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Synthesis and screening of angularly fused pyrido[3',2':4,5]furo[3,2-*d*]pyrimidin-4(3*H*)-ones

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ABSTRACT

In the present study, a series of novel pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-ones were synthesised by condensing 3-amino-6-hydroxy-4-methyl furo[2,3-b]pyridine-2-carboxamide with different aromatic acids. The structure of the synthesized compounds was confirmed by spectral studies and the purity was checked by Thin layer chromatographic analysis. All the compounds (PFP-HM 1-7) were screened for their In vitroantioxidant and In vivoanti-inflammatory activities. Antioxidant activity of the synthesised compounds was assessed by DPPH method. Compound PFP-HM2 was found to be a potent antioxidant agent (IC_{50}: 0.129 μM). The anti-inflammatory activity was performed by rat paw oedema method. Ibuprofen was used as standard (100mg/kg body mass). Except compound PFP-HM3, all other compounds showed very good anti-inflammatory activity when compared with the standard. Compound PFP-HM6, at 1^{st} h did not show any inhibition of inflammation. But after 2^{nd} h, it showed 100% protection from inflammation. Compound PFP-HM 2 also showed very good percentage inhibition of inflammation from 1^{st} h to 5^{th} h (76-98.8%). These data revealed that the presence of 4-trifluoromethylphenyl, 4-chlorobenzyl, 3, 5-dimethoxyphenyl and 3-fluorophenyl substitutions in the fused pyrimidine systemplayed a major role in their biological activities.

Key words: angularly fused pyrimidine; furo[2,3-b]pyridine-2-carboxamide; antioxidant;anti-inflammatory; rat paw oedema.

INTRODUCTION

Fused pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acid to their current use in the chemotherapy of AID. Fused Pyrimidines have been the subject of substantial attention by synthetic and medicinal chemists because of the role of this heteroaromatic ring in many biological systems. Heterofused pyrimidines exhibit promising antiviral [1], antibacterial [2], anti-AIDS [3], and antinociceptive [4] activities. Fused pyrimidines are extensively used in neurology, particularly

in the treatment of neurodegenerative disorders such as Parkinson's disease [5], antianxiety disorders [6], and depression [7]. Fused pyrimidines are selective inhibitors for multidrug resistance (MDR) [8]. Folate metabolism has long been recognized as an attractive target for cancer chemotherapy because of indispensable role of fused pyrimidine antifolates as antitumor agents [9].

Usage of microwave irradiation (MWI) is well known for the synthesis of variety of compound wherein chemical reactions are accelerated because of selective absorption of microwave by polar molecules. The coupling of MWI with solid supported reagents under solvent free conditions provides unique chemical processes with special attribute such as enhanced reaction rate, higher yield and greater selectivity. But this technique requires an appreciable amount of solvent for adsorption of reactants and elution of products. In the view of ongoing research on neat synthesis, when the no solvent reactions are coupled with MWI prove to be advantageous for environmental reasons as well as due to their uniform heating effect and shorter reaction times.

In view of the involvement of biological activities of fused pyrimidine and the eco-friendly synthesis, the purpose of the present work is to construct some novel pyrido[3',2':4,5]furo[3,2-*d*]pyrimidin-4(3*H*)-onesas potentantioxidant and anti-inflammatory agents.

MATERIALS AND METHODS

Chemistry

The melting point of the synthesised compounds was determined in open capillary tubes and was uncorrected. The IR spectra of the compounds were recorded in the range of 500-4000 cm^{-1} on Shimadzu FT-IR 8310 using KBr pellets. The ^{1}H-NMR spectra were recorded on Joel, model GSV-400 MHz spectrometer using $CDCl_3$/DMSO-d_6 as solvent. The chemical shifts were reported as parts per million downfield from tetramethylsilane (Me_4Si). Mass spectra were recorded on the Shimadzu GC-MS QP5050. The purity of the compounds were checked by TLC on SiO_2 gel (HF_{254}, 200 mesh) coated glass plates. The spots were visualized by UV light.

Synthesis of N-phenyl chloroacetamide

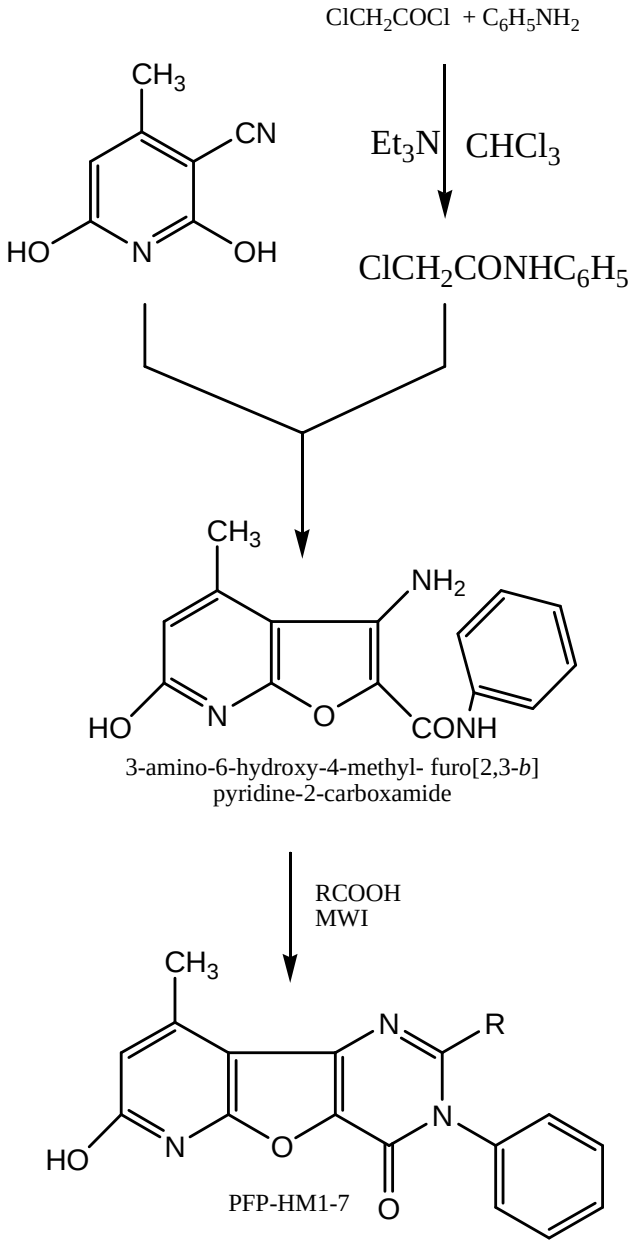
To a solution of chloroacetyl chloride (1mmol) in chloroform, aniline (1.2mmol), and triethylamine (2mmol) were added in succession and the reaction mixture was allowed to be stirred at room temperature for half an hour. Then the reaction mixture was diluted with dichloromethane (20ml) and washed with water. The organic layer was dried with sodium sulphate and concentrated to dryness under reduced pressure to yield solid compounds which were crystallized to furnish N-phenyl chloroacetamide derivative.

Synthesis of pyridine fused furano-2-carboxamides

To a solution of 4, 6-disubstituted 2-hydroxypyridine-3-carbonitrile (0.02mol) in dry acetone, N-phenyl chloroacetamide (0.02mol) and anhydrous potassium carbonate (0.2mol) were added and the reaction mixture was refluxed for about 8h. The potassium salt was filtered off and subsequent trituration with ethanol gave pyridine fused furano-2-carboxamides.

Synthesis of angularly fused pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-ones
Equi molar amount of pyridine fused furano-2-carboxamides was irradiated with various carboxylic acids for 4-7minutes to give the aimed compounds.

Figure 1: Synthesis of angularly fused pyrimidine derivatives



Spectral data of the representative compounds
2-(4-fluorophenyl)-7-hydroxy-9-methyl-3-phenylpyridofuro[3,2-d]pyrimidin-4(3H)-one(PFP-HM4): IR (KBr) (cm^{-1}): 3026 (aromatic -CH-, Str.), 1680 (C=O, Str.), 1232 (C – F, Str.), 2924 (-CH_3-, -CH- Str.), 3381 (-OH-, Str.). ^{1}H-NMR (DMSO-*d*6): 6.8-7.8 (multiplet, 9H, aromatic-H),

4.3 (singlet, 1H, -OH-), 3.5 (singlet, 3H, -CH_3-), 6.6 (singlet, 1H, ring -CH=, C_8). MS: 387(M^{+1}), 342, 273, 245, 148, 136, 122, 110, 96, 61.

*7-hydroxy-2-(3,5-dimethoxyphenyl)-9-methyl-3-phenyl pyridofuro[3,2-d]pyrimidin-4(3H)-one (PFP-HM7):*IR (KBr) (cm^{-1}): 3097 (aromatic -CH-, Str.), 1676 (C=O, Str.), 3186 (-OH-, Str.), 2837 (-OCH_3, Str.), 1595 (ring C=N str.), 1433 (ring C-N str.), 2937 (-CH_3-, -CH- Str.),. ^{1}H-NMR (DMSO-*d*6): 6.5-7.8 (multiplet, 8H, aromatic-H), 4.3 (singlet, 1H, -OH-), 3.1 (singlet, 3H, -CH_3-), 3.9 (OCH_3, 3H, singlet). MS: 429 (M^{+1}), 402, 272, 250, 239, 221, 180, 147, 136, 109, 95, 81, 60.

Biological activity

Antioxidant activity by DPPH method

The assay was carried out in a 96 well micro titre plate. To 100 μL of the DPPH solution, 100 μL of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentrations of the test and standard solutions used were 1000 μg/ml to 7.8125 μg/ml. The plates were incubated at 37 ^{0}C for 20 minutes and the absorbance of each solution was measured at 540 nm, using ELISA micro titre plate reader. The experiment was performed in triplicate and the percentage scavenging activity was calculated using formula given below. IC_{50} (Inhibitory Concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals and it was calculated from the graph, % scavenging Vs concentration [10].

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

Anti-inflammatory activity

Animals

Albino rats of *Wistar* strain of either sex, weighing 150-180g, were selected by random sampling technique and used in the study of anti-inflammatory activity. The animals had free access to standard commercial diet and water *ad libitum*and rats were kept in rooms maintained at 22±1ºC with a 12 h light / dark cycle. Prior approval for this study was obtained from Institutional Animal Ethical Committee, MAHE (F. No: IAEC/KMC/45/2009-2010).

Carrageenan induced paw oedema

The anti-inflammatory activity of the test compounds was carried out by carrageenan induced paw oedema method [11]. Carboxy methyl cellulose (0.5 % w/v CMC) was selected as vehicle to suspend the standard drug and the test compounds. The animals were weighed, marked for identification and divided into 9groups each group containing 6 animals. Oedemawas induced in the left hind paw of all rats by subcutaneous injection of 0.1 ml of 1% (W/V) carrageenan in normal saline (0.9%) intotheir footpads. The 1stgroup was kept as control and was given the respective volume of the solvent. The 2ndto 8thgroups were given an aqueous suspension of the synthesised compounds in a dose of 100 mg/kg body mass. The 9th group was administered ibuprofen in a dose of 100 mg/kg (standard). All the test compounds and the standard drug were administered orally, 1 hour before the carrageenean injection. The paw volume of each rat was measured using a digital plethysmometer (UGO Basil, Italy), just before the carrageenean injection (0 h) and then hourly for 5 hours post administration of the carrageenan injection. The

percentage inhibition of paw volume for each test group was calculated using the following equation.

$$\text{Percentage of inhibition (\%)} = 100(1- (a-x/b-y))$$

Where a = mean paw volume of treated animals after carrageenan injection
x = mean paw volume of treated animals before carrageenan injection
b = mean paw volume of control animals after carrageenan injection
y = mean paw volume of control animals before carrageenan injection

RESULTS AND DISCUSSION

Chemistry

The synthesis of hitherto unreported title compounds were prepared as outlined in Figure 1.The list of the synthesised compounds and the substitutions are mentioned in Table 1. The MWI reaction time, percentage yield, melting point and R_f value were given in Table 2.

Table 1: List of the angularly fused pyrido furo[3,2-d]pyrimidin-4(3H)-ones synthesized

Compound code	R	IUPAC name
PFP-HM1	4-bromobenzyl	2-(4-bromobenzyl)-7-hydroxy-9-methyl-3-phenylpyrido furo[3,2-d]pyrimidin-4(3H)-one
PFP-HM2	4-chlorobenzyl	2-(4-chlorobenzyl)-7-hydroxy-9-methyl-3-phenyl pyrido furo[3,2-d]pyrimidin-4(3H)-one
PFP-HM3	(4-chlorophenoxy)methyl	2-((4-chlorophenoxy)methyl)-7-hydroxy-9-methyl-3-phenylpyridofuro[3,2-d]pyrimidin-4(3H)-one
PFP-HM4	4-fluorophenyl	2-(4-fluorophenyl)-7-hydroxy-9-methyl-3-phenylpyridofuro[3,2-d]pyrimidin-4(3H)-one
PFP-HM5	3-fluorophenyl	2-(3-fluorophenyl)-7-hydroxy-9-methyl-3-phenyl pyridofuro[3,2-d]pyrimidin-4(3H)-one
PFP-HM6	4-trifluoromethylphenyl	2-(4-(trifluoromethyl)phenyl)-7-hydroxy-9-methyl-3-phenylpyridofuro[3,2-d]pyrimidin-4(3H)-one
PFP-HM7	3,5-dimethoxyphenyl	7-hydroxy-2-(3,5-dimethoxyphenyl)-9-methyl-3-phenyl pyridofuro[3,2-d]pyrimidin-4(3H)-one

Table 2: Physico-chemical properties of the synthesised compounds PFP-HM 1-7

Compound code	Mol. Weight	Yield (%)	Reaction time (min)	Melting point (ºC)	R_f
PFP-HM1	461	93	6	98	0.64
PFP-HM2	417	91	5	101	0.58
PFP-HM3	433	74	5	121	0.72
PFP-HM4	387	69	7	123	0.82
PFP-HM5	387	75	5	114	0.52
PFP-HM6	437	70	5	102	0.75
PFP-HM7	429	78	4	86	0.85

The postulated structures of the synthesised compounds were in accordance with IR, ^{1}H-NMR, Mass spectral studies. In the IR spectra of compounds PFP-HM 1-7,the carbonyl absorption bands were observed in the region of 1670-1690cm^{-1}. The ring C-H stretching band was observed around 2929-3052cm^{-1}. Whereas the ring –C=N- stretching was observed around 1556-1600cm^{-1}. The presence of -OH and –CH_3groups in all the synthesised compounds were producing absorption bands at 3180-3381cm^{-1}and 2922-2927cm^{-1}respectively.

In the ^{1}H-NMR spectra of these compounds, the –OH proton appeared as singlet at of δ 4.3, while the aromatic protons were seen as multiplet in the range of δ 6.5 -8.10. The methyl group showed a singlet in the range of δ3.5 – 3.1. The –CH= proton in the pyrido[3',2':4,5]furo[3,2-d]pyrimidin-4(3H)-ones(C_8) showed a singlet in the range between of δ 6.6 – 6.3. The –OCH_3

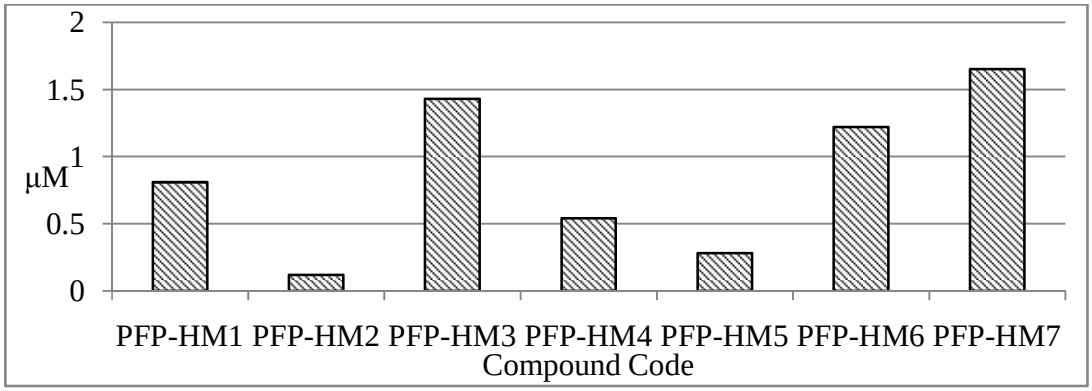
group present in the compound PFP-HM7 showed a strong singlet at δ3.9. Mass spectra showed the corresponding molecular ion peaks for all the compounds.

The antioxidant activity of the synthesised compounds (PFP-HM 1-7) was assessed by DPPH method. The percentage scavenging of free radicals and the IC_{50} values were summarized in Table 4.According to the structure activity relationship (SAR) studies, almost all these compounds have shown very good antioxidant activity. Among these tested compounds, compound2-(4-chlorobenzyl)-7-hydroxy-9-methyl-3-phenyl pyrido furo[3,2-d]pyrimidin-4(3H)-one (PFP-HM 2) showed pronounced antioxidant activity. The IC_{50} value of this compound was found to be 53.81µg/ml (0.129 µM; Figure 2).

Table 3: Percentage antioxidant activity of the synthesised compounds

Conc. (µg/ml)	Percentage antioxidant activity							
	PFP-HM1	PFP-HM2	PFP-HM3	PFP-HM4	PFP-HM5	PFP-HM6	PFP-HM7	Standard
1000	63.7100	75.5204	73.0769	72. 0000	70. 0000	56.5217	51.7723	90.3172
500	58.3400	70.9492	65.7762	65.0501	59. 0000	57.2135	59.2178	89.1566
250	50.1236	64.5507	51.1953	63.3965	53. 0000	53.5058	54.8355	89.7021
125	46.2850	47.4916	34.0549	55.9533	51. 0000	46.2114	45.4816	89.6969
62.5	44.1898	44.4536	23.4042	44.9263	49.2635	33.5515	34.5063	89.5993
31.25	29.8841	14.1903	13.9900	23.6755	47.2682	15.6457	22.4431	65.9246
IC_{50}(µg)	373.6	53.81	620.54	210.39	110.929	535.04	709.47	99.68

Figure 2: IC_{50} values of the angularly fused pyrido furo[3,2-d]pyrimidin-4(3H)-ones (PFP-HM 1-7).



Followed by this, compounds PFP-HM 5, 4 and 1 showed potent free radical scavenging activity. Their IC_{50} values were 110.9, 210.3 and 373.6 µg/ml respectively. These data revealed that the chloro or fluoro substituted phenyl ring in the 2^{nd}position played an important role in their antioxidant activity.

All the synthesised compounds (PFP-HM 1-7) were screened for their anti-inflammatory activity by carrageenan induced rat paw oedema method. Carrageenan is a pro-inflammatory agent, which induces the inflammation by releasing inflammatory mediators such as histamine, serotonin and cytokines. They lead cascade inflammation reactions in short and later (up to 6 h). Further augmentation of inflammation reaction is mediated by prostaglandins (PGs) derived from arachidonic acid by the action of prostaglandin H synthase [also referred as cyclooxygenase (COX)]. The present study illustrated that all the compounds are having anti-inflammatory

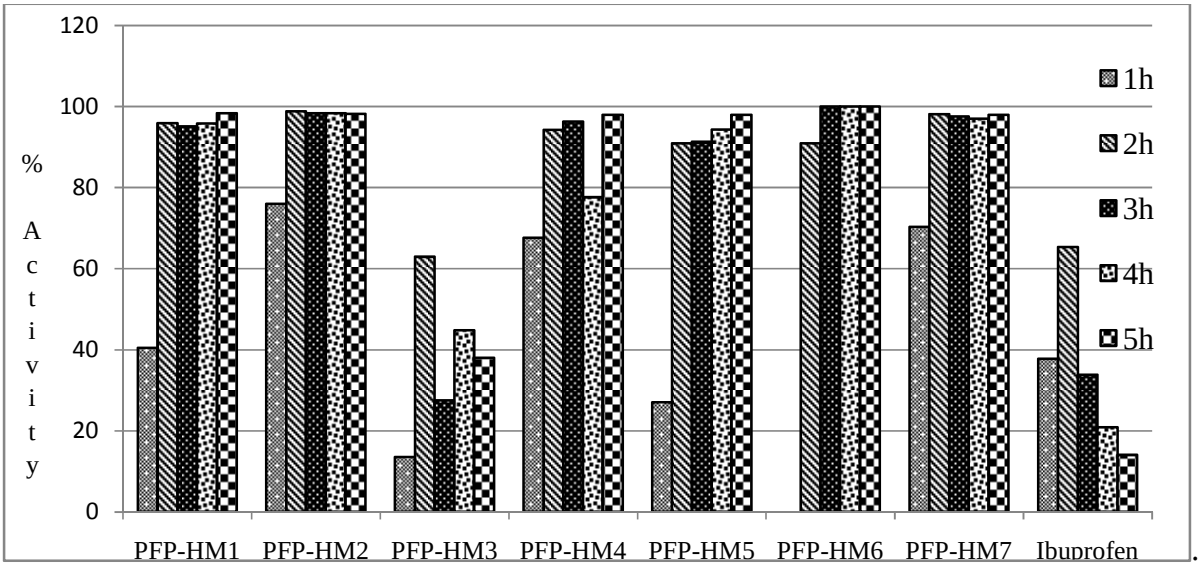
activity. The mean increase in paw volume is summarized in Table 4 and the percentage inhibition of paw oedema is represented in Figure 3.

Table 4: Anti-inflammatory activity of the synthesised compounds

Compound code	Mean increase in paw volume (mean ± S.E.M)				
	1h	2h	3h	4h	5h
PFP-HM1	0.0550±0.0122	0.0125±0.0077	0.0098±0.0192	0.0070±0.0170	0.0020±0.0537
PFP-HM2	0.0222±0.0375	0.0035±0.0412	0.0038±0.0175	0.0020±0.0264	0.0022±0.0881
PFP-HM3	0.0800±0.0118	0.1121±0.0196	0.1450±0.0010	0.0925±0.0113	0.0775±0.0156
PFP-HM4	0.0300±0.0180	0.0175±0.0207	0.0075±0.0221	0.0375±0.0435	0.0025±0.0136
PFP-HM5	0.0675±0.0090	0.0275±0.1148	0.0175±0.0660	0.0095±0.2918	0.0025±0.0900
PFP-HM6	0.1000±0.0093	0.0275±0.0286	NI	NI	NI
PFP-HM7	0.0275±0.0453	0.0056±0.0082	0.0050±0.0548	0.0050±0.0321	0.0025±0.0877
Control	0.0925±0.0032	0.3025±0.1494	0.2000±0.0334	0.1675±0.0425	0.1250±0.0317
Ibuprofen	0.0575±0.0019	0.1050±0.0026*	0.1325±0.0037*	0.1325±0.0039**	0.1425±0.0106**

*NI: No increase in paw volume; * p <0.05; ** p<0.01*

Figure 3: Percentage anti-inflammatory activity of the test compounds



All the compounds showed good anti-inflammatory activity after 1st h. Compound PFP-HM 3 showed moderate anti-inflammatory activity. Compound PFP-HM6, at 1st h did not show any inhibition of inflammation. But after 2nd h, it showed 100% protection from the inflammation. Compound 2-(4-chlorobenzyl)-7-hydroxy-9-methyl-3-phenyl pyrido furo[3,2-d]pyrimidin-4(3H)-one(PFP-HM 2) showed very good percentage inhibition of inflammation from 1sth to 5th h (76-98.8%). Other compounds like PFP-HM1, PFP-HM 4, PFP-HM 5 and PFP-HM 7 also showed good anti-inflammatory activity.

Compound 2-((4-chlorophenoxy)methyl)-7-hydroxy-9-methyl-3-phenylpyridofuro[3,2-d]pyrimi -din-4(3H)-one(PFP-HM3) was not very active when compared with other test compounds and it showed maximum inhibition of oedema at 2nd h (62.9%). These data revealed that the presence of4-trifluoromethylphenyl, 4-chlorobenzyl, 3,5-dimethoxyphenyl and 3-fluorophenyl

substitutions in the fused pyrimidine systemplayed a major role in theirantioxidant and anti-inflammatory activities.

CONCLUSION

Some novel pyrido[3',2':4,5]furo[3,2-*d*]pyrimidin-4(3*H*)-oneshave been synthesised by adapting a modified strategy which has the advantage of employing non-drastic reaction conditions to give high yields.All the compounds were screened for their antioxidant and anti-inflammatory activity. The results revealed that the groups like, 4-trifluoromethylphenyl, 4-chlorobenzyl, 3,5-dimethoxyphenyland 3-fluorophenyl substitutions at 2rd position in nucleus led to increase their biological activities.

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