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Novel dihydropyrimidines and its pyrazole derivatives: Synthesis and pharmacological screening

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ABSTRACT

In the present study, we have synthesized novel dihydropyrimidines (**1a**–**j**), their dimethylated adducts (**2a**–**j**), and hydrazine derivatives (**3a**–**j**) of **2a**–**j** and subsequently their pyrazole derivatives (**4a**–**j**). Elemental analysis, IR, ^{1}H NMR and mass spectral data elucidated structure of newly synthesized compounds. Some of these novel derivatives showed moderate to potent in vitro antioxidant, anti-inflammatory, antibacterial, antifungal and anthelmintic activity.

1. Introduction

Pyrimidine, being an integral part of DNA and RNA, have imparts diverse pharmacological properties as effective bactericide and fungicide [1–3]. Certain pyrimidine derivatives were also known to exhibit antimalarial [4], antifilarial [5], antioxidant [6,7], anthelmintic [8] and anti-HIV activities [9]. Some of the dihydropyrimidines (DHPM) have emerged as integral backbones of several calcium channel blockers, antihypertensive agents, adrenergic and neuropeptide antagonist [10]. Several alkaloids containing dihydropyrimidine have been isolated from marine sources and among them the *batzelladine* alkaloids were found to be potent HIV-gp-120-CD_4 inhibitors [11–13].

In addition to the diverse biological activities of pyrimidine, other heterocycles in association with pyrimidines play an essential role in several biological processes and have a considerable chemical and pharmacological importance. Pyrimidines in association with pyrazole have occupied prominent place in medicinal chemistry because of their significant properties as therapeutics in clinical application [14–16]. A survey of literature revealed that pyrazole derivatives of pyrimidine have received much attention during recent years on account of their prominent utilization as analgesic, anti-inflammatory, ulcerogenic [17], antibacterial, antifungal [18,19], antitubercular [20], antimalarial [21], antitumor [18,22,23], antioxidant [23], antiproliferative [24], antihypertensive [25], hypnotic [26] and vasodilator [27].

In the view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents, we have synthesized some new dihydropyrimidines **2**–**3** and its pyrazole derivatives **4**. The novel derivatives were characterized by spectral data and elemental analysis and these compounds were tested for their antioxidant, anti-inflammatory, antimicrobial and anthelmintic screening.

The required 6-oxo-4-substituted aryl-2-sulfanyl-1,6-dihydropyrimidine-5-carbonitrile (**1a**–**j**) were prepared in moderate to good yield by using the method of Ramesh et al. [28]. Ethyl 5-amino-1-(5-cyano-1-methyl-6-oxo-4-substituted-1,6-dihydropyrimidin-2-yl)-1*H*-pyrazole-4-carboxylates (**4a**–**j**) were afforded from **1a**–**j** through their dimethylated adducts (**2a**–**j**) and subsequently their hydrazine derivatives (**3a**–**j**) following a three step reaction sequence (Scheme 1).

2. Results and discussion

2.1. Chemistry

In the present work the title compounds were synthesized by the cyclization of three-components like arylaldehydes, thiourea

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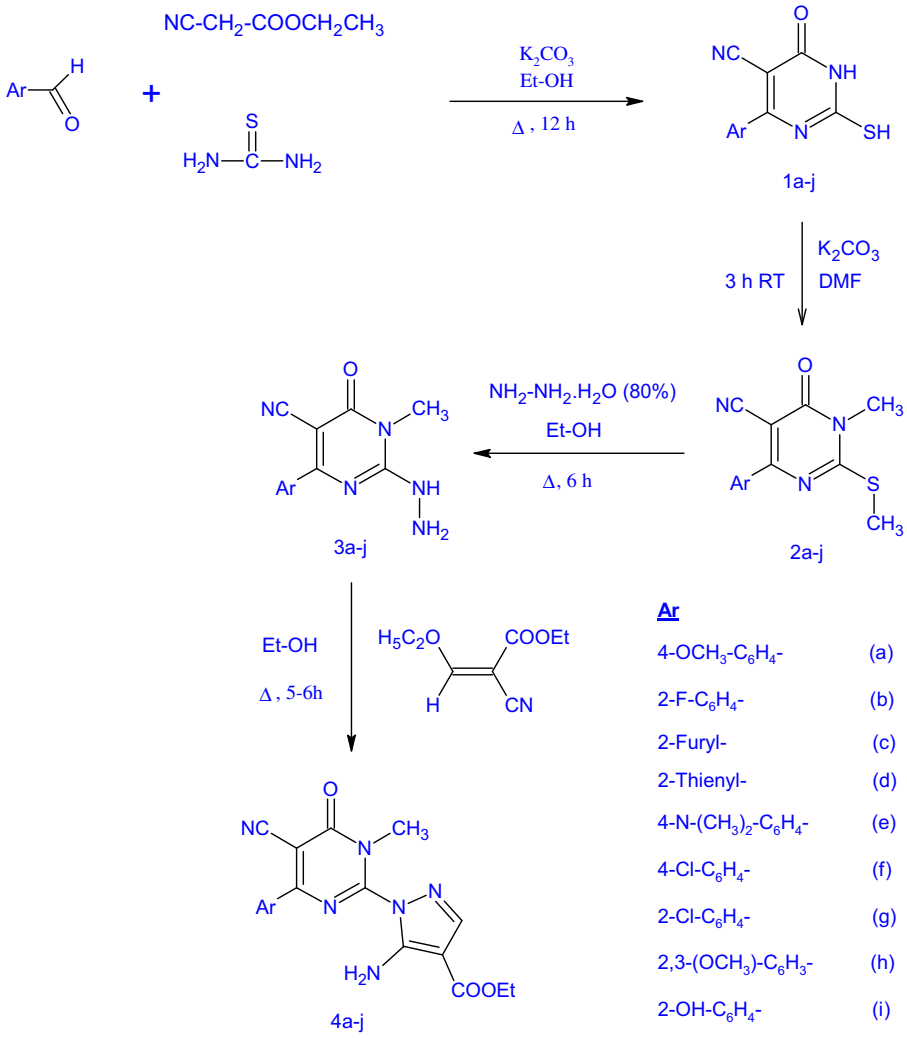

Et-OH = ethanol, DMF = N,N- dimethyl formamide, RT = room temperature

Scheme 1. Schematic representation of synthesis of compounds (**2**–**4**).

and ethyl cyanoacetate in ethanol using potassium carbonate to form 6-oxo-4-substituted aryl-2-sulfanyl-1,6-dihydropyrimidine-5-carbonitriles (**1a**–**j**). While, room temperature stirring of **1a**–**j** with methyl iodide in DMF in presence of potassium carbonate yielded 1-methyl-2-(methylthio)-5-cyano-6-oxo-4-substituted-1,6-dihydropyrimidines (**2a**–**j**). The 2-hydrazino derivatives, 2-hydrazino-1-methyl-5-cyano-6-oxo-4-substituted-1,6-dihydropyrimidines (**3a**–**j**) was obtained by heating **2a**–**j** with hydrazine hydrate (80%) in ethanol. Condensation of latter with ethyl-2-cyano-3-ethyl acrylate in alcohol and in the presence of acetic acid afforded the corresponding ethyl 5-amino-1-(5-cyano-1-methyl-6-oxo-4-substituted-1,6-dihydropyrimidin-2-yl)-1*H*-pyrazole-4-carboxylates (**4a**–**j**) (Scheme 1). The physical and analytical data have been given in Table 1.

The structures assigned to the compounds were substantiated by their analytical and other spectral data. The IR spectra of all the synthesized compounds showed characteristic signals at 2252–2200 cm^{-1} for C≡N, 1740–1633 cm^{-1} for C=O, 1658–1557 cm^{-1} for C=N and 1589–1464 cm^{-1} for C=C. Similarly the ^{1}H NMR spectra showed peaks due to N–CH_3 proton in the range of δ 2.96–4.46. The **2a**–**j** has showed peak in the range of δ 2.28–3.31 for S–CH_3 in the ^{1}H NMR and peak for C–S in the range of 774–758 cm^{-1} in the IR spectra. The hydrazine derivatives (**3a**–**j**) showed peak at 3403–3298 cm^{-1} for the NH–NH_2 in the IR and δ 2.06–2.48 for NH and δ 2.62–2.95 for NH_2 in the ^{1}H NMR spectra. IR spectrum of the compounds **4a**–**j** showed the appearance of absorption bands at 3467–3310 cm^{-1} for the NH_2 and absorption bands at 1772–1672 cm^{-1} for the C=O of ester group, their ^{1}H NMR spectrum showed the presence of characteristic peaks at δ 5.83–6.92 for NH_2, δ 7.46–8.13 for CH of pyrazole, δ 3.26–4.65 for CH_2 of ester and at δ 1.11–1.56 for CH_3 of ester. The mass spectrum of all the compounds showed molecular ion peak at M+1 corresponding to its molecular formula, which confirmed its chemical structure. The IR, ^{1}H NMR, mass spectra and elemental analysis supported the structure of various synthesized pyrimidines and its pyrazole derivatives (Table 2).

2.2. Pharmacological screening

2.2.1. Antioxidant activity

All the synthesized compounds **2**–**4** were screened for their in vitro antioxidant activity by various methods such as scavenging of

Table 1
Physical and analytical characterization of compounds (**2**–**4**).

Compounds	Chemical name	Ar	Yield (%)	M.P. (°C)	Mol. Formula	Rf value
2a	4-(4-Methoxyphenyl)-1-methyl-2-(methylthio)-5-cyno-6-oxo-1,6-dihydropyrimidine	4-$OCH_3 \cdot C_6H_4$	46	197	$C_{14}H_{13}N_3O_2S$	0.56
2b	4-(2-Fluorophenyl)-1-methyl-2-(methylthio)-5-cyno-6-oxo-1,6-dihydropyrimidine	2-F–C_6H_4	51	158	$C_{13}H_{10}FN_3OS$	0.64
2c	4-(2-Furyl)-1-methyl-2-(methylthio)-5 cyno-6-oxo-1,6-dihydropyrimidine	2-Furyl	58	95	$C_{11}H_9N_3O_2S$	0.72
2d	1-Methyl-2-(methylthio)-5 cyno-6-oxo-4-thien-2-yl-1,6-dihydropyrimidine	2-Thienyl	61	256	$C_{11}H_9N_3OS_2$	0.46
2e	4-[4-(Dimethylamino)phenyl]-1-methyl-2-(methylthio)-5-cyno-6-oxo-1,6-dihydropyrimidine	4-N–$(CH_3)_2$–C_6H_4	40	219	$C_{15}H_{16}N_4OS$	0.68
2f	4-(4-Chlorophenyl)-1-methyl-2-(methylthio)-5-cyno-6-oxo-1,6-dihydropyrimidine	4-Cl–C_6H_4	61	209	$C_{13}H_{10}ClN_3OS$	0.39
2g	4-(2-Chlorophenyl)-1-methyl-2-(methylthio)-5-cyno-6-oxo-1,6-dihydropyrimidine	2-Cl–C_6H_4	56	190	$C_{13}H_{10}ClN_3OS$	0.43
2h	4-(3,4-Dimethoxyphenyl)-1-methyl-2-(methylthio)-5-cyno-6-oxo-1,6-dihydropyrimidine	2,3–$(O–CH_3)_2$–C_6H_3	44	115	$C_{15}H_{15}N_3O_3S$	0.52
2i	4-(2-Hydroxyphenyl)-1-methyl-2-(methylthio)-5-cyno-6-oxo-1,6-dihydropyrimidine	2-OH–C_6H_4	48	190	$C_{13}H_{11}N_3O_2S$	0.61
2j	4-(2,4-Dihydroxyphenyl)-1-methyl-2-(methylthio)-5-cyno-6-oxo-1,6-dihydropyrimidines	2,3-$(OH)_2$–C_6H_3	68	205	$C_{13}H_{11}N_3O_3S$	0.49
3a	4-(4-Methoxyphenyl) 2-hydrazino–1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	4-$OCH_3 \cdot C_6H_4$	55	269	$C_{13}H_{13}N_5O_2$	0.58
3b	4-(2-Fluorophenyl)-2-hydrazino-1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	2-F–C_6H_4	59	218	$C_{12}H_{10}FN_5O$	0.45
3c	4-(2-Furyl)-2-hydrazino-1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	2-Furyl	61	S.S. [Ψ]	$C_{10}H_9N_5O_2$	0.60
3d	2-Hydrazino-1-methyl-5-cyno-6-oxo-4-thien-2-yl-1,6-dihydropyrimidine	2-Thienyl	68	277	$C_{10}H_9N_5OS$	0.68
3e	4-[4-(Dimethylamino)phenyl]-2-hydrazino-1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	4-N–$(CH_3)_2$–C_6H_4	69	222	$C_{14}H_{16}N_6O$	0.56
3f	4-(4-Chlorophenyl)-2-hydrazino-1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	4-Cl–C_6H_4	51	260	$C_{12}H_{10}ClN_5O$	0.69
3g	4-(2-Chlorophenyl)-2-hydrazino-1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	2-Cl–C_6H_4	63	118	$C_{12}H_{10}ClN_5O$	0.48
3h	4-(3,4-Dimethoxyphenyl)-2-hydrazino-1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	2,3-$(O–CH_3)_2$–C_6H_3	69	S.S.[Ψ]	$C_{14}H_{15}N_5O_3$	0.53
3i	2-Hydrazino-4-(2-hydroxyphenyl)-1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	2-OH–C_6H_4	51	155	$C_{12}H_{11}N_5O_2$	0.63
3j	4-(2,4-Dihydroxyphenyl)-2-hydrazino-1-methyl-5-cyno-6-oxo-1,6-dihydropyrimidine	2,3-$(OH)_2$–C_6H_3	54	248	$C_{12}H_{11}N_5O_3$	0.70
4a	5-Amino-4-etheylcorboxylaty -1-[5-cyano-4-(4-methoxyphenyl)-1-methyl-6-oxo-1, 6-dihydropyrimidin-2-yl]-1*H*-pyrazole	4-$OCH_3 \cdot C_6H_4$	55	210	$C_{19}H_{18}N_6O_4$	0.59
4b	5-Amino-4-etheylcorboxylaty -1-[5-cyano-4-(-methoxyphenyl)-1-methyl-6-oxo-1, 6-dihydropyrimidin-2-yl]-1*H*-pyrazole	2-F–C_6H_4	44	90	$C_{18}H_{15}FN_6O_3$	0.48
4c	Ethyl 5-amino-1-[5-cyano-4-(2-furyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]-1 *H*-pyrazole-4-carboxylate	2-Furyl	49	192	$C_{16}H_{14}N_6O_4$	0.64
4d	Ethyl 5-amino-1-(5-cyano-1-methyl-6-oxo-4-thien-2-yl-1,6-dihydropyrimidin-2-yl)-1 *H*-pyrazole-4-carboxylate	2-Thienyl	56	210	$C_{16}H_{14}N_6O_3S$	0.44
4e	5-Amino-1-[5-cyano-4-(4-dimethylamino-phenyl)-1-methyl-6-oxo-1,6-dihydro-pyrimidin-2-yl]-1 H-pyrazole-4-carboxylic acid ethyl ester	4-N–$(CH_3)_2$–C_6H_4	49	295	$C_{20}H_{21}N_7O_3$	0.55
4f	Ethyl 5-amino-1-[4-(4-chlorophenyl)-5-cyano-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]-1 *H*-pyrazole-4-carboxylate	4-Cl–C_6H_4	60	240	$C_{18}H_{15}ClN_6O_3$	0.69
4g	Ethyl 5-amino-1-[4-(2-chlorophenyl)-5-cyano-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]-1 *H*-pyrazole-4-carboxylate	2-Cl–C_6H_4	44	90	$C_{18}H_{15}ClN_6O_3$	0.64
4h	5-Amino-1-[5-cyano-4-(3,4-dimethoxy-phenyl)-1-methyl-6-oxo-1,6-dihydro-pyrimidin-2-yl]-1 *H*-pyrazole-4-carboxylic acid ethyl ester	2,3–$(O–CH_3)_2$–C_6H_3	48	80	$C_{20}H_{20}N_6O_5$	0.43
4i	Ethyl 5-amino-1-[5-cyano-4-(2-hydroxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]-1 *H*-pyrazole-4-carboxylate	2–OH–C_6H_4	59	162	$C_{18}H_{16}N_6O_4$	0.58
4j	Ethyl 5-amino-1-[5-cyano-4-(2,4-dihydroxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]-1 *H*-pyrazole-4-carboxylate	2,3-$(OH)_2$–C_6H_3	42	183	$C_{18}H_{16}N_6O_5$	0.41

[Ψ] S.S. = semisolid.

Table 2
Spectral characterization and elemental analysis of compounds (**2**–**4**).

Compounds	IR (KBr) ν (cm^{-1})	^1H NMR DMSO-δ (ppm)	Mass *m/z*	% Calculated (Found)		
				C	H	N
2a	2227 (C≡N), 1723 (C=O), 1625 (C=N), 1589 (C=C), 1250 (C–N), 769 (C–S), 1263 (C–O–C)	7.18–8.07 (m, 4H, Ar–H), 2.72 (s, 3H, S–CH_3), 3.46 (s, 3H, N–CH_3), 3.87 (s, 3H, O–CH_3)	288.3 M + 1	58.52 (58.53)	4.56 (4.51)	14.62 (14.61)
2b	2252 (C≡N), 1689 (C=O), 1613 (C=N), 1508 (C=C), 1358 (C–N), 766 (C–S), 1018 (C–F)	6.98–7.68 (m, 4H, Ar–H), 2.58 (s, 3H, S–CH_3), 3.32 (s, 3H, N–CH_3)	276.3 M + 1	56.72 (56.71)	3.66 (3.62)	15.26 (15.21)
2c	2216 (C≡N), 1740 (C=O), 1609 (C=N), 1546 (C=C), 1317 (C–N), 765 (C–S), 1255 (C–O–C)	6.96–8.18 (m, 3H, Furan), 3.31(s, 3H, S–CH_3), 4.24 (s, 3H, N–CH_3)	248.3 M + 1	53.43 (53.41)	3.67 (3.63)	16.99 (16.91)
2d	2232 (C≡N), 1714 (C=O), 1617 (C=N), 1544 (C=C), 1340 (C–N), 762 (C–S of S–CH_3), 782 (C–S of Thiophene)	7.20–8.13 (m, 3H, Thiophene), 2.77 (s, 3H, S–CH_3), 4.46 (s, 3H, N–CH_3)	264.4 M + 1	50.17 (50.13)	3.44 (3.41)	15.96 (15.93)
2e	2223 (C≡N), 1716 (C=O), 1606 (C=N), 1568 (C=C), 1354 (C–N), 759 (C–S), 1338 (dimethyl amino)	7.62–8.04 (m, 4H, Ar–H), 2.96 (s, 3H, S–CH_3), 4.22 [s, 6H, N–$(CH_3)_2$=, 3.38 (s, 3H, N–CH_3),	301.3 M + 1	59.98 (59.93)	5.37 (5.33)	18.65 (18.61)
2f	2212 (C≡N), 1694 (C=O), 1610 (C=N), 1538 (C=C), 1352 (C–N), 760 (C–S), 770 (C–Cl)	7.18–7.42 (m, 4H, Ar–H), 3.06 (s, 3H, S–CH_3), 3.96 (s, 3H, N–CH_3)	292.6 M + 1	53.52 (53.51)	3.45 (3.42)	14.40 (14.42)
2g	2222 (C≡N), 1715 (C=O), 1628 (C=N), 1552 (C=C), 1326 (C–N), 758 (C–S),776 (C–Cl)	6.96–7.57 (m, 4H, Ar–H), 2.87 (s, 3H, S–CH_3), 3.76 (s, 3H, N–CH_3)	292.5 M + 1	53.52 (53.51)	3.45 (3.42)	14.40 (14.41)
2h	2225 (C≡N), 1704 (C=O), 1618 (C=N), 1528 (C=C), 1354 (C–N), 760 (C–S), 1214 (C–O–C)	6.78–7.35 (m, 3H, Ar–H), 2.28 (s, 1H, S–CH_3), 2.92 (s, 1H, N–CH_3), 3.68 (s, 6H, 2.O–CH_3)	318.4 M + 1	56.77 (56.72)	4.76 (4.73)	13.24 (13.21)
2i	2219 (C≡N), 1714 (C=O), 1616 (C=N), 1538 (C=C), 1314 (C–N), 774 (C–S), 3412 (OH)	7.04–7.72 (m, 4H, Ar–H), 2.96 (s, 3H, S–CH_3), 4.22 (s, 3H, N–CH_3), 5.72 (s, 1H, OH)	274.3 M + 1	57.13 (57.12)	4.06 (4.10)	15.37 (15.31)
2j	2236 (C≡N), 1722 (C=O), 1632 (C=N), 1555 (C=C), 1316 (C–N), 765 (C–S), 3466 (2.OH)	6.28–7.27 (m, 3H, Ar–H), 3.11 (s, 3H, S–CH_3), 3.88 (s, 3H, N–CH_3), 5.21 (s, 2H, 2.OH)	290.3 M + 1	53.97 (53.93)	3.83 (3.79)	14.52 (14.51)
3a	3325 (NH–NH_2), 2225 (C≡N), 1738 (C=O), 1618 (C=N), 1532 (C=C), 1221 (C–O–C)	7.05–7.74 (m, 4H, Ar–H), 3.24 (s, 3H, N–CH_3), 4.17 (s, 3H, O–CH_3), 2.21 (s, 1H, NH), 2.62(s, 2H, NH_2)	272.3 M + 1	57.56 (57.51)	4.83 (4.81)	25.82 (25.80)
3b	3300 (NH–NH_2), 2200 (C≡N), 1690 (C=O), 1600 (C=N), 1500 (C=C), 1005 (C–F)	7.24–7.74 (m, 4H, Ar–H), 3.35 (s, 3H, N–CH_3), 2.42 (s, 1H, NH), 2.84(s, 2H, NH_2)	260.3 M + 1	55.60 (55.51)	3.89 (3.91)	27.02 (27.03)
3c	3348 (NH–NH_2), 2218 (C≡N), 1728 (C=O), 1658 (C=N), 1554 (C=C), 1217 (C–O–C)	6.68–7.56 (m, 3H, Furan), 3.22 (s, 3H, N–CH_3), 2.23 (s, 1H, NH), 2.65 (s, 2H, NH_2)	232.3 M + 1	51.95 (51.91)	3.92 (3.91)	30.29 (30.21)
3d	3298 (NH–NH_2), 2212 (C≡N), 1658 (C=O), 1606 (C=N), 1553 (C=C), 716 (C–S)	7.34–8.08 (m, 3H, Thiophene), 3.24 (s, 3H, N–CH_3), 2.48 (s, 1H, NH), 2.78(s, 2H, NH_2)	248.2 M + 1	48.57 (48.51)	3.67 (3.71)	28.32 (28.31)
3e	3327 (NH–NH_2), 2222 (C≡N), 1657 (C=O), 1608 (C=N), 1510 (C=C), 1350 (dimethyl amino)	6.84–7.52 (m, 4H, Ar–H), 3.32 (s, 3H, N–CH_3), 3.84 [s, 6H, N–$(CH_3)_2$=,2.28 (s, 1H, NH), 2.62(s, 2H, NH_2)	288.3 M + 1	59.14 (59.13)	5.67 (5.62)	29.56 (29.51)
3f	3313 (NH–NH_2), 2218 (C≡N), 1678 (C=O), 1610 (C=N), 1537 (C=C), 710 (C–Cl)	7.21–7.46 (m, 4H, Ar–H), 4.06 (s, 3H, N–CH_3), 2.34 (s, 1H, NH), 2.88 (s, 2H, NH_2)	276.4 M + 1	52.28 (52.10)	3.66 (3.62)	25.40 (25.32)
3g	3337 (NH–NH_2), 2226 (C≡N), 1712 (C=O), 1654 (C=N), 1574 (C=C), 718 (C–Cl)	7.11–7.53 (m, 4H, Ar–H), 4.42 (s, 3H, N–CH_3), 2.32 (s, 1H, NH), 2.95 (s, 2H, NH_2)	276.4 M + 1	52.28 (52.21)	3.66 (3.63)	25.40 (25.41)
3h	3403 (NH–NH_2), 2237 (C≡N), 1724 (C=O), 1646 (C=N), 1578 (C=C), 1232 (C–O–C)	6.92–7.26 (m, 3H, Ar–H), 2.96 (s, 1H, N–CH_3), 4.16 (s, 6H, 2.O–CH_3), 2.18 (s, 1H, NH), 2.68 (s, 2H, NH_2)	302.4 M + 1	55.81 (55.72)	5.02 (5.07)	23.24 (23.21)
3i	3342 (NH–NH_2), 2214 (C≡N), 1633 (C=O), 1557 (C=N), 1508 (C=C), 3405 (OH)	6.75–7.68 (m, 4H, Ar–H), 3.38 (s, 3H, N–CH_3), 5.42 (s, 1H, OH), 2.33 (s, 1H, NH), 2.78 (s, 2H, NH_2)	290.3 M + 1	56.03 (56.08)	4.31 (4.32)	27.22 (27.21)
3j	3368 (NH–NH_2), 2220 (C≡N), 1635 (C=O), 1578 (C=N), 1512 (C=C), 3450 (2.OH)	6.65–7.34 (m, 3H, Ar–H), 2.96 (s, 3H, N–CH_3), 5.22 (s, 2H, 2.OH), 2.06 (s, 1H, NH), 2.84 (s, 2H, NH_2)	274.3 M + 1	52.75 (52.77)	4.06 (4.07)	25.63 (25.61)
4a	3452 (NH), 2228 (C≡N), 1722 (C=O, ester), 1648 (C=O, pyrimidine), 1578 (C=N), 1520 (C=C), 1218 (C–O–C)	7.28–7.77 (m, 4H, Ar–H), 3.46 (s, 3H, N–CH_3), 3.82 (s, 3H, O–CH_3), 6.92 (s, 2H, NH_2), 7.96 (s, 1H, CH of pyrazole), 4.38 (q, 2H, CH_2 of ester), 1.28 (t, 3H, CH_3 of ester)	395.3 M + 1	57.86 (57.91)	4.60 (4.58)	21.31 (21.33)
4b	3425 (NH), 2208 (C≡N), 1710 (C=O, ester), 1650 (C=O, pyrimidine), 1606 (C=N), 1548 (C=C), 1010 (C–F)	7.32–7.65 (m, 4H, Ar–H), 3.18 (s, 3H, N–CH_3), 5.83 (s, 2H, NH_2), 7.89 (s, 1H, CH of pyrazole), 3.96 (q, 2H, CH_2 of ester), 1.11 (t, 3H, CH_3 of ester)	383.3 M + 1	56.54 (56.52)	3.95 (3.92)	21.98 (21.91)
4c	3344 (NH), 2214 (C≡N), 1687 (C=O, ester), 1619 (C=O, pyrimidine), 1600 (C=N), 1464 (C=C), 1290 (C–O–C)	6.73–7.45 (m, 3H, Furan), 2.99 (s, 3H, N–CH_3), 6.38 (s, 2H, NH_2), 7.72 (s, 1H, CH of pyrazole), 4.16 (q, 2H, CH_2 of ester), 1.31 (t, 3H, CH_3 of ester)	355.4 M + 1	54.24 (54.19)	3.98 (4.01)	23.72 (23.61)
4d	3467 (NH), 2218 (C≡N), 1702 (C=O, ester), 1682 (C=O, pyrimidine), 1637 (C=N), 1545 (C=C), 720 (C–S)	7.11–7.38 (m, 3H, Thiophene), 3.22 (s, 3H, N–CH_3), 6.13 (s, 2H, NH_2), 8.07 (s, 1H, CH of pyrazole), 4.65 (q, 2H, CH_2 of ester), 1.24 (t, 3H, CH_3 of ester)	371.4 M + 1	51.88 (51.82)	3.81 (3.84)	22.69 (22.61)
4e	3450 (NH), 2219 (C≡N), 1772 (C=O, ester), 1679 (C=O, pyrimidine), 1623 (C=N), 1528 (C=C), 1340 (dimethyl amino)	6.68–7.27 (m, 4H, Ar–H), 3.07 (s, 3H, N–CH_3), 3.72 [s, 6H, N–$(CH_3)_2$=, 5.95 (s, 2H, NH_2), 7.68 (s, 1H, CH of pyrazole), 3.97 (q, 2H, CH_2 of ester), 1.18 (t, 3H, CH_3 of ester)	408.3 M + 1	58.96 (58.99)	5.20 (5.25)	24.07 (24.08)
4f	3460 (NH), 2225 (C≡N), 1672 (C=O, ester), 1609 (C=O, pyrimidine), 1594 (C=N), 1561 (C=C), 710 (C–Cl)	7.18–7.32 (m, 4H, Ar–H), 3.33 (s, 3H, N–CH_3), 6.38 (s, 2H, NH_2), 7.59 (s, 1H, CH of pyrazole), 3.86 (q, 2H, CH_2 of ester), 1.45 (t, 3H, CH_3 of ester)	399.7 M + 1	54.21 (54.26)	3.79 (3.82)	21.07 (21.01)
4g	3378 (NH), 2212 (C≡N), 1732 (C=O, ester), 1648 (C=O, pyrimidine), 1575 (C=N), 1510 (C=C), 714 (C–Cl)	7.26–7.63 (m, 4H, Ar–H), 3.54 (s, 3H, N–CH_3), 6.23 (s, 2H, NH_2), 8.13 (s, 1H, CH of pyrazole), 3.72 (q, 2H, CH_2 of ester), 1.39 (t, 3H, CH_3 of ester)	308.7 M + 1	54.21 (54.26)	3.79 (3.81)	21.07 (21.10)
4h	3408 (NH), 2232 (C≡N), 1726 (C=O, ester), 1669 (C=O, pyrimidine), 1576 (C=N), 1520 (C=C), 1224 (C–O–C)	6.74–7.17 (m, 3H, Ar–H), 2.89 (s, 1H, N–CH_3), 3.73 (s, 6H, 2.O–CH_3), 5.87 (s, 2H, NH_2), 7.52 (s, 1H, CH of pyrazole), 4.21 (q, 2H, CH_2 of ester), 1.38 (t, 3H, CH_3 of ester)	425.3 M + 1	56.60 (56.56)	4.75 (4.76)	19.80 (19.81)
4i	3310 (NH), 2220 (C≡N), 1700 (C=O, ester), 1650 (C=O, pyrimidine), 1600 (C=N), 1520 (C=C), 3300 (OH)	6.56–7.08 (m, 4H, Ar–H), 3.07 (s, 3H, N–CH_3), 5.18 (s, 1H, OH), 6.12 (s, 2H, NH_2), 7.46 (s, 1H, CH of pyrazole), 3.85 (q, 2H, CH_2 of ester), 1.56 (t, 3H, CH_3 of ester)	381.3 M + 1	56.84 (56.80)	4.24 (4.23)	22.10 (22.11)
4j	3334 (NH), 2196 (C≡N), 1677 (C=O, ester), 1624 (C=O, pyrimidine), 1561 (C=N), 1543 (C=C), 3350 (2.OH)	6.43–7.48 (m, 3H, Ar–H), 2.82 (s, 3H, N–CH_3), 5.01 (s, 2H, 2.OH), 6.28 (s, 2H, NH_2), 7.74 (s, 1H, CH of pyrazole), 3.26 (q, 2H, CH_2 of ester), 1.38 (t, 3H, CH_3 of ester)	397.3 M + 1	54.54 (54.50)	4.07 (4.11)	21.20 (21.19)

hydrogen peroxide, scavenging of nitric oxide radical, lipid peroxidation inhibitory activity and reducing power determination. 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, hydrazine derivatives (**3a**–**j**) showed more promising antioxidant activity as compared to that of standard, ascorbic acid. This could be due the availability of free hydrazine group. In scavenging of nitric oxide radical techniques compounds **3j** and **3i** shown low IC_{50} value than the standard. While, **3j**, **3i** and **3a** has shown more potent activity by scavenging of hydrogen peroxide. This may be due to additional OH group present on benzene ring in the structure. All the compounds showed higher IC_{50} value than the standard by lipid peroxidation inhibitory activity and reducing power determination. Derivatives with OH group on benzene ring having good antioxidant activity compared with the other compounds in their series. **2j**, **3h**, **3a**, **2i**, **4j**, **3f**, **3b**, **3g** and **3d** having moderate to good antioxidant activity.

2.2.2. Anti-inflammatory activity

All of the newly obtained compounds **2**–**4** were tested for in vitro anti-inflammatory activity. Compared to the standard, diclofenac sodium, they have shown acceptable anti-inflammatory activity. In vitro anti-inflammatory activity of compounds is summarized in Table 4. The results revealed that the compounds, **2f**, **4g**, **2g** and **2b** exhibited moderate anti-inflammatory activities. Amongst all the tested compounds **2f** found to be more potent. The compounds **4f**, **2d**, **3f**, **4j**, **2c**, **4i**, **3g** and **2i** have showed good activity. While other having weak to moderate activities.

Table 3
Antioxidant activity (IC_{50} values) of compounds (**2**–**4**).

Compounds	IC_{50} (Mean ± S.D.) µg/mL			
	Scavenging of nitric oxide radical	Scavenging of hydrogen peroxide	Lipid peroxidation inhibitory activity	Reducing power determination
2a	73 ± 0.087	48 ± 0.121	53 ± 0.333	58 ± 0.333
2b	78 ± 0.121	63 ± 0.024	58 ± 0.183	61 ± 0.183
2c	108 ± 0.318	79 ± 0.318	90 ± 0.453	68 ± 0.453
2d	87 ± 0.058	72 ± 0.087	83 ± 0.121	71 ± 0.121
2e	93 ± 0.780	84 ± 0.279	98 ± 0.066	78 ± 0.066
2f	71 ± 0.082	56 ± 0.333	54 ± 0.024	64 ± 0.024
2g	81 ± 0.162	67 ± 0.453	72 ± 0.121	76 ± 0.121
2h	67 ± 0.082	43 ± 0.066	48 ± 0.162	54 ± 0.162
2i	58 ± 0.162	39 ± 0.279	44 ± 0.333	52 ± 0.333
2j	53 ± 0.082	37 ± 0.087	41 ± 0.183	48 ± 0.183
3a	56 ± 0.052	32 ± 0.318	39 ± 0.066	41 ± 0.066
3b	60 ± 0.066	49 ± 0.121	43 ± 0.318	50 ± 0.318
3c	65 ± 0.453	58 ± 0.318	78 ± 0.045	64 ± 0.045
3d	63 ± 0.183	53 ± 0.066	68 ± 0.087	61 ± 0.087
3e	69 ± 0.318	66 ± 0.162	63 ± 0.162	81 ± 0.162
3f	59 ± 0.453	41 ± 0.087	46 ± 0.453	44 ± 0.453
3g	62 ± 0.045	46 ± 0.024	57 ± 0.279	58 ± 0.279
3h	54 ± 0.024	36 ± 0.121	37 ± 0.318	44 ± 0.318
3i	46 ± 0.333	30 ± 0.183	34 ± 0.087	38 ± 0.087
3j	44 ± 0.279	29 ± 0.087	31 ± 0.121	34 ± 0.024
4a	79 ± 0.318	74 ± 0.333	68 ± 0.121	59 ± 0.087
4b	87 ± 0.183	86 ± 0.082	94 ± 0.024	69 ± 0.453
4c	108 ± 0.318	104 ± 0.333	116 ± 0.183	91 ± 0.333
4d	98 ± 0.058	109 ± 0.024	97 ± 0.318	79 ± 0.024
4e	115 ± 0.780	119 ± 0.162	111 ± 0.045	98 ± 0.279
4f	87 ± 0.279	94 ± 0.453	88 ± 0.453	64 ± 0.121
4g	93 ± 0.066	98 ± 0.121	102 ± 0.066	78 ± 0.318
4h	69 ± 0.453	67 ± 0.453	57 ± 0.087	54 ± 0.318
4i	64 ± 0.066	58 ± 0.162	57 ± 0.045	48 ± 0.183
4j	58 ± 0.087	53 ± 0.279	49 ± 0.162	41 ± 0.087
Standard	47 ± 0.087	33 ± 0.121	26 ± 0.333	31 ± 0.183

S.D. = standard deviation (Average of three determination), Standard = Ascorbic acid.

Table 4
Anti-inflammatory activity (in vitro) of compounds (**2**–**4**).

Compounds	Mean Absorbance ± S.D.	% Inhibition of denaturation
Control	0.1980 ± 0.024	
2a	0.2401 ± 0.001	21.26
2b	0.3353 ± 0.008	69.34
2c	0.3112 ± 0.020	57.17
2d	0.3210 ± 0.010	62.12
2e	0.2363 ± 0.025	19.34
2f	0.3547 ± 0.004	79.14
2g	0.3409 ± 0.026	72.17
2h	0.2736 ± 0.046	38.18
2i	0.3008 ± 0.007	51.92
2j	0.2853 ± 0.016	44.09
3a	0.2387 ± 0.014	20.56
3b	0.2814 ± 0.019	42.12
3c	0.2626 ± 0.046	36.63
3d	0.2700 ± 0.005	36.36
3e	0.2229 ± 0.006	12.58
3f	0.3191 ± 0.009	61.16
3g	0.3040 ± 0.020	53.54
3h	0.2467 ± 0.018	24.60
3i	0.2661 ± 0.024	34.39
3j	0.2550 ± 0.008	28.79
4a	0.2250 ± 0.026	13.64
4b	0.2949 ± 0.016	48.94
4c	0.2816 ± 0.020	42.22
4d	0.2864 ± 0.009	44.65
4e	0.2384 ± 0.014	20.40
4f	0.3260 ± 0.024	64.64
4g	0.3450 ± 0.003	74.24
4h	0.2651 ± 0.025	33.89
4i	0.3058 ± 0.026	54.44
4j	0.3121 ± 0.016	57.63
Standard	0.3630 ± 0.003	83.33

S.D. = standard deviation (Average of three determination), Standard = Diclofenac sodium.

2.2.3. Antimicrobial activity

The antimicrobial activities of the compounds **2**–**4** were tested against *Escheria coli*, *Pseudonmonas aeruginosa* (gram-negative bacteria), *Bacillus subtillis* and *Staphylococcus aureus* (gram-positive bacteria) and two fungi *Candida albicans* and *Aspergillus niger* and the results were reported as zone of inhibition. The results of preliminary antibacterial testing of compounds **2**–**4** are shown in Table 5. The results revealed that, all the pyrazole derivatives of pyrimidines (**4a**–**j**) were showing good to potent antibacterial activity against all the tested strains of bacteria. While the entire derivatives showed moderate to potent activity against *Bacillus subtilis*. Amongst all the derivatives in series i.e. **2**, **3** and **4**, the halogenated derivatives exhibited potent antibacterial activity. As compare to standard streptomycin, compounds **4b**, **4f** and **4g** exhibited good to potent activity against all the tested strains. While compounds **4i**, **4j**, **4c**, **4d**, **4e** and **4h** were moderately active. Compound **2b** is the most potent against *B. subtilis*. While compounds **2g**, **2f**, **2j**, **2i** and **3b** exhibited moderate to good activity against *B. subtilis*. All the above data may reveal that the pyrazole ring is responsible for the good antibacterial activity. While the pyrimidine ring may responsible for the good activity against *B. subtilis*. The halogenated substituent on benzene ring in the structure of the synthesized compounds revealed in the increased antibacterial activity. Moreover, the other compounds were weakly active against the tested organism.

The results of preliminary antifungal testing of the compounds **2**–**4** are shown in Table 6. All the compounds exhibited moderate to good antifungal activity. Compounds **3g** and **2c** exhibited potent

Table 5
Antibacterial activity of the compounds[a](**2**–**4**).

Compounds	*Escheria coli*			*Pseudomonas aeruginosa*			*Bacillus subtilis*			*Staphylococcus aureus*		
	25 μg mL^{-1} ±S.D.	50 μg mL^{-1} ±S.D.	100 μg mL^{-1} ±S.D.	25 μg mL^{-1} ±S.D.	50 μg mL^{-1} ±S.D.	100 μg mL^{-1} ±S.D.	25 μg mL^{-1} ±S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ±S.D.	25 μg mL^{-1} ±S.D.	50 μg mL^{-1} ±S.D.	100 μg mL^{-1} ±S.D.
2a	5.08 ± 0.26	7.19 ± 0.58	8.79 ± 0.45	6.19 ± 0.26	8.84 ± 1.16	9.17 ± 2.09	11.67 ± 0.61	13.1 ± 0.58	19.11 ± 1.53	8.76 ± 0.58	12.80 ± 0.17	14.84 ± 0.58
2b	10.86 ± 1.16	13.78 ± 0.58	14.23 ± 0.58	9.17 ± 1.16	10.39 ± 0.26	11.84 ± 0.58	16.90 ± 0.45	20.16 ± 0.61	29.39 ± 1.16	12.16 ± 0.47	15.33 ± 0.58	20.92 ± 0.61
2c	5.74 ± 1.16	6.12 ± 1.53	7.82 ± 2.09	–	–	–	11.12 ± 0.26	12.23 ± 1.53	15.59 ± 1.53	7.06 ± 1.16	9.12 ± 2.09	12.55 ± 1.53
2d	5.86 ± 1.73	6.29 ± 2.52	8.78 ± 2.65	–	–	–	10.36 ± 0.47	12.90 ± 1.16	18.52 ± 1.53	6.29 ± 0.45	8.92 ± 0.61	11.10 ± 0.37
2e	–	–	–	6.06 ± 0.58	8.52 ± 0.47	10.63 ± 0.61	10.26 ± 1.71	13.95 ± 0.61	20.26 ± 0.45	6.38 ± 0.58	8.25 ± 0.17	9.38 ± 1.16
2f	7.08 ± 1.16	9.82 ± 0.58	11.65 ± 1.53	8.85 ± 1.16	9.62 ± 0.61	11.95 ± 0.58	13.76 ± 2.09	18.13 ± 0.58	27.29 ± 2.89	9.68 ± 0.26	11.68 ± 0.58	15.76 ± 2.09
2g	9.64 ± 0.58	10.21 ± 0.58	12.39 ± 1.53	8.52 ± 1.16	10.37 ± 1.53	12.02 ± 0.58	14.16 ± 1.16	19.33 ± 0.58	28.80 ± 1.73	9.40 ± 0.58	12.32 ± 0.61	16.79 ± 0.26
2h	–	–	–	5.86 ± 0.26	8.25 ± 0.58	9.93 ± 1.16	10.38 ± 1.16	14.36 ± 1.73	20.27 ± 0.45	–	–	–
2i	6.25 ± 1.16	9.16 ± 0.47	11.27 ± 0.00	6.36 ± 0.45	8.16 ± 0.58	10.79 ± 1.16	12.85 ± 2.09	16.88 ± 0.45	25.49 ± 1.53	8.14 ± 0.17	9.33 ± 1.16	11.18 ± 0.26
2j	7.28 ± 0.58	8.78 ± 1.16	10.39 ± 2.00	7.67 ± 0.58	8.12 ± 1.16	12.68 ± 2.09	13.04 ± 1.53	15.82 ± 0.45	24.37 ± 1.71	8.70 ± 0.61	10.60 ± 0.58	13.61 ± 0.26
3a	6.28 ± 0.58	7.71 ± 1.16	7.49 ± 0.58	5.00 ± 1.00	6.96 ± 1.53	7.21 ± 0.54	9.26 ± 1.53	10.33 ± 1.16	13.62 ± 1.53	–	–	–
3b	9.85 ± 0.26	13.48 ± 0.58	15.35 ± 1.16	9.00 ± 2.09	13.85 ± 0.17	15.39 ± 0.58	13.12 ± 1.16	15.66 ± 0.58	21.62 ± 1.16	13.58 ± 0.58	17.30 ± 1.16	22.18 ± 0.61
3c	–	–	–	–	–	–	10.84 ± 1.53	13.33 ± 0.58	14.19 ± 1.16	8.02 ± 0.58	10.52 ± 1.16	12.21 ± 0.58
3d	–	–	–	–	–	–	9.27 ± 1.16	10.10 ± 0.17	15.37 ± 1.53	9.12 ± 0.26	10.61 ± 0.58	13.38 ± 0.26
3e	5.74 ± 0.17	7.82 ± 0.58	8.28 ± 1.16	5.78 ± 1.16	6.27 ± 0.58	8.83 ± 1.73	8.10 ± 1.16	10.48 ± 0.58	14.48 ± 2.62	–	–	–
3f	8.18 ± 2.09	10.95 ± 2.00	12.42 ± 0.58	7.64 ± 1.16	10.18 ± 2.09	14.90 ± 0.58	10.15 ± 1.53	15.98 ± 0.17	24.15 ± 0.63	11.19 ± 0.58	15.53 ± 1.16	20.60 ± 1.16
3g	9.26 ± 0.45	10.11 ± 0.58	13.10 ± 1.16	8.41 ± 0.26	12.65 ± 1.16	15.36 ± 0.58	11.06 ± 1.16	15.10 ± 1.53	22.68 ± 1.53	12.82 ± 0.58	15.94 ± 0.63	19.32 ± 0.26
3h	7.38 ± 0.47	7.16 ± 0.58	8.12 ± 1.16	6.15 ± 1.16	8.12 ± 0.17	9.15 ± 1.73	9.90 ± 1.16	12.24 ± 0.17	16.67 ± 0.47	8.72 ± 0.58	11.15 ± 1.76	15.75 ± 0.26
3i	8.39 ± 0.61	9.58 ± 1.16	11.22 ± 2.09	7.27 ± 1.16	9.17 ± 1.53	13.04 ± 0.58	9.81 ± 1.53	13.78 ± 0.58	22.21 ± 0.63	10.64 ± 0.26	12.24 ± 1.16	17.68 ± 0.45
3j	8.72 ± 0.26	8.16 ± 0.58	10.18 ± 1.16	8.10 ± 0.45	10.10 ± 0.58	12.92 ± 0.61	11.48 ± 1.16	14.49 ± 2.09	22.13 ± 1.53	–	–	–
4a	10.48 ± 0.47	14.72 ± 0.58	17.39 ± 1.16	8.21 ± 2.09	10.23 ± 0.58	16.12 ± 1.73	10.19 ± 1.16	14.04 ± 0.58	18.14 ± 1.16	10.12 ± 0.37	15.05 ± 0.26	18.65 ± 1.53
4b	15.10 ± 2.09	22.28 ± 1.16	27.49 ± 0.47	12.67 ± 0.26	16.06 ± 0.45	26.25 ± 0.61	14.78 ± 1.53	19.26 ± 0.58	28.00 ± 1.16	15.76 ± 2.09	20.84 ± 0.45	26.67 ± 1.16
4c	12.96 ± 0.17	14.18 ± 0.45	18.04 ± 1.16	9.19 ± 1.16	12.12 ± 2.09	19.85 ± 0.26	12.92 ± 1.16	14.11 ± 1.16	18.30 ± 1.53	10.92 ± 0.45	14.10 ± 1.16	17.71 ± 1.53
4d	12.54 ± 1.71	13.12 ± 0.58	16.11 ± 1.16	9.67 ± 1.53	12.72 ± 0.58	18.82 ± 1.73	12.88 ± 1.16	15.05 ± 0.58	18.67 ± 0.61	11.82 ± 0.17	12.18 ± 1.71	16.49 ± 0.61
4e	11.10 ± 0.58	10.18 ± 0.47	14.03 ± 0.17	8.29 ± 1.16	11.92 ± 0.26	16.73 ± 0.58	11.21 ± 1.53	14.82 ± 0.47	20.90 ± 0.47	10.12 ± 0.58	13.48 ± 0.58	16.52 ± 0.58
4f	14.72 ± 0.45	19.48 ± 1.71	25.02 ± 1.16	11.79 ± 0.61	17.12 ± 2.09	25.67 ± 0.26	14.18 ± 2.09	16.71 ± 1.16	25.39 ± 1.53	13.07 ± 0.63	17.06 ± 1.16	22.00 ± 0.47
4g	13.16 ± 0.61	20.48 ± 0.58	26.90 ± 1.16	12.15 ± 1.16	16.31 ± 0.58	23.88 ± 1.73	13.42 ± 1.16	17.16 ± 0.58	26.18 ± 2.00	14.60 ± 2.09	18.26 ± 0.61	24.32 ± 0.58
4h	10.26 ± 0.26	12.36 ± 0.47	16.18 ± 0.61	9.90 ± 1.16	12.26 ± 0.58	17.42 ± 0.58	11.62 ± 1.53	15.10 ± 0.45	19.25 ± 1.16	10.33 ± 0.58	14.10 ± 0.58	18.67 ± 0.17
4i	13.48 ± 0.58	17.27 ± 0.58	24.42 ± 1.16	10.16 ± 0.17	15.00 ± 2.09	21.12 ± 0.58	13.92 ± 1.16	14.23 ± 1.16	23.06 ± 1.53	12.33 ± 0.61	17.38 ± 1.16	22.82 ± 0.47
4j	13.12 ± 0.47	16.00 ± 0.58	22.90 ± 1.16	10.28 ± 1.16	12.82 ± 0.58	20.33 ± 1.73	14.42 ± 1.16	15.10 ± 2.09	21.80 ± 0.61	12.67 ± 0.71	16.12 ± 0.45	20.30 ± 0.17
Standard	15.78 ± 0.17	21.10 ± 1.16	28.71 ± 0.58	13.36 ± 1.53	18.70 ± 0.45	30.33 ± 0.61	15.14 ± 0.78	20.23 ± 0.47	31.82 ± 1.53	15.39 ± 0.58	22.41 ± 2.09	29.48 ± 0.17

S.D. = standard deviation; Standard = Streptomycin.
[a] Zone of inhibition.

Table 6
Antifungal activities of compounds[a](**2**–**4**).

Compounds	*Candida albicans*			*Aspergillus niger*		
	25 μg mL^{-1} ± S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ± S.D.	25 μg mL^{-1} ± S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ± S.D.
2a	9.33 ± 0.58	11.18 ± 1.53	19.79 ± 0.47	11.59 ± 0.58	12.02 ± 0.53	18.04 ± 0.61
2b	10.76 ± 0.58	12.14 ± 1.16	16.00 ± 1.00	12.67 ± 0.53	14.92 ± 0.58	22.67 ± 1.53
2c	13.00 ± 0.00	16.82 ± 1.73	18.44 ± 1.53	12.27 ± 0.58	15.94 ± 0.61	23.48 ± 0.58
2d	10.64 ± 1.16	13.84 ± 1.73	21.78 ± 1.53	13.04 ± 0.58	15.63 ± 0.58	22.58 ± 1.16
2e	11.82 ± 0.58	14.37 ± 0.58	17.95 ± 1.73	11.14 ± 0.58	14.54 ± 1.16	19.72 ± 1.53
2f	9.27 ± 0.58	13.84 ± 1.16	18.36 ± 1.53	9.92 ± 0.58	11.00 ± 2.00	18.67 ± 0.58
2g	9.18 ± 1.16	13.82 ± 0.58	20.75 ± 0.61	10.62 ± 0.47	10.62 ± 0.58	17.54 ± 0.27
2h	10.48 ± 0.58	13.00 ± 1.00	19.48 ± 1.53	9.25 ± 0.58	12.00 ± 1.00	19.72 ± 0.27
2i	8.84 ± 0.53	12.62 ± 0.58	20.00 ± 1.00	8.00 ± 0.00	9.46 ± 0.58	15.42 ± 1.16
2j	9.48 ± 0.58	13.92 ± 1.73	21.00 ± 1.00	8.40 ± 0.61	10.85 ± 0.58	17.10 ± 1.53
3a	8.14 ± 0.58	10.66 ± 0.47	15.82 ± 0.27	8.32 ± 0.61	10.00 ± 2.00	17.00 ± 0.00
3b	11.82 ± 0.58	17.00 ± 1.00	19.24 ± 1.53	11.14 ± 0.58	14.00 ± 1.00	19.62 ± 0.58
3c	8.26 ± 0.58	10.15 ± 0.58	16.00 ± 1.00	9.82 ± 0.58	10.06 ± 0.47	17.62 ± 1.16
3d	10.68 ± 0.47	13.82 ± 0.61	15.88 ± 1.53	10.16 ± 1.16	12.84 ± 1.16	15.88 ± 1.16
3e	9.14 ± 0.58	9.00 ± 2.00	15.28 ± 1.53	7.14 ± 0.58	9.48 ± 0.58	15.84 ± 0.58
3f	12.82 ± 0.58	15.72 ± 0.58	17.28 ± 0.47	14.64 ± 0.58	16.84 ± 1.73	21.42 ± 1.16
3g	13.44 ± 0.58	17.88 ± 0.61	21.36 ± 0.61	11.23 ± 0.58	14.00 ± 0.00	19.38 ± 0.27
3h	10.12 ± 0.47	11.00 ± 2.00	17.86 ± 1.53	8.38 ± 0.27	10.26 ± 0.58	16.66 ± 0.58
3i	9.62 ± 0.58	12.46 ± 1.16	17.68 ± 1.16	8.24 ± 0.58	10.57 ± 0.58	16.48 ± 1.73
3j	9.35 ± 0.58	13.75 ± 0.58	18.72 ± 1.53	7.06 ± 0.58	8.44 ± 0.58	14.96 ± 0.58
4a	9.62 ± 0.58	12.58 ± 0.58	19.76 ± 1.53	11.56 ± 0.58	13.00 ± 0.00	20.66 ± 0.58
4b	12.04 ± 0.58	15.20 ± 0.58	21.16 ± 1.16	11.90 ± 1.73	14.12 ± 0.61	21.16 ± 0.61
4c	10.86 ± 0.58	12.30 ± 0.47	18.00 ± 2.00	10.14 ± 0.47	13.10 ± 0.58	20.36 ± 1.53
4d	9.10 ± 0.58	13.83 ± 0.61	21.46 ± 1.53	12.10 ± 0.58	15.80 ± 0.61	22.10 ± 0.58
4e	10.38 ± 0.58	13.72 ± 1.16	17.72 ± 1.16	10.24 ± 2.08	12.24 ± 1.16	18.26 ± 2.08
4f	12.19 ± 0.58	16.02 ± 2.08	21.76 ± 1.16	11.60 ± 0.58	15.58 ± 1.16	23.78 ± 1.53
4g	11.48 ± 0.58	14.86 ± 1.16	20.16 ± 1.53	12.16 ± 0.58	15.06 ± 0.63	22.40 ± 0.58
4h	09.41 ± 0.58	13.48 ± 1.63	17.00 ± 1.53	10.80 ± 0.58	12.12 ± 0.27	18.94 ± 0.47
4i	10.58 ± 0.61	14.46 ± 0.47	15.46 ± 1.53	9.21 ± 0.58	12.62 ± 0.27	18.12 ± 0.58
4j	10.43 ± 0.58	13.30 ± 0.27	17.72 ± 0.63	9.48 ± 0.58	11.74 ± 0.61	17.16 ± 1.16
Standard	12.89 ± 0.58	15.26 ± 1.73	23.10 ± 1.53	14.89 ± 0.47	17.13 ± 0.58	26.14 ± 0.61

S.D. = standard deviation; Standard = Amphotericin-B.
[a] Zone of inhibition.

activity against *C. albicans* and compounds **3f**, **4f**, **2e**, **3b** and **4g** exhibited good activity than the standard. Compound **3f** showed potent activity against *A. niger*. While the compounds **2d**, **4f**, **4g**, **4d**, **2c**, **2b**, **2a**, **2e**, **3b**, **3g**, **4a**, and **4b** exhibited moderate to good activity.

2.2.4. Anthelmintic

Synthesized compounds **2**–**4** were also tested for in vitro anthelmintic activity. All the compounds showed moderate to potent anthelmintic activity. In vitro anthelmintic activity of synthesized compounds is summarized in Table 7. Comparison of anthelmintic activity data revealed that hydrazine derivatives of the pyrimidines are slightly more active than the others. Compounds **3e**, **3d**, **3i**, **2e**, **2d**, **2i** and **4e** showed more potent paralyzing effect than the standard, albendazole. While in addition to these compounds **4i**, **4c**, **3f**, **2f** and **2c** exhibits less time for death than the standard. Moreover, compounds **3a**, **3c**, **4f**, **2j**, **3j**, **3g** and **3b** showed anthelmintic activity comparable to that of reference compound. While other compounds showed moderate activity. This whole data revealed that dihydropyrimidine ring is responsible for the anthelmintic activity and the compounds with additional hetero atom in the structure may responsible for increase in the activity.

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 [29] in methanol were added to 2 mL of hydrogen peroxide solution in phosphate buffer saline. The absorbance was measured at 230 nm after 10 min [30].

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 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 640 nm [31].

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 or standard, ascorbic acid (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 [32].

Table 7
Anthelmentic activity of compounds (**2**–**4**).

Compounds	Time (in minutes) ± S.D.					
	For paralysis % Concentration (w/v)			For death % Concentration (w/v)		
	0.1	0.2	0.5	0.1	0.2	0.5
Control	–	–	–	–	–	–
2a	54.56 ± 0.98	46.56 ± 0.45	42.65 ± 1.44	78.22 ± 1.87	72.67 ± 0.82	62.21 ± 0.82
2b	72.34 ± 0.59	71.40 ± 0.84	55.17 ± 1.88	90.40 ± 1.43	85.73 ± 0.49	69.54 ± 0.93
2c	42.68 ± 1.12	36.59 ± 0.72	32.55 ± 0.23	60.18 ± 2.17	55.49 ± 1.32	53.98 ± 0.44
2d	39.78 ± 0.21	38.48 ± 0.91	33.35 ± 1.65	45.24 ± 1.04	47.82 ± 0.58	42.34 ± 0.62
2e	35.25 ± 0.44	44.52 ± 1.43	40.22 ± 1.24	47.57 ± 1.02	55.59 ± 0.41	54.10 ± 0.60
2f	48.15 ± 0.52	39.29 ± 0.76	40.51 ± 1.12	60.96 ± 1.04	57.38 ± 0.69	55.12 ± 0.71
2g	55.29 ± 0.89	40.27 ± 0.92	40.61 ± 1.91	72.39 ± 0.20	55.80 ± 0.85	50.24 ± 0.55
2h	75.54 ± 0.83	57.18 ± 1.28	41.47 ± 1.47	83.02 ± 1.18	60.44 ± 0.89	56.08 ± 0.38
2i	40.28 ± 0.67	39.46 ± 0.28	35.14 ± 1.23	50.82 ± 0.78	48.34 ± 0.56	45.30 ± 0.49
2j	58.04 ± 0.93	62.28 ± 0.71	50.10 ± 1.08	65.29 ± 0.39	65.50 ± 0.81	61.02 ± 0.96
3a	47.23 ± 1.56	45.23 ± 0.88	42.60 ± 1.33	62.32 ± 0.87	60.22 ± 1.75	57.45 ± 0.78
3b	54.93 ± 0.80	51.34 ± 0.77	48.77 ± 1.56	68.45 ± 1.86	65.43 ± 0.67	61.02 ± 0.59
3c	43.73 ± 1.90	40.73 ± 0.49	37.52 ± 2.23	61.58 ± 1.63	57.48 ± 0.59	50.98 ± 0.97
3d	32.35 ± 0.43	29.13 ± 0.43	26.13 ± 1.31	58.24 ± 0.31	44.42 ± 0.35	40.30 ± 1.13
3e	28.25 ± 0.14	26.35 ± 0.24	25.64 ± 0.46	54.22 ± 1.22	46.44 ± 0.56	43.34 ± 0.35
3f	42.10 ± 0.23	40.13 ± 0.92	37.67 ± 1.90	58.30 ± 0.49	46.45 ± 0.88	42.54 ± 0.76
3g	50.65 ± 1.56	52.38 ± 2.03	45.38 ± 0.33	67.33 ± 0.28	66.37 ± 1.44	60.86 ± 1.21
3h	55.23 ± 0.24	55.57 ± 0.34	51.42 ± 1.31	71.45 ± 1.76	70.25 ± 0.35	67.22 ± 1.10
3i	35.42 ± 0.45	33.85 ± 0.67	30.13 ± 1.06	50.13 ± 1.44	37.10 ± 0.83	44.10 ± 1.02
3j	52.22 ± 0.37	48.56 ± 0.72	45.65 ± 1.25	66.22 ± 1.29	63.98 ± 0.86	60.27 ± 0.81
4a	52.09 ± 1.44	49.46 ± 0.69	45.02 ± 0.53	67.41 ± 1.28	63.37 ± 0.40	57.93 ± 1.44
4b	70.67 ± 0.17	68.48 ± 1.95	48.38 ± 0.74	88.45 ± 1.55	80.74 ± 0.65	60.77 ± 0.87
4c	47.26 ± 1.46	42.22 ± 0.94	45.98 ± 1.17	58.76 ± 0.41	55.23 ± 0.79	54.82 ± 0.48
4d	42.48 ± 0.82	40.12 ± 1.63	43.59 ± 1.01	47.38 ± 1.03	49.67 ± 0.37	47.44 ± 1.42
4e	41.45 ± 2.20	46.58 ± 1.08	42.87 ± 0.49	49.79 ± 0.97	57.55 ± 1.02	58.72 ± 0.88
4f	49.28 ± 0.62	41.03 ± 1.34	34.76 ± 0.51	64.72 ± 0.45	58.23 ± 1.26	56.33 ± 0.53
4g	58.08 ± 0.45	42.05 ± 0.65	46.18 ± 0.44	72.26 ± 1.12	57.50 ± 0.38	51.70 ± 0.70
4h	75.14 ± 1.12	55.10 ± 0.51	43.90 ± 1.02	87.55 ± 0.56	62.12 ± 0.61	52.69 ± 0.31
4i	43.09 ± 0.49	40.03 ± 1.23	38.52 ± 1.55	55.20 ± 1.69	49.54 ± 0.20	47.23 ± 1.69
4j	60.22 ± 0.67	65.54 ± 0.90	53.34 ± 1.12	70.56 ± 1.40	75.78 ± 0.87	60.74 ± 0.56
Standard	50.38 ± 0.52	35.58 ± 0.59	30.57 ± 1.26	60.36 ± 1.02	55.25 ± 0.93	53.34 ± 1.62

S.D. = standard deviation (Average of three determination), Standard = Albendazole.

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.

3.1.4. Reducing power determination

The reducing power of test samples was determined according to the method of Oyaizu [33]. The test compounds and standard drug, ascorbic acid were dissolved in *N*,*N*-dimethyl formamide (DMF) to get different concentrations (12.5, 25, 50, 100 μg/mL). This was mixed with 2.5 mL of (pH 6.6) 0.2 M phosphate buffer and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. 2.5 mL of 10% Trichloroacetic acid was added to the mixture, which was then centrifuged for 10 min at 1000 rpm. 2.5 mL upper layer of solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The blank was also carried out in similar manner.

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

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 Kabayashi [34] 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, 100 μg/mL) 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-vis 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 [35,36]. 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.

3.3. Antimicrobial activity

Applying the agar plate diffusion technique [37] all of the newly synthesized compounds were screened in vitro for antibacterial activity against *E. coli*, *P. aeruginosa* (Gram-negative), *S. aureus*, *B. subtilis* (Gram-positive) at 25, 50 and 100 μg/mL concentrations,

respectively. Streptomycin (binds to the 16SrRNA of the bacterial ribosome, interfering with the binding of formylmethionyl-tRNA to the 30S subunit therefore prevents initiation of protein synthesis and leads to death of microbial cell) was chosen as a standard drug [38]. Streptomycin is an antibiotic that inhibits both gram-positive and gram-negative bacteria, and is therefore a useful broad spectrum antibiotic. Similarly, the antifungal screening of the compounds was carried out in vitro by paper disc method against two fungi *A. niger* and *C. albicans* by using Amphotericin-B (binds to plasma membrane sterols like ergosterol. The fungi cells have large amount of ergosterol in plasma membrane. The ergosterol facilitates the attachment of amphotericin B, which act as ionophores and cause leakage of cations like K^+) as a standard [35,39].

3.4. Anthelmintic activity

The synthesized compounds were screened for anthelmintic activity by using earthworms, *Perituma posthuma* [40]. Six earthworms of nearly equal size were placed in standard drug solution and test compound solutions at room temperature. Normal saline used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and adjusted the volume up to 15 mL with normal saline solution to get the concentration of 0.1, 0.2 and 0.5% w/v. Albendazole was used as a standard drug [41]. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive. The anthelmintic activity data was revealed in the form of mean lethal time and paralysis time of the earthworms for different test compounds and the standard drug.

4. Conclusion

In conclusion, we have described simple and efficient protocol for the synthesis of novel dihydropyrimidines (**1a**–**j**), its dimethylated adducts (**2a**–**j**), and hydrazine derivatives (**3a**–**j**) of **2a**–**j** and its pyrazole derivatives (**4a**–**j**) with moderate to good yields. All the synthesized compounds **2**–**4** have been investigated for their in vitro antioxidant, anti-inflammatory, antibacterial, antifungal and anthelmintic activity. In our newly synthesized compounds, it is cleared that the highest antioxidant activity for compounds **3j** and **3i**, anti-inflammatory activity for compound **2f**, antibacterial activity for compound **4b**, antifungal activity for compounds **3g** and **2c** against *C. albicans* and **3f** against *A. niger* and anthelmintic activity for compounds **4e** and **2d** were observed. Accordingly, these novel classes of dihydropyrimidines and its pyrazole derivatives presented in our laboratory emerged as a valuable lead series that might be useful as antioxidant, anti-inflammatory, antibacterial, antifungal and anthelmintic agents and hence promising candidates for further efficacy evaluation.

5. Experimental section

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were determined by Micro control based melting point instrument and are uncorrected. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel (60GF-254) plates, 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-MSMS (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$1.1% of the theoretical values.

6. Material and methods

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

A 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 for 12 h. 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.

6.2. General procedure for the synthesis 1-methyl-2-(methylthio)-5-cyano-6-oxo-4-substituted-1,6-dihydropyrimidine (*2a–j*)

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 for 3 h 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 creamish crystals.

6.3. General procedure for the synthesis of 2-hydrazino-1-methyl-5-cyano-6-oxo-4-substituted-1,6-dihydropyrimidine (*3a–j*)

A mixture of compound 1-methyl-2-(methylthio)-5-cyano-6-oxo-4-substituted-1,6-dihydropyrimidine (**2a**–**c**, 10 mmol) and hydrazine hydrate (80%, 0.96 g, 30 mmol) in absolute alcohol was refluxed for 6 h. The reaction mixture was poured into crushed ice. Then the product was isolated and recrystallized from ethanol/DMF mixture as yellow crystals.

6.4. General procedure for the synthesis of ethyl 5-amino-1-(5-cyano-1-methyl-6-oxo-4-substituted-1,6-dihydropyrimidin-2-yl)-1H-pyrazole-4-carboxylate (*4a–j*)

A mixture of compound 2-hydrazino-1-methyl-5-cyano-6-oxo-4-substituted-1,6-dihydropyrimidine (**3a**–**c**, 10 mmol) and ethyl-2-cyano-3-ethyl acrylate (1.69 g, 10 mmol) in absolute alcohol and few drops of glacial acetic acid was refluxed for 5–6 h. Excess of solvent is removed and mixture was added to crushed ice to get above said products.

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