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Synthesis, characterization, antimicrobial and antitubercular activity of some new pyrimidine derivatives

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Pyrimidine heterocycles are proven to be biologically active heterocycles, found in many biological systems, displaying a broad spectrum of biological activities including anticancer, anxiolytic, antioxidant, antiviral, antifungal, anticonvulsant, antidepressant and antibacterial activities. New substituted 2-oxopyrimidines have been synthesized from the chalcones linked *via* indane-1,3-dione moity by the methods discussed in experimental section. We have synthesized some new 2-[6-(substituted phenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione by reacting chalcones (step-1 compounds have been reported by us) with urea.

Keywords: Chalcones**,** Pyrimidines**,** Antimicribial activity, Antitubercular activity**,** Microplate Alamar Blue Assay

Chalcones are well known intermediates for synthesizing various heterocyclic compounds. The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial[1], anti-inflammatory[2], analgesic[3], antiulcerative, antimalarial, anticancer, antiviral, antitubercular, antihyperglycemic, immunomodulatory etc. Pyrimidine derivatives in medicinal chemistry have been well known for their therapeutic application. One possible reason for their activity is the presence of a pyrimidine base in thymine, cytosine and uracil, which are essential building blocks of nucleic acids, DNA, and RNA[4]. One important class of pyrimidine is 2-thiopyrimidine and its derivatives, which are also called as 2-mercaptopyrimidine compounds. The sulfur in 2-thiopyrmidine act as an interesting replacement for the existing oxygen atom bonded to C-2 in uridine base. Considering this assumption, 2-thiopyrimidine has attracted substantial interest of synthetic biochemists. The literature indicated that compounds having pyrimidine nucleus possess broad range of biological activities like analgesic, anti-inflammatory[4,5], anticancer[6], and antimicrobial activities[7-9] etc. Thiopyrimidine also possess biological activities like antiparkinsonism[10], analgesic, anti-inflammatory, antioxidant, antimicrobial activities[11-13], anticancer[14-16], and anti-HIV activity[17].

Based on these observations, we planed to synthesize some new substituted pyrimidines and thiopyrimidines from the chalcones linked via indane-1,3-dione moiety by the methods discussed below, in order to consolidate the results in the substituted pyrimidine series.

Experimental Details

Materials and methods

All the chemicals used in the synthesis were obtained from standard commercial sources. Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography. The separation of the compounds was checked on TLC under UV lamp and also by spraying the plates with 10% sulphuric acid or phosphomolybdic acid or ninhydrin solution.

All the melting points were determined in open capillaries, using Elico digital melting point apparatus, expressed in °C and are uncorrected. Infrared spectra (IR) were recorded on BRUKER ALPHA FT-IR

spectrophotometer with sodium chloride optics. Samples were screened in potassium bromide (KBr) pellets and the values are expresses in cm^{-1}. The ^1H NMR spectra of the compounds were recorded on BRUKER Avance 400 MHz Nuclear magnetic resonance spectrophotometer using tetramethylsilane (TMS) as an internal standard and the values are expressed in δ ppm. The standard abbreviations s, d, t, q, m, dd, dt, and brs refer to singlet, doublet, triplet, quartet, multiplet, doublet of a doublet, doublet of a triplet, and broad singlet, respectively.

General procedure for synthesis of new pyrimidine derivatives (3a-3f)[18-22]

The condensation of the chalcones with urea in an alkaline medium viz., in sodium hydroxide in the presence of ethanol as a solvent, at reflux temperatures (2 to 6 h) resulted in the formation of corresponding pyrimidines (Scheme 1). Completion of the reaction was established by TLC using silica gel-G. After completion of the reaction, the reaction mixture was poured onto crushed ice with constant stirring. The solid that separated was filtered, dried and purified by recrystalliation in ethanol. The purified pyrimidine derivatives were obtained as light to bright yellow coloured powders (Scheme 1). Table 1 shows the IUPAC names of the synthesized compound (**3a-3f**).

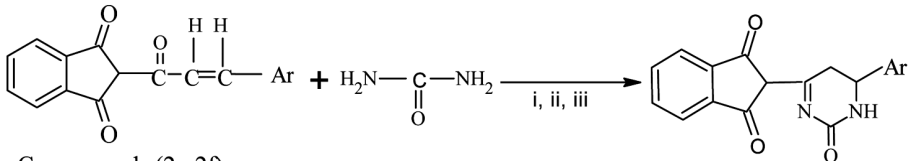
Synthesis of 2-[6-(4-Fluorophenyl)-2-oxo-1,2,5,6-tetrahydro Pyrimidin-4yl]-indane-1,3- dione, (3a)

A mixture of 2-[3(4-flourophenyl)-prop-2-enoyl]-indane-1,3-dione (**2a**) (0.01 mol) and urea (0.01 mol) was reacted using ethanol (25 mL) as a solvent in a round bottomed flask. The reaction mixture was heated under reflux on a water bath for 5 h with 10 mL sodium hydroxide (0.01 mol). The mixture was concentrated by distilling out the solvent under reduced pressure. Then the content was poured into a beaker containing crushed ice by mixing thoroughly, where upon a yellow to brown coloured solid separated out. This was filtered under vacuum, dried and purified by recrystalliation in ethanol (Scheme 1).The remaining compounds **3b**, **3c**, **3d**, **3e** and **3f** have been synthesized in the same manner as above by reacting with compounds (chalcones) - **2b**, **2c**, **2d**, **2e** and **2f**, respectively. The spectral data of the newly synthesized are depicted in Table 2 and physicochemical parameters are depicted in Table 3.

Results and Discussion

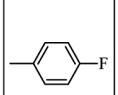
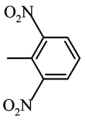
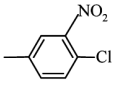
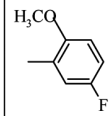
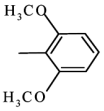
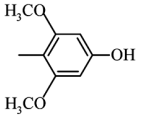
Antibacterial activity

The antibacterial activity was tested by disc diffusion method[23]. The antibacterial activity of the new synthesized compounds (**3a-3f**) were tested and compared with the standard (Ciprofloxacin) solution at concentration of 50 µg/mL and 100 µg/mL. DMSO



Coumpounds (2a-2f)

New Compounds (3a-3f)

Compound Code	3a	3b	3c	3d	3e	3f
Ar-						

Scheme 1 — Synthesis of new pyrimidine derivatives (**3a-3f**)

Table1 — IUPAC names of pyrimidines of chalcones.(**3a-3f**)

Compound	IUPAC Name
3a	2-[6-(4-Fluorophenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4yl]-indane-1,3- dione
3b	2-[6-(2,6-dinitro phenyl)-2-oxo-1,2,5,6-tetrahydro pyrimidin-4yl]-indane-1,3- dione
3c	2-[6-(4-Chloro-3-nitro phenyl)-2-oxo-1,2,5,6-tetrahydro pyrimidin-4yl]-indane-1,3- dione
3d	2-[6-(3-Fluoro-6-methoxy phenyl)-2-oxo-1,2,5,6-tetrahydro pyrimidin-4yl]-indane-1,3- dione
3e	2-[6-(2,6-dimethoxyphenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4yl]-indane-1,3- dione
3f	2-[6-(4-Hydroxy-2,6-dimethoxy phenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4yl]-indane-1,3- dione

was used as a solvent and control. Two gram positive bacteria *Staphylococcus aureus* (ATCC 12598), *Bacillus subtilis* (ATCC 6051) and two gram negative bacteria *Eshcherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25619) were the organisms used. All the compounds have shown considerable activity against *B. subtilis,* activity of which is equal with the standard drug, where as all compounds of this work exhibited moderate to good activity at a concentration of 25 µg/mL against *S. aureus*. Overall all the new compounds have shown favourable anti-bacterial activity (Table 4 & 5).

Antifungal activity

The antifungal activity of all the new pyrimidine derivatives (**3a-3f**) was assessed by following the same procedure of disc diffusion method[23] as described in the antibacterial activity section. But one major difference is that, in this anti-fungal disc diffusion method, Sabouraud agar medium is used instead of Brain heart infusion agar medium. Fluconazole was employed as standard drug at a concentration of 30 µg/mL to compare the results of the test compounds. The fungi strains used were *Candida albicans* (ATCC 2091) and *Aspergillus niger* (ATCC 9029). The compounds **3a**(p-F) and **3c**(p-Cl) have more inhibition at less concentration among the series. Among the series of compounds **3a**(p-Cl) is more potent against *C. albicans* and the compound **3f**(o-OCH_3, o-OCH_3, p-oH) is more potent against *A. niger*. Overall all the new compounds have shown significant anti-fungal activities (Table 6).

Table 2 — Spectroscopic data of synthesized compounds

Compound	FT-IR (KBr, ν,cm^{-1})	^{1}H NMR spectral data (δ)	Mass (LC-MS) m/z value
3a	3239.50(N-H); 3016.32(Ar C-H); 1672.36(C=O); 1621.20(C=N); 1569.39(Ar C=C); 1228.37(C-N); 867.45(C-F)	10.7(s,1H, N-H Srt); 7.4-8.0(m, 8 H, Ar); 6.2(dd,J=9.8 Hz, J=4.2 Hz, 1H); 3.5(s,1H), 1.7(d, 2H)	335 (M+ peak)
3b	3251.44(N-H); 1691.47(C=O); 1614.87(C=N); 1571.31 (Ar C=C),; 1216.19(C-N)	10.8(s,1H,N-H Srt), 7.2-7.9(m,7H,Ar); 6.0(dd, J=10.1 Hz,J=4.3 Hz,1H); 3.3 (s,1H), 1.6(d, 2H)	408 (M+ peak)
3c	3246.51(N-H); 1700.14(C=O); 1614.86(C=N); 1562.24(Ar C=C); 1211.62(C-N); 831.49(C-Cl)	10.6 (s,1H, N-H Srt); 7.1-7.8(m, 7 H, Ar); 6.1(dd, J=10.2 Hz, J=4.6 Hz, 1H); 3.4 (s,1H),1.8(d, 2H)	396 (M+ peak)
3d	3259.50(N-H); 3021.27(Ar C-H); 1682.45(C=O); 1620.30(C=N); 1231.15(C-N)	10.2 (s,1H, N-H); 7.3-7.9 (m, 7 H, Ar); 6.3(dd, J=9.9 Hz, J=8.3 Hz, 1H); 3.4 (s,1H), 1.8(d, 2H)	365 (M+ peak)
3e	3243.49(N-H); 1678.19 (C=O); 1620.37 (C=N); 1562.25 (C=C); 1215.18(C-N); 1157.34(O-CH_3); 879.45(C-F)	10.5 (s,1H, N-H); 7.2-7.8(m, 7 H, Ar); 6.2(dd, J=10.1 Hz, J=9.3 Hz, 1H); 3.2 (s,1H), 1.6 (d, 2H)	377 (M+ peak)
3f	3433.70(O-H); 3252.41(N-H); 1673.14(C=O); 1619.21(C=N); 1552.16(C=C Str); 1221.60 (C-N Srt); 1163.25(O-CH_3)	10.3 (s,1H, N-H); 7.4-8.0(m, 6 H, Ar); 6.5(dd, J=9.8 Hz, J=4.1 Hz, 1H); 3.5 (s,1H), 1.5 (d, 2H)	393 (M+ peak)

Table 3 — Physicochemical parameters of synthesized compounds (**3a-f**)

Compound	Molecular Formula	Melting point (°C)	Yield (%)	R_f value
3a	$C_{19}H_{11}N_2O_3F$	150-152	55	0.70
3b	$C_{19}H_{10}N_4O_7$	156-157	58	0.68
3c	$C_{19}H_{10}N_3O_5Cl$	169-171	58	0.68
3d	$C_{20}H_{13}N_2O_5F$	160-162	57	0.72
3e	$C_{21}H_{16}N_2O_5$	179-181	59	0.72
3f	$C_{21}H_{16}N_2O_6$	163-165	54	0.75

Table 4 — Antibacterial activity of synthesized compounds (**3a-3f**) against gram+ve bacteria

Compound	Zone of inhibition (mm)							
	Bacillus subtilis				*Staphylococcus aureus*			
	75 µg/mL	50 µg/mL	25 µg/ml	10 µg/mL	75 µg/mL	50 µg/mL	25 µg/mL	10 µg/mL
3a	46	40	37	34	16	13	-	-
3b	40	36	32	30	22	20	18	-
3c	39	36	30	28	25	23	20	-
3d	42	38	35	32	20	18	16	-
3e	43	40	37	34	23	21	18	-
3f	43	41	38	35	25	22	19	-
Ciprofloxacin				30				26

- indicates absence of zone of inhibition

Table 5 —Antibacterial activity of synthesized compounds against gram –ve bacteria

Compound	Zone of inhibition (mm)							
	Pseudomonas aeruginosa				*Eshcherichia coli*			
	75 µg/mL	50 µg/mL	25 µg/mL	10 µg/mL	75 µg/mL	50 µg/mL	25 µg/mL	10 µg/mL
3a	42	38	36	32	22	19	-	-
3b	44	37	39	35	27	20		-
3c	43	40	38	33	34	28	18	-
3d	44	41	35	30	26	18		-
3e	46	42	41	32	25	22	15	-
3f	40	38	35	30	27	24	15	-
Ciprofloxacin				21				32

- indicates absence of zone of inhibition

Table 6 — Antifungal activity of synthesized compounds

Compound	Zone of inhibition (mm)							
	Aspergilus niger				*Candida albicans*			
	75 µg/mL	50 µg/mL	25 µg/mL	10 µg/mL	75 µg/mL	50 µg/mL	25µg/ml	10 µg/mL
3a	45	40	38	34	32	28	24	20
3b	42	41	39	34	31	25	-	-
3c	46	41	38	36	23	16	15	-
3d	47	40	38	35	32	26	-	-
3e	46	41	37	36	17	14	-	-
3f	48	42	39	37	20	15	14	-

- indicates absence of zone of inhibition

Flucanazole (30 µg/mL) : 26 mm against *Candida albicans* (ATCC 2091) and 24 mm against *Aspergillus niger* (ATCC 9029)

Table 7 — Anti-tubercular activity

Compound	MIC (µg/mL)
3a	6.25
3b	6.25
3c	12.50
3d	6.25
3e	12.50
3f	6.25
Pyrazinamide	3.125
Streptomycin	6.25
Ciprofloxacin	3.125

Antitubercular activity

All compounds, synthesized in the present investigation were screened for antitubercular activity against *Mycobacterium tuberculosis* ATCC 27294 H37 RV strain in the Middlebrook 7H9 (MB 7H9 broth) by using Streptomycin, Pyrazinamide and Ciprofloxacin as standard drug those have MIC at a concentration of 6.25 µg/mL, 3.125 µg/mL and 3.125 µg/mL, by following the procedure using Microplate Alamar Blue Assay (MABA)[24,25] of serial dilution method. The compounds **3a**(p-fluoro), **3b**(o,o-dinitro), **3d**(o-methoxy,m-fluoro), **3f**(o,o-dimethoxy, p-hydroxy) have moderate activity against ATCC 27294 H37 RV strain and showed MIC at 6.25 µg/mL (Table 7).

Conclusion

This work is an endeavor in the direction of synthesis and characterization of new heterocyclic compounds based on their IR, ^{1}HNMR and mass spectral data including their pharmacologcal and antimicrobial screening. The resulted new compounds, after purification and characterization by physical and spectral methods have been successfully converted into their pyrimidine derivatives by reaction with urea which then were identified by spectral and chemical methods and screened for selected biological activities based on the reported literature.

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