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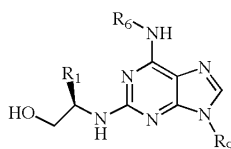
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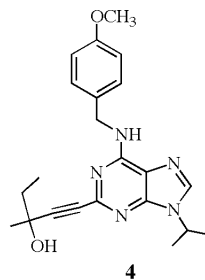
The synthesis of new purine derivatives designed to inhibit cell cycle regulating cyclin-dependent kinases (CDKs), is reported. These compounds, related to olomoucine and roscovitine, are characterised by the presence of a pyrrolidine methanol substituent at C-2 and a variety of *ortho*, *meta* and/or *para* substituents on the C-6 arylamino group.

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Cyclin-dependent Kinases (CDK) have raised considerable interest in view of their essential role in the regulation of the cell cycle division [1-3]. Following the identification of 6-dimethylaminopurine and isopentenyladenine as CDK1 [4] inhibitors, Vesely *et al.* described in 1994 [5] the purine Olomoucine **1** as a first lead compound which specifically inhibited the CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclin E kinases and the brain CDK5/p35 kinase among 35 kinases tested ( $IC_{50}$ 's: 7, 7, 7 and 3  $\mu M$ , respectively). An improved compound, Roscovitine **2**, described in 1997 [6] inhibited the same enzymes with  $IC_{50}$  values of 0.65, 0.7, 0.7 and 0.16  $\mu M$ , respectively. Reviews on chemical inhibitors of CDKs have recently appeared [7].



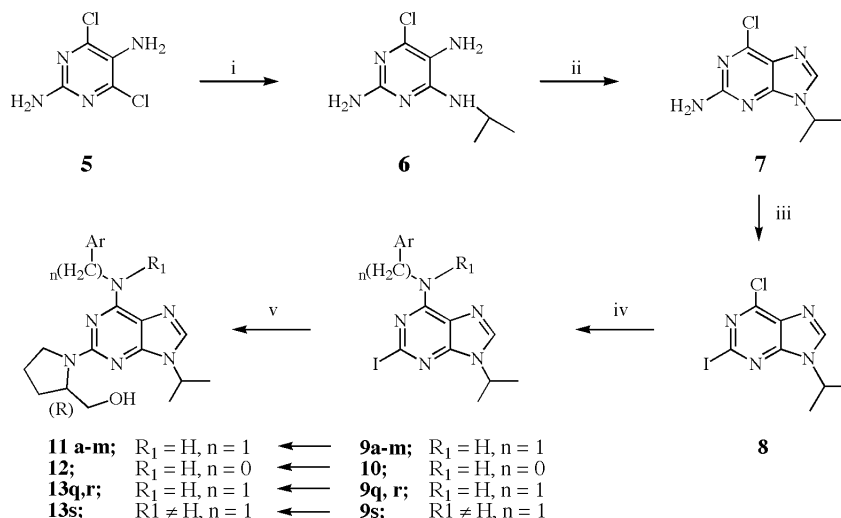
**1, Olomoucine** ( $R_1 = H$ ;  $R_9 = Me$ ;  $R_6 = CH_2Ph$ )  
**2, Roscovitine** ( $R_1 = Et$ ;  $R_9 = i-Pr$ ;  $R_6 = CH_2Ph$ )  
**3, Purvalanol A** ( $R_1 = R_9 = isopropyl$ ;  $R_6 = m-Cl-Ph$ )



The preparation of combinatorial purine libraries using solid-phase methods has been recently reported [8-10]. We have previously described [11] the synthesis and CDK1/cyclin B inhibitory activity of various C2-amino ethanol substituted purines, as well as C2 alkynylated purines like **4** [12,13] with potent anti-CDK activity *in vitro*. It has been established by several groups that this type of activity can be improved by varying the position and the nature of the substituent on the N6 phenyl ring. We describe in the present paper the synthesis of new N6 substituted purine derivatives with their CDK inhibitory activity *in vitro*. The purpose of this work is to study the effect of modifications of the anilino or benzylamino group at C-6 while keeping substituents at C-2 and N-9 unchanged (pyrrolidine methanol and isopropyl respectively).

## Results.

The synthesis of the compounds described in this article, was achieved from 6-chloro-2-iodo-9-isopropyl-(9*H*)-purine, itself obtained from 2-amino-6-chloro-9-alkyl(9*H*)-purine as previously described [14, 15]. Substitution of the 2-amino group in **7**, by a 2-iodo atom was performed by nitrosation using isoamyl nitrite in the presence of iodide



i)  $(CH_3)_2CH-NH_2$ , *n*-BuOH, 100°C ii)  $CH(OEt)_3$ ,  $H^+$ ,  $(Me)_2NCOCH_3$ , RT iii) isoamyle nitrite (3 equivalents),  $CH_2I_2$  (10 equivalents), CuI (1 equivalent),  $I_2$  (1 equivalent), THF reflux iv) amine, Ethanol, 45-50°C v) pyrrolidine methanol 120-140°C

sources (diiodomethane, cuprous iodide, iodine), according to the original method of Nair and Richardson [16].

Selective nucleophilic substitution of the 6-chloro group was obtained below 50 °C, whereas nucleophilic substitution of the 2-iodo atom by (*R*)-pyrrolidine methanol was achieved around 140 °C. The structures as well as the results of *in vitro* inhibitory experiments (concentrations giving 50% inhibition of starfish oocytes cyclin-dependent kinase, IC<sub>50</sub>) for compounds **11**, **12**, and **13** are given in Tables 1 and 2. Physical data are given in Table 3 and 4. CDK1/cyclin B was purified and assayed as previously described [6,13].

Table 1  
Inhibition of CDK1/cyclin B Activity by Compounds **11** and **12**

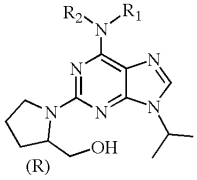
**11**

**12**

| Compound   | R                                         | IC <sub>50</sub><br>(μM) | Compound   | R                          | IC <sub>50</sub><br>(μM) |
|------------|-------------------------------------------|--------------------------|------------|----------------------------|--------------------------|
| <b>11a</b> | <i>p</i> -OCH <sub>3</sub>                | 0.21                     | <b>12a</b> | H                          | 0.30                     |
| <b>11b</b> | <i>m</i> , <i>p</i> -OCH <sub>2</sub> O   | 0.35                     | <b>12b</b> | <i>m</i> -Cl               | 0.43                     |
| <b>11c</b> | <i>m</i> , <i>p</i> -di-Cl                | 0.43                     | <b>12c</b> | <i>m</i> , <i>p</i> -di-Cl | 0.43                     |
| <b>11d</b> | <i>p</i> -Cl                              | 0.33                     | <b>12d</b> | <i>m</i> -Br               | 0.33                     |
| <b>11e</b> | H                                         | 0.65                     | <b>12e</b> | <i>p</i> -Br               | 0.30                     |
| <b>11f</b> | <i>m</i> , <i>m'</i> -di-OCH <sub>3</sub> | 0.3                      |            |                            |                          |
| <b>11g</b> | <i>m</i> -I                               | 0.45                     |            |                            |                          |
| <b>11h</b> | <i>m</i> -CF <sub>3</sub>                 | 0.7                      |            |                            |                          |
| <b>11i</b> | <i>p</i> -CF <sub>3</sub>                 | 0.8                      |            |                            |                          |
| <b>11j</b> | <i>m</i> , <i>m'</i> -di-CF <sub>3</sub>  | 1                        |            |                            |                          |
| <b>11k</b> | <i>o</i> -OCH <sub>3</sub>                | 1.8                      |            |                            |                          |
| <b>11l</b> | <i>o</i> -CF <sub>3</sub>                 | 4                        | <b>1</b>   | -                          | 7                        |
| <b>11m</b> | <i>m</i> -OCH <sub>3</sub>                | 0.3                      | <b>2</b>   | -                          | 0.65                     |

The *o* = ortho, *m* = meta, *p* = para substituents of the phenyl group.

Table 2  
Inhibition of CDK1/cyclin B Activity by Compounds **13**

|  <p><b>13</b></p> |                                                                            |                       |
|------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------------------|
| Compound                                                                                             | R <sub>1</sub> , R <sub>2</sub>                                            | IC <sub>50</sub> (μM) |
| <b>13q (S)</b> [a]                                                                                   | R <sub>1</sub> = H; R <sub>2</sub> = CH(CH <sub>2</sub> OH)Phe             | 0.8                   |
| <b>13r (R)</b> [a]                                                                                   | R <sub>1</sub> = H; R <sub>2</sub> = CH(CH <sub>2</sub> OH)Phe             | >10                   |
| <b>13s</b>                                                                                           | R <sub>1</sub> = CH <sub>2</sub> Phe; R <sub>2</sub> = CH <sub>2</sub> Phe | >10                   |

[a] *S* and *R* refer to stereochemical configuration at N-6.

Table 3  
Physical Data

| Compounds  | Yields (%) | Mp (°C) | Molecular Formula                                                 | Analyses %   |      |       |
|------------|------------|---------|-------------------------------------------------------------------|--------------|------|-------|
|            |            |         |                                                                   | Calcd./Found | C    | H     |
| <b>9f</b>  | 67         | 150-153 | C <sub>17</sub> H <sub>20</sub> N <sub>5</sub> O <sub>2</sub> I   | 45.05        | 4.45 | 15.45 |
| <b>9h</b>  | 69         | 192-194 | C <sub>16</sub> H <sub>15</sub> N <sub>5</sub> F <sub>3</sub> I   | 44.89        | 4.38 | 15.37 |
| <b>9i</b>  | 80         | 198-200 | C <sub>16</sub> H <sub>15</sub> N <sub>5</sub> F <sub>3</sub> I   | 41.67        | 3.28 | 15.18 |
| <b>9j</b>  | 70         | 153-156 | C <sub>17</sub> H <sub>14</sub> N <sub>5</sub> F <sub>6</sub> I   | 41.86        | 3.47 | 15.14 |
| <b>9k</b>  | 72         | 186-188 | C <sub>16</sub> H <sub>18</sub> N <sub>5</sub> OI                 | 41.67        | 3.28 | 15.18 |
| <b>9l</b>  | 85         | 200-202 | C <sub>16</sub> H <sub>15</sub> N <sub>5</sub> F <sub>3</sub> I   | 41.71        | 3.44 | 15.15 |
| <b>9m</b>  | 84         | 136-140 | C <sub>16</sub> H <sub>18</sub> N <sub>5</sub> OI                 | 38.58        | 2.67 | 13.23 |
| <b>9q</b>  | 78         | 140-142 | C <sub>16</sub> H <sub>18</sub> N <sub>5</sub> OI                 | 39.07        | 2.78 | 12.98 |
| <b>9r</b>  | 98         | 175-177 | C <sub>16</sub> H <sub>18</sub> N <sub>5</sub> OI/1/5 cyclohexane | 45.40        | 4.29 | 16.55 |
| <b>9s</b>  | 78         | 140-142 | C <sub>22</sub> H <sub>22</sub> N <sub>5</sub> I                  | 45.34        | 4.34 | 16.58 |
| <b>10d</b> | 65         | 124-126 | C <sub>14</sub> H <sub>13</sub> N <sub>5</sub> BrI                | 41.67        | 3.28 | 15.18 |
| <b>10e</b> | 67         | 174-176 | C <sub>14</sub> H <sub>13</sub> N <sub>5</sub> BrI                | 41.93        | 3.47 | 15.33 |
| <b>11a</b> | 86         | 153-156 | C <sub>21</sub> H <sub>28</sub> N <sub>6</sub> O <sub>2</sub>     | 45.40        | 4.29 | 16.55 |
| <b>11b</b> | 87         | 149-151 | C <sub>21</sub> H <sub>26</sub> N <sub>6</sub> O <sub>3</sub>     | 45.36        | 4.34 | 16.41 |
| <b>11c</b> | 64         | 120-124 | C <sub>20</sub> H <sub>24</sub> N <sub>6</sub> OCl <sub>2</sub>   | 45.40        | 4.29 | 16.55 |
| <b>11d</b> | 57         | 141-143 | C <sub>20</sub> H <sub>25</sub> N <sub>6</sub> OCl                | 45.03        | 4.46 | 16.47 |
| <b>11f</b> | 88         | 145-147 | C <sub>22</sub> H <sub>30</sub> N <sub>6</sub> O <sub>3</sub>     | 46.94        | 4.67 | 15.91 |
| <b>11h</b> | 55         | 130-133 | C <sub>21</sub> H <sub>25</sub> N <sub>6</sub> OF <sub>3</sub>    | 46.52        | 4.53 | 16.35 |
| <b>11i</b> | 83         | 138-140 | C <sub>21</sub> H <sub>25</sub> N <sub>6</sub> OF <sub>3</sub>    | 54.67        | 4.59 | 14.49 |
| <b>11j</b> | 79         | 165-167 | C <sub>22</sub> H <sub>24</sub> N <sub>6</sub> OF <sub>6</sub>    | 54.63        | 4.66 | 14.36 |
| <b>11k</b> | 80         | 90-91   | C <sub>21</sub> H <sub>28</sub> N <sub>6</sub> O <sub>2</sub>     | 36.71        | 2.86 | 15.29 |
| <b>11l</b> | 62         | 125     | C <sub>21</sub> H <sub>25</sub> N <sub>6</sub> OF <sub>3</sub>    | 36.68        | 2.93 | 15.31 |
| <b>11m</b> | 98         | 130-133 | C <sub>21</sub> H <sub>28</sub> N <sub>6</sub> O <sub>2</sub>     | 36.71        | 2.86 | 15.29 |
| <b>12a</b> | 40         | 151-153 | C <sub>19</sub> H <sub>24</sub> N <sub>6</sub> O                  | 36.83        | 2.91 | 15.04 |
| <b>12b</b> | 64         | 135-137 | C <sub>19</sub> H <sub>23</sub> N <sub>6</sub> OCl                | 63.62        | 7.12 | 21.20 |
| <b>12c</b> | 65         | 179-181 | C <sub>19</sub> H <sub>22</sub> N <sub>6</sub> OCl <sub>2</sub>   | 63.59        | 7.08 | 21.15 |
| <b>12d</b> | 49         | 125-126 | C <sub>19</sub> H <sub>23</sub> N <sub>6</sub> OBr                | 61.45        | 6.38 | 20.47 |
| <b>12e</b> | 31         | 182-185 | C <sub>19</sub> H <sub>23</sub> N <sub>6</sub> OBr                | 61.61        | 6.23 | 20.47 |
| <b>13q</b> | 53         | 90-92   | C <sub>21</sub> H <sub>28</sub> N <sub>6</sub> O <sub>2</sub>     | 55.18        | 5.56 | 19.30 |
| <b>13r</b> | 74         | 160-162 | C <sub>21</sub> H <sub>28</sub> N <sub>6</sub> O <sub>2</sub>     | 54.98        | 5.26 | 19.14 |
| <b>13s</b> | 82         | 139-141 | C <sub>27</sub> H <sub>32</sub> N <sub>6</sub> O                  | 59.92        | 6.29 | 20.96 |

Table 4

<sup>1</sup>H NMR Data for compounds **9f-s**, **10d**, **10e**, **11a-m**, **12a-e**, and **13q-s** (δ values in ppm, Tetramethylsilane as the Internal Standard, in Deuterated Chloroform, 200 MHz)

| Compounds  | <sup>1</sup> H NMR                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Compounds  | <sup>1</sup> H NMR                                                                                                                                                                                                                                                                                                                                                                                              |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>9f</b>  | 7.65 (s, 1H, H8), 6.53 (d, 2H, Phe, J = 2.1), 6.36 (t, 1H, Phe, J = 2.1), 6.08 (br s, 1H, NH), 4.83-4.73 (m, 3H, CH(CH <sub>3</sub> ) <sub>2</sub> , CH <sub>2</sub> Phe), 3.77 (s, 6H, 2xOCH <sub>3</sub> ), 1.54 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                                                                                                                                                                                                                                    | <b>11d</b> | 7.49 (s, 1H, H-8), 7.30 (m, 4H, Phe), 6.80 (br s, 1H, NH), 4.77 (m, 2H, CH <sub>2</sub> Phe), 4.65-4.55 (m, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> ), 4.35-4.25 (m, 1H, H2'), 3.90-3.40 (m, 4H, CH <sub>2</sub> OH, H5'), 2.15-1.60 (m, 4H, H3', H4'), 1.53 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 5.8).                                                                                                |
| <b>9h</b>  | 7.88 (br s, 1H, H8), 7.68-7.47 (m, 4H, Phe), 6.8 (br s, 1H, NH), 4.94-4.80 (m, 3H, CH(CH <sub>3</sub> ) <sub>2</sub> , CH <sub>2</sub> Phe), 1.59 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                                                                                                                                                                                                                                                                                                     | <b>11f</b> | 6.54 (d, 2H, Ho,o'-Phe, J = 2.2), 6.36 (t, 1H, Hp-Phe, J = 2.2), 3.76 (s, 6H, 2xOCH <sub>3</sub> ), other protons are identical to <b>11a</b> .                                                                                                                                                                                                                                                                 |
| <b>9i</b>  | 7.84 (s, 1H, H8), 7.63-7.48 (m, 4H, Phe), 6.8 (br s, 1H, NH), 4.92-4.82 (m, 3H, CH(CH <sub>3</sub> ) <sub>2</sub> , CH <sub>2</sub> Phe), 1.58 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                                                                                                                                                                                                                                                                                                        | <b>11h</b> | 7.69 (s, 1H, H-8), 7.70-7.45 (m, 4H, Phe), other protons are identical to <b>11a</b> .                                                                                                                                                                                                                                                                                                                          |
| <b>9j</b>  | 7.89-7.70 (m, 4H, H-8, Phe), 6.85 (br s, 1H, NH), 4.95-4.78 (m, 3H, CH(CH <sub>3</sub> ) <sub>2</sub> , CH <sub>2</sub> Phe), 1.59 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                                                                                                                                                                                                                                                                                                                    | <b>11i</b> | 7.64-7.43 (m, 5H, Phe, H-8), 4.98-4.75 (m, 2H, CH <sub>2</sub> Phe), other protons are identical to <b>11a</b> .                                                                                                                                                                                                                                                                                                |
| <b>9k</b>  | 7.66 (s, 1H, H-8), 7.44-7.39 (m, 1H, Phe), 7.27-7.22 (m, 1H, Phe), 6.95-6.85 (m, 2H, Phe), 6.2 (br s, 1H, NH), 4.85-4.65 (m, 3H, CH(CH <sub>3</sub> ) <sub>2</sub> , CH <sub>2</sub> Phe), 3.87 (s, 3H, OCH <sub>3</sub> ), 1.59 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                                                                                                                                                                                                                      | <b>11j</b> | 7.89 (unresolved d, 2H, Ho,o'-Phe), 7.76 (s, 1H, H-8), 7.56 (unresolved t, 1H, Hp-Phe), 5.00-4.75 (br d, 2H, CH <sub>2</sub> Ph), other protons are identical to <b>11a</b> .                                                                                                                                                                                                                                   |
| <b>9l</b>  | 7.72-7.68 (m, 3H, H-8, Phe), 7.52 (t, 1H, Phe, J = 7.3), 7.38 (t, 1H, Phe, J = 7.50), 6.09 (br s, 1H, NH), 5.00 (br s, 2H, CH <sub>2</sub> Phe), 4.81 (sept, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> ), 1.56 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                                                                                                                                                                                                                                            | <b>11k</b> | 7.43-6.82 (m, 4H, Phe), 3.87 (s, 3H, OCH <sub>3</sub> ), other protons are identical to <b>11a</b> .                                                                                                                                                                                                                                                                                                            |
| <b>9m</b>  | 7.63 (s, 1H, H-8), 7.25 (t, 1H, Phe, J = 8.1), 6.96-6.93 (m, 2H, Phe), 6.85-6.81 (m, 1H, Phe), 5.20 (br s, 2H, CH <sub>2</sub> Phe), 4.88-4.70 (m, 3H, CH(CH <sub>3</sub> ) <sub>2</sub> , CH <sub>2</sub> Phe), 3.80 (s, 3H, OCH <sub>3</sub> ), 1.56 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                                                                                                                                                                                                | <b>11l</b> | 7.70-7.30 (m, 4H, Phe), 5.10-4.92 (br d, 2H, CH <sub>2</sub> Ph), other protons are identical to <b>11a</b> .                                                                                                                                                                                                                                                                                                   |
| <b>9q</b>  | identical to <b>9r</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <b>11m</b> | 7.28-7.20 (m, 1H, Ho-Phe), 6.99-6.78 (m, 3H, Ho', m, p-Phe), 3.79 (s, 3H, OCH <sub>3</sub> ); other protons are identical to <b>11a</b> .                                                                                                                                                                                                                                                                       |
| <b>9r</b>  | 7.90 (s, 1H, H-8), 7.51-7.28 (m, 5H, Phe), 5.45 (br s, 1H, NH), 4.81 (m, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> ), 4.05 (d, 1H, CHOH, J = 5.5), 3.70-3.30 (br m, 2H, OH, CHPhe), 1.60-1.54 (dd, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> ).                                                                                                                                                                                                                                                                     | <b>12a</b> | 7.83 (d, 2H, o,o'-Phe, J = 7.8), 7.63 (s, 1H, H-8), 7.37 (t, 2H, m,m'-Phe, J = 7.8), 7.09 (t, 1H, p-Phe, J = 7.3); 4.64 (sept, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.6), 4.45-4.30 (m, 1H, H-2'), 3.98-3.65 (m, 4H, CH <sub>2</sub> OH, CH <sub>2</sub> -5'), 2.30-1.65 (m, 4H, 2xCH <sub>2</sub> ), 1.58 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7).                                         |
| <b>9s</b>  | 7.67 (s, 1H, H-8), 7.30-7.26 (m, 10H, 2xPhe), 5.45 (br s, 2H, CH <sub>2</sub> Phe), 4.89-4.82 (m, 3H, CH <sub>2</sub> Phe, CH(CH <sub>3</sub> ) <sub>2</sub> ), 1.55 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7).                                                                                                                                                                                                                                                                                  | <b>12b</b> | 8.23 (s, 1H, H-8), 7.95 (br s, 1H, NH), 7.64 (s, 1H, Phe), 7.47-7.43 (m, 1H, Phe), 7.27-7.20 (m, 1H, Phe), 7.04-7.00 (m, 1H, Phe), 4.64 (sept, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.6), 4.35 (m, 1H, H-2'), 3.9-3.7 (m, 4H, CH <sub>2</sub> OH, CH <sub>2</sub> -5'), 2.10-1.70 (m, 4H, 2xCH <sub>2</sub> ), 1.56 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7).                                |
| <b>10d</b> | 8.19 (s, 1H, H-8), 7.96 (br s, 1H, NH), 7.79 (m, 1H, Phe), 7.75-7.68 (m, 1H, Phe), 7.23 (d, 2H, Phe), 4.86 (sept, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7), 1.59 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7).                                                                                                                                                                                                                                                                              | <b>12c</b> | 8.38 (s, 1H, H-8), 8.22 (br s, 1H, NH), 7.67 (s, 1H, Phe), 7.45-7.34 (m, 2H, Phe), 4.65 (sept, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.6), 4.37 (m, 1H, H-2'), 3.79 (m, 4H, CH <sub>2</sub> OH, CH <sub>2</sub> -5'), 2.18-2.01 (m, 5H, OH, 2xCH <sub>2</sub> ), 1.58 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                                                                               |
| <b>10e</b> | 7.80 (s, 1H, H-8), 7.68-7.46 (m, 4H, Phe), 4.86 (sept, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7), 1.60 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7).                                                                                                                                                                                                                                                                                                                                         | <b>12d</b> | 7.95-7.10 (m, 4H, Phe), 7.62 (s, 1H, H-8), other protons are identical to <b>12a</b> .                                                                                                                                                                                                                                                                                                                          |
| <b>11a</b> | 7.47 (s, 1H, H-8), 7.30 (d, 2H, Hm,m'-Phe, J = 8.6), 6.85 (d, 2H, Ho, o'-Phe, J = 8.6), 5.95-5.75 (m, 1H, NH), 4.8-4.65 (m, 2H, CH <sub>2</sub> Ph), 4.59 (septet, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7), 4.40-4.24 (m, 1H, H <sub>2</sub> '), 3.79 (s, 3H, OCH <sub>3</sub> ), 3.85-3.55 (m, 4H, CH <sub>2</sub> OH, CH <sub>2</sub> -5'), 2.22-2.02 (m, 1H, H-3'), 2.00-1.80 (m, 2H, CH <sub>2</sub> -4'), 1.75-1.60 (m, 1H, H-3'), 1.53 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7). | <b>12e</b> | 7.73 (d, 2H, Phe, J = 8.6), 7.64 (s, 1H, H-8), 7.45 (d, 2H, Phe, J = 8.5), other protons are identical to <b>12a</b> .                                                                                                                                                                                                                                                                                          |
| <b>11b</b> | 7.50 (s, 1H, H-8), 6.89-6.73 (m, 3H, Phe), 5.93 (s, 2H, OCH <sub>2</sub> O), 4.70-4.60 (m, 3H, CH <sub>2</sub> Phe, CH(CH <sub>3</sub> ) <sub>2</sub> ), 4.32 (m, 1H, H2'), 3.72-3.68 (m, 4H, H5', CH <sub>2</sub> OH), 2.07-1.89 (m, 4H, H3', H4'), 1.54 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7).                                                                                                                                                                                             | <b>13q</b> | 7.66 (s, 1H, H-8), 7.60-7.20 (m, 5H, Phe), 5.60-5.15 (m, 1H, CHPhe), 4.62 (sept, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8); 4.4-4.2 (br m, 1H, H-2' pyrrolidine), 4.10-3.94 (m, 2H, HOCH <sub>2</sub> CHPhe), 3.80-3.55 (m, 4H, CH <sub>2</sub> OH, CH <sub>2</sub> -5' pyrrolidine), 2.25-1.50 (m, 4H, 2xCH <sub>2</sub> ), 1.54 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8).                  |
| <b>11c</b> | 7.54 (s, 1H, H-8), 7.51-7.26 (m, 3H, Phe), 6.30 (br s, 1H, NH), 4.80 (br s, 2H, CH <sub>2</sub> Phe), 4.63 (m, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> ), 3.72-3.60 (m, 4H, CH <sub>2</sub> OH, H2'), 2.1-1.6 (m, 5H, H3', H4', OH), 1.55 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.7).                                                                                                                                                                                                               | <b>13r</b> | identical to <b>13q</b> .                                                                                                                                                                                                                                                                                                                                                                                       |
|            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <b>13s</b> | 7.53 (s, 1H, H-8), 7.45-7.15 (m, 10H, 2xPhe), 5.66-5.35 (br s, 2H, CH <sub>2</sub> Phe), 5.06-4.80 (br s, 2H, CH <sub>2</sub> Phe), 4.88-4.65 (br m, 1H, CH(CH <sub>3</sub> ) <sub>2</sub> ), 4.45-4.25 (br m, 1H, H-2' pyrrolidine), 3.85-3.50 (m, 4H, CH <sub>2</sub> OH, CH <sub>2</sub> -5' pyrrolidine); 2.25-1.55 (m, 4H, 2xCH <sub>2</sub> ), 1.56 (d, 6H, CH(CH <sub>3</sub> ) <sub>2</sub> , J = 6.8). |

### Concluding Remarks.

Interestingly, with the exception of *ortho*-substitution, most of the substituents on the 6-benzylamino series increase activity with respect to the unsubstituted derivative **11e** (Table 1 and 2). The best activity is observed for *para*-methoxy and *para*-chloro derivatives **11a** and **11d**. *Meta* substituted derivatives are intermediate between *ortho* and *para* substituted compounds. The influence of *meta* and/or *para* substituents in the anilino series is less important. The drop of activity of the N6-disubstituted compounds **13** should be noticed, and may be explained by the loss of the capacity of hydrogen bond formation between NH-6 and the enzyme [13]. However, most of *meta/para* substituted compounds are more inhibitory than the lead compounds, Olomoucine (**1**) and Roscovitine (**2**). Further modifications at N9 and C2 combined with the best substituents at C6 should lead to improved anti-cyclin dependent kinase inhibitory activities.

### EXPERIMENTAL

The melting points were taken on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were performed by the "Service de Microanalyse", CNRS, ICSN, 91198 Gif sur Yvette, France. Proton nuclear magnetic resonance ( $^1\text{H}$ -nmr) spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer in deuteriochloroform (unless otherwise specified). Chemical shifts were reported in  $\delta$  ppm referenced to tetramethylsilane. Coupling constants were indicated in hertz.

2-Amino-6-chloro-9-isopropyl-9H-purine (**7**) was synthesized from pyrimidine **5** [14] as previously described [15].

6-Chloro-2-iodo-9-isopropyl-9H-purine (**8**).

A solution of compound **7** (1 g, 4.72 mmol) in tetrahydrofuran (40 mL) was stirred under reflux for one hour in the presence of isoamylnitrite (1.6 g, 14.2 mmol), diiodomethane (12.6 g, 47 mmol), iodine (1.2 g, 4.7 mmol) and cuprous iodide (0.9 g, 4.8 mmol). The mixture was cooled to room temperature, filtered, and the filtrate was evaporated to dryness before purification by column chromatography eluting with 5% ethanol in dichloromethane. Compound **8** was obtained in 80% yield; mp 108-109 °C;  $^1\text{H}$  nmr (dimethylsulfoxide- $d_6$ )  $\delta$  8.76 (s, 1H), 4.83 (m, 1H), 1.55 (d, 6H,  $J = 6.7$  Hz).

Anal. Calcd. for  $\text{C}_8\text{H}_8\text{N}_4\text{ClI}$ : C, 29.79; H, 2.50; N, 17.37. Found: C, 30.07; H, 2.65; N, 17.58.

General Procedure for the Preparation of 6-Substituted Purines **9** and **10**.

Nucleophilic substitution of the 6-chloro atom in **8** was achieved by reaction with 1.5 equivalents of the appropriate amine (4-methoxybenzylamine, piperonylamine, 3,4-dichlorobenzylamine, 4-chlorobenzylamine, 3,5-dimethoxybenzylamine, 3-methoxybenzylamine, 3-trifluoromethylbenzylamine, 4-trifluoromethylbenzylamine, 3,5-bis(trifluoromethyl)benzylamine, 2-methoxybenzylamine, 2-trifluoromethylbenzylamine, benzylamine, aniline, 4-bromoaniline, 3-bromoaniline, 3-chloroaniline, 3,4-dichloroaniline, (S)-2-phenylglycinol,

(R)-2-phenylglycinol, dibenzylamine, purchased from Aldrich) in ethanol containing triethylamine (4 equivalents) under inert atmosphere at 50 °C for 3-4 hours. After evaporation of the volatile material *in vacuo*, the residue was dissolved in dichloromethane and washed twice with water. The organic phase was dried (magnesium sulfate), evaporated to dryness and subjected to column chromatography on silica gel eluting with 1-5% ethanol in dichloromethane. The crude products obtained after column chromatography were sufficiently pure for the next step. Yields refer to chromatographically purified products. Unless otherwise stated, products were recrystallized from heptane to provide an analytical sample. Melting points (mp) refer to recrystallized samples.

Compounds **9a**, **9b**, **9c**, **9d**, **10a**, **10b** and **10c** have already been described [13] as well as **11e**, and **11g** [11].

General Procedure for the Preparation of Compounds **11** and **12**.

A mixture of iodo-purines **9** or **10** (2 mmol), in *N,N*-dimethylacetamide (40 mL), tripropylamine (1 mL), and 1.5 equivalents of (R)-2-pyrrolidine methanol was stirred at 140 °C for 24 hours. After evaporation to dryness (oil pump), the residue was dissolved in dichloromethane, washed with water (twice), adsorbed on silica gel and chromatographed on silica gel column, eluting successively with dichloromethane and 2-5% ethanol in dichloromethane. Yields refer to purified compounds after chromatography. When necessary, analytical samples were obtained after recrystallization from heptane. Melting points refer to analytical samples.

Biological Assays.

Starfish CDK1/cyclin B was purified from M oocytes by affinity chromatography on p9<sup>CKShs1</sup>-Sephacrose beads, from which it was eluted by free p9<sup>CKShs1</sup>. It was assayed with 1 mg histone H1 (Sigma type III-S)/mL, in the presence of 15  $\mu\text{M}$  [ $\gamma$ - $^{32}\text{P}$ ] ATP (3,000 Ci/mmol; 1 mCi/mL) in a final volume of 30  $\mu\text{L}$ . After 10 minutes incubation at 30°C, 25  $\mu\text{L}$  aliquots of supernatant were spotted onto 2.5 x 3 cm pieces of Whatman P81 phosphocellulose paper, and, after 20 seconds, the filters were washed five times (for at least 5 minutes each time) in a solution of 10 mL phosphoric acid/liter of water. The wet filters were transferred into 6 mL plastic scintillation vials, and counted in the presence of 2 mL ACS (Amersham) scintillation fluid. The kinase activity was expressed in pmoles phosphate incorporated in histone H1/10 minute incubation or in % of maximal activity. IC<sub>50</sub>'s were calculated from inhibitor dose-response curves.

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