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ORIGINAL ARTICLE

Novel pyrimidine and its triazole fused derivatives: Synthesis and investigation of antioxidant and anti-inflammatory activity

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Abstract In the present study, we have carried out the synthesis of novel dihydropyrimidinecarbonitrile (**1a–c**), its dimethylated adduct (**2a–c**), and hydrazine derivative (**3a–c**) of **2a–c** and its triazole fused derivatives (**4a–c, 5a–c** and **6a–c**). The structure of newly synthesized compounds was confirmed by IR, ^{1}H NMR, mass spectral data and elemental analysis. Further the novel derivatives were investigated for their in vitro antioxidant and anti-inflammatory activity. The results revealed that some of the tested compounds showed potent antioxidant and anti-inflammatory activity. The mass spectral pattern of **6a** has been investigated in order to elucidate the structure.

1. Introduction

Pyrimidine, being an integral part of DNA and RNA, imparts to diverse pharmacological properties as effective bactericide and fungicide (Williams and Cline, 1936; Reidlinger and Dworczak, 1994; Hardtman and Otto, 1972). Certain pyrimidine derivatives were also known to exhibit antimalarial (Brown and Evans, 1985), antifilarial (Brown and Rees, 1984), antioxidant (Vanessa et al., 2010; Prasenjit et al., 2010) and anti-HIV activities (Okabe et al., 1991). Some of the 3,4-dihydropyrimidines (DHPM) have emerged as integral backbones of several calcium channel blockers, antihypertensive agents, adrenergic and neuropeptide antagonist (Pasha et al., 2005). Several alkaloids containing 3,4-dihydropyrimidine have been isolated from marine sources and among them the *batzelladine* alkaloids are found to be potent HIV-gp-120-CD_4 inhibitors (Kappe, 2000; Kappe et al., 2005; Patil et al., 1995).

Along with the varied biological activities of pyrimidine, other heterocycles fused with pyrimidines play an essential role in several biological processes and have a considerable chemical and pharmacological importance. Triazole in association

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with the pyrimidine has shown good antifungal (Singh et al., 2004) and hypoglycemic action (Agarwal, 1991). [1,2,4]Triazole fused pyrimidine exhibit good antimicrobial activity (Fathy et al., 2004), antitumour activity (Swelam, 1998), analgesic, anti-inflammatory and ulcerogenic activities (Hend et al., 2008).

In the view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents. Hence, we have synthesized some new dihydropyrimidinecarbonitrile and its triazole fused derivatives. The novel derivatives were characterized by spectral data and elemental analysis and these compounds were used for their antioxidant and anti-inflammatory screening. Compound **6a** is one of the final triazole derivatives of pyrimidine and its intermediates **1a** and **3a** have shown good antioxidant activity so we have described the electron spray ionization mass spectral fragmentation of **6a**.

2. Materials and methods

2.1. Materials and reagents

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting point was determined by Micro control based melting point instrument and is uncorrected. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel (60GF-254) plates, by using ethyl acetate: butanol: chloroform in the ratio of [1:2:1] as mobile phase and visualized with UV light. Column chromatography was performed on silica gel (200–300 mesh). Infra red (IR) spectra was recorded by using KBr disk on a Thermo Nicolate IR-400 FTIR spectrophotometer, ^{1}H NMR spectra was recorded on Bruker Avance-300F spectrometer (300 MHz) using tetramethylsilane as internal standard (chemical shift in δ ppm). Mass spectra were recorded on a Triple Quadrupole LC–MS–MS (Sciex with ESI source) spectrometer. The elemental analysis was carried out by using Heraus CHN rapid analyzer. All the compounds gave C, H and N analysis within $\pm 1.2\%$ of the theoretical values. Spectra facilities and elemental analysis were carried out by Department of University scientific instrument centre, Karnatak University, Dharwad, India and Suven Life Sciences, Hyderabad India.

2.2. Synthesis

2.2.1. General procedure for the synthesis of 6-oxo-4-substituted aryl-2-sulfanyl-1,6-dihydropyrimidine-5-carbonitrile (*1a–c*)

Mixture equimolar quantities of ethyl cyanoacetate (5.7 g, 50 mmol), thiourea (3.8 g, 50 mmol), appropriate aromatic aldehyde (50 mmol) and potassium carbonate (6.9 g, 50 mmol) in absolute ethanol (50 ml) was gently refluxed till the completion of reaction. The reaction mixture was neutralized with glacial acetic acid to precipitate out the product. The product was isolated and recrystallized from ethanol as yellow crystals.

2.2.2. General procedure for the synthesis of 1-methyl-2-(methylsulfanyl)-6-oxo-4-substituted aryl-1,6-dihydropyrimidine-5-carbonitrile (*2a–c*)

To a solution of 6-oxo-4-substituted aryl-2-sulfanyl-1,6-dihydropyrimidine-5-carbonitrile (**1a–c**, 20 mmol) in *N*,*N*-dimethyl formamide (DMF, 30 ml), potassium carbonate (5.52 g, 40 mmol) and methyl iodide (5.68 g, 40 mmol) were added and stirred till the completion of reaction at room temperature. Then the reaction mixture was diluted with cold water and neutralized by glacial acetic acid. The product was filtered off and recrystallized from ethanol as creamy crystals.

2.2.3. General procedure for the synthesis of 2-hydrazinyl-1-methyl-6-oxo-4-substituted aryl-1,6-dihydropyrimidine-5-carbonitrile (*3a–c*)

A mixture of compound 1-methyl-2-(methylsulfanyl)-6-oxo-4-substituted aryl-1,6-dihydropyrimidine-5-carbonitrile (**2a–c**, 10 mmol) and hydrazine hydrate (80%, 1.90 g, 30 mmol) in absolute alcohol was refluxed till the completion of reaction. The reaction mixture was poured into crushed ice. Then the product was isolated and recrystallized from ethanol/DMF mixture as yellow crystals.

2.2.4. General procedure for the synthesis of 8-methyl-7-oxo-5-substituted aryl-7,8-dihydro[1,2,4]triazolo[4,3-α]pyrimidine-6-carbonitrile (*4a–c*)

A mixture of compound 2-hydrazinyl-1-methyl-6-oxo-4-substituted aryl-1,6-dihydropyrimidine-5-carbonitrile (**3a–c**, 5 mmol) in 20 ml formic acid was refluxed till the completion of reaction. The excess of formic acid was distilled. The reaction mixture after cooling was poured into crushed ice. Then the product was isolated and recrystallized from DMF as yellow crystals.

2.2.5. General procedure for the synthesis of 3,8-dimethyl-7-oxo-5-substituted aryl-7,8-dihydro[1,2,4]triazolo[4,3-α]pyrimidine-6-carbonitrile (*5a–c*)

A mixture of compound 2-hydrazinyl-1-methyl-6-oxo-4-substituted aryl-1,6-dihydropyrimidine-5-carbonitrile (**3a–c**, 5 mmol) in 20 ml acetic anhydride was refluxed till the completion of reaction. The excess of acetic anhydride was distilled. The reaction mixture after cooling was poured into crushed ice. Then the product was isolated and recrystallized from DMF as yellow crystals.

2.2.6. General procedure for the synthesis of 8-methyl-7-oxo-3-phenyl-5-substituted aryl-7,8-dihydro[1,2,4]triazolo[4,3-α]pyrimidine-6-carbonitrile (*6a–c*)

A mixture of compound 2-hydrazinyl-1-methyl-6-oxo-4-substituted aryl-1,6-dihydropyrimidine-5-carbonitrile (**3a–c**, 5 mmol) in 20 ml benzoyl chloride was refluxed till the completion of reaction. The excess of benzoyl chloride was distilled. The reaction mixture after cooling was poured into crushed ice. Then the product was obtained as semisolid.

The physical constants and spectral (IR, ^{1}H NMR, mass) characterization and elemental analysis supported the structure of various synthesized compounds (Tables 1 and 2).

3. Pharmacological screening

3.1. Antioxidant screening: (in vitro)

3.1.1. Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4). Various concentrations (12.5, 25, 50, 100 μg/ml) of 1 ml of the test samples or standard, ascorbic acid (Ismaili et al., 2008; Rang et al., 2003) in methanol were added to 2 ml of hydrogen peroxide solution

Table 1 Physical constant of synthesized compounds.

Compound	Chemical name	Ar	Reaction time (h)	Yield (%)	M.P. (°C)	Mol. formula	R_f value#
1a	6-Oxo-4-phenyl-2-sulfanyl-1,6-dihydropyrimidine-5-carbonitrile	C_6H_5	10	53	235–238	$C_{11}H_7N_3OS$	0.45
1b	4-(3-Methoxyphenyl)-6-oxo-2-sulfanyl-1,6-dihydropyrimidine-5-carbonitrile	3-OCH_3·C_6H_4	14	61	237–240	$C_{12}H_9N_3O_2S$	0.49
1c	6-Oxo-2-sulfanyl-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile	3,4,5-(OCH_3)C_6H_2	12	63	220–222	$C_{14}H_{13}N_3O_4S$	0.52
2a	1-Methyl-2-(methylsulfanyl)-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile	C_6H_5	3	62	148–150	$C_{13}H_{11}N_3OS$	0.56
2b	4-(3-Methoxyphenyl)-1-methyl-2-(methylsulfanyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile	3-OCH_3·C_6H_4	4	66	175–178	$C_{14}H_{13}N_3O_2S$	0.58
2c	1-Methyl-2-(methylsulfanyl)-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile	3,4,5-(OCH_3)C_6H_2	4	59	210–212	$C_{16}H_{17}N_3O_4S$	0.6
3a	2-Hydrazinyl-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile	C_6H_5	6	71	275–277	$C_{12}H_{11}N_5O$	0.58
3b	2-Hydrazinyl-4-(3-methoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile	3-OCH_3·C_6H_4	5	70	280–283	$C_{13}H_{13}N_5O_2$	0.59
3c	2-Hydrazinyl-1-methyl-6-oxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile	3,4,5-(OCH_3)C_6H_2	7	66	252–255	$C_{15}H_{17}N_5O_4$	0.61
4a	8-Methyl-7-oxo-5-phenyl-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	C_6H_5	6	72	250–252	$C_{13}H_9N_5O$	0.59
4b	5-(3-Methoxyphenyl)-8-methyl-7-oxo-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	3-OCH_3·C_6H_4	9	67	258–260	$C_{14}H_{11}N_5O_2$	0.57
4c	8-Methyl-7-oxo-5-(3,4,5-trimethoxyphenyl)-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	3,4,5-(OCH_3)C_6H_2	7	55	296–298	$C_{16}H_{15}N_5O_4$	0.62
5a	3,8-Dimethyl-7-oxo-5-phenyl-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	C_6H_5	7	69	265–268	$C_{14}H_{11}N_5O$	0.61
5b	5-(3-Methoxyphenyl)-3,8-dimethyl-7-oxo-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	3-OCH_3·C_6H_4	7	62	251–254	$C_{15}H_{13}N_5O_2$	0.63
5c	3,8-Dimethyl-7-oxo-5-(3,4,5-trimethoxyphenyl)-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	3,4,5-(OCH_3)C_6H_2	8	57	287–290	$C_{17}H_{17}N_5O_4$	0.68
6a	8-Methyl-7-oxo-3,5-diphenyl-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	C_6H_5	7	61	S.S.$^{\Psi}$	$C_{19}H_{13}N_5O$	0.54
6b	5-(3-Methoxyphenyl)-8-methyl-7-oxo-3-phenyl-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	3-OCH_3·C_6H_4	9	48	S.S.$^{\Psi}$	$C_{20}H_{15}N_5O_2$	0.55
6c	8-Methyl-7-oxo-3-phenyl-5-(3,4,5-trimethoxyphenyl)-7,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile	3,4,5-(OCH_3)C_6H_2	8	54	S.S.$^{\Psi}$	$C_{22}H_{19}N_5O_4$	0.57

S.S.$^{\Psi}$ – semisolid.

Table 2 Spectral characterization and elemental analysis of synthesized compounds.

Compound	IR (KBr) ν (cm^{-1})	^{1}H NMR DMSO-δ (ppm)	Mass m/z	% Calculated (found)		
				C	H	N
1a	3655 (NH), 2204 (C≡N), 1633 (C=O), 1514 (C=N)	7.46–7.87 (m, 5H, Ar-H), 11.60 (s, 1H, NH), 1.46 (s, 1H, SH)	230.4 M + 1	57.63 (57.60)	3.08 (3.05)	18.33 (18.30)
1b	3645 (NH), 2214 (C≡N), 1636 (C=O), 1581 (C=N), 1234 (C–O–C)	6.81–7.18 (m, 4H, Ar-H), 3.68(s, 3H, OCH_3), 10.93 (S, 1H, NH), 1.74 (s, 1H, SH)	260.3 M + 1	55.59 (55.60)	3.50 (3.50)	16.21 (16.20)
1c	3631 (NH), 2203 (C≡N), 1628 (C=O), 1509 (C=N), 1243 (C–O–C)	7.12 (s, 2H, Ar-H), 3.53 (s, 9H, OCH_3), 11.17 (s,1H,NH), 1.61 (s, 1H, SH)	320.3 M + 1	52.66 (52.58)	4.10 (4.07)	13.16 (13.15)
2a	2219 (C≡N), 1681 (C=O), 1551 (C=N), 1365 (C–N)	7.59–7.97 (m, 5H, Ar-H), 2.63 (s, 3H, S–CH_3), 4.11 (s, 3H, N–CH_3)	258.3 M + 1	60.68 (60.69)	4.31 (4.30)	16.33 (16.35)
2b	2217 (C≡N), 1670 (C=O), 1598 (C=N), 1368 (C–N), 1257 (C–O–C)	6.92–7.15 (m, 4H, Ar-H), 1.96 (s, 3H, S–CH_3), 2.98 (s, 3H, N–CH_3), 3.72 (s, 3H, OCH_3)	288.4 M + 1	58.52 (58.46)	4.56 (4.53)	14.62 (14.60)
2c	2210 (C≡N), 1668 (C=O), 1587 (C=N), 1365 (C–N), 1242 (C–O–C)	7.08 (s, 2H, Ar-H), 2.14 (s, 3H, S–CH_3), 3.21 (s, 3H, N–CH_3), 4.11 (s, 9H, OCH_3)	348.4 M + 1	55.32 (55.34)	4.93 (4.92)	12.10 (12.12)
3a	3339 (NH–NH_2), 2206 (C≡N), 1695 (C=O), 1619 (C=N)	7.51–7.91 (m, 5H, Ar-H), 2.09 (s, 1H, NH), 2.50 (s, 2H, NH_2), 3.21 (s, 3H, N–CH_3)	242.4 M + 1	59.74 (59.72)	4.60 (4.59)	29.03 (29.04)
3b	3261 (NH–NH_2), 2218 (C≡N), 1667 (C=O), 1599 (C=N), 1253 (C–O–C)	6.72–7.04 (m, 4H, Ar-H), 1.94 (s, 1H, NH), 2.17 (s, 2H, NH_2), 2.91 (s, 3H, N–CH_3), 3.88 (s, 3H, OCH_3)	272.3 M + 1	57.56 (57.50)	4.83 (4.80)	25.82 (25.76)
3c	3326 (NH–NH_2), 2214 (C≡N), 1664 (C=O), 1595 (C=N), 1246 (C–O–C)	6.56 (s, 2H, Ar-H), 1.83 (s, 1H, NH), 2.08 (s, 2H, NH_2), 3.09 (s, 3H, N–CH_3), 4.01 (s, 9H, OCH_3)	333.3 M + 1	54.38 (54.22)	5.17 (5.11)	21.14 (21.09)
4a	2230 (C≡N), 1680 (C=O), 1624 (C=N), 1320 (C–N)	7.40–7.92 (m, 5H, Ar-H), 8.28 (s, 1H, CH-triazole), 2.41 (s, 3H, N–CH_3)	252.3 M + 1	62.15 (62.08)	3.61 (3.57)	27.87 (21.78)
4b	2219 (C≡N), 1668 (C=O), 1599 (C=N), 1322 (C–N), 1251(C–O–C)	7.03–7.40 (m, 4H, Ar-H), 8.32 (s, 1H, CH-triazole), 2.27 (s, 3H, N–CH_3), 3.83 (s, 3H, O–CH_3).	282.4 M + 1	59.78 (59.82)	3.94 (3.92)	24.90 (24.86)
4c	2217 (C≡N), 1670 (C=O), 1593 (C=N), 1340 (C–N), 1246 (C–O–C)	6.88 (s, 2H, Ar-H), 7.94 (s, 1H, CH-triazole), 2.13 (s, 3H, N–CH_3), 3.64 (s, 9H, O–CH_3).	342.4 M + 1	56.30 (56.22)	4.43 (4.44)	20.52 (20.50)
5a	2229 (C≡N), 1674 (C=O), 1626 (C=N), 1321 (C–N)	7.32–7.86 (m, 5H, Ar-H), 2.38 (s, 3H, N–CH_3), 1.89 (s, 3H, C–CH_3)	266.3 M + 1	63.39 (63.29)	4.18 (4.15)	26.40 (26.36)
5b	2219 (C≡N), 1667 (C=O), 1593 (C=N), 1319 (C–N), 1251 (C–O–C)	6.94–7.47 (m, 4H, Ar-H), 2.41 (s, 3H, N–CH_3), 2.76 (s, 3H, C–CH_3), 3.62 (s, 3H, O–CH_3).	296.3 M + 1	61.01 (60.78)	4.44 (4.39)	23.72 (23.65)
5c	2217 (C≡N), 1670 (C=O), 1594 (C=N), 1340 (C–N), 1246 (C–O–C)	7.26 (s, 2H, Ar-H), 2.50 (s, 3H, N–CH_3), 2.09 (s, 3H, C–CH_3), 3.75 (s, 9H, O–CH_3).	356.3 M + 1	57.46 (57.38)	4.82 (4.79)	19.71 (19.66)
6a	2361 (C≡N), 1668 (C=O), 1619 (C=N), 1318 (C–N)	7.03–8.13 (m, 10H, 2Ar-H), 2.50 (s, 3H, N–CH_3)	328.3 M + 1	69.71 (69.59)	4.00 (4.02)	21.39 (21.28)
6b	2372 (C≡N), 1786 (C=O), 1598 (C=N), 1322 (C–N), 1214 (C–O–C)	6.82–7.96 (m, 9H, 2Ar-H), 2.34 (s, 3H, N–CH_3), 4.22 (s, 3H, O–CH_3).	358.4 M + 1	67.22 (67.18)	4.23 (4.20)	19.60 (19.57)
6c	2361 (C≡N), 1687 (C=O), 1595 (C=N), 1324 (C–N), 1289 (C–O–C)	6.65–7.88 (m, 7H, 2Ar-H), 2.27 (s, 3H, N–CH_3), 3.62 (s, 9H, O–CH_3).	418.4 M + 1	63.30 (63.32)	4.59 (4.56)	16.78 (16.72)

in phosphate buffer saline. The absorbance was measured at 230 nm after 10 min (Jayaprakasha et al., 2004).

3.1.2. 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 various concentrations (12.5, 25, 50, 100 μg/ml) was incubated at 25 °C for 150 min. After incubation, 0.5 ml of reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic 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 640 nm (Marcocci et al., 1994).

3.1.3. Lipid peroxidation inhibitory activity

Egg lecithin (3 mg/ml phosphate buffer, pH 7.4) was sonicated in an ultrasonic sonicator for 10 min to ensure proper liposome formation. Test samples (100 μl) of different concentrations (12.5, 25, 50, 100 μg/ml) were added to liposome mixture (1 ml); the control was without test sample. Lipid peroxidation was induced by adding ferric chloride (10 μl, 400 mM) and L-ascorbic acid (10 μl, 200 mM). After incubation for 1 h at 37 °C the reaction was stopped by adding hydrochloric acid (2 ml, 0.25 N) containing trichloroacetic acid (150 mg/ml), thiobarbituric acid (3.75 mg/ml) and butylated hydroxy anisole (0.50 mg/ml). The reaction mixture was subsequently boiled for 15 min, cooled, centrifuged at 1000 rpm for 15 min and the absorbance of the supernatant was measured at 532 nm and compared with that of ascorbic acid (Duh and Yen, 1997).

For all the above antioxidant methods, experiments were done in triplicate and average is taken, the % inhibition at different concentration was calculated by the following formula

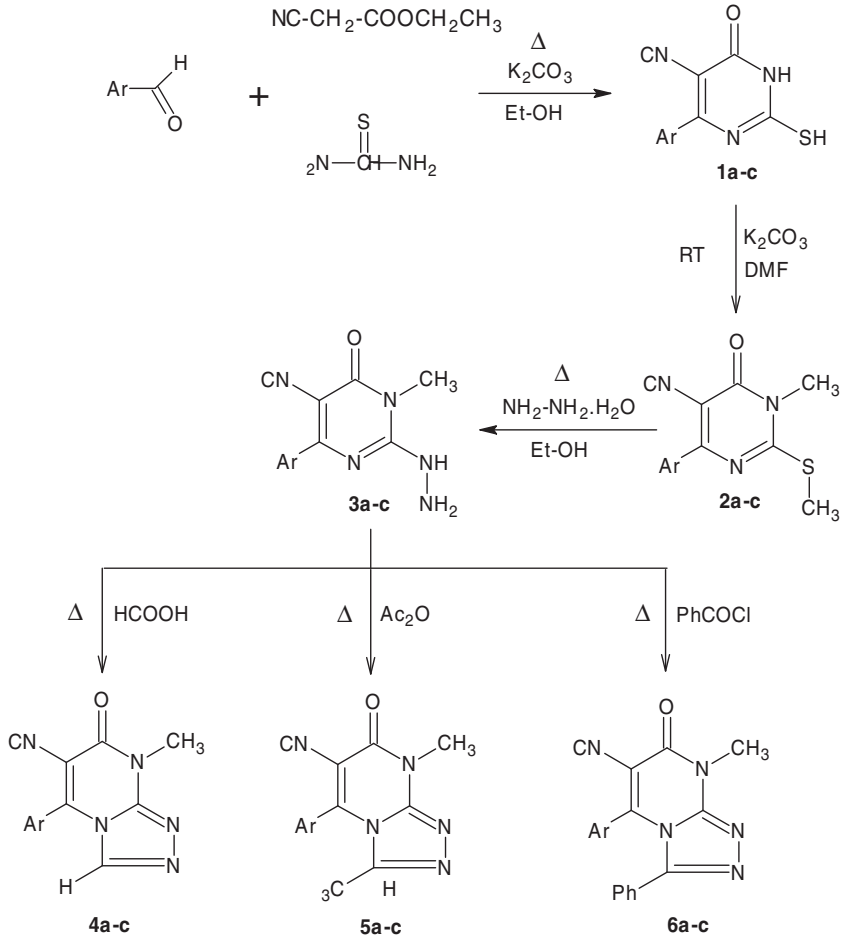
$$\% \text{ Inhibition} = [1 - (V_t/V_c)] \times 100$$

where, V_t = mean absorption of test compound, V_c = mean absorption of control.

The IC-50 value was derived from the% inhibition at different concentration.

3.2. Anti-inflammatory screening (in vitro)

The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Muzushima and



Ar - C_6H_5 (a), 3-OCH_3-C_6H_4 (b), 2,3,4-$(OCH_3)_3$-C_6H_2 (c)

Figure 1 Scheme 1. Synthetic route of compounds 1–6.

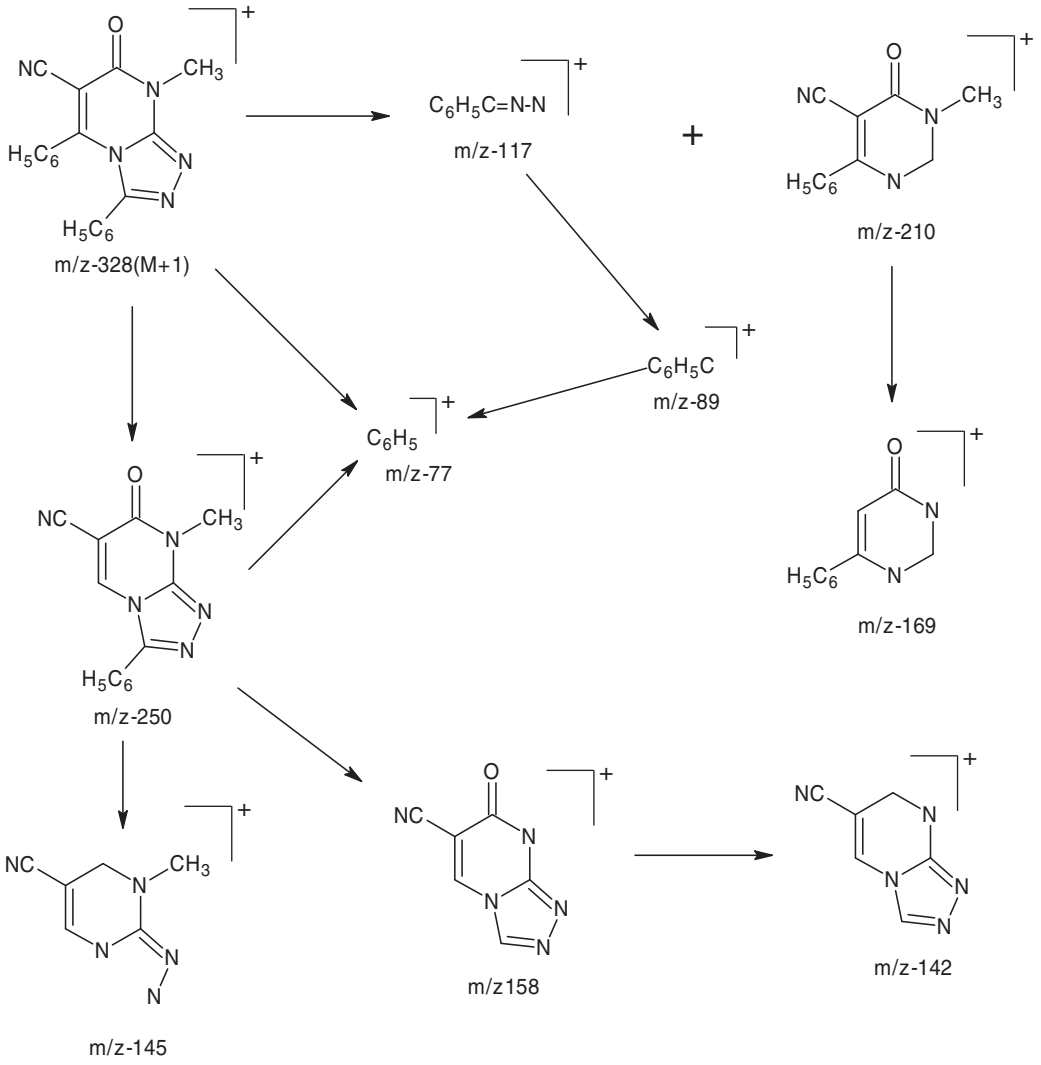


Figure 2 Scheme 2. MS fragmentation diagram of 8-methyl-7-oxo-3,5-diphenyl-7,8-dihydro[1,2,4] triazolo [4,3-*a*]pyrimidine-6-carbonitrile (**6a**).

Kobayashi (1968) with slight modification. The standard drug and test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer saline (pH 7.4) in such a way that concentration of DMF in all solutions was less than 2.5%. Test solution (1 ml) containing concentration (100 μg/ml) of drug was mixed with 1 ml of 1% albumin solution in phosphate buffer saline and incubated at 27 ± 1 °C in an incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 °C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm with UV–visible spectrophotometer. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is taken. The diclofenac was used as standard drug. (Robert and Morrow, 2001; Rang et al., 2003). The percentage of inhibition was calculated using the formula

$$\% \text{ Inhibition of denaturation} = [(V_t/V_c) - 1] \times 100$$

where, V_t = mean absorption of test compound, V_c = mean absorption of control.

4. Results and discussion

4.1. Chemistry

In the present work the title compounds were synthesized by the cyclization of three-components like arylaldehydes, thiourea and ethyl cyanoacetate in ethanol by using potassium carbonate to form 6-oxo-4-substituted aryl-2-sulfanyl-1,6-dihydropyrimidine-5-carbonitrile (**1a–c**), at room temperature stirring of **1a–c** with methyl iodide in DMF in presence of potassium carbonate yields 1-methyl-2-(methylsulfanyl)-6-oxo-4-substituted aryl-1,6-dihydropyrimidine-5-carbonitrile (**2a–c**). The 2-hydrazino derivatives, 2-hydrazinyl-1-methyl-6-oxo-4-substituted aryl-1, 6-dihydropyrimidine-5-carbonitrile (**3a–c**) was obtained by heating **2a–c** with hydrazine hydrate in ethanol. Condensation of latter with formic acid afforded the corresponding 8-methyl-7-oxo-5-substituted aryl-7, 8-dihydro[1,2,4]triazolo[4,3-α] pyrimidine-6-carbonitrile (**4a–c**). On the other hand, the 3-methyl derivatives 3,8-dimethyl-7-oxo-5-substituted aryl-7,8-dihydro[1,2,4]triazolo[4,3-α]pyrimidine-6-carbonitrile (**5a–c**) and 3-phenyl derivatives, 8-methyl-7-

oxo-3-phenyl-5-substituted aryl-7,8-dihydro[1,2,4]triazolo[4,3-α]pyrimidine-6-carbonitrile (**6a–c**) were prepared by the condensation with acetic anhydride and benzoyl chloride, respectively (Fig. 1).

All the pyrimidines and their [1,2,4]triazole fused pyrimidine derivatives were synthesized and confirmed by physical data, IR, ^{1}H NMR, mass spectra and elemental analysis. All the compounds have shown C≡N peak in 2220–2200 cm^{-1}, C═O peak in 1700–1625 cm^{-1}, C═C peak in 1555–1470 cm^{-1}. While the methoxy and trimethoxy derivatives have showed the C–O–C peak in 1255–1230 cm^{-1}. The **1a–c** compounds showed peak in 3631–3653 cm^{-1} for the N–H stretch in IR and 10.93–11.17 (s, 1H, NH═SH) peak in ^{1}H NMR, which is absent in **2a–c** compounds. While **2a–c** has shown 2.98–4.11 peak for N–CH_3 and 1.96–2.63 peak for S–CH_3 in ^{1}H NMR. The **3a–c** compounds showed the peak in 3340–3280 cm^{-1} for the NH–NH_2 in IR and 1.83–2.09 for NH and 2.08–2.50 for NH_2. The fused triazoles (**4a–c, 5a–c** and **6a–c**) were not showing the N–H stretch in IR and NH, NH_2 peak in ^{1}H NMR. The mass spectrum of **6a** showed an intense molecular ion peak at *m/z* 328 (M + 1) corresponding to molecular formula $C_{19}H_{13}N_5O$. This was found to be the base peak. M + 1 ion of **6c** underwent fragmentation to produce a peak at *m/z* 250 by losing C_6H_5 (*m/z* 77). Simultaneously a peak at *m/z* 210 was observed by losing C_6H_5C═N–N (*m/z* 117). C_6H_5C═N–N ion underwent further fragmentation to give peak at *m/z* 89 for C_6H_5C ion. The ion at *m/z* 89 underwent loss C to give peaks at *m/z* 77 (C_6H_5). Fragmentation pattern of **6a** has been shown in Fig. 2.

4.2. Pharmacological screening

4.2.1. Antioxidant activity

All the synthesized compounds were screened for in vitro antioxidant activity by various methods as scavenging of hydrogen peroxide, scavenging of nitric oxide radical, lipid peroxidation inhibitory activity. In vitro antioxidant activity of synthesized compound is summarized in Table 3.

The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. Particularly, compounds**3a, 3c, 1a, 1c, 3b** and **3c** have shown more promising antioxidant activity as compare to standard, ascorbic acid, while other derivatives are moderately active. **1c** has shown good antioxidant activity as compare to standard by scavenging of nitric oxide radical

Table 4 Anti-inflammatory activity of the compounds and standard diclofenac sodium.

Compound	Mean absorbance ± S.D.[a]	Inhibition of denaturation (%)
Control	0.0468 ± 0.0003	
Standard	0.0861 ± 0.0001	83.97
1a	0.0559 ± 0.0008	19.44
1b	0.0499 ± 0.0020	66.24
1c	0.0498 ± 0.0010	64.10
2a	0.0529 ± 0.0025	13.03
2b	0.0794 ± 0.0004	69.66
2c	0.0554 ± 0.0026	18.38
3a	0.0584 ± 0.0046	24.79
3b	0.0824 ± 0.0007	76.07
3c	0.0615 ± 0.0016	31.41
4a	0.0556 ± 0.0014	18.80
4b	0.0671 ± 0.0019	43.38
4c	0.0533 ± 0.0046	13.89
5a	0.0487 ± 0.0005	04.06
5b	0.0619 ± 0.0006	32.27
5c	0.0709 ± 0.0009	51.50
6a	0.0567 ± 0.0020	21.15
6b	0.0579 ± 0.0018	23.72
6c	0.0507 ± 0.0024	08.34

[a] Average of three determination.

Table 3 Antioxidant activity (IC-50 values) of synthesized compounds and standard ascorbic acid.

Compound	IC-50 (Mean ± S.D.)[a] (μg/ml)		
	Scavenging of nitric oxide radical	Scavenging of hydrogen peroxide	Lipid peroxidation inhibitory activity
Standard	56 ± 0.087	38 ± 0.121	26 ± 0.333
1a	67 ± 0.121	43 ± 0.024	37 ± 0.183
1b	78 ± 0.318	52 ± 0.318	42 ± 0.453
1c	51 ± 0.058	41 ± 0.087	40 ± 0.121
2a	108 ± 0.78	63 ± 0.279	59 ± 0.066
2b	87 ± 0.082	59 ± 0.333	70 ± 0.024
2c	85 ± 0.162	55 ± 0.453	64 ± 0.121
3a	74 ± 0.081	41 ± 0.066	34 ± 0.162
3b	47 ± 0.052	52 ± 0.279	43 ± 0.333
3c	53 ± 0.066	45 ± 0.087	48 ± 0.183
4a	97 ± 0.453	57 ± 0.318	56 ± 0.066
4b	88 ± 0.183	68 ± 0.121	60 ± 0.318
4c	91 ± 0.318	83 ± 0.318	65 ± 0.045
5a	99 ± 0.453	82 ± 0.066	58 ± 0.087
5b	85 ± 0.045	84 ± 0.162	61 ± 0.162
5c	92 ± 0.024	89 ± 0.087	64 ± 0.453
6a	112 ± 0.333	103 ± 0.024	71 ± 0.279
6b	104 ± 0.279	92 ± 0.121	73 ± 0.318
6c	110 ± 0.318	85 ± 0.183	63 ± 0.087

[a] Average of three determination.

and scavenging of hydrogen peroxide method, while **3b** showed most potent antioxidant activity by scavenging of nitric oxide radical method.

4.2.2. Anti-inflammatory activity

Synthesized compounds were also tested for anti-inflammatory activity. Compared to the standard Diclofenac sodium, they have shown acceptable anti-inflammatory activity. In vitro anti-inflammatory activity of synthesized compounds is summarized in Table 4 and Fig. 6. Among the tested compounds, **3b, 2b, 1b** and **1c** showed better anti-inflammatory activity. Amongst all **3b** found with most potent activity.

5. Conclusion

The synthesized compounds were tested for antioxidant and anti-inflammatory activity. In antioxidant activity **1a–c** and **3a–c** derivatives have shown more promising results. This may be due to the available NH and SH group present in **1a–c** and $NH–NH_2$ present in **3a–c**. Few synthesized compounds have shown good anti-inflammatory action, amongst **3b** has shown excellent activity. Observed good anti-inflammatory activity may be due to 3-OCH_3 group present in structure and may be reduced in trimethoxy compound due to its OCH_3 group at para position. This observation may promote a further development of this group of pyrimidines may lead to compounds with better pharmacological profile than standard antioxidant and anti-inflammatory drugs.

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