Research Article

Synthesis of Novel 5-Carboxynaphthanilide Dihydropyrimidinone Derivatives and Evaluation of Their Biological Activity

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ABSTRACT
Citric acid mediated synthesis of naphthalene derived Biginelli compounds have been prepared successfully in good to excellent yields. The sixteen compounds synthesized were evaluated for antimicrobial, anti-inflammatory and anti-tumor activities. Compound 5c and 5f showed potent activity and compounds 5d, 5g, 6e, 6d, 6g showed moderately potent activity antibacterial activity. Compounds 5c, 5f, 6d showed potent and compounds 5d, 5g, 6f, 6g and showed moderately potent antifungal activity. Compounds 5c, 6d, 6f, 6g at 100 mg/kg showed significant reduction in paw oedema. Compounds 5c, 5h, 6d showed potent and compounds 6f, 6g showed moderately potent activity. From this, it was concluded that the compounds bearing nitro and chloro group show significant activity when compared to compounds without these groups. It was also confirmed that the groups in para position showed better activity when compared to the groups in ortho position.

Keywords: citric acid, naphthanilide DHPMs, biological activity, multi component reaction.

INTRODUCTION

Multicomponent reactions (MCRs) are of increasing importance in organic and medicinal chemistry for various reasons[1]. One such important MCR that belongs in this category is the venerable Biginelli dihydropyrimidine synthesis. Dihydropyrimidinones (DHPMs) always lie in the forefront due to their therapeutic and pharmacological properties. Biginelli DHPMs are reported to be physiologically and pharmacologically important compounds and have attracted considerable interest because, they exhibit promising activities such as α-1a-antagonists, antihypertensive, HIV gp-120-CD4 inhibitors, antiviral, antitumor, antimalarial, antifilarial, antiinflammatory, antitubercular, antimicrobial and also possess anti oxidant activity[2].

There are plethora of reports concerning to the synthesis of Biginelli compounds using Bronsted acids, Lewis acids, ionic liquids, microwave irradiation, polymer-supported catalysts, solid phase reagents, heteropoly acids, heterogeneous catalysts and organic acids[3]. In 2003, we reported synthesis of Biginelli compounds using $Bi(OTf)_3$ at room temperature for the first time according to literature reports[3a]. Recently, we explored citric acid as an efficient organic acid for the synthesis of Biginelli compounds[3b].

The biodynamic property of this ring system prompted us to design pyrimidine derivatives stimulating pharmacophores and substituents responsible for diverse pharmacological activities. In continuation of the search of molecules which gratifies the requirements of dihydropyrimidines and based on the report by

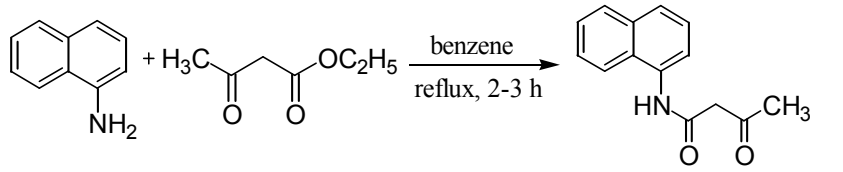
Coutinho[4], compounds with novelty in the structure were synthesized for the first time using the classical Biginelli reaction for the synthesis of DHPMs. In the present work, the novelty of the structure lies in the fifth carbon atom where the acetyl or ester group of the classical DHPM is replaced by the naphthyl carboxamide moiety.

In continuation of our interest in developing carbon-carbon, carbon-hetero bond formations[5], herein we report our brief findings using citric acid as promoter to synthesize 5-carboxynaphthanilide DHPMs and most importantly, biological evaluation has been carried out extensively.

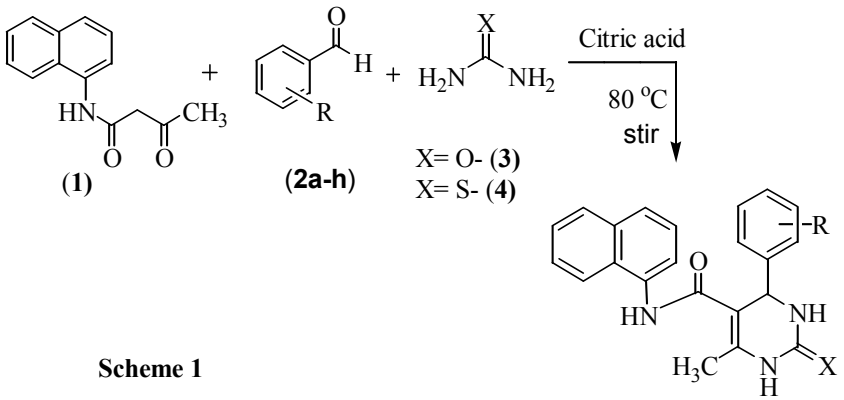
RESULTS AND DISCUSSION

Initially, we reacted 1-naphthyl amine with ethyl acetoacetate in presence of benzene to afford *N*-(naphthalene-1-yl)-3-oxobutanamide according to the literature procedure[4]. *N*-Napthyl-3-oxobutanamide (1 equiv.), electronically and structurally divergent benzaldehydes/hetero aromatic aldehyde (1 equiv.), urea or thiourea (1.2 equiv.) and citric acid (0.5 equiv.) under solvent-free conditions were taken in a round bottom flask under stirring at 80 ^{o}C for appropriate time to afford the corresponding DHPM.

All the synthesized compounds were characterized using IR, ^{1}H NMR and mass spectrometry (entries 1-16, **Table 1**).



N-(Naphthalen-1-yl)-3-oxobutanamide
(**1**)



Scheme 1

5a-h (X=O-)
6a-h (X=S-)

To mention, IR spectrum of **5a** showed the characteristic bands as 3492 (N-H), 1680 (C=C), 1237(C-N) cm^{-1}. The ^{1}H NMR spectrum of compound **5a** showed the characteristic values at δ 2.11 for methyl protons as a singlet, 3.94 ppm for methylene protons, the addition proton CH appeared as a singlet at 5.61 ppm, aromatic protons as multiplet at 7.24-7.97 ppm and different NH protons at 7.76, 8.91 and 9.68 ppm. The mass spectrum of compound entry 1 further confirms the structure. The molecular ion peak of the compound was appeared at 358 (M^{+}). The fragmentation peaks were appeared at *m/z* 83, 142, 226 and 268. Based on the above spectral data, the synthesized compound **5a** was confirmed as 6-methyl-*N*-(naphthalen-1-yl)-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxamide. By adopting the above synthetic procedure, compounds (entries 2-16) were synthesized by using substituted aromatic aldehydes, urea or thiourea. In all cases, the three-component reaction proceeded smoothly and rapidly to give the

corresponding 5-carboxynaphthanilide dihydropyrimidinones in high yield (65-97%).

Antimicrobial activity

All The synthesized compounds were evaluated for *in vitro* antibacterial activity against various *Gram*-positive and *Gram*-negative bacteria by well diffusion method. All the synthesized compounds were evaluated for antibacterial activity of which compounds possessed potent activity against *Gram*-positive and *Gram*-negative bacteria when compared with that of the standard ciprofloxacin (30 µg/ml). The zone of inhibition of various concentrations of the synthesized compounds against gram positive and gram negative bacteria was measured and were tabulated. All the compounds possess potent to moderately potent activity against *Gram*-positive and *Gram*-negative bacteria.

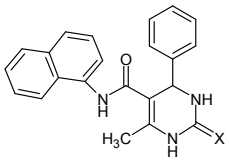
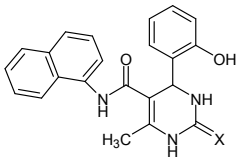
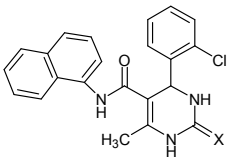
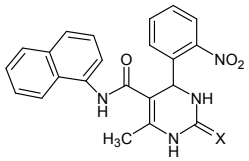
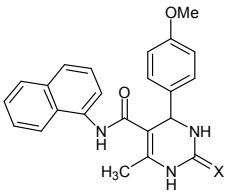
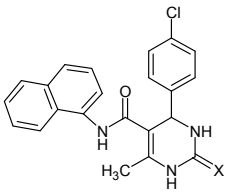
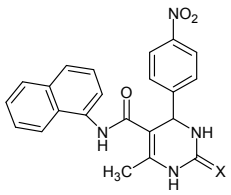
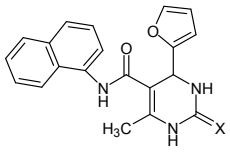
All the synthesized compounds were evaluated for *in vitro* antifungal activity by well diffusion method against *Aspergillus niger and Aspergillus flavus*, and compared with that of the standard fluconzole (50 µg/ml).

Acute oral toxicity was performed based on the guidelines for the synthesized compounds NDC 1-16. No toxicity was observed for all the compounds on administration of 200 mg/kg body weight. So, further anti-inflammatory activity was carried out at 100 mg/kg body weight in which no death was observed and considered as safe. The synthesized compounds were evaluated for anti-inflammatory activity by carrageenan induced paw edema method using indomethacin as standard drug. Compounds 5g and 6g have shown significant reduction in paw oedema.

Anti-inflammatory activity of the synthesized compounds (Carragenan induced Paw Oedema)

Group	1 hr	2 hr	3 hr	4 hr	5 hr
Control 1% Water (1 ml/kg)	0.31±0.01	0.55±0.04	0.63±0.02	0.72±0.01	0.84±0.02
Compound –5e (100 mg/kg)	0.24±0.02 (7.28%)	0.46±0.01 (20.26%)	0.36±0.03 (38.06%)	0.26±0.04 (52.24%)	0.22±0.02 (64.38%)
Compound –5f (100 mg/kg)	0.23±0.03 (8.26%)	0.48±0.02 (20.86%)	0.36±0.03 (38.42%)	0.24±0.01Z (66.46%)	0.17±0.04** (72.64%)
Compound –5g (100 mg/kg)	0.25±0.02 (8.42%)	0.52±0.04 (09.10%)	0.32±0.02 (40.48%)	0.28±0.03 (65.29%)	0.16±0.02* (79.42%)
Compound – 6c (100 mg/kg)	0.26±0.01 (06.52%)	0.50±0.03 (28.46%)	0.36±0.04 (34.28%)	0.26±0.02 (58.14%)	0.18±0.01** (75.28%)
Compound – 6d (100 mg/kg)	0.23±0.02 (10.24%)	0.52±0.03 (28.21%)	0.34±0.02 (47.60%)	0.28±0.01 (64.64%)	0.18±0.02** (73.84%)
Compound –6f (100 mg/kg)	0.22±0.03 (10.86%)	0.53±0.04 (29.64%)	0.35±0.02 (40.52%)	0.29±0.02 (63.86%)	0.17±0.03** (76.52%)
Compound – 6g (100 mg/kg)	0.23±0.01 (10.64%)	0.50±0.03 (32.10%)	0.32±0.04 (41.48%)	0.27±0.03 (64.29%)	0.16±0.01* (78.22%)
Compound –5c (100 mg/kg)	0.28±0.02 (7.62%)	0.50±0.01 (22.92%)	0.34±0.02 (32.68%)	0.28±0.04 (62.82%)	0.16±0.03** (74.04%)
Standard-Indomethacin (10 mg/kg)	0.26±0.01 (15.55%)	0.52±0.02 (36.92%)	0.32±0.01** (62.40%)	0.24±0.03*** (76.66%)	0.15±0.02*** (82%)

Table 1. Synthesis of 5-carboxynaphthanilide dihydropyrimidinones

Entry	Product		Time (min)	Yield (%)[a]	Mp (^{0}C)^{new}
5a		X=O	15	72	150-152
6a		X=S	15	83	142-144
5b		X=O	15	65	156-158
6b		X=S	20	92	150-152
5c		X=O	15	76	154-156
6c		X=S	15	80	145-147
5d		X=O	20	92	196-198
6d		X=S	15	90	190-193
5e		X=O	20	88	212-214
6e		X=S	15	95	205-207
5f		X=O	20	87	154-156
6f		X=S	15	82	148-150
5g		X=O	15	93	196-198
6g		X=S	15	97	190-192
5h		X=O	15	85	145-146
6h		X=S	15	94	140-142

[a] Isolated yields

Anti-oxidant activity

All the synthesized compounds were evaluated for *in vitro* antioxidant activity by radical scavenging method and compared with that of the standard ascorbic acid at 440 nm. The percentage of inhibition of various concentrations of the synthesized compounds along with the standard were measured and tabulated.

Antioxidant activity of synthesized compounds by radical scavenging method

Compound	% inhibition by radical scavenging method Concentrations mg/ml					IC 50 mg/ml
	0.5 mg/ml	1 mg/ml	1.5 mg/ml	2 mg/ml	2.5 mg/ml	
5a	3.9	6.2	11.29	15.53	20.55	6.1
5b	0.78	3.07	10.61	20.55	30.63	3.88
5c	26.8	36.7	54.5	58.9	59.95	1.6
5e	0.98	6.48	22.54	38.56	40.9	2.75
5d	11.23	15.36	22.45	35.43	55.5	2.09
5h	27.5	40.5	52.95	53.47	99	1.35
5f	8.1	16.52	17.21	17.22	26.81	2.00
5g	4.7	10.30	20.34	41.71	53.13	2.43
6a	11.1	19.3	22.43	35.7	41.6	3.02
6b	12.6	19.5	28.4	45.5	55.8	2.23
6c	10.3	15.46	20.34	41.71	53.13	2.47
6e	15.35	20.47	35.46	45.64	65.34	2.13
6d	23.35	50.43	57.85	60.23	66.75	1.41
6h	4.3	7.0	10.93	29.51	35.4	3.42
6f	6.6	26.6	41.5	57.65	58.79	1.93
6g	13.24	25.53	50.58	52.17	59.63	1.90
Ascorbic acid	23.3	66.78	80.45	82.45	85.46	0.64

Brine shrimp lethality test (BSLT)

Some of the synthesized compounds were evaluated for *in vitro* cytotoxic activity by brine shrimp lethality test and compared with that of the standard podophyllotoxin. Among the compounds screened for cytotoxic activity, compounds 5c, 5h, 5d showed potent and compounds 6f and 6g showed moderately potent activity.

CONCLUSION

In summary, a new method for the preparation of 5-carboxynaphthanilide-DHPMs was discovered that utilizes a multicomponent coupling reaction catalyzed by citric acid, with a rapid and high yielding cyclocondensation to afford the corresponding DHPMs.

Antimicrobial studies were performed for all the synthesized compounds 5a-6h by well diffusion method and compared to standard ciprofloxacin (30 µg/mL) and fluconazole (50 µg/ml) for antibacterial and antifungal studies respectively. The zone of inhibition of various concentrations of the synthesized compounds against *gram* positive and *gram* negative bacteria was measured and were tabulated.

All the compounds showed potent to moderately potent antibacterial activity. Among the compounds synthesized, compound 5c and 5f showed potent activity and compounds 5d, 5g, 6e, 6d and 6g showed moderately potent activity antibacterial activity. Compounds 5d, 5g, 6f, 6g showed potent and compounds showed moderately potent antifungal activity.

Among the compounds screened for antioxidant activity, compounds 5c, 5h, 5d showed potent and compounds 6f, 6g showed moderately potent activity.

The compounds tested have shown potent to moderately potent anti-inflammatory activity. Among the tested compounds, 5c, 6d, 6g compounds at 100 mg/kg showed significant reduction in paw oedema when compared to compounds 5h and 6h. The compound 5c, 6d, 6g showed 76-79% protection. Compounds 5h and 6h showed 71-75% protection.

Among the compounds screened for cytotoxic activity, compounds 5c, 5h, 5d showed potent and compounds 6f and 6g showed moderately potent activity.

From this, it was concluded that the compounds bearing nitro and chloro group show significant activity when compared to compounds without these groups. It was also confirmed that the groups in *para*- position showed better activity when compared to the groups in *ortho*- position.

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EXPERIMENTAL SECTION

Experimental

Melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected. Thin layer chromatography was performed using precoated aluminium plates coated with silica gel GF_{254} [E. Merck] using solvent system *n*-hexane:ethyl acetate in the ratio of 7:3. The spots were visualized in the iodine chamber. IR spectra were recorded on ELICO FTIR spectrometer using potassium bromide pellets. ^{1}H-NMR spectra of the compounds in deutiriated dimethyl sulfoxide, which was recorded on BRUKER AV 400 spectrometer. Mass spectra was recorded on GCMS QP 5000 shimadzu.

Synthetic Protocols

a)Synthesis of *N*-(Naphthalen-1-yl)-3-oxobutanamide

1-Amino naphthalene (10mmol) was dissolved in 30ml of benzene and then added to ethylacetoacetae (10mmol) taken in a round bottomed flask with continuous stirring. This reaction mixture was refluxed for 2 hrs. Completion of the reaction was confirmed by TLC. Then it was transferred to a china dish and the solvent was evoaporated at room temperature which resulted in the formation of crystals. Filtered and washed with alcohol. recrystallized from ethanol.

b) Synthesis of entries 5a-h and 6a-h

A mixture of benzaldehyde (1 mmol), naphthyl acetoacetamide (1.3 mmol), urea or thiourea (1.5 mmol) and citric acid (0.5 mmol), were taken in a round bottomed flask and heated the reaction mixture at 80 ^{0}C under stirring for 1 h. Completion of the reaction was confirmed by TLC. To the reaction mixture cold water was added, stirred for 10 min, and then filtered. The obtained filtrate was washed with water and dried in vacuum and the obtained product was further recrystallized from ethanol.

Biological evaluation

Anti-inflammatory studies

The toxicity of the compounds was tested by Acute oral toxicity- acute toxic class method[6] using a stepwise procedure, each step using three rats of single sex. The rats were fasted prior to dosing (food was with held but not water) for 3-4 hours. After 4 hours, the synthesized compounds were suspended in Tween-80 and administered orally in a dose of 2000 mg/kg body weight. The animals were kept under observation for 14days. As no mortality was observed with the above dose, a dose of 200 mg/kg body weight was selected for the evaluation of anti inflammatory activity.

Acute inflammatory model-carrageenan induced rat paw oedema assay

The anti-inflammatory activity of the test compounds was evaluated in Wistar male albino rats. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of six animals. The different test compounds were administered to the animals in the test group at the dose of 200 mg/kg by oral route. Animals in the standard group received indomethacin at the dose of 10 mg/kg by oral route. All test and standard compounds were administered as 1% sodium carboxy methyl cellulose. Rats in the control group received the vehicle solution without drugs. One hour after test drugs administration, rats in all the groups were challenged with 0.1 ml of 1% carrageenan in the subplantar region of right hind paw. A zero hour paw volume was measured for the rats using digital Plethysmometer immediately after the administration of carrageenan for all groups. Paw volumes were again measured one hrs after the challenge of carrageenan. The percent inhibition of paw volume for each rat in treated groups was calculated by comparing with mean paw volume of control group and expressed as mean (± SE) percent inhibition of paw volume for each test group.

Percent edema inhibition = $100(1-V_t/V_c)$
V_c = Volume of edema in the control group
V_t = Volume of edema in the treated group

Anti bacterial activity

The standard antibiotics used in the present study were ciprofloxacin[7]. In the present study, the well diffusion method[8] was used to evaluate the antimicrobial activities of the synthesized compounds in vitro. The test organisms were sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain.

Antifungal activity

The antifungal activities of the synthesized compounds were studied by disc diffusion method.

The standard drug selected for antifungal activity was fluconazole[9].

Antioxidant Activity By P-NDA (P-Nitroso Dimethyl Aniline) Radical Scavenging Method[10]

To a solution containing ferric chloride (0.1mM, 0.5 ml), EDTA (0.1mM, 0.5 ml), ascorbic acid (0.1mM, 0.5ml), hydrogen peroxide (2mM, 0.5ml) and p-nitroso dimethyl aniline (0.01mM, 0.5 ml) in phosphate buffer (p^H 7.4, 20Mm) were added various concentrations of the test compounds in distilled DMSO or dissolving solvent or alcohol to produce final volume of 3ml. Absorbance was measured at 440 nm.

$$\textit{p}\text{-NDA radical scavenging activity}(\%) = \frac{[\text{Abs(sample)}-\text{Abs(standard)}]}{[\text{Abs (sample)}]} \times 100$$

Cytotoxic studies

Brine shrimp lethality assay[11-12] was used according to method of *Meyer et. al*. Brine Shrimp (*Artima salina*) nauplii were hatched in sterile brine solution (prepared using sea salt 38 g/l and adjusted the pH to 8.5 using 1N NaOH) under constant aeraton for 38 h. After hatching, 10 nauplii were placed in each vial and added various concentrations of drug solution in a final volume of 5 ml, maintained at 37 ^{0}C for 24h under the light of incandescent lamps and surviving larvae were counted (Krishnaraju et al). Each experiment was conducted along with control (vehicle treated), at various concentrations of the test substances. Percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. The ED_{50} values were obtained using fenny probed analysis software. The result for the test compound was compared with the positive control podophyllotoxin.

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