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Synthesis of Dihydropyrimidone Derivatives by Application of Organic Red Clay and their Anti-Microbial Screening

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ABSTRACT
The organic red clay used as heterogenous catalyst for the one-pot Biginelli reaction in mild conditions; good to excellent yield of the desired 3,4-dihydropyrimidone derivatives was obtained in short reaction time. Powder XRD (X-ray Diffractometer), SEM-EDX (Scanning Electron Microscope- Energy Dispersion X-ray) analysis was carried out to characterize the red clay. Crystallite size of clay calculated from XRD spectra. The synthesized compounds examine using proton NMR, carbon NMR & FTIR spectroscopic studies. The reaction was carried out under solvent-free condition, using microwave heating. An environmentally benign procedure, recovery of catalyst, & short reaction time are some of the important features of this novel protocol. In addition, synthesized compounds were screened for their anti-microbial properties against two bacterial pathogens & one fungi. Most of products demonstrated higher to moderate anti-microbial actions.

Keywords: Dihydropyrimidone; Solvent-free; Microwave heating; Organic Red Clay; Antimicrobial Screening

INTRODUCTION
Clays are the nanoparticles with layered structures and hydrophilic nature Fig. 1. They are used as Bronsted and Lewis acids or as bases to catalyze various types of organic reactions[1]. Otherwise, hydrophobic modification of the clay intrasurface allows many organic guest molecules to be easily intercalated[2]. Biginelli synthesis of dihydropyrimidones are important class of compounds which attracts high attention due to their therapeutic and pharmacological properties[3,4] and they exhibit intresting biological effects[10-18]. 3,4 Dihydropyrimidin-2(1H) –ones derivatives (DHPMs) are one of these compounds which can be synthesized by acid catalyzed three component cyclocondensation reaction constituting aldehyde, urea and an easily enolizable carbonyl compound[19,20]. The simple and direct method originally reported by Biginelli involving three component condensation reactions (i.e. aldehydes, β-keto ester, and urea) often suffer from low yields practically in case of substituted aromatic aldehydes[21].

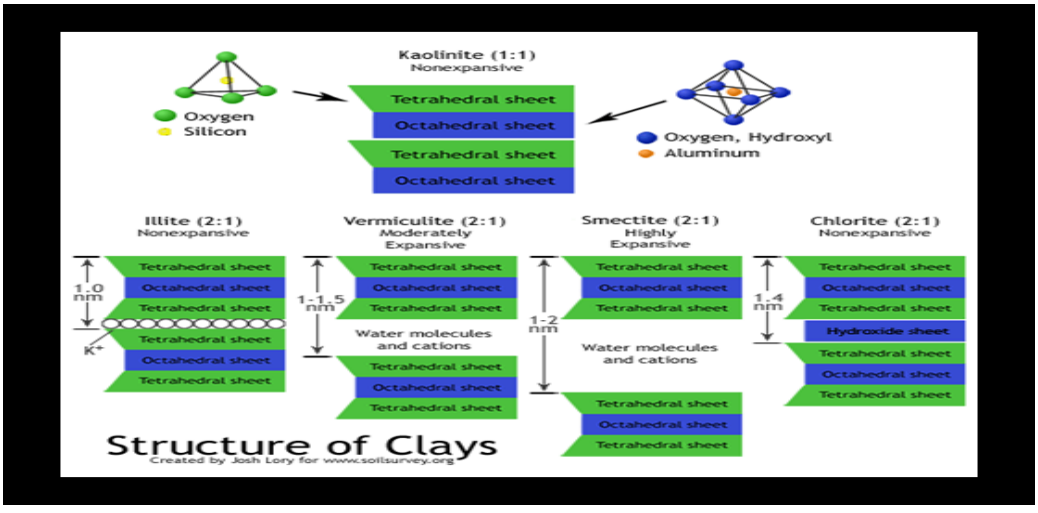

Fig. 1: shows this clay minerals absorb water between their layers, which move apart and the Clay's swells.

Several modifications and improvements have been sought. Although high yields could be achieved by following complex multi-step procedures, these methods lack the simplicity of the original one-pot Biginelli protocol[22].
Therefore, there is a need to develop versatile, simple and environmentally friendly processes for the synthesis of 3, 4-dihydropyrimidin-2(1H)-ones. The development of alternative methods would extend the scope of this Biginelli reaction. Currently, application of microwave in many fields of organic synthesis has many advantages such as shorter

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reaction times, improve reaction yields, and easier work-up matching with 'green chemistry' protocols. Studies to scale up the microwave-assisted reactions from the laboratory to industrial scale without changing the laboratory optimized reaction conditions have been investigated. The few reports describing the improvements brought about by the use of microwaves in the synthesis of 3, 4-dihydropyrimidin-2(1H)-ones have been published so far. In particular, clays and catalysis seem to have a very promising future, and even if many catalytic application have already been found day by day (in the laboratory & on an industrial scale), with a focus mainly towards establishing new environmentally-friendly technologies. Herein as part of our continued efforts to develop green and new catalysts systems with a reduced environmental impact[23-26].

EXPERIMENTAL

Materials

Reactions were monitored by thin layer chromatography on 0.2 mm silica gel F-252 (Merck) plates. All β-keto esters, aldehydes, and urea derivatives were obtained from Aldrich Chemical Co. and used without further purification, with the exception of benzaldehyde, which was distilled in vacuo prior to use. Organic red clay sample were collected from Panvel region, purified via simple washing and analysed by characterisation techniques. All solid components were employed as grained powders. Infrared spectral studies were carried out using KBr discs on a Perkin Elmer FTIR/4000 spectrophotometer. 1H NMR and 13C spectra were recorded in DMSO-d6 on Bruker Advance II 400 NMR spectrometer. Microwave oven used is of Sineo MAS II Plus. All products were characterized by
FT-IR, 1H NMR, 13C NMR spectroscopic studies and by comparison of physical characteristics with authentic samples. In this work we report in vitro study of antimicrobial activity of synthesized dihydropyrimidones against Gram + ve bacterium (Staphylococcus aureus- ATCC 6538), Gram – ve bacteria (Escherichia coli - ATCC 25922) and fungi (Candida albicans- MTCC 183).

One-pot synthesis of Dihydropyrimidone derivatives

A mixture of appropriate aromatic aldehydes (0.01 mol), acetyl acetone/ethyl acetoacetate/substituted acetophenone (0.01 mol, 1.3 g), urea (0.015 mol, 0.9 g) / thiourea (0.01 mol, 0.76 g) and red clay as a catalyst (0.2 g) (Scheme 1, Scheme 2) was subjected to microwave irradiation for appropriate time without solvent. Cool the reaction mixture and quenched with crushed ice. The solid separated out was filtered, washed with cold water, dried and recrystallized from 95% ethanol to give pure products and structures confirmed by FT-IR, Proton, Carbon spectroscopic studies. The spent catalyst were collected by filtration and then washed with hot ethanol.

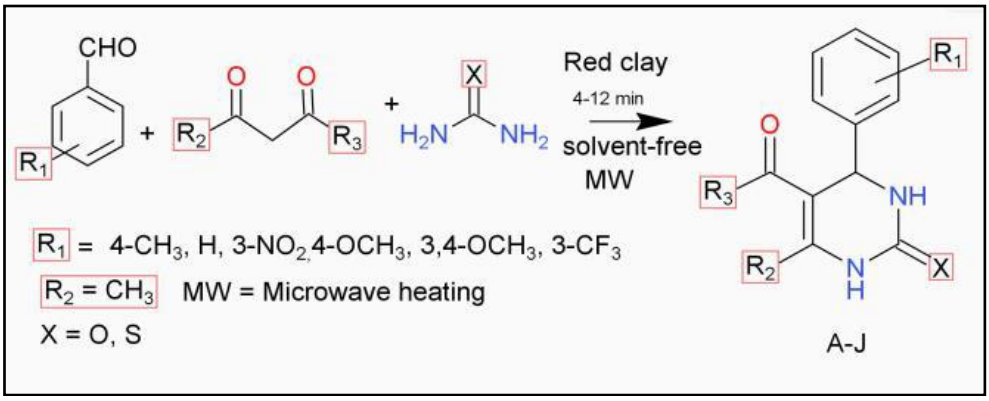

Fig. 2: Scheme 1

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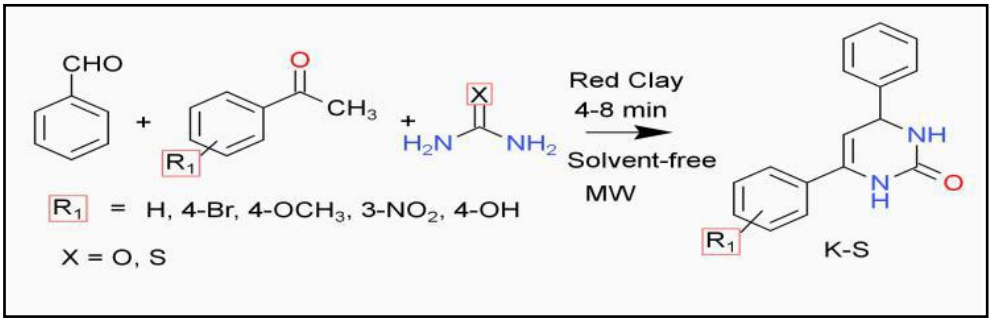

Fig. 3: Scheme 2

Characterisation of Organic Red Clay

X-ray diffraction (XRD) pattern of organic red clay was recorded using an X-ray Diffractometer (Rigaku; model Miniflex –II) with monochromatic Cu K-α radiation (λ = 1.54178 Ao). The XRD data was collected with a scan rate of 3o per minute. Energy dispersive x-ray analyzer (EDXA), Oxford instrument was used in conjuction with SEM (Scanning Electron Microscope) model JEOL JSM-6010 to measure the elemental composition of clay. To check the pH of clay Digital Equiptronics (Model EQ - 610) pH meter was used.

Antimicrobial assay of Dihydropyrimidone/thione derivatives

Antibacterial analysis was followed by using standard agar well diffusion method[27-29] to study the antimicrobial activity of the compounds. Each bacterial strain were grown in Luria bertani broth whereas fungal strain was grown in Sabouraud broth providing respective time incubation then used forthe study. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 0.1ml (10 mg compound in 1ml DMSO) of the sample solution were poured into the wells. The plates were incubated for 24 h at 37 ˚C for bacterial and fungal culture was incubated for 7 days. Antimicrobial activity was evaluated by measuring the zone of inhibition in mm against the test microorganisms. DMSO was used as solvent control. Kanamycin was used as reference antibacterial agent and antifungal agent. The results of in vitro study of antimicrobial activity of DHPM against each of the two bacterial species (Escherichia coli, Staphylococcus aureus) and fungal species (Candida albicans) are reported in the Table 3.

RESULTS AND DISCUSSION

This novel technique offers a clean and easy method for the preparation of the target molecules. The reaction provided additional advantages such as an easy work-up and is carried out in absence of the solvent.

Table 1: Solvent-free microwave-assisted synthesis of 3, 4-dihydropyrimidin-2(1H)-ones derivatives catalysed by Red clay (Scheme 1)

Entry	R_1	R_2	R_3	X	Product	M.P. (˚C)	Yield (%)	Time (min)	Power (w)
1	4-CH_3	CH_3	OC_2H_5	O	**A**	170-172	80	10	250
2	H	CH_3	OC_2H_5	O	**B**	200-203	65	8	250
3	H	CH_3	CH_3	O	**C**	211-213	82	8	250
4	H	CH_3	OC_2H_5	S	**D**	190-194	69	8	250
5	3-NO_2	CH_3	OC_2H_5	O	**E**	225-228	36	12	250
6	4-OCH_3	CH_3	OC_2H_5	O	**F**	205-208	40	8	400
7	3,4-OCH_3	CH_3	OC_2H_5	O	**G**	175-177	38	8	250
8	3-CF_3	CH_3	CH_3	O	**H**	312-316	75	7	250
9	3-CF_3	CH_3	OC_2H_5	O	**I**	160-164	80	6	250

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10	X	CH_3	CH_3	O	**J**	206-210	65	4	400

X = m – Thiophene

Table 2: Solvent-free microwave assisted synthesis of 4, 6-diphenyl-3,4-dihydropyrimidin-2(1H) - one derivatives by Red clay (Scheme 2)

Entry	R_1	X	Product	M.P. (°C)	Yield (%)	Time (min)	Power (w)
1	H	O	**K**	206-210	68	4	400
2	4-Br	O	**L**	180-184	85	5	400
3	H	S	**M**	150- 153	56	5	400
4	4-OCH_3	O	**N**	215-217	51	5	400
5	4-NO_2	O	**O**	225-228	74	7	400
6	3-NO_2	O	**P**	210-213	63	4	400
7	4-OH	O	**Q**	235-237	39	5	400
8	4-Br	S	**R**	145-147	58	5	400
9	4-NO_2	S	**S**	116-118	69	4	400

The electronic effect of aromatic aldehyde moiety may influence the nature, such as yield, reaction time as reported by sweet et al[30]. From the Table 1. it is clearly observed that the electronic effect of the different aromatic aldehyde (Scheme 1) has significant impact on the nature of reaction. From the data reported in Table 1, compound A, C & I obtained in very good yield (80-82%). Compound I bearing meta –CF3 group (R1) required less time (6 min) as compared to compound A (10 min) bearing para substituted –CH3 (R1) group & compound C (8 min) bearing –H (R1). It is intresting to note that by incorporating –CF3 group into the phenyl ring of the aromatic benzaldehyde, there is no significant difference observed in the percent yield of these compounds but product (Compound I) formed in shorter period of time compared to simple benzaldehyde (compound C). Compound E having meta substituted -NO2 group, compound F having para substituted –OCH3 & 3, 4 –OCH3 disubstituted compound G obtained in less yield (36-40%) as compound to other compounds. The reaction time was higher for nitro benzaldehyde (compound E) compared to simple benzaldehyde and other substituted benzaldehyde. Compound D, H & J obtained in moderate yield (65-75%) as compared to other compounds. Thiophene group substituted Compound J formed in shorter period of time compared to other compounds. In Scheme 2, reaction carried out between the benzaldehyde and substituted acetophenones. From Table 2. it was observed that electronic effect of different acetophenone has significant impact on nature of reaction such as yield, reaction time. Compound L having para –Br substituent obtained in very good (85%) as compared to simple acetophenones. It is evident that substituent X will affect the yield of reaction. Para bromo substituted acetophenone (compound L) given the more yield when reacted with urea than the reaction with thiourea (compound R), it is may be due to electronegavity of oxygen is more than sulphur. Compound K, M, N, P, R & S obtained in almost moderate yield (51- 69%). Compound Q having para –OH substituent obtained in less yield (39%) as compared to other compounds. The reaction time was higher (7 min) for para nitro substituted acetophenone while for meta nitro substituted acetophenone was shorter (4 min) than the simple and other substituted acetophenone.

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Analysis of Red clay

To test the recovery of catalyst model reaction was carried out with organic red clay (0.2 g) for synthesis of product A. There is negligible loss (less than 0.02g) was found with 3 cycle of reaction. The pH of organic red clay was calculated by using pH meter (Digital Equiptronics, Model EQ - 610) which is 5.65 (acidic).

EDX study of Red Clay

Elemental composition of organic red clay determined by EDS pattern recorded in the binding energy region of 1-10 KeV was shown in Fig. 4.

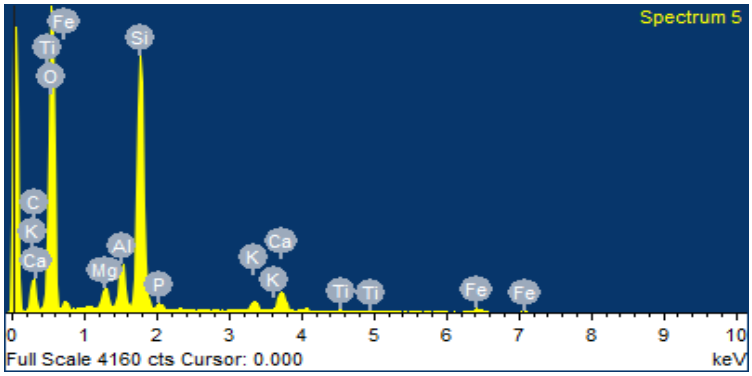

Fig. 4: EDX spectra of Organic Red Clay

From the EDX spectra Fig.4 it was cleared that red clay contains Oxygen (O), Aluminium (Al), Silicon (Si), Iron (Fe) elements in large percentage as compared to other elements. (Main Element Composition). Results are summerised in Table 3.

Table 3: Elemental Composition of Organic Red Clay

Elements	Red clay (Wt%)
Oxygen(O)	48.19
Sodium (Na)	0.37
Magnesium (Mg)	0.84
Aluminium (Al)	8.87
Silicon (Si)	14.66
Potassium (K)	0.37
Titanium (Ti)	0.70
Iron (Fe)	18.08

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X-ray diffraction study of Red clay

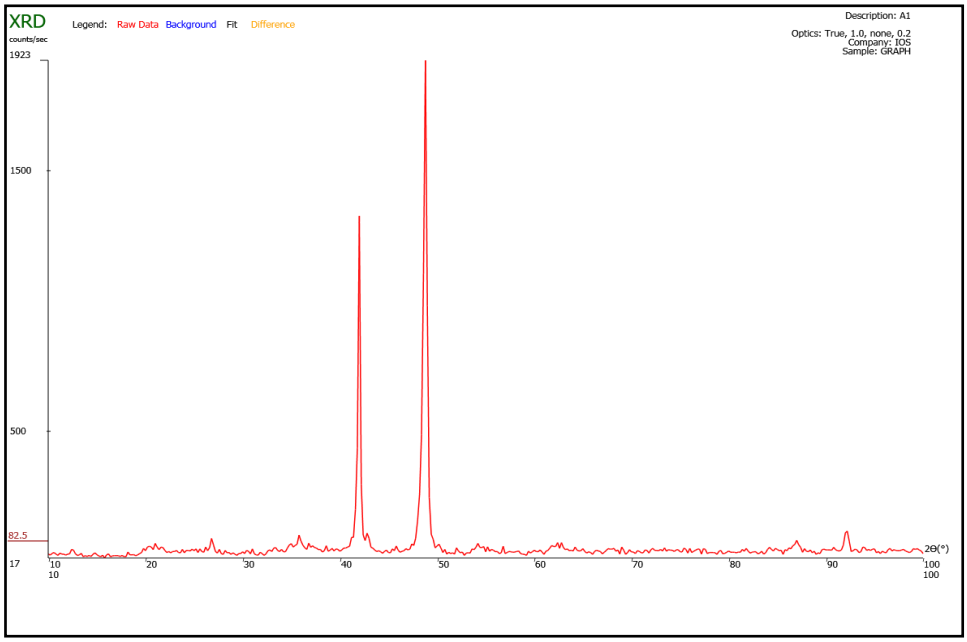

Fig. 5: XRD spectra of Organic Red Clay

Fig. 5 shows XRD Spectra of Organic red clay. The sharpness of peaks determined the degree of crystallinity. Two strong sharp peaks indicate good crystallinity of red clay. XRD plot Fig. 5 shows two well defined peaks at 2Θ = 42o and 48.8o which indicate good crystallinity. From XRD pattern, crystallite size of the red clay is calculated by using Debye-Scherrer equation,

$$D = 0.90\ \lambda / \beta \cos \theta \qquad \text{Equation 1}$$

Where, β = broadening of the diffraction line (Bragg peak) measured at full width at half of its maximum intensity (FWHM) (in radian), λ = wavelength of target, D = diameter of the crystal particle, θ = angle of diffraction

The average crystallite size distribution can be determined independently from X-ray diffraction line broadening which for the organic red clay is 20.40 nm. Surface area increases as the size of the clay particles decreases. Surface area and the pore volume in the clay structure also add to the efficiency of the catalyst. Because of their large surface area and the presence of surface defects and dislocations, nanoparticles in soil are very reactive towards external solute molecules.

Table 4: Antibacterial and antifungal activity of DHPMs

Compounds	Zone of Inhibition (mm)		
	Escherichia coli (-) Bacteria	Staphylococcus aureus (+) Bacteria	Candida albicans Fungi
Kanamycin	29	19	27
A	13	10	14
D	13	12	14
I	12	11	14

E	14	11	13
J	12	13	13
M	13	12	13
G	14	12	12
H	12	12	12

From Table 4. it was observed that all compounds showed moderate activity when compared with the standard kanamycin. Among all compounds tested for antimicrobial activity. Compound E having meta substituted nitro group showed the good moderate activity against gram negative bacteria Escherichia coli compared to other compounds. Amongst compounds which are tested for antibacterial activity all compounds showed good activity against gram positive bacterial species Staphylococcus aureus compared to Compound A which have methyl substituent at para position. Compound J having thiophene moiety showed good activity against gram positive bacteria Staphylococcus aureus compared to other compounds. Compounds A, compound D and compound I which has meta substituted trifluoro carbon group showed good activity compared to other compounds against fungal species candida albicans.

CONCLUSION

We have described a novel method for the preparation of substituted dihydropyrimidinones /thiones derivatives catalyzed by red organic clay as a catalyst under solvent-free conditions. Moderate to excellent yields of the corresponding DHPMs were obtained from readily available starting materials. The antimicrobial screening suggests that the newly synthesized Biginelli compounds tested positive for antibacterial and anti-fungal activity against microorganisms. Average crystallite size of red clay was 20.40 nm and pH was 5.65. As the organic chemist is becoming more aware of the clay's efficacy, its uses in organic synthesis are bound to increase, especially because it helps in developing eco-friendly chemical processes.

Acknowledgements

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