Research Article

DBN catalyzed one- pot efficient synthesis and antioxidant activity of pyrano [2, 3-*d*] pyrimidine derivatives

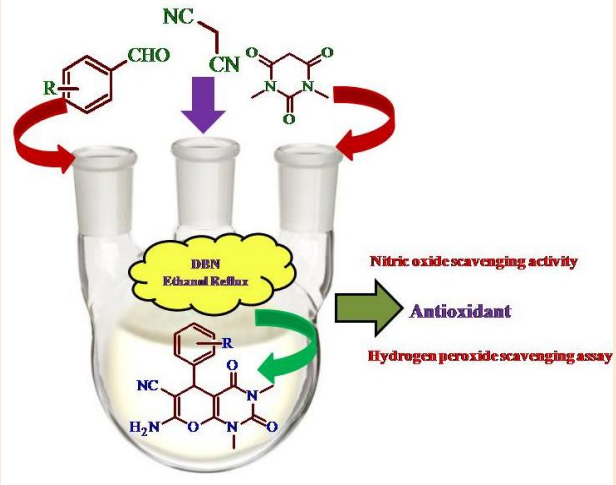
Reshma Patil[1], Digambhar Kumbhar[1], Priyanka Mohire[1], Sunetra Jadhav[1], Ajinkya Patravale[2] and Madhukar Deshmukh[1,2*]

[1]Chemistry Research Laboratory, Department of agrochemicals & Pest Management,Shivaji University,Kolhapur-416004, Maharashtra, India.
[2]Heterocyclic Chemistry Laboratory, Department of Chemistry,Shivaji University, Kolhapur-416004, Maharashtra, India

Abstract

Free radicals are unpaired electrons on an open shell configuration & imbalance causes high reactivity which will lead to tissue damage and oxidative stress. Title molecules have been synthesized by using substituted aldehydes, barbituric acid and malanonitrile in DBN and study their antioxidant potential radical scavenging assay. Some of the tested compounds show moderate to good antioxidant activity. The structural elucidation of synthesized compounds ensured on the basis of their elemental and spectral studies.

Keywords: Pyrano [2, 3-d] pyrimidine, antioxidant activities



***Correspondence**
Author: Madhukar Deshmukh
Email: m_deshmukh1@rediffmail.com

Introduction

Free radicals causes imbalances and get participated in the pathogenesis of various disorders such as cancers, atherosclerosis, diabetes, Alzehimer, Parkinson and diseases related to aging process [1-3]. Furthermore, the oxygen consumption inherent in cell growth leads to the generation of a series of oxygen free radicals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated in aerobic organisms as part of the normal physiological and metabolic processes or from exogenous factors and agents [4-5]. These ROS are capable of damaging a wide range of essential macromolecules in the membrane of lipids, proteins, nucleic acids etc and are directly or indirectly associated in the pathogenesis of various diseases [6-8] .There is need to produce effective antibiotic which prevent the body for suspicious infections.

Pyrimidine and their derivatives is a unique template and associated with several biological activities such as antimicrobial [9-14], anti-inflammatory [15-17], antioxidant [18-22], anticancer [23-27], insecticidal [28-30] activities. An exhaustive literature search revealed that pyrimidines have shown most significant bioactivities and certain kinds of tumors and have been suggested as possible pharmacophore too. Prompted by the varied biological activities,we have reported a green and efficient protocol for the synthesis of pyrano pyrimidines derivatives using DBN as catalyst and study their invitro antioxidant activity.

Materials and Methods

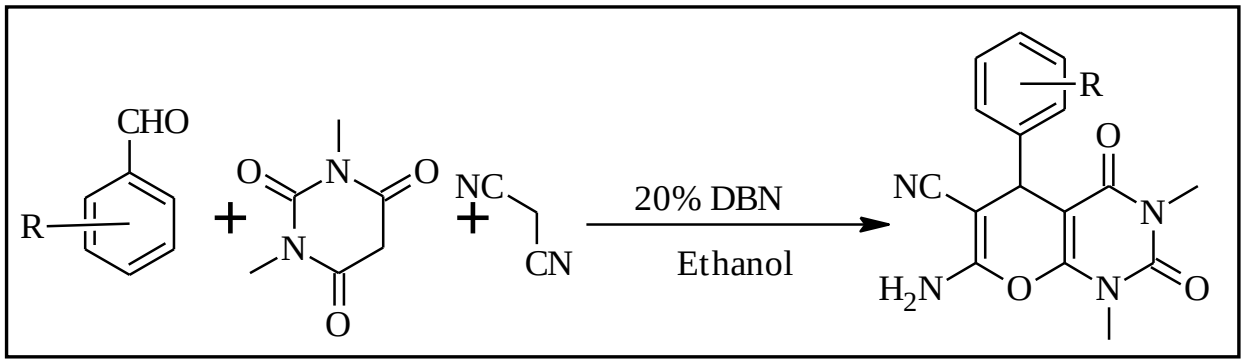
All chemicals used were commercially available and purchased from Sigma Aldrich. Melting points were taken on a melting point apparatus and are uncorrected. The reactions were monitored by thin layer chromatography (TLC). Proton nuclear magnetic resonance ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 300 MHz and 75 MHz spectrophotometer in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. Infrared (IR) spectra

were recorded on a FTIR spectrophotometer. Elemental analysis was done on a Flash elemental analyzer EURO EA-3000. The solutions were prepared by using DMSO as a solvent.

Experimental

Typical procedure for synthesis of 7-amino-5-phenyl-2, 4-dioxo-1, 3, 4, 5-tetrahydro-2H-pyrano [2, 3-d] pyrimidine-6-carbonitrile derivatives

To the mixture of 5 ml ethanol containing 20 mol% DBN, barbituric acid (1mmol), malononitrile (1mmol) and aryl aldehyde (1 mmol) was added, and the mixture was continued the reflux condition for 30-35 min. The progress of the reaction was monitored by TLC using ethyl acetate- petroleum ether (8:2 v/v). After completion, reaction mixture was cooled at room temperature. The product was precipitated in round bottom flask was collected by filtration, washed with water (20 ml). Finally, the crude product was recrystalised with ethanol to obtain the pure product(Scheme.1)



Scheme 1 DBN catalyzed synthesis of 7-amino-5-phenyl-2, 4-dioxo-1, 3, 4, 5-tetrahydro-2H-pyrano [2, 3-d] pyrimidine-6-carbonitrile derivatives

Spectral data of the representative compounds

7-amino-1,3-dimethyl-5-(3-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile: IR(KBr, vmax cm-1): 3392, 3314, 3189, 3094, 3010, 2193, 1701, 1662, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.06 (s, 3H, -CH_3), 3.39 (s, 3H, CH_3), 5.23 (s, 1H, -CH), 7.01 (s, 2H, Ar- H), 7.29-7.34(m, 2H, Ar- H), 7.48-7.53(t, 1H, Ar-H), 7.70-7.73(d, 2H, Ar-H), Anal.Calc: C(54.09%) H (3.69%) N(19.71%) O(22.52%).Found: C(54.01%) H (3.59%) N(19.61%)

7-amino-1,3-dimethyl-5-(4-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile: IR(KBr, vmax cm-1):3304,3201,3094,2192,1660. ^{1}H NMR (300 MHz, DMSO-d6): δ3.09(s,3H), 3.39(s,3H), 4.48(s,1H), 7.25(s,2H), 7.44-7.47(d,2H), 8.07-8.10(d,2H). Anal.Calc: C(54.09%) H(3.69%) N(19.71%) O (22.52%) Found: C(54.04%) H(3.63%) N(19.65%)

7-amino-5-(4-chlorophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile: IR(KBr, vmax cm-1): 3359, 3094, 3033, 2225, 1716, 1683, 1576, 1557,1H NMR (300 MHz, DMSO-d6): δ 4.23 (s, 1H, -CH), 7.05 (.s, 1H, Ar- H), 7.18-7.28(m, 4H, Ar- H), 11.04 (s, 1H, -NH), 12.04(s, 1H, -NH) Anal.Calc: C(53.09%) H(2.86%) Cl(11.19%) N(17.69%),Found: C(53.10%) H(2.76%) Cl(11.12%) N(17.70%)

7-amino-5-(2-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile: IR(KBr, vmax cm-1): 3468, 3364, 3175, 3010, 2829, 2197, 1698, 1650, 1595, 1557.1H NMR (300 MHz, DMSO-d6): δ 4.44 (s, 1H, -CH), 7.21 (s, 2H, Ar- H), 7.56-7.71(dd, 2H, Ar- H), 8.04-8.07(d, 2H, Ar-H), 11.09(s, 1H, -NH), 12.13(s, 1H, -NH). Anal.Calc: C(51.38%) H(2.77%) N(21.40%), Found: C(51.31%) H(2.72%) N(21.38%) .

7-amino-5-(3-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d] pyrimidine-6-carbonitrile: IR(KBr, vmax cm-1): 3304, 3201, 2034, 2192, 1705, 1660, 1558.1H NMR (300 MHz, DMSO-d6):δ4.42(s,1H),7.099 (s,2H),7.64(s,1H),8.01 (s,3H),11.07(s,1H),12.08(s,1H). Anal.Calc: C(51.38%) H(2.77%) N(21.40%), Found: C(51.33%) H(2.74%) N(21.32%)

7-amino-5-(4-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d] pyrimidine-6-carbonitrile: IR(KBr, vmax cm-1): 3349,3094,3033,2225,1715, 1683,1673. 1H NMR (300 MHz, DMSO-d6): 4.41(s,1H), 7.24(s,2H), 7.50-7.52(d,2H),8.14-8.14(d,2H),11.12(s,1H), 12.16 (s,1H). Anal.Calc: C(51.38%) H(2.77%) N(21.40%) O(24.45%), Found: C(51.34%) H(2.72%) N(21.35%) O(24.42%)

Antioxidant activity

Hydrogen peroxide scavenging assay

This method is based on the ability of a compound to convert hydrogen peroxide to water. A 40 mM solution of hydrogen peroxide was prepared in saline phosphate buffer (pH 7.4). 100 µl DMSO solutions of the test compounds or standards at the concentrations of (100 µg/ml) were separately added to 2 ml of the prepared hydrogen peroxide solution and the absorbance was measured at 230 nm after 10 min against a blank solution. The blank solution was composed of 100 µl DMSO solutions of test compounds or standards and 2 ml of saline phosphate buffer. The hydrogen peroxide scavenging activity for compounds and standards was calculated using the following equation:

$$H_2O_2 \text{ scavenging activity (\%)} = [(Ac-At) / Ac] \times 100$$

Where, Ac is the absorbance of the control and At is the absorbance of the tested compounds or standards. Gallic acid at the concentration rang of (100 µg/ml) was used as the standard [31].

Nitric oxide scavenging activity

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (pH 7.4, 1 ml) and test samples or standard, ascorbic acid solution in dimethyl sulphoxide (1 mL) at concentration (100 µg/ ml) was incubated at 25^0C for 150 min. After incubation, 0.5 mL of reaction mixture containing nitrite ion was removed, 1 ml of sulphanillic acid reagent was added to this, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at λ 640 nm24 using spectrophotometer [32].

$$\% \text{ of scavenging} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Where A control is the absorbance of the control reaction (containing all reagents and Ascorbic acid), A sample is the absorbance of the test compound (containing all reagents and test compound). Tests were carried out in triplicate. The results obtained from antioxidant assay shows (**Table 4** and **Figure 1**)

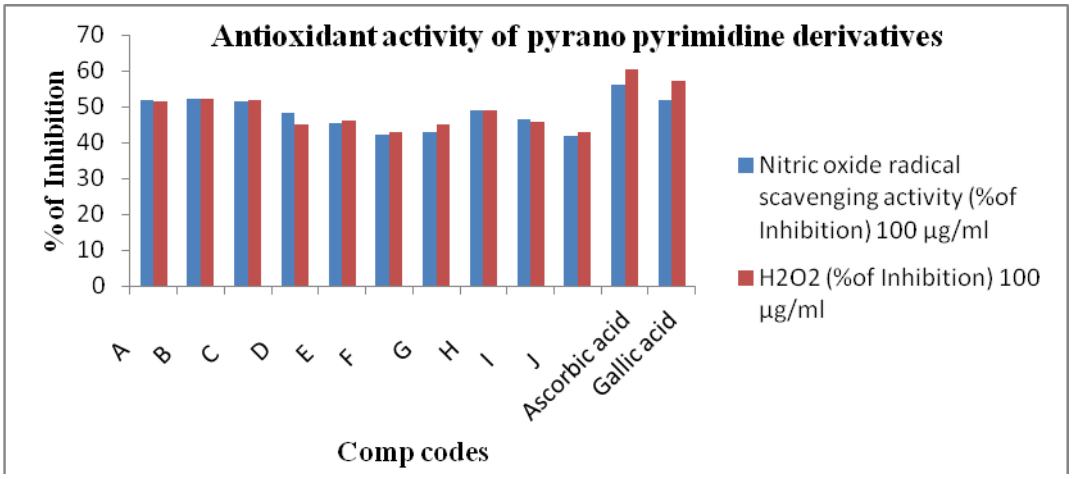


Figure 1 Antioxidant activity of 7-amino-2, 4-dioxo-5-phenyl-1, 3, 4, 5-tetrahydro-2H-pyrano [2, 3-d] pyrimidine-6-carbonitriles

Results and Discussion

Chemistry

The investigation of this study was started by a model reaction of 3-notrobenzaldehyde 0.150g (1 mmol), barbituric acid 0.128g (1 mmol), and malanonitrile 0.066g (1 mmol) were charged in water at room temperature for 3-4 hours without use of any catalyst. However it has been found that the expected results were not obtained since reaction was not proceed beyond the knoevenagel condensation. This reduced yield was assumed to be a result of non-homogenized mixing of the reactants occurred due to poor solubility and hydrophobicity. Then we subjected a reaction of barbituric acid, benzaldehyde and malanonitrile in ethanol solvent under reflux condition. The reaction go efficiently yielding of pyrano [2, 3-d]pyrimidine derivatives in 30-60 min with appropriately higher yields (90-95%) (**Table 1**).

To explore the efficiency of the catalyst with other one, the various basic catalysts like Triethyl Amine, NaOH, $NaOC_2H_5$ and DBN were used under same reaction condition. Among these, the DBN was afforded resultant 7-amino-5-(3-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile in excellent yield with shorter reaction time (**Table 2**).

Table1 Solvent optimization for synthesis of 7-amino-5-(3-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile

Entry	Solvent	Yield (%)a	Time(min)	Temp. (0C)
1	Ethanol	90-95	30	Reflux
2	Methanol	87-90	110	Reflux
3	Water	20	320	Reflux
4	Ethanol:Water 50:50	40	85	70-80
5	Ethanol:Water 70:30	40	90	70-80

[a] Isolated yield.

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

Entry	Catalyst	Time (min.)	Yield(%)[a]
1	Uncatalyzed	120	45
2	**DBN**	**30**	**90**
4	Triethyl Amine	310	70
5	NaOH	100	75
6	$NaOC_2H_5$	150	80
1	Uncatalyzed	120	45

[a] Isolated yield

Antioxidant activity

Due to their unique adaptability and derivatizations the structural elements of these series are correlates with such type of activities. To this end, a number of radical scavenging tests were carried out against Nitric oxide radical scavenging and H_2O_2 assay assay. The results demonstrate that synthesized all pyrano [2, 3-*d*] pyrimidine s possesses significant activity at 100µg/ml (**Table 4** and Figure 1)

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

Entry	R	R^1	Product	Time (min)	Yield (%)[d]
1.	3-Nitro	CH_3	A	60	85
2.	4-Nitro	CH_3	B	45	81
3.	4-Chloro	CH_3	C	60	84
4.	3-Chloro	CH_3	D	60	87
5.	2-Choro	CH_3	E	45	81
6.	3-Bromo	CH_3	F	60	70
7.	3-Nitro	H	G	35	85
8.	4-Nitro	H	H	45	80
9.	4-Chloro	H	I	60	81
10.	2-Choro	H	J	60	83
11.	3-Bromo	H	K	60	75

Table 4 Antioxidant activity of 7-amino-2, 4-dioxo-5-phenyl-1, 3, 4, 5-tetrahydro-2H-pyrano [2, 3-d] pyrimidine-6-carbonitriles

Comp code	Nitric oxide radical scavenging activity (% of Inhibition) 100 µg/ml	H2O2 (% of Inhibition) 100 µg/ml
A	51.85	51.46
B	52.31	52.26
C	51.52	52.01
D	48.17	45.09
E	45.42	46.12
F	42.10	42.98
G	43.02	45.01
H	49.10	49.08
I	46.45	45.62
J	41.86	43.08
Ascorbic acid	56.18	60.48
Gallic acid	51.70	57.04

Conclusions

In summary, we have developed an efficient and green method for the synthesis of pyrano [2, 3-d] pyrimidine derivative DBN. A mild reaction conditions, moderate to high yields and the ethanol mediated reaction conditions are the main features of this synthetic route. The synthesized derivatives possesses moderate to good antioxidant activity, which may be taken into consideration in the design of new theruptic agents.

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