A COMPARATIVE STUDY: CLASSICAL AND ULTRASOUND MEDIATED SYNTHESIS, ANTIMICROBIAL SCREENING AND MOLECULAR MODELLING OF PYRIMIDINE-CHALCONE HYBRIDS

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Abstract— An expeditious, efficient and eco-friendly method for one pot synthesis of pyrimidine-chalcones under ultrasound irradiations using Alumina supported Na_2CO_3 as a green catalyst system has been developed and reported. A present study describes classical and ultrasound mediated Synthetic route. The ultrasonic method is comparatively safe, mild and effective affording excellent yields of pyrimidine-chalcones within shorter reaction times. All the synthesized compounds were characterized using melting point, IR, 1H-NMR, 13C-NMR, Mass spectral studies and X-ray crystallography. Newly synthesized compounds were screened for their in vitro antibacterial activities against S. abony, S. epidermidis and E. coli microorganisms. Molecular modelling studies viz., Lipinski rule of five, drug likeness, drug scores, toxicity profiles and other physico-chemical properties of drugs were performed using Molinspiration and Osiris software available online and verified experimentally

Keywords— Ultrasound Irradiation, Solid Support, Pyrimidine-Chalcones, X-ray Crystallography, Molecular Modelling

I. INTRODUCTION

The molecular hybridization is current concept of rational drug design approach. With the help of this approach, new potent chemical entities can be developed from the combination of two or more pharmacophore skeletons from different bioactive compounds into a single molecular setting. By using this concept, medicinal chemists hope that the new hybrid molecules will possess better selectivity, affinity, efficacy, multiple modes of action, less undesirable side effects, least interactions between different drug molecules, reduced drug resistance and lower cost as compared with the individual parent drugs molecules, [1]. This concept motivated us to design and synthesize pyrimidine-chalcone hybrids using mild, green and clean chemical technology. Chalcone are obtainable in plentiful in the plant kingdom. They are α, β unsaturated ketones having reactive keto ethylene moiety in their skeleton that provides an active binding sites to different microorganisms. It is well-known concept that most natural or synthetic chalcones are highly active with extensive pharmaceutical and medicinal applications [2-3]. Recently, the members of chalcone family signified pharmacological and medicinal properties like anti-bacterial [4], anti-fungal, anti-malarial [5], anti-oxidant [6], anti-inflammatory [7], anti-cancer [8], anti-microbial [9], anti-protozoal [10], anti-HIV, anti-viral [11], anti-diabetic, cardiovascular, anti-allergic [12], anti-ulcer, anti-leishmanial [13], and anti-tubercular activities [14]. The Pyrimidine skeleton is one of great important core to chemists as well as biologists as it is available in a large variety of naturally occurring compounds and in clinically useful molecules having diverse biological activities [15-16]. These include anti-cancer [17] and anti-viral [18-19] activities.

Several methods and reagents over the years have been reported for classical Claisen Schmidt condensation to synthesize chalcone skeletons. Those were alkaline alumina [20], zinc chloride, Lewis acid such as dry HCl gas, BF_3, $AlCl_3$

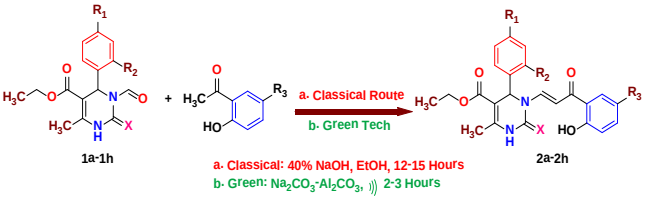
[21], Mg–Al-OBu hydro calcite [22], Phase transfer catalysts [23], Ba (OH)2 [24], calcined $NaNO_3$/phosphate [25], potassium phosphate, microwave [26] and ultrasonic reaction conditions [27]. Recently, many scientist reported synthesis of chalcones using p-TSA catalyst [28], catalyst free [29] and by using other traditional methods [30]. However, these methodologies suffer from one or more drawbacks like harsh reaction conditions, use of corrosive catalyst, longer reaction times, tedious work up or low atom economy. Currently, there is a growing interest in application of heterogeneous catalysts for the synthesis of chalcones [31]; however there is no report of the use of Na_2CO_3/Al_2O_3 as a catalyst in combination with ultrasound.

Thus, to explore mild, better, cheap, safe, and environmentally friendly experimental techniques to carry out chemical transformations under an important premise of green chemistry is necessary. One such technique includes application of ultrasonic irradiation on reaction mixtures and on solid surfaces, which has emerged as a useful methodology for achieving better yields of the products, a significant reduction in reaction time, and elimination of environmentally detrimental solvents. For these reasons, ultrasonic assisted synthesis has clearly become a rapidly growing field of study especially for various organic conversions. Ultrasonic irradiation leads to the acceleration of numerous catalytic reactions as well as in homogeneous and heterogeneous systems and we had previously reported one such conversion using K_2CO3/Al_2O3 mediated ultrasound synthetic system [32]. Thus, due to the varied pharmacological profile of this class of compounds and in continuation of our research in this area [32-33] and our interest in green chemistry lead us to synthesized differently substituted Pyrimidine-Chalcones (2a-2h) as shown in Scheme1.

II. RESULTS AND DISCUSSION

A. *Chemistry*

We have reported the efficient green synthesis, antimicrobial activity and molecular modelling of the substituted ethyl 1,2,3,6-tetrahydro-1-(3-(2-hydroxyphenyl)-3-oxo-1-propenyl)-4-methyl-2-oxo/thioxo-6-phenyl pyrimidine-5-carboxylates (Chalcone) 2a-k, using Sodium carbonate over solid supported alumina, under ultrasound irradiation. The obtained results, which are in the form of comparative study between classical and presented green technology, are given in **Table 1**. There was no progress of reaction, when a base catalyzed reaction was carried out at room temperature. It was witnessed that pyrimidines skeleton did not undergo nucleophilic addition reaction easily at ambient temperature.



Scheme 1: Comparative synthesis of Pyrimidine-chalcone hybrids

To develop and activate the course of reaction in frontward path, we amplified the reaction timings and conditions. The preferred products were obtained by the classical method with average yields and more time was needed for the completion of reaction. These results forced and encouraged us to explore novel, ecofriendly, efficient and quick method for the synthesis of Pyrimidine-chalcone hybrids. The solid supported catalyst, Na_2CO_3 is readily available, low-priced, mild and safe which can be easily handled and removed from the reaction mixture. Thus, the extraordinary catalytic activities along with its operating simplicity make this process suitable for the synthesis of medicinally important chalcone hybrids. It is found that the condensation reaction in solvent-free conditions in the presence of ultrasonic irradiation worked well and the products were obtained in excellent yields within less reaction time and no by products were formed. The plausible mechanistic pathway appears to involve basic media of Na_2CO_3 which activates both carbonyl function, thereby making carbonyl and methyl groups readily enolisable which in turn undergoes condensation and derived α- β unsaturated carbonyl group with loss of water molecule to produce products 2a-2h **(Scheme 2)**.The structural composition of synthesized substituted Ethyl-6-methyl/chloro-2-oxo/thioxo-3-(3-oxo-3-(m-tolyl)-prop-1-en-1-yl)-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylates

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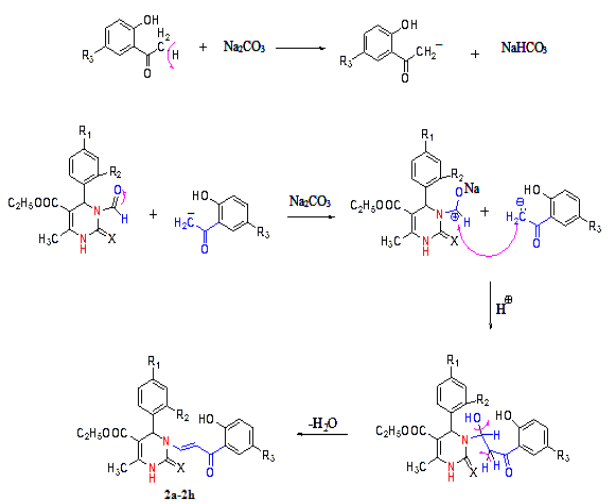
(2a-2h) were confirmed by using IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopic techniques. The IR spectrum of compound 2a displayed sharp absorption bands at 3355 (O-H str.), 3195 (N-H str.), 3105 (=C-H str.), 3068 (C-H, Ar), 2930 (C-H, Methyl), 1675 (C=O str.), 1605 (C=C str.), 1475 (C=C str.). The ^{1}HNMR spectrum of 2a explained the presence of two methyl group by appearance of the two different peaks, triplet at ∂ 1.14 & singlet at ∂ 1.50 which integrating for three protons respectively.



Scheme 2: The plausible mechanistic pathway in synthesis of Pyrimidine-chalcone hybrids

A singlet at ∂ 2.23 indicated the presence of methyl protons on aromatic ring integrating for three protons. The C-H_{α} and C-H_{β} protons of 2a appeared as two doublets at ∂ 5.20 and ∂ 8.25 integrating for one proton each. Compound 2a showed singlets at ∂ 5.42 and 8.31 (N-H) for the protons in pyrimidine ring. A characteristic singlet at δ 7.65 indicated the presence of Ar–OH. It also presents double doublets integrating for nine protons from ∂ 7.24-42 in an aromatic region. In the ^{13}C-NMR spectrum of the compound 2a, the signals belonging to the same carbon groups were recorded at 182.8, 166.8, 162.9, 158.8, 153.9, 141.4, 127.9, 127.7, 126.2, 102.9, 62.8, 56.9, 36.1, 22.1, 13.8 and 13.7. The product 2a was analyzed for $C_{24}H_{22}N_2O4_5$, which exhibited molecular ion peak at m/z 420 [M^{+}].

III. EXPERIMENTAL SECTION

All chemicals were obtained from Sigma Aldrich and S. D. Fine chemical companies and used without further purification. All solvents were distilled prior to use. The purity of the synthesized compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by iodine vapors. Developing solvents were n-Hexane-ethylacetate (7:3). Melting points were determined by an open capillary method and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded from CDCl3 solution on a Brucker Avance II 400 (400 MHz) NMR Spectrometer. Chemical shifts are reported in ppm using TMS as an internal standard. IR spectra were obtained on a Shimadzu FTIR spectrophotometer using KBr discs. Mass spectra were recorded by using Shimadzu gas chromatograph coupled with QP5050 Spectrometer at 1-1.5 eV. Spectra lab model UCB 40D ultrasonicator with a frequency of 40 kHz and a nominal power of 250 Watt was used for the ultrasonic mediated synthesis of pyrimidine-chalcone hybrids. Antimicrobial screening was performed at the Department of Microbiology & Biotechnology, S. P. College, Chandrapur, Maharashtra, India.

Classical Method for general synthesis of substituted Ethyl-6-methyl/chloro-2-oxo/thioxo-3-(3-oxo-3-(m-tolyl)-prop-1-en-1-yl)-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates (2a-2h)

In a 50 mL RB Flask, Substituted acetophenones (0.011mol) and ethyl 1-formyl-1, 2, 3, 6-tetrahydro-4-methyl-2-oxo/thioxo-6-phenylpyrimidine-5-carboxylates **(1a-1h)** (0.01mol) were dissolved in the 20 mL ethanol by warming the mixture and it was stirred for 05 Minutes. To this, 40% solution of 0.03 mol NaOH was added and the reaction mass was stirred at room temperature for 12-15 hours. After the completion of reaction, as monitored by TLC, the reaction was subsequently quenched by ice cold 1:1 HCl, until the reaction mixture became acidic. The formed precipitate was filtered, washed and dried. The products were recrystallized from rectified spirit to give crystal of chalcone **(2a-2h)**. The purity of the synthesized compounds were checked by TLC using Benzene: ethyl acetate (7:3) as eluent.

Ultrasound stimulated Na_2CO_3-Al_2O_3 catalyzed general synthesis of substituted Ethyl-6-methyl/chloro-2-oxo/thioxo-3-(3-oxo-

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3-(m-tolyl)-prop-1-en-1-yl)-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates (2a-2h)

Substituted acetophenones (0.011mol), ethyl 1-formyl-1, 2, 3, 6-tetrahydro-4-methyl-2-oxo/thioxo-6-phenylpyrimidine-5-carboxylates **(1a-1h)** (0.01mol) and 15 mol% Na_2CO_3 was dissolved in 5 mL dichloromethane. The reaction mixture was absorbed on 1.5 g neutral Al_2CO_3 and air-dried. The mixture was irradiated in the water bath of Spectra lab model UCB 40D ultrasonicator with a frequency of 40 kHz and a power of 250 Watt for the time slots given in Table 1. After completion of reaction, as monitored by TLC, 20 mL ethanol was added to the reaction mixture. Inorganic material was filtered out and the filtrate was poured in ice cold 1:1 HCl solution. Obtained solid products were separated by filtration and recrystallized from rectified spirit to get the desired pyrimidine-chalcones in 75-86 % yields. The purity of the products was checked using TLC from Benzene: ethyl acetate (7:3) as eluent.

Ethyl-1, 2, 3, 6-tetrahydro-1-(3-(2-hydroxy-5-methylphenyl)-3-oxoprop-1-enyl)-4-methyl-2-oxo-6-phenylpyrimidine-5-carboxylate (2a). Pale Yellow crystals**;** mp 140-145^{0}C; IR (KBr disc), ν, cm^{-1}: 3455 (O-H), 3195 (N-H), 3105 (=C-H), 3068 (C-H, Ar), 2930 (C-H, Methyl), 1675 (C=O), 1605 (C=C), 1475 (C=C), 1090 (C-O); ^{1}H-NMR ($CDCl_3$, 400MHz) δ 1.14 (t, 3H), 1.50 (s, 3H), 2.23 (s, 3H), 4.14-4.21 (q, 2H), 5.20 (d, 1H), 5.41 (s, 1H), 7.26-7.42 (m, 8H), 7.65 (s, O-H), 8.25 (d, 1H), 8.31 (s, 1H, N-H); ^{13}C-NMR (400 MHz, CDCl3, δ ppm): 166.8, 162.9, 153.9, 141.4, 127.9, 127.7, 126.24 102.9, 62.8, 56.9, 36.2, 22.5, 13.8, 13.7, 13.6; DI-MS: m/z 420 [M]$^+$

Ethyl-6-(4-chlorophenyl)-1, 2, 3, 6-tetrahydro-1-(3-(2-hydroxy-5-methyl phenyl)-3-oxoprop-1-enyl)-4-methyl-2-oxopyrimidine-5-carboxylate (2b). Yellow crystals**;** mp 125-130^{0}C; IR (KBr disc), ν, cm^{-1}: 3460 (O-H), 3190 (N-H), 3100 (=C-H), 3065 (C-H, Ar), 2935 (C-H, Methyl), 1670 (C=O), 1610 (C=C), 1470 (C=C), 1095 (C-O), 680 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400MHz) δ 1.14 (t, 3H), 1.70 (s, 3H), 2.32 (s, 3H), 4.13-4.20 (q, 2H), 5.20 (d, 1H), 5.64 (s, 1H), 7.24-7.42 (m, 7H), 7.59 (s, O-H), 8.21 (d, 1H), 8.48 (s, 1H, N-H); ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 166.8, 162.9, 153.9, 141.4, 127.9, 127.7, 126.4, 102.9, 62.8, 56.9, 36.1, 22.8, 13.7, 13.6; DI-MS: m/z 454 [M]$^+$

Ethyl-1, 2, 3, 6-tetrahydro-1-(3-(2-hydroxy-5-methylphenyl)-3- oxoprop-1-enyl)-4-methyl-6-phenyl -2-thioxopyrimidine-5-carboxylate (2c). Brown crystals**;** mp 135-140^{0}C; 3460 (O-H), 3200 (N-H), 3110 (=C-H), 3070 (C-H, Ar), 2935 (C-H, Methyl), 1665 (C=O), 1615 (C=C), 1475 (C=C), 1110 (C=S), 1095 (C-O), ^{1}H-NMR (DMSO, 400MHz) δ 1.16 (t, 3H), 1.80 (s, 3H), 2.30 (s, 3H), 3.99-4.05 (q, 2H), 5.42 (d, 1H), 6.80 (s, 1H), 7.20-7.41 (m, 8H), 7.85 (s, O-H), 8.10 (d, 1H), 8.87 (s, 1H, N-H); ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 166.7, 162.2, 153.9, 141.2, 127.1, 127.2, 126.9, 102.8, 62.8, 56.9, 36.2, 22.5, 13.7, 13.5; DI-MS: m/z 436 [M]$^+$

Ethyl-6-(4-chlorophenyl)-1, 2, 3, 6-tetrahydro-1-(3-(2-hydroxy-5-methyl phenyl)-3-oxoprop-1-enyl)-4-methyl-2-thioxopyrimidine-5-carboxylate (2d). Yellow crystals**;** mp 115-120^{0}C; IR (KBr disc), ν, cm^{-1}: 3345 (O-H), 3192 (N-H), 3107 (=C-H), 3067 (C-H, Ar), 2930 (C-H, Methyl), 1678 (C=O), 1615 (C=C), 1475 (C=C), 1105 (C=S), 1092 (C-O), 686 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400MHz) δ 1.14 (t, 3H), 1.50 (s, 3H), 2.32 (s, 3H), 4.13-4.14-4.22 (q, 2H), 5.20 (d, 1H), 5.34 (s, 1H), 7.26-7.42 (m, 7H), 7.69 (s, O-H), 8.25 (d, 1H), 8.45 (s, 1H, N-H); ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 166.9, 162.9, 153.9, 141.4, 127.9, 127.7, 126.4, 102.9, 62.8, 56.5, 36.1, 22.1, 13.9, 13.7; DI-MS: m/z 470 [M]$^+$

Ethyl-1-(3-(5-chloro-2-hydroxyphenyl)-3-oxoprop-1-enyl)-1, 2, 3, 6-tetrahydro-4-methyl-2-oxo-6-phenylpyrimidine-5-carboxylate (2e). Yellow crystals**;** mp 175-180^{0}C; IR (KBr disc), ν, cm^{-1}: 3420 (O-H), 3198 (N-H), 3105 (=C-H), 3068 (C-H, Ar), 1677 (C=O), 1613 (C=C), 1478 (C=C), 1090 (C-O), 676 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400MHz) δ 1.14 (t, 3H), 4.15-4.2o (q, 2H), 5.20 (d, 1H), 5.40 (s, 1H), 7.26-7.42 (m, 8H), 7.69 (s, O-H), 8.26 (d, 1H), 8.70 (s, 1H, N-H); ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 166.1, 162.9, 153.9, 141.4, 127.9, 127.7, 126.2, 102.9, 62.8, 56.9, 36.1, 22.9, 13.8, 13.7; DI-MS: m/z 440 [M]$^+$

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Ethyl-1-((E)-3-(5-chloro-2-hydroxyphenyl)-3-oxoprop-1-enyl)-6-(4-chlorophenyl)-1, 2, 3, 6-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate (2f). Dark yellow crystals; mp 130-135^{0}C; 3350 (O-H), 3205 (N-H), 3108 (=C-H), 3070 (C-H, Ar), 1680 (C=O), 1615 (C=C), 1480 (C=C), 1095 (C-O), 678, 682 (C-Cl);); ^{1}H-NMR ($CDCl_3$, 400MHz) δ 1.14 (t, 3H), 2.32 (s, 3H), 4.14-4.22 (q, 2H), 5.20 (d, 1H), 5.34 (s, 1H), 7.26-7.42 (m, 7H), 7.69 (s, O-H), 8.25 (d, 1H), 8.43 (s, 1H, N-H); ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 166.2, 162.8, 153.9, 141.4, 127.8, 127.4, 126.4, 102.8, 62.8, 56.8, 36.2, 22.1, 13.8, 13.6; DI-MS: m/z 475 [M] $^+$

Ethyl-1-(3-(5-chloro-2-hydroxyphenyl)-3-oxoprop-1-enyl)-1, 2, 3, 6-tetrahydro-4-methyl-6-phenyl -2-thioxopyrimidine-5-carboxylate (2g). Yellow crystals; mp 115-120^{0}C; 3420 (O-H), 3197 (N-H), 3107 (=C-H), 3065 (C-H, Ar), 1677 (C=O), 1619 (C=C), 1476 (C=C), 1109 (C=S), 1097 (C-O), 685 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400MHz) δ 1.14 (t, 3H), 4.12-4.20 (q, 2H), 5.11 (d, 1H), 5.44 (s, 1H), 7.26-7.42 (m, 8H), 7.59 (s, O-H), 8.25 (d, 1H), 8.48 (s, 1H, N-H); ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 166.9, 162.8, 153.9, 141.4, 127.9, 127.4, 126.6, 102.1, 62.8, 56.9, 36.2, 22.1, 13.8, 13.6; DI-MS: m/z 456 [M] $^+$

Ethyl-1-((E)-3-(5-chloro-2-hydroxyphenyl)-3-oxoprop-1-enyl)-6-(4-chlorophenyl)-1, 2, 3, 6-tetrahydro-4-methyl-2-thioxopyrimidine-5-carboxylate (2h). Yellow crystals; mp 100-105^{0}C; 3465 (O-H), 3192 (N-H), 3101 (=C-H), 3072 (C-H, Ar), 1685 (C=O), 1618 (C=C), 1485 (C=C), 1105 (C=S), 1094 (C-O), 675, 689 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400MHz) δ 1.14 (t, 3H), 2.23 (s, 3H), 4.14-4.21 (q, 2H), 5.20 (d, 1H), 5.41 (s, 1H), 7.26-7.42 (m, 7H), 7.65 (s, O-H), 8.25 (d, 1H), 8.81 (s, 1H, N-H); ^{13}C-NMR (400 MHz, DMSO-d6, δ ppm): 166.7, 162.8, 153.9, 141.4, 127.5, 127.4, 126.4, 102.1, 62.83, 56.9, 36.2, 22.5, 13.8, 13.7; DI-MS: m/z 491 [M] $^+$

IV. CONCLUSIONS

A simple, mild, expeditious, efficient and eco-friendly method for one pot synthesis of novel pyrimidine-chalcone hybrids under ultrasonic irradiations using Alumina supported Na_2CO_3 as a green catalyst with excellent yields has been developed. Various reported drawbacks like harsh reaction conditions, use of corrosive catalyst, longer reaction times, and tedious work up procedures or low atom economy have been wiped out through this method. It is established that the presence of –Cl substituents at R_1 and R_3 along with X = S, are accountable for the potency of synthesized pyrimidine-chalcone hydrids and this is in good agreement with the calculated data obtained through molecular modelling.

A. Antibacterial Screening Test

The antibacterial activity of the synthesized compounds 2a-2h was studied against three human pathogenic bacteria, viz *E.coli* (ATCC No. 8739), *S.abony* (NCTC No. 6017) and *S.epidermidis* (NCTC No. 8853, ATCC No. 12228). For the detection of antibacterial activities, Kirby aurer method was employed. Chloromphenicol and Streptomycin were used as standard antibiotics for the antibacterial test. Nutrient agar (NA) was used as the basal medium for test bacteria. These agar media were inoculated with 1mL of the 24 hr liquid cultures containing 10^7 microorganisms/mL. The incubation time was 12 hr at 37^{0}C for bacteria. Discs with only DMSO were used as control. The diameter (in mm) of the observed inhibition zones were taken as a measure of inhibitory activity.

B. Antifungal Screening Tests

The antifungal activity of compound 2a-2h was evaluated towards plant pathogenic and mold fungi viz *Candida albicans* and *Aspergillas niger*. The antifungal activity was assessed by Disc diffusion methods. Griesofulvin was used as standard fungicide for the antifungal test. Potato Dextrose agar (PDA) was used as basal medium for test fungi. Glass Petri dishes were sterilized and 15mL of sterilized melted PDA medium (45^{0}C) was poured into each Petri dish (90mm). After solidification of the medium, small portion of mycelium of each fungus were spread carefully over the center of each PDA plate with the help of sterilized needle. Each fungus was transferred to a number of PDA plates. The PDA plates were incubated at (25+/-2^{0}C) and after five days of incubation, they were ready for use. The prepared disc of test samples were placed gently on the solidified agar plates freshly seeded with the test organism with sterile forceps. Control discs were also placed on the test plates to compare

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the effect of the test samples and to nullify the effect of solvent. The plates were kept in a refrigerator at 4^0C for 24 hours, so that the materials had sufficient time to diffuse to a considerable area of the plates. Afterwards, the plates were incubated at 37.5^0C for 72hours. Dimethyl sulphoxide (DMSO) was used as a solvent to prepare desired solution (10mg/mL) of the compound initially.

The antibacterial activity of compounds 2a-2h has been assayed at the concentration of 100-μg $disc^{-1}$ against strains of pathogenic bacteria. Initially, susceptibility testing was carried out by measuring the inhibitory zone diameters on nutrient agar (NA) with conventional paper disc method; and the inhibitory zone diameter were read and rounded off to the nearest whole numbers (mm) for analysis. The results of inhibitory effects of compounds 2a-2h against these organisms are given in **Table 2**. The results were compared with standard Streptomycin. The antibacterial screening results revealed that *E. coli* is highly sensitive to the all the tested compounds (2a-2h), followed by S. *epidermis* and S. *ebony*. It has been observed that the presence of substituent at R_1 and the variation of X (O and S) of pyrimidine ring played a vital role in the potency of antibacterial activities. From the mentioned data in table 1, it is observed that all the pyrimidine-chalcone hydrids showed excellent activities against *E. Coli,* more than that for Streptomycin. Moreover, compound 2h possessed broad spectrum of biological activities against all the tested organisms and its activities are more or close to the drug used for comparison. In general, It can be concluded that the presence of –Cl substituents at R_1 and R_3 along with X = S, are responsible for the potency of synthesized pyrimidine-chalcone hydrids.

C. Antifungal Activity

As the sensitivity was not observed at concentration < 100μg $disc^{-1}$, the antifungal activities of compounds 2a-2h have been assayed in vitro at a concentration 100μg disc-1 against C. *albicans* and A. niger. Susceptibility testing was carried out by measuring the inhibitory zone diameters on Potato Dextrose agar (PDA) with conventional paper disc method. The plates were then incubated at 37.5^0C for 24hr. The inhibitory zone diameters were read and rounded off to the nearest whole numbers (mm) for analysis. The inhibitory effects of compounds 2a-2h against these organisms are given in table 5. Griesofulvin used as a standard fungicide. The antifungal studies revealed that the fungi i.e. C. albicans and A. niger both were found to be resistant to the all tested compounds. All the compounds were inactive against the tested fungal strains.

D. Molecular Modelling

Calculation and analysis of toxicity profiles and drug likeness scores by Osiris [35-36]

We have calculated and reported the toxicity risk and other drug relevant properties like ClogP, solubility, drug likeness and drug score of the synthesized compounds **(**2a-2h) using Osiris software available online. Toxicity risk alerts are a sign that the drawn structure could be harmful concerning the risk category specified. Moreover, overall drug score values indicate the qualification of considered compound as a drug. Osiris Property Explorer predicts the molecule's potential mutagenic, tumorigenic, reproductive, or other risks. It helped to calculate various drug relevant properties of chemical structures likewise. From the data evaluated in **Table 3** indicates that all the compounds are non-toxic, non-mutagenic, non-tumorigenic, non-irritating with no reproductive effects when run through the mutagenicity assessment system comparable with standard drugs used. Low hydrophilicities and so high log P values may cause poor absorption or permeation. Log P value, which must not be greater than 5.0. On this basis, all the compounds are having log P values are under the appropriate criteria. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, an occasional solubility goes together with a nasty absorption and so the overall aim is to avoid poorly soluble compounds. There are quite 80% of the drugs on the market which have an (estimated) log S value greater than -4 and compounds 2a-2h are well under this value. **Table 3** shows drug likeness and drug score of compounds (2a-2h). The reported compounds showed acceptable solubility, moderate to good drug likeness and drug score as compared with standard drug used. It is observed based on Osiris data that most potent compound is 2h and least potent compounds are 2e, 2d and 2a. It

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was supported by the antibacterial activities data in Table 2.

Calculations of Molecular Properties (QSAR) and Drug likeness Score against receptors by Molinspiration [36]

Prediction of biological activities by Molinspiration software which calculates drug likeness score against GPCR ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI) and other enzyme inhibitors (EI) and molecular properties. Molecular Polar area (TPSA) has been shown to be a good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration. Prediction results of compounds (2a-2h) and molecular properties (TPSA, NV, GPCR ligand, KI, NRL, PI, EI and ICM) are valued in **Table 4**. Polar area (PSA) values are important properties for the prediction of per oral bioavailability of drug molecules Therefore, we have got calculated PSA values for compounds (2a-2h) and compared them with the values obtained for traditional drugs Chloramphenicol and Streptomycin. Molecules with PSA values of 140 A^0 or more are expected to exhibit poor intestinal absorption. **Table 4** shows that every compound is within this limit. To support this contention, note that only few compounds have just one violation of the Rule of 5. Two or more violations of the Rule of 5 suggest the probability of problems in bioavailability. Few compounds have just one violation of the Rule of 5 with only a few compounds have zero violation. Drug likeness includes properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and adaptability and presence of miscellaneous pharmacophores features influence the behavior of molecule on living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and plenty of others. Activity of all eight compounds and standard drugs were rigorously analyzed under six criteria of known successful drug activity within the areas of GPCR ligand activity, ion channel modulation, kinase inhibition activity, and nuclear receptor ligand activity. Results are shown for all compounds in **Table 4** by means of numerical assignment. Likewise all compounds have consistent negative values altogether categories and numerical values conforming and similar to that of normal drugs used for comparison. Therefore, it has readily seen that each one the compounds are expected to possess near similar activity to the standard drug used based upon these six rigorous criteria of drug likeness, which was supported by the antibacterial activities data in Table 2.

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Table 1: Comparative study of classical synthesis and green technology in the synthesis of Pyrimidine-chalcone hybrids

Compound	R1	R2	R3	X	Classical Synthesis 40% NaOH, EtOH		Green Technology Na2CO3/Al2O3-Ultrasonics	
					Time (Hours)	Yield (%)	Time (Hours)	Yield (%)
2a	H	H	CH3	O	14	75	3.0	84
2b	Cl	H	CH3	O	13.5	72	2.5	81
2c	H	H	CH3	S	12	69	2.0	82
2d	Cl	H	CH3	S	12.5	71	2.0	86
2e	H	H	Cl	O	14	68	2.5	84
2f	Cl	H	Cl	O	14	64	3.0	75
2g	H	H	Cl	S	13.5	65	2.5	83
2h	Cl	H	Cl	S	13.5	62	3.0	75

Table 2. Antimicrobial-Screening Results of Synthesized Compound (2a-2h)

Entry	S. abony	S. epidermidis	E. coli
2a	-	16mm	15mm
2b	12mm	15mm	24mm
2c	12mm	12mm	21mm
2d	11mm	-	13mm
2e	-	-	22mm
2f	18mm	14mm	26mm
2g	14mm	13mm	25mm
2h	21mm	26mm	27mm
SD	25mm	-	29 mm

SD – Streptomycin, (-) No zone

Table 3: Toxicity risks and Osiris calculations

Compd.	MW	Toxicity risk				Osiris calculations			
		MUT	TUMO	IRRI	REP	CLP	S	D-L	D-S
2a	404					3.47	-4.74	-0.19	0.58
2b	439					4.08	-5.48	+0.94	0.62
2c	421					3.83	-8.82	-1.25	0.46
2d	455					4.44	-5.56	-0.06	0.50
2e	425					3.74	-5.13	-0.07	0.56
2f	459					4.34	-5.87	+1.00	0.58
2g	441					4.10	-5.21	-1.12	0.45
2h	475					4.70	-5.95	+0.05	046
SD	581					-7.83	-0.96	0.83	0.43

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: Non-toxic; : Slightly toxic; : Highly toxic. MUT: Mutagenic; TUMO: Tumorigenic; IRRI: irritant; REP: reproductive effective. CLP: cLogP, S: Solubility, DL: Drug-likeness, DS: Drug-Score. SD: Streptomycin

Table 4: Physico-chemical properties and molinspiration calculations

Compd	Molinspiration calculations					Drug-likeness					
	TPSA	N/OH	NV	Nrotb	VOL	GPCRL	ICM	KI	NRL	PI	EI
2a	95.94	2	0	7	381.90	-0.27	-0.31	-0.78	-0.33	-0.54	-0.34
2b	95.94	2	1	7	395.43	-0.27	-0.30	-0.77	-0.33	-0.56	-0.35
2c	78.87	2	0	7	390.77	-0.49	-0.48	-0.96	-0.58	-0.73	-0.48
2d	78.64	2	1	7	404.31	-0.48	-0.47	-0.94	-0.58	-0.74	-0.49
2e	95.94	2	1	7	378.87	-0.24	-0.25	-0.78	-0.33	-0.53	-0.32
2f	95.94	2	1	7	392.41	-0.24	-0.24	-0.75	-0.32	-0.52	-0.31
2g	78.87	2	1	7	387.75	-0.47	-0.42	-0.95	-0.59	-0.72	-0.46
2h	78.87	2	1	7	401.28	-0.45	-0.41	-0.92	-0.57	-0.70	-0.45
SD	336	16	3	9	497.00	-0.09	-0.16	-0.17	-0.18	-0.65	-0.38

TPSA: Total polar surface area, O/NH: O—HN interaction, NV: Number of violation, VOL: Volume, ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand. PI: Protease inhibitor; EI: Enzyme inhibitor. SD: Streptomycin

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