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MULTICOMPONENT ONE-POT FACILE SYNTHESIS OF PYRIMIDINE DERIVATIVES UNDER MICROWAVE IRRADIATION TECHNIQUE AND STUDY OF THEIR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT: Multicomponent one-pot synthetic protocol is applied for the efficient preparation of a series of pyrimidine derivative under microwave irradiation method. The synthetic process proceeds via Knoevenagel condensation initially with subsequent Michael addition reaction followed by cyclization of equimolar quantities of substituted bezaldehydes, ethylcyanoacetate and guanidine in the presence of ethanolic sodium hydroxide solution to produce corresponding pyrimidine derivatives. The reaction mixture was refluxed under microwave radiation for 7-12min at power level-2 which corresponds to 210watt. Microwave heating provides cleaner reaction condition with shorter reaction time and improved product yield as compared to conventional heating method. The structures of the newly synthesized compounds were elucidated by their FT-IR, ^{1}H-NMR and LC-MS spectral data. The newly synthesized compounds were screened for their anti-inflammatory activity *in-vitro* by using membrane stabilization method (heat induced hemolytic technique). Some of the tested compounds were found to possess significant anti-inflammatory potential as compared to the standard drug (Diclofenac).

KEYWORDS: Microwave, multicomponent, one-pot, synthesis, pyrimidine, anti-inflammatory.

1. INTRODUCTION

Heterocyclic compounds play vital role for the generation of new biologically active compounds in the area of drug development[i]. The heterocyclic framework provides opportunity for the preparation of privileged scaffolds to construct target compounds with suitable therapeutic properties[ii]. Among the nitrogen containing heterocyclic compounds, pyrimidine derivatives have received considerable attention due to their wide spectrum of therapeutic potentials such as anticancer, antihypertensive, anticonvulsant, antibacterial, antifungal, antiviral, anti-tubercular, anthelmintic, anti-diabetic, anti-Alzheimer, analgesic and anti-inflammatory activities etc[iii-vii].

Inflammation is a vital physiological response to different types of stimulus such as infection, trauma, burns, surgery and injury. So, non-steroidal anti-inflammatory drugs (NSAIDs) are

used to treat pain and inflammation via inhibition of cyclooxygenase enzyme (COX). COX is responsible for biosynthesis of prostaglandins (PGs) from arachidonic acid. But NSAIDs possess side effects like ulcers and bleeding. So, it was thought of interest to develop safer anti-inflammatory agents containing pyrimdine scaffold[viii-x].

Pyrimidine is a six membered heterocyclic compound containing two nitrogen atoms present at positions 1 and 3 of the ring system with molecular formula $C_4H_4N_2$ as presented in figure 1. The name of the pyrimidine was first coined by Pinner from combination of two words such as pyridine and amidine. Pyrimidine was first isolated by Gabriel and Colman in 1899. Pyrimidines are generally resistant to ring opening but the presence of electron-withdrawing groups at C_5 decrease the stability of the pyrimidine ring[xi-xiv].

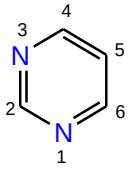


Figure1. Nomenclature of pyrimidine

Various clinically available drugs containing pyrimidine scaffold include Rosuvastatin (Anti-lipidemic), Buspirone (Anti-psychotic), Pyrimethamine (Antimalarial), Octotiamine(Anti-inflammatory), Phenobarbitone (Sedative-hypnotics), Stavudine (Anti-HIV), Azacitidine (Antineoplastic), Sulphamidine (Antibacterial), Enazadrem (Anti-psoriatic) etc as depicted in figure **2**. Ceritinib was identified as a potential anti-tubercular agent that contains pyrimidine pharmacophore in its structure[xv-xvii].

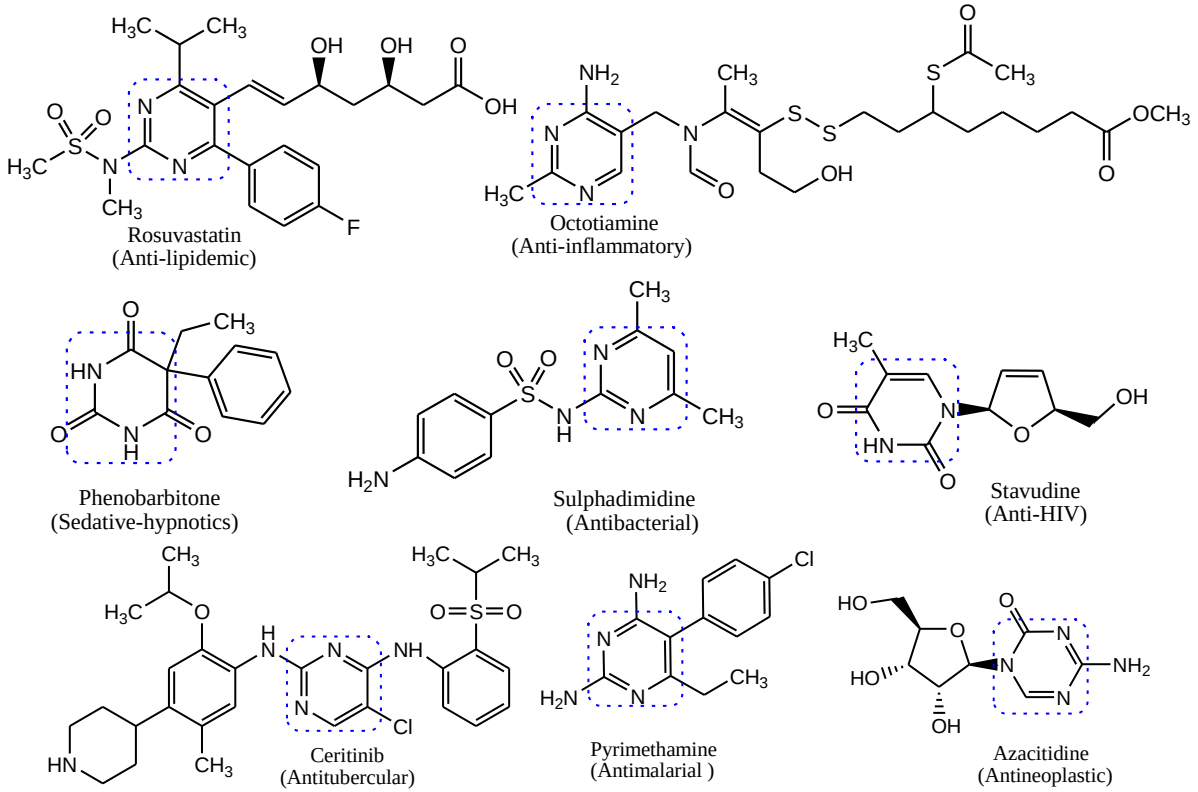


Figure 2. Structures of clinically available drugs containing pyrimidine scaffold.

In the present study, microwave heating method is applied to facilitate the multicomponent one-pot synthesis of pyrimidine derivatives under optimized reaction condition[xviii]. Microwave promotedsynthesis is considered as environmentally benign method that usually provide cleaner products with improved selectivity, reduced reaction time, efficient rate of the reaction

and high product yield[xix]. Multicomponent reactions (MCR) are the efficient synthetic protocol that involves the reactions where at least three reacting materials react to produce a single product[xx]. MCR is beneficial in relation to mainstream synthetic frameworks in terms of easy set up, simple reaction design, cost-effectiveness and atom-economy. This method acts as powerful tool to generate highly complex molecules with diverse structures and wide range of biological potentials.It is a focused approach in organic synthesis due to creation of new C–H, C–C, C–O and C-N bonds for the construction of molecular functionality from three or more starting materials through one-pot reaction condition[xxi,xxii].

So, the multi-component one-pot synthesis is performed under microwave irradiation by reacting equimolar mixture of substituted benzaldehydes, ethylcyanoacetate and guanidine [xxiii]. Mechanistically, the formation of the pyrimidine derivatives is asequence of reactions which involves Knoevenagel condensationof ethylcyanoacetate with substituted benzaldehydes by loss of watermolecule, followed by Michael addition of guanidine and intramolecular heterocyclization in presence of ethanolic sodium hydroxidesolution[xxiv]. The newly synthesized compounds are characterized by FT-IR, ^{1}H-NMR and LC-MS spectral data. The prepared compounds are subjected to *in-vitro* screening for their anti-inflammatory activity[xxv].

2. EXPERIMENTAL

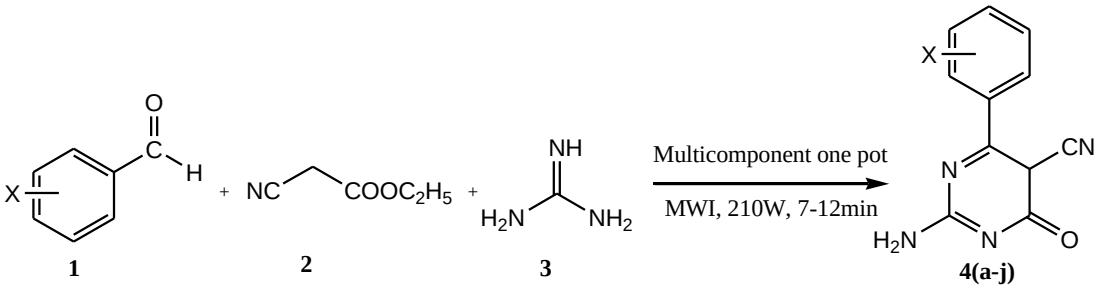
2.1. Materials

The chemicals and solvents used for the present study were of commercially grade. The melting points of the synthesized compounds were determined by open capillary tube method and are uncorrected. The purity of the synthesized compounds and completions of the reaction were checked by TLC on pre-coated Silica gel-aluminum plates (Type 60 F254, Merck) and were visualized by exposure to UV-light (254 nm) or iodine vapor. Ethyl acetate and n-hexane (40: 60) were used as mobile phase for monitoring TLC. The IR spectra of the titled compounds were recorded on FT-IR Spectrophotometer, model IR Affinity-1 (SHIMADZU), using KBr powder and the values are expressed in cm^{-1}. ^{1}H-NMR spectra of the selected compounds were recorded on FT-NMR Spectrometer; model Advance-II (Bruker), (400 MHz) using tetramethylsilane (TMS) as an internal standard. The multiplicities of the signals are denoted with the symbols *s, d, t* and *m* for singlet, doublet, triplet and multiplet respectively. The microwave induced synthesis was performed in scientific microwave oven, Catalyst System (operating between 140-700W). All the synthetic reactions were carried out at power level-2 which corresponds to 210W[xxvi].

2.2. Methods

General synthesis of 2-amino-(substituted phenyl)-4-oxo-1,4-dihydropyrimidine-5-carbonitrile(4a-j)

Equimolar mixture of substituted benzaldehydes (1a-j), ethylcyanoacetate (2) and guanidine (3) in ethanolic sodium hydroxide solution were refluxed under microwave irradiation at power level-2 (210W) for 7-12 min. The completion of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice cold water followed by acidification with dilute hydrochloric acid to get the solid product of pyrimidine derivatives (4a-j).



1 **2** **3** **4(a-j)**

Scheme 1. Synthetic route of the titled compounds (4a-j).

3. ANTI-INFLAMMATORY ACTIVITY

The newly synthesized compounds were screened for their anti-inflammatory activity based on the following *in-vitro* methods[xxvii].

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected from healthy human volunteer who had not received any NSAIDs for 2 weeks prior to the experiment and transferred to the centrifuge tubes containing heparinto prevent coagulation. The tubes were centrifuged at 3000 rpm for 10 min and the supernatant was carefully removed with a sterile pipette, washed three times with equal volume of isotonic saline solution and again centrifuged. The process was repeated three times until the supernatants became clear. The volume of blood was measured and reconstituted as 10% v/v suspension with isotonic saline solution.

Heat induced hemolysis

1.5 ml of 10% v/v RBCs suspension was added separately to 1.5 ml of test, control and standard solutions. Normal saline and Diclofenac sodium were used as control and standard respectively. All the centrifuge tubes containing mixture of test, control and standard solution were incubated separately at 56°C for 30 min. After incubation, all the tubes were cooled under running tap water. Then all the incubated tubes were centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants were measured at 560 nm. The experiment was performed in triplicates for all the test samples. Percent inhibition of hemolysis was calculated by the following formula.

$$\%\ \text{Inhibition of hemolysis} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

4. RESULTS AND DISCUSSION

In the present work, 2-amino-(substitutedphenyl)-4-oxo-1,4-dihydropyrimidine-5-carbonitrile (4a-j) were synthesized based on the multi-component one-pot synthesis by reacting equimolar mixture of substituted benzaldehydes, ethylcyanoacetate and guanidine under microwave irradiation. With the help of microwave induced synthesis, the rate of reaction was increased with improved yield and environmental-friendly reaction as compared to conventional heating method. For this purpose, ethylcyanoacetate undergoes Knoevenagel condensation reaction with substituted benzaldehydes followed by Michael addition reaction of guanidine and intramolecular cyclization in presence of ethanolic sodium hydroxidesolution.

The yield of final product obtained was 65-84% by using microwave irradiation technology. The melting points of the newly synthesized compounds were determined by open capillary tube method and were found to be in the range of 154-183^{0}C. Purity of the compounds was checked by TLC using pre-coated silica gel-G. The mobile phase used was n-hexane and ethylacetate (70:30). The spots were visualized under U.V light and the R_f values were found in the range of 0.54-0.67 (Table **1**).

Table 1. Physical data of newly synthesized compounds (4a-f).

Comp. Code	X	Molecular Formula	mp (°C)	R_f	C		M	
					RT (hour)	Yield (%)	RT (min)	Yield (%)
4a	H	$C_{11}H_8N_4O$	170-172	0.54	2	65	6	74
4b	4-CH_3	$C_{12}H_{10}N_4O$	172-174	0.67	4	69	8	74
4c	4-OCH_3	$C_{12}H_{10}N_4O_2$	180-183	0.65	3	58	7	67
4d	2-NO_2	$C_{11}H_7N_5O_3$	162-164	0.56	2	65	10	73
4e	4-NO_2	$C_{11}H_7N_5O_3$	160-162	0.58	4	72	9	84
4f	4-F	$C_{11}H_7FN_4O$	156-158	0.52	3	67	12	78
4g	4-OH	$C_{11}H_8N_4O_2$	154-157	0.56	2	54	7	68
4h	2-Cl	$C_{11}H_7ClN_4O$	166-169	0.58	4	62	11	76
4i	4-Cl	$C_{11}H_7ClN_4O$	174-177	0.54	3	66	9	72
4j	2,3-Cl_2	$C_{11}H_6Cl_2N_4O$	166-169	0.59	4	57	12	65

* **C:** Conventional; **M**: microwave

The IR spectroscopy was performed with IR Affinity-1 (SHIMADZU), using KBr powder and the values are expressed in cm^{-1}.The stretching vibrations in the range of 1290-1346cm^{-1},1422-1568cm^{-1}, 1576-1620cm^{-1}, 1674-1736cm^{-1}, 2190-2318cm^{-1}, 3034-3124cm^{-1} and 3487-3546cm^{-1}indicates the presence of -OCH_3, -C=C, -C=N,C=O, -CN, Ar-CH, -NH respectively. Further, the IR spectrum of nitro group (-NO_2) exhibited absorption with λ_{max} at 1620-1546 and 1440-1360cm^{-1}. Similarly, pyrimidine derivatives substituted with halogens exhibited the IR absorption bands in theregion 1426-1024cm^{-1}, 837-647cm^{-1}which corresponds to C-F str., C-Cl str. respectively. Whereas the presence of Ar-OH is confirmed by IR absorption bands in theregion of 3640-3235cm^{-1} and the C-H stretching in case of –CH_3 exhibited at 2945-2857cm^{-1}. The ^{1}H-NMR was performed on Advance-II (Bruker), (400MHz) using tetramethylsilane (TMS) as an internal standard. The chemical shift (δ ppm) in the range of 7.14-8.47 indicates the presence of aromatic proton (Ar-H) and was observed as multiplet. Similarly, the ^{1}H-NMR spectrum of –NH_2 was observed as singlet at δ 8.58. The mass spectra of the pyrimidine derivative exhibited molecular ion peak that corresponds to their molecular formula. Compound 4b,4c, 4d, 4e, 4f, 4g, 4h showed molecular ion peak at *m/z* 226.06, 242.04,257.06, 230.08, 228.02and 246.07respectively.

All the newly synthesized pyrimidine derivatives (4a-j) were evaluated for their anti-inflammatory activity using membrane stabilization method. The results of this evaluation study were compared with reference standard (Diclofenac sodium). The anti-inflammatory activity results were presented in table **2**and figure **3**. Among the tested compounds, 4c, 4d, 4f, 4g, 4h and 4i displayed promising activity as compared to standard drug (Diclofenac).

Table 2. Anti-inflammatory activity of synthesized compounds (4a-j)

Treatment groups	Absorbance	% Inhibition
4a	0.43	54.25
4b	0.38	59.57
4c	0.34	63.82
4d	0.36	61.70
4e	0.42	55.31
4f	0.32	65.95

4g	0.37	60.63
4h	0.33	64.89
4i	0.35	62.76
4j	0.38	59.57
Control	0.94	-
Diclofenac (Standard)	0.18	80.85

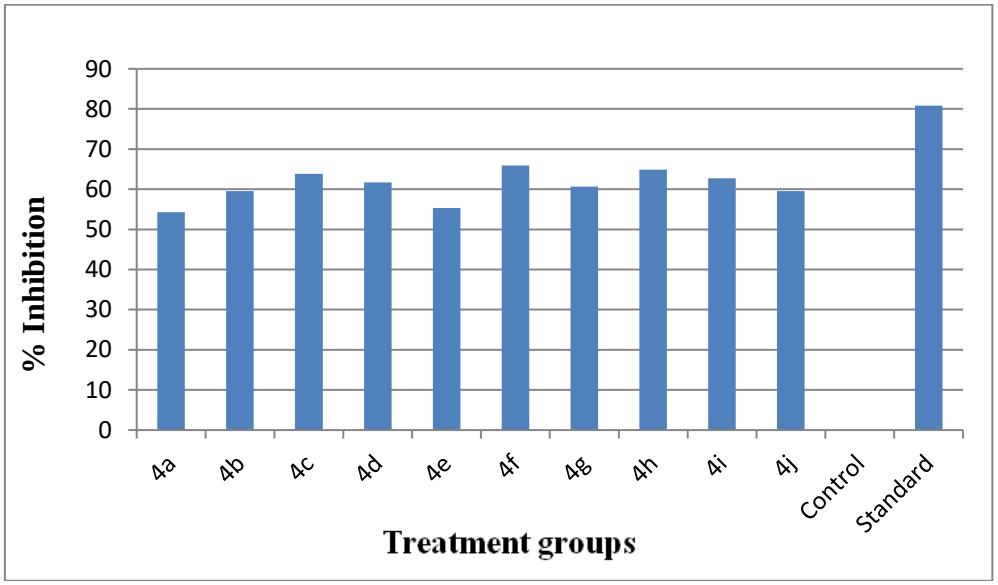


Figure3. Anti-inflammatory activity of titled compounds (4a-j).

5. STRUCTURE ACTIVITY RELATIONSHIPS (SAR) STUDY

The structure activity relationship study of pyrimidine derivatives is mainly discussed based the results obtained from the biological activity. SAR studies provide insights into molecular properties that are related to binding affinity and selectivity of compounds towards receptor. It represents the effect of the structure and functionalization of the pyrimidine derivatives on their activity (Fig. **4**). The promising biological activity of the pyrimidine derivatives may be attributed to the substitutions on the hydrophobic domain (aryl ring).The presence of pyrimidine moiety imparts significant biological activity.The presence of aryl part (substituted phenyl) on pyrimidine moiety increases the lipophilicity of drug molecule. The presence of electron withdrawing group (nitro, chloro, flouro, hydroxyl, methoxy) on ring of pyrimidine derivatives exhibits better anti-inflammatory activityas compared to other derivatives[xxviii-xxx].

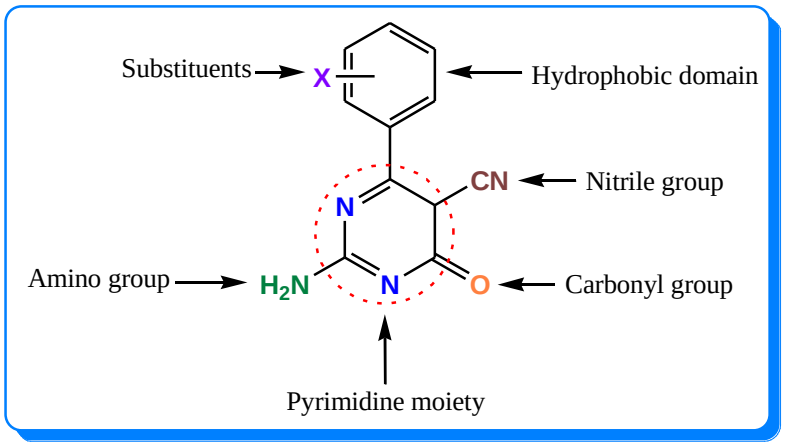


Figure4.SAR Study of Pyrimidine derivatives.

CONCLUSION

The new series of 2-amino-(substitutedphenyl)-4-oxo-1,4-dihydropyrimidine-5-carbonitrile (4a-j) derivatives were prepared based on the protocol of multicomponent one-pot synthesis. The mixture of substituted bezaldehydes, ethylcyanoacetate and guanidine were allowed to react in presence of ethanolic sodium hydroxide solution under microwave irradiation. Microwave radiation has been applied as alternative energy source to accelerate the rate of reaction and improve the yields with formation of the pure products. The newly synthesized compounds were screened for their anti-inflammatory activity *in-vitro* by using membrane stabilization method (heat induced hemolytic technique). Most of the compounds exhibited promising activity due to presence of electron withdrawing groups at *para* positions of the phenyl ring of pyrimidine derivatives. Hence, it can be concluded that, this new series of pyrimidine derivatives certainly holds a greater promise in designing potential anti-inflammatory agents.

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REFERENCES

i) Katritzky, A.R.; Ramsden, C.A.; Scriven, E.F.V.; Taylor, R.J.K. Comprehensive heterocyclic chemistry III. Eds. Pergamon: Oxford, U.K., 2008, 1-13.

ii) Katritzky, A.R.; Ress, C.W.; Scriven, E.F.V. Comprehensive heterocyclic chemistry-II. Eds. Pergamon: Oxford, U.K., 1996, 1-9.

iii) Dansena, H.; Dhongade, H.J.; Chandrakar, K. Asian J. Pharm Clin. Res., 2015, **8**(4), 171-7.

iv) Jainey, P.J.; Bhat, K.I. Ind. J. of Het. Chem., 2011, **20**, 309-312.

v) Rashmi, P.; Nargund, L.G.; Hazra, K. Der. Chemica Sinica, 2011, **2**, 165-71.

vi) Gupta, J.K.; Chaudhary, A.; Dudhe, R.; Varuna, K.A. Int. J. of Pharmac. Sci. and Res., 2010, **1**(5), 34-49.

vii) Verma, A.; Sahu, L.; Chaudhary, N.; Dutta, T.; Dewangan, D.; Tripathi, D.K.Asian J. of Biochem. and Pharmac. Res.,2012, **1**(2), 1-15.

viii) Bhat, I.; Kumar, A. Pyrimidines as potent cytotoxic and Anti-inflammatory Agents. *Asian* J. Pharm. Clin. Res., 2017, **10**(6), 237-239.

ix) Medzhitov, R. *Nature*, 2008, **454**(7203), 428-435.

x) Meade, E. A.; Smith, W. L.; DeWitt, D. L. J. Biol. Chem., 1993, **268**, 6610–6614.
xi) Arora, P.; Arora, V.; Lamba, H. S.; Wadhwa, D. *Int. J. of Pharma. Sci. And Res.*, 2012, **3**(9), 2947-2954.
xii) Brown, D.J.; Mason, S.F. Chemistry of heterocyclic compounds: The pyrimidines. 2008,**16**, 31-81.
xiii) Lagoja, I. M. Chem. & Biodivers. 2005, **2**, 1-50.
xiv) Michael, B.S.; March, J. March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure. Wiley-Int. sci., 2007, **6**, 102-110.
xv) Mariya,D.; Geeta, E.; Krishna K. K. Asian J. of Pharmac. Anal. Med. Chem., 2019, **7**(3), 84-91.
xvi) Morgan, J.; Rachada, H.; Keller, P. A. Bioorg. & Med. Chem. Lett., 2003, **13**, 1755-1757.
xvii) Selvam, T. P.; James, C. R.; Dniandev, P. V.; Valzita, S. K. Res. in Pharmacy, 2012, **2**(4), 01-09.
xviii) Kidwai, M.; Singhal, K.; Kukreja, S. Zeitschrift fur Naturforschung B., 2007, **62**(5), 732–736.
xix) Mahato,A. K.; Sahoo, B.M.; Banik, B. K. J. Indian Chem. Soc., 2018, **95**, 1-13.
xx) Sahoo, B. M.; Ravi Kumar, B.V.V.; Panda, J.; Dinda, S.C. J. of Nanoparticles, 2013,ID 780786.
xxi) Kappe, C.O. Angew Chem. Int. Ed., 2004, **43**(46), 6250-84.
xxii) De, R. M.; Gising, J.; Odell, L.R.; Larhed, M.Upsala j. of med. sci., 2014, **119**(2), 181-91.
xxiii) Gaba, M.; Dhingra, N. Ind. J. of Pharma. Edu. Res., 2011, **45**(2), 175-83.
xxiv) Gedye, R.; Smith, R.; Westaway, K.; Ali, H.; Baldisera, L. Tetrahedron Lett. 1986, **27**, 279-282.
xxv) Sahoo, B. M.; Rajeswari, M.; Panda, J.; Sahoo, B. Ind. J. of Pharma. Edu. and Res., 2017, **51**(4S), 136-43.
xxvi) Sadler, S.; Moeller, A.R.; Jones, G.B. Expert opin. on Drug Dis., 2012, **7**(12),1107, 28.
xxvii) Anosike, C. A.; Obidoa, O.; Ezeanyika, L. U. *DARU J. of Pharm. Sci.*, 2012, 20(1), 76.
xxviii) Pore, Y.; Kuchekar, B.; Bhatia, M.; Ingale, K. Digest. J. of Nanomat. and Biostr., 2009, **4**(2), 373-382.
xxix) Pouplana, R.; Lozano, J. J.; Perez, C.; Ruiz, J. J. of Comp. Aided Mole. Des., 2002, **16**(10), 683-709.
xxx) Mohamed, T.; Xiaobei, Z.; Habib, L. K.; Jerry, Y.; Rao, P.P.N. Bioorg. Med. Chem., 2011, **19**(7), 2269–2281.

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