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Selective synthesis of fluorinated benzyl- or phenylpyrimidines from the heterocyclisation of α-trifluoroacetylarylpropanenitriles

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Abstract

Some 5-benzyl-6-trifluoromethyl-2,4-diaminopyrimidines analogous to trimethoprim and 6-aryl-5-trifluoroethyl-2,4-diaminopyrimidines analogous to pyrimethamine were prepared from the same synthons, trifluoromethylated β-ketonitriles. The heterocyclisation between enol ether of β-ketonitrile and guanidine leading to these compounds was studied.

These fluorinated novel compounds were tested for their *in vitro* activity against *Toxoplasma gondii*, a widespread apicomplexan protozoa responsible for congenital toxoplasmosis and cerebral toxoplasmosis in immunocompromised patients.

Keywords: Diaminopyrimidine; Solvent and fluorine effects on heterocyclisation; Benzylpyrimidine; Phenylpyrimidine; Toxoplasmosis

1. Introduction (Fig. 1)

A surprisingly vast number of fluorine-containing molecules are marketed as drugs or are in clinical trials [1,2] considering organo-fluorine compounds are very rare in Nature, the traditional source of bioactives (only 13 secondary metabolites identified) [3]. The special properties of the fluorine atom can have considerable impacts on the behaviour of a molecule in a biological environment [4–6]. The various uses of fluorine to increase metabolic stability and lipophilicity are examples of directed strategies of fluorine substitution [1–6].

These compounds result from the impressive development of synthetic methodologies in organic fluorine chemistry and efforts in this way are in constant progress [6,7]. Two general strategies are employed: the direct fluorination method [7a] and the building-block method [7a,b].

Along the second route, we have developed syntheses of trifluoromethylated β-enaminoketones and demonstrated their usefulness in the preparation of various CF_3 nitrogen heterocycles [8].

Furthermore, the introduction of fluorine significantly alters the physico-chemical properties of a molecule (due in part to its large electronegativity) and thus can have surprising effects on chemical reactivity [5,6].

In a previous paper concerning the preparation of fluorinated aminopyrimidines analogous to trimethoprim, we have described an unexpected synthesis of 5-trifluoroethyl-2,4-diaminopyrimidines from the heterocyclisation of α-trifluoroacetylpropanenitriles [8d]. These compounds can be considered as inverted (5,6-substituents) pyrimethamine derivatives.

Diaminopyrimidines trimethoprim (tmp) and pyrimethamine (pyr) are the reference drugs used for the treatment of opportunistic infections due to *Toxoplasma gondii*.

T. gondii is an apicomplexan protozoa, which is characterized by a bifunctional enzyme, called dihydrofolate reductase-thymidylate synthase (DHFR-TS) which plays a crucial role in pyrimidine biosynthesis [9]. Recently, more attention has been given to *T. gondii* as a frequent cause of toxoplasmic encephalitis in immunocompromized patients with AIDS or in immunosuppressed organ transplant recipients [9].

These DHFR inhibitors (tmp and pyr) are always used in combination for synergistic effect with sulfonamides such as sulfadiazine (with pyr) that is known to induce severe allergic reactions in many patients [9a]. Despite the continuing success

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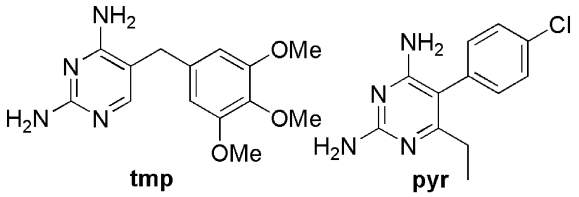


Fig. 1. Structure of trimethoprim (tmp) and pyrimethamine (pyr).

of these compounds, there is currently a need for more effective derivatives for the treatment of toxoplasmosis as this drug combination is frequently responsible for adverse side effects.

To complete this work, we now wish to report: (i) the synthesis of novel fluorinated pyrimidines, 6-trifluoromethyl-2,4-diaminopyrimidines analogous to tmp and 5-trifluoroethyl-2,4-diaminopyrimidines analogous to pyr obtained from heterocyclisation between the same trifluoromethylated synthons and guanidine; (ii) the study of the effect of the trifluoromethyl group on the heterocyclisation and the role played by the solvent; (iii) the evaluation of anti-*toxoplasma* activity of these compounds.

2. Results and discussion

For the synthesis of these 2,4-diaminopyrimidines we have employed a general method that consists of condensing enol ethers of β-ketonitriles with guanidine.

2.1. Synthesis of 5-benzyl-6-trifluoromethylpyrimidines analogous to tmp (Scheme 1)

Benzylpyrimidines **3a**,**b** were prepared from β-ketonitriles **1a**,**b** isolated in their more stable hydrated forms. Compounds **1a**,**b** were converted into enol ethers **2a**,**b** (78 and 88%, respectively) as *Z*,*E* isomeric mixtures by reaction with triethyl orthoformate. The heterocyclisation with guanidine as a free base in acetonitrile at reflux gave **3a**,**b** (91 and 66%, respectively).

2.2. Synthesis of 6-aryl-5-trifluoroethylpyrimidines analogous to pyr (Scheme 1)

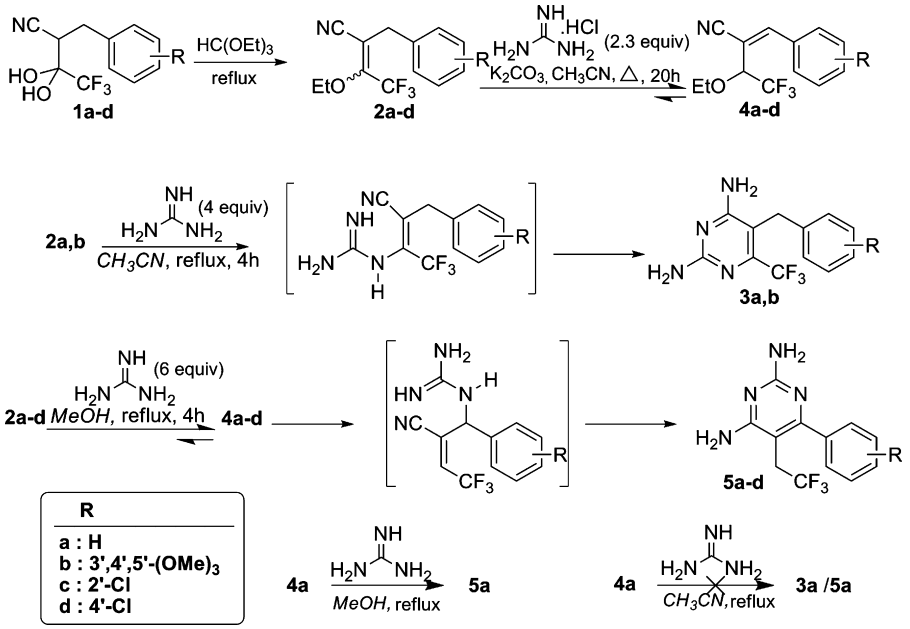
When methanol was used instead of acetonitrile as the solvent of the reaction, phenylpyrimidines **5c**,**d** were isolated with good yields (51 and 63%, respectively) by procedure described previously for **5a**,**b** [8d].

In our previous paper, a mechanism was also proposed for this unexpected heterocyclisation after observing the tautomerization of enol ether **2a** to enenitrile **4a** when **2a** was treated with guanidine hydrochloride in the presence of potassium carbonate at reflux in acetonitrile [8d].

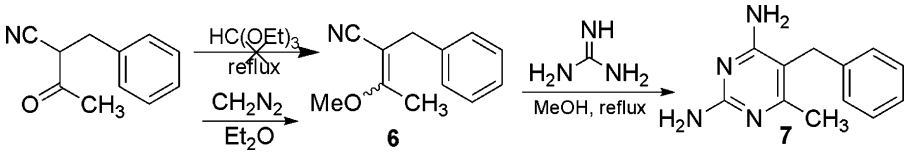
Indeed, tautomerization occurs in strong basic conditions since guanidine acts as a base. This reaction is also very slow: after 20 h at reflux **4a** was obtained (42%) and **2a** was recovered (25%). Furthermore, in order to study the equilibrium displacement of the tautomerization, isolated enenitrile **4a** was treated in the same conditions as above mentioned; surprisingly, after 20 h at reflux, isomerization of **4a**–**2a** did not occur (**4a** was recovered). So the equilibrium is favourably but slowly displaced from **2** toward **4** with these base-catalysed conditions.

Thus, phenylpyrimidines no doubt originate from a 1,4 Michaël addition of guanidine on the tautomer **4**, followed by ethanol elimination, then cyclisation on the nitrile carbon [8d].

To confirm this, the isolated enenitrile **4a** was treated with guanidine ''base'' in methanol at reflux and phenylpyrimidine **5a** was obtained (30%).



Scheme 1.



Scheme 2.

2.3. Solvent effect on heterocyclisation and role of the CF_3 group (Schemes 1–3)

Depending on the solvent used, and starting from the same trifluoromethylated synthon, benzylpyrimidine or phenylpyrimidine was obtained by condensation with guanidine.

In order to elucidate the mechanism and especially the role played by the trifluoromethyl group, we achieved the reaction in the nonfluorinated series by reacting enol ether **6** with guanidine in methanol at reflux (Scheme 2). In this case, only benzylpyrimidine **7** [10] was obtained with good yields (73%) instead of phenylpyrimidine as observed in the fluorinated series. It is also interesting to note that enol ether **6** was only obtained by reaction between diazomethane (a strong methylating agent) and the corresponding β-ketonitrile (the attempt with triethyl orthoformate as in the fluorinated series was unsuccessful). And so probably, tautomerization of enol ether **6** to the corresponding enenitrile does not occur with these basic conditions if we compare enolization in these two series.

Evidently, trifluoromethyl group has a great influence on tautomerization and heterocyclisation when methanol, a polar and protic solvent, was used.

This solvent is able to form hydrogen bonds with the oxygen of the ethoxy group of **4a** which induces ethanol elimination. Thus two types of mechanism can be considered (Scheme 3): formation of stable allyl/benzyl carbocations (the positive charge is also delocalized around the benzene ring) or a concerted allylic S_N2' process. In the first case, a S_N1 mechanism is possible because such cations (with a CF_3 group placed on the α-position) can be generated, studied, and used in synthetic applications [11].

And in both cases, the cyclocondensation with guanidine starts preferentially on the benzyl carbon (Scheme 3) because nucleophilic substitution on a CF_3-substituted carbon atom is not an easy process. The difficulty is attributed to destabilization of the transition state by fluorine and also to electrostatic repulsion between a nucleophile and the lone pairs of electrons of the fluorine atoms [6,7].

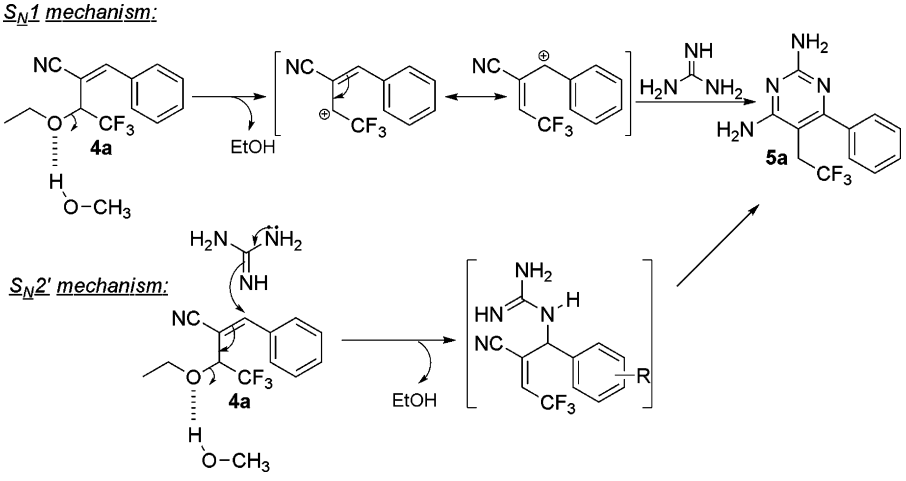
Since no hydrogen bonding is possible between acetonitrile, a polar and aprotic solvent, and enenitrile **4a**, the heterocyclisation can only occur with enol ether **2a** in a Michaël addition/elimination mechanism. To check this, **4a** was treated with guanidine "base" in acetonitrile at reflux (20 h) and neither benzylpyrimidine **3a** nor phenylpyrimidine **5a** was obtained, only **4a** was recovered with 11% yields (Scheme 1).

We have also noted that the reaction of guanidine with **2a** in dimethylformamide at room temperature gave no product and we observed total destruction of the reagent **2a**.

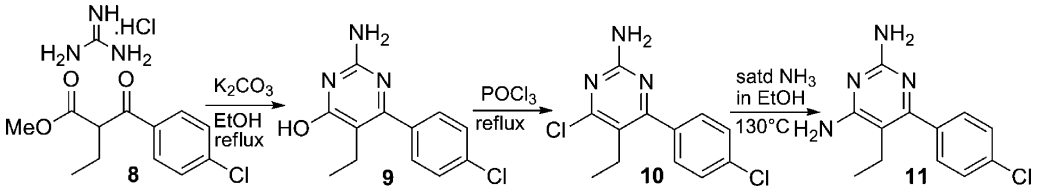
According to these results, we can conclude that the tautomerization equilibrium is very slow and displaced from **2** toward **4** in guanidine base-catalysed conditions. And when a large excess of guanidine as a free base (4 equiv.) is used in acetonitrile, isomerization occurs but slowly and, as **4** is not activated in this solvent, guanidine acts also as a nucleophile and prefers reacting with **2**. Even though in methanol, **4** is activated (by hydrogen bonding) and becomes more reactive than **2** and so reacts with guanidine after base-catalysed tautomerization.

2.4. Synthesis of the non fluorinated inverted pyr derivative (Scheme 4)

Compound **11** was prepared in order to compare the bioactivity of **5d** with the non fluorinated derivative and thus to evaluate the influence of fluorine on the bioactivity.



Scheme 3.



Scheme 4.

The synthetic approach used to prepare **11** was adapted from the method developed by Hitchings [12]. It was synthesized from the corresponding 4-hydroxy derivative **9** which was obtained by refluxing an ethanolic solution of guanidine hydrochloride with keto ester **8** in the presence of potassium carbonate (70%). The hydroxy aminopyrimidine **9** was then converted into the chloro derivative **10** by treatment with excess phosphoryl chloride under reflux conditions with low yields (30%). The 4-chloroaminopyrimidine **10** was finally reacted with saturated solution of ethanolic ammonia to give diaminopyrimidine **11** (56%).

2.5. Biological results: in vitro activity of tmp/pyr derivatives on T. gondii (Table 1)

Anti-*toxoplasma* activities of derivatives **3a**,**b** and **5c**,**d** and **11** were assessed *in vitro*.

All these compounds were tested individually at 10 concentrations ranging from 0.01 to 40 mg L^{-1} (final concentration in the culture). Each concentration was tested in eight replicate wells and in two replicate culture plates. A reference drug (*i.e.* pyrimethamine or trimethoprim) was tested in each set of experiments.

In vitro, all the compounds started to show inhibitory effects on *Toxoplasma* growth, at concentrations >1 mg L^{-1} for compounds **5c**, >2 mg L^{-1} for **3b** and **5d** and >5 mg L^{-1} for **3a** and **11**. A cytotoxic effect on the monolayers was recorded for compounds **3b**, **5d** and **11** at concentrations ≥20 mg L^{-1}. Estimated IC_{50} by linear regression analysis ranged between 3.5 mg L^{-1} (**5d**) and 16 mg L^{-1} (**5c**) (Table 1). In comparison, the IC_{50} of trimethoprim and pyrimethamine were estimated at 2.8 and 0.06 mg L^{-1}, respectively, *i.e.* concentrations in agreement with our previously reported results using the same assay [13].

Surprisingly, the tmp analogue **3b** revealed itself to be less active than tmp and cytotoxic at concentrations >40 mg L^{-1}.

Table 1
Activities of diaminopyrimidines against *T. gondii* on MRC5 fibroblast tissue cultures

Compound	*T. gondii* IC_{50} (mg L^{-1})	Cytotoxicity (mg L^{-1})
3a	15.4	100
3b	9.7	40
5c	16	100
5d	3.5	40
11	5	20
pyr	0.06	>50
tmp	2.8	>100

So the introduction of a trifluoromethyl group at the 6-position of the pyrimidine ring, seems to decrease the inhibitory activity while slightly enhancing the cytotoxicity. The weak activity of the benzyl derivative **3a** can also be explained by the absence of substituents on the aromatic group. Indeed, structure–activity relationships studies generally showed that the presence of one or more substituent(s) on the benzyl group can play an important role in the activity (by binding process) [14].

For compounds related to pyr (**5c**,**d** and **11**), a marked decrease of the inhibitory effect was noted, about 100-fold lower compared to pyr; however estimated IC_{50} were close to that of tmp. Moreover, these results show that there is no influence of fluorine on the activity, when comparing IC_{50} of **5d** and **11**. Compound **5c** is the less active because of the *ortho*-position of Cl on the aromatic ring. In this series, activities were described only for molecules with substituent(s) in the *meta* or *para* position [15].

3. Conclusion

Herein we have described a novel method which allowed the preparation of fluorinated benzyl- or phenylpyrimidines from the same reagents, only by varying the nature of the solvent. The presence of the CF_3 group induces this unusual reactivity, which may be ascribed to the strong electron withdrawing effect of this substituent.

Concerning their bioactivity on *T. gondii*, they were found of the same range of activity than the reference drug tmp, even if less active than pyr. Moreover the most active pyr derivative **5d** (IC_{50} = 3.5 mg L^{-1}) revealed to be two to threefold more cytotoxic than pyr. Comparing **5d** with **11**, no influence of the CF_3 group on bioactivity could be highlighted.

4. Experimental

4.1. General experimental techniques

Melting points were taken on a Stuart Scientific melting point apparatus (smp3) and were uncorrected. IR spectra were recorded on a BOMEM MB Series apparatus. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker AC 300 spectrometer. Chemical shifts are expressed in ppm from TMS as internal reference. Mass spectra (EIMS and HRMS) were recorded on a Micromass GCT spectrometer. Thin layer chromatography (TLC) was carried out on aluminium-baked Merck silica gel 60 F254. Column chromatography was performed on silica gel.

4.2. General procedure for the preparation of **2a–d**

A solution of **1a–d** in $HC(OEt)_3$ (3 mL/1.5 mmol of **1**) was refluxed (140 °C) and the reaction was monitored by TLC. The excess of $HC(OEt)_3$ was removed by distillation in vacuo and **2a–d** as *Z,E* isomeric mixtures were obtained by purification on column chromatography.

4.2.1. 2-Benzyl-3-ethoxy-4,4,4-trifluorobut-2-enenitrile (**2a**)

Oil; 78%; IR (KBr); ν 2992, 2223, 1638, 1331, 1186, 1140, 1008, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.39 (3H, m, CH_3), 3.66 (2H, s, CH_2), 4.10 and 4.25 (2H, q, J = 7.0 Hz, CH_2O), 7.15–7.39 (5H, m, H-ar); ^{13}C NMR ($CDCl_3$): δ 15.1 and 15.2 (CH_3), 33.7 and 34.5 (CH_2), 70.5 and 71.8 (CH_2O), 104.7 (Cq), 114.8 and 115.7 (CN), 119.2 (q, $^1J_{C,F}$ = 279.9 Hz, CF_3), 127.5 and 127.6 (CH-ar), 128.5 and 128.6 (2× CH-ar), 128.9 (2× CH-ar), 135.2 and 135.5 (Cq-ar), 152.8 (q, $^2J_{C,F}$ = 33.3 Hz, Cq). EIMS, *m/z*: 255 $[M]^+$ (92), 226 (26), 158 (100), 130 (87). HRMS, calcd for $C_{13}H_{12}F_3NO$: 255.0871. Found: 255.0871.

4.2.2. 3-Ethoxy-4,4,4-trifluoro-2-(3′,4′,5′-trimethoxybenzyl)-but-2-enenitrile (**2b**)

Oil; 88%; IR (KBr); ν 3000, 2946, 2215, 1638, 1593, 1510, 1462, 1424, 1333, 1248, 1184, 1130, 1011, 847, 824, 716 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.42 (3H, t, J = 7.0 Hz, CH_3), 3.61 (2H, s, CH_2), 3.86 (3H, s, CH_3O), 3.87 (6H, s, 2× CH_3O), 4.15 and 4.28 (2H, q, J = 7.0 Hz, CH_2O), 6.46 (2H, s, H-ar); ^{13}C NMR ($CDCl_3$): δ 15.1 and 15.3 (CH_3), 34.8 (CH_2), 56.0 (2× CH_3O), 60.8 (CH_3O), 70.5 and 71.8 (CH_2O), 104.6 (Cq), 105.5 and 105.6 (2× CH-ar), 114.9 and 115.8 (CN), 119.2 (q, $^1J_{C,F}$ = 279.7 Hz, CF_3), 130.7 and 131.1 (Cq-ar), 137.4 (Cq-ar), 152.7 (q, $^2J_{C,F}$ = 33.0 Hz, Cq-CF_3), 153.4 (2 × Cq-ar). EIMS, *m/z*: 346 $[M + H]^+$ (18), 345 (100), 330 (22), 181 (36).

4.2.3. 2-(2′-Chlorobenzyl)-3-ethoxy-4,4,4-trifluorobut-2-enenitrile (**2c**)

Oil, 69%, IR (KBr); ν 2989, 2225, 1636, 1475, 1446, 1332, 1184, 1141, 1010, 748 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.40 (3H, m, CH_3), 3.83 (2H, s, CH_2), 4.12 and 4.30 (2H, q, J = 7.1 Hz, CH_2O), 7.18–7.49 (4H, m, H-ar); ^{13}C NMR ($CDCl_3$): δ 15.2 and 15.3 (CH_3), 31.3 and 32.2 (CH_2), 70.4 and 72.0 (CH_2O), 102.7 (Cq), 114.4 and 115.2 (CN), 119.1 (q, $^1J_{C,F}$ = 280.0 Hz, CF_3), 127.3 (CH-ar), 129.1 (CH-ar), 129.8 (CH-ar), 130.6 (CH-ar), 133.2 (Cq-ar), 134.2 (Cq-ar), 153.5 (q, $^2J_{C,F}$ = 33.2 Hz, Cq-CF_3). EIMS, *m/z*: 291 $[M]^+$ (4), 289 $[M]^+$ (22), 226 (100), 218 (53), 131 (25), 125 (23). HRMS, calcd for $C_{13}H_{11}F_3NO^{35}Cl$: 289.0481. Found: 289.0488; and for $C_{13}H_{11}F_3NO^{37}Cl$: 291.0452. Found: 291.0466.

4.2.4. 2-(4′-Chlorobenzyl)-3-ethoxy-4,4,4-trifluorobut-2-enenitrile (**2d**)

Oil; 73%; ^1H NMR ($CDCl_3$): δ 1.42 (3H, m, CH_3), 3.66 (2H, s, CH_2), 4.13 and 4.28 (2H, q, J = 7.0 Hz, CH_2O), 7.18 (2H, d, J = 8.4 Hz, H-ar), 7.32 (2H d, J = 8.4 Hz, H-ar); ^{13}C NMR ($CDCl_3$): δ 15.2 and 15.3 (CH_3), 33.2 and 34.0 (CH_2), 70.5 and 71.9 (CH_2O), 104.0 (Cq), 114.7 and 115.7 (CN), 119.2 and 120.0 (q, $^1J_{C,F}$ = 278.3 Hz, CF_3), 129.1 (CH-ar), 129.2 (CH-ar), 129.9 (CH-ar), 130.0 (CH-ar), 133.6 (Cq-ar), 134.1 (Cq-ar), 153.0 (q, $^2J_{C,F}$ = 33.7 Hz, Cq-CF_3). EIMS, *m/z*: 291 $[M]^+$ (16), 289 $[M]^+$ (100), 260 (16), 254 (16). HRMS, calcd for $C_{13}H_{11}F_3NO^{35}Cl$: 289.0481. Found: 289.0482; and for $C_{13}H_{11}F_3NO^{37}Cl$: 291.0452. Found: 291.0455.

4.3. General procedure for the synthesis of **3a,b**

A solution of guanidine hydrochloride (4 equiv.) in anhydrous methanol (0.3 mL $mmol^{-1}$ of guanidine hydrochloride) was added to a stirred solution of sodium methoxide (4 equiv.) prepared in situ in anhydrous methanol (0.3 mL $mmol^{-1}$ of sodium methoxide) under an atmosphere of nitrogen at rt.

The mixture was stirred for 5 min, filtered, and MeOH was evaporated. Guanidine (4 equiv.) as a free base was dissolved in CH_3CN (4 mL $mmol^{-1}$ of enol ether **2**) and **2a**,**b** was added. The reaction mixture was heated to reflux and was monitored by TLC. It was then cooled at rt, concentrated in vacuo and the residue was purified by column chromatography to yield compounds **3a**,**b**.

4.3.1. 5-Benzyl-2,4-diamino-6-(trifluoromethyl)pyrimidine (**3a**)

Solid; 91%; mp 169–170 °C; IR (KBr); ν 3500, 3328, 3192, 1645, 1565, 1466, 1451, 1420, 1177, 1130, 723 cm^{-1}; ^1H NMR (CD_3OD): δ 3.90 (2H, s, CH_2), 7.00–7.30 (5H, m, H-ar); ^{13}C NMR (CD_3OD): δ 30.9 (CH_2), 104.4 (C-5), 123.3 (q, $^1J_{C,F}$ = 276.5 Hz, CF_3), 127.5 (CH-ar), 128.7 (2× CH-ar), 129.6 (2× CH-ar), 139.4 (Cq-ar), 153.1 (q, $^2J_{C,F}$ = 31.1 Hz, C-6), 163.1 and 166.7 (C-2, C-4). EIMS, *m/z*: 268 $[M]^+$ (100), 267 (46), 218 (29), 191 (25). HRMS, calcd for $C_{12}H_{11}F_3N_4$: 268.0936. Found: 268.0921.

4.3.2. 2,4-Diamino-5-(3′,4′,5′-trimethoxybenzyl)-6-(trifluoromethyl)pyrimidine (**3b**)

Solid; 66%; mp 235–237 °C; IR (KBr); ν 3459, 3424, 3343, 3221, 1667, 1640, 1595, 1472, 1457, 1422, 1230, 1178, 1127, 725 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.63 (3H, s, CH_3O), 3.71 (6H, s, 2× CH_3O), 3.79 (2H, s, CH_2), 6.36 (2H, s, NH_2), 6.44 (2H, s, H-ar), 6.58 (2H, br s, NH_2); ^{13}C NMR (DMSO-d_6): δ 29.7 (CH_2), 56.0 (2× CH_3O), 60.2 (CH_3O), 102.0 (C-5), 105.2 (2× CH-ar), 122.5 (q, $^1J_{C,F}$ = 277.4 Hz, CF_3), 134.9 (Cq-ar), 136.0 (Cq-ar), 151.2 (q, $^2J_{C,F}$ = 30.3 Hz, C-6), 152.9 (2 × Cq-ar), 161.8 and 165.0 (C-2, C-4). EIMS, *m/z*: 358 $[M]^+$ (100), 343 (29), 327 (26), 311 (24), 219 (48), 69 (23). HRMS, calcd for $C_{15}H_{17}F_3N_4O_3$: 358.1253. Found: 358.1266.

4.4. 3-Ethoxy-4,4,4-trifluoro-2-[1-phenyl-methylidene]-butyronitrile (**4a**)

Enol ether **2a** (165 mg, 0.65 mmol) was added to a solution of guanidine hydrochloride (143 mg, 1.5 mmol) and K_2CO_3 (270 mg, 1.95 mmol) in CH_3CN (10 mL). The reaction mixture was heated to reflux for 20 h, the solvent was then evaporated. The residue was purified by column chromatography (CH_2Cl_2/cyclohexane; 50/50) to give 30 mg of **4a** and 80 mg of a mixture of **4a** and **2a** (49/51).

Oil; 42%; IR (KBr); ν 2917, 2218, 1620, 1454, 1358, 1266, 1184, 1143, 1121, 1101, 756, 688 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.32 (3H, t, J = 7.0 Hz, CH_3), 3.76 (2H, m, CH_2O), 4.34 (1H, q, $^3J_{H,F}$ = 5.9 Hz, C*H*CF_3), 7.39 (1H, s, CH), 7.46 (3H, m, H-ar), 7.85 (2H, m, H-ar); ^{13}C NMR ($CDCl_3$): δ 14.9 (CH_3), 67.3 (CH_2O), 78.6 (q, $^2J_{C,F}$ = 32.1 Hz, *C*HCF_3), 103.7 (Cq), 116.1 (Cq), 123.0 (q, $^1J_{C,F}$ = 283.0 Hz, CF_3), 129.0 (2× CH-ar), 129.6 (2× CH-ar), 131.7 (CH-ar), 132.1 (Cq-ar), 148.6 (CH). EIMS, *m/z*: 255 $[M]^+$ (26), 186 $[M - CF_3]^+$ (54), 158 (100), 140 (49). HRMS, calcd for $C_{13}H_{12}F_3NO$: 255.0871. Found: 255.0873.

4.5. General procedure for the synthesis of **5c**,**d** and **7** was previously described [8d]

4.5.1. 6-(2′-Chlorophenyl)-2,4-diamino-5-(2′,2′,2′-trifluoroethyl)pyrimidine (**5c**)

Solid; 51%; mp 182 °C; IR (KBr); ν 3356, 3183, 1620, 1557, 1447, 1261, 1141, 1100, 760 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.3 (2H, m, CH_2), 6.13 (2H, br s, NH_2), 6.60 (2H, br s, NH_2), 7.23–7.59 (4H, m, H-ar); ^{13}C NMR (DMSO-d_6): δ 30.1 (q, $^2J_{C,F}$ = 29.5 Hz, CH_2), 93.3 (C-5), 126.7 (q, $^1J_{C,F}$ = 277.2 Hz, CF_3), 127.1 (CH-ar), 129.3 (CH-ar), 129.9 (CH-ar), 130.9 (CH-ar), 131.0 (Cq-ar), 138.0 (Cq-ar), 162.2, 164.0 and 165.0 (C-2, C-4, C-6). EIMS, *m/z*: 304 $[M]^+$ (39), 302 $[M]^+$ (100), 267 (35), 235 (28), 233 (84), 198 (56), 69 (33). HRMS, calcd for $C_{12}H_{10}F_3N_4Cl$: 302.0546. Found: 302.0562.

4.5.2. 6-(4′-Chlorophenyl)-2,4-diamino-5-(2′,2′,2′-trifluoroethyl)pyrimidine (**5d**)

Solid; 63%; mp 208–209 °C; ^1H NMR (DMSO-d_6): δ 3.41 (2H, q, $^3J_{H,F}$ = 11.0 Hz, CH_2), 6.10 (2H, br s, NH_2), 6.58 (2H, br s, NH_2), 7.37 (2H, d, J = 8.5 Hz, H-ar), 7.48 (2H, d, J = 8.5 Hz, H-ar); ^{13}C NMR (DMSO-d_6): δ 29.6 (q, $^2J_{C,F}$ = 29.2 Hz, CH_2), 92.5 (C-5), 126.8 (q, $^1J_{C,F}$ = 277.5 Hz, CF_3), 128.3 (2× CH-ar), 130.2 (2× CH-ar), 132.7 (Cq-ar), 138.8 (Cq-ar), 162.1, 164.2 and 166.3 (C-2, C-4, C-6). EIMS, *m/z*: 304 $[M]^+$ (21), 303 (31), 302 $[M]^+$ (72), 301 (100), 235 (9), 233 (28), 198 (19). HRMS, calcd for $C_{12}H_{10}F_3N_4Cl$: 302.0546. Found: 302.0548.

4.5.3. 5-Benzyl-2,4-diamino-6-methylpyrimidine (**7**)

Solid; 73%; IR (KBr); ν 3375, 3150, 1665, 1575, 1408, 881, 618, 557 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.03 (3H, s, CH_3), 3.76 (2H, s, CH_2), 5.72 (2H, br s, NH_2), 6.08 (2H, br s, NH_2), 7.06–7.36 (5H, m, H-ar); ^{13}C NMR (DMSO-d_6): δ 21.5 (CH_3), 30.5 (CH_2), 102.6 (C-5), 125.9 (CH-ar), 128.0 (2× CH-ar), 128.4 (2× CH-ar), 140.7 (Cq-ar), 161.5, 161.3, 163.5 (C-2, C-4, C-6). EIMS, *m/z*: 214 $[M]^+$ (100), 213 (36). HRMS, calcd for $C_{12}H_{14}N_4$: 214.1218. Found: 214.1223.

4.6. 2-Amino-6-(4′-chlorophenyl)-4-hydroxy-5-ethylpyrimidine (**9**)

8 (1 g, 4.16 mmol) was refluxed with guanidine hydrochloride (400 mg, 4.19 mmol) and K_2CO_3 (400 mg, 2.90 mmol) in EtOH for 24 h. The mass was diluted with water (15 mL) and then made acid with acetic acid (5 mL). The crystalline mass was filtered off and washed with EtOH to yield 729 mg of **9** as a white solid (70%). ^1H NMR (DMSO-d_6): δ 0.99 (3H, t, J = 7.1 Hz, CH_3), 2.20 (2H, q, J = 7.1 Hz, CH_2), 6.41 (2H, br s, NH_2), 7.41 (2H, d, J = 8.3 Hz, H-ar), 7.50 (2H, d, J = 8.3 Hz, H-ar), 10.99 (1H, br s, OH); ^{13}C NMR (DMSO-d_6): δ 14.2 (CH_3), 19.2 (CH_2), 113.6 (C-5), 128.2 (2× CH-ar), 130.0 (2× CH-ar), 133.0 (Cq-ar), 138.8 (Cq-ar), 153.4 (C-6), 160.6 and 163.4 (C-2, C-4).

4.6.1. 2-Amino-4-chloro-6-(4′-chlorophenyl)-5-ethylpyrimidine (**10**)

The above compound **9** (111 mg, 0.445 mmol) was refluxed with $POCl_3$ (5 mL) for 2 h. The excess $POCl_3$ was removed in vacuo and the residue was poured on to ice and ammonia, then filtered off. The solid was purified by column chromatography (CH_2Cl_2/MeOH, 98/2) to yield 36 mg of **10** as a pale yellow solid (30%). ^1H NMR ($CDCl_3$): δ 1.10 (3H, t, J = 7.4 Hz, CH_3), 2.59 (2H, q, J = 7.4 Hz, CH_2), 5.72 (2H sl, NH_2), 7.39 (2H, d, J = 8.4 Hz, H-ar), 7.45 (2H, d, J = 8.4 Hz, H-ar); ^{13}C NMR ($CDCl_3$): δ 14.0 (CH_3), 21.8 (CH_2), 121.9 (C-5), 128.7 (2× CH-ar), 129.4 (2× CH-ar), 135.5 (Cq-ar), 135.9 (Cq-ar), 159.7 (C-6), 163.1 and 166.6 (C-2, C-4).

4.6.2. 2,4-Diamino-6-(4′-chlorophenyl)-5-ethylpyrimidine (**11**)

The chloro compound **10** (35 mg, 0.13 mmol) was heated in a closed system at 135 °C with 10 mL saturated solution of ethanolic ammonia for 20 h. The solvent was then evaporated and the residue was washed with aqueous EtOH to give 18 mg of **11** as a white solid (56%). IR (KBr); ν 3301, 3156, 1680, 1634, 1514, 1113, 1091 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.94 (3H, t, J = 7.3 Hz, CH_3), 2.28 (2H, q, J = 7.3 Hz, CH_2), 7.27 (2H, sl, NH_2), 7.55 (2H, d, J = 8.5 Hz, H-ar), 7.65 (2H, d, J = 8.5 Hz, H-ar), 7.94 (2H, sl, NH_2); ^{13}C NMR (DMSO-d_6): δ 13.4 (CH_3), 18.2 (CH_2), 108.4 (C-5), 129.0 (2× CH-ar), 130.6 (2× CH-ar), 131.6 (Cq-ar), 135.1 (Cq-ar), 155.2 (C-6), 164.4 (C-2, C-4). EIMS, *m/z*: 250 $[M]^+$ (23), 248 $[M]^+$ (66), 235 (33), 233 (100), 198 (37), 130 (31). HRMS, calcd for $C_{12}H_{13}N_4Cl$: 248.0829. Found: 248.0825.

4.7. Biological studies: anti-T. gondii activity

Briefly, following a previously described method [13] the virulent RH strain of *T. gondii* was maintained in mice by intraperitoneal passage every 2 days. For each experiment, tachyzoites were collected from the peritoneal cavity of infected mice then resuspended in physiological saline. Tissue cultures and drug tests were carried out using MRC5 fibroblast tissue cultures. Confluent monolayers prepared in 96-well tissue culture plates were inoculated with 2000 fresh tachyzoites. After 4 h, 10 serial dilutions of each drug, ranging form 0.01 to 40 mg L^{-1} for test compounds and 0.001–5 for pyrimethamine were added into the culture medium. Each culture plate comprised eight negative control (without *T. gondii*) and eight positive control wells (without drug). After 72 h of incubation, the plates were examined microscopically for cytopathic effects then fixed with cold methanol for 5 min. *Toxoplasma* growth was assessed by enzyme linked immunoassay (ELISA) performed

directly on the fixed cultures using a peroxydase-labeled monoclonal antibody directed against the SAG-1 surface protein of *T. gondii*. After addition of the substrate, spectrophotometric readings were performed at 405 nm with blanking on the negative control well. For each wells, the results were expressed as optical density (OD) values. The effect of each drug at various concentrations was described by plotting the OD values as a function of the logarithm of the concentration and a linear regression model was used to summarize the concentration–dose effect relationship and to determine the IC_{50}.

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References

[1] R. Filler, Y. Kobayashi, L.M. Yagupolskii, Organofluorine Compounds in Medicinal Chemistry and Biomedical Application, Elsevier, Amsterdam, 1993.
[2] (a) F.M.D. Ismail, J. Fluorine Chem. 118 (2002) 27–33;
(b) C. Isanbor, D. O'Hagan, J. Fluorine Chem. 127 (2006) 303–319;
(c) J.P. Bégué, D. Bonnet-Delpon, J. Fluorine Chem. 127 (2006) 992–1012;
(d) K.L. Kirk, J. Fluorine Chem. 127 (2006) 1013–1029.
[3] D. O'Hagan, D.B. Harper, J. Fluorine Chem. 100 (1999) 127–133.
[4] B.E. Smart, J. Fluorine Chem. 109 (2001) 127–133.
[5] ChemBioChem 5 (2005) 557–726 (special issue).
[6] T. Kitazume, T. Yamazaki, Experimental Methods in Organic Fluorine Chemistry, Kodansha, Tokyo, 1998.
[7] For recent reviews, see:
(a) M. Shimizu, T. Hiyama, Angew. Chem. Int. Ed. 44 (2005) 214–231;
(b) M. Schlosser, Angew. Chem. Int. Ed. 45 (2006) 2–17.
[8] (a) M. Soufyane, C. Mirand, J. Lévy, Tetrahedron Lett. 34 (1993) 7737–7740;
(b) M. Soufyane, S. Van den Broek, L. Khamliche, C. Mirand, Heterocycles 51 (1999) 2445–2451;
(c) H. Berber, M. Soufyane, C. Mirand, S. Schmidt, A.-M. Aubertin, Tetrahedron 57 (2001) 7369–7375;
(d) H. Berber, M. Soufyane, M. Santillana-Hayat, C. Mirand, Tetrahedron Lett. 43 (2002) 9233–9235;
(e) A. Touzot, M. Soufyane, H. Berber, L. Toupet, C. Mirand, J. Fluorine Chem. 125 (2004) 1299–1304.
[9] (a) A.C. Anderson, DDT 10 (2005) 121–128, and cited references;
(b) I.M. Kompis, K. Islam, R.L. Then, Chem. Rev. 105 (2005) 593–620, and cited references.
[10] Benzylpyrimidine (7) was previously reported:
E.A. Falco, S. Dubreuil, G.H. Hitchings, J. Am. Chem. Soc. 73 (1951) 3758–3762.
[11] X. Creary, Chem. Rev. 91 (1991) 1625–1638.
[12] H. Hitchings, Tuckaohoe, P.B. Russel, E.A. Falco, US Patent 2,688,019 (1954).
[13] F. Derouin, C. Chastang, Antimicrob. Agents Chemother. 33 (1989) 1753–1759.
[14] (a) R.D. King, S. Muggleton, R.A. Lewis, M.J.E. Sternberg, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 11322–11326;
(b) J.D. Hirst, J. Med. Chem. 39 (1996) 3526–3532;
(c) C.D. Selassie, W.-X. Gan, L.S. Kallander, T.E. Klein, J. Med. Chem. 41 (1998) 4261–4272;
(d) D. Pez, I. Leal, F. Zucotto, C. Boussard, R. Brun, S.L. Croft, V. Yardley, L.M. Ruiz Perez, D.G. Pacanowska, I.H. Gilbert, Bioorg. Med. Chem. 11 (2003) 4693–4711;
(e) A. Rosowsky, R.A. Forsch, C.H. Sibley, C.B. Inderlied, S.F. Queener, J. Med. Chem. 47 (2004) 1475–1486.
[15] (a) P.B. Russel, G.H. Hitchings, J. Am. Chem. Soc. 73 (1951) 3763–3770;
(b) E.A. Bliss, R.J. Griffin, M.F.G. Stevens, J. Chem. Soc. Perkin Trans. I (1987) 2217–2228;
(c) B. Tarnchompoo, C. Sirichaiwat, W. Phupong, C. Intaraudom, W. Sirawaraporrn, S. Kamchonwongpaisan, J. Vanichtanankul, Y. Thebtaranonth, Y. Yuthavong, J. Med. Chem. 45 (2002) 1244–1252.