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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 3-AMINO-11-CYANO-4-IMINO- PYRAZOLO [4, 5-E]-*4H*-PYRIMIDO [2, 1-B] QUINOLINE AND THEIR SUBSTUITED DERIVATIVES

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Abstract
2-Amino-3-cyano quinoline (**1**) and bis (methylthio) methylene malononitrile **(2)** were refluxed in N,N-dimethyl formamide (DMF) in presence of catalytic amount of anhydrous potassium carbonate to afforded 3, 11-dicyano-4-imino-2-methylthio -*4H*-pyrimido [1, 2-*a*] quinoline (**3**). The latter were further reacted with different substituted hydrazino. Afforded to 3-amino-11-cyano-4-imino pyrazolo [4, 5-*e*]-*4H*-pyrimido [2, 1-*b*] quinoline and their 2-substuited derivatives **(4a-j)**. All these newly synthesized compounds were characterized by elemental analysis and spectral data, and screened for their antimicrobial activities.

Keywords : N,N'-dimethyl formamide, Potassium carbonate, hydrazino benzothiazole .

Introduction

The pyrazolo pyrimido quinoline system has shown many interesting biological and pharmacological properties, such as antitubercular activity[1,5], various derivatives of pyrazole possess significant antibacterial[6], antifungal[7] and antiinflammatory [8] activities. The Pharmacolg--ical properties of some pyrazolo quinolines as Antiasthmatic[9], antitumor [10-11]. Very recently, some derivatives of pyrazolo [3,4-b]quinoline were reported as antineoplastic for treatment of precancerous lesions.[13]. A literature survey shows that a number of quinoline derivatives have been synthesized by using various method but not single reference have been found where 2-substuited-3-amino-11-cyano-4-imino pyrazolo[4,5-*e*]-*4H*-pyrimido[2,1-*b*] quinoline is used . A view to obtaining more active heterocyclic system containing two biologically active moieties quinoline[14-17]. The most suitable protocol for the synthesis of functionalized organic compounds would be a one pot reaction due to the fact that the synthesis can performed without the isolation of the intermediates, without discharging any functional groups in short reaction time. Hence in present investigation, we report synthesis of 3,11-dicyano-4-imino-2-methylthio-*4H*-pyrimido[1,2-*a*]quinoline, imino pyrazolo pyrimido quinoline nucleus which have also been recognized as promising antimicrobial activity.

Result and discussion

In our research approach to synthesize title compounds, we have performed reaction to connect 2-amino-3-cyano-quinoline **1** and bis (methylyhio) methylene malononitrile **2** in presence of N,N'-dimethyl formamide catalytic amount of anhydrous Potassium carbonate to form 3,11-dicyano-4-imino-2-methylthio-*4H*-pyrimido[1,2-*a*]quinoline **3** (scheme-1). A plausible mechanism for the formation of parent compound **3** can be adduced as shown in (scheme-2).

The compound **3** possesses replaceable active methylthio group at 2- position which is activated by the ring 1-nitrogen atom and reactive 3-cyano group. Compound **3** was condensed with hydrazine hydrate / phenyl hydrazine / 4-nitro phenyl hydrazine / 2,4-dinitro phenyl hydrazine / 2-hydrazino benzothiazole / 6-methyl -2-hydrazino benzothiazole / 4,6-methyl -2-hydrazino benzothiazole / 6-methoxy -2-hydrazino benzothiazole / 6-chloro -2- hydrazino benzothiazole / 6-nitro -2-hydrazino benzothiazole in presence of N,N'-dimethyl formamide catalytic amount of anhydrous Potassium carbonate afforded the new fused heterocyclic compound **4a-j** (scheme-3).

The confirmation of the product was determined by IR, ^{1}H NMR, Mass and ^{13}C NMR spectral studies. The IR spectrum of compounds shows absorption bands in the region 3100-3400cm^{-1} due to =NH group indicate that the ring gets cyclized. Stretching absorption band in the range of 2190-2230 cm^{-1} for –CN, The presence of ^{1}H-NMR spectra of compounds exhibited singlet peak in the region δ 8.6-9.3 due to=NH proton and one singlet peak observe in the region δ 3.7-4.3 due to $-NH_2$ proton. The MS spectra showed the molecular ion peaks which correspond to molecular weight of respective compounds. The mechanism for the formation can be represented as shown in (scheme-3).

The elemental analysis values are in good agreement with theoretical data. Similarly, all these compounds were characterized on the basis of spectral studies. All the compounds were screened for their antibacterial and antifungal activity.

Experimental Section

Melting points were determined by in an open capillary method and are uncorrected. The chemicals and solvents used for laboratory grade and were purified. IR spectra were recorded (in KBr pallets) on Shimadzu spectrophotometer. ^{1}H NMR spectra were recorded (in DMSO-d_6) on Avance-300 MHz spectrometer using TMS as an internal standard. The mass were recorded on EI-Shimadzu GC–MS spectrometer. Elemental analyses were performed on a Heraeus CHN-O rapid analyzer.

3, 11-Dicyano-4-imino-2-methylthio-*4H*-pyrimido [1, 2-*a*] quinoline (3)

A mixture of 2-anino-3-cyano-quinoline **1** (2.91g**,** 0.01 m mol) and bis (methylthio) methylene malononitrile **2** (1.70 g, 0.01 m mol) was refluxed in the presence of N, N'-dimethyl formamide catalytic amount of anhydrous Potassium carbonate for 4 hrs. The reaction mixture was cooled to room temperature and poured in to ice cold water. The separated solid was filtered, washed with water and recrystallized from N, N' –dimethyl formamide-ethanol mixture to afford compound **3**.

Brown powder, (88% yield), Mp: above 300 ^{o}C; EI-MS (m/z-RA%) : 291 (M^{+}) ,IR (cm^{-1}, KBr) : 3236 (=NH), 2216-2218 (CN), ^{1}H NMR (DMSO-d_6, ppm) : 2.4 (s, 3H, SCH_3), 6.3-7.8 (m, 5H, Ar-H),8.8(s,1H,=NH), ^{13}C NMR (300 MHz, DMSO-d_6, ppm) 15,80,96, 110,115.9, 115.9, 116.2, 118.7,124.7,124.9,127.2,128.7,139.2,156.7,164.9; Anal. Calcd. For: $C_{15}H_9N_5S$; C, 61.84; H, 3.11; N, 24.04; Found: C, 61.38; H, 2.83; N, 23.62.

3-Amino-11-cyano-4-imino-2-(H/phenyl/4'-nitrophenyl / 2,4'-dinitrophenyl/ 2'-benzothiazolyl / 6'-methyl-2'-benzothiazolyl/4,6'-dimethyl-2'-benzothiazolyl/ 6'-methoxy-2'-benzothiazolyl /6'-chloro-2'-benzothiazolyl/6'-nitro-2'-benzothiazolyl) pyrazolo [4, 5 -*e*]-*4H*-pyrimido [2, 1-*b*] quinoline (4a-j)

A mixture of **3** (0.01m mol) and independently with hydrazine hydrate (80 %), phenyl hydrazine, 4-nitro phenyl hydrazine, 2,4-dinitro phenyl hydrazine, 2-hydrazino benzothiazole, 6-methyl 2-hydrazino benzothiazole, 2,4-dimethyl 2-hydrazino benzothiazole, 6-methoxy 2-hydrazino benzothiazole, 6-chloro 2-hydrazino benzothiazole, 6-nitro 2-hydrazino benzothiazole, (0. 01m mol) in DMF and catalytic amount of Anhydrous Potassium carbonate was refluxed for 4 hrs. The reaction mixture was cooled to room temperature and poured in to 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-j.**

3-Amino-11-cyano-4-imino-2-(H) pyrazolo [4, 5 -*e*]-*4H*-pyrimido [2, 1-*b*] quinoline (4a)

Brown powder, (91% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3380 (=NH); NH_2 asymetric and symetric 3320 , 3214, 2219 (CN), ^{1}H NMR (DMSO-d_6, ppm): 4.0 (s, 2H, Ar-NH_2) , 6.4-7.1 (m, 4H), 7.8 (s, 1H, CH=C), 9.1 (br s, 1H, =NH), 13.8 (s, 1H, NH) . EI-MS (m/z RA%):276 (M+**1**) Anal. Calcd : $C_{14}H_9N_7$; C, 61.09; H, 3.30; N, 35.62; Found: C, 60.54 ; H, 2.98; N, 35.12.

3-Amino-11-cyano-4-imino-2-(phenyl) pyrazolo [4, 5 -*e*]-*4H*-pyrimido [2, 1-*b*] quinoline (4b)

Brown powder, (84% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3384 (=NH);NH_2 asymetric and symetric 3325 , 3216, 2216 (CN), ^{1}H NMR (DMSO-d_6, ppm): 4.1 (s, 2H, Ar-NH_2), 6.2-7.1 (m, 4H), 7.3 (s, 5H,Ar-H),7.6 (s, 1H,CH=C), 9.2 (br s, 1H, =NH), EI-MS (m/z RA%): 351 (M^{+}) Anal. Calcd : $C_{20}H_{13}N_7$; C, 68.37; H, 3.73; N, 27.90; Found: C, 68.01; H, 2.91; N, 27.22.

3-Amino-11-cyano-4-imino-2-(4'-nitrophenyl)pyrazolo[4,5-*e*]-*4H*-pyrimido[2,1-*b*]quinoline (4c)

Brown powder, (89% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3378 (=NH); NH_2 asymetric and symetric 3328 , 3219, 2211 (CN), ^{1}H NMR (DMSO-d_6, ppm): 4.0 (s, 2H, Ar-NH_2), 6.3-7.0 (m, 4H), 7.5 (s, 2H, Ar-H), 7.7 (s, 1H,CH=C), 8.2 (s, 2H,Ar-H), 9.2 (br s, 1H, =NH), EI-MS (m/z RA%): 396 (M^{+}) Anal. Calcd : $C_{20}H_{12}N_8O_2$; C, 60.60; H, 3.05; N, 28.27; Found: C, 60.19; H, 2.74; N, 27.86.

3-Amino-11-cyano-4-imino-2-(2,4'-dinitrophenyl)pyrazolo[4,5-*e*]-*4H*-pyrimido[2,1-*b*] quinoline (4d)

Brown powder, (82% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3388 (=NH); NH_2 asymetric and symetric 3321 , 3212, 2214 (CN), ^{1}H NMR (DMSO-d_6, ppm): 4.3 (s, 2H, Ar-NH_2), 6.5-7.0 (m, 4H), 7.6 (s, 1H,CH=C), 7.9 (s, 1H,Ar-H), 8.7-9.1 (s, 2H,Ar-H), 9.3 (br s, 1H, =NH), EI-MS (m/z RA%): 441 (M^{+}) Anal. Calcd : $C_{20}H_{11}N_9O_4$; C, 54.43; H, 2.51; N, 28.56; Found: C, 54.01; H, 2.14; N, 27.16.

3-Amino-11-cyano-4-imino-2-(2'-benzothiazolyl) pyrazolo[4,5-*e*]-*4H*-pyrimido[2,1-*b*] quinoline (4e)

Brown powder, (78% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3381 (=NH); NH_2 asymetric and symetric 3324 , 3218, 2205 (CN), ^{1}H NMR (DMSO-d_6, ppm): 4.0 (s, 2H, Ar-NH_2), 6.4-6.9 (m, 4H), 7.7 (s, 1H,CH=C), 7.8-8.1 (s, 4H, Ar-H), 9.3 (br s, 1H, =NH), EI-MS (m/z RA%): 408 (M^+) Anal. Calcd : $C_{21}H_{12}N_8S$; C, 61.75; H, 2.96; N, 27.43; Found: C, 61.31; H, 2.24; N, 27.02.

3-Amino-11-cyano-4-imino-2-(6'-methyl-2'-benzothiazolyl) pyrazolo[4,5-*e*]-*4H*-pyrimido [2,1-*b*] quinoline (4f)

Brown powder, (83% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3389 (=NH); NH_2 asymetric and symetric 3321 , 3212, 2196 (CN), ^{1}H NMR (DMSO-d_6, ppm): 2.3 (s, 3H, Ar-CH_3), 4.2 (s, 2H, Ar-NH_2), 6.3-7.1 (m, 4H), 7.7 (s, 1H,CH=C), 7.6-7.9 (s, 3H, Ar-H), 9.1 (br s, 1H, =NH),EI-MS (m/z RA%): 422 (M^+) Anal. Calcd : $C_{22}H_{14}N_8S$; C, 62.55; H, 3.34; N, 26.52;Found: C, 62.09; H, 3.01; N, 26.12.

3-Amino-11-cyano-4-imino-2-(4,6'-dimethyl-2'-benzothiazolyl) pyrazolo[4,5-*e*]-*4H*-pyrimido [2,1-*b*] quinoline (4g)

Brown powder, (79% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3384 (=NH);NH_2 asymetric and symetric 3328 , 3216, 2222 (CN), ^{1}H NMR (DMSO-d_6, ppm): 2.3 (s, 6H, Ar-CH_3), 4.0 (s, 2H, Ar-NH_2), 6.4-7.0 (m, 5H), 7.6 (s, 1H,CH=C), 7.8 (s, 1H, Ar-H), 8.8 (br s, 1H, =NH),EI-MS (m/z RA%): 436 (M^+) Anal. Calcd : $C_{23}H_{16}N_8S$; C, 60.26; H, 3.22; N, 25.56; Found: C, 60.00; H, 3.01; N, 25.04.

3-Amino-11-cyano-4-imino-2-(6'-methoxy-2'-benzothiazolyl) pyrazolo[4,5-*e*]-*4H*-pyrimido [2,1-*b*] quinoline (4h)

Brown powder, (76% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3384 (=NH);NH_2 asymetric and symetric 3328 , 3216, 2212 (CN), ^{1}H NMR (DMSO-d_6, ppm): 3.6 (s, 3H, Ar-OCH_3), 4.1 (s, 2H, Ar-NH_2), 6.5-7.0 (m, 5H), 7.5 (s, 1H,CH=C), 7.8-8.1 (s, 2H, Ar-H), 8.6 (br s, 1H, =NH); EI-MS (m/z RA%): 439 (M+1) Anal. Calcd : $C_{22}H_{14}N_8OS$; C, 60.26; H, 3.22; N, 25.56; Found: C, 60.02; H, 2.97; N, 25.03.

3-Amino-11-cyano-4-imino-2-(6'-chloro-2'-benzothiazolyl) pyrazolo[4,5-*e*]-*4H*-pyrimido [2,1-*b*] quinoline (4i)

Brown powder, (84% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3376 (=NH);NH_2 asymetric and symetric 3318 , 3224, 2205 (CN), ^{1}H NMR (DMSO-d_6, ppm): 4.0 (s, 2H, Ar-NH_2), 6.4-7.5 (m, 5H), 7.7 (s, 1H,CH=C), 8.1 (s, 2H, Ar-H), 8.6 (br s, 1H, =NH),EI-MS (m/z RA%): 442 (M+1); Anal. Calcd : $C_{21}H_{11}ClN_8S$; C, 56.95; H, 2.50; N, 25.30; Found: C, 56.42; H, 2.08; N, 24.92.

3-Amino-11-cyano-4-imino-2-(6'-nitro-2'-benzothiazolyl) pyrazolo[4,5-*e*]-*4H*-pyrimido[2,1-*b*] quinoline (4j)

Brown powder, (89% yield), Mp: above 300^{o}C; IR (cm^{-1},KBr) : 3388 (=NH);NH_2 asymetric and symetric 3311 , 3218, 2218 (CN), ^{1}H NMR (DMSO-d_6, ppm): 4.2 (s, 2H, Ar-NH_2), 6.4-7.0 (m, 4H), 7.7 (s, 1H,CH=C), 8.5-8.9 (s, 3H, Ar-H), 9.2 (br s, 1H, =NH),EI-MS (m/z RA%): 453 (M+1); Anal. Calcd : $C_{21}H_{11}N_9O_2S$; C, 55.63; H, 2.45; N, 27.80; Found: C, 55.12; H, 2.10; N, 27.32.

Antimicrobial activity

All the compounds were for their antifungal and antibacterial activity against species *Aspergillus flavus, Aspergillus niger* and *E. coli* and *B. Subtilis* by paper disc diffusion method [18] . Control for fungal and bacterial species using Disc diffusion method, solvent DMSO was used, Fungi- Czapek's dox agar, Bacteria- Nutrient agar. Incubation period for fungi 4 days (24+/- 2 ^{0}C) and for bacteria 24 hrs (37 ^{0}C). The synthesized compounds exhibited zone of inhibition of 10-31 mm in diameter, where as standard Fluconazole exhibited zone of inhibition of 26 and 25 mm .

Streptomycin exhibited zone of inhibition of 32 and 30 mm in diameter against *E. coli* and *B. Subtilis* respectively. Amongst the synthesized compounds 3, compound 4h (18, 19 mm) , 4i (20, 18 mm) and 4j (16,18 mm) showed higher zone of inhibition against *Aspergillus flavus , Aspergillus niger* respectively. And 4h (07, 12 mm) , 4i (30, 28 mm) and 4j (22,08 mm) showed higher zone of inhibition against *E. coli* , *B. Subtilis* It seems that the presence of methoxy, nitro and chloro group at 6- position 4i increases antifungal activity.

Sr. No	**code**	**Zone of inhibition in mm**			
		Fungal species		**Bacterial species**	
		Af	***An***	***Ec***	***Bs***
1	3	14	15	13	22
2	4a	--	12	--	17
3	4b	11	10	12	16
4	4c	14	--	11	18
5	4d	13	12	15	16
6	4e	14	**--**	--	10
7	4f	15	15	17	20
8	4g	16	10	11	16
9	4h	18	19	07	12
10	4i	20	18	30	28
11	4j	16	18	22	08
Positive control		26	25	32	30
		Fluconazole		**Streptomycin**	

Conclusion

In this communication all synthesized compounds reported first time and describe the simple route of their synthesis in mild condition with good yield. The present study showed that all the newly synthesized compounds were exhibiting significant antibacterial and antifungal activities. However, further studies are required to establish the mechanism of action of the title compounds. From the screening data it was found that 4h,4i,4j derivative have encouraging antifungal activity, which need to be further investigation to get better antifungal and antibacterial agents.

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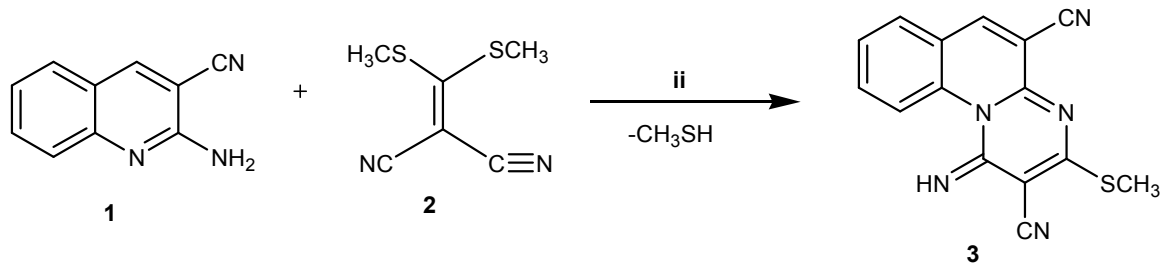
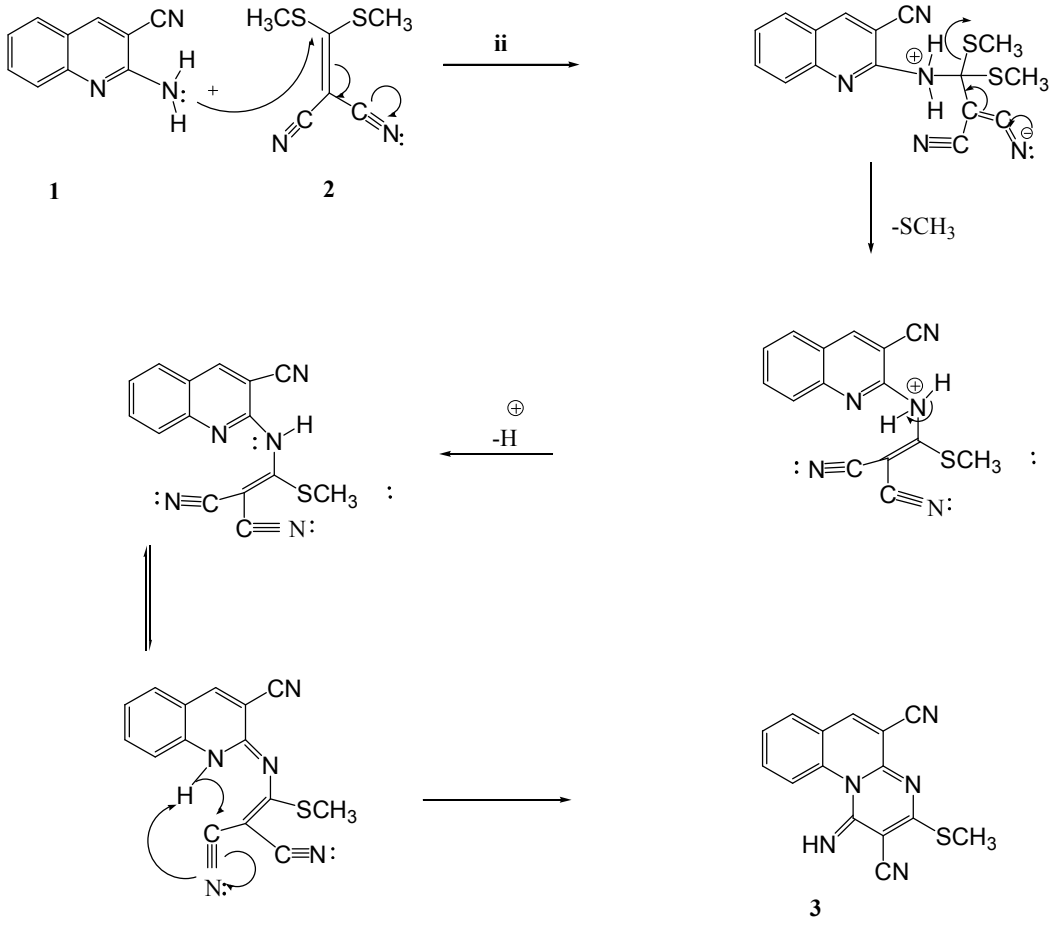


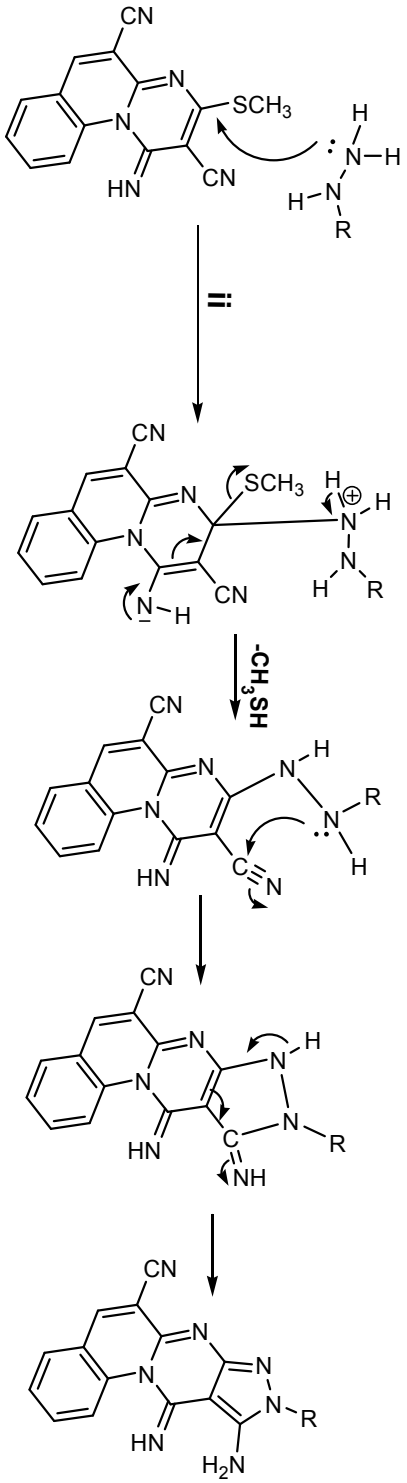
Reaction and condition ii] DMF / Anhy.K_2CO_3 4 hrs reflux

Scheme 1



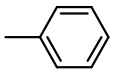
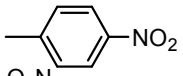
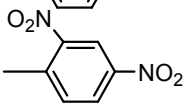
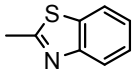
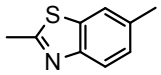
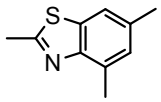
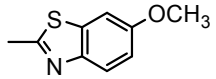
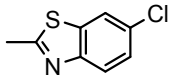
Scheme 2.

Mechanism: Plausible mechanism 3, 11-Dicyano-4-imino-2-methylthio -4H-pyrimido [1, 2-a] quinoline



4(a- j)

Scheme 3

	R
4a :	-H
4b :	
4c :	
4d :	
4e :	
4f :	
4g :	
4h :	
4i :	
4j: