Designing and Synthesis of New Fluorine Substituted Pyrimidine-Thion-5-Carbonitriles and the Related Derivatives as Photochemical Probe Agents for Inhibition of Vitiligo Disease

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ABSTRACT
A new biocidal agents fluorine substituted-3-thioxopyrimidine-5-carbonitriles (2-9) and/or the related fluorine substituted pyrimido [4,5-d]pyrimidines (10-14) were synthesized by the cycloaddition of fluorinated β-arylidene malononitriles (1a-c) followed by a nucleophilic attack against α,β-bifunctional reagents in different conditions. Structures of the fluorine targets were characterized by their elemental analysis and spectral data (UV, IR, 1H NMR, 13C NMR and mass measurements) and further evaluated as photochemical probe for inhibition of Vitiligo, it was found that compounds 5, 9, 11 and 12 exhibited high potency over the investigated compounds.

Keywords: Synthesis; Fluoropyrimidines; Photochemical Probes

1. Introduction
In the past few years fluorinated heterocyclic systems have been incorporated into drug discovery research [1-12] to improve the drug physicochemical properties and the antibacterial potency.

The DNA polymerase inhibitors Fludarabine (I) (F-ara-A), Clofarabine (II) and Tezacitabine (III) are used as cancer chemotherapeutic agents [13] while Gleevec (IV) is used as a molecule catalytic inhibitor of imatinib mesylate [14]. In addition, BX-1382BS (V) showed a significant effect as a Protein Kinase inhibitor in cancer patients [15,16] and that cyanopyrimidine scaffold JNJ-17029259 (VI) is an oral inhibitor of VEGF-mediated signal transduction (Figure 1) [17].

The 4-amino-2-thioxopyrimidine-5-carbonitrile (2) and/or 2,5,7-trithioxopyrimido[4,5-d]pyrimidine (3) were synthesized further reactions of 2 and 3 with α,β-bifunctional reagents gave the fluorocompounds (4-13) (Schemes 1-3).

2. Results and Discussion
2.1. Chemistry
From cyclocondensation of fluorinated β-arylidene malononitrile (1a-c) with thiourea in boiling ethanol [18] and in the presence of anhydrous K₂CO₃ (Scheme 1) 4-Amino-6-fluoroaryl-1H-pyrimidine-5-carbonitriles (2a-e) were obtained. UV spectrum structure of 2a showed λmax at 370 and 314 nm assigned to higher heteroconjugation systems combined with π-π* electronic transition. Also, IR spectrum showed ν at 3358 and 2226 cm⁻¹ assigned to the amino and cyano groups respectively. 1H NMR spectrum showed resonated signals at δ 3.4 and 8.7 ppm assigned to NH₂ and NH protons. 13C NMR spectrum of 2a showed signals at δ 166, 114 and 180 ppm assigned to the C-CN, C≡N and C=S carbons with 165, 130, 115 ppm of C-F aromatic carbons. It was interesting to note that refluxing compound 2 with carbon disulfide in DMF [18] afforded 4-fluoroaryl-2,5,7-trithioxo-1,6,8-trihydropyrimido[4,5-d]pyrimidine (3) (Scheme 1).

The electronic conjugated molecules of compound 3 exhibited λmax at 317 nm. Due to the 3NH, IR spectrum showed vibration bands at ν 3358, 3170 and 3095 cm⁻¹ with 1298 and 1222 cm⁻¹ which were attributed to cyclic NCS and C=S groups. 1H NMR spectrum showed resonated signals at δ 8.5, 12.01 and 13.2 ppm for 3NH different types of carbons were recorded by 13C NMR spectrum at δ 178, 180 ppm and 194 ppm assigned to 3C=S with δ at 165, 128 and 118 ppm of aryl carbon-fluorine. The base peak for compound 3 was recorded at m/z 95 as 4-fluorophenyl cation (Scheme 5).

4-Thiazolidinones possess biological and pharmacol-
ogical activities as plant protecting, anticancer and anti AIDS agents [19]. Based on these activities, condensation of compound 2a with 4-fluorobenzaldehyde in ethanol yielded the Schiff base 4, which upon cycloaddition reacted with mercaptoacetic acid to give 4-[2′-(4″-fluorophenyl-4′-oxothiazolidin-3′-yl)]-6-(4-fluorophenyl)-1H-pyrimidine-5-acetonitrile (5) (Scheme 2).

The OH and NH absorption bands of structure 5 which was deduced from both elemental analysis and spectral measurements of IR spectrum were shown at 3373 and 3329 cm$^{-1}$ respectively, with 2229, 1647 (C≡N & C=O) and at 1222 cm$^{-1}$ (C-S). $^1$H NMR spectrum resonated signals at δ 4.7 (CH= of thiazolidinone) 9.7 (NH of pyrimidine) and at 10.2 (3-OH of thiazole) with aromatic protons at δ 7.9 - 7.1 ppm. Resonated signals were recorded by $^{13}$C NMR spectrum at δ 180, 163, 162 due to the presence of C=S, C=O, and C-F and 114.3, 130.1, 163.89 ppm of imidazole moiety. The signals which were observed at δ 115.5, 114.88, 128, 130 ppm also, were assigned to C=N and aromatic carbons respectively.

Condensation of 4-amino-6-(4′-fluorophenyl)-1-H-pyrimidine-5-carbonitrile (2a) with oxazol-5-one derivative 6 in boiling dry pyridine produced 4[4′-(4″-fluorobenzylidene)-2-phenyl-5-oxo-imidazol-1′-yl]-6-(4′-fluoro-phenyl)-1H-pyrimidine-5-carbonitrile (7) (Scheme 2). UV absorption of 7 recorded $\lambda_{\text{max}}$ at 360 nm assigned to n-π* electronic transition with a conjugated system of imidazole. Absorption bands at ν 3329, 2225 and 1650 cm$^{-1}$ attributed to NH, C≡N and C=O functional groups, in addition at 1248, and 1130 cm$^{-1}$ assigned to NCS and C-S with 677 cm$^{-1}$ characterized by C-F group were shown by the IR spectrum. $^1$H NMR spectrum exhibited a resonated signal at δ 13.3 and with 8.6 ppm due to NH and exo CH protons of imidazolone, with aromatic protons between 7.8 - 6.8 ppm. Similarly, cyclocondensation of 2,3-pyridinedicarboxylic acid anhydride (8) with compound 2a in boiling dry pyridine, afforded 4-(2′,3′-phthalimidopyridin-1′-yl)-6-(4′-fluorophenyl)-1H-pyrimidine-5-carbonitrile (9) (Scheme 2).

Due to n-π* electronic system UV absorption spectrum of 9 exhibited $\lambda_{\text{max}}$ at 302 nm. Peaks were recorded by IR spectrum at ν 3334, 2216 and 1689 cm$^{-1}$ which were
Scheme 2. Condensation of compound 2a.

Scheme 4. The formation of fluoropyrimidine 3.

Scheme 5. Mass fragmentation pattern of compound 3a.

assigned to NH, C≡N and C=O functional groups. In addition, peaks at 810, 770 and 750 cm\(^{-1}\) were assigned to aromatic ring. \(^1\)H NMR showed a resonated signal at \(\delta\) 10.1 ppm assigned to 3NH with aromatic protons at 8.1 - 6.8 ppm.

An amylolytic activity against some fungi [20] was shown by 1,2,4-Triazines bearing carboxymethylthia groups. Accordingly, alkylation of 3 using chloroacetic acid in aqueous NaOH yielded 8-aryl-2,5,7-tri(carboxymethylthia) pyrimido[4,5-d]pyrimidine (10) (Scheme 3). Formation of 3 is shown in (Scheme 4). Structure of 10 was derived from its elemental analysis and spectral data. UV absorption spectrum was recorded \(\lambda_{\text{max}}\) at 269 nm, while IR spectrum showed absorption bands at 3400, 1700 and 1480 cm\(^{-1}\) which were attributed to OH, C=O and CH\(_2\) groups in addition \(\nu\) 1180 and 690 cm\(^{-1}\) were assigned to C-S and C-F groups. Due to the presence of 3(COOH) protons and aromatic protons \(^1\)H NMR spectrum recorded signals at \(\delta\) 9 - 7.2 (m) ppm in addition, at 3.3 - 3.6 ppm for active methylene protons. \(^13\)C NMR spectrum showed a resonated signal at \(\delta\) 171.15, 171, 163.85 ppm which were assigned to C-S, C=O, C-F
and at 162.5, 162.11, 154.15, 114 ppm attributed to 2C=N of substituted pyrimidine, C=N of condensed pyrimidine. In addition, at 33.88 ppm of C(S-CH2-CO) with 130.15, 115.35 of aromatic carbons.

In the treatment of HIV and cancer diseases fluorinated heterocyclic nitrogen systems possess specific and unique properties as drugs [21-23]. Based on these observations, a simple nucleophilic attack for removal of sulfur atoms of compound \( 3a \) yielded 4-aryl-2,5,7-tri(4'-sulfonamophenylamino)-pyrimido[4,5-d]-pyrimidine (11) (Scheme 3). Also, treatment of \( 3a \) with sulfa-drug as sulfadiazine in boiling DMF yielded 4-aryl-2,5,7-tri(4'-sulfonamophenylamino)-pyrimido[4,5-d]-pyrimidine (12) (Scheme 3).

UV absorption of 11 showed band at \( \lambda_{\text{max}} \) at 271 nm, IR bands at \( \nu \) 3197, 1575, 1219 and 776 cm\(^{-1}\) assigned to NH, C=N, NCS and C-F functional groups. Resonated signals were shown by \( ^1H \) NMR spectrum at \( \delta \) 10.1 and in the range \( \delta 7.2 - 7.9 \) ppm of 3NH of pyrimidines with aromatic protons respectively.

Hydrazino groups are used as starting materials for bioactive isolated heterocyclic systems [24-26]. Refluxing \( 3a \) with hydrazine hydrate in ethanol produced 4-aryl-2,5,7-tri(hydrazinopyrimido[4,5-d]pyrimidine (13)

Ring closure reactions of 13 with malononitrile in boiling ethanol with a few drops of piperidine as a catalyst via cycloaddition led to the direct formation of 8-(4'-fluorophenyl)-2,5,7-tri(3',5'-diaminopyrazol-1'-yl) pyrimido[4,5-d]pyrimidine (14) (Scheme 3).

Structure of 14 was deduced from elemental analysis and spectral data. UV absorption was recorded \( \lambda_{\text{max}} \) at 350 and 310 run assigned to a rich n-\( \pi^* \) and n-\( \sigma^* \) electronic transition. IR spectrum showed bands at \( \nu \) 3240, 1664 cm\(^{-1}\) due to NH2 group with other peaks at 1609, 1574 cm\(^{-1}\) for C=N with 776 cm\(^{-1}\) of C-F functional groups. \(^1H \) NMR spectrum showed a signal at 3.3 - 3.6 ppm attributed to 6 NH2 protons, with aromatic protons at \( \delta 7.2 \) and 8.18 - 8.0 ppm of three CH of 4-pyrazoles.

\(^13C \) NMR showed signals at \( \delta 162.45, 162.13 \) ppm of C-C and two C=N of substituted pyridine in addition at \( \delta 152.55, 148.85 \) ppm attributed to 2 C\(_2\)-NH\(_2\) of pyrazole with chemical shifts at 155.9 and 77.85 of C\(_e\)-C\(_s\) of fused pyrimidopyrimidine. Other signals of pyrazole were observed at \( \delta 162.45, 77.85 \) of C-F and C=C besides aromatic carbons at \( \delta 130, 127, 115.88 \) ppm which confirmed the proposed structure. The molecular ion was recorded by the mass spectrum at m/z 514 (5%) with a base peak at m/z 132 (100%) attributed to a delocalized pyrimidopyrimidine radical. Synthesized single fluorine which was attached to phenyl ring in all the fluorinated systems appeared in the region at \( \delta 120 - 126 \) ppm.

### 2.2. Pharmacology

Vitiligo is an acquired disorder which is characterized by patchy progressive depigmentation of the skin. About 2% of the world population is affected by it. Thus, by using the disk diffusion method the antimicrobial activity of the prepared fluorinated pyrimidine compounds was performed [27,28] at concentrations of 100, 75 and 50 \( \mu \)g/disk with the interference drugs, Chloroamphenicol (10 \( \mu \)g/disk) and Nalidixic acid (20 \( \mu \)g/disk) for bacteria and Nystatin (30 unit/disk = 0.12 \( \mu \)g/disk) for fungi. The disks were placed on the surface of the cold medium and incubated with Bacillus subtilis and Staphylococcus aureus (Gram + ve bacteria) Escherichia coli and Pseudomonas aeruginosa (Gram – ve bacteria) and Candida albicans (fungi) at 25°C for one hour to permit good diffusion and were then transferred to an incubator at 37°C for 24 hours. The results are summarized in (Table 1). Before transferring it to the incubator the photochemical screening employing UV light at 366 nm was also carried out following the same procedure without UV for 3 hours. The results are given in (Table 2). The sensitivity of fluorinated compounds against microorganisms revealed the following observations:

1) High MIC for the tested compounds against positive bacteria was achieved at 50 \( \mu \)g/disk concentration, while that of negative bacteria at 100 \( \mu \)g/disk and for fungi at 50 \( \mu \)g/disk.

2) After using UV light at 50 \( \mu \)g/mL\(^{-1}\) all the targets exhibited high effects.

3) Most of the tested compounds were highly active (IZ 12 - 15) at 50 - 100 \( \mu \)g/mL\(^{-1}\) and others showed a moderate activity (IZ 9 - 12) at the same concentration in comparison to the standard antibiotics.

4) The presence of the fluorine atoms enhanced the activity until the concentration is 50 \( \mu \)g/disk.

5) Increasing fluorine atoms led to increasing of biocidal effects. Also, introducing sulfa moieties increased the biocidal effects.

6) The compounds which combined heterocyclic systems with both the fluorine and sulfa drugs moiety exhibited higher biocidal effects before and after using UV light especially with compounds 5, 9, 11 and 12 which can be used in antimicrobial and photochemical probe agents especially towards +ve bacteria.

Through Quantitative-structure activity relationship (QSAR) for the tested compounds it was shown that the cytotoxicity of these compounds is controlled by electronic and hydrophobic factors as well as by steric factor which is due to the large size of molecular formula and the electronegativities (5, 9, 11, 12). Thus, the electron density on the active center of the tested target increased the biocidal effect.

In comparison with the standard antibiotics e.g. chloroamphenicol, Nalidixic acid and Nystatin compounds 11 and 12 can be used as antimicrobial and anti-
Table 1. Preliminary screening antimicrobial activity of the fluorinated compounds 2-12.

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<tr>
<th>Compd NO.</th>
<th>Conc.</th>
<th>Bacillus s.</th>
<th>Staphylococcus a.</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginase</th>
<th>Candida albicans</th>
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Table 2. Preliminary screening antimicrobial activity of the fluorinated compounds 2-12 after using UV-light (366 nm).

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<th>Compd NO.</th>
<th>Conc.</th>
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Chloroamphenical: for Bacteria; IZ = 15; Nalidixic: Non-Selective for Bacteria; Nystatin for Fungi.

The prominent role of fluorine substituent effects on bioactivity is due to the effect of fluorination on C-H acidity which normally is predictable and depends on several factors, including the site of fluorination and geometry of the conjugate carbanion. Thus, \( \alpha \)-fluorine can increase or decrease acidity. The latter is the case when the conjugate carbanion is close to planar since this geometry maximizes lone-pair repulsions. \( \beta \)-Fluorine invariably increases C-H acidity through inductive and hyper conjugative resonance stabilization of the carbanion (Figure 2) [8].

3. Conclusion

Fluorinated pyrimidines and pyrimido[4,5-d] pyrimidine derivatives 2-14 were efficiently synthesized beginning from fluorinated \( \beta \)-arylidine malononitriles 1. A significant activity was shown by all of these targets against Basillus subtilis, and Staphylococcus aureus (Gram + ve bacteria) Escherichia coli and Pseudomonas aeruginase (Gram – ve bacteria) and Candida albicans as fungi. In general the presence of fluorine atom increased the antimicrobial activity at 50 mg/disk and especially increased the biocidal effects towards +ve bacteria. After using UV-light, the biocidal effects of the tested compounds also increased towards +ve bacteria, especially the compounds 5, 9, 11 and 12. On the other hand, the high effects of these compounds may be attributed to the three signal excitation states of oxygen atom. Thus, for inhibition of Vitiligo disease the fluorinated pyrimidine derivatives can be used as photochemical probe agents.

4. Experimental Section

4.1. General Procedures

Melting points were determined with an electrothermal Bibby Stuart Scientific melting point Smpl (US). A Perkins Elmer model RXI-FT-IR system 55529 was used for
recording the IR spectra of the prepared compounds. A Brucker advance DPX 400 MHz model using TMS as an internal standard was used for recording the $^1$H and $^{13}$C NMR spectra of the compounds on deuterated DMSO. A GC-MS-QP 1000-Ex model was used for recording the mass spectra of the compounds. Electronic spectra were recorded in ethanol on Shimadzu UV and visible 3101 PC Spectrophotometer. Microanalysis (Molecular weight determination) was performed by the Microanalytical Center of Cairo University, Egypt. M/S spectroscopy was determined on a GCMS-Q 1000-Ex spectrometer at 75 eV in m/z. Hexafluorobenzene was used as external standard for $^{19}$F NMR at 8425 MHz (Chemical shift in $\delta$, ppm).

4.1.1. Fluorinated $\beta$-Arylidene Malononitriles (1a-c)

1a: Crystallized from dioxan as faint yellow crystals. Yield = 85%, m.p. 85°C - 87°C. Analytical data: Found: C = 69.36, H = 2.8, N = 16.01, and F = 10.72%. Calculated for C$_{10}$H$_4$N$_2$F (172): C = 69.76, H = 2.90, N = 16.27 and F = 11.04%. IR (KBr disk) $\nu$ cm$^{-1}$: 3049 (C-H arom.), 1350 (NCS), 1298, 1222, 758 (2 NH), 7.4 - 7.6 (d, 2 H, aromatic protons). 13C NMR (DMSO) $\delta$: 13.2, 8.5 (each s, 3 H of NH), 7.8 - 7.7, 7.6 - 7.55 (each d, 2 H, aromatic protons).

1b: Crystallized from dioxan to give pale yellow crystals. Yield = 62%, m.p. 65°C - 67°C. Analytical data: Found: C = 58.01, H = 1.91, N = 13.44, Cl = 16.81 and F = 8.88%. Calculated for C$_{10}$H$_4$N$_2$F$_2$ (206.5): C = 58.25, H = 1.94, N = 13.59, Cl = 16.99 and F = 9.2%.

1c: Crystallized from dioxan to give pale yellow crystals. Yield = 90%, m.p. 119°C - 120°C. Analytical data: Found: C = 62.95, H = 1.99, N = 14.64, and F = 19.88%. Calculated for C$_{10}$H$_4$N$_2$F$_2$ (190): C = 63, H = 2.10, N = 14.73 and F = 20.0%.

4.1.2. 4-Amino-6-fluoroaryl-2-thioxo-1H-pyrimidine-5-carbonitriles (2a-c)

A mixture of compound 1a-c (0.01 mol), thiourea (0.01 mol), anhydrous K$_2$CO$_3$ (0.01 mol), in ethanol (30 ml) was refluxed for 3 h. The precipitate obtained was washed with water and crystallized to give 2a-c.

2a: Crystallized from ethanol to give orange crystals. Yield = 75%, m.p. 238°C - 240°C. Analytical data: Found: C = 53.56, H = 2.59, N = 22.46, S = 12.75 and F = 7.25%. Calculated for C$_{11}$H$_4$N$_2$SF (246.5): C = 53.65, H = 2.84, N = 22.76, S = 11.01 and F = 7.72%. IR (KBr disk) $\nu$ cm$^{-1}$: 3358 (NH$_2$), 3120 (NH), 3010 (C-H aromatic), 2226 (C=N), 1630 (deform. NH$_2$), 1585 (C=N), 1230 (C-F), 1180 (C=S), 810 (substituted phenyl). UV (EtOH) $\lambda$$_{max}$ (nm) 370.

1H NMR (DMSO) $\delta$: 8.7 (s, NH), 3.4 (s, NH$_2$). 7.4 - 7.8, 7.0 - 7.1 (each d, 4H of aryl protons). $^{13}$C NMR (DMSO) $\delta$: 180 (C=S), 164 (C-CN), 116 (C=N), 92.7 (C-CN), 129.11, 129.57, 132.58, 134.79 (aromatic carbons). MS (m/z, %): 248 (M$^+$, 1.15), 246 (5.11), 174 (100), 80 (13.17).

2b: Crystallized from ethanol to give pale yellow crystals. Yield = 59%, m.p. 175°C - 177°C. Analytical data: Found: C = 46.81, H = 2.11, N = 19.79, S = 11.1, Cl = 12.51 and F = 7.25%. Calculated for C$_{11}$H$_4$N$_2$SF$_2$ (281): C = 46.97, H = 2.13, N = 19.92, S = 11.38, Cl = 12.63, F = 6.76%.

2c: Crystallized from ethanol to give pale yellow crystals. Yield = 78%, m.p. 300°C - 302°C. Analytical data: Found: C = 44.43, H = 1.9, N = 18.66, S = 21.21 and F = 12.15%. Calculated for C$_{11}$H$_4$N$_2$SF$_2$ (296): C = 44.59, H = 2.02, N = 18.91, S = 21.62, and F = 12.15%.

4.1.3. 4-Fluoroaryl-2,5,7-trithioxo-1,6,8-trihydro-pyrimido [4,5-d] pyrimidines (3a-c)

To a solution of 2a-c (0.01 mol) in DMF (30 mL) or alcoholic KOH (5 gm/100 ml EtOH), CS$_2$ (20 mL) was added dropwise. The reaction mixture was refluxed on a water-bath for 12 hr. The solvent was evaporated and mass obtained was triturated with water then crystallized to give 3a-c.

3a: Crystallized from DMF to give orange crystals. Yield = 65%, m.p. 250°C - 252°C. Analytical data: Found: C = 44.52, H = 1.97, N = 17.01, S = 29.69 and F = 5.66%. Calculated for C$_{11}$H$_4$N$_2$SF$_3$ (322): C = 44.72, H = 2.17, N = 17.39, S = 29.81 F = 5.90% IR (KBr disk) $\nu$ cm$^{-1}$: 3305, 3170, 3095 (3 NH) 1350 (NCS), 1298, 1222, 1180 (C-F, C=S, C-S). UV (EtOH) $\lambda$$_{max}$ (nm) 317 (log $\varepsilon$ = 3.01). $^{13}$C NMR (DMSO) $\delta$: 13.2, 8.5 (each s, 3 H of NH), 7.8 - 7.7, 7.6 - 7.55 (each d, 2 H, aromatic protons). $^{19}$F NMR (DMSO) $\delta$: 13.2, 8.5 (each s, 3 H of NH), 7.8 - 7.7, 7.6 - 7.55 (each d, 2 H, aromatic protons). MS (m/e, %): 346 (M$^+$, 100), 306 (85), 266 (35), 226 (35), 186 (15), 146 (15), 106 (5), 66 (5), 28 (5).

Figure 2. Hyper-conjugation between F-atoms and C-H.
3b: Crystallized from DMF to give orange crystals.
Yield = 71%, m.p. 300°C - 303°C. Analytical data:
Found: C = 39.96, H = 1.49, N = 15.21, S = 26, 25, Cl = 26.25 and F = 5.01. Calculated for C_{12}H_{12}N_{12}S_{12}F_{12} (365.5): C = 40.39, H = 1.68, N = 15.70, S = 26, 25, Cl = 26.25 and F = 5.39.

3c: Crystallized from dilute DMF to give orange crystals. Yield = 60%, m.p. 298°C - 300°C. Analytical data:
Found: C = 41.88, H = 1.6, N = 16.47, S = 28.23 and F = 11.17. Calculated for C_{12}H_{12}N_{12}S_{12}F_{12} (365.5): C = 42.35, H = 1.76, N = 16.47, S = 28.23 and F = 11.17%.

4.2. Formation of Schiff Base 4

Equimolar mixture of 2a and 4-fluorobenzaldehyde in dry ethanol (20 ml) was refluxed for 1h and cooled. The solid obtained was crystallized to give 4.

4: Crystallized from acetic acid to give yellow crystals.
Yield = 85%, m.p. 299°C - 300°C. Analytical data:
Found: C = 60.20, H = 2.77, N = 15.55, S = 8.55, F = 10.22%. Calculated for C_{12}H_{12}N_{12}S_{12}F_{12} (365.5): C = 61.36, H = 2.84, N = 15.90, S = 9.09, F = 10.79%.

4.2.1. 1-(4'-Oxo-thiazolidin-3'-yl)-6-(4'-fluorophenyl)-2-thioxopyrimidine-5-carbonitrile (5)
A mixture of compound 4 (0.01 mol), thioglycollic (0.05 mol), in THF (50 ml) was refluxed for 12 h, cooled and then neutralized with acetic acid. The product was filtered and crystallized from THF to give 5 as yellow crystals. Yield = 68%, m.p. 198°C - 200°C. Analytical data:
Found: C = 55.93, H = 2.59, N = 13.01, S = 14.38 and F = 8.80%. Calculated for C_{12}H_{12}N_{12}S_{12}F_{12} (365.5): C = 56.33, H = 2.81, N = 13.14, S = 15.02 and F = 8.92%.

4.2.2. 4-[4'-(4''-Fluorobenzylidene)-2-phenyl-5-oxoimidazol-1-yl]-6-(4'-fluorophenyl)-2-thioxo-1H-pyrimidine-5-carbonitrile (7)
Equimolar amount of 2a and 6 in dry pyridine (100 ml) was refluxed for 12 h, cooled and then poured onto ice-HCl. The solid obtained was filtered off and crystallized from THF to give 7 as brown crystals. Yield = 66%, m.p. 258°C - 260°C. Analytical data:
Found: C = 66.13, H = 2.99, N = 14.01, S = 6.60 and F = 7.32%. Calculated for C_{27}H_{15}N_{5}S_{5}F_{5} (553): C = 65.45, H = 3.03, N = 14.14, S = 6.46, F = 7.67%. IR (KBr disk) ν cm\(^{-1}\): 3373 (OH), 3329 (NH), 2229 (C=N), 1980 (C=O), 1590, 1580 (2 C=N), 1480 (deform. CH\(_2\)). UV (EtOH) λ\(_{max}\) (nm) 3020 (aromatic CH), 2910 (aliphatic CH), 2225 (C-F), 1700 (C=O), 1590, 1580 (substituted pyrimidine and aromatic protons).

4.2.3. 8-(Phthalimido-1'-yl)-6-(4'-fluorophenyl)-2-thioxo-1H-pyrimidine-5-carbonitrile (9)
A mixture of 2a (0.01 mol) and pyridine-2,3-dicarboxylic anhydride (8) (0.01 mol) in dry pyridine (50 ml) was refluxed for 10 h., cooled and then poured onto ice-HCl. The produced solid was filtered and crystallized from THF to give 9 as deep brown crystals. Yield 55%, m.p. 299°C - 300°C. Analytical data:
Found: C = 57.10, H = 1.98, N = 18.41, S = 7.84 and F = 4.90%. Calculated for C_{18}H_{10}N_{4}S_{3}F_{2} (340): C = 42.35, H = 2.84, N = 15.90, S = 9.09, F = 10.79%.

4.2.4. 2-(2'-Chloro-6'-fluorophenyl)-2,5,7-tri(carboxymethylthia)-3H,4H-pyrimido[4,5-d]pyrimidine (10)
A mixture of 3a (0.01 mol) and chloroacetic acid (0.03 mol) in DMF (50 ml) was refluxed for 2 h, and then poured onto ice. The solid thus obtained was filtered off and crystallized from THF to give 10 as yellow crystals. Yield = 80%, m.p. 110°C - 132°C. Analytical data:
Found: C = 43.41, H = 2.33, N = 11.31, S = 19.39 and F = 3.55%. Calculated for C_{18}H_{10}N_{4}S_{3}F_{2} (495): C = 43.63, H = 2.42, N = 11.11, S = 18.39, F = 3.83%. IR (KBr disk) ν cm\(^{-1}\): 3400 (OH), 3050 (aromatic CH), 2280 (aliphatic CH\(_2\)). UV (EtOH) λ\(_{max}\) (nm) 271 (log ε = 1.2). 1H NMR (DMSO) δ: 10.2 (s, 1 H, OH of thiazole), 9.7 (s, 1 H, NH of pyrimidine), 7.7 - 7.4, 7.2 - 7.14 (each m, 8H, aromatic protons), 4.25 (s, 1H, CH= of thiazole).

4.2.5. 8-(2'-Chloro-6'-fluorophenyl)-2,5,7-tri(carboxymethylthia)-3H,4H-pyrimido[4,5-d]pyrimidine (11)
A mixture of 3a (0.01 mol) and 4-fluoroaniline (0.03 mol) in abs. EtOH (100 ml) was refluxed for 12 h and cooled. The resulted solid was filtered and crystallized from THF to give 11 as yellow crystals. Yield = 78%, m.p. 115°C - 117°C. Analytical data:
Found: C = 64.69, H = 3.33, N = 17.55, and F = 13.45%. Calculated for C_{30}H_{19}N_{7}F_{4} (553): C = 65.09, H = 3.43, N = 17.72 and F = 13.74%. UV (EtOH) λ\(_{max}\) (nm) 271 (log ε = 1.2). 1H NMR (DMSO) δ: 10.1 (s,
and F = 3.48%. Calculated for C42H30N16S3F2O6 (988): C 51.01, H = 3.03, N = 22.67, S = 9.71, F = 3.84%. IR (KBr disk) ν cm⁻¹: 3100 - 3080 (NH2), 3020 (aromatic CH), 1595, 1580 (C=N), 1250 (C-F). UV (EtOH) λmax (nm) 350 (log ε = 2.5). 1H NMR (DMSO) δ: 8.18 - 8.00 (each s, 3H of C₄-pyrazole), 7.8 - 7.2 (m, 4H, aromatic protons), 3.6 - 3.3 (m, 12H of 6 NH₂ of pyrimidine), 7.9 - 7.2 (m, 16 H of aromatic protons).

4.2.7. 8-(4'-Fluorophenyl)-2,5,7-tri-(hydrazine)-pyrimido[4,5-d]pyrimidine (13)
A mixture of compound 3a (0.01 mol) and hydrazine hydrate (0.03 mol) in abs. EtOH (100 ml) was refluxed for 12 hr, cooled and then concentrated. The obtained solid was crystallized from ethanol to give 13 as yellow crystals. Yield = 78%, m.p. 170°C - 172°C. Analytical data: Found: C = 45.14, H = 3.95, N = 44.30 and F = 5.88%. Calculated for C₃₂H₂₃N₁₆F (316): C = 45.56, H = 4.11, N = 44.01 and F = 6.01%. IR (KBr disk) ν cm⁻¹: 3240 (NH₂), 1609, 1574 (C=N), 1230 (C-F). MS (m/e, % = 223 (M-291, 13.11), 128 (C=N), 120 (C-F). −CN, 7.9 - 7.2 (m, 16 H of aromatic protons).

4.2.8. 8-(4'-Fluorophenyl)-2,5,7-tri(3',5'-diaminopyrazol-l'-yl)pyrimido[4,5-d]pyrimidine (14)
A mixture of 13 (0.01 mol) and malononitrile (0.03 mol) in ethanol (50 ml) with a few drops of piperidine was refluxed for 4hr and cooled. The resulted solid was crystallized from dioxin as yellow crystals to give 14. Yield = 66%, m.p. 235°C - 237°C. Analytical data: Found: C = 48.48, H = 3.61, N = 43.17 and F = 3.19%. Calculated for C₃₃H₁₉N₁₈F (514): C = 49.02, H = 3.69, N = 43.57 and F = 3.69%. IR (KBr disk) ν cm⁻¹: 3240 (NH₂), 1609, 1574 (C=N), 1230 (C-F). UV (EtOH) λmax (nm) 350 (log ε = 2.5). 1H NMR (DMSO) δ: 8.18 - 8.00 (each s, 3H of C₄-pyrazole), 7.8 - 7.2 (m, 4H, aromatic protons), 3.6 - 3.3 (m, 12H of 6 NH₂ of pyrazole). MS (m/e, % = 223 (M-291, 13.11), 223 (5.19), 128 (17.33), 97 (25.18) and 95 (100).

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