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ORIGINAL ARTICLE

Synthesis and evaluation of 3-cyano-4-imino-2-methylthio-4*H*-pyrido[1,2-*a*] pyrimidine derivatives as potent antioxidant agents

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3-Cyano-4-imino-2-methylthio-4*H*-pyrido[1,2-*a*] pyrimidine

Abstract The bis (methylthio) methylene malononitrile (**1**) on treatment with 2-amino pyridine (**2**) in *N*,*N*-dimethyl formamide (DMF) and anhydrous potassium carbonate, gives 3-cyano-4-imino-2-(methylthio)-4*H*-pyrido[1,2-*a*]pyrimidine (**3**). The latter were further reacted with selected N-, O- and C-nucleophiles such as aryl amines, hetryl amines, substituted phenols and compounds containing active methylene groups.

1. Introduction

Several types of pyrido[1,2-*a*]pyrimidines have aroused much interest due to their valuable pharmacological properties, such as antiviral properties (Albert, 1986), as antioxidants which can neutralize free radicals (Blois, 1958), and as antimalarials (Chan et al., 1993; Chem et al., 1993). Thus, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and propagation of oxidative diseases like autoimmune diseases, cardiovascular diseases, neurovascular diseases (Eberlein et al., 1994), show hypoglycemic (George et al., 1971), and antibacterial properties (Gupta et al., 1971). The homeostatic balance between the reactive oxygen species (ROS) and endogenous antioxidants is important in maintaining healthy tissues. Excessive ROS states are important in diseases such as acute respiratory distress syndrome and idiopathic pulmonary fibrosis (Halliwell, 1994). They are also used as synthetic intermediates or as additives to photographic materials and dyes (Heseltine and Brooker, 1966). Most living organisms possess enzymatic and non enzymatic defence systems against excessive production of the reactive oxygen species. However, different external factors (smoke, diet, alcohol and some drugs) and aging decrease the efficiency of such protecting systems, resulting in disturbances of the redox equilibrium established under healthy conditions (Leutner et al., 2001). Free radical scavenging activity of mushroom polysaccharide (Liu et al.,

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Table 1 Antioxidant potential of the tested compounds.

S. No.	Compound (1 mM)	DPPH radical scavenging activity (%)	OH radical scavenging activity (%)	SOR scavenging activity (%)	Reducing activity (%)
1	**3**	17.24	17.96	NR	250
2	**4a**	00.49	NR	1.80	205
3	**4b**	01.97	79.64	14.37	177
4	**4c**	08.62	59.88	12.57	211
5	**4d**	NR	95.21	45.51	250
6	**4e**	NR	71.86	09.58	102
7	**4f**	02.71	NR	NR	250
8	**5a**	NR	84.43	20.36	202
9	**5b**	NR	NR	NR	250
10	**5c**	NR	71.26	07.19	158
11	**5d**	06.16	78.44	09.58	181
12	**6a**	06.90	65.27	31.14	235
13	**6b**	NR	72.46	20.96	250
14	**6c**	NR	60.48	29.34	222
15	**6d**	01.48	61.08	NR	250
16	**6e**	14.04	46.71	11.38	217
17	**6f**	NR	50.30	NR	174
18	**7a**	03.69	62.87	14.97	116
19	**7b**	NR	97.01	13.17	250
20	**7d**	NR	99.40	13.17	240
21	Ascorbic Acid	82.54 ± 0.021	02.82 ± 0.021	51.24 ± 0.028	155.7 ± 0.91

Results presented here are the mean values from three independent experiments ± S.D.,
NR = No reaction under experimental condition.

1997), as antimalarial (Meszaros et al., 1976) and as antihypertensive is noted (Mosby, 1961), redox imbalance increases the breakdown of the extracellular matrix component hyaluronan into lower molecular weight fragments that in turn activate the innate immune responses and perpetuate tissue injury (Horton, 2008), antioxidative activities are obtained with glucosamine (Oyaizu, 1986), antihypertensive activities are also seen (Bhuyan et al., 2003). Therefore, antioxidants, which can neutralize free radicals may be of central importance in the prevention of cardiovascular and neurodegenerative changes associated with aging (Roberta et al., 2006), antioxidants are antiallergic (Roth and Cheng, 1982), as hydroxyl radicals act as potential intracellular mediators of polymorpho nuclear neutrophil apoptosis (Rollet-Labelle et al., 1998), act as Anticancer agents (EL-Assiery et al., 2004), and as anti-HIV agents (Sheng-Hui et al., 2010). Recently, more attention has been paid to the role of natural antioxidants, mainly phenolic compounds, which may have higher antioxidant activities than those of conventional vitamins C, E and β-carotene (Vinson et al., 1995). Antimalarial (Yale and Sheehan, 1973, 1977) effects have been observed in a number of pyrido[1,2-*a*]pyrimidines. The development of physiologically highly potent fused pyrimidine has been of great interest in the synthesis of facile and general routes to these molecules in synthetically useful yields.

Considerable scientific work in this direction to synthesize these compounds and the evaluation of novel synthetic antioxidant compounds have been in progress. Therefore, in the present study an attempt has been made to investigate newly synthesized imino pyrido pyrimidine derivatives for their antioxidant potential. In the present work we report the details of the synthesis of novel imino pyrido pyrimidine derivatives and their antioxidant properties.

2. Materials and methods

Melting points were determined by open capillary tubes and were uncorrected. All the reactions monitored by thin layer chromatography, were carried out on 0.2 mm silica gel-C plates using iodine vapors for detection. Infrared spectra were recorded in Nujol or as potassium bromide pellets on an infrared spectrophotometer, nuclear magnetic resonance spectra were obtained on Brukner advance spectrophotometer. 400 MHz mass spectra were recorded on FT-VC-7070 H Mass spectrometer using the EI technique at 70 eV. All the reactions were carried out under ambient atmosphere. Elemental analysis was performed on a Heraeus CHN–O rapid analyzer.

*2.1. 3-Cyano-4-imino-2-(methylthio)-4H-pyrido [1,2-a]pyrimidine (**3**)*

A mixture of 2-amino pyridine (**2**) (0.01 mol) and bis (methylthio) methylene malononitrile (**1**) (0.01 mol) in 15 mL of *N*,*N*′-dimethyl formamide and anhydrous potassium carbonate (10 mg) was refluxed for 5 h. The reaction mixture was cooled to room temperature and poured into ice cold water. The separated solid product was filtered, washed with water and recrystallized from a *N*,*N*′-dimethyl formamide–ethanol mixture to give pure (**3**).

*2.2. 2-Substituted derivatives of 3-cyano-4-imino-2-(methylthio)-4H-pyrido[1,2-a]pyrimidine (**4a–f**, **5a–d**, **6a–d** and **7a–d**)*

A mixture of (**3**) (0.001 m mol) and independently, various aromatic amines, hetryl amines, substituted phenols or compounds containing an active methylene group (0.001 m mol)

in *N*,*N*′-dimethyl formamide (10 mL) and anhydrous potassium carbonate (10 mg) was refluxed for 4–6 h. The reaction mixture was cooled to room temperature and poured into ice cold water. The separated solid product was filtered, washed with water and recrystallized from *N*,*N*′-dimethyl formamide–ethanol mixture to give pure **4a–f, 5a–d, 6a–f** and **7a–d**.

*2.3. 3-Cyano-4-imino-2-(methylthio)-4H-pyrido[1,2-a]pyrimidine (**3**)*

Orange powder, yield 60%, m.p. 230 °C (dec.). IR (KBr/cm^{-1}) 3350 (=NH), 2225 (CN); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.59 (s, 3H, SCH_3), 6.2–6.5 (d, 4H, CH=CH), 9.2 (br s, 1H, =NH). EI-MS (*m*/*z*: RA%): 217 (M + I), 100%), 215 (35). ^{13}C NMR (300 MHz, $CDCl_3$): δ = 15.07, 79, 109, 115.9, 122, 133, 138, 150, 164, 164.09. Anal. Calcd. for $C_{10}H_8N_4S$: C, 60.35; H, 3.55; N, 24.85. Found: C, 60.28; H, 3.21; N, 24.62.

*2.4. 3-Cyano-4-imino-2-(4-methoxy anilino)-4H-pyrido[1,2-a]pyrimidine (**4a**)*

Brown powder, yield 50%, m.p. 212 °C (dec.). IR (KBr/cm^{-1}) 3385 (=NH), 2207 (CN).^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.6 (s, 3H, Ar-OCH_3), 4.2 (s, 1H, –NH), 5.2–5.4 (d, 2H, CH=CH), 6.2–6.7 (m, 6H, Ar-H), 8.9 (br s, 1H, =NH) EI-MS (*m*/*z*: RA%): 292 (M + I), 273, 239, 219, 217, 215, 201, 160, 120, 109, 107, 102 . Anal. Calcd. for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.46; N, 24.05. Found: C, 65.92; H, 4.41; N, 24.00.

*2.5. 3-Cyano-4-imino-2-(p-methyl anilino)-4H-pyrido[1,2-a]pyrimidine (**4b**)*

Brown powder, yield 50%, m.p. 212 °C (dec.). IR (KBr/cm^{-1}) 3384 (=NH), 2196 (CN). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.5 (s, 3H, Ar-CH_3), 4.0 (s, 1H, –NH–), 5.1–5.3 (d, 2H, CH=CH), 6.1–6.8 (m, 6H, Ar-H), 9.1 (br s, 1H, =NH). EI-MS (*m*/*z*: RA%) : 276 (M + I), 230, 217. Anal. Calcd. for $C_{16}H_{13}N_5$; C, 69.80; H, 4.76; N, 25.44; Found: C, 69.42; H, 4.31; N, 25.02.

*2.6. 3-Cyano-4-imino-2-(3-methoxy anilino)-4H-pyrido[1,2-a]pyrimidine (**4c**)*

Brown powder, yield 74%, m.p. 216 °C (dec.). IR (KBr/cm^{-1}) 3382 (=NH), 3314 (NH); 2207 (CN), Anal. Calcd. for $C_{16}H_{13}N_5O$; C, 65.97; H, 4.50, N, 24.04; Found: C, 65.38; H, 4.15; N, 23.66.

*2.7. 3-Cyano-4-imino-2-(4-chloro anilino)-4H-pyrido[1,2-a]pyrimidine (**4d**)*

Brown powder, yield 68%, m.p. 212 °C (dec.). IR (KBr/cm^{-1}) 3388 (=NH), 3312 (NH). 2196 (CN), EI-MS (*m*/*z*:RA%): 296 (M + I). Anal. Calcd. for $C_{15}H_{10}ClN_5$; C, 69.80; H, 4.76; N, 25.44. Anal. Calcd. for $C_{15}H_{10}ClN_5$; C, 60.92; H, 3.41, N, 23.68; Found: C, 59.87; H, 2.95; N, 23.10.

*2.8. 3-Cyano-4-imino-2-(4-nitro anilino)-4H-pyrido[1,2-a]pyrimidine (**4e**)*

Brown powder, yield 58%, m.p. 213 °C (dec.). IR (KBr/cm^{-1}) 3380 (=NH), 3315 (NH), 2210 (CN). Anal. Calcd. for $C_{15}H_{10}N_6O_2$; C, 58.82; H, 3.29; N, 27.44; Found : C, 57.98; H, 2.95; N, 26.85.

*2.9. 3-Cyano-4-imino-2-(o,p-dichloro anilino)-4H-pyrido[1,2-a]pyrimidine (**4f**)*

Brown powder, yield 62%, m.p. 208 °C (dec.). IR (KBr/cm^{-1}) 3374 (=NH), 3311 (NH), 2207(CN), Anal. Calcd. for $C_{15}H_9Cl_2N_5$; C, 54.57; H, 2.75, N, 21.21. Found: C, 53.91; H, 2.15; N, 20.85.

*2.10. 3-Cyano-4-imino-2-(pyrolidino)-4H-pyrido[1,2-a]pyrimidine (**5a**)*

Brown powder, yield 58%, m.p.196 °C (dec.). IR (KBr/cm^{-1}) 3388 (=NH), 2204 (CN), ^{1}H NMR (400 MHz, DMSO-d_6) : δ = 1.6 (t, 4H, 2-CH_2–), 2.9 (t, 4H, 2-NCH_2), 5.2–5.6 (d, 2H, CH=CH), 6.3–6.6 (d, 2H, CH=CH), 9.2 (br s, 1H, =NH), EI-MS (*m*/*z* : RA%): 239 (100%), 217, 215, Anal. Calcd. for $C_{13}H_{13}N_5$; C, 65.25; H, 5.48; N, 29.27; Found: C, 64.92; H, 4.85; N, 28.86.

*2.11. 3-Cyano-4-imino-2-(piperidino)-4H-pyrido[1,2-a]pyrimidine (**5b**)*

Brown powder, yield 70%, m.p. 190 °C (dec.). IR (KBr/cm^{-1}) 3380 (=NH), 2210 (CN), Anal. Calcd. for $C_{14}H_{15}N_5$; C, 66.38; H, 5.97; N, 27.85. Found: C, 65.88; H, 5.05; N, 26.86.

*2.12. 3-Cyano-4-imino-2-(morpholino)-4H-pyrido[1,2-a]pyrimidine (**5c**)*

Brown powder, yield 64%, m.p. 214 °C (dec.). IR (KBr/cm^{-1}) 3387 (=NH), 2198 (CN), ^{1}H NMR (400 MHz, DMSO-d_6) : δ = 2.9 (t, 4H, 2-NCH_2), 3.7 (t, 4H, 2-OCH_2-), 5.1–5.5 (d, 2H, CH=CH), 6.2–6.5 (d, 2H, CH=CH), 9.1 (br s, 1H, =NH), EI-MS (*m*/*z*: RA%): 256 (M + I), Anal. Calcd. for $C_{13}H_{13}N_5O$; C, 61.17; H, 5.13; N, 27.43. Found: C, 60.93; H, 4.85; N, 26.95.

*2.13. 3-Cyano-4-imino-2-(piperazino)-4H-pyrido[1,2-a]pyrimidine (**5d**)*

Brown powder, yield 54%, m.p. 200 °C (dec.). IR (KBr/cm^{-1}) 3378 (=NH), 2203 (CN), Anal. Calcd. for $C_{13}H_{14}N_6$; C, 61.40; H, 5.55; N, 33.05. Found: C, 60.92; H, 4.95; N, 32.63.

*2.14. 3-Cyano-4-imino-2-(phenoxy)-4H-pyrido[1,2-a]pyrimidine (**6a**)*

Brown powder, yield 54%, m.p. 210 °C (dec.). IR (KBr/cm^{-1}) 3380 (=NH), 2198 (CN), Anal. Calcd. for $C_{15}H_{10}N_4O$; C, 68.69; H, 3.84; N, 21.36. Found: C, 68.02; H, 3.25; N, 20.69.

*2.15. 3-cyano-4-imino-2-(4-methoxyphenoxy)-4H-pyrido[1,2-a]pyrimidine (**6b**)*

Brown powder, yield 70%, m.p. 202 °C (dec.). IR (KBr/cm^{-1}) 3384 (=NH), 2214 (CN), ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.7 (s, 3H, Ar-OCH_3), 5.1–5.3 (d, 2H, CH=CH), 6.3–6.5 (d, 2H, CH=CH), 6.6–6.8 (m, 4H, Ar-H), 8.9 (br s, 1H, =NH); EI-MS (*m*/*z*: RA%): 292 (M + I), 270, 261, 249, 191,

123, Anal. Calcd. for $C_{16}H_{12}N_4O_2$; C, 65.75; H, 4.14; N, 19.17. Found: C, 65.02; H, 3.75; N, 18.63.

*2.16. 3-Cyano-4-imino-2-(p-methyl phenoxy)-4H-pyrido[1,2-a]pyrimidine (**6c**)*

Brown powder, yield 54%, m.p. 216 °C (dec.). IR (KBr/cm^{-1}) 3382 (=NH), 2206 (CN), EI-MS (*m*/*z*: RA%): 276 (M). Anal. Calcd. for $C_{16}H_{12}N_4O$; C, 69.55; H, 4.38; N, 20.28. Found: C, 69.02; H, 3.95; N, 19.63.

*2.17. 3-Cyano-4-imino-2-(4-chloro phenoxy)-4H-pyrido[1,2-a]pyrimidine (**6d**)*

Brown powder, yield 59%, m.p. 204 °C (dec.). IR (KBr/cm^{-1}) 3381 (=NH), 2210 (CN). Anal. Calcd. for $C_{15}H_9Cl\ N_4O$; C, 60.72; H, 3.06; N, 18.88. Found : C, 60.12; H, 2.75; N, 18.23.

*2.18. 3-Cyano-4-imino-2-(o-methyl phenoxy)-4H-pyrido[1,2-a]pyrimidine (**6e**)*

Brown powder, yield 54%, m.p. 200 °C (dec.). IR (KBr/cm^{-1}) 3385 (=NH), 2210 (CN). Anal. Calcd. for $C_{16}H_{12}N_4O$; C, 69.55; H, 4.38; N, 20.28. Found: C, 69.02; H, 3.95; N, 19.63.

*2.19. 3-Cyano-4-imino-2-(o-phenyl phenoxy)-4H-pyrido[1,2-a]pyrimidine (**6f**)*

Brown powder, yield 54%, m.p. 194 °C (dec.). IR (KBr/cm^{-1}) 3380 (=NH), 2210 (CN), EI-MS (*m*/*z*: RA%): 338 (M). Anal. Calcd. for $C_{21}H_{14}N_4O$; C, 74.54; H, 4.17; N, 16.56. Found: C, 74.01; H, 3.75; N, 16.03.

*2.20. 3-Cyano-4-imino-2-(acetyl acetonyl)-4H-pyrido[1,2-a]pyrimidine (**7a**)*

Brown powder, yield 68%, m.p. 241 °C (dec.). IR (KBr/cm^{-1}) 3382 (=NH), 2211 (CN), EI-MS (*m*/*z*: RA%): 238 (M + I), 218, 217, 216, Anal. Calcd. for $C_{15}H_{14}N_4O_3$; C, 60.40; H, 4.73; N, 18.78. Found: C, 60.92; H, 4.95; N, 32.63.

*2.21. 3-Cyano-4-imino-2-(α-ethyl acetoacetyl)-4H-pyrido[1,2-a]pyrimidine (**7b**)*

Brown powder, yield 64%, m.p.245 °C (dec.). IR (KBr/cm^{-1}) 3384 (=NH), 2205 (CN), EI-MS (*m*/*z*: RA%): 298(M + I). Anal. Calcd. for $C_{15}H_{14}N_4O_3$; C, 60.40; H, 4.73; N, 18.78. Found: C, 59.82; H, 4.45; N, 18.13.

*2.22. 3-Cyano-4-imino-2-(α-ethyl cyano acetyl)-4H-pyrido[1,2-a]pyrimidine (**7c**)*

Brown powder, yield 64%, m.p. 194 °C (dec.). IR (KBr/cm^{-1}) 3375 (=NH), 2199 (CN), EI-MS (*m*/*z*:RA%) : 282(M + 1), 270, 247, 214 (100). Anal. Calcd. for $C_{14}H_{11}N_5O_2$; C, 60.92; H, 4.95; N, 32.63. Found: C, 60.38; H, 4.53; N, 32.19.

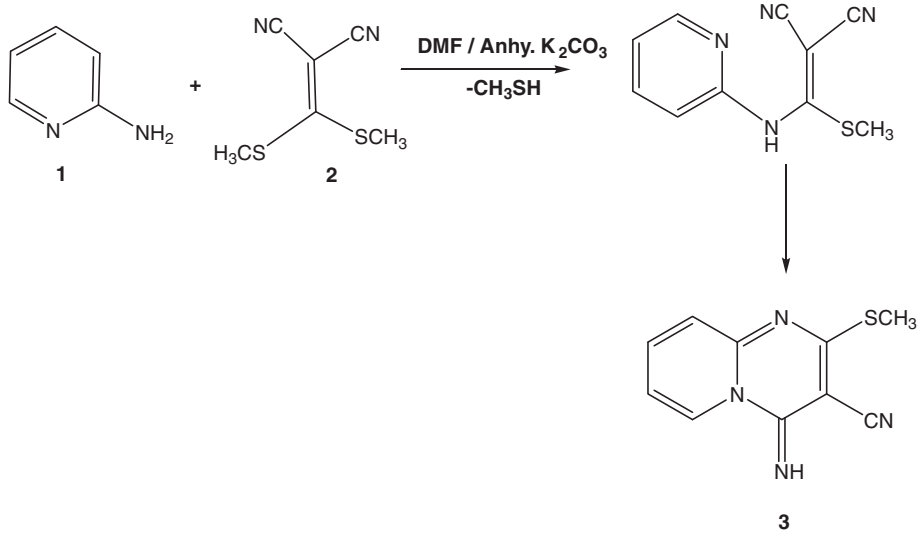
*2.23. 3-Cyano-4-imino-2-(malonyl)-4H-pyrido[1,2-a]pyrimidine (**7d**)*

Brown powder, yield 70%, m.p. 244 °C (dec.). IR (KBr/cm^{-1}) 3382 (=NH), 2207 (CN), ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.2 (s, 1H, –CH), 5.1–5.3 (d, 2H, CH=CH), 7.6–7.7 (d, 2H, CH=CH), 8.9 (br s, 1H, =NH). EI-MS (*m*/*z*: RA%): 334 (M), 324, 323, 297, 286, 248, 246, 186 (100), 178, 154, 119. Anal. Calcd. for $C_{12}H_6N_6$; C, 61.54; H, 2.58; N, 35.88. Found: C, 61.09; H, 2.25; N, 35.23.

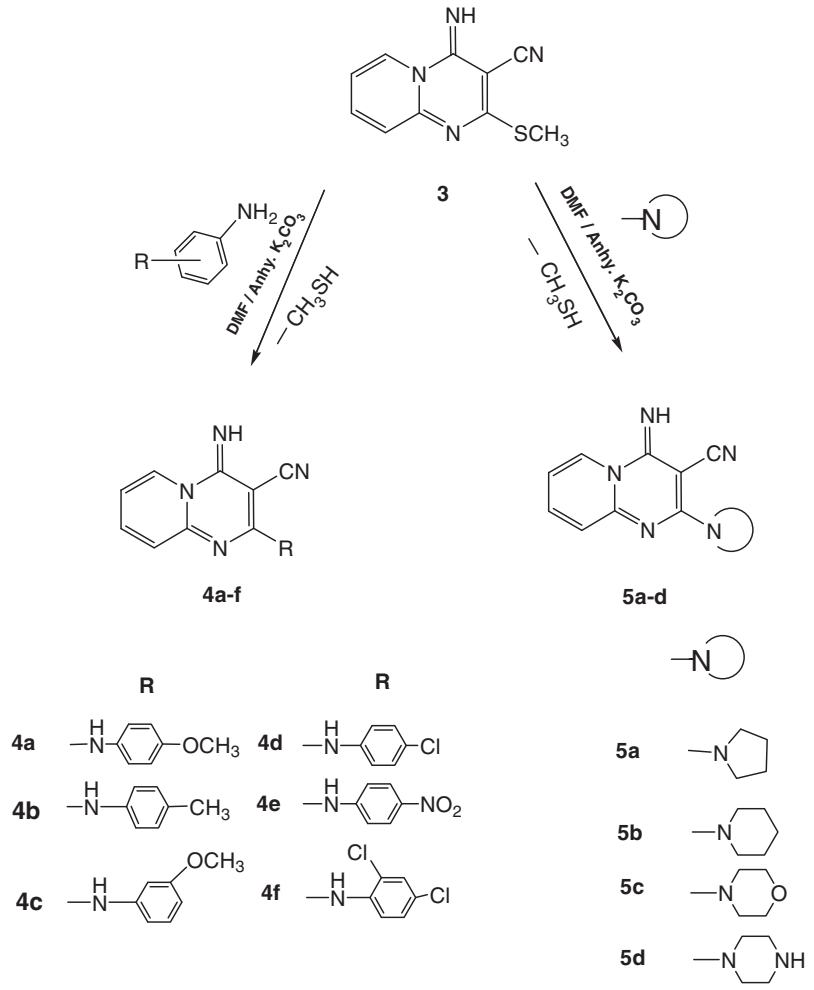
3. Result and discussion

Compound (**3**) was prepared from the reaction of 2-amino pyridine (**1**) bis (methylthio) methylene malanonitrile (**2**) with the presence of catalytic amounts of anhydrous potassium carbonate in *N*,*N*′-dimethyl formamide (Scheme 1). The yield of compound (**3**) is 60% the structure of this compound with m.p. 230 °C and was confirmed on the basis of elemental analysis, IR, PMR and MASS spectral data spectral studies of these compounds indicate that all newly synthesized compounds are stable and do not exhibit any tautomerism.

Compound (**3**) posses a replaceable active methylthio group at the 2-position which is activated by a ring 1-nitrogen atom



Scheme 1 Formation of fused pyrido[1,2-*a*]pyrimidine.



Scheme 2 3-Cyano-4-imino-2-(substituted derivative)-4*H*-pyrido[1,2-*a*]pyrimidine (**4a**–**f**, **5a**–**d**).

and electron withdrawing 3-cyano group. Compound (**3**) reacted with selected N-, O-, C-nucleophiles like aryl amines hetryl amines, substituted phenols and compounds containing an active methylene group. The compound (**3**) on independent reaction with *p*-methoxy aniline, *p*-methyl aniline, o-methyl aniline, *p*-chloro aniline, *p*-nitro aniline, m-methoxy aniline, o-*p*-dichloro aniline in *N*,*N*′-dimethyl formamide and catalytic amounts of anhydrous potassium carbonate, afforded 3-cyano-4-imino-2-(*p*-methoxy anilino/*p*-methyl anilino/*p*-chloro anilino/*p*-nitro anilino/*m*-methoxy anilino/o, *p*-dichloro anilino)-4*H*-pyrido-[1,2-*a*]pyrimidine (**4a**–**f**) (Scheme 2).

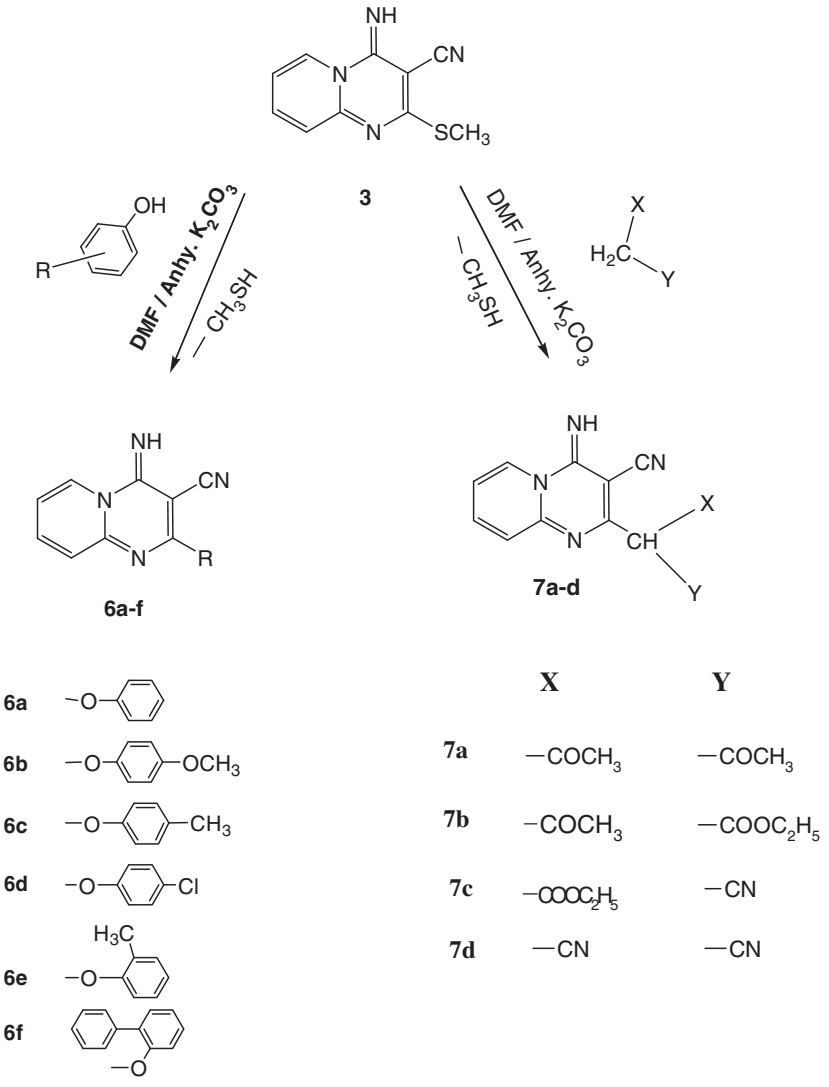
Under similar experimental conditions compound (**3**) reacted independently with hetryl amines like pyrolidine, piperidine, morpholine and piperazine to yield 3-cyano-4-imino-2-(pyrolidino/piperidino/morpholino/piperazino)-4*H*-pyrido[1,2-*a*]pyrimidine(**5a**–**d**) (Scheme 2).

3-Cyano-4-imino-2-(phenoxy/*p*-methoxy phenoxy/*p*-methyl phenoxy/*p*-chloro phenoxy/o-methyl phenoxy/o-phenyl phenoxy)-4*H*-pyrido[1,2-*a*]pyrimidine (**6a**–**f**), respectively (Scheme 3) were obtained by condensation of (**3**) with phenol, *p*-methoxy phenol, *p*-methyl phenol, *p*-chloro phenol, *o*-methyl phenol, *o*-phenyl phenol, in *N*,*N*′-dimethyl formamide and catalytic amounts of anhydrous potassium carbonate. The compounds on reaction with acetyl acetone, ethyl acetoacetate, ethyl cyano acetate, malanonitrile in presence of dimethyl formamide and catalytic amount of anhydrous potassium carbonate yielded compounds 3-cyano-4-imino-2(acetyl acetonyl/α-ethyl acetoacetyl/α-ethyl cyano acetyl/malonyl)-4*H*-pyrido[1,2-*a*]pyrimidine (**7a**–**d**), respectively (Scheme 3). Compounds **4a**–**f**, **5a**–**d**, **6a**–**f** and **7a**–**d** show absorption bands in their IR spectra in the range of 3350–3400 cm^{-1} and 2185–2215 cm^{-1} due to =NH and CN stretching, respectively ^1H NMR and Mass spectral data are also in agreement with structures of newly synthesized compounds **4a**–**f**, **5a**–**d**, **6a**–**f** and **7a**–**d**.

4. Antioxidant activity studies

4.1. DPPH radical scavenging assay

The DPPH radical scavenging assay has been used for preliminary screening of the samples for antioxidant activity. The proton radical scavenging action is known as an important mechanism of antioxidants. The odd electron in the DPPH radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow when the odd electron of the DPPH radical becomes paired with hydrogen from free radical scavenging antioxidants to form



Scheme 3 3-Cyano-4-imino-2-(substituted derivative)-4*H*-pyrido[1,2-*a*]pyrimidine (**6a**–**f**, **7a**–**d**).

reduced DPP-H. The results of DPPH reduction are summarized in Table 1. It is clear from the results that very few samples (1 mM) were found to interact with the stable free radical DPPH, which indicates their radical scavenging ability. The overall range of DPPH radical scavenging activity was found to be 00.49–17.24.

4.2. OH radical scavenging assay

The hydroxyl (OH) radicals are the most reactive among relative oxygen species and that every type of molecule found in living system cells: sugars, amino acids, phospholipids, DNA bases, organic acids may change the normal physiological function of cells (Barry and John, 1984). The summary of OH radical scavenging activities has been shown in (Table 1). In general, except the tested compounds **4f**, 5b and **4a** all the other compounds showed effective OH radical stabilizing potentials in a range of 17.96–99.40%. The compound **7d (99.40%)** was found to be hyper reactive toward OH radicals followed by **7b (97.01%)** and **4d (95.21%), whereas** the compounds **4f**, **5b** and **4a** showed no effect on OH radicals.

4.3. Superoxide radical (SOR) scavenging assay

The superoxide radical scavenging activity was measured for the test compound and presented in Table 1. The perusal of Table 1 clearly indicates that except **3**, **4f**, **6d**, 5b and **4f** all the tested compounds showed superoxide radical scavenging activity. The potential of SOR scavenging activity of all other tested compounds were not significant. However, **4d exhibits** at par SOR scavenging potential as compared with the standard (vitamin C).

4.4. Reducing activity

The reducing ability (%) of the tested compounds (1 mM) exhibited very prominent results as shown in Table 1. The overall range of reducing ability of the tested compounds is 102–250. The compounds **3**, **6b**, **4d**, **4f**, **6d**, 5b and **7b** are very promising

reducing agents because they showed significant rise (250%) in their reducing ability as compared with the vitamin C (150%).

5. Antioxidant properties

5.1. DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was carried out as per reported method with slight modifications (Blois, 1958; Roberta et al., 2006). In brief, 1 ml (1 mM) of the test sample is added to equal quantity of 0.1 mM solution of DPPH in ethanol. After 20 min of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid (1 mM) was used as the reference compound.

5.2. OH radical scavenging assay

The OH radicals scavenging activity was demonstrated with Fenton's reaction (Rollet-Labelle et al., 1998). The reaction mixture contained, 60 µl of $FeCl_2$ (1 mM), 90 µl of 1–10 phenanthroline (1 mM), 2.4 ml of phosphate buffer (0.2 M, pH 7.8), 150 µl of H_2O_2 (0.17 M) and 1.5 ml of individual compound (1 mM). The reaction was started by adding H_2O_2. After 5 min. incubation at room temperature, the absorbance was recorded at 560 nm. Ascorbic acid (1 mM) was used as the reference compound.

5.3. Superoxide radical (SOR) scavenging assay

The superoxide anion scavenging assay was performed by the published method (Liu et al., 1997). Superoxide anion radicals were generated in a non-enzymatic phenazine methosulphate–nicotinamide adenine dinucleotide (PMS–NADH) system through the reaction of PMS, NADH and Oxygen. It was assayed by the reduction of Nitroblue tetrazolium (NBT). In this experiment superoxide anion was generated in 3 ml of Tris–HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 mM), 0.75 ml of NADH (936 mM), and 0.3 ml of sample (1 mM). The reaction was initiated by adding 0.75 ml of PMS (120 mM) to the mixture. After 5 min. of incubation at room temperature the absorbance at 560 nm was measured in a spectrophotometer. Ascorbic acid (1 mM) was used as the reference compound.

5.4. Reducing activity

The principle of the reduction assay is that the reducing agents reduce Fe^{3+} to Fe^{2+}. Higher absorbances (as compared to control) of the reaction mixture indicate greater reducing power (Oyaizu, 1986). The reducing power of the tested compounds was determined by the method of Oyaizu. In brief, the test compounds (1 mM) were mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate (K3Fe(CN)6) (1% w/v) followed by incubating at 50 °C in a water bath for 20 min. The reaction was terminated by adding 0.75 ml of trichloroacetic acid solution (10%) and then centrifuged at 800*g* for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1% w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Ascorbic acid (1 mM, 155.7%) was used as a reference compound. The values of absorbances obtained were multiplied by a factor of 100 for the calculation of % reducing power.

6. Conclusion

In conclusion, we have synthesised a series of novel imino pyrido[1,2-*a*]pyrimidine derivatives and have been screened as potent antioxidant agents. The results from our biological activity studies proved that compound **4d** having a *p*-chloro aniline group on the imino pyrido[1,2-*a*]pyrimidine moiety shows good activity when compared with standard ascorbic acid (vitamin C).

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