Synthesis and Chemical Reactivity of some new Fluorine Substituted Pyrazolopyrimidine Derivatives and Their Effect on Cellobiase Activity Produced by Fungi

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Abstract Various fluorine substituted fused heteropolycyclic nitrogen systems containing a pyrazolopyrimidine moiety have been synthesized from the interaction between 6-mercaptopyrazolo[3,4-d]pyrimidines with bifunctional nitrogen and halogen compounds. The effect of these fluorinated systems on the activity of the cellobiase produced by Aspergillus Nidulans was evaluated. The outcome of the study discloses that, some of the compounds have shown promising activity in low concentration.

Keywords: fused heteropolycyclic, Aspergillus Nidulans, pyrazolopyrimidine, cellobiase fungi, 6-mercaptopyrazolo, fluoro heteropolycyclic


1. Introduction

The chemistry of fluorine substituted pyrimidines has received more interest recently and regarded further development as a novel class of Antimycobacterium tuberculosis agents [1], stronger antibacterial and antifungal activities [2,3] and inhibition of the human epidermal growth factor receptors [4]. In addition, the combination of pyrazoles with pyrimidines had a potential therapeutic application. [5,6,7] Therefore, this work reports an attempt to combine both edaravone and pyrimidine with substituted fluorine infused heteropolycyclic nitrogen systems in hope to achieve a purine analogue with a view of their biological properties towards some fungi as an enzymatic effect.

2. Material and Method

2.1. Chemistry

Fluoroacylation and fluorobenzylation of compound 1 afforded 4-trifluoroacetyl-4,5-dihydro-3-methyl-1-phenyl pyrazol-5-one (2) and 4-(4′-fluorobenzyl)-4,5-dihydro-3-methyl-1-phenylpyrazol-5-one (3) respectively. Ring closure reactions of both 2 and 3 with thiourea furnished 6-mercaptopyrazolo[3,4-d]pyrimidines derivatives 4 and 5 via cycloaddition reaction (Scheme 1). [8] Both compounds 4 and 5 are used as starting materials for the synthesis of new fluorine substituted fused heteropolycyclic nitrogen systems.

Scheme 1. The synthetic route to 6-mercaptopyrazolo[3,4-d]pyrimidines derivatives 4 and 5
Consequently a simple nucleophilic displacement of the SH group of compounds 4 and 5 by primary amines was studied. Thus, reflux 4 and 5 with ethanolamine in ethanol produce N-substituted amino derivatives 6 and 7. Reflux of compound 6 and 7 with Ac₂O/EtOH led to the formation of 7,8-tetrahydro[4,3-[6,5] imidazolines 8 and 9 respectively. (Scheme 2).

The interaction between compounds 4 and 5 with primary aromatic amines yielded 6-arylamino-4-(trifluoromethyl)-3-methyl-1-phenylpyrazolo[3,4-d]pyrimidines (10 and 11) respectively (Scheme 3). In addition, reaction of both 4 and 5 with piperazine as bisecondary amine [9] yield 1,4-(diheteroaryl)piperazines 12 and 13 respectively, (Scheme 4).

### 2.2. Experimental Section

Melting points determined by an electrothermal Bibby Stuart Scientific melting point sample (UK). A Perkin Elmer Model RXI-FT IR system 55529 used for IR spectra of the prepared compounds (cm⁻¹). A Bruker advance DPX 400 MHz model uses TMS as internal standard was used for recording the ¹H and ¹³C NMR spectra of the compounds on deuterated DMSO-d₆ (ppm). A GC-MS-GP 1000 Ex model used for recording the mass spectra of the compounds (MH⁺).Electronic spectra recorded in ethanol on Shimadzu UV and visible 310 IPC Spectrophotometer (nm). Elemental analysis was performed in micro analytical Center of Cairo University, Cairo, Egypt.

#### 4-(Trifluoroacetyl)-4,5-dihydro-1-phenyl-3-methylpyrazol-5-one (2)

A mixture of 1 (1.7g, 0.01 mol) and trifluoroethyl acetate (1.4g, 0.01 mol) in toluene (20 ml) was reflux for 6 h, cooled. The resulting solid collected by filtration and crystallized from EtOH to give compound in 70% yield, mp116 - 118°C. ¹H NMR (400 MHz, DMSO) δ: 13.59 (s, 1H, OH), 7.97-7.13 (m,5H, ph), 1.25 (s,3H, CH₃), ¹³C NMR (100 MHz, DMSO) δ: 157 (C=O), 148 (C-F), 141 (C=N), 137 (C-N), 129.71-125.20 (Ar=C), 119.118 (C=C), 14.14 (CH₃). IR(cm⁻¹) V: 3500-3400(b, OH), 1680 (C=O), 1448 (CH₃), 1230 (C-F), 800 (ph): Anal. Calcd. For
C$_2$H$_4$N$_2$F$_2$O$_2$: C, 53.33; H, 3.33; N, 10.37; F, 21.00 %
Found: C, 53.01; H, 3.21; N, 10.17; F, 21.11 %.

4-[4'-Fluorobenzoyl]-1-phenyl-3-methyl-pyrazol-5-one (3)

A mixture of 1 (1.7g, 0.01 mol) and 4-fluorobenzoyl chloride (1.6g, 0.01 mol) in DMF (15 ml) refluxed for 5h, cooled. The resulting solid collected by filtration and crystallized from EtOH to give 3 in 80% yield, $^1$H NMR (400 MHz, DMSO) δ: 9.78 (s, 1H, OH).

4,5-[Hydroxyethanylamino]-4-[4'-(fluorophenyl)]-1-phenyl-3-methyl-pyrazolo[3,4-d]pyrimidines (7)

Yield 71%, UV (nm) $\lambda_{max}$: 443 (s, 0.163).

4-Mercapto-4-(trifluoromethyl)-1-phenyl-3-methyl-pyrazolo[3,4-d]pyrimidines (4)

General procedure for 4 and 5: A mixture of equal molar of 2 or 3 and thiourea, in MeOH / NaOH (5%, 25 ml) were refluxed for 4 h, after cooled 1ml of Conc. HCI was added. The resulting solid was collected by filtration and crystallized from EtOH to give 4 in 73% yield, IR (cm$^{-1}$) ν: 3500-3300 (NH), 1600-1520 (C=C), 1445 (CH$_2$-methyl), 1364, 1290, 1280, 1260, 1240, 1210 shook xylene (363); C, 66.54; H, 4.63 %.

6-Arylamino-4-(trifluoromethyl)-1-phenyl-3-methylpyrazolo[3,4-d]pyrimidines (10a-c)

A mixture of compound 4 or 5 (0.6g, 0.002 mol) and a 3-chloro-4-fluoroaniline (0.30g, 0.002 mol), (b) 3,4-dichloroaniline (0.33g, 0.002 mol) and (c) sulfanilamide (0.34g, 0.002 mol) in DMF (15 ml) refluxed for 7 h, cooled. The solids obtained is collected by filtration and crystallized from DMF to give compounds 10a-c and 11a-c, respectively. $^{10}$a: in 93% yield. IR (cm$^{-1}$) ν: 3120 (NH2), 2920 (aliphatic CH), 1586 (C=O), 1240 (C-N), 1160, 782 (phenyl), 679 (C=C), 633 (C-F), mp 139-140°C. Anal. Calcd. For C$_{12}$H$_9$F$_4$N$_2$S (319); C, 54.51; H, 2.85; N, 16.62; F, 18.05; Cl, 8.31 %. Found: C, 54.31; H, 2.60; N, 17.85; F, 18.10; S, 9.11 %.

6-[Hydroxyethanylamino]-4-(trifluoromethyl)-1-phenyl-3-methylpyrazolo[3,4-d]pyrimidines (6)

General procedure for compound 6 and 7 synthesis a mixture of equal molar of compound 4 or 5 and ethanolamine in EtOH (25 ml) were refluxed for 6 h and cooled, 2 ml of acetic acid was added. The solid is collected by filtration and crystallized from EtOH to give compound 6 in 73% yield, IR (cm$^{-1}$) ν: 3500-3300, 1610-1520, 1445, 1290, 1260, 1240, 1210 shook xylene (363); C, 54.51; H, 3.34; F, 12.72; S, 7.14 %. Found: C, 50.76; H, 3.14; N, 18.55; F, 12.48; S, 7.01 %.

5,5'-[Hydroxyethanylamino]-5,5'-dihexylalanyl-pyrazolopyrimidine (10a-c)

The structure of the new synthesized systems 5-13 was established from numerous spectrum data, the UV absorption gave confirming information about structures of compounds 5-9. The shift in wavelength to $\lambda_{max}$ at 277 (5) 443 (7) and 269 (9) nm are indicative of a chromophore conjugated of pyrazolopyrimidine 7, while a lower $\lambda_{max}$ of 9 refer to inhibition of heteroconjugation.

NMR (100 MHz, DMSO) δ: 147 (C-F), 141 (C=N), 138 (C-N), 129, 128, 126, 124,121,118 (aromatic carbons),
109,104 (cyclic C-C), 12.9 (CH₃). IR (cm⁻¹) v: 31200 (NH), 2918 (aliphatic CH), 1591 (C=O), 1487 (CH₃), 1249(C-F), 1313 (NHSO₂), 843, 815 (phenyl). mp 168-170°C. Anal. Calcd. For C₂H₂N₂F₂Cl (454): C, 63.43; H, 4.84; N, 15.41; F: 8.37; Cl, 7.70 %. Found: C, 63.25; H, 4.58; N, 15.21; F, 8.11; Cl, 7.49 %. **11** in 94% yield, mp 160-162°C. Anal. Calcd. For C₂H₁₄N,FCl₂ (464): C, 62.06; H, 3.44; N, 15.08; F, 4.09; Cl, 15.30 %. Found: C, 61.89; H, 3.15; N, 14.91; F, 3.88; Cl, 15.11 %. **11c** in 87% yield, mp 179-180°C. Anal. Calcd. For C₂H₂H₄N₈F₂SO₂ (474): C, 60.75; H, 4.0; N, 17.22; F, 4.00; S, 6.75 %. Found: C, 60.55; H, 3.89; N, 17.42; F, 3.90; S, 6.6 %.

1,4-di(Trifluoromethyl)piperazine (12 and 13)

A mixture of compound 4 or 5 (0.6g, 0.002mol) and piperazine (0.34g, 0.004 mol) in EtOH (25 ml) refluxed for 6h, cooled. The solids were collected by filtration and crystallized from EtOH to give the compound 12 or 13.

12 in 78% yield, IR (cm⁻¹) v: 2800, 2790 (aliphatic CH), 1615, 1584 (C=C, C=N), 1488 (CH₃), 1210 (C-F), 892, 814 (phenyl).¹H NMR (400 MHz) δ: 12.0-11.0 (b,2H,NH-NH), 7.9, 7.8, 7.7, 7.43-7.41, 7.39-7.27, 7.26-7.19, 7.16-7.14 (aryl and arH). 12.4 (3, 18H, CH₃).¹³C NMR (100 MHz, DMSO) δ: 166 (C-Npip), 152 (C=C), 138 (C=N), 137 (=C-NH), 128.9-121 (Ar-C), 118.8 (CF₃),109 (C-C), 58.2 (4CH₃ pip) 12.93 (C-CH₃) mp 158-160°C. Anal. Calcd. For C₆H₆N₂F₂ (638): C, 56.42; H, 3.76; N, 21.94; F, 17.86 %. Found: C, 56.12; H, 3.49; N, 21.80; F, 17.60.

1,4-di(4-fluorophenyl)piperazine 13

At 82% yield.¹H NMR (400 MHz, DMSO) δ: 7.45-7.43 (m, 8H, Ar), 7.34-7.24 (m, 10H, ph). 3.23 (s, 8H, 4CH₂ph), 2.85 (s, 3H, CH₃).¹³C NMR (100 MHz, DMSO) δ: 167.42 (=C-Npiprazin), 162 (ar-CF), 152 (N=C-CF), 138 (C=N), 137 (=C-NH), 129.9-121 (Ar-C), 128.6, 116, 132 (pCH₂HF), 107 (C=C), 7.21 (4Cpip), 11.93 (C-CH₃). mp 188-190°C. Anal. Calcd. For C₆H₄N₁F₆ (690): C, 69.56; H, 4.63; N, 20.28; F, 5.50 %. Found: C,69.31; H, 4.45; N, 19.89; F, 5.01 %. m/z (% Int. %): 640 (M+2, 1.11),277 (23), 92 (2.1), 85 (41), 69 (100).

3. Result and Discussion

Aliphatic-CF₃ is more active in comparison with aromatic C-F, thus, -CF₃ often improve the physical, chemical and biological properties of their systems [10,11].

The structure of the new synthesized systems 5-13 were established from numerous spectrum data. The UV absorption gave confirming information about the structures of compounds 5-9. The shift in wavelength to λmax at 277 (5), 443 (7) and 269 (9) nm are indicative of a chromophore conjugated of pyrazolopyrimidine 7, while a lower λmax of 9 refers to inhibition of heterocoujugation Skelton. While the ¹H NMR spectrum of compound 7 showed a signal at δ 5.5, 10.9, 7.90-7.35, 2.8, 2.5 and 1.25 ppm attribute to the OH, NH, aromatic-H, CH₂-CH₂ and Me protons respectively, compound 9 showed leak of both OH and NH proton due to cyclized polyheterocyclic nitrogen system formed. Similarly, compound 12 exhibits a different type of aliphatic protons Me, piperazine CH₂CH₂.¹³CNMR showed the expected aromatic carbon at the range of δ 120 in addition to the characteristic C-S carbon at δ 161, the disappearance of this beak gives clear indication on the nucleophilic reaction. While the C-F carbon appears in same rang at δ 152 in most synthesis compounds. The Mass spectroscopy of synthesis compound shows a base peak at m/e 69 and 95 attribute to CF₃ and 4-fluorophenyl radicals as example compound 4 and 5 respectively.

3.1. Biological Activity

The interesting synthesized system that combined between pyrazole and pyrimidine moiety were primarily evaluated on the activity of cellobiase produced by Aspergillus Nidulans fungi. The fungus was grown in Gzapeck medium (pH=4) and incubated for 8 days at 45°C. The filtrate was then assayed for cellobiase activity, according to the described method [12]. Each compound was dissolved in DMF to obtain different concentrations (Table 1) and then added to the assay mixture consisting of the enzyme solution and the substrate (cellobiase) dissolved in phosphors citrate buffer at pH= 4.8-5 and incubated at 50°C for 1 h. The released reducing sugar was estimated calorimetrically at 540 nm as an indication of the enzymatic activity.

Table 1 showed that compounds with more fluorine character exhibited high activity over the other compounds at various concentrations in the order of 12> 10a> 10b> 4> 6> 8 at concentration 1000 µg/ml in comparison with the blank standard 0.35 µg/ml. on other hand, all tested compound showed close (±1) activity to the standard at low concentration 10 µg/ml.

Compound 12 shows the highest activity in comparison with other tested compounds at 1000 and 100 µg/ml and can be used as a biodynamic agent in the cellobiase activity, followed by 10a and 10b respectively. This could give an preliminary result that dimer (bis) structure with strong trifluoro group for example 12 is more active in both tested concentration 1000 and 100 µg/ml, while the fluoro chloro derivative 10a is the more reactive compound at low concentration in comparison with other tested compounds. (Table 1)

| Table 1. The effect on cellobiase activity produced by Aspergillus Nidulans |
|---------------------------|---------------------|------------------------|
| Compound number | Concentrations | |
| | 1000 µg/ml | 100 µg/ml | 10 µg/ml |
| 2 | 0.38 | 0.36 | 0.35 |
| 4 | 0.42 | 0.40 | 0.35 |
| 6 | 0.40 | 0.38 | 0.36 |
| 8 | 0.38 | 0.37 | 0.35 |
| 10a | 0.51 | 0.40 | 0.38 |
| 10b | 0.48 | 0.38 | 0.36 |
| 10c | 0.45 | 0.40 | 0.35 |
| 12 | 0.55 | 0.45 | 0.36 |
| 13 | 0.36 | 0.35 | 0.32 |

* Blank: 0.35 µg/ml (without substance or DMF ), DMF: 0.04 µg/ml (as solvent) is better solvent in our compounds.
** Other tested compounds recorded 0.30-0.35 µg/ml reduces sugar at all concentrations.
Conclusions

This work explored the new route toward the synthesis of fluorine substituted pyrazolopyrimidine and fused polyheterocyclic nitrogen systems under the action of mercaptopyrazolo[3,4-d]pyrimidines as a key reagent for the synthesis of various fluorinated heteropolycyclic systems as enzymatic agents towards some fungi.

References


