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Efficient synthesis of antimicrobial active annulated uracil Derivatives

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ABSTRACT

1, 4-diazabicyclo [2.2.2] octane (DABCO) as an efficient organocatalyst were used for synthesis of annulated uracil derivatives via one-pot three component condensation reactions of substituted aromatic aldehydes, malononitrile and barbituric acid in aqueous ethanol carried at NTP. This is rapid, efficient synthetic route with several advantages viz; operational simplicity, mild reaction conditions, high yields of the biological active products, uses less toxic solvents and cheap catalyst. The synthesized products were screened for antimicrobial activity. Therefore annulated uracil derivatives are excellent antimicrobial agents for globally alarming drug resistance issues in clinically used therapeutics.

Keywords: Multi-component, Pyrano [2, 3-d] pyrimidine, DABCO, Antimicrobial activity

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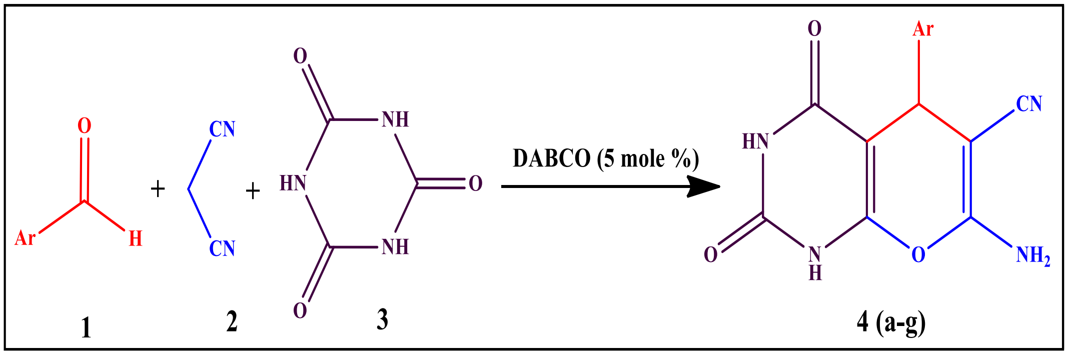
INTRODUCTION

Multicomponent reactions (MCRs) by virtue of their convergence, productivity, ease of execution and generally high yields of products have attracted considerable attention from the point of view of combinatorial chemistry [1, 2] Solid-phase organic synthesis is a subject of recent interest in the context of generating libraries of molecules for the discovery of biologically active molecules and also for the optimization of potent drug candidates. The potential application of microwave technology in organic synthesis [3] particularly in the solid state is increasing rapidly, because of its reaction simplicity, less pollution, and minimum reaction time providing rapid access to large libraries of diverse molecules. Many organic solvents are harmful and their use should therefore be minimized as far as possible or even excluded altogether. Green alternatives under investigation for organic reactions are water [4], supercritical fluids, in particular CO2 [5] and solvent-free condition (SFC) [6]. The use of water as the reaction medium exhibits a remarkable benefit because this green solvent is highly polar and therefore immiscible with most organic compounds [7]. Moreover the water-soluble catalyst resides and operates in the aqueous media, and separation of organic compounds is thus easy. Reactions in aqueous media are generally environmentally safe, devoid of any carcinogenic effects, simple work up, comparatively cheaper to operate and especially important in industry [8]. Thus, there is a need for developing multicomponent reactions (MCR's) in aqueous ethanol and without the use of many harmful organic solvent.

The synthesis of fused heterocycles has attracted considerable interest in heterocyclic chemistry as the fusion of biodynamic hetero systems has proved to be a very attractive and useful for the design of new molecular framework of potential drugs with varying pharmacological activities. Recently, organo catalysis has increased spectacularly in the last few years as a result of both the novelty of the concept and more importantly, the fact that the efficiency and the selectivity of many organ catalytic reactions meet the standards of established organic reactions. One of these organo catalysts is the 1,4-diazabicyclo[2.2.2]octane (DABCO) which has received considerable attention as an inexpensive, eco-friendly, high reactive and non-toxic base catalyst for various organic transformations, affording the corresponding products in excellent yields with high selectivity. Pyrano[2,3-d]pyrimidine consisting of one oxygen atom and two nitrogen atoms respectively, showed resultant pharmaceutical activity as abundant in biologically active compounds such as antitumour [9], cardiotonic[10], antibronchitic [11] and antifungal activity [12]. Some of them exhibit antiallergic [13], antimalarial [14] analgesic [15] and used as drug for insomnia treatment [16].Therefore, for the preparation of these complex molecules large efforts have been directed towards the synthetic manipulation of annulated uracils. Therefore we describe here a rapid, energy efficient, green, economically viable and easy protocol for synthesis of annulated uracil derivatives by using DABCO as an efficient organocatalyst (Scheme 1).



Scheme 1: General Synthesis of substituted annulated uracil derivatives.

MATERIALS AND METHOD

All chemicals were obtained from Aldrich Chemical Co. and used without further purification. Melting points were determined by open capillary method and were uncorrected.^{1}H NMR spectra were obtained on a BRUKER instrument (300 MHz). IR spectra were recorded on a Perkin–Elmer 298 spectrophotometer and ^{13}C-NMR (100 MHz) spectra were recorded in DMSO-d_6 as solvent with TMS as internal standard. Chemical shifts are reported in ppm and Mass spectra were measured using a high resolution GC-MS (DFS) thermo spectrometers with EI (70 EV). Reactions have been monitored by thin layer chromatography on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor.

General procedure for the preparation Pyrano [2, 3-*d*] pyrimidines

Aromatic aldehydes (**1**), malononitrile (**2**), barbituric acid (**3**) (2 mmol each) & 5 mol% 1, 4-diazabicyclo [2, 2, 2] octane (DABCO) taken in RB flask with 15 mL solvent ethanol: water mixture and stirred for 30-40 minutes at room temperature. The reaction was monitored by TLC (thin layer chromatography). The Crude solid was filtered, washed with cold water and recrystallization from ethanol to obtain pure product annulated uracil derivatives.

Spectral data

Synthesis of 7-amino-2, 4-dioxo-5-phenyl-1, 3, 4, 5-tetrahydro-2*H*-pyrano [2, 3-*d*] pyrimidine-6-carbonitrile (4a)

IR (KBr) νcm^{-1}: 3299, 3168, 2989, 2202, 1708, 1460; ^{1}H NMR (DMSO-d$_6$): 4.19 (1H, s, H-5), 7.10 (2H, br, s, NH$_2$), 6.51 (2H, d, *J*= 7.2 Hz, H-Ar), 7.13 (2H, d, *J* = 7.2 Hz, H-Ar), 11.12 (1H, br, s, NH), 12.14 (1H, br, s, NH) PPm; ^{13}C NMR (60 MHz, DMSO-d$_6$) 30.02, 129.8, 156.3, 152.8, 135.4, 128.6, 60.2, 69.2 ppm. EI- MS: ($C_{14}H_{10}N_4O_3$) (m/z) = 281 (M+), 256, 236, 205, 173, 141 etc.

Synthesis of 7-amino-5-(4-methoxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano [2,3-*d*] pyrimidine- 6-carbonitrile (4b)

IR (KBr) *v*cm^{-1}: 3306,3188, 2197, 1708, 1459 ,1336.53; ^{1}H NMR (DMSO-d$_6$): 3.8 (s, 3H, OCH$_3$), 4.21 (1H, s, H-5), 7.38 (2H, br, s, NH$_2$), 7.13 (2H, d, *J* = 7.8 Hz, H-Ar), 7.91 (2H, d, *J* = 7.6 Hz, H-Ar), 12.09 (1H, br, s, NH); ^{13}C NR (60 MHz, DMSO-d$_6$) 33.03, 130.8,64.3, 112.8,135.4, 67.6, 143.2, 152.3 ppm. EI-MS: ($C_{15}H_{12}N_4O_4$) (m/z) = 89 (M+), 269, 232, 221, 201, 176, 149, 110;

Synthesis of 7-amino-5-(3,4-dimethoxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (4c)

IR (KBr) *v*cm^{-1}: 1262.72, 1576.09, 1662.63, 1734.21, 2223.44, 3103.23,3195.06; ^{1}H NMR (DMSO-d$_6$); 3.6 (s, 3H, OCH$_3$), 7.02 (3H, s, OCH$_3$), 4.2 (s, 1H, H-5), 7.1 (S,2H, NH$_2$), 11.1 (s,1H,NH), 11.4 (s,1H,NH), 8.27 (2H, d, *J* = 7.6 Hz, H-Ar), 8.47 (2H, d, *J* = 7.7 Hz, H-Ar) ; ^{13}C NMR (60 MHz, DMSO-d$_6$) 37.9, 135.8, 56.3, 163.8,57.4, 79.7, 150.1, 114.2,146.9,149.4 ppm. EI-MS: ($C_{16}H_{14}N_4O_5$) (m/z) =342 (M+), 312, 295, 279, 249, 243, 220,217,149 etc

Synthesis of 7-amino-5-(4-hydroxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitril (4d)

IR (KBr) *v*cm^{-1}: 1555.2, 1676.34, 1729.86, 2293.22 , 3131.64, 3260.32,3457.99 ; ^{1}H NMR (DMSO-d$_6$); 3.316 (s, 1H, OH), 7.315 (s, 2H, NH$_2$), 10.512-11.103 (S,1H, NH), 6.88,7.782,8.213,8.46 (s,4H, H-Ar); ^{13}C NMR (60 MHz, DMSO-d$_6$) 29.03, 130.5, 162.3, 112.8,126.4, 68.5, 150.2, 151.1 ppm. EI-MS:($C_{14}H_{10}N_4O_4$) (m/z) =298 (M+), 249, 232, 188, 142, 128, 115 etc

Synthesis of 7-amino-5-(3-hydroxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitril (4e)

IR (KBr) *v*cm-1: 3439, 3337, 3193, 3028, 2206, 1677, 1625; ^{1}H NMR (300 MHz, DMSO-d$_6$) 4.10 (1H, s, H-5), 6.56 (2H, br s, NH$_2$), 6.59 (1H, m, H-Ar), 7.04-7.10 (m, 3H, H-Ar), 9.33 (1H, br s, OH), 11.09 (1H, br s, NH), 12.07 (1H, br s, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d$_6$) 35.6, 59.9, 89.5, 114.7, 114.9, 118.8, 120.1, 130.1, 146.5, 150.4, 153.1, 158.1, 158.5, 163.3 ppm; MS: ($C_{14}H_{10}N_4O_4$) *m/z* (%) = 298 (M·+), 249 (6), 232 (17), 188 (12), 142 (100), 128 (30), 115 (33).

Synthesis of 7-amino-5-(4-bromophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitril (4f)

IR (KBr) cm^{-1}: 3370, 3340, 3189, 3080, 2220, 1684, 1567; ^{1}H NMR (300 MHz, DMSO-d$_6$) 4.26 (1H, s, H-5), 7.17 (2H, br s, NH$_2$) 7.20 (2H, d, *J* = 8.2 Hz, H-Ar), 7.48 (2H, d, *J* = 8.2 Hz, H-Ar), 12.45 (1H, br s, NH), 13.66 (1H, br s, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d$_6$) 35.3, 58.5, 82.8, 119.0,120.0, 129.9, 132.2, 132.7, 143.0, 157.4, 160.3, 174.0 ppm.

7.Synthesis of 7-amino-5-(4-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitril (4g)

IR (KBr) cm^{-1}: 3380, 3321, 3182, 2196, 1696, 1640,1519, 1348; ^{1}H NMR (300 MHz, DMSO-d$_6$) 4.42 (1H, s, H-5),7.26 (2H, br s, NH$_2$), 7.52 (2H, d, *J* = 7.2 Hz, H-Ar), 8.14 (2H, d, *J* = 7.2 Hz, H-Ar), 11.12 (1H, br s, NH), 12.17 (1H, br s, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d6) 35.7, 57.5, 87.5, 119.0, 124.3, 130.7, 146.4, 149.6, 151.9, 152.7, 157.8, 162.6 ppm.

Biological evaluation of synthesized annulated uracil derivatives

The specific strains used for antimicrobial activity are Pseudomonas aureus, Klebshiella pneumonia, E. Coli and Staphylococcus aureus respectively. Antimicrobial activity of annulated uracil derivatives **4a, 4b, 4c, 4d, 4e, 4f** and **4g** were tested by the disk diffusion method using Whatman No. 1 filter paper. All the products were dissolved in N,N-dimethylformamide(DMF) for dilution to prepare stock solutions of 20 mg/mL for antimicrobial assay. Agar plates were uniformly surface inoculated with fresh broth culture of Pseudomonas aureus, Klebshiella pneumonia, E. Coli and Staphylococcus aureus. These impregnated disks were placed on medium suitably spaced apart and plates were incubated at 30 °C for 1 h to permit good diffusion and were then transferred to an incubator at 37±2 °C for 24 hrs. The zones of inhibition were measured on mm scale. Streptomycin was used as standard antimicrobial drug and dimethylsulphoxide used as solvent (Table 1).

Minimum inhibitory concentration (MIC) is the lowest product concentration preventing visible bacterial growth. MICs of selected products **4a, 4b, 4c**, **4d, 4e, 4f and 4g** were determined by taking different concentrations of the product in DMF. The different concentrations were added by using sterilized pipettes to different test tubes containing sterilized broth medium inoculated with test organism. DMF alone showed no inhibition zone. Then all the test tubes were incubated at 37^{0}C for 24 h and after incubation period, the presence of growth (turbidity) was observed. Antimicrobial screening of product **4a** exhibited best broad-spectrum activity against E. coli and Pseudomonas aureus bacteria, product **4b** exhibited best against Staphylococcus aureus bacteria, product **4c** exhibited best against Klebsiella, E. Cololi bacteri, product **4d** is best against Pseudomonas bacteria, product **4e** exhibited best against Staphylococcus aureus as well for Pseudomonas aureus bacteria, Product **4f** is best against E. Cololi and Staphylococcus aureus bacteria and the Product **4g** exhibited best broad-spectrum activity against E. coli, Staphylococcus aureus and Pseudomonas aureus bacteria.

Table 1: Antimicrobial activity of annulated uracil derivatives

Entry	Products	E. Coli	Staphylococcus aureus	Klebsiella pneumonia	Pseudomonas aureus
1	4a	+ + +	+ +	+	+ + +
2	4b	+ +	+ + +	+ +	+
3	4c	+ + +	+ +	+ + +	+
4	4d	+ +	+ +	+	+ + +
5	4e	+ +	+ + +	+ +	+ + +
6	4f	+ + +	+ + +	+ +	+ +
7	4g	+ + +	+ +	+ + +	+ + +
8	Streptomycin (Reference)	+ + +	+ + +	+ + +	+ + +

+++High sensitive (zone area = 11-15 mm), ++ Faire in sensitive (zone area =6- 10mm), + Slightly sensitive (zone area =1-5 mm

RESULTS AND DISCUSSSION

The experimental procedure is simple. Solutions of equimolar amounts of aromatic aldehyde **1**, malononitrile **2** and barbituric **3** in H2O-EtOH (1:1), at room temperature, are mixed thoroughly in the presence of a catalytic amount of DABCO (5 mol%) to afford various functionalized annulated uracil derivatives **4a-g** in good yields Table 2. Results are more attractive in efficient one pot synthesis of annulated uracil derivatives of biological importance via initial Knoevenagel, subsequent Michael and final heterocyclization reactions. DABCO catalyze synthetic route is easy way to handle, non-corrosive in environmentally benign aqueous media, cheap, nontoxic and commercial availability with an advantageous high product's selectivity and yields.

Table 2: Synthesis of some annulated uracil derivatives 4(a-g).

S.N.	Ar	Product	Colour	Time (min)	Yield (%)[a]	M.P. (in° C)
1	C_6H_5	**4a**	Yellow Solid	40	94	206- 208
2	4-MeOC_6H_4	**4b**	Dark yellow solid	35	97	290-293
3	3,4-MeOC_6H_4	**4c**	Yellow Solid	40	91	305-308
4	4-OH-C_6H_4	**4d**	Yellow Solid	30	94	158-160
5	3-OH-C_6H_4	**4e**	Yellow Solid	35	91	169-173
6	4-Br-C_6H_4	**4f**	White Solid	40	90	228-232
7	4-NO_2-C_6H_4	**4g**	White Solid	40	85	235–236

[a]Yields refer to those of pure isolated products characterized by Mass Spectrometry and IR, ^{1}H & ^{13}C NMR Spectroscopic data.

These formulated annulated uracil products as antimicrobial agents may become excellent derivatives for globally alarming drug resistance issues in clinically used therapeutics. The effect of different concentration of catalyst for the synthesis of **4a** in EtOH–H2O at room temperature and it was found DABCO shows equal or more efficient catalytic activity in terms of reaction times and yields of the product. In the absence of the catalyst, the reaction was rather sluggish and resulted in poor yield (56%) even after 4 hrs time under same reaction conditions, thus confirming 5% DABCO role as an efficient catalyst Table 3. DABCO is bicyclo amine basic in character, so facilitates proton removal from active methylene group of malononitrile there by increases reaction rate yields annulated uracil derivatives. Catalyst gets easily removed by aqueous washing due to its solubility in water; hence no need of further neutralization and work-up is accomplished by simple filtration and recrystallization by ethanol.

Prepared derivatives furnishes motivating model for studying interaction of annulated uracil derivatives with antimicrobials target as possible charge modification of substituent and O/N of pharmacophore groups present in skeleton. The antimicrobial strains reveal that the heteroaryl-substituted products (**4a, 4b, 4c,4d,4e,4f and 4g)** showed differential activities The presence of heteroaryl ring and cyano and amino groups on pyran ring make these more basic which increases its penetrating power on bacterial cell wall (protein) and the compounds becomes more active. In these cases, the hydroxyl, methoxy, nitril and bromil heteroaryl part is associated with the bacterial cell wall which makes them more active. Future flexible pharmacophore sites geometric conformation enables to prepare derivatives for multi-therapeutic annulated uracil derivatives products with high selectivity.

Table 3: Optimization mol % of DABCO during the synthesis 4a in aqueous ethanol.

Entry	**Mole % of DABCO**	**Time (min)**	**Yield (%)[a]**
1	5	40	94
2	10	40	81
3	15	35	73
4	Without catalyst	4hrs	56

[a] Isolated yield

.CONCLUSION

We have demonstrated a rapid and an efficient synthetic route for DABCO catalyzed one-pot three component synthesis of annulated uracil derivatives in aqueous ethanol: water (1:1) solvent mixture at room temperature and the current synthetic route has the advantages of operational simplicity, mild reaction conditions and good to high yield of the biological active products. Prepared annulated uracil derivatives owe antimicrobials activity and motivates model to study interaction with target drugs. This is due to possible charge modification of substituent and O/N of pharmacophore groups present in annulated skeleton.

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