Insilico toxicity prediction, synthesis, characterization, antimicrobial and antioxidant activity of different di substituted chalcones.

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Abstract

In this we reported the synthesis of novel series of 4'-chloro-3'-nitro-phenyl-(3-keto-1,2diene-1-flurobenzene), 4'-chloro-3'nitro-phenyl-(3-keto-1,2diene-1-chlorobenzene), 4'-chloro-3'-nitro- phenyl-(3-keto-1,2diene-1-thiophene), 4'-chloro-3'nitro-phenyl-(3-keto-1,2diene-1-pyridine), 4'-chloro-3'nitro-phenyl-(3-keto-1,2diene-1-furon). The target compounds were synthesized by the Claisen-schimidt condensation using acid and base as a reaction catalyst. Insilico optimization methods by use of different data base like molin spiration for molecular properties, bioactivity, ochem for biological data, pre ADME for pharmacokinetic properties, preToxicity for toxicity profile of the compounds, swiss ADME for pharmacokinetic property, Swiss toxicity for biological toxicity of the compounds, and Osiris (datawarrior) for toxicity prediction for active lead molecules identification. From the result compounds indented as active leads they used to evaluate different biological activity. The compounds also pass the amees test mainly for mutagenecity detection that compounds shows mutagencity towards the test organism they also safe to inhibit cytochrome P450 enzyme subunits like cyp1A2, cyp2C9, cyp2C19 which are located in endoplasmic reticulum. Alpha beta unsaturated chalcones have excellent antioxidant and antimicrobial activity so the synthesized compounds were screened for their antimicrobial by using five bacterial strains and three fungal strains and antioxidant activity by DPPH method. From anti-oxidant results compound 3a, 3b, 3c show significant activity the assay values are nearer to standard drug value. Antimicrobial activity results the compound 3a, 3c, 3e showed significant activity against *Bacillus subtilis, E Coli, Salmonella tyhphi, Pseudomonas aeruginosa* whereas the compound 3b, 3d showed moderate activity compared to standard drug vale. Compound 3b, 3d shown excellent antifungal activity against Candida albicans, Aspergillus niger, Alternaria alternata. The compounds 3a, 3c, 3e exhibit moderate antifungal activity when compared to standard value.

Keywords: Molinspiration, Osiris, Ochem, PreADME, Antioxidant, Antimicrobial activity.

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Introduction

Chalcones are aromatic ketone they are central core for synthesized variety of biological compounds. Claisen-schimidt condensation between aromatic ketone and aromatic aldehyde to give chalcone the reaction catalysed by acid and base under homogeneous or heterogeneous conditions they are open chain precursor molecule for biosynthesis of flavanoids, isoflavnoids and poly phenolic derivatives. They exist as two isomeric forms cis and trans. Trans are more stable than cis form [1]. The compounds have large no of replaceable hydrogen atoms that allow to synthesized a large variety of derivatives like pyrazoles, imidazole, thiazole, triazole, oxazole etc, they exhibit different type of biological activities like antigout, anti histamine, antioxidant, antiodesity, hypotics, antispasmodic, antiprotozal etc. Nowadays, a number of comparative pharmacological investigations of the chalcones have showed good antioxidant activity with low side effects. In previous reports most of the chalcones synthesized by using single substituted ketone with single or di substituted aldehydes but in present work using di substituted ketone with different substituted aldehydes used for synthesis of different chalcone derivatives [2].

Materials and Methods

All the chemicals and reagents were obtained in synthetic grade from commercial sources. Microwave irradiation was carried out in LG domestic microwave oven. Reaction was monitorby TLC. 4'Chloro-3'nitro MKBX1257V Sigma Aldrich Acetophenone, Potassium hydroxide N1400361 Avra synthesis (85%) P-Chlorobenzaldehyde L126101406 Loba chemic, 2-Fluorobenzaldehyde A301696 Spectro chem., 2-Furaldehyde (99%) N1601838 Avra synthesis Pyridine-4-carboxalde N1602027 Avra synthesis-hyde (98%) 3-Fluorobenzaldehyde N1610254 Avra synthesis (98%) 3-Chlorobenzaldehyde B300201 Spectro chem., 2-Thiophenecarboxalde N1703574 Avra synthesis-hyde (98%), 2-Chlorobenzaldehyde N1810073 Avra synthesis,4-Fluorobenzaldehyde N1800433 Avra synthesis (98%) 3-Hydroxy benzaldehyde N1701409 Avra synthesis (97%) 2-Hydroxybenzaldehyde N1800578 Avra synthesis (98%) 4-Hydroxybenzaldehyde N1800160 Avra synthesis [3].

Synthesis of chalcones

A mixture of ketone (0.05 mol), appropriate aldehyde (0.05 mol) and 10% aqueous sodium hydroxide (10 ml) in ethanol

(30 ml) should be stirred at room temperature for about 3-24 h. The reaction mixture after achieving the desired spot in the TLC should transfer into crushed ice to form the precipitate. The precipitate should be filtered, washed with distilled water and recrystallized with chloroform (Figure 1) [4,5].

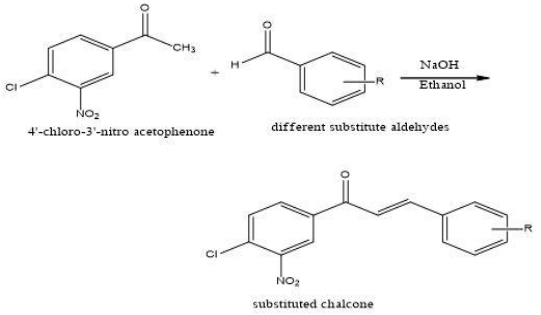


Figure 1. *R=4-F, 2-C1, thiophen 2-carboxaldehyde, pyridine-2-carboxaldehyde-2-furaldehyde.*

Antibacterial activity of the synthesized compounds was tested against five bacterial strains and three fungal strains using agar well diffusion method. Dimethyl sulfoxide was used as solvent control. The bacterial culture was inoculated on nutrient agar and fungal culture was inoculated on potato dextrose agar media (20 ml). The test compounds were dissolved in DMSO to get a concentration of 12.79 M and 100 µ L of this sample was loaded into the wells of agar plates directly. Plates inoculated with the bacteria were incubated at 37°C for 24 h and the fungal culture was incubated at 25°C for 72 h [6]. All determinations were done in triplicates. The Streptomycin (1.71 M and 0.85 M) and Griseofulvin (3.26 M and 1.6 M) were used as standard drugs for antibacterial and antifungal activities respectively. After the incubation period, the minimum inhibition zone at which the microorganism growth was inhibited was measured in µg/mL [7].

Free radicalscavenging capacities of synthesized compounds were determined according to the reported procedure. The newly synthesized compounds at different concentrations (25-100 µg/mL) were added to each test tube and volume was made up to 4 ml using methanol. To this, 3 ml of 0.004% DPPH in methanol was added and the mixtures were incubated at room temperature under dark condition for 30 min. The absorbance was recorded at 517 nm using UVVisible spectrophotometer [8].

Where Acontrol is the absorbance of the control sample (DPPH solution without test sample) and Atest is the absorbance of the test sample (DPPH solution+test compound) (Tables 1-12).

Results and Discussion

Physicochemical properties

Table 1. *Insilico toxicity prediction for synthesized compounds.*

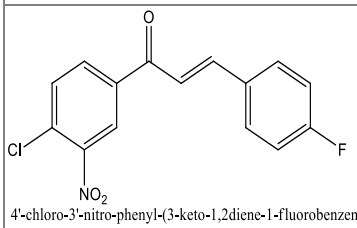
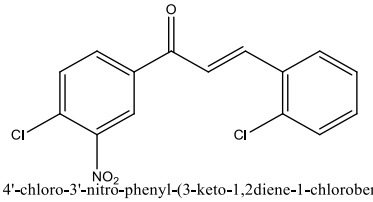
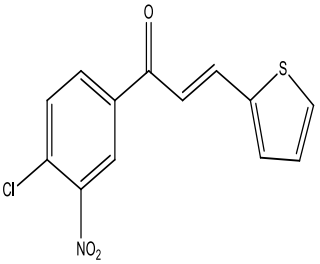
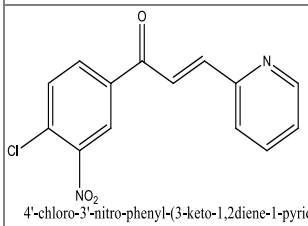
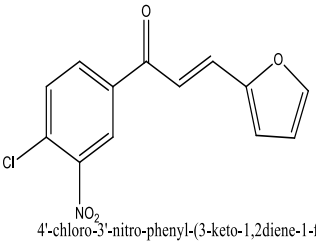
Name of the compound	Percentage weight	Rf value	Elution ratio	Molecular weight	Melting point(oc)
 4'-chloro-3'-nitro-phenyl-(3-keto-1,2diene-1-fluorobenzene	86%	0.8	8.2	304	142
 4'-chloro-3'-nitro-phenyl-(3-keto-1,2diene-1-chlorobenzene	0.94	0.7	8.2	322	132
 4'-chloro-3'-nitro-phenyl-(3-keto-1,2diene-1-thiophene	0.68	0.8	8.2	293	121
 4'-chloro-3'-nitro-phenyl-(3-keto-1,2diene-1-pyridine	0.74	0.7	8.2	288	96
 4'-chloro-3'-nitro-phenyl-(3-keto-1,2diene-1-furon	0.8	0.7	8.2	277	91

Table 2. *Molinspiration results of physicochemical properties of di substituted chalcones.*

COMP ID	MW	clogP	n AH	n DH	TPSA	n rotb
3A	305	4.03	4	0	62.9	4
3B	322	4.31	3	0	62.8	4

3C	293	3.58	3	0	62.8	4
3D	288	2.51	4	0	88.19	4
3E	277	2.94	4	0	75.7	4
MW, molecular weight; nDH, number of H-bond donors; nAH, number of H-bond acceptors; TPSA, topological polar surface area; cLogP, Partition coefficient.						

Table 3. *Molinspiration bioactive score value of disubstituted chalcones.*

COMP ID	GPCR ligand	Ion channel modulator	kinase inhibitor	Nuclear receptor ligand	protease inhibitor	enzyme inhibitor
3A	-0.4	-0.29	-0.4	-0.26	-0.45	-0.18
3B	-0.43	-0.32	-0.56	-0.27	-0.52	-0.28
3C	-0.7	-0.55	-0.58	-0.55	-0.69	-0.36
3D	-0.2	-0.19	-0.24	-0.44	-0.39	-0.03
3E	-0.77	-0.76	-0.86	-0.62	-0.92	-0.46
If bioactivity score is >0, it is an active compound while <-5.0 is an inactive compound and range between -5.0 to 0.0 is moderately active compounds.						

Table 4. *Pre*ADMET *results of disustituted chalcones.*

COMP ID	HIA (%)	*Invitro* Caco2(nm/sec)	MDCK (nm/sec)	PPB	BBB	CYP 2C9, 19
3a	98.4	15.1	0.43	96.8	0.25	INHIBITOR
3b	98.4	7.2	6.94	95.5	0.039	INHIBITOR
3c	96.6	1.18	1.47	96.8	1.44	INHIBITOR
3d	97.1	8.4	4.93	94.6	0.03	INHIBITOR
3e	95.3	2.16	3.55	98.8	1.38	INHIBITOR
HIA(%), Percentage human intestinal absorption; PCaco2 (nm/sec), Caco2 cell permeability in nm/sec; MDCK (nm/sec), Madin-Darby canine kidney cell permeabity in nm/sec; PPB(%), *in vitro* plasma protein binding (percentage); BBB, in vivo Blood-Brain Barrier penetration.						

Table 5. *PreADMET toxicity values of di substituted chalcones.*

Comp ID	algae_at	Ames_test	TA100_10RLI	TA100_NA	TA1535_10RLI	TA1535_NA	Carcino_Mouse	Carcino_Rat
3a	0.026515	mutagen	Positive	negative	Negative	Positive	Positive	Negative
3b	0.015208	mutagen	Positive	negative	Positive	Positive	Positive	Negative
3c	0.0310418	mutagen	Positive	negative	Negative	Negative	Positive	Negative
3d	0.0651226	mutagen	Positive	positive	Negative	Positive	Positive	Negative
3e	0.0611501	mutagen	Positive	negative	Positive	Negative	Positive	Negative
Mutagenicity of ames salmonealla TA100, TA98, TA1535 Species result positive (+) shows compound is mutagenic negative (-) means non mutagenic. In rodent carcinogenicity 2 years assay of rat and mouse by backward elimination and Rprop neural net method result Negative (-) indicate non carcinogenic, positive (+) indicate carcinogenic.								

Table 6. *OCHEM chemical and biological data of di substituted chalcones.*

Comp ID	Ames	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4	Melting point(0c)	pyrolsis point (celsius)
3a	Active	+	+	+	+	-	140	180
3b	Active	+	+	+	-	-	130	180
3C	Active	+	+	+	-	-	120	180
3d	Active	+	+	+	-	-	95	180
3C	Active	+	+	+	-	-	92	170
Ames test used to determine mutagenicity of the sample result inactive means not shows any mutational change in test organism, CYP1A2, 2C9, 2C19, 2D6, 3A4 enzyme inhibition from the above results positive (+) means enzyme inhibiting property, negative (-) means drug does not inhibit the enzyme, by using these software tool also determine the melting point and pyrolsis point (chemical decomposition of organic material by application of heat) of the compounds.								

Table 7. *Swiss physicochemical properties and bioactive score values of substituted chalcones.*

Comp ID	MW	No of heavy atoms	No of hydrogen acepters	No of hydrogen donars	No of rotable bonds	Log P	Log S	TPSA
3A	305	21	4	0	4	4.03	-4.57	62.9
3b	322	21	3	0	4	4.31	-5.82	62.8
3c	293	19	3	0	4	3.58	-5.02	88.19
3d	288	20	4	0	4	2.51	-4.37	75.7
3e	277	19	4	0	4	2.94	-4.82	76
Swiss ADME web tool predict the molecular weight, no of heavy atoms present in the molecule, no ofhydrogen acceptors, hydrogen donar, Log P (partition coeffient) , Log S(Solubility), TPSA, topological polar surface area. Lipinski, Ghose and Veber rules states that most molecules with good membrane permeability have logP ≤ 5, logS ≤ 5, molecular weight ≤500, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors ≤ 5, topological polar surface area (TPSA)<140 Å2 and number of rotatable bonds (n rotb)<10.								

Table 8. *Swiss percentage inhibition of di substituted chalcones.*

Comp ID	Enzyme	GPCR	Oxidoreductase	Protease	Ligand gated ion channel	Primary active transport	Other cytosolic protein	Nuclear receptor	Kinase	Higase	Cytochrome P450	Voltage gated ion channel	Surface antigen

3a	20	20	13.3	6.7	26.7	6.7	6.7	0	0	0	0	0	0
3b	20	20	13.3	6.7	26.7	6.7	6.7	0	0	0	0	0	0
3c	20	20	13.3	6.7	26.7	6.7	6.7	0	0	0	0	0	0
3d	13.3	6.7	20	6.7	13.3	6.7	0	0	6.7	0	20	0	0
3e	13.3	6.7	20	6.7	13.3	6.7	0	0	6.7	0	20	0	0

Table 9. *Osiris (data warrior) molecular properties of di substituted chalcones.*

Comp ID	MW	No of hydrogen acceptors	No of hydrogen donars	No of rotable bonds	cLog P	cLog S	TPSA	DL
3A	305	4	0	4	4.03	-4.57	62.9	5.03
3b	322	3	0	4	4.31	-5.82	62.8	4.94
3c	297	3	0	4	3.58	-5.02	88.19	3.58
3d	288	4	0	4	2.51	-4.37	75.7	5.01
3e	277	4	0	4	2.94	-4.82	76	4.92

Table 10. *Osiris (data warrior) toxicity prediction of di substituted chalcones.*

Comp code	MU	TU	RE	IR
3A	None	None	None	None
3B	None	None	None	None
3C	None	None	None	None
3D	None	None	None	None
3E	None	None	None	None

Spectral analysis

IR (KBr disk) 1538 and 1346.89 (NO_2 streaching), 1555.43 (C-C=CH streaching), 1664 (C=O streaching), 1601 (C-chlorine), 1040 (C-F), 1537 (NO_2), 1693 (C=O), 1554 (C-C=CH), 1666 (C-Cl), 1540(NO_2), 1594(C-C=CCH Streaching), 2337 (C-Cl),1600(C=O streaching), 1538(C-C=CH streaching), 1555 (C- NO_2 Streaching), 2854 (C-Cl), MASS M+H= , HNMR=spectra displayed two doublet each of one proton intensity one at δ 7.45-7.68, second δ 7.91-8.2513, CNMR of α, βunsaturated carbonyl system of chalcone by the presence of three peaks δ 120.3-126.8, 145-147.9, 190.2-192.5 corresponding C1 propenone, C2 prpenone, C=O respectively [9,10].

Table 11. *Antifungal activity results of synthesized compounds (3a-3e).*

Comp ID	Concentration(mg/ml)	C. a ± S.D*	A. n ± S.D*	A. a ± S.D*
3a	1	10 ± 0.14	11 ± 0.16	8 ± 0.12
3b	1	12 ± 0.15	16 ± 0.18	10 ± 0.15
3c	1	10 ± 0.17	11 ± 0.19	12 ± 0.12
3d	1	13 ± 0.16	17 ± 0.11	13 ± 0.19
3e	1	10 ± 0.15	12 ± 0.10	8 ± 0.11
griseoflavin		14 ± 0.12	19 ± 0.15	16 ± 0.13
Each value is the mean of three replicate determinations ± standard deviation; C. a - Candida albicans; A. n - Aspergillus niger; A. a-Alternaria alternate.				

Table 12*. IC50 values DPPH radical scavenger results of synthesized compound (3a-3e).*

Test compound	IC 50 µg/ml values DPPH assay
3a	85.03 ± 0.19
3b	88.62 ± 0.10
3c	91.25 ± 0.05
3d	61.88 ± 0.01
3e	64.43 ± 0.00
BHT	95.25 ± 0.05
Each value is expressed as mean ± SD of three replicates; Stda BHT used as standard for DPPH radical scavenging activity.	

The active lead molecules detected by using insilico databases. The above results of insilico databases the compound act as active lead molecule so they used to synthesis so many heterocyclic derivatives, directly evaluate the biological activity [11,12]. From the antimicrobial results compounds shows excellent anti-bacterial activity, excellent anti-fungal activity remain compounds shows moderate activity, good anti-oxidant activity by DPPH method the values are nearer to standard drug value [13,14].

Conclusion

Disubstituted chalcones were synthesized from the starting material of disubstituted ketone, it have one electron withdrawing group and one electron releasing group. These compounds characterized by IR, NMR, MASS. the results of insilico databases all the compound act as active lead molecule so they directly evaluate for its biological activity The compounds have excellent antimicrobial and anti-oxidant activity the values are almost nearer to standard drug value. Finally conclude that in future these chalcone taken as an intermediate to synthesis pyrazole, imidazole, quinoline, thiazole derivatives and evaluate different biological activities.

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