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Synthesis and biological investigation of *S*-aryl-*S*-DABO derivatives as HIV-1 inhibitors

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Abstract—*S*-Aryl-*S*-DABO derivatives, a novel subclass of *S*-DABO anti-HIV-1 agents, were synthesized via Ullmann type reaction starting from the corresponding 2-thiouracils by the aid of microwave irradiation. The results of their evaluation as inhibitors of RT are reported together with their antiviral activity in cellular assays.

The reverse transcriptase (RT) of the human immunodeficiency virus type 1 (HIV-1 RT) is one of the main targets of drugs used in the treatment of AIDS.[1,2] Several RT inhibitors have been developed and approved by the FDA and are currently in clinical use. In particular, the non-nucleoside RT inhibitors (NNRTIs)[3–5] are highly effective drugs with few side effects. However, RT mutations rapidly emerge that confer resistance to all known NNRTIs, including the clinically established drugs, such as nevirapine and efavirenz (Chart 1), thus reducing their effectiveness.[6,7] Therefore, new NNRTIs that are effective against the existing drug-resistant viral strains are urgently needed.

In line with these requirements, new classes of NNRTIs have been recently described, among which DAPY derivatives, such as TMC125,[8] show high inhibitory potency against several clinically relevant mutant strains. According to Arnold et al.,[9] this aspect is due to their high structural flexibility that allows for multiple binding conformations and hence confers the capability of targeting the RT of different mutants.

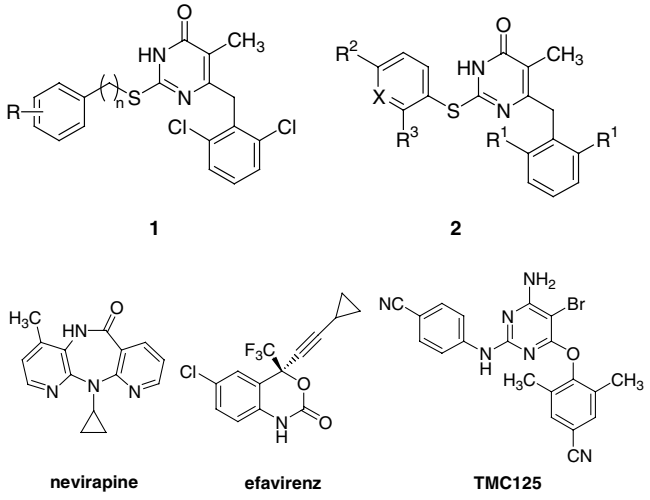

Chart 1.

During the course of our investigations on potential inhibitors of RT, we became involved in the preparation of a number of dihydro-alkylthio-benzyl-oxopyrimidines (*S*-DABOs)[10,11] of general structure **1**,[12] characterized by the presence of an arylalkylthio substituent at position 2 which was widely varied both in terms of substitution pattern and length of the alkyl spacer. Some of these compounds displayed potent RT inhibiting

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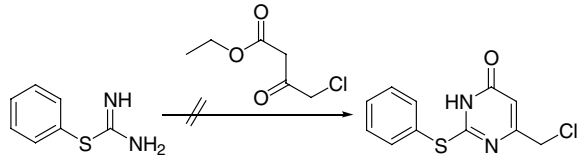
activity and antiviral properties on cell lines infected with either wild type or mutant HIV-1.

As an extension of our research, we planned to investigate the antiviral properties of new compounds of structure **2** which, bearing an aromatic/heteroaromatic ring directly linked to the sulfur atom, can be regarded as hybrids between DAPY and *S*-DABO compounds.[13]

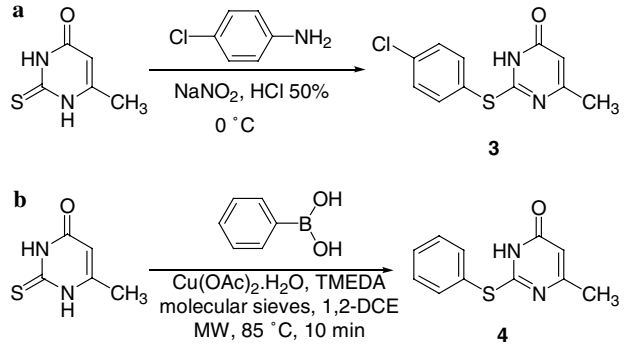
The target compounds **2** can, in principle, be obtained according to two different approaches: the condensation between an *S*-arylthiourea and a β-ketoester, or arylation of the appropriate 2-thiouracil. The first approach, used on a model reaction, gave no positive results. Indeed, when *S*-phenylisothiourea[14] was treated with the highly reactive 4-chloroacetoacetate in a range of different conditions,[15,16] complex reaction mixtures were always obtained, accompanied by decomposition of the starting material in alkaline media[17] (Scheme 1). According to this result, we decided to prepare first differently 6-substituted 2-thiouracils,[12,13] and then to introduce an aromatic or heteroaromatic substituent in position 2.

To this aim, the arylation via diazonium salts[18] was compared to an Ullmann type reaction[19–21] on a model substrate. Commercially available 2-thio-6-methyluracil was thus reacted in parallel with (a) the diazonium salt of *p*-chloroaniline and (b) phenylboronic acid in the presence of $Cu(OAc)_2{\cdot}H_2O$ under microwave irradiation (Scheme 2).

In the first reaction the corresponding 2-arylthiopyrimidinone **3** was obtained in 25% yield after a laborious chromatographic purification followed by crystallization from EtOH. Analogous results in terms of yield were obtained following the second approach and using TMEDA as a base and 1,2-DCE as the solvent in the presence of molecular sieves. This time, compound **4** could be obtained in 23% yield after chromatographic purification. Despite the low yield, the Ullmann type condensation was selected as the best methodology for a number of reasons: (i) its high reproducibility; (ii) the availability of a large number of arylboronic acids, much easier to handle than the diazonium salts of the corresponding anilines; (iii) the dramatic reduction of reaction times by the use of microwave irradiation.



Scheme 1.



Scheme 2.

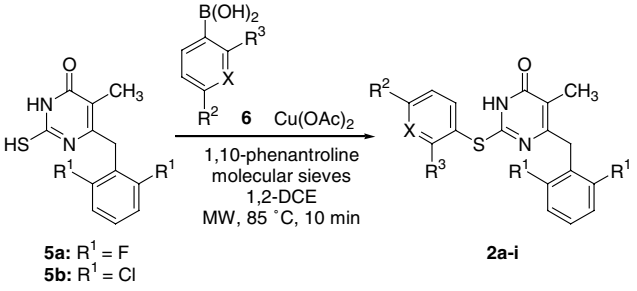
Having chosen this procedure for the synthesis of compounds of interest, we proceeded with the optimization of the reaction conditions. The use of 1,10-phenantroline[22] instead of TMEDA and an excess (3 equivalents) of the arylboronic acid instead of a stoichiometric amount resulted to be particularly fruitful. Also the purification step, which proved to be particularly troublesome because of the similar chromatographic behaviour of the arylboronic acids and the target compounds, needed some adjustments.

In particular, a filtration on Celite® of the reaction mixture followed by treatment with an alkaline solution to remove the excess of arylboronic acid gave compounds **2a**–**d** in acceptable purity; conversely, for compounds **2e**–**i**, a rapid filtration on silica gel of the reaction mixture, followed by recrystallization, gave the best results.

In order to verify the influence of the reaction temperature and, in particular, microwave irradiation, we performed the reaction between **5a** (Scheme 3) and phenylboronic acid under different conditions. When the reaction was carried out at room temperature, compound **2b** was obtained in 50% yield after 24 h; working at reflux temperature, the reaction time was reduced to 30 min with a dramatic loss in reaction yield (27%). Thus, the irradiation with microwaves at 85 °C proved to be the best option, providing the highest yield (64%) of **2b** in the shortest reaction time (10 min).

Accordingly, compounds **2a**–**i** were obtained in acceptable to good yield by the use of this copper-mediated arylation procedure[23] starting from 2-thiouracils **5a**,**b** and arylboronic acids **6** (Scheme 3 and Table 1). In spite of some attempts, the yield of compound **2a** could not be improved over 30%.

To the best of our knowledge, this is the first example of the synthesis of *S*-arylpyrimidinones by the use of an Ullmann type reaction. In fact, only a single example



Scheme 3.

Table 1.

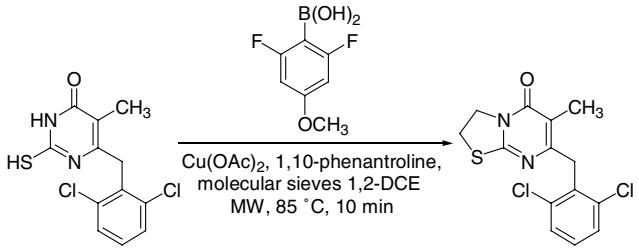
Compound	R^1	R^2	R^3	X	Mp[a] (°C)	Yield (%)
2a	F	F	F	CH	193–194	30
2b	F	H	H	CH	178–179	64
2c	F	Cl	H	CH	208–210	88
2d	F	OCH_3	H	CH	201 (dec)	85
2e	Cl	OCH_3	H	CH	220–221	49
2f	Cl	H	H	CH	208–209	73
2g	Cl	F	F	CH	223–224	59
2h	Cl	CN	H	CH	233–234	51
2i	Cl	OCH_3	H	N	222–223	76

[a] After recrystallization from MeOH.

of C–S coupling catalyzed by $Cu(OAc)_2$ on a 2-phenylthio-1,4-dihydropyrimidine[21] has been reported in the literature so far.

However, when applied to **5b** and 2,6-difluoro-4-methoxyphenylboronic acid, the reaction gave unexpected results (Scheme 4).

In fact, the bicyclic compound **7** was obtained instead of the expected *S*-arylpyrimidinone as a result of alkylation of the sulfur atom by DCE, used as the reaction solvent, followed by cyclization. Attempts to obtain the *S*-arylpyrimidinone by reacting **5b** and 2,6-difluoro-4-methoxyphenylboronic acid in a different solvent (CH_2Cl_2, THF) proved to be unsatisfactory, leading to complex reaction mixtures.



5b **7**

Scheme 4.

Compounds **2a–i** were evaluated in enzymatic tests for their ability to inhibit either wild type (wt) or mutated RTs as well as on MT-4 cells for cytotoxicity and anti-HIV activity, in comparison with nevirapine and efavirenz used as reference drugs.[12] In particular, the following mutants were used: K103N and Y181I for enzymatic tests, K103N, Y188L, Y181C and IRLL98 (bearing the K101Q, Y181C and G190A mutations conferring resistance to nevirapine, delavirdine and efavirenz) for tests on cell lines. The results of these assays are reported in Table 2.

As a general consideration, compounds **2a–d**, characterized by the presence of 2,6-difluorobenzyl moiety at C-6, show interesting antiviral activity with an EC_{50} ranging from 0.67 to 0.087 μM, while the activity of the 2,6-dichlorobenzyl-substituted derivatives **2e–i** is more modest. This aspect is particularly evident if we consider the couples **2a/2g**, **2b/2f** and **2d/2e**: in all cases the 2,6-difluorobenzyl-substituted partner is, at least, one order of magnitude more active than the 2,6-dichloro-substituted counterpart, as far as the activity against wt strain is concerned. No substantial difference in antiviral activity against mutant strains can be observed within the two series of compounds.

In line with the results of the cellular tests, the enzymatic activity of compounds **2a–i** is limited to the wt RT, regardless of the substitution pattern. Nevertheless, compound **2i**, bearing a pyridylthio substituent at C-2, merits some attention as the most active compound in the enzymatic assay. Quite unexpectedly, all compounds **2**, and in particular compounds **2a–d**, proved to be more active in cellular tests than in enzymatic assays.

Table 2.

Compound	ID_{50}[a,b] (μM)			EC_{50}[a,c] (μM)					CC_{50}[d]
	wt	K103N	Y181I	NL4-3 wt	IRLL98	K103N	Y181C	Y188L	
2a	30	na[e]	na	0.31	25.8	>65.8	—	>65.8	>65.8
2b	32	na	na	0.09	16.7	42.5	—	>72.7	>72.7
2c	22	na	na	0.10	>66.1	8.01	—	>66.1	>66.1
2d	na	na	na	0.67	>57.5	>57.5	—	>57.5	57.5
2e	na	na	na	26.5	>61.4	>61.4	>61.4	>61.4	>61.4
2f	36.0	na	na	1.80	4.91	>66.3	12.1	>66.3	>66.3
2g	10.0	na	na	3.36	44.7	>60.5	—	>60.5	>60.5
2h	23.0	na	na	2.49	>27.6	>27.6	5.10	>27.6	27. 6
2i	3.5	na	na	2.72	8.68	>61.7	6.52	>61.3	>61.3
Nevirapine	0.4	8.0	20	0.052	>7.50	3.90	—	>7.50	>7.50
Efavirenz	0.04	0.4	0.1	0.001	0.20	0.057	—	0.30	>0.32
AZT				0.003	0.008	0.003	—	0.003	3.70

[a] Data represent mean values of at least two experiments.
[b] ID_{50}, inhibiting dose 50 or needed dose to inhibit 50% of enzyme.
[c] EC_{50}, effective concentration 50 or needed concentration to inhibit 50% HIV-induced cell death, evaluated with MTT method in MT-4 cells.[24,25]
[d] CC_{50}, cytotoxic concentration 50 or needed concentration to induce 50% death of non-infected cells evaluated with the MTT method in MT-4 cells.
[e] na, not active at 400 μM (the highest concentration tested).

Accordingly, it may be difficult to explain their antiviral activity only in terms of RT inhibition. This result may reflect the interference of the test compounds with viral replication steps other than reverse transcription.

Finally, it should be noted that all the new compounds are less cytotoxic than reference drugs, with compound **2b** showing a selectivity index ($SI = CC_{50}/EC_{50wt}$) > 835, higher than those of nevirapine and efavirenz.

In conclusion, we have described the microwave-assisted synthesis of new *S*-DABO analogues characterized by the presence of an aromatic/heteroaromatic portion directly linked to the sulfur atom via an Ullmann type *S*-arylation procedure. Some of the new compounds displayed anti-HIV-1 activity in the submicromolar range along with low cytotoxicity.

Acknowledgments

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23. General procedure for the preparation of compounds **2a**–**i**: thiouracil **5** (0.70 mmol), arylboronic acid **6** (2.10 mmol), $Cu(OAc)_2$ (0.70 mmol) and 1,10-phenantroline (1.40 mmol) were mixed in dry 1,2-DCE in the presence of molecular sieves (2.00 g) under N_2. The reaction mixture was irradiated at 85 °C for 10 min, then cooled down and filtered on silica gel. The organic solvent was evaporated at reduced pressure to give a crude product which was recrystallized from MeOH.As an example, spectroscopic data for compound **2d** are reported. ^1H NMR [$(CD_3)_2CO$, 200 MHz]: δ 7.26–7.22 (m, 3H), 6.85–6.81 (m, 4H), 3.85 (s, 3H), 3.79 (s, 2H), 2.04 (s, 3H). IR ($CHCl_3$) ν_{max}: 3452, 1650 cm^{-1}. ESI/MS: *m/z* 375 [M+1], 397 [M+Na]. Elemental analysis: Calcd for $C_{19}H_{16}F_2N_2O_2S$: C, 60.95; H, 4.31; N, 7.48. Found: C, 61.03; H, 4.28; N, 7.57.
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