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Microwave Assisted ZnO Nanocatalysed biginelli Synthesis of Pyrazolopyrimidione Derivatives and Evaluation of Their Bioactivity

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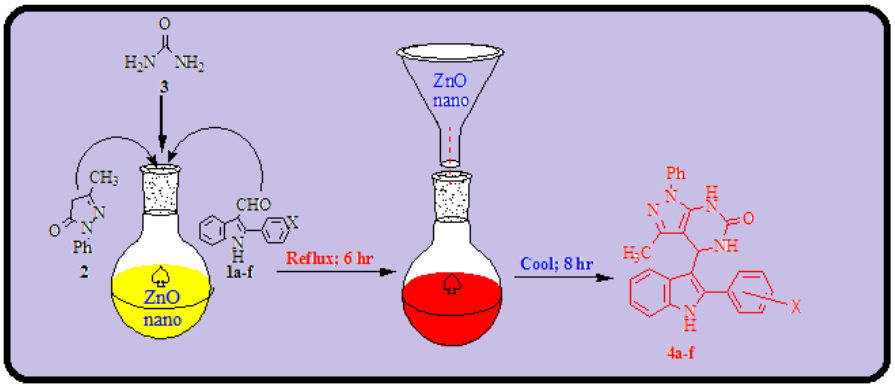
ABSTRACT

A new series of 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives **(4a-f)** by using multicomponent one pot reaction of 3-formylindole **(1a-f)**, 3-methyl-1-phenyl-5-pyrazolone **2** and urea **3** catalyzed by ZnO nanoparticles under microwave irradiation has been reported. Use of ZnO nanoparticles in this environmentally benign protocol takes less time and gives better yield than classical approach. Recyclability and reusability of this nanocatalyst is a paradigm shift directed to greener and rate efficient way. All the synthesized compounds **(4a-f)** are confirmed on the basis of elemental analyses and spectral data. Representative compounds were also evaluated for their antimicrobial activity against 10 pathogens and anti-inflammatory activity at different concentration. Some of them showed promising anti-inflammatory activity.

Graphical Abstract:



Keywords: Green Chemistry, Microwave irradiation, ZnO nanocatalyst, Biginelli Reaction, Multi-component reaction.

INTRODUCTION

Multi-component reactions (MCRs) have emerged as a promising approach and an integral part for the diversity oriented synthesis of biologically relevant scaffolds having dihydropyrimidinone (DHPM) moiety. It involves the rapid generation of complexity in molecules in a limited number of reaction steps. The functionalized scaffolds DHPM are the key element of various research efforts those have a huge value from the side of pharmaceutical **[1-3]** as it is the integral backbone of calcium channel blocker, antihypertensive **[4,5]**, potassium channel antagonists **[6]**, anti-HIV **[7]**,anti-epileptics **[8]**, antimalarials **[9,10]**, anti-inflammatory **[11]**and antimicrobial agents **[12,13]**. They are the building blocks of numerous drugs and natural products as well as display the properties as anti-metabolite in purine based biochemical reaction **[14]**.

Classical Biginelli reaction involves acid catalyzed three component condensation of ethyl acetoacetate, benzaldehyde and urea/thiourea in ethanol **[15]**. Virtually most of the research publications are flooded with the catalyst variation, asymmetric synthesis, scaffold variations etc. **[16].** A number of catalysts like Bronsted acid **[17-21]**, organocatalysts**[22-28]**, Lewis acid **[29-33]**, biocatalyst **[34-36]** and nanocatalyst **[37-41]** have been employed in Biginelli and Biginelli kind of scaffoldsto achieve high yields and time economy. Catalytic alteration without doing any major structural variationsin this privileged scaffold have been made through green chemical technologies including nanoparticles, mechanochemical route, ultrasonication, microwave irradiations, etc. Recently, a number of reviews have appeared in literature revealed that nanosized particles are of considerable interest due to their high surface area to volume ratio, non-toxicity, reusability, availability, efficiency, eco-friendly behavior. In addition to this, it also offers controllable selectivity in Biginelli reaction by using sulfonic acid group functionalized nano and micro silica structures **[42]**.

In continuation to our earlier research work **[43]**, we have synthesized 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives **(4a-f)** by reacting substituted 3-formylindole **(1a-f)**, 3-methyl-1-phenyl pyrazole-5-one **2**and urea **3** in absolute alcohol under microwave irradiation in the presence of ZnO nanocatalyst. Use of ZnO nanoparticles as catalyst make this synthetic protocol a greener and feasible approach as it enhances rate of reaction, yield and selectivity. The primary crux is to fulfill the aspects of eco-friendly chemistry by considering the intriguing value and prime importance of nanocatalysts and increase the selectivity to achieve the desired products **(4a-f)**.

MATERIALS AND METHODS

General: Melting points are reported uncorrected and were determined in open glass capillaries. The IR absorption spectra (ν_{max}. in $cm.^{-1}$) were reported on PERKIN ELMER FTIR Spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR were reported on JEOL-AL 300 Spectrophotometer using $CDCl_3$/DMSO-d_6 as solvents. TMS was taken as internal standard. Chemical shifts were reported in parts per million (ppm, δ) downfield from TMS. DART-MS spectra were recorded on Q-TOF micro mass spectrophotometer having a DART source. The DART mass spectra and CHN analyses were recorded at IIT, Bombay, India. The purity of compounds was assured by TLC (precoated silica gel 60 mesh, MERCK, as absorbent, UV light or iodine accomplished chamber). All common reagents and solvents were used as obtained from commercial suppliers without further purification. Various substituted 3-formylindoles **(1a-f)**, ZnO nanoparticles and 3-methyl-1-phenyl-5-pyrazolone **2**were prepared according to literature method **[47-52]**.

Animals: Swiss albino mice (25-35 gm) of either sex were used in this experimental protocol. The animals housed in standard rat cages (6 per cage) under standard laboratory conditions maintained at 25 ± 3ºC in 14/10 dark/light cycle. These mice fed with a standard laboratory chow (Aashirwaad food industries, Chandigarh) and water *ad libitum*. The animals were kept according to the guidelines of the Committee Designed for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations.

The experiment protocols were cleared by the institutional ethic committee of Dept. of Pharmacy, MGIMS, Jaipur.

Acute toxicity pilot study: Pilot study of acute toxicity of 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives **(4a-f)** were studied in Swiss rats with the standard method described by Winter *et. al.*1962)**[43]**. Initially pretreatment of mice was done by injecting standard drug celecoxib, followed by the injection of 0.1 ml. of Carregeenan (in 1 % CMC) solution into sub-plantar region of right hind paw. Paw edema volume was measured by means of a plethysmometer in form of dislocation of water (in ml.) at 0 hr and re-measured in the interval of 1, 2, 3 & 4 hr. after administration of carrageenan. It was recorded immediately after applying carrageenan at 0 hr then after the interval of 0.5 hr. Anti-inflammatory response is considered only when there is a reduction in edema volume in compare to that of control treated animals. Second group of animals was treated with the suspension of standard drug at a dose level of 10 mg/kg body weight.

Multicomponent One pot domino synthesis of3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives **(4a-f)**

Method A: Conventional refluxing method in the presence of ZnOnano as catalyst: A mixture of 3-formylindoles **(1a-f)** (1mmol), 3-methyl-1-phenyl-5-pyrazolone **2** (1mmol) and urea **3** (2mmol) in absolute alcohol with ZnO nanocatalyst was refluxed for 1.5-2.0 h at 120 ºC in a 100 mL round bottom flask. As the reaction proceeds, color of reaction mixture darkens to red indicating the formation of products **(4a-f)**. Progress of the reaction was monitored *via* Thin Layer Chromatography. After the completion of the reaction, the reaction mixture was filtered to recover ZnO nanocatalyst. Further it was washed several times with cold pet ether and then dried in muffle furnace for reusability. The filtrate was cooled to room temperature and then kept in deep freezer for 6 hours till solid precipitate was obtained. The red solid precipitate so obtained was filtered off, dried and recrystallized with ethanol to give pure desired products. Yield=85%

Method B: Microwave assisted green synthetic method in the presence of ZnO nanocatalyst: A mixture of 3-formylindoles **(1a-f)** (1mmol), 3-methyl-1-phenyl-5-pyrazolone **2** (1mmol) and urea **3** (2mmol) in absolute alcohol with ZnO nanocatalyst was taken in a beaker and absorbed on silica (320 mesh). The mixture was stirred well, dried in the air and then subjected to MWI for 18-24 min at power 300W. As the reaction proceeds, color of reaction mixture darkens to red indicating the formation of products **(4a-f)**. Progress of the reaction was monitored *via* Thin Layer Chromatography. Final temperature of the reaction was measured at the end of the reaction with the help of thermometer. After the completion of the reaction, the product was extracted with ethanol (2×10 ml) and the reaction mixture was filtered to recover ZnO nanocatalyst. The filtrate was cooled to room temperature and then kept in deep freezer for 6 hours till solid precipitate was obtained. The red solid precipitate so obtained was filtered off, dried and recrystallized with ethanol to give pure desired products, Further ZnO nanocatalyst was washed several times with cold ethyl acetate and dried in muffle furnace for reuse. Yield=97%

General procedure for the synthesis of 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives **(4a-f)**

4a.3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one: m.p. 246ºC IR (cm^{-1}, KBr): 3442 (-OH str.), 3170 (-NH str.), 3101, 3066 (phenyl ring), 2924 (-CH str.), 1627(C=C str.),^{1}HNMR (300 MHz., $CDCl_3$, 30ºC in δ ppm) 8.09 (1H, NH_{indole}), 7.18-7.91 (18H, ArH), 6.61 (-CH), 2.51 ($-NH_{pyrimidinone}$), 0.82 ($-CH_3$), ^{13}CNMR (75 MHz., $CDCl_3$, 30ºC in δ ppm) 38, 39, 40, 76, 77, 112, 117, 122, 123, 125, 128, 131, 133, 138, 150, 159, 164, MS (ES+) m/z = 420.1169 [M+1], molecular formula $C_{26}H_{21}N_5O$ Anal. Calc. C 74.43 (74.44), H 5.09 (5.05) N 16.67 (16.70) O 3.83 (3.81)

4b.4-(2-(4-fluorophenyl)-1H-indol-3-yl)-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin -6(3aH)-one : m.p. 252ºC IR (cm^{-1}, KBr): 3448 (-OH str.), 3185 (-NH str.), 3110, 3068 (phenyl ring), 2928 (-CH str.), 2876 ($-CH_2$ str.), 1630(C=C str.) ^{1}HNMR (300 MHz., $CDCl_3$, 30ºC in δ ppm) 8.02 (1H, NH_{indole}), 7.24-7.91 (18H, ArH), 5.90 (-CH), 2.54 ($NH_{pyrimidinone}$), 1.04 ($-CH_3$), MS (ES+) m/z = 438.1866 [M+1], molecular formula $C_{26}H_{20}FN_5O$ Anal. Calc. C 71.31 (71.36) H 4.58 (4.61) F 4.35 (4.34) N 16.04 (16.01) O 3.67 (3.66)

4c.4-(2-(4-chlorophenyl)-1H-indol-3-yl)-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin -6 (3aH)-one : m.p. 235ºC IR (cm^{-1}, KBr): 3442 (-OH str.), 3170 (-NH str.), 3101, 3066 (phenyl ring), 2924 (-CH str.), 2866 ($-CH_2$ str.), 1627(C=C str.) ^{1}HNMR (300 MHz., $CDCl_3$, 30ºC in δ ppm) 8.03 (1H, NH_{indole}), 7.19-7.92 (ArH), 5.97 (-CH methine), 2.51 $NH_{pyrimidinone}$), 1.01 ($-CH_3$), MS (ES+) m/z = 454.1866 [M+1], molecular formula $C_{26}H_{20}ClN_5O$ Anal. Calcd. C 68.84 (68.80), H 4.41 (4.44) Cl 7.80 (7.81) N 15.46 (15.43) O 3.53 (3.52)

4d.4-(2-(4-bromophenyl)-1H-indol-3-yl)-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d] pyramid in -6(3aH)-one : m.p. 286ºC IR (cm^{-1}, KBr): 3435 ($-NH_{indole}$ str.), 3175 ($-NH_{pyrimidinone}$ str.), 3078 (phenyl ring), 2922 (-CH str.), 2862 ($-CH_2$ str.), 1635(C=C str.) ^{1}HNMR (300 MHz., $CDCl_3$, 30ºC in δ ppm) 8.01 (1H, NH_{indole}), 7.11-7.85 (ArH), 5.79 (-CH), 2.39 ($NH_{pyrimidinone}$), 0.90 ($-CH_3$), MS (ES+) m/z = 498.1232 [M+1], molecular formula $C_{26}H_{20}BrN_5O$ Anal. Calc. C 62.67 (62.66) H 4.07 (4.04) Br 16.05 (16.03) N 14.05 (14.02) O 3.18(3.21)

4e. 3-methyl-1-phenyl-4-(2-p-tolyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one : m.p. 266ºCIR (cm^{-1}, KBr): 3422 (-OH str.), 3165 (-NH str.), 3108, 3086 (phenyl ring), 2928 (-CH str.), 2876 ($-CH_2$ str.), 1625(C=C str.) ^{1}HNMR (300 MHz., $CDCl_3$, 30ºC in δ ppm) 8.01 (1H, NH_{indole}), 7.17-7.96 (ArH), 5.85 (-CH), 2.49 ($NH_{pyrimidinone}$), 0.92 ($-CH_3$), MS (ES+) m/z = 434.29 [M+1], molecular formula $C_{27}H_{23}N_5O$ Anal. Calcd. C 74.83 (74.81), H 5.33 (5.35) N 16.14 (16.16), O 3.70 (3.69)

4f.-(2-(2,4-dichlorophenyl)-1H-indol-3-yl)-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyram idin-(3aH)-one : m.p. 272ºCIR (cm^{-1}, KBr): 3446 (-OH str.), 3178 (-NH str.), 3111, 3069 (phenyl ring), 2929 (-CH str.), 2869 ($-CH_2$ str.), 1634(C=C str.) ^{1}HNMR (300 MHz., $CDCl_3$, 30ºC in δ ppm) 8.14 (1H, NH_{indole}), 7.23-7.95 (18H, ArH), 5.92 (-CH), 2.59 ($-NH_{pyrimidinone}$), 1.12 ($-CH_3$), MS (ES+) m/z = 488.1363 [M+1], molecular formula $C_{26}H_{19}Cl_2N_5O$ Anal. Calcd. C 63.91 (63.94) H 3.92 (3.89) N 14.31 (14.34) Cl 14.31 (14.34) O 3.24 (3.26)

RESULTS AND DISCUSSION

Titled compounds **(4a-f)** were obtained by Multicomponent Biginellireaction of 3-formylindoles**(1a-f)**, 3-methyl-1-phenyl-5-pyrazolone **2** and urea **3**catalyzed by ZnO nanoparticles. Conventional refluxing ofequimolar ratio of 3-formylindole **(1a)**, 3-methyl-1-phenyl-5-pyrazolone **2**, urea **3**and 1-2 drops of piperidine as catalyst resulted in the formation of two compounds which were identified as the minor condensed product 3-methyl-1-phenyl-4-((2-phenyl-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-one **5a** (confirmed by condensing 3-formylindole **(1a)** and 3-methyl-1-phenyl pyrazole-5-one **2**, ^{1}H NMR and IR) along with the desired major product 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one **4a**butin low overall yield.

To enhance the reaction rate, yield and selectivity towards the formation of only the desired product **4a**, different ratio of reactants and ZnO nanoparticles/bulk catalyzed reaction as well as microwave irradiation of 3-formylindole **(1a)**, 3-methyl-1-phenyl-5-pyrazolone **2** and urea **3** were studied. Best results were obtained when 3-formylindole **(1a)**, 3-methyl-1-phenyl-5-pyrazolone **2** and urea **3**in 1:1:2 molar ratio,respectively were irradiated in the presence of ZnO nanoparticles (10 mol %) by microwave irradiation.**(Table 1)**The optimized reaction conditions were extended to the synthesis of other titled

derivatives of 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one **(4a-f)**.

In the IR spectra of compounds (**4a-f),** absorbance from 3443-3430 $cm^{-1}$is assigned to –NH stretching vibration of indole moiety. A new broad absorbance from 3195-3169cm^{-1} is assigned to $–NH_{pyrimidinone}$ stretching vibration. The -CO stretching vibrations appeared from 1690-1710 cm^{-1} and the absorption bands from 1628-1614 cm^{-1} have been assigned to –C=N stretching vibrations. In the PMR spectra of compounds **(4a-f),** indolic-NH proton appeared as a broad singlet from δ 8.01-8.10 ppm (D_2O exchangeable). A complex multiplet from δ 7.37-7.72 ppm due to the aromatic protonsis observed. Methine proton of dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one moiety appeared as doublet from δ 6.48-6.60 ppm. Disappearance of indolicformyl peakfrom δ 10.1 ppm and appearance of a new characteristic sharp singlet from δ 1.96- 2.23 ppm due to $–NH_{pyrimidinone}$ and a singlet from δ 1.00-1.15 ppm due to methyl protons of pyrazolone moiety assured the formation of products **(4a-f)**. Further confirmation was obtained by high resolution mass spectra (HRMS) of compounds **(4a-f)** which displayed M+1 peak at 420**(4a)**, 438**(4b)**, 454**(4c)**, 498**(4d)**, 434**(4e)**, and 488**(4f)** that agreed well with their corresponding molecular formulae.

Scheme 1: Synthesis of 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives **(4a-f)**

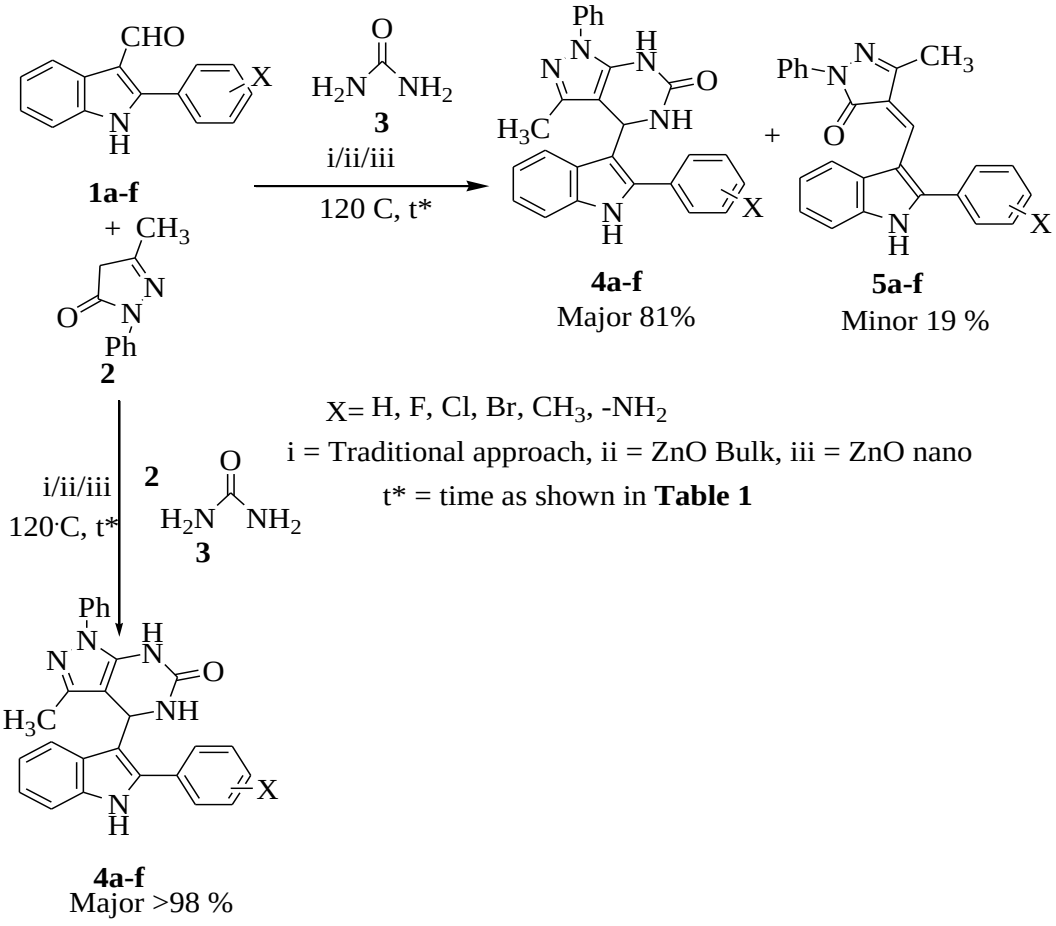


Table 1:% Yield and time for the synthesis of 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives(4a-f)

Compound	X	Without Catalyst		ZnO Bulk		ZnO Nano		
						Conventional		Microwave
		Yield (%)	Time (hr)	Yield (%)	Time (hr)	Yield (%)	Time (hr)	Yield (%) Time (min)
4a	$4\text{-}CH_3$	72	4.0	81	1.8	85	1.4	89 20
4b	4-Cl	74	5.2	90	1.7	92	1.5	94 23
4c	4-F	71	4.5	88	1.4	89	1.1	93 18
4d	4-Br	69	4.0	87	1.5	90	1.2	94 24
4e	H	73	4.2	89	1.5	95	1.3	96 20
4f	2,5-di-Cl	74	5.0	84	1.6	87	1.3	92 20

Disc Diffusion Assay (DDA)

Disc Diffusion Assay (DDA) **[44]** was performed to examine antimicrobial activities of a series of 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives **(4a-f)** on selected 10 pathogens. Out of these 10 pathogens, 6 are bacterial strains (*Klebisella, Pseudomonas, E. coli, Proteus, S. aureus, Shigella*) and 4 are fungal strains *(C. albicans, A.niger, A.flavus, T. rubrum)*. Concentration of the compounds **(4a-f)** varies from 10^{-5} mg/ml to 10^{-1} mg/ml per disc of the test compounds. All the synthesized compounds **(4a-f)** to be tested were put on radiation sterilized disc of 6 mm diameter. Discs were placed on the surface of agar plates and placed on the surface of agar plates and then inoculated at standard temperature condition for time period 10-12 hrs. Developed zone of inhibition (IZ) and activity index (AI) were calculated. The Minimum Inhibitory Concentration (MIC) was calculated by plotting the curve between the natural logarithm of the concentration of standard drug used against the square of the value of zone of inhibition respective to tested compounds **(4a-f)**. MIC value is the antilogarithm of the intercept on the logarithm of the concentration axis which was obtained by regression line drawn through the points **[46]**. Zone of Inhibition (IZ), Activity Index (AI) (**Table 2)** and Minimum Inhibitory Concentration (MIC) value are displayed in **Table 3.**

Anti-microbial activity

All the synthesized compounds **(4a-f)** were screened for their antimicrobial activityon selected 10 pathogens. Out of these 10 pathogens, 6 are bacterial strains (*Klebisella, Pseudomonas, E. coli, Proteus, S. aureus, Shigella*) and 4 are fungal strains *(C. albicans, A.niger, A.flavus, T. rubrum)*. Concentration of the compounds **(4a-f)** varies from 10^{-5} mg/ml to 10^{-1} mg/ml per disc of the test compounds **(Figure 1,2,3)**. Compound **4a** having methyl group as substituent in the formylindole moiety shows excellent activity against *Klebisella and E.coli*even at its lower concentration and it showed mild activity against *Shigellaflexneri*. In compound **4b** and **4c,** introduction of chlorine and fluorine group, respectively in the indolicaryl ring decreases their activity against *Shigellaflexneri bacteria*. Compound **4c** also deliberated promising activity against *A. niger and T. rubrum*at all its concentration. It is highly potent than standard drug tetracyclin against *KlebisellaPneumoniae* (MIC 0.1218) and *T.rubrum* (MIC 0.38) **(Table 3)** Compound **4e** does not exhibit any activity against *Shigellaflexneri*and was found highly active against *C. albicans*. In compound **4f,** the presence of two chlorine atoms in indolic aryl ring resulted in significantly good activity against all pathogens at its higher as well as lower concentration. All the synthesized compounds **(4a-f)** were found to be far more potent than the standard reference drug tetracyclin. **(Table 2)** and need to be rigoursly explored further.

Table 3: Antimicrobial activity of various 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives(4a-f)

Compounds	Mean value of area of inhibition in mmIZ[a] (AI)[b]																	
	4a						4b						4c					
Concentration (in ppm) Bacterial sps.	**Standard**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-2}**	**10^{-1}**	**Standard**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-2}**	**10^{-1}**	**Standard**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-2}**	**10^{-1}**
Klebisella	51	40.78	38.74	36.71	38.74	39.76	51	30.59	28.55	29.57	30.59	32.63	50	10.20	12.24	15.30	20.40	15.30
Pseudomonas	53	27.51	31.58	31.58	35.66	37.70	50	29.58	18.36	15.30	16.32	30.60	51	37.72	40.78	32.63	37.72	30.59
E.coli	49	33.67	33.67	35.71	37.75	41.84	50	15.30	17.34	18.36	20.40	15.30	50	38.76	40.80	42.84	38.76	38.76
Proteus	53	31.58	32.60	35.66	36.68	37.70	45	24.53	15.55	27.60	27.60	28.62	50	10.20	12.24	15.30	13.26	14.28
S.aureus	47	30.64	33.70	34.72	34.72	36.77	45	29.64	30.66	32.71	32.71	35.77	46	11.24	20.43	38.83	15.33	20.43
Shigella	44	15.41	21.47	22.50	24.54	25.57	55	18.33	20.36	20.36	21.38	25.45	50	12.24	15.30	13.26	14.26	14.28
C.albicans	52	28.54	29.56	31.60	31.60	33.65	52	32.61	35.67	39.75	39.75	40.77	52	15.29	35.67	30.58	30.58	10.19
A.niger	50	23.	25.	25.	26.	27.	49	28.	29.	28.	30.	31.	47	39.	42.	40.	40.	39.

		46	50	50	52	54		57	59	59	61	63		83	89	85	85	83
A.flavus	49	18.37	18.37	18.39	20.41	21.43	50	28.52	26.52	28.56	32.64	40.80	55	27.49	27.49	28.51	28.51	27.49
T.rubrum	52	25.48	28.54	29.56	31.60	34.65	54	39.72	38.70	35.65	35.65	38.70	54	47.87	42.78	40.74	45.83	49.91

Compounds	**Mean value of area of inhibition in mmlZ[a] (AI)[b]**																	
	4d						**4e**						**4f**					
Concentration (in ppm) Bacterial sps.	**Standard**	**10-5**	**10-4**	**10-3**	**10-2**	**10-1**	**Standard**	**10-5**	**10-4**	**10-3**	**10-2**	**10-1**	**Standard**	**10-5**	**10-4**	**10-3**	**10-2**	**10-1**
Klebisella	50	40.80	40.80	40.80	43.86	44.88	54	11.20	20.37	31.57	25.46	26.48	58	41.70	38.65	30.52	33.57	36.62
Pseudomonas	48	31.65	32.67	38.79	40.83	40.83	53	10.19	12.23	12.23	13.25	14.26	48	35.73	34.71	36.75	37.77	37.77
E.coli	48	28.58	28.58	30.63	33.69	33.69	50	30.06	32.64	32.64	35.70	40.80	55	36.65	38.69	39.71	41.74	40.73
Proteus	51	30.59	31.61	31.61	33.65	32.63	53	20.38	34.64	35.66	38.72	42.79	50	30.60	33.66	33.66	32.64	32.64
S.aureus	50	26.52	27.54	30.60	32.64	31.62	50	25.50	27.54	28.56	31.62	35.70	45	25.55	27.60	28.62	32.71	33.73
Shigella	50	33	34	37	37	39	55	Ni	Ni	Ni	Ni	15	49	29	32	32	36	39

		.66	.68	.74	.74	.78		Nil	Nil	Nil	Nil	.27		.59	.65	.65	.73	.79
C.albicans	53	40.75	40.75	37.70	38.72	37.70	51	40.78	41.80	45.88	48.94	49.94	51	37.72	39.76	37.72	38.74	39.76
A.niger	47	29.62	28.59	29.62	31.66	31.66	46	32.70	34.74	35.76	35.76	35.76	52	34.65	36.69	37.71	38.73	41.79
A.flavus	50	27.54	25.50	28.56	29.58	28.56	50	28.56	34.68	37.74	37.74	39.78	47	39.83	39.83	38.81	35.74	33.70
T.rubrum	51	37.72	39.76	39.76	40.78	40.78	51	25.49	35.69	37.73	41.80	40.78	53	39.74	40.75	43.81	41.77	41.77

Table 4. Minimum Inhibitory Concentration (MIC) value of 3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-one derivatives**(4a-f)**

Compd	**MIC (mg/disc)**									
	Bacterial Species						**Fungi**			
	Klebsiella Pneumoniae	***Pseudomonas***	***E. coli***	***Proteus vulgaris***	***S. aureus***	***Shigella flexneri***	***Aspergillusniger***	***A. flavus***	***Candida albicans***	***T. rubrum***
4a	1.979	0.5790	0.7645	0.8920	1.001	0.5621	1.0548	0.9285	0.9868	0.6073
4b	1.367	2.060	1.3684	0.9622	0.9160	0.5811	2.127	0.1255	0.8140	----------
4c	0.1218	1.137	1.029	0.6708	1.022	1.338	1.130	2.125	1.165	0.3802
4d	1.194	0.6181	0.8546	1.366	0.8400	0.9579	1.256	1.302	1.429	1.400

4e	0.06 78	0.59 54	0.62 44	----- ---	0.48 27	------ --	1.33 9	0.63 83	0.82 99	------ --
4f	------ --	1.35 40	1.17 14	1.72 44	0.59 67	0.60 12	0.93 39	0.94 07	1.78 25	1.58 60
Tetracyclin	1.22 26	1.70 48	1.42 93	1.97 50	1.92 55	1.54 54	0.92 10	2.69 58	0.13 5	2.12 97

Disc diameter: 6 mm

Anti-inflammatory Activity

Carrageenan induced hind paw edema is the commonly used standard model of acute inflammation. Carrageenan is generally used as standard agent of choice for testing anti-inflammatory drugs due to its antigenic property and it is away from apparent systemic effects. Biphase response from 1st to 4th hour for calculating % inhibition in form of carrageenan induced edema was followed. In first phase (0-3 hrs. after injecting carrageenan to hind paw), histamine, serotonin and kinins**[45]** are released and it is mediated through the release of these chemicals. On the other hand, second phase (3-4 hrs) is corresponds to the release of prostaglandins and bradykinins**[46]**.

All the synthesized compounds **(4a-f)** were screened for anti-inflammatory activity using Carragenan induced hind paw edema model. Compounds **(4a-f)** showed significant activity ranging from 25% to 83.33%and standard drug celecoxib showed 25 % and 66.67 % inhibition rate after 1 hr and 3 hr, respectively.**(Table 4)** The structural similarity of the standard drug celecoxib and potent compound **4a** (pyrazolone ring and methyl group substituted by trifluoro methyl) showed maximum anti-inflammatory activity among all the synthesized compounds. Especially, synthesized compounds **4a** and **4e** revealed a marked anti-inflammatory activity, subsequently may omit itself as a good anti-inflammatory drug. Compound **4c** did not show remarkable inhibitory rate with time whereas compound **4b,4f** are found to be least potent for this activity among the series. Still further scrutiny of pharmacological profile of the drug needs to be looked into.

Table 5:Anti-inflammatory activity of3-methyl-1-phenyl-4-(2-phenyl-1H-indol-3-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3aH)-onederivatives **(4a-f)**

Compound Code	**Mean Paw Edema Volume (in ml)**					**% Inhibition rate**				
	0hr	1hr	2hr	3hr	4 hr	0hr	1hr	2hr	3hr	4 hr
Control	1.2	1.2	1.2	1.2	1.2	-	-	-	-	-
Standard (Celecoxib)	0.9	0.7	0.6	0.5	0.4	25	41.66	50	58.33	66.67
4a	0.7	0.7	0.5	0.3	0.2	41.66	41.66	58.33	75	83.33
4b	0.8	0.8	0.7	0.7	0.6	33.33	33.33	41.66	41.66	50
4c	0.7	0.6	0.6	0.6	0.4	41.66	50	50	50	66.66
4d	0.8	0.7	0.7	0.6	0.5	33.33	41.66	41.66	50	58.33
4e	0.6	0.6	0.4	0.4	0.2	50	50	66.66	66.66	83.33
4f	0.9	0.8	0.8	0.7	0.6	25	33.33	33.33	41.66	50

Dose Level: 10 mg / kg

% Inhibition rate = V_c-V_t/V_c ×100

V_c = Edema Volume of Control

V_t = Edema Volume of test compounds

CONCLUSIONS

Suitable ZnOnanocatalyst is imported to turn the face of this motif towards greener eco-friendly sideto this reaction, In this research study, it has been concluded that the rate enhancement and removal of isolation of intermediate adds quality in domino one pot synthetic pathway. Therefore, it is widely acceptable rather linear transformation which is followed by subsequent nucleophilic attack of urea molecules over condensed product 3-methyl-1-phenyl-4-((2-phenyl-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-one **5a**.

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