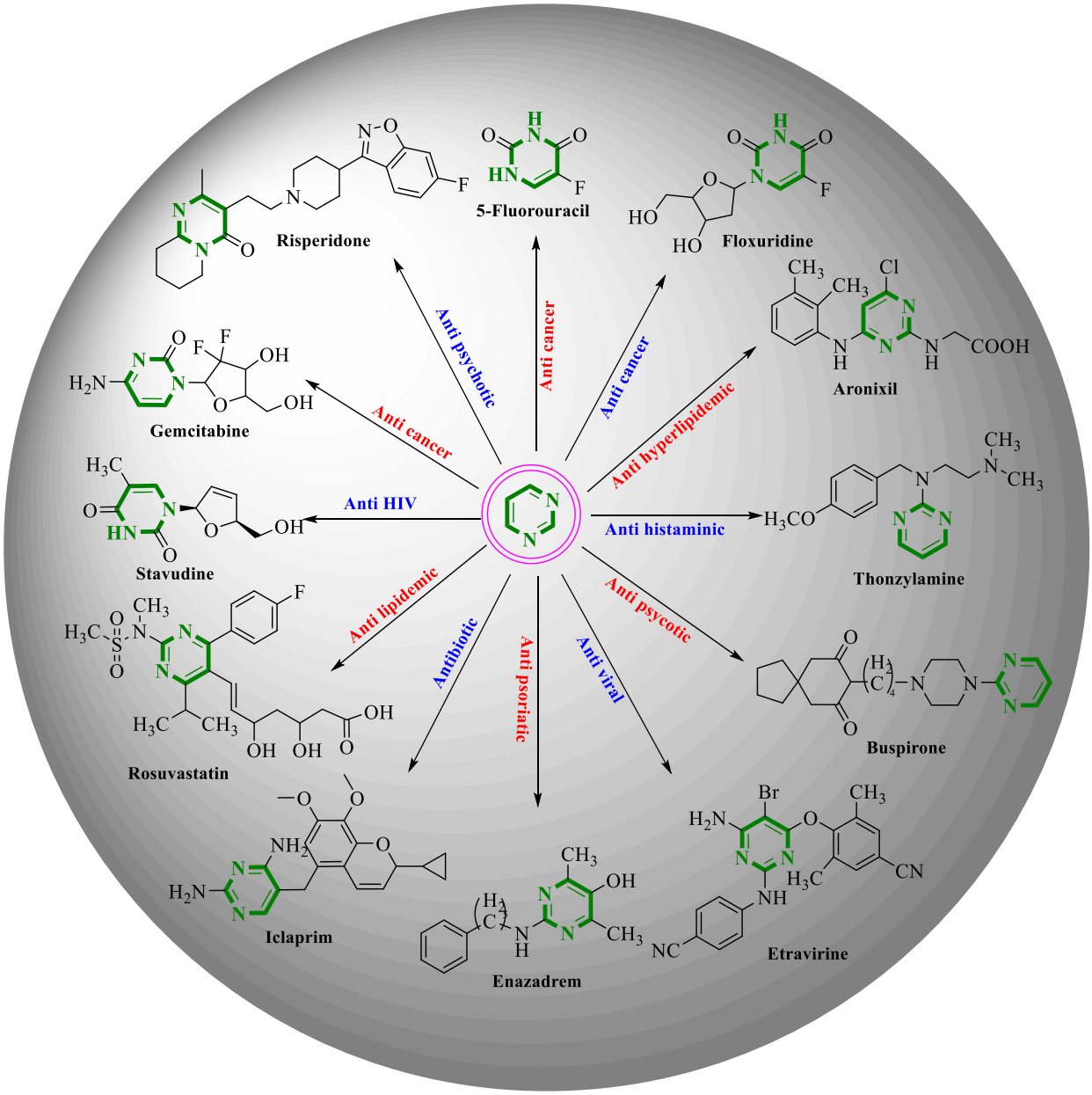


Figure-2: Marketed available drugs containing pyrimidine pharmacophore.

Many pyrimidinyl-thiazolidinone hybrid compounds were effective in causing a marked increase in growth inhibition activity against various types of bacteria, fungal and viruses. Effect of different substituent like morpholine is also of great interest due to its diverse activities such as antimicrobial, anthelmintic, bacterial and insecticidal activities [29]. Motivated by previously mentioned and in continuation of our enduring studies on pyrimidine as a versatile reagent heterocycles of pharmacological importance, it was considered worthwhile to design the synthesis of some bioactive derivatives of pyrimidinyl-hydrazinyl-thiazolidinone, which can be envisaged as the new hybrid lead compounds shown in **Figure-3**.

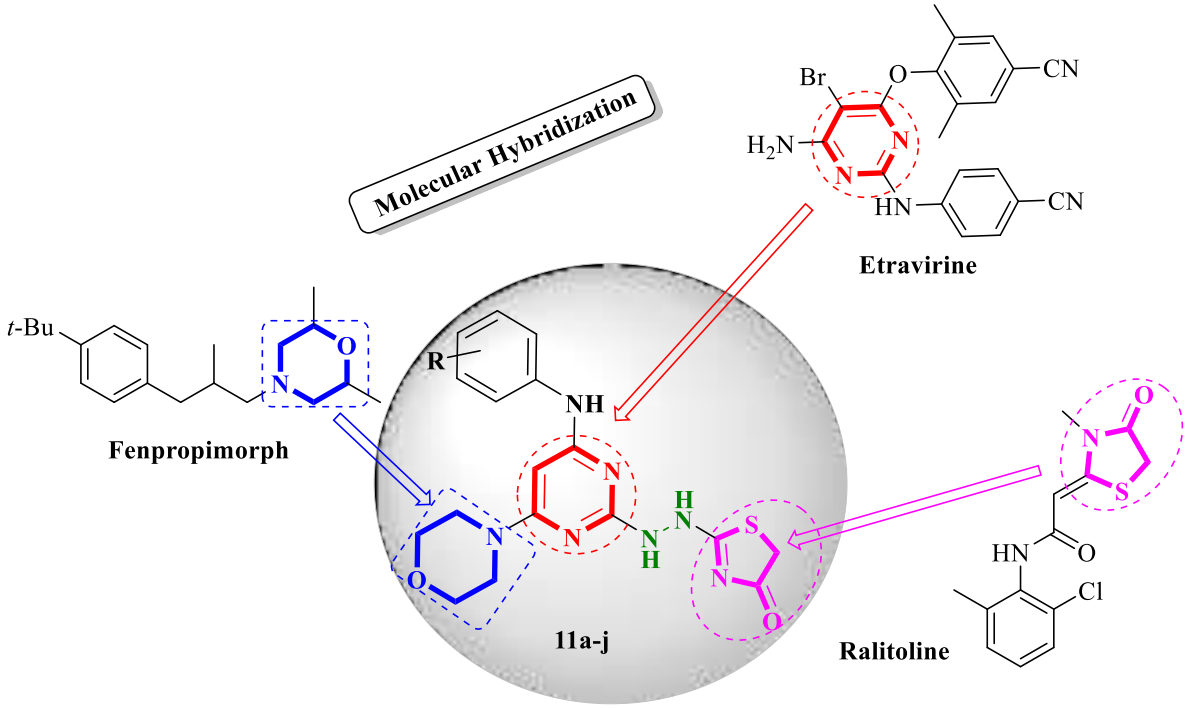


Figure-3: The design of pyrimidine-morpholine-thiazolidinone hybrid derivatives

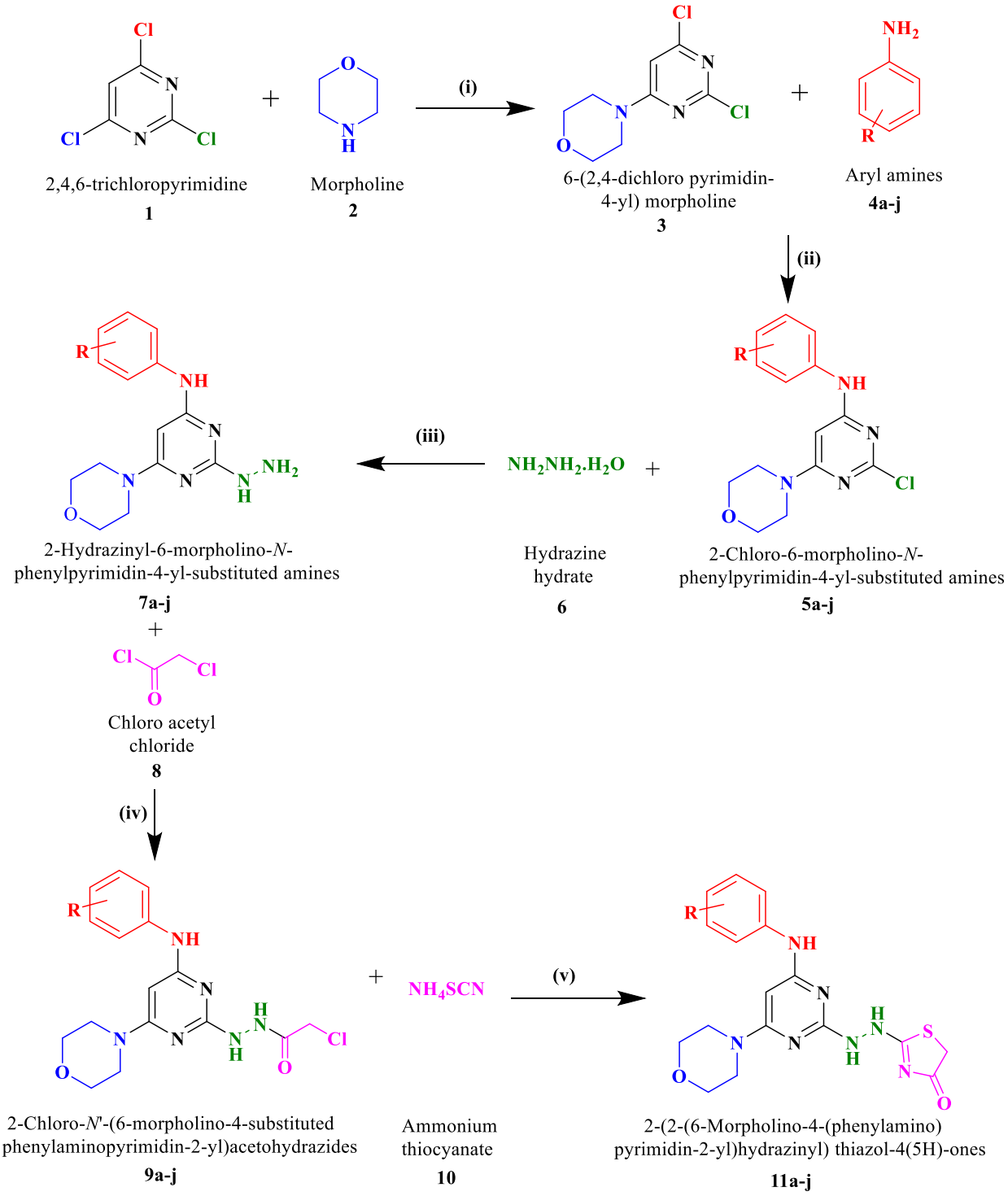
As the literature survey revealed that pyrimidine and thiazolidinone rings possess potential antimicrobial activity [30, 31]. It was thought that is worthwhile to link pyrimidinyl-thiazolidinone conjugated system to get enhanced bioactivity. In view of the above mentioned results and in a continuation of our research on pyrimidine derivatives an attempt has been made to identify new lead compounds that might be of value addition to generate a new class of antimicrobial agents. We report herein the synthesis and antimicrobial evaluation of a new series of pyrimidinyl-thiazolidinone derivatives linked through hydrazide function (**Scheme-1**) in order to achieve further knowledge of the structure-activity-relationship. Led by the previous facts and coupled with ongoing project aimed at investigating new bioactive molecules, pharmacophores, we report our results related to site-selective stepwise reaction of 2,4,6-trichloropyrimidine and provide convenient access to a variety of trisubstituted aminopyrimidines that are not readily available by other methods. The present work involves the synthesis and antimicrobial activity of new hybrid pyrimidinyl-thiazolidinone derivatives.

Chemistry:

A new series of pyrimidine based thiazolidinone derivatives have been synthesized by the temperature controlled reaction. This property allows substitution of three different nucleophiles into the same pyrimidine which provides a vast array of possible ((pyrimidin-2-yl)hydrazinyl)thiazolidin-4-one derivatives. The most promising methods for the synthesis of 2-(2-(6-morpholino-4-substituted(phenyl

amino)pyrimidin-2-yl)hydrazinyl)thiazol-4(5*H*)-one derivatives are multistep synthesis, starting from 2,4,6-trichloropyrimidine which is commercially available compound. The chlorine atom of 2,4,6-trichloro pyrimidine can be replaced in a stepwise process at different temperature by different nucleophiles. The reactivity order of three chlorine atoms in 2,4,6-trichloro pyrimidine during nucleophilic aromatic substitution reaction (SNAr) towards nucleophilic reagents are: $C_6 \geq C_4 > C_2$. [32,33] The first chlorine atom is replaced at 0-5°C temperature from 6th position of pyrimidine [34], second chlorine atom is replaced at 35-40°C temperature from 4th position of pyrimidine [35] and third chlorine atom is replaced at 70-100°C temperature form 2nd position of pyrimidine [36]. However, our synthetic strategy commences from 2,4,6-trichloropyrimidine **1** compound that treated with morpholine **2** led to the construction of requisite 6-(2,4-dichloropyrimidin-4-yl)morpholine **3** was achieved as per standard condensation reaction. These key compounds were stirred for 4 hour in an equimolar quantity with freshly prepared mixture in solvent THF under conditions 0-5°C to give compound 6-(2,4-dichloropyrimidin-4-yl)morpholine **3**, subsequently this compound **3** was allowed to reflux with various arylamines **4a-j** in solvent THF for 45-50°C affording 2-chloro-6-morpholino-*N*-phenylpyrimidin-4-yl-amine **5a-j** in good yield. Than compounds **5a-j** were further refluxed with hydrazine hydrate **6** to get the compounds 2-hydrazinyl-6-morpholino-*N*-phenylpyrimidin-4-yl-substituted amine **7a-j** which upon adding chloro acetyl chloride **8** at reflux conditions in THF to get compounds 2-chloro-*N'*-(6-morpholino-4-substituted phenyl aminopyrimidin-2-yl)acetohydrazide **9a-j**. Finally the corresponding target compounds 2-(2-(6-morpholino-4-substituted(phenylamino) pyrimidin-2-yl)hydrazinyl)thiazol-4(5*H*)-one **11a-j** were obtained by the addition of ammonium thiocyanate through intermolecular cyclization between compound **9a-j** and ammonium thiocyanate **10** under specific conditions. The confirmation of synthesized compounds **11a-j** were proved on the basis of IR, ^{13}C NMR & ^{1}H NMR spectral analysis and the purity was as certain by elemental analysis. Herein we also report log *P* value of the synthesized title compounds which is used in QSAR studies and rational drug design as a measure of molecular hydrophobicity which affects drug bioavailibity, adsorption, hydrophobic drug-receptor interactions, metabolism of molecules as well as toxicity of the compounds. Log *P* value must not be more than 5.0.

Scheme-1: Synthesis of target compounds.



Synthesis of final pyrimidine derivatives, Reagents and condition: (i) THF, 10% $NaHCO_3$, 0-5°C /4 h (ii) THF, 10% $NaHCO_3$, 45-50°C /6 h (iii) THF, 10% $NaHCO_3$, 80-90°C /4 h (iv) 2-3 drops TEA, Toluene, 0-5°C /3-4 h (v) Reflux, Ethanol, 2 h.

Where, R=

11a	H
11b	3-NO_2
11c	4-NO_2
11d	2-Cl
11e	3-Cl
11f	4-Cl
11g	2-CH_3
11h	3-CH_3
11i	4-CH_3
11j	4-OCH_3

Medicinal chemistry part:

***In vitro* evaluation of antimicrobial activity:**

In order to study the antimicrobial properties of the novel hybrid pyridinyl-hydrazinyl-thiazolidinone derivatives, several bacterial (*Staphylococcus aureus* MTCC 96, *Staphylococcus pyogenus* MTCC 442, *Pseudomonas aeruginosa* MTCC 741, *Escherichia coli* MTCC 443) and fungal (*Candida albican* MTCC 227, *Aspergillus niger* MTCC 282, *Aspergillus clavatus* MTCC 1323) species were selected and minimum inhibitory concentration (MIC) of the compound was determined by the agar streak dilution method. A stock solution of the tested compound (100 µg/mL) in dimethyl sulfoxide was prepared and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar, i.e. nutrient agar for the evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test compound was poured into a petri dish at a depth of 4-5 mm and allowed to solidify under aseptic conditions. A suspension of the respective microorganism of approximately 105 CFU/mL was prepared and applied to plates with serially diluted compounds with concentrations in the range of 3.125-100 µg/mL in dimethyl sulfoxide and incubated at (37±1)°C for 24 hours. The lowest concentration of the substance which prevents the development of visible growth is considered to be the MIC value.

Material & Methodology:

Melting points were determined in open capillaries on a veego electronic apparatus VMP-D and are uncorrected. IR spectra of synthesized compounds were recorded on a shimadzu 8400-S FT-IR spectrometer using KBr pellets. Thin layer chromatography was performed on object glass slides (2×4 cm) coated with silica gel-G and spots were visualized under UV irradiation and also by ninhydrin solution to prove the presence of -NH_2 functional group. ^{1}H NMR spectra were recorded on a varian 400 MHz model spectrometer using dimethyl sulfoxide as a solvent and chemical shifts (δ) were reported in parts per million (ppm) with reference to tetramethylsilane as internal standard and

coupling constants (*J*) were reported in Hertz (Hz). ^{13}C NMR spectra were obtained on a Bruker 100 MHz AC-300 spectrometer in dimethyl sulfoxide. Elemental analysis (C, H, N) were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany). The log P values of the compounds were determined by ChemBioDraw Ultra 12.0 program.

Experimental Section:

(i) 6-(2,4-Dichloro pyrimidin-4-yl)morpholine (3):-

Morpholine **2** (0.85 ml, 0.00981 mol) in THF (5 ml) was slowly added to a well stirred slurry of 2,4,6-trichloro pyrimidine **1** (2.0 g, 0.01090 mol) in THF (5 ml) at 0-5°C and stirred for 4 hours. pH of the solution was adjusted to neutral by the gradual addition of 10% $NaHCO_3$ solution. Progress of the reaction was monitored by TLC using hexane: ethyl acetate (7:3) as a solvent system. After the completion of reaction, it was poured into crushed ice. The solid product obtained which was filtered, washed with water and dried. The crude product was purified by crystallization from ethanol to get the title compound **3**. White solid, 90 % Yield, M.P. = 138-140°C

(ii) General procedure of 2-chloro-6-morpholino-*N*-phenylpyrimidin-4-yl-substituted amine (5a-j):-

To a stirred solution of 6-(2,4-dichloro pyrimidin-4-yl) morpholine **3** (2.0 g, 0.00854 mol) in THF (5 ml), substituted amines **4a-j** (0.00769 mol) in THF (3 ml) was added at 5-10°C, the temperature was gradually raised to 45-50°C and stirred for 6 hours. The pH of reaction mixture was adjusted to neutral by the gradual addition of 10% $NaHCO_3$ solution. Progress of the reaction was monitored by TLC using hexane: ethyl acetate (8:2) as a solvent system. After the completion of reaction the reaction mixture was poured into crushed ice. The solid product obtained was filtered washed with the copious quantity of water and dried. The crude product was purified by crystallization from ethanol to get the title compound **5a-j**.

(iii) General procedure of 2-hydrazinyl-6-morpholino-*N*-phenylpyrimidin-4-yl-substituted amine (7a-j):-

A mixture of 2-chloro-6-morpholino-N-phenyl pyrimidin-4-yl- substituted amine **5a-j** (0.00687 mol) and hydrazine hydrate **6** (0.344 ml, 0.00687 mol) in THF (15 ml) was heated on water bath at 40°C for 1 hour than the temperature was gradually raised to 80-90°C and refluxed for 4 hours. The pH was adjusted to neutral by the addition of 10% $NaHCO_3$ solution. Progress of the reaction was monitored by TLC using hexane: ethyl acetate (5:5) as a solvent system. After the completion of reaction the reaction mixture was added to cold water. The solid product obtained was filtered washed with water

and dried. The crude product was purified by crystallization from ethanol to get the title compound **7a-j**.

(iv) General procedure of 2-chloro-*N'*-(6-morpholino-4-substituted phenylaminopyrimidin- 2-yl)acetohydrazide (9a-j):

A mixture of chloro acetyl chloride **8** (0.55 ml, 0.00698 mol) and toluene (2 ml) were stirred at 0-5°C for 1 hour. To this solution 2-hydrazinyl-6-morpholino-*N*-phenyl pyrimidin-4-yl-substituted amine **7a-j** (0.00698 mol) in toluene (5 ml) along with 3-4 drops of triethyl amine was added drop wise and temperature was gradually raised to 60-70°C and then refluxed for 3-4 hours. Progress of the reaction was monitored by TLC using hexane: ethyl acetate (9:1) as a solvent system. After the completion of reaction the reaction mixture was cooled to room temperature then dumped in normal water to get the product, which was filtered, washed with toluene and recrystallized from ethanol to get the title compound **9a-j**.

(v) General procedure of 2-(2-(6-morpholino-4-substituted(phenylamino)pyrimidin-2-yl)hydrazinyl)thiazol-4(5*H*)-one (11a-j):

To a solution of 2-chloro-*N*'-(6-morpholino-4-substituted amino pyrimidin-2-yl) acetohydrazide **9a-j** (0.05512 mol) in ethanol (5 ml), ammonium thiocyanate **10** (0.42 g, 0.05512 mol) in ethanol (10 ml) was added & refluxed for 4 hours and allowed to stand overnight so the precipitates were observed. Progress of the reaction was monitored by TLC using hexane: ethyl acetate (8:2) as a solvent system. After the completion of reaction, the resulting precipitates were filtered out washed with copious quantity of water and then crystallized by ethanol to get the title compound **11a-j**. Based on the above method the other substituted analogous were prepared, their physical and analytical data are mentioned in **Table-1.**

Table-1: Physical and Analytical data of Compounds 11a-j

Comp. No.	-R	Molecular Formula	M.P. °C	Yield %	Log P Value	Elemental analysis			
							% C	% H	% N
11a	**H**	$C_{17}H_{19}N_7O_2S$	180	70	2.95	R F	52.97 52.94	4.97 4.92	25.44 25.41
11b	**3-NO_2**	$C_{17}H_{18}N_8O_4S$	196	65	0.97	R F	47.44 47.41	4.21 4.18	26.03 26.00
11c	**4-NO_2**	$C_{17}H_{18}N_8O_4S$	199-201	56	0.97	R F	47.44 47.40	4.21 4.19	26.03 26.05
11d	**2-Cl**	$C_{17}H_{18}N_7O_2SCl$	193	62	3.51	R F	48.63 48.59	4.32 4.30	23.35 23.38
11e	**3-Cl**	$C_{17}H_{18}N_7O_2SCl$	182-184	58	3.51	R F	48.63 48.60	4.32 4.29	23.35 23.31
11f	**4-Cl**	$C_{17}H_{18}N_7O_2SCl$	184	64	3.51	R F	48.63 48.65	4.32 4.36	23.35 23.33

11g	**2-CH_3**	**$C_{18}H_{21}N_7O_2S$**	186	62	3.43	R F	54.12 54.09	5.30 5.26	24.54 24.51
11h	**3-CH_3**	**$C_{18}H_{21}N_7O_2S$**	191-193	60	3.43	R F	54.12 54.14	5.30 5.27	24.54 24.49
11i	**4-CH_3**	**$C_{18}H_{21}N_7O_2S$**	194	62	3.43	R F	54.12 54.10	5.30 5.25	24.54 24.50
11j	**4-OCH_3**	**$C_{18}H_{21}N_7O_3S$**	197-199	54	2.82	R F	52.04 52.01	5.09 5.04	23.60 23.56

Characterisation of Products:

Compound 11a*: 2-(2-(6-Morpholino-4-(phenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* White solid, Yield= 70 %, M.P.= 180°C. IR (8400-S, KBr): 734 cm^{-1} (-C-S- stretching in thiazolidinone), 1160 cm^{-1} (-C-O-C-), 1335 cm^{-1} (-C-NH-), 1495 cm^{-1} (-C=C-C-), 1509 cm^{-1} (-C-C-), 1540 cm^{-1} (-C=C-C-), 1613 cm^{-1} (-NH-), 1635 cm^{-1} (-C=O), 3330 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 3.387 (t, *J*= 3.8 Hz, 4H), 3.482 (t, *J*= 4.2 Hz, 4H), 3.635 (d, *J*= 6.0 Hz, 1H), 3.786 (d, *J*= 5.6 Hz, 1H), 3.983 (s, 2H), 6.423 (s, 1H), 6.944-7.242 (m, 5H) and 9.003 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 35.15, 45.92, 65.59, 77.60, 120.50, 123.06, 129.04, 141.27, 157.44, 161.12, 163.48, 182.42, 184.67. Elemental analysis For $C_{17}H_{19}N_7O_2S$: C: 52.97, H: 4.97, N: 25.44 *Found: C: 52.94, H: 4.92, N: 25.41*. ESIMS: m/z for $(M+H)^+$ 386.10

Compound 11b*: 2-(2-(6-Morpholino-4-(3-nitrophenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* Light yellow, Yield= 65 %, M.P.= 196°C. IR (8400-S, KBr): 741 cm^{-1} (-C-S-), 1171 cm^{-1} (-C-O-C-), 1340 cm^{-1} (-C-NH-), 1497 cm^{-1} (-C=C-C-), 1520 cm^{-1} (-C-C-), 1542 cm^{-1} (-C=C-C-), 1555 cm^{-1} ($-NO_2$), 1614 cm^{-1} (-NH-), 1630 cm^{-1} (-C=O), 3323 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 3.388 (t, *J*= 3.2 Hz, 4H), 3.763 (t, *J*= 3.0 Hz, 4H), 3.771 (d, *J*= 6.4 Hz, 1H), 3.789 (d, *J*= 5.2 Hz, 1H), 3.980 (s, 2H), 6.428 (s, 1H), 6.941-7.202 (m, 5H) and 9.036 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 35.15, 45.92, 65.59, 77.60, 115.14, 115.92, 124.12, 129.85, 144.48, 149.21, 157.44, 161.12, 163.48, 182.42, 184.67. Elemental analysis For $C_{17}H_{18}N_8O_4S$: C: 47.44, H: 4.21, N: 26.03 *Found: C: 47.41, H: 4.18, N: 26.00*. ESIMS: m/z for $(M+H)^+$ 431.12

Compound 11c*: 2-(2-(6-Morpholino-4-(4-nitrophenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* Yellow solid, Yield= 56 %, M.P.= 199-201°C. IR (8400-S, KBr): 732 cm^{-1} (-C-S-), 1165 cm^{-1} (-C-O-C-), 1330 cm^{-1} (-C-NH-), 1480 cm^{-1} (-C=C-C-), 1510 cm^{-1} (-C-C-), 1530 cm^{-1} ($-NO_2$), 1533 cm^{-1} (-C=C-C-), 1623 cm^{-1} (-NH-), 1639 cm^{-1} (-C=O), 3331 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 3.398 (t, *J*= 3.4 Hz, 4H), 3.575 (t, *J*= 3.8 Hz, 4H), 3.715 (d, *J*= 6.6 Hz, 1H), 3.780 (d, *J*= 5.4 Hz, 1H), 3.991 (s, 2H), 6.418 (s, 1H), 6.461-7.187 (m, 5H) and 9.146 (s, 1H). ^{13}C

NMR (100 MHz, DMSO) δppm: 35.15, 45.92, 65.59, 77.60, 119.71, 127.10, 142.45, 148.13, 157.44, 161.13, 163.48, 182.42, 184.67. Elemental analysis For $C_{17}H_{18}N_8O_4S$: C: 47.44, H: 4.21, N: 26.03 *Found: C: 47.40, H: 4.19, N: 26.05.* ESIMS: m/z for $(M+H)^+$ 431.19

Compound 11d*: 2-(2-(6-Morpholino-4-(2-chlorophenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* Off-white solid, Yield= 62 %, M.P.= 193-195°C. IR (8400-S, KBr): 747 cm^{-1} (-C-S-), 815 cm^{-1} (-Cl), 1173 cm^{-1} (-C-O-C-), 1333 cm^{-1} (-C-NH-), 1482 cm^{-1} (-C=C-C-), 1530 cm^{-1} (-C-C-), 1543 cm^{-1} (-C=C-C-), 1627 cm^{-1} (-NH-), 1641 cm^{-1} (-C=O), 3337 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 3.388 (t, *J*= 3.0 Hz, 4H), 3.762 (t, *J*= 3.2 Hz, 4H), 3.771 (d, *J*= 6.8 Hz, 1H), 3.795 (d, *J*= 5.6 Hz, 1H), 4.001 (s, 2H), 6.522 (s, 1H), 6.933-7.282 (m, 5H) and 9.015 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 35.15, 45.92, 65.59, 77.97, 122.55, 124.41, 127.01, 127.68, 131.25, 140.31, 156.49, 160.91, 163.42, 183.42, 184.67. Elemental analysis For $C_{17}H_{18}N_7O_2SCl$: C: 48.63, H: 4.32, N: 23.35 *Found: C: 48.59, H: 4.30, N: 23.38.* ESIMS: m/z for $(M+H)^+$ 421.11

Compound 11e*: 2-(2-(6-Morpholino-4-(3-chlorophenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* White solid, Yield= 58 %, M.P.= 182-184°C. IR (8400-S, KBr): 733 cm^{-1} (-C-S-), 824 cm^{-1} (-Cl), 1169 cm^{-1} (-C-O-C-), 1341 cm^{-1} (-C-NH-), 1483 cm^{-1} (-C=C-C-), 1511 cm^{-1} (-C-C-), 1530 cm^{-1} (-C=C-C-), 1621 cm^{-1} (-NH-), 1637 cm^{-1} (-C=O), 3334 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 3.395 (t, *J*= 3.8 Hz, 4H), 3.763 (t, *J*= 3.2 Hz, 4H), 3.778 (d, *J*= 5.6 Hz, 1H), 3.797 (d, *J*= 4.8 Hz, 1H), 4.407 (s, 2H), 6.519 (s, 1H), 6.814-7.199 (m, 4H) and 9.002 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 35.15, 45.92, 65.59, 77.60, 120.29, 120.32, 122.21, 130.18, 134.47, 144.23, 157.44, 161.12, 163.48, 182.42, 184.67. Elemental analysis For $C_{17}H_{18}N_7O_2SCl$: C: 48.63, H: 4.32, N: 23.35 *Found: C: 48.60, H: 4.29, N: 23.31*. ESIMS: m/z for $(M+H)^+$ 421.14

Compound 11f*: 2-(2-(6-Morpholino-4-(4-chlorophenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* Off-white solid, Yield= 64 %, M.P.= 184°C. IR (8400-S, KBr): 742 cm^{-1} (-C-S-), 834 cm^{-1} (-Cl), 1173 cm^{-1} (-C-O-C-), 1343 cm^{-1} (-C-NH-), 1491 cm^{-1} (-C=C-C-), 1521 cm^{-1} (-C-C-), 1531 cm^{-1} (-C=C-C-), 1628 cm^{-1} (-NH-), 1639 cm^{-1} (-C=O), 3339 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 3.346 (t, *J*= 3.0 Hz, 4H), 3.641 (t, *J*= 4.8 Hz, 4H), 3.763 (d, *J*= 6.4 Hz, 1H), 3.783 (d, *J*= 5.8 Hz, 1H), 4.233 (s, 2H), 6.559 (s, 1H), 6.925-7.153 (m, 4H) and 9.079 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 35.15, 45.92, 65.59, 77.60, 121.53, 128.96, 128.97, 140.50, 157.44, 161.13, 163.48, 182.42, 184.67. Elemental analysis For $C_{17}H_{18}N_7O_2SCl$: C: 48.63, H: 4.32, N: 23.35 *Found: C: 48.65, H: 4.36, N: 23.33.* ESIMS: m/z for $(M+H)^+$ 421.09

Compound 11g*: 2-(2-(6-Morpholino-4-(2-methylphenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* Light brown solid, Yield= 62 %, M.P.= 186°C. IR (8400-S, KBr): 753 cm^{-1} (-C-S-), 1180 cm^{-1} (-C-O-C-), 1345 cm^{-1} (-C-NH-), 1499 cm^{-1} (-C=C-C-), 1531 cm^{-1} (-C-C-), 1561 cm^{-1} (-C=C-C-),

1641cm^{-1} (-NH-), 1651 cm^{-1} (-C=O), 2854 cm^{-1} (-CH_3), 3328 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 2.278 (s, 3H), 3.336 (t, *J*= 4.8 Hz, 4H), 3.440 (t, *J*= 3.6 Hz, 4H), 3.761 (d, *J*= 6.6 Hz, 1H), 3.794 (d, *J*= 4.6 Hz, 1H), 4.538 (s, 2H), 5.017 (s, 1H), 6.943-7.153 (m, 5H) and 9.079 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 17.35, 35.15, 45.92, 65.59, 77.97, 121.66, 122.96, 126.68, 129.67, 131.17, 141.69, 156.49, 160.91, 163.42, 182.42, 184.67. Elemental analysis For $C_{18}H_{21}N_7O_2S$: C: 54.12, H: 5.30, N: 24.54 *Found: C: 54.09, H: 5.26, N: 24.51.* ESIMS: m/z for (M+H)$^+$ 399.98

Compound 11h*: 2-(2-(6-Morpholino-4-(3-methylphenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* Brown solid, Yield= 60 %, M.P.= 191-193°C. IR (8400-S, KBr): 750 cm^{-1} (-C-S-), 1175 cm^{-1} (-C-O-C-), 1347 cm^{-1} (-C-NH-), 1482 cm^{-1} (-C=C-C-), 1523 cm^{-1} (-C-C-), 1545 cm^{-1} (-C=C-C-), 1630 cm^{-1} (-NH-), 1649 cm^{-1} (-C=O), 2871 cm^{-1} (-CH_3), 3327 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 2.360 (s, 3H), 3.347 (t, *J*= 4.0 Hz, 4H), 3.442 (t, *J*= 4.4 Hz, 4H), 3.763 (d, *J*= 5.8 Hz, 1H), 3.809 (d, *J*= 5.2 Hz, 1H), 4.282 (s, 2H), 5.904 (s, 1H), 6.798-7.204 (m, 5H) and 8.865 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 21.20, 35.15, 45.92, 65.59, 77.60, 118.29, 119.89, 123.12, 127.81, 139.64, 143.72, 157.44, 161.12, 163.48, 182.42, 184.67. Elemental analysis For $C_{18}H_{21}N_7O_2S$: C: 54.12, H: 5.30, N: 24.54 *Found: C: 54.14, H: 5.27, N: 24.49.* ESIMS: m/z for (M+H)$^+$ 400.12

Compound 11i*: 2-(2-(6-Morpholino-4-(4-methylphenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* Light brown solid, Yield= 62 %, M.P.= 194°C. IR (8400-S, KBr): 754 cm^{-1} (-C-S-), 1177 cm^{-1} (-C-O-C-), 1351 cm^{-1} (-C-NH-), 1489 cm^{-1} (-C=C-C-), 1524 cm^{-1} (-C-C-), 1547 cm^{-1} (-C=C-C-), 1634 cm^{-1} (-NH-), 1651 cm^{-1} (-C=O), 2891 cm^{-1} (-CH_3), 3329 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 2.321 (s, 3H), 3.348 (t, *J*= 3.0 Hz, 4H), 3.443 (t, *J*= 4.2 Hz, 4H), 3.763 (d, *J*= 6.8 Hz, 1H), 3.807 (d, *J*= 5.0 Hz, 1H), 4.277 (s, 2H), 5.903 (s, 1H), 6.928-7.133 (m, 4H) and 9.277 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 21.12, 35.15, 45.92, 65.59, 77.60, 119.60, 129.07, 132.50, 138.82, 157.44, 161.12, 163.48, 182.42, 184.67. Elemental analysis For $C_{18}H_{21}N_7O_2S$: C: 54.12, H: 5.30, N: 24.54 *Found: C: 54.10, H: 5.25, N: 24.50.* ESIMS: m/z for (M+H)$^+$ 400.19

Compound 11j*: 2-(2-(6-Morpholino-4-(4-methoxyphenylamino) pyrimidin-2-yl)hydrazinyl) thiazol-4(5H)-one.* White solid, Yield= 54 %, M.P.= 197-199°C. IR (8400-S, KBr): 763 cm^{-1} (-C-S-), 1179 cm^{-1} (-C-O-C-), 1209 cm^{-1} (-O-), 1353 cm^{-1} (-C-NH-), 1492 cm^{-1} (-C=C-C-), 1531 cm^{-1} (-C-C-), 1551 cm^{-1} (-C=C-C-), 1639 cm^{-1} (-NH-), 1654 cm^{-1} (-C=O), 3333 cm^{-1} (-NH-NH-). ^{1}H NMR (400 MHz, DMSO) δppm: 3.369 (t, *J*= 3.8 Hz, 4H), 3.427 (t, *J*= 4.4 Hz, 4H), 3.735 (d, *J*= 6.8 Hz, 1H), 3.769 (s, 3H), 3.774 (d, *J*= 5.6 Hz, 1H), 4.780 (s, 2H), 5.677 (s, 1H), 6.830-7.921 (m, 4H) and 8.910 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δppm: 35.15, 45.92, 56.03, 65.59, 77.60, 115.58, 121.53, 134.81, 156.26, 157.44, 161.12, 163.48, 182.42, 184.67. Elemental analysis For $C_{18}H_{21}N_7O_3S$: C: 52.04, H: 5.09, N: 23.60 *Found: C: 52.01, H: 5.04, N: 23.56.* ESIMS: m/z for (M+H)$^+$ 416.22

Result and Discussion:

The entire tested compounds contained pyrimidine as the core unit structure substituted with derivatives of morpholine, various substituted amines and 4-thiazolidinone through hydrazine precursor. The antimicrobial activities of bi-heterocyclic entities i.e. pyrimidine, 4-thiazolidinone and their derivatives have already been discussed. Urged by their findings and further exploration of the thiazolidinone ring system as a promising nucleus in search of new antimicrobial agent, it was of interest to hybridize this nucleus with various substituted pyrimidine attached at position-2 of the thiazolidinone ring through hydrazine spacer.

The antimicrobial potency in terms of MIC value are summarized in **Table-II**. Results of MIC values of the compounds are observed in the varied range (62.5 to 500µg/ml) to antibacterial activities against all the tested bacterial strains. For Gram negative strains compounds **11g** & **11h** with electron donating group like 2 & 3-CH_3 respectively and electron withdrawing group like **11b** (3-NO_2) to phenyl nucleus appeared with MIC value 100 and 62.5 respectively. While against Gram positive strains **11d** with electron withdrawing group like 2-Cl and **11h** with electron donating group like 3-CH_3 to phenyl nucleus appeared with MIC 100. Rest of other compounds appeared with moderate to good activity profile.

The antifungal potency in terms of MIC values are summarized in **Table-II**. Results of MIC values of the compounds are observed in the varied range (250 to >1000 µg/ml) against studied fungal strains. No significant deviation of activities are observed against all studied fungal strains except some electron donating group like 2, 3 & 4-CH_3 (**11g, 11h & 11i)** group and one electron withdrawing group shows activity to some extent against *C. albicans* only. Rest of other compounds appeared with moderate to good activity profile.

Table-2: *In vitro* antibacterial & antifungal activities of compounds 11a-j

Comp. No.	Minimum Inhibitory Concentration							
	Antibacterial activity					Antifungal activity		
	R	Gram Positive		Gram Negative		---		
		S.aureus **MTCC 96 µg/ml**	***S.pyogenus*** **MTCC 443 µg/ml**	***P.aeruginosa*** **MTCC 741 µg/ml**	***E.coli*** **MTCC 442 µg/ml**	***C.albicans*** **MTCC 227 µg/ml**	***A.niger*** **MTCC 282 µg/ml**	***A.clavatus*** **MTCC 1323 µg/ml**
11a	H	500	500	250	250	500	250	250
11b	3-NO_2	250	250	62.5	100	1000	500	1000
11c	4-NO_2	250	200	200	125	500	500	1000
11d	2-Cl	100	250	500	500	500	500	500
11e	3-Cl	200	250	500	500	1000	500	1000
11f	4-Cl	250	250	125	200	1000	250	500
11g	2-CH_3	200	200	100	100	250	>1000	>1000

11h	3-CH_3	100	100	100	125	250	>1000	>1000
11i	4-CH_3	200	200	250	250	250	500	500
11j	4-OCH_3	200	200	100	100	250	>1000	500
Standard Drug	**Ciprofloxacin**	**50**	**50**	**25**	**25**	**--**	**--**	**--**
	Nystatin	**--**	**--**	**--**	**--**	**100**	**100**	**100**

Note* ***S.aureus:*** *Staphylococcus aureus,* ***S.pyogenus:*** *Streptococcus pyogenus,* ***P.aeruginosa:*** *Pseudomonas aeruginosa,* ***E.coli:*** *Escherichia coli,* ***C.albicans:*** *Candida albicans,* ***A.niger:*** *Aspergillus niger,* ***A.clavatus:*** *Aspergillus clavatus*

Frontier molecular orbital analysis (HOMO–LUMO concept):

Molecular orbitals (HOMO and LUMO) and their properties such as energy are a very useful tool for physicists and chemists [37]. The calculated HOMO and LUMO energies show that charge transfer occurs within the molecule. They are also very important parameters for quantum chemistry. Both HOMO and LUMO are the main orbitals taking part in chemical stability [38]. This electronic absorption corresponds to the transition from the ground state to the first excited state, and is mainly described by one electron excitation from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The HOMO– LUMO energy gap of the compound reveals that the energy gap reflects the biological activity of the molecule. [39–40] The HOMO represents the ability to offer an electron and LUMO as an electron-acceptor represents the ability to gain an electron. The atomic orbital compositions for **11a, 11b, 11f, 11i** and **11j** of the frontier molecular orbitals are sketched in **Figure-4**.

i) In the case of compound **11a,** the HOMO is located over pyrimidine and the N-atom of morphine and the HOMO-LUMO transition implies an electron density transfer to the N-atom of pyrimidine ring.

ii) In the case of compound **11b,** the HOMO is located over the C-atoms of pyrimidine and the N-atom of morphine and the HOMO-LUMO transition implies an electron density transfer to the $–NO_2$ group of substituted aniline ring and N-atom of pyrimidine ring.

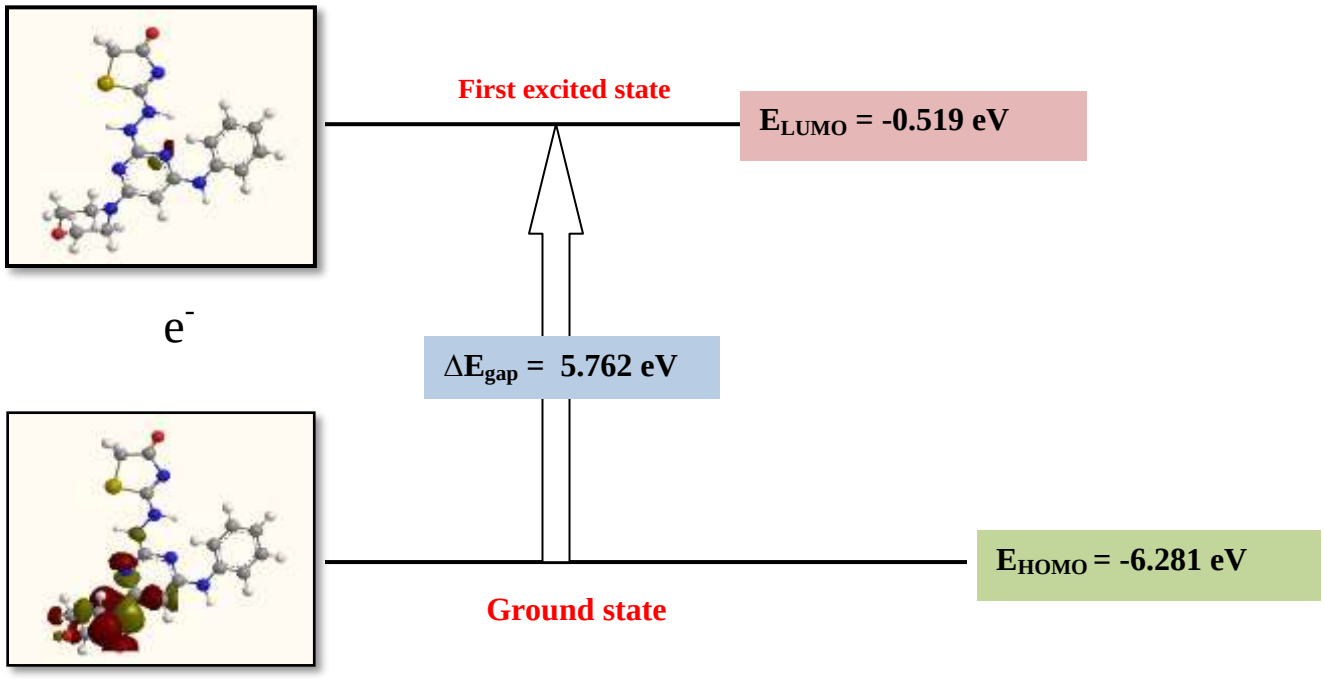
iii) In the case of compound **11f,** the HOMO is located over the C-atoms of pyrimidine and the N-atom of morphine and the HOMO-LUMO transition implies an electron density transfer to the C-atoms of substituted aniline ring and slightly at N-atom of pyrimidine ring.

iv) In the case of compound **11i,** the HOMO is located over the C & N--atoms of pyrimidine and the N-atom of morphine and the HOMO-LUMO transition implies an electron density transfer to the C-atoms of substituted aniline ring and slightly at N-atom of pyrimidine ring.

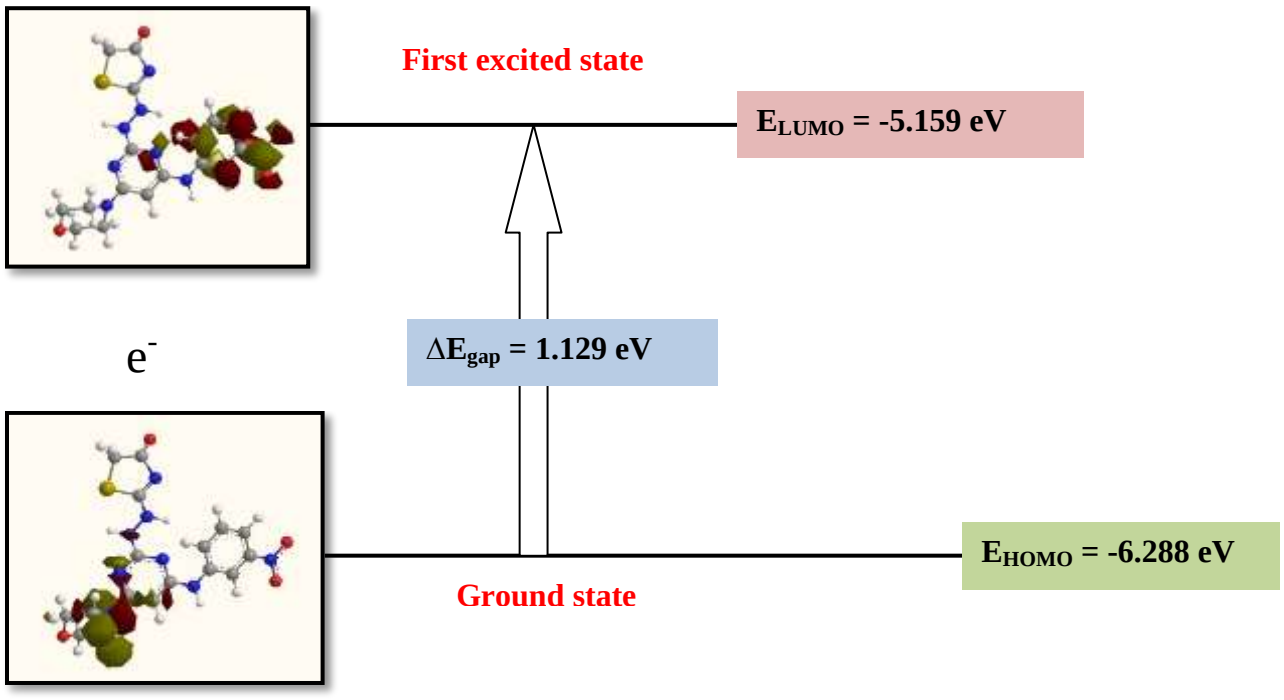
v) In the case of compound **11j,** the HOMO is located over the C & N--atoms of pyrimidine and the N-atom of morphine and N-atom of hydrazide and the HOMO-LUMO transition implies an electron density transfer to the C-atoms of substituted aniline ring and slightly at N-atom of pyrimidine ring.

So, in summary it is conclude that the substitution of different group of aniline ring is matter to the activity of compound. From this study it is proved that the HOMO-LUMO transition is affected to the structure activity relationship of compound. The energy gap between HOMO and LUMO explains the biological activity of the molecule, which is due to the change in partial charge and to the change in total dipole moment. The energy of the HOMO directly relates to the ionization potential while the energy of the LUMO directly relates to the electron affinity. The calculated HOMO and LUMO energies are given in **Table 3**.

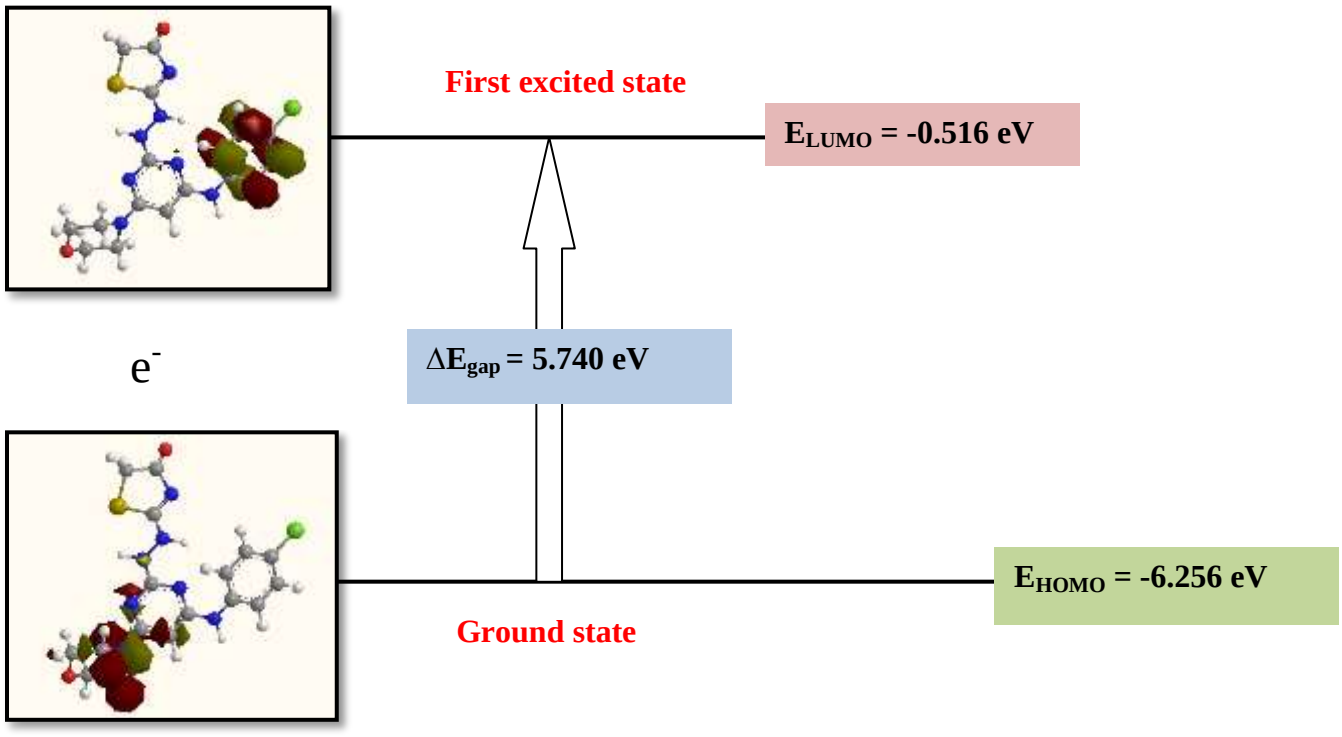
Compound 11a (HOMO-LUMO plot):



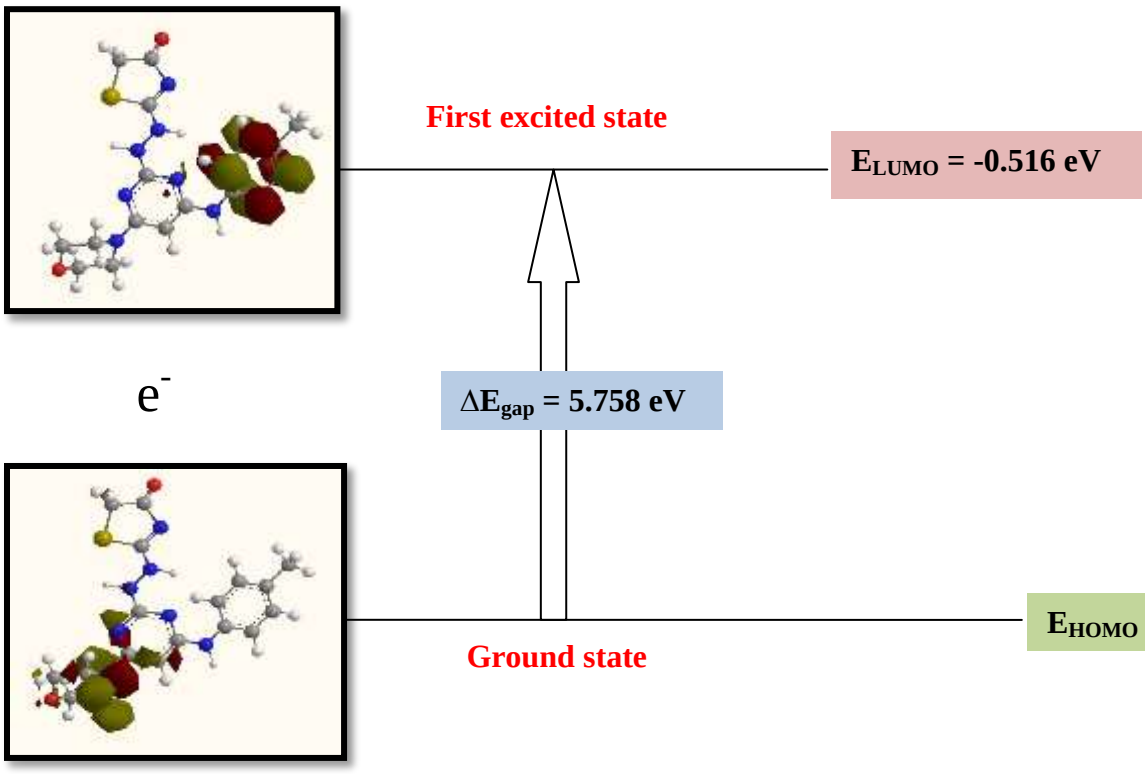
Compound 11b (HOMO-LUMO plot):



Compound 11f (HOMO-LUMO plot):



Compound 11i (HOMO-LUMO plot):



Compound 11j (HOMO-LUMO plot):

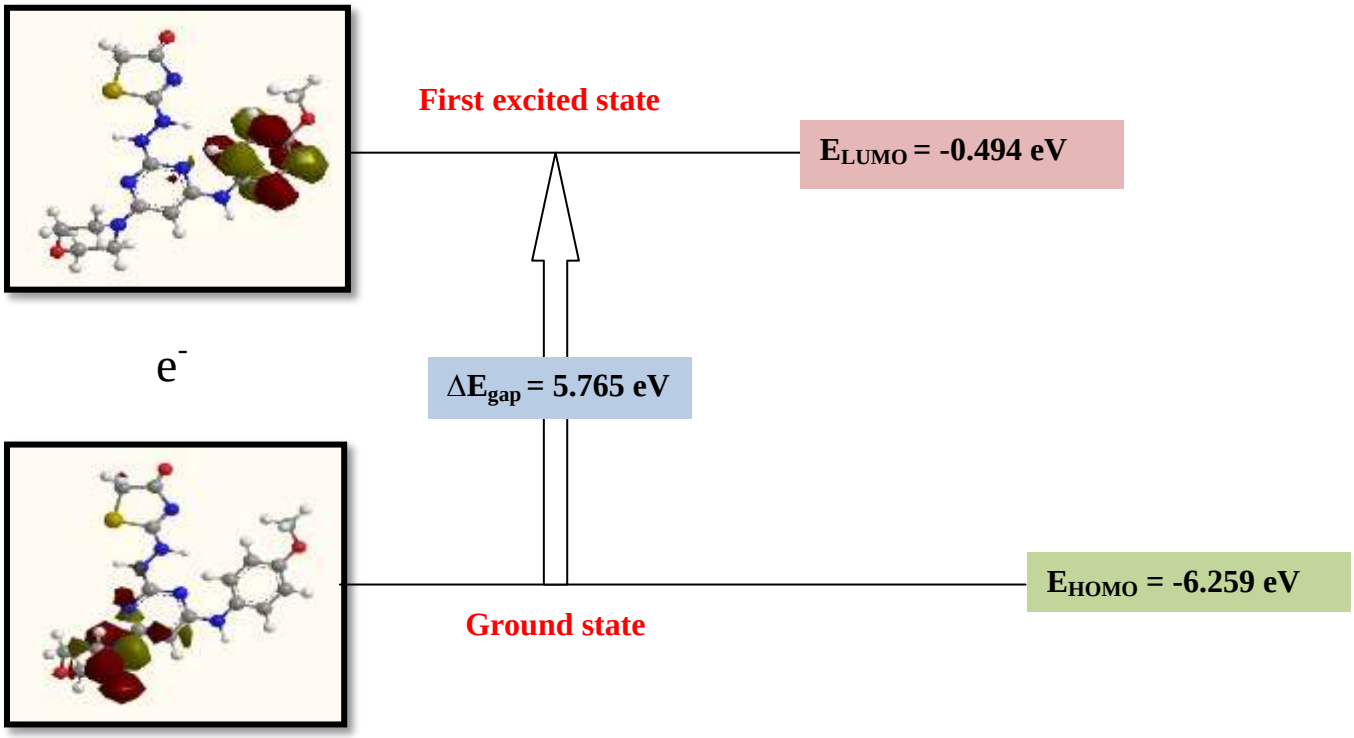


Figure-4: Atomic orbital compositions of the FMO for Compound **11a, 11b, 11f, 11i** and **11j**

Table 3: Energy gap of HOMO-LUMO of the compounds

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	Energy gap $\Delta E = E_{LUMO–HOMO}$ (eV)
11a	-6.281	-0.519	5.762
11b	-6.288	-5.159	1.129
11f	-6.256	-0.516	5.740
11i	-6.274	-0.516	5.758
11j	-6.259	-0.494	5.765

Conclusion:

The results obtained reveals that the nature of substituent and substitution pattern on the pyrimidine ring may have a considerable impact on the biological activities of the target product. The variation of antimicrobial activity is related to the tested microorganisms as well as to the chemical structure of the tested compound. In the present study better potency has been observed with the final compounds bearing morpholine substitution at 6^{th} position of pyrimidine nucleus. The results obtained by the substitution of aryl amines reveals that the nature of substituents and substituent of substituted pyrimidine-thiazolidinone ring may have a considerable impact on the biological activities. In general, it is observed that some of the compounds containing electron donating group like $-CH_3$ **11g, 11h, & 11i** and electron withdrawing group like $-NO_2$ **11b & 11c** favour the increase in activity. As usual hyperconjugation of $–CH_3$ group favors the increase in activity of lead molecule. The structural variations such as methyl group at o & m position to the aromatic amine linkage resulted in increase in activity due to the crowding effect of $–CH_3$ group. As usual halogen substituents like –Cl also favours the activity in positive manner. Surprisingly no proper conclusion can be derived off for better activity associated with electron withdrawing group only at position-3. Based on a computational study of HOMO–LUMO, it is concluded that, the lower the value of energy gap, the better the biological activity against various strains. i.e.,**11b**. The biological profile of the chosen pharmacophore is such that it is worth proceeding with further research on the functionalization and annelation of other bioactive motifs with anticipation of the development of chemistry and biological profiles.

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SUPPORTING INFORMATION:

Additional supporting information may be found online in the Supporting Information section as an individual file of this article.

References:

1. Gamir, J., Darwiche, R., Hof, P., Choudhary, V., Stumpe, M., Schneiter, R., & Mauch, F. (2017). The sterol-binding activity of PATHOGENESIS-RELATED PROTEIN 1 reveals the mode of action of an antimicrobial protein. *The Plant Journal*, *89*(3), 502-509.
2. Runyoro, D. K., Ngassapa, O. D., & Kamugisha, A. (2017). Antimicrobial Activity of Propolis from Tabora and Iringa Regions, Tanzania and Synergism with Gentamicin. *Journal of Applied Pharmaceutical Science Vol*, *7*(01), 171-176.
3. da Silva Ferreira, V., ConzFerreira, M. E., Lima, L. M. T., Frasés, S., de Souza, W., & Sant'Anna, C. (2017). Green production of microalgae-based silver chloride nanoparticles with antimicrobial activity against pathogenic bacteria. *Enzyme and microbial technology*, *97*, 114-121.
4. Wagener, J., MacCallum, D. M., Brown, G. D., & Gow, N. A. (2017). Candida albicans Chitin Increases Arginase-1 Activity in Human Macrophages, with an Impact on Macrophage Antimicrobial Functions. *MBio*, *8*(1), e01820-16.
5. Schmitz, F. J., Fluit, A. C., Lückefahr, M., Engler, B., Hofmann, B., Verhoef, J., ... & Jones, M. E. (1998). The effect of reserpine, an inhibitor of multidrug efflux pumps, on the in-vitro activities of ciprofloxacin, sparfloxacin and moxifloxacin against clinical isolates of Staphylococcus aureus. *The Journal of antimicrobial chemotherapy*, *42*(6), 807-810.
6. Schmidt, R. M., & Rosenkranz, H. S. (1970). Antimicrobial activity of local anesthetics: lidocaine and procaine. *The Journal of infectious diseases*, 597-607.
7. Fleming, A. (1944). The discovery of penicillin. *British Medical Bulletin*, *2*(1), 4-5.
8. Hill, M. D., & Movassaghi, M. (2008). New strategies for the synthesis of pyrimidine derivatives. *Chemistry-A European Journal*, *14*(23), 6836-6844.
9. Mahfoudh, M., Abderrahim, R., Leclerc, E., & Campagne, J. M. (2017). Recent Approaches to the Synthesis of Pyrimidine Derivatives. *European Journal of Organic Chemistry*, *2017*(20), 2856-2865.
10. Lagoja, I. M. (2005). Pyrimidine as constituent of natural biologically active compounds. *Chemistry & Biodiversity*, *2*(1), 1-50.

11. Lenzen, S., & Panten, U. (1988). Alloxan: history and mechanism of action. *Diabetologia*, *31*(6), 337-342.
12. Selvam, T. P., James, C. R., Dniandev, P. V., & Valzita, S. K. (2015). A mini review of pyrimidine and fused pyrimidine marketed drugs. *Research in Pharmacy*, *2*(4).
13. Shanmuga Priya, S., & Aanandhi, V. (2012). Synthesis and Anticonvulsant activity of Thiazolidinone derivatives. *International Journal of ChemTech Research*, *4*(1), 01-04.
14. Ergenc, N., Capan, G., Günay, N. S., Oezkirimli, S., Guengoer, M., Özbey, S., & Kendi, E. (1999). Synthesis and Hypnotic Activity of New 4-Thiazolidinone and 2-Thioxo-4, 5-imidazolidinedione Derivatives. *Archiv der Pharmazie*, *332*(10), 343-347.
15. Ali, Y., Alam, M. S., Hamid, H., Husain, A., Dhulap, A., Bano, S., & Kharbanda, C. (2017). Novel 2, 4-dichlorophenoxy acetic acid substituted thiazolidin-4-ones as anti-inflammatory agents: Design, synthesis and biological screening. *Bioorganic & medicinal chemistry letters*, *27*(4), 1017-1025.
16. Deep, A., Narasimhan, B., Lim, S. M., Ramasamy, K., Mishra, R. K., & Mani, V. (2016). 4-Thiazolidinone derivatives: synthesis, antimicrobial, anticancer evaluation and QSAR studies. *RSC Advances*, *6*(111), 109485-109494.
17. Lakshmi Ranganatha, V., Begum, A. B., Naveen, P., Zameer, F., Hegdekatte, R., & Khanum, S. A. (2014). Synthesis, xanthine oxidase inhibition, and antioxidant screening of benzophenone tagged thiazolidinone analogs. *Archiv der Pharmazie*, *347*(8), 589-598.
18. Kanagarajan, V., & Gopalakrishnan, M. (2010). One-pot synthesis and in vitro microbiological evaluation of pyrimidinyl thiazolidinones catalyzed by activated fly ash in dry media. *Pharmaceutical chemistry journal*, *44*(7), 374-380.
19. Dhumal, S. T., Deshmukh, A. R., Khillare, L. D., Arkile, M., Sarkar, D., & Mane, R. A. (2017). Synthesis and Antitubercular activity of New Thiazolidinones with Pyrazinyl and Thiazolyl Scaffolds. *Journal of Heterocyclic Chemistry*, *54*(1), 125-130.
20. Rao, T. Srinivas, H. G. Akkamma, and B. S. Vikram. "Synthesis and In Vitro Antifungal and Anthelmintic Activity Studies of Some Substituted Aryloxy-4-Thiazolidinones." *Asian Journal of Research in Chemistry* 3.2 (2010): 508-512.
21. Singh, N., Aggarwal, R. C., & Singh, C. P. (2014). Cardiovascular activity of Indole derivatives by incorporating Oxadiazole, Azetidinone and Thiazolidinone Moieties: A Review. *International Journal of Drug Development and Research*.

22. Omar, K., Geronikaki, A., Zoumpoulakis, P., Camoutsis, C., Soković, M., Ćirić, A., & Glamočlija, J. (2010). Novel 4-thiazolidinone derivatives as potential antifungal and antibacterial drugs. *Bioorganic & medicinal chemistry*, *18*(1), 426-432.
23. Khan, S. A., & Yusuf, M. (2009). Synthesis and biological evaluation of some thiazolidinone derivatives of steroid as antibacterial agents. *European journal of medicinal chemistry*, *44*(6), 2597-2600.
24. Nitsche, C., Schreier, V. N., Behnam, M. A., Kumar, A., Bartenschlager, R., & Klein, C. D. (2013). Thiazolidinone–peptide hybrids as dengue virus protease inhibitors with antiviral activity in cell culture. *Journal of medicinal chemistry*, *56*(21), 8389-8403.
25. Mobinikhaledi, A., Foroughifar, N., Kalhor, M., & Mirabolfathy, M. (2010). Synthesis and antifungal activity of novel 2-benzimidazolylimino- 5-arylidene- 4-thiazolidinones. *Journal of Heterocyclic Chemistry*, *47*(1), 77-80.
26. Shaikh, M. U. M., Mudaliar, S. S., & Chikhalia, K. H. (2016). An efficient alkynylation of 4-thiazolidinone with terminal alkyne under C–H functionalisation. *RSC Advances*, *6*(56), 50780-50785.
27. Mudaliar, S. S., Shaikh, M. M., & Chikhalia, K. H. (2017). An Efficient Synthetic Strategy for sp3 (C)-N Amination on 4-thiazolidinone with Primary Heteroaryl Amines. *Chemistry Select*, *2*(4), 1689-1693.
28. Patel, A., Patel, J., & Chikhalia, K. H. (2017). Palladium-catalyzed acid-free Fujiwara–Moritani alkenylation of 4-thiazolidinones. *Molecular Diversity*, 1-10.
29. Naik, T. A., & Chikhalia, K. H. (2007). Studies on synthesis of pyrimidine derivatives and their pharmacological evaluation. *Journal of Chemistry*, *4*(1), 60-66.
30. Patel, R. B., Desai, P. S., Desai, K. R., & Chikhalia, K. H. (2006). Synthesis of pyrimidine based thiazolidinones and azetidinones: antimicrobial and antitubercular agents. *Indian Journal of Chemistry*, *45*B, 773-778.
31. Jambu, S. P., Patel Y. S. (2016). Synthesis, characterization & biological studies of novel fused thiazolidinone derivatives. *Journal of Chemical & Pharmaceutical Research*, *8*(1), 750-754.
32. Linton, M. A., Burke, B. J., Johnson, T. W., Ninkovic, S., Gajiwala, K. S., Richardson, P., & Le, P. T. (2015). Effect of water solvation on the lipophilicity of isomeric pyrimidine-carboxamides. *Bioorganic & medicinal chemistry*, *23*(13), 3408-3413.
33. Radi, M., Tintori, C., Musumeci, F., Brullo, C., Zamperini, C., Dreassi, E., ... & Laurenzana, I. (2013). Design, synthesis, and biological evaluation of pyrazolo [3, 4-d] pyrimidines active in vivo on the Bcr-Abl T315I mutant. *Journal of medicinal chemistry*, *56*(13), 5382-5394.

34. Engelhardt, H., Schultes, S., de Graaf, C., Nijmeijer, S., Vischer, H. F., Zuiderveld, O. P., ... & Scharn, D. (2013). Bispyrimidines as potent histamine H4 receptor ligands: delineation of structure–activity relationships and detailed H4 receptor binding mode. *Journal of medicinal chemistry*, *56*(11), 4264-4276.
35. Han, Y. T., Choi, G. I., Son, D., Kim, N. J., Yun, H., Lee, S., ... & Kim, Y. H. (2012). Ligand-based design, synthesis, and biological evaluation of 2-aminopyrimidines, a novel series of receptor for advanced glycation end products (RAGE) inhibitors. *Journal of medicinal chemistry*, *55*(21), 9120-9135.
36. Laot, Y., Petit, L., & Zard, S. Z. (2010). Unusual radical addition on a heteroaromatic nitrogen. A convenient access to new pyrimidine derivatives. *Chemical Communications*, *46*(31), 5784-5786.
37. Ahmed A. A., Abdul A. H. K., Abu B. M., (2012), -------*Molecules*, *17*, 5713-5723.
38. Akira N., Kenji H., Sho H., Susumu S., (1993) *Quantitative structure activity relationships*, *12*, 251-255.
39. Sajan D., Lakshmi K. U., Erdogdu Y., Joe I. H., (2011) *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 78(1),* 113-121.
40. Sudha S., Karabauck M., Kurt M., Anar M., Sundaraganesan N., (2011) *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 84(1),*184-195.