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Synthesis of some novel pyrazolo[3,4-*d*]pyrimidine derivatives as potential antimicrobial agents

Bantwal Shivarama Holla,[a,*] Manjathuru Mahalinga,[a] Mari Sitambaram Karthikeyan,[a] Padiyath Mohamed Akberali[b] and Nalilu Sucheta Shetty[c]

[a]*Department of Chemistry, Mangalore University, Mangalagangothri 574199, India*
[b]*Strides Arcolab Limited, 120 A&B, Industrial Area, Baikampady, New Mangalore, India*
[c]*Department of Biochemistry, Justice K. S. Hegde Medical Academy, Deralakatte, India*

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Abstract—The reaction of 4-hydrazino-8-(trifluoromethyl)quinoline (**2**) with ethoxymethylenecyanoacetate afforded ethyl 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylate (**3**) and that with ethoxymethylenemalononitrile afforded 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carbonitrile (**5**). Compounds **3** and **5** were hydrolyzed to get 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylic acid and then reacted with acetic anhydride to afford 6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]pyrazolo[3,4-*d*]oxazin-4-one (**6**), which was condensed with different aromatic amines to give a series of 5-substituted 6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones (**7**). Compounds **3** and **5** also reacted with formamide, urea, and thiourea affording the corresponding pyrazolo[3,4-*d*]pyrimidines (**8**–**13**), respectively. Structures of the products have been determined by chemical reactions and spectral studies. All compounds of the series have been screened for their antibacterial and antifungal activity studies. The results are summarized in Tables 1 and 2.

1. Introduction

Pyrazole and pyrimidine derivatives attracted organic chemists very much due to their biological and chemotherapeutic importance. Pyrazolopyrimidines and related fused heterocycles are of interest as potential bioactive molecules. They are known to exhibit pharmacological activities such as CNS depressant,[1] neuroleptic[2], and tuberculostatic.[3] Pyrazolo[3,4-*d*]pyrimidines were identified as a general class of adenosine receptors.[4,5] There is not much difference in the basic structures of pyrazolopyrimidines and purines. In the literature, we have found that the replacement of 1*H* of pyrazole of pyrazolo[3,4-*d*]pyrimidine ring system by some other bioactive moiety drastically alters its pharmacological properties. Keeping this in mind, we have contemplated on the synthesis of pyrazolo[3,4-*d*]-pyrimidine derivatives bearing a fluorinated heterocyclic moiety, particularly 8-trifluoromethylquinoline.

Moreover, in recent years, fluorinated compounds find much importance in the pharmaceutical field.[6] Fluorinated compounds in general, fluorinated heterocycles in particular, are those focused much in modern-day medicinal chemistry. Incorporation of a fluorine atom instead of a hydrogen one can alter the course of the reaction as well as biological activities.[7–9] Further introduction of a fluorine atom as the CF_3 group provides a more lipophilically and pharmacologically interesting compound compared to their non-fluorinated analogues. The trifluoromethyl substituted compounds have been reported to possess biological activities as herbicides,[10] fungicides,[11] analgesic,[12] antipyretic[13], and inhibitors for platelet aggregation.[14]

Prompted by the varied biological activities of pyrazolo[3,4-*d*]pyrimidine derivatives and trifluoromethyl-bearing compounds, we envisioned our approach toward the synthesis of a novel series of pyrazolo-[3,4-*d*]pyrimidine derivatives bearing a trifluoromethyl

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* Corresponding author. Tel.: +91 0824 2287262; fax: +91 0824 2287367/2287424; e-mail: hollashivarama@yahoo.co.in

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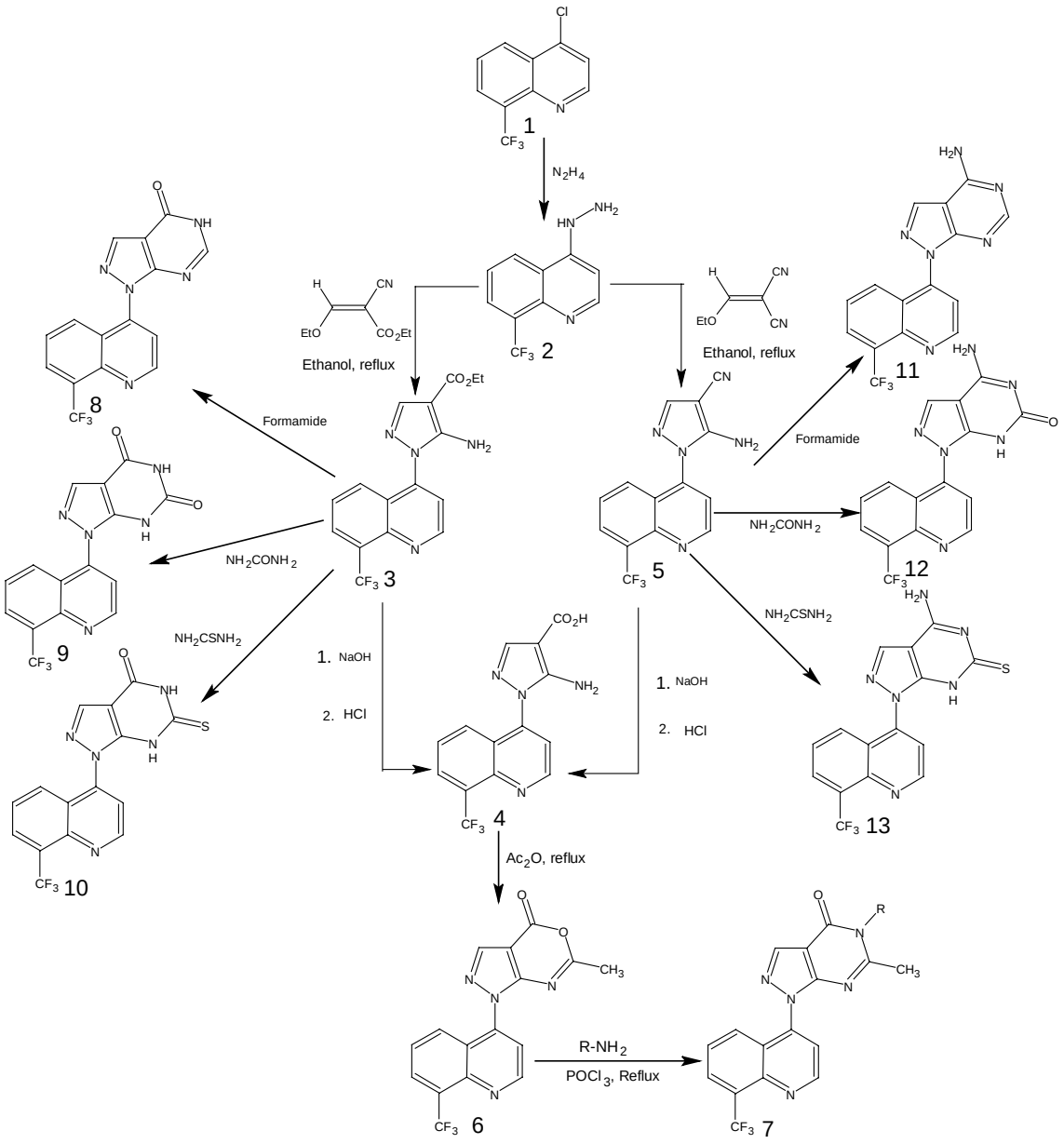

Figure 1. Synthesis of pyrazolo[3,4-*d*]pyrimidines.

quinoline moiety and to study the biological activity (Fig. 1).

2. Results and discussion

2.1. Chemistry

Condensation of 4-hydrazino-8-(trifluoromethyl)quinoline (**2**) with ethoxymethylenecyanoacetate afforded ethyl-5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylate (**3**). The 400 MHz ^{1}H NMR of (**3**) showed the signal at δ 1.4 triplet (3H) and 4.3 quartet (2H) indicating the presence of an ethyl group of the ester, 5.3 singlet corresponding to two protons indicating the presence of NH_2 group in the pyrazole ring (which disappeared in D_2O exchange), and 7.9 singlet, which corresponds to the pyrazole ring proton and quinoline ring protons, appeared as four doublets and one triplet integrating for five protons. In the mass spectrum of (**3**), the molecular ion peak (MH^+) appeared at *m*/*z* 351 (MH^+, 100%), which is in accordance with its molecular formula. Condensation of 4-hydrazino-8-(trifluoromethyl)quinoline (**2**) with ethoxymethylenemalononitrile afforded 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carbonitrile (**5**). Formation of compound (**5**) was very clear from its spectral data. Thus, ^{1}H NMR (400 MHz DMSO-d_6) showed peaks at δ 6.92, a singlet, integrating for two protons indicating the presence of a NH_2 group in the pyrazole ring (which disappeared in D_2O exchange) and all the aromatic protons exactly matching the structure. The formation of compound (**5**) was also confirmed by recording its mass spectrum. The molecular ion peak *m*/*z* appeared at 304 (MH^+, 100%), which is in accordance with its molecular formula. Compounds (**3**) and (**5**) underwent basic hydrolysis to

give compound (**4**) in excellent yield. The spectral data revealed the formation of the compound (**4**). Compound (**4**), when heated with acetic anhydride, gave a cyclized product, 6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]-pyrazolo[3,4-*d*]oxazin-4-(1*H*)-one (**6**). ^{1}H NMR spectrum revealed that the disappearance of both NH_2 and OH peaks, and the appearance of a singlet at δ 2.4, which corresponds to methyl protons, confirm the formation of the compound (**6**). Mass spectrum of compound (**6**) exhibited a molecular ion peak at *m*/*z* 346, which appeared as a base peak and strongly indicated the formation of the compound. The compound (**6**) was treated with substituted aromatic amines in the presence of phosphorus oxychloride to give title compounds (**7a**–**m**). The newly synthesized compounds were analyzed for N content and their structures were confirmed by IR, NMR, and mass spectral data.

Compounds (**3**) and (**5**) were fused with formamide, urea, and thiourea to afford the corresponding pyrazolo-[3,4-*d*]pyrimidines (**8**)–(**13**), respectively. The formation of fused pyrimidines was confirmed by recording their spectra. Thus, the 400 MHz ^{1}H NMR spectrum of compound (**8**) showed a downfield signal at δ 12.56 corresponding to the NH peak, and the NH_2 and CH_2CH_3 signals were absent in the spectrum. Thus, it confirmed the formation of a cyclized product (**8**). Mass spectrum of the compound (**8**) showed a molecular ion peak at m/z 332 (MH^+) which appeared as a base peak strongly supporting the formation of the compound (**8**). Compound (**5**), on reacting with formamide, afforded fused pyrazolopyrimidine (**11**). The formation of the compound (**11**) was confirmed by its spectral data. Thus, the 400 MHz ^{1}H NMR of (**11**) showed a signal at δ 7.3 corresponding to the NH_2 peak and all other peaks exactly matching its structure. In the IR spectrum, the absence of a CN peak indicated that the cyclization was completed. The mass spectrum of the compound (**11**) also confirmed the formation of the molecule. The compounds (**9**), (**10**), (**12**), and (**13**) also confirmed the same by recording their spectra. The structural elucidations of all compounds were carried out on the basis of elemental analysis and spectral data. The spectral data of all these compounds supported the assigned structures.

2.2. Biological activity

2.2.1. Antibacterial activity. The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATTC-27853), and *Bacillius subtilis* (recultured) bacterial stains by the disk diffusion method.[15,16] Disks measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottles and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using *N*,*N*-dimethylformamide. 1 ml containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 discs. Disks of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Streptomycin was used as a standard drug at a concentration of 10 μg/ml. Solvent and growth controls were kept and zones of inhibition were noted. The results of such studies are given in Table 1.

The above data showed that compound 1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**8**) was the most potent compound, which exhibited very good activity against the four organisms. The compounds 6-thioxo-1-[8-(trifluoro-

Table 1. Antibacterial activity data of prepared compounds

Compound	*Escherichia coli* (ATTC-25922)	*Staphylococcus aureus (ATTC-25923)*	*Pseudomonas aeruginosa* (ATTC-27853)	*Bacillius subtilis* (recultured)
7a	12	12	13	9
7b	12	22	10	8
7c	9	—	—	—
7d	9	13	8	10
7e	14	13	17	14
7f	—	13	14	14
7g	—	—	13	13
7h	18	15	12	12
7i	14	—	10	—
7j	14	19	9	9
7k	—	19	10	—
7l	15	18	8	11
7m	14	—	—	11
8	28	25	24	26
9	22	22	18	17
10	26	26	22	23
11	20	18	17	18
12	19	19	20	21
13	24	22	20	20
Standard (Streptomycin)	20	21	24	24

Zone of inhibition in millimeters.

methyl)quinolin-4-yl]-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**10**) and 4-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thione (**13**) exhibited very good activity against *E. coli* and *S. aureus*. The remaining compounds were found to have slight or moderate activity against the tested organisms and some of the compounds were found to be inactive (indicated — sign).

2.2.2. Antifungal activity. Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* (NICM No. 524), *Aspergillus fumigatus* (NCIM No. 902), *Candida albicans* (NCIM No. 300), *Penicillium marneffei* (recultured), and *Trichophyton mentagrophytes* (recultured) in DMSO by the serial plate dilution method.[17,18] Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of the corresponding species. Agar media (20 ml) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with that of flucanazole as standard.

The antifungal data showed that the newly prepared compounds have moderate to good activity against the above-mentioned organisms. The compound 4-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (**11**) exhibited very good activity against *A. flavus*, *A. fumigatus*, and *T. mentagrophytes*. Compound 4-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thione (**13**) showed very good activity toward *A. flavus* and *A. fumigatus*. The remaining compounds were found to have slight or moderate activity against the tested organisms and some of the compounds were found to be inactive (indicated — sign) (Table 2).

3. Conclusion

We have successfully synthesized a series of 6-methyl-5-trifluromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones and fused pyrimidines containing C═O/C═S/NH_2 functional groups. The antibacterial and antifungal activity data of the prepared compounds showed that the fused pyrimidines (**8**–**13**) showed very good antimicrobial activity compared to those of the compounds (**7a**–**m**). Basically pyrazolo[3,4-*d*]pyrimidines are analogs of purine nucleus and various structural changes of the natural purines have resulted in potent antagonists in biological systems. In our study, we have replaced the H atom of the pyrazole ring by 8-trifluoromethylquinoline as an active moiety these structural changes made fused pyrimidines (**8**–**13**) more active towards tested organisms. Further, incorporation of CF_3 group in the quinoline ring increased lipophilicity as well as biological activity.

4. Experimental

Melting points were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Perkin-Elmer 157 IR spectrophotometer. PMR spectra were recorded in DMSO-d_6 on either a Bruker AC-300F (300 MHz) or an amx 400 (400 MHz) NMR spectrometer using TMS as an internal standard. Chemical shift values are given in δ scale. The mass spectra of some of the selected compounds were recorded on a MASPEC low resolution

Table 2. Antifungal activity data of prepared compounds (**6a**–**m**)

Compound	*Aspergillus flavus* (NICM No. 524)	*Aspergillus fumigatus* (NCIM No. 902)	*Penicillium marneffei* (recultured)	*Candida Albicans* (NCIM No. 300)	*Trichophyton Mentagrophytes* (recultured)
7a	—	—	18	15	17
7b	13	—	12	10	10
7c	12	15	—	—	9
7d	20	15	18	14	15
7e	—	—	—	—	—
7f	12	10	13	18	—
7g	15	16	21	17	16
7h	—	17	—	—	18
7i	18	17	18	15	19
7j	—	17	—	—	—
7k	19	21	14	16	14
7l	16	18	15	18	19
7m	15	—	—	—	—
8	18	17	15	20	18
9	17	20	18	17	17
10	20	18	17	16	19
11	25	22	19	18	24
12	15	18	23	16	17
13	25	24	20	17	19
Standard (Flucanazole)	21	18	21	20	19

Zone of inhibition in millimeters.

mass spectrometer operating at 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on a silica gel plate.

4.1. Synthesis of 4-hydrazino-8-(trifluoromethyl)quinoline (2)

To 4-Chloro-8-trifluoromethyl quinoline (**1**) (25 g, 0.1 mol) in 100 ml ethanol was added hydrazine hydrate (100%) (10 ml). After being stirred at 80 °C for 8 h in the absence of light, the reaction mixture was diluted with water, the resulting precipitate collected and recrystallized from ethanol to give compound (**2**) as light yellow needles (20.2 g, 82.5%); mp 198–200 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.54 (br s, 2H, NH_2), 7.03 (d, 1H, J = 8.1 Hz), 7.46 (t, 1H, J = 7.7 Hz), 7.98 (d, 1H, J = 7.2 Hz), 8.41 (d, 1H, J = 8.3 Hz), 8.66 (d, 1H, J = 4.5 Hz), 8.71 (s, 1H, NH); IR (KBr) ν: 3388, 3394 (NH str), 3038, 2942 (Ar–H str), 1028 (C–F str) cm^{-1}; MS: (MH^+, %) m/z 228 (MH^+, 100), 227 (50), 192 (10), 166 (5), 120 (5), 107 (10).

4.2. Synthesis of ethyl 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylate (3)

Ethoxymethylenecyanoacetate (14.8 g, 0.09 mol) was carefully added in small portions to (20 g, 0.09 mol) of 4-hydrazino-8-(trifluoromethyl)quinoline. The reaction mass was heated to 80 °C for 1 h on a water bath, the completion of the reaction monitored by TLC. Cooled to room temperature and to the reaction mass added 100 ml water, stirred for 2 h, the solid obtained was filtered, washed with water, and suck dried. Crystallization from ethanol gave compound (**3**) as colorless crystals (18 g, 58.4%); mp 138–140 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.4 (t, 3H, CH_3), 4.3 (q, 2H, CH_2), 5.3 (s, 2H, NH_2), 7.6 (d, 1H, J = 8.0 Hz), 7.7 (t, 1H, J = 8.8 Hz), 7.9 (s, 1H, pyrazole ring), 8.1 (d, 1H, J = 7.9 Hz), 8.2 (d, 1H, J = 8.2 Hz), 9.3 (d, 1H, J = 4.5 Hz); IR (KBr) ν: 3380 (NH str), 3035, 2940 (Ar–H str), 1720 (CO str), 1026 (C–F str) cm^{-1}; MS: (MH^+, %) m/z 351 (MH^+, 100), 350 (20), 323 (8), 305 (50), 273 (10), 218 (5), 196 (2), 107 (10).

4.3. Synthesis of 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carbonitrile (5)

Ethoxymethylenemalononitrile (10.8 g, 0.09 mol) was carefully added in small portions to (20 g, 0.09 mol) of 4-hydrazino-8-(trifluoromethyl)quinoline. The reaction mass was heated to 80 °C for 1 h on a water bath, the completion of the reaction monitored by TLC. Cooled to room temperature and to the reaction mass added 100 ml of water, stirred for 2 h, the solid obtained was filtered and washed with water. Crystallization from ethanol gave, compound (**5**) as colorless crystals (15.5 g, 58%); mp 238–240 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ: 6.98 (s, 2H, NH_2), 7.79 (d, 1H, J = 8.2 Hz), 7.82 (t, 1H, J = 8.0 Hz), 7.98 (s, 1H, pyrazole ring), 8.02 (d, 1H, J = 7 Hz), 8.30 (d, 1H, J = 8.5 Hz), 9.20 (d, 1H, J = 4.6 Hz); IR (KBr) ν: 3038, 2950 (Ar–H str), 2240 (CN str), 1022 (C–F str) cm^{-1}; MS: (MH^+, %) m/z 304 (MH^+, 100), 303 (10), 277 (5), 218 (5), 196 (6), 107 (8).

4.4. Synthesis of 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylic acid (4) (method A)

A mixture of ethyl 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylate (**3**) (17 g, 0.05 mol) and sodium hydroxide (4.2 g, 0.1 mol) was dissolved in 65 ml of methanol and 21 ml water. The contents were heated to reflux on a water bath for 5 h and the completion of the reaction was monitored by TLC. Cooled to room temperature and the reaction mass poured into 340 ml ice water, then adjusting pH of the reaction mass to four using concentrated hydrochloric acid, the solids obtained were filtered, washed with water, and crystallized from methanol yielding (**4**) (14.2 g, 78%); mp 234–36 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.6 (s, 2H, NH_2), 7.4 (d, 1H, J = 8.4 Hz), 7.6 (t, 1H, J = 8.0 Hz), 7.7 (s, 1H, pyrazole ring), 8.0 (d, 1H, J = 7.8 Hz), 8.2 (d, 1H, J = 8.2 Hz), 9.2 (d, 1H, J = 4.5 Hz); IR (KBr) ν: 3410 (OH str), 3382 (NH str), 3035, 2940 (Ar–H str), 1678 (C=O str) 1026 (C–F str); MS: (MH^+, %) m/z 323 (M^+, 65), 322 (15), 307 (40), 279 (5), 228 (100), 196 (6), 120 (10), 107 (8).

4.5. Synthesis of 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylic acid (4) (method B)

5-Amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carbonitrile (**5**) (10 g, 0.03 mol) and sodium hydroxide (4 g, 0.1 mol) in 20 ml water were heated on a water bath for 15 h. After cooling to 10 °C the reaction mixture was acidified using acetic acid and the precipitated product was filtered, washed with water, and recrystallized from methanol to yield (**4**) (6.5 g, 61%); mp 234–36 °C. Spectral data showed that the product obtained in method B is exactly matching the product obtained in method A.

4.6. Synthesis of 6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]pyrazolo[3,4-*d*]oxazin-4(1*H*)-one (6)

5-Amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylic acid (**4**) (13 g 0.04 mol) taken in 26 ml acetic anhydride was heated to reflux on an oil bath for 8 h. It was then cooled to room temperature and poured into ice-cold water, stirring for 30 min. The solid was filtered, washed with water, and then crystallized from ethyl acetate to obtain (11.3 g, 81.2%) pure product (**6**); mp 220–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.4 (s, 3H, CH_3), 7.65 (d, 1H, J = 8.1 Hz), 7.69 (t, 1H, J = 8.8 Hz), 8.14 (d, 1H, J = 8.5 Hz), 8.19 (d, 1H, J = 7.4 Hz), 8.31 (s, 1H, pyrazole ring), 9.26 (d, 1H, J = 4.8 Hz); IR (KBr) ν: 3035, 2940 (Ar–H str), 1710 (C=O str), 1026 (C–F str) cm^{-1}; MS: (M^+, %) m/z 346 (M^+, 100), 303 (80), 276 (40), 210 (10), 177 (40), 149 (35), 77 (12), 60 (30), 52 (20).

4.7. Synthesis of 5-(5-chloro-2-methylphenyl)-6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7a)

To the compound (**6**) (1.0 g, 0.003 mol) taken in 10 ml phosphorus oxychloride was added one molar equivalent of 5-chloro-2-methyl aniline and heated to 100–105 °C for

8 h on an oil bath. It was then cooled to room temperature and poured into ice-cold water, neutralized with sodium bicarbonate solution, the solid filtered, and washed with water. The crude product was recrystallized from ethyl acetate to obtain 0.8 g, 57.1 % of pure product; mp 291–293 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.12 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 7.21 (s, 1H), 7.38 (d, 1H, J = 8.2 Hz), 7.44 (d, 1H, J = 8.1 Hz), 7.69 (t, 1H, J = 7.9 Hz), 7.81 (d, 1H, J = 8.5 Hz), 8.19 (d, 1H, J = 7.1 Hz), 8.33 (d, 1H, J = 8.5 Hz), 8.4 (s, 1H, pyrazole ring), 9.26 (d, 1H, J = 4.7 Hz); IR (KBr) v: 3035, 2940 (Ar–H str), 1710 (C=O str), 1026 (C–F str), 816 (C–Cl str) cm^{-1}; MS: (M^+, %) m/z 471 (M^{2+}, 2), 469 (M^+, 5) 454 (100), 370 (35), 305 (5), 227 (30), 196 (20), 149 (20), 60 (16). Anal. Calcd for N: 14.88. Found: 14.90.

4.8. Synthesis of 5-(3-chloro-4-fluorophenyl)-6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7b)

Obtained from compounds (**6**) and 2-chloro-4-fluoro aniline; light yellow needles (0.75 g, 55%); mp 249–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.13 (s, 3H, CH_3), 7.22–8.00 (m, 7H, Ar–H), 8.2 (s, 1H, pyrazole ring), 9.25–9.26 (d, 1H, J = 4.7 Hz); IR (KBr) v: 3030, 2942 (Ar–H str), 1713 (C=O str), 1026 (C–F str), 876 (C–Cl str) cm^{-1}; MS: (M^+, %) m/z 473 (M^+, 5), 455 (5), 377 (6), 340 (40), 304 (11), 278 (10), 236 (10), 170 (35), 149 (100), 109 (10), 6 (17); Anal. Calcd for N: 14.75. Found: 14.78.

4.9. Synthesis of 6-methyl-5-(4-methylphenyl)-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7c)

Obtained from compounds (**6**) and 4-methyl aniline; light yellow needles (0.8 g, 64%); mp 245-48 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.7 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 7.25–8.20 (m, 9H, Ar–H), 9.27–9.29 (d, 1H, J = 4.5 Hz); IR (KBr) v: 3033, 2945 (Ar–H str), 1703 (C=O str), 1021 (C–F str) cm^{-1}; MS: (M^+, %) m/z 435 (M^+, 15), 379 (6), 342 (10), 304 (10), 235 (10), 147 (30), 57 (100); Anal. Calcd for N: 16.05. Found: 15.88.

4.10. Synthesis of 6-methyl-5-[2-(trifluoromethyl)phenyl]-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7d)

Obtained from compounds (**6**) and 2-trifluoromethyl aniline; amorphous powder (0.78 g, 52%); mp 240–42 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.16 (s, 3H, CH_3), 7.26–8.20 (m, 9H, Ar–H), 9.25–9.27 (d, 1H, J = 4.6 Hz); IR (KBr) v: 3039, 2935 (Ar–H str), 1713 (C=O str), 1021, 1049 (C–F str) cm^{-1}; Anal. Calcd for N: 14.30. Found: 14.31.

4.11. Synthesis of 6-methyl-5-phenyl-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7e)

Obtained from compounds (**6**) and aniline; colorless solid (0.65 g, 50%); mp 255–257 °C; IR (KBr) v: 3038, 2955 (Ar–H str), 1708 (C=O str), 1021 (C–F str) cm^{-1}; MS: (M^+, %) m/z 421 (M^+, 5), 378 (100), 335 (10), 312 (5), 244 (10), 184 (5), 118 (15); Anal. Calcd for N: 16.55. Found: 16.58.

4.12. Synthesis of 6-methyl-5-(4-methyl-2-nitrophenyl)-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7f)

Obtained from compounds (**6**) and 4-methyl-2-nitro aniline; yellow solid (0.80 g, 54%); mp 267-269 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.43 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 6.89–77.69, (m, 7H, Ar–H), 8.1 (s, 1H, pyrazole ring), 9.19–9.21 (d, 1H, J = 4.7 Hz); IR (KBr) v: 3035, 2932 (Ar–H str), 1723 (C=O str), 1076 (C–F str) cm^{-1}. Anal. Calcd for N: 18.74. Found: 18.74.

4.13. Synthesis of 5-(2,5-dichlorophenyl)-6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo-[3,4-*d*]pyrimidin-4-one (7g)

Obtained from compounds (**6**) and 2,5 dichloro aniline; colorless crystals (0.78 g, 52.7%); mp 280–282 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 7.39 (s, 1H), 7.51 (d, 1H, J = 8.6 Hz), 7.60 (d, 1H, J = 8.7 Hz), 7.70 (t, 1H, J = 7.9 Hz), 7.81 (d, 1H, J = 4.7 Hz), 8.19 (d, 1H, J = 7.2 Hz), 8.32 (d, 1H, J = 8.3 Hz), 8.43 (s, 1H, pyrazole ring), 9.27 (d, 1H, J = 4.7 Hz). IR (KBr) v: 3030, 2952 (Ar–H str), 1713 (C=O str), 1106 (C–F str), 845 (C–Cl str) cm^{-1}; MS: (M^+, %) m/z 490 (M^+, 100), 454 (10), 412 (15), 342 (5), 321 (10), 279 (5), 186 (15); Anal. Calcd for N: 14.26. Found: 14.28.

4.14. Synthesis of 6-methyl-5-(2,4,5-trichlorophenyl)-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo-[3,4-*d*]pyrimidin-4-one (7h)

Obtained from compounds (**6**) and 2,4,5 trichloro aniline; colorless crystals (0.78 g, 49%); mp 272–274 °C; IR (KBr) v: 3035, 2955 (Ar–H str), 1716 (C=O str), 1126 (C–F str), 845, 855 (C–Cl str) cm^{-1}. Anal. Calcd for N: 13.37. Found: 13.34.

4.15. Synthesis of 6-methyl-5-(2-methylphenyl)-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7i)

Obtained from compounds (**6**) and 2-methyl aniline; colorless solid (0.88 g, 66%); mp 260–262 °C; IR (KBr) v: 3043, 2965 (Ar–H str), 1736 (C=O str), 1128 (C–F str), cm^{-1}. Anal. Calcd for N: 16.05. Found: 16.08.

4.16. Synthesis of 5-(4-chlorophenyl)-6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7j)

Obtained from compounds (**6**) and 4-chloro aniline; colorless solid (0.85 g, 60%); mp 280–282 °C; IR (KBr) v: 3040, 2960 (Ar–H str), 1730 (C=O str), 1118 (C–F str), 824 (C–Cl str) cm^{-1}. Anal. Calcd for N: 15.35. Found: 15.36.

4.17. Synthesis of 6-methyl-5-(4-nitrophenyl)-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7k)

Obtained from compounds (**6**) and 4-nitro aniline; yellow solid (0.75 g, 52%); mp 244–246 °C; IR (KBr) *v*: 3044, 2966 (Ar–H str), 1730 (C=O str), 1138 (C–F str), cm^{-1}; MS: (M^+, %) *m*/*z* 467 (MH^+, 100), 466 (M^+, 35), 451 (10), 421 (18), 365 (20), 279 (10), 163 (25), 149 (60); Anal. Calcd for N: 18.00. Found: 18.02.

4.18. Synthesis of 5-(3,4-dichlorophenyl)-6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo-[3,4-*d*]pyrimidin-4-one (7l)

Obtained from compounds (**6**) and 3, 4-dichloro aniline; colorless solid (0.85 g, 56%); mp 278–280 °C; IR (KBr) *v*: 3033, 2944 (Ar–H str), 1736 (C=O str), 1148 (C–F str), 834 (C–Cl str) cm^{-1}. Anal. Calcd for N: 14.24. Found: 14.28.

4.19. Synthesis of 5-(4-fluorophenyl)-6-methyl-1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (7m)

Obtained from compounds (**6**) and 4-fluoro aniline; colorless solid (0.81 g, 60%); mp 270–272 °C; IR (KBr) *v*: 3055, 2946 (Ar–H str), 1716 (C=O str), 1138, 1234 (C–F str)cm^{-1}. Anal. Calcd for N: 15.96. Found: 15.94.

4.20. Synthesis of 1-[8-(trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8)

Five grams of ethyl 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylate (**3**) and 10 ml formamide were heated at 180–190 °C for 1.5 h. The cooled solution was diluted with 100 ml of ice-cold water and the precipitate obtained was filtered to yield 2.8 g of the crude product. The product was purified by dissolving it in 10% sodium hydroxide solution and reprecipitating using acetic acid to obtain pure product (**8**). Yield 2.1 g (44.6%); mp 270–273 °C dec; ^1H NMR (400 MHz, DMSO-d_6): δ 7.82 (d, 1H, J = 7.84 Hz), 7.97 (t, 1H, J = 7.5 Hz), 8.18 (s, 1H, proton on of pyrazole ring), 8.22 (d, 1H, J = 8.4 Hz), 8.33 (d, 1H, J = 6.9 Hz), 8.57 (s, 1H, proton on C-1 of pyrimidine ring), 9.28 (d, 1H, J = 4.5 Hz) and 12.56 (br s, 1H, NH, of pyrimidine ring); MS: (MH^+, %) *m*/*z*, 332 (MH^+, 100), 331 (25), 251 (70), 175 (35), 136 (60), 120 (5), 107 (25). IR (KBr) *v*: 3385 (NH str), 1690 (C=O str) cm^{-1}. Anal. Calcd for N: 21.14. Found: 21.12.

4.21. Synthesis of 1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*, 7*H*)-dione (9)

Five grams of ethyl 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carboxylate (**3**) and 10 g urea were heated together at 150–160 °C for 30 min. The clear solution went mushy and heating was continued for another 10 min at 170 °C. The resulting solid was dissolved in dilute sodium hydroxide and then carefully acidified with acetic acid to obtain 5.5 g of crude product (**9**). Further purification was accomplished by reprecipitation from dilute sodium hydroxide solution with acetic acid. Yield, 1.8 g (36.7%); mp > 300 °C; MS: (*m*/*z*, %) 347 (M^+, 100), 325 (55), 177 (35), 152 (40), 120 (5). IR (KBr) *v*: 3389, 3396, (NH str), 1675, 1680, (C=O str) cm^{-1} Anal. Calcd for N: 20.17 Found: 20.20.

4.22. Synthesis of 6-thioxo-1-[8-(trifluoromethyl)quinolin-4-yl]-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10)

The reaction was done in the same manner as in the preparation of (**9**). The product was isolated as in the preparation of (**9**) to yield 34% after purification; mp 280–282 °C; IR (KBr) *v*: 3385 (NH), (1690) C=O cm^{-1}. Anal. Calcd for N: 19.27. Found: 19.20.

4.23. Synthesis of 4-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (11)

Five grams of 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carbonitrile (**5**) and 10 ml formamide were heated at 180–190 °C for 30 min. The cooled solution was diluted with 100 ml ice-cold water and the precipitate obtained was filtered to yield 2.2 g of the crude product. The product was purified by dissolving it in 10% HCl, the insolubles removed by filtration. The filtrate was taken and pH was adjusted to 8 with concentrated ammonium hydroxide. This solution was allowed to cool to 10–15 °C and then filtered, washed with water to give pure compound (**11**), yield 1.5 g (32.6%); mp 280–282 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 5.63 (s, 2H, NH_2), 7.83 (d, 1H, J = 7.8 Hz), 7.98 (t, 1H, J = 7.6 Hz), 8.19 (s, 1H, proton on of pyrazole ring), 8.22 (d, 1H, J = 8.5 Hz), 8.35 (d, 1H, J = 6.9 Hz), 8.39 (s, 1H, proton on C-1 of pyrimidine ring), 9.24 (d, 1H, J = 4.5 Hz); IR (KBr) *v*: 3385 (NH) cm^{-1}; MS: (M^+, %) *m*/*z* 331 (MH^+, 100), 330 (10), 307 (40), 242 (5), 196 (9), 165 (13); Anal. Calcd for N: 25.44. Found: 25.38.

4.24. Synthesis of 4-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1,7-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (12)

Five grams of 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carbonitrile (**5**) and 10 g urea were heated together at 180–185 °C for 10 min. The resulting solid was dissolved in dilute sodium hydroxide and then carefully acidified with acetic acid to obtain 4.2 g of crude product (**12**). Further purification was accomplished by reprecipitation from dilute sodium hydroxide with acetic acid. Yield 2.5 g (43.8%); mp > 300 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 7.4 (s, 2H, NH_2), 7.81 (d, 1H, J = 7.8 Hz), 7.96 (t, 1H, J = 7.5 Hz), 8.15 (s, 1H, proton on of pyrazole ring), 8.20 (d, 1H, J = 8.2 Hz), 8.33 (d, 1H, J = 7 Hz), 9.27 (d, 1H, J = 4.5 Hz), 13.44 (br s, 1H, NH). IR (KBr) *v*: 3385, 3390 (NH) cm^{-1}. Anal. Calcd for N: 24.27. Found: 24.30.

4.25. Synthesis of 4-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thione (13)

Five grams of 5-amino-1-[8-(trifluoromethyl)quinolin-4-yl]-1*H*-pyrazole-4-carbonitrile (**5**) and 10 g thiourea

were heated together at 180 °C for 30 min until the clear solution became mushy. The cooled melt was dissolved in dilute sodium hydroxide and then carefully acidified with acetic acid to obtain 3.1 g crude product (**13**). Further purification was accomplished by reprecipitation from dilute sodium hydroxide with acetic acid. Yield 1.8 g (30%); mp 288–290 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.4 (s, 2H, NH_2), 7.82 (d, 1H, J = 7.6 Hz), 7.97 (t, 1H, J = 7.5 Hz), 8.16 (s, 1H, proton on of pyrazole ring), 8.21 (d, 1H, J = 8.5 Hz), 8.34 (d, 1H, J = 7 Hz), 9.28 (d, 1H, J = 4.5 Hz), 13.55 (br s, 1H, NH/SH). IR (KBr) v: 3388, 3395 (NH) cm^{-1}. Anal. Calcd for N: 23.19. Found: 23.15.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.10.053.

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