



# Synthesis and Anticonvulsant Properties of Triazolo- and Imidazopyridazinyl Carboxamides and Carboxylic Acids

Stéphane Moreau,<sup>a</sup> Pascal Coudert,<sup>a</sup> Catherine Rubat,<sup>b</sup> Danielle Vallee-Goyet,<sup>c</sup> Daniel Gardette,<sup>c</sup> Jean-Claude Gramain<sup>c</sup> and Jacques Couquelet<sup>a,\*</sup>

<sup>a</sup>Groupe de Recherche en Pharmacochimie, Laboratoire de Chimie Thérapeutique, Faculté de Pharmacie, Université d'Auvergne, 28 Place Henri Dunant, 63 001 Clermont-Ferrand Cedex, France

<sup>b</sup>Laboratoire de Pharmacologie, Faculté de Pharmacie, Université d'Auvergne, 28 Place Henri Dunant, 63 001 Clermont-Ferrand Cedex, France

<sup>c</sup>Laboratoire de Chimie des Substances Naturelles, Université Blaise Pascal CNRS UMR 6504, 63 177 Aubière Cedex, France

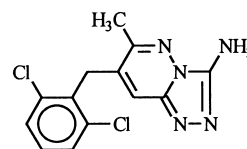
Received 12 May 1997; accepted 10 February 1998

**Abstract**—Analogues of 3-amino-7-(2,6-dichlorobenzyl)-6-methyltriazolo[4,3-*b*]pyridazine **PC25** containing amide or carboxylic acid function were synthesized and tested for anticonvulsant activity. The compounds having the imidazole ring substituted with an amide group have been found to be generally more active against maximal electroshock-induced seizures in mice ( $15.2 \leq \text{ED}_{50} \leq 37.5 \text{ mg kg}^{-1}$  orally). Furthermore, maximum activity was generally associated with a 2,6-dichlorobenzyl substitution pattern. 3-Amido-7-(2,6-dichlorobenzyl)-6-methyltriazolo[4,3-*b*]pyridazine **4b** was also protective in the pentylenetetrazole-induced seizures test ( $\text{ED}_{50} = 91.1 \text{ mg kg}^{-1}$  orally) and blocked strychnine-induced tonic extensor seizures ( $\text{ED}_{50} = 62.9 \text{ mg kg}^{-1}$  orally). Moreover, calculated electrostatic isopotential maps of the whole active compounds were quite similar and, consequently, could be associated to optimum anticonvulsant activity. © 1998 Elsevier Science Ltd. All rights reserved.

## Introduction

Previous works realized on 3-amino-7-(2,6-dichlorobenzyl)-6-methyltriazolo[4,3-*b*]pyridazine **PC25** and analogues have shown some of these compounds to be anticonvulsant agents with potent activity against maximal electroshock-induced seizures (MES) in mice.<sup>1</sup> In order to increase this anticonvulsant activity, firstly an amide group or a carboxylic acid moiety was introduced on the triazole or on the isosteric imidazole ring of these molecules. Such substituents were chosen owing to their presence in many anticonvulsants<sup>2–6</sup> and specially in currently antiepileptic drugs namely carbamazepine and valproic acid. Secondly, taking into account our previous results,<sup>1</sup> benzyl moiety attached to the pyridazine ring was either unsubstituted or substituted by two chlorine atoms at the 2,6-position. We report herein the synthesis and the anticonvulsant

activity of these new compounds. Moreover, structure–activity relationships have been examined for these molecules.

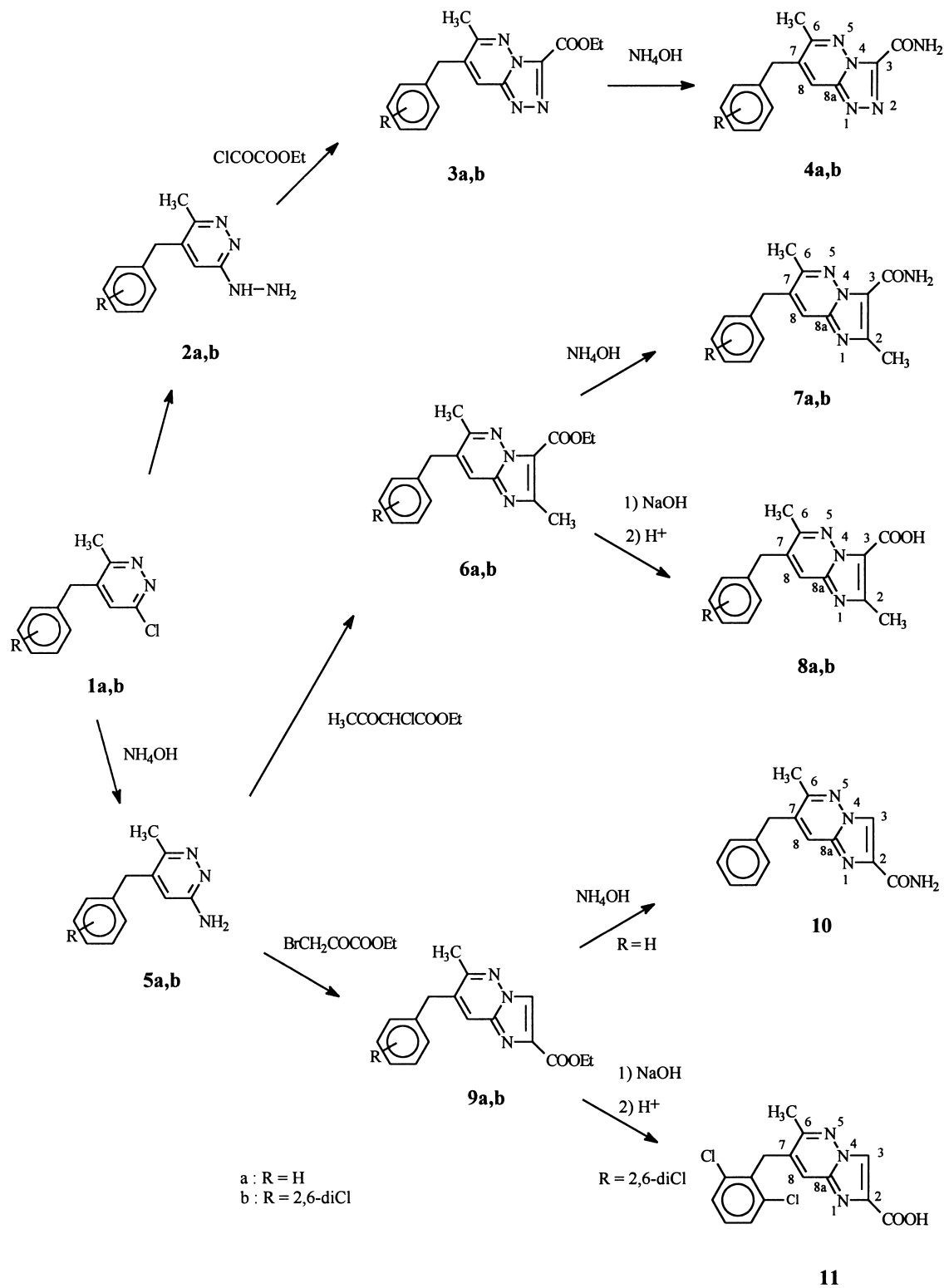


Triazolopyridazine **PC25**

## Chemistry

Triazolo[4,3-*b*]pyridazines and imidazo[1,2-*b*]pyridazines were synthesized according to Scheme 1. Starting material **1** and key intermediates **2** and **5** were prepared using reported methods.<sup>1,7</sup> Hydrazinopyridazines **2** were treated with ethyloxalyl chloride and the resulting bicyclic esters **3** were hydrolysed in aqueous ammonia to

\*Corresponding author. Tel.: 33 4 7360 8016; Fax: 33 4 7328 2707.



Scheme 1.

lead to the amides **4**. Synthesis of esters **6** and **9** was achieved by reacting 3-aminopyridazines **5** with ethyl 2-chloroacetoacetate and ethyl bromopyruvate respectively. Treatment of **6** and **9** with concentrated aqueous ammonia furnished amides **7** and **10**. Base-catalyzed hydrolysis of esters **6** and **9**, followed by acidification to pH 2 with 6 N hydrochloric acid, afforded acids **8** and **11**, respectively. Physical constants and spectral data of compounds **4**, **7**, **8**, **10** and **11** were reported in Tables 1 and 2.

### Results and Discussion

The anticonvulsant activities of pyridazine derivatives **4**, **7**, **8**, **10** and **11** were first determined using the maximal electroshock seizure (MES) test. The results were compared to those obtained for **PC25**<sup>1</sup> and the clinically proven antiepileptic agents: phenytoin, phenobarbital, sodium valproate, carbamazepine, diazepam and lamotrigine. All compounds were orally administered to mice and produced significant anticonvulsant activity with ED<sub>50</sub>'s that ranged from 12.5 to 101 mg kg<sup>-1</sup> (Table 3). Compared with sodium valproate whose ED<sub>50</sub> value is 112 mg kg<sup>-1</sup>, pyridazine derivatives were more potent. However, they were less active than **PC25** and other reference drugs in this screen. In this new series of compounds, presence of an amide moiety compared with a carboxylic acid function (e.g. **7a** or **7b** vs **8a** or **8b** and **10** vs **11**) resulted in an equipotent or an increase of anticonvulsant activity. Furthermore, our previous works<sup>1</sup> have proved that the potencies of compounds greatly increased if the benzyl moiety was substituted in the *ortho* position with a chlorine atom. In the series of amides and acids derivatives, the presence of such substituents also has, in two of the three cases, a significant impact on activity. Pyridazines **4a** and **8a** are respectively at least five- and threefold less active than the 2,6-dichloro analogues **4b** and **8b**. In addition, changing the triazolopyridazine structure of **PC25** or **4** into the imidazo[1,2-*b*]pyridazine ring system present in **7**, **8**, **10** and **11**, resulted in compounds having good activity against MES. Thus, the imidazo[1,2-*b*]pyridazine bicycle

serves as an excellent bioisostere of the triazolo[4,3-*b*]pyridazine system with these anticonvulsant agents. On the other hand, spontaneous motor activity was measured as a parameter of the sedative action of compounds in the central nervous system (Table 3). Only compound **10** was significantly active, inducing a 38% decrease in motor activity. In the other cases, either a very slight increase or a decrease were observed in spontaneous motor activity at 100 mg kg<sup>-1</sup>, contrary to many anticonvulsant drugs (i.e., phenytoin, phenobarbital, diazepam, lamotrigine) that affect motor movements at therapeutic doses.

Contained in Table 3 are the median doses for neurological impairment (TD<sub>50</sub>) using the rotarod test. Compared with antiepileptic reference drugs, most pyridazine derivatives were free of important neurotoxicity. Except compounds **7b** and **8a**, the protection indices of test drugs were largely greater than that of currently used reference drugs. Considering their activity on the one hand and their neurotoxicity on the other, the most promising compounds were **4b**, **7a**, and **10** with PI values of 20, over 52 and 17, respectively. Thus, these three pyridazines were selected for determining their ability to protect animals against chemically-induced seizures. Among the possible mechanisms known to inhibit seizure activity are the enhancement of inhibitory (principally GABA-mediated) processes and the reduction of excitatory (particularly glutamate-mediated) transmission.<sup>8,9</sup> Involvement of one or two of these mechanisms in anticonvulsant properties of the three selected drugs was explored through classical empirical animal models. Anticonvulsants which block seizures induced by strychnine, bicuculline and *N*-methyl-D,L-aspartate (NMDLA) do so by acting on glycine,  $\gamma$ -aminobutyric acid (GABA) and NMDA receptors, respectively.<sup>10</sup> With regard to anticonvulsants effective in the yohimbine test, they may act at one and the same time on GABA and NMDA receptors.<sup>10</sup>

In the pentylenetetrazole (PTZ) test, hindlimb extension was abolished by all pyridazine derivatives (Table 4). In this test situation, ED<sub>50</sub> values indicated that **4b**, **7a**, and **10** were less potent than **PC25** and reference drugs such as phenobarbital, carbamazepine, diazepam and lamotrigine. But they were more effective than the clinically used drugs phenytoin and sodium valproate. The sc PTZ test is fairly nonspecific and could clearly lead to considerable ambiguity on the way of a drug exercising its anticonvulsant effect. It can nevertheless be inferred that test drugs may be active in treating generalized absence seizures.<sup>11</sup>

Against strychnine-induced seizures, only **4b** and **10** were active with ED<sub>50</sub> values equal to 61.2 and 93.1 mg kg<sup>-1</sup> respectively, whereas phenytoin, phenobarbital and

**Table 1.** Physical constants of compounds **4**, **7**, **8**, **10** and **11**

| Compd     | R        | mp<br>(°C) | Yield<br>(%) | Formula                                                                                          |
|-----------|----------|------------|--------------|--------------------------------------------------------------------------------------------------|
| <b>4a</b> | H        | 135        | 98           | C <sub>14</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub> H <sub>2</sub> O                   |
| <b>4b</b> | 2,6-diCl | 243        | 41           | C <sub>14</sub> H <sub>11</sub> N <sub>5</sub> Cl <sub>2</sub> O <sub>2</sub> ·2H <sub>2</sub> O |
| <b>7a</b> | H        | 230        | 57           | C <sub>16</sub> H <sub>16</sub> N <sub>4</sub> O                                                 |
| <b>7b</b> | 2,6-diCl | 250        | 33           | C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> Cl <sub>2</sub> O                                 |
| <b>8a</b> | H        | 260        | 90           | C <sub>16</sub> H <sub>15</sub> N <sub>3</sub> O <sub>2</sub>                                    |
| <b>8b</b> | 2,6-diCl | 225        | 62           | C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> Cl <sub>2</sub> O <sub>2</sub>                    |
| <b>10</b> |          | 254        | 68           | C <sub>15</sub> H <sub>14</sub> N <sub>4</sub> O                                                 |
| <b>11</b> |          | 194        | 39           | C <sub>15</sub> H <sub>11</sub> N <sub>3</sub> Cl <sub>2</sub> O <sub>2</sub>                    |



**Table 3.** Locomotor activity, anticonvulsant activity (maximal electroshock seizure test), rotorod test and protective index for pyridazine derivatives

| Compd            | Locomotor activity at<br>100 mg kg <sup>-1</sup> orally <sup>a</sup><br>[(-) decrease, (+) increase] | MES ED <sub>50</sub> <sup>a,b,c</sup><br>(mg kg <sup>-1</sup> orally) | Rotorod TD <sub>50</sub> <sup>a,b</sup><br>mg kg <sup>-1</sup> orally | PI <sup>d</sup> |
|------------------|------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------|-----------------|
| <b>4a</b>        | +6 ± 3 (NS)                                                                                          | 69.7 (46.2–105.1)                                                     | 800.0 (560.2–1142.4)                                                  | 11.5            |
| <b>4b</b>        | -27 ± 5 (NS)                                                                                         | 12.5 (9.2–17.0)                                                       | 253.3 (171.2–374.8)                                                   | 20.3            |
| <b>7a</b>        | -13 ± 7 (NS)                                                                                         | 15.2 (8.3–27.8)                                                       | > 800                                                                 | > 52.6          |
| <b>7b</b>        | +4 ± 2 (NS)                                                                                          | 37.5 (29.8–47.1)                                                      | 294.7 (150.2–578.2)                                                   | 7.9             |
| <b>8a</b>        | -9 ± 5 (NS)                                                                                          | 101.0 (67.4–151.4)                                                    | 881.5 (726.7–1069.3)                                                  | 8.7             |
| <b>8b</b>        | -13 ± 6 (NS)                                                                                         | 35.2 (20.3–60.9)                                                      | 483.3 (331.7–704.2)                                                   | 13.7            |
| <b>10</b>        | -38 ± 8 <sup>e</sup>                                                                                 | 19.7 (12.3–31.3)                                                      | 330.2 (282.9–385.3)                                                   | 16.8            |
| <b>11</b>        | -24 ± 6 (NS)                                                                                         | 23.1 (13.4–39.9)                                                      | 294.7 (150.2–578.2)                                                   | 12.8            |
| <b>PC25</b>      | +16 ± 4 (NS)                                                                                         | 9.2 (6.3–13.1)                                                        | 410.0 (332.3–505.9)                                                   | 44.5            |
| Phenytoin        | -16 ± 5 <sup>e</sup>                                                                                 | 4.0 (3.0–5.3)                                                         | 30.5 (22.7–40.9)                                                      | 7.6             |
| Phenobarbital    | +33 ± 4 <sup>e</sup>                                                                                 | 4.6 (3.0–7.0)                                                         | 33.0 (29.3–37.2)                                                      | 7.2             |
| Sodium valproate | -5 ± 2 (NS)                                                                                          | 112.0 (88.2–142.2)                                                    | 208.0 (173.8–248.9)                                                   | 1.9             |
| Carbamazepine    | +7 ± 3 (NS)                                                                                          | 5.5 (4.6–6.5)                                                         | 40.0 (38.3–41.8)                                                      | 7.3             |
| Diazepam         | -42 ± 6 <sup>e</sup>                                                                                 | 4.0 (3.4–4.8)                                                         | 2.8 (1.8–4.2)                                                         | 0.7             |
| Lamotrigine      | -34 ± 5 <sup>e</sup>                                                                                 | 3.9 (2.2–7.1)                                                         | 59.1 (46.3–75.5)                                                      | 15.1            |

<sup>a</sup>Drugs were administered 30 min before testing.<sup>b</sup>MES (Maximal Electroshock Seizure Test).<sup>c</sup>Numbers in parentheses are 95% confidence intervals.<sup>d</sup>PI (Protective Index) = TD<sub>50</sub>/ED<sub>50</sub>. PI values are for MES test.<sup>e</sup>The level of significance was  $p < 0.05$ . NS = not significant.**Table 4.** Anticonvulsant activity (ED<sub>50</sub>, mg kg<sup>-1</sup> orally) of pyridazine derivatives in the chemically induced seizures tests

| Compd            | Pentylenetetrazole <sup>a</sup><br>(85 mg kg <sup>-1</sup> sc) | Strychnine <sup>a</sup><br>(1 mg kg <sup>-1</sup> sc) | Bicuculline <sup>a</sup><br>(0.8 mg kg <sup>-1</sup> iv) | Yohimbine <sup>a</sup><br>(45 mg kg <sup>-1</sup> sc) | NMDLA <sup>a</sup><br>(345 mg kg <sup>-1</sup> sc) |
|------------------|----------------------------------------------------------------|-------------------------------------------------------|----------------------------------------------------------|-------------------------------------------------------|----------------------------------------------------|
| <b>4b</b>        | 91.1 (54.9–151.1) <sup>b</sup>                                 | 61.2 (32.8–114.1) <sup>b</sup>                        | 62.9 (50.3–78.6) <sup>b</sup>                            | 10(NS) <sup>c</sup>                                   | 20(NS) <sup>c</sup>                                |
| <b>7a</b>        | 130.1 (57.9–292.2) <sup>b</sup>                                | 0 <sup>c</sup>                                        | 56.0 (40.0–78.4) <sup>b</sup>                            | 0 <sup>c</sup>                                        | 40 <sup>c,f</sup>                                  |
| <b>10</b>        | 114.5 (69.6–188.4) <sup>b</sup>                                | 93.1 (50.7–171.2) <sup>b</sup>                        | 0 <sup>c</sup>                                           | 10(NS) <sup>c</sup>                                   | 0 <sup>c</sup>                                     |
| <b>PC25</b>      | 76.0 (64.4–89.7) <sup>b</sup>                                  | 34.5 (26.0–45.7) <sup>b</sup>                         | 93.0 (55.2–156.7) <sup>b</sup>                           | 76.0 (66.5–86.8) <sup>b</sup>                         | 14(NS) <sup>d</sup>                                |
| Phenytoin        | 0 <sup>c</sup>                                                 | 0 <sup>c</sup>                                        | 0 <sup>c</sup>                                           | 10(NS) <sup>d</sup>                                   | 0 <sup>d</sup>                                     |
| Phenobarbital    | 15.0 (13.4–16.8) <sup>b</sup>                                  | 0 <sup>c</sup>                                        | 20.0 (14.7–27.2) <sup>b</sup>                            | 17.0 (12.1–23.9) <sup>b</sup>                         | 30.0 (12.8–70.5) <sup>b</sup>                      |
| Sodium valproate | 20 (NS) <sup>c</sup>                                           | 0 <sup>c</sup>                                        | 14(NS) <sup>c</sup>                                      | 10(NS) <sup>d</sup>                                   | 0 <sup>d</sup>                                     |
| Carbamazepine    | 17.5 (12.1–25.2) <sup>b</sup>                                  | 8.5 (7.6–9.6) <sup>b</sup>                            | 0 <sup>c</sup>                                           | 10(NS) <sup>d</sup>                                   | 20(NS) <sup>d</sup>                                |
| Diazepam         | 0.5 (0.3–0.8) <sup>b</sup>                                     | 1.1 (0.6–1.8) <sup>b</sup>                            | 0.4 (0.2–0.6) <sup>b</sup>                               | 2.3 (1.4–3.7) <sup>b</sup>                            | 30 <sup>c,f</sup>                                  |
| Lamotrigine      | 42.1 (23.5–75.4) <sup>b</sup>                                  | 13.0 (4.7–35.8) <sup>b</sup>                          | 21.3 (16.3–27.8) <sup>b</sup>                            | 10(NS) <sup>c</sup>                                   | 60 <sup>c,f</sup>                                  |

<sup>a</sup>Drugs were administered 30 min before testing.<sup>b</sup>95% confidence intervals.<sup>c</sup>Percentage of protection at 100 mg kg<sup>-1</sup> orally.<sup>d</sup>Percentage of protection at 150 mg kg<sup>-1</sup> orally.<sup>e</sup>Percentage of protection at 30 mg kg<sup>-1</sup> orally.<sup>f</sup>The level of significance was  $p < 0.05$ . NS = not significant.

sodium valproate failed to protect mice from seizures. But carbamazepine, diazepam and lamotrigine provided better protection than the pyridazines did. In the bicuculline test, **4b** and **7a** were equipotent with ED<sub>50</sub>'s of 62.9 and 56.0 mg kg<sup>-1</sup>, respectively, and were more effective than **PC25** (ED<sub>50</sub> = 93.0 mg kg<sup>-1</sup>). Compound **10**, as well as phenytoin, sodium valproate and carbamazepine were inactive in this animal seizure model.

Like phenytoin, sodium valproate, carbamazepine and lamotrigine, **4b**, **7a** and **10** were unable to prevent seizures induced by yohimbine when phenobarbital and diazepam were very potent with ED<sub>50</sub>'s values of 17.0 and 2.3 mg kg<sup>-1</sup>, respectively.

Finally, at the maximum doses employed, no protection against seizures induced by NMDLA was noted, except

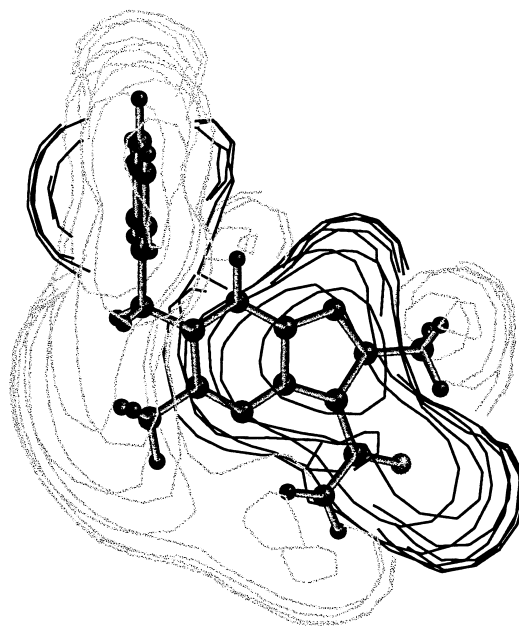
for marginal activity in the case of **7a**. Only phenobarbital and lamotrigine exhibited prominent inhibition of NMDLA-induced convulsions in mice.

Therefore, except yohimbine and NMDLA induced seizures, **4b** was active against all of the seizure models with a relatively narrow dose range and appeared to be the most interesting anticonvulsant drug together with compound **PC25**. Thus the anticonvulsant profile observed for **4b** was obviously somewhat better than those observed for either phenytoin or sodium valproate.

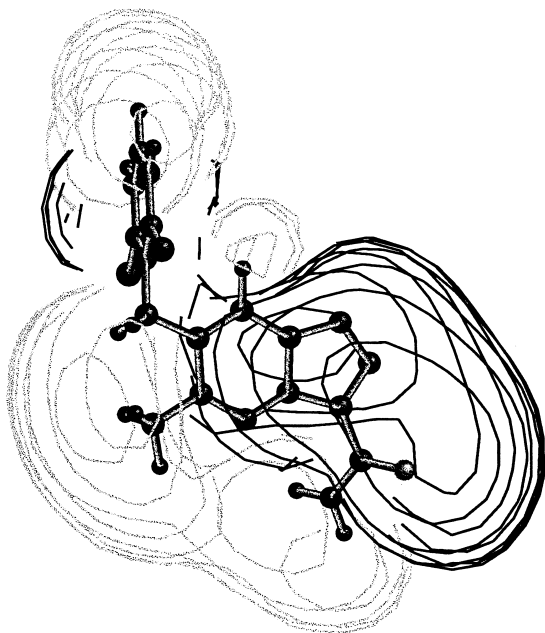
As it has been demonstrated in a series of purine analogues,<sup>12</sup> the differences in activity of **4b**, **7a**, **10** and **PC25** could be related to the electrostatic potential on the surface of the molecules. In order to provide support for this hypothesis, electrostatic isopotential maps of pyridazines were generated using SYBYL 6.3 software<sup>13</sup> from MOPAC charges (Figures 1–4) as described in Experimental section. The major differences in the shapes of the contour maps are observed near the 2-position of the bicyclic pyridazine system. Optimum activity could be associated with the electrostatic potential surface presented by **PC25**. Triazolopyridazine **4b**, which has a surface somewhat similar to that of **PC25**, with a negative electrostatic isopotential near the 2-position of the pyridazine ring (see Scheme 1), is the most active compound of this new series, contrary to

imidazopyridazines **7a** and **10** whose isopotential surfaces are quite different because of their positive electrostatic zone at the same position.

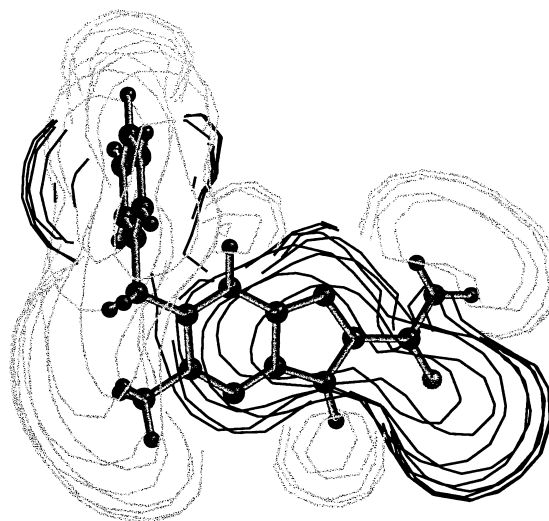
In conclusion, most of the compounds described in this report have significant activity as anticonvulsant drugs in the MES screen, associated with high protection



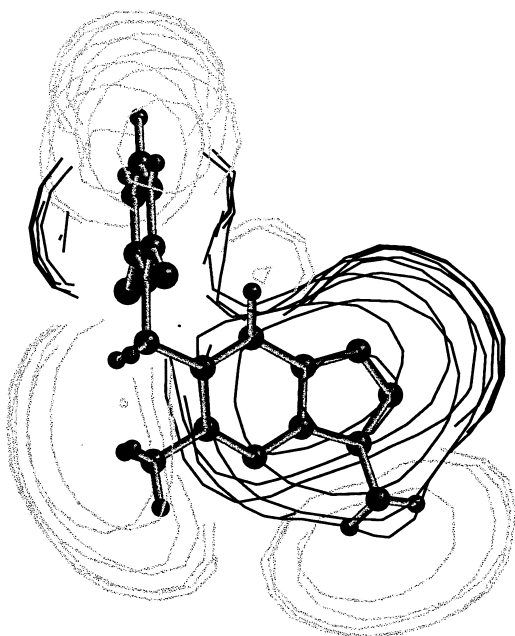
**Figure 2.** Stereoview of electrostatic isopotential surfaces ( $E$ ) of compound **7a**.  $E \leq -5 \text{ kcal mol}^{-1}$ ; bold solid.  $E \geq 5 \text{ kcal mol}^{-1}$ ; solid.



**Figure 1.** Stereoview of electrostatic isopotential surfaces ( $E$ ) of compound **4b**.  $E \leq -5 \text{ kcal mol}^{-1}$ ; bold solid.  $E \geq 5 \text{ kcal mol}^{-1}$ ; solid.



**Figure 3.** Stereoview of electrostatic isopotential surfaces ( $E$ ) of compound **10**.  $E \leq -5 \text{ kcal mol}^{-1}$ ; bold solid.  $E \geq 5 \text{ kcal mol}^{-1}$ ; solid.



**Figure 4.** Stereoview of electrostatic isopotential surfaces (E) of compound **PC25**.  $E \leq -5 \text{ kcal mol}^{-1}$ ; bold solid.  $E \geq 5 \text{ kcal mol}^{-1}$ ; solid.

indices. An amino and amido substituent in the 2-position of the pyridazine derivatives fulfills the structural requirements for activity in the sc PTZ screen as well as in the bicuculline and the strychnine tests. Furthermore, electrostatic potential surfaces seem to be a useful tool for selecting new synthetic targets, potentially active, taking **4b** or **PC25** surfaces as a model.

## Experimental

### Chemistry

Melting points were taken on a Reichert apparatus and were uncorrected. Infrared (M) spectra were run as potassium bromide disks on a Beckman 4240 spectrophotometer. The proton nuclear magnetic resonance spectra were performed on a Bruker AC 400 (400 MHz). Chemical shifts were reported in ppm related to internal standard, tetramethylsilane. TLC was carried out on Merck silica gel 60 F254 plates. Structures were confirmed by spectral data. Elemental analyses were performed by the Service Central d'Analyses, Centre National de la Recherche Scientifique, 69 390 Vernaison, France, and all analytical results were within  $\pm 0.4\%$  of the theoretical values.

Representative methods used to prepare pyridazine derivatives are described for following examples.

**7-Benzyl-6-methyltriazolo[4,3-*b*]pyridazine-3-ethyl carboxylate (3).** To an anhydrous pyridine solution (25 mL) of appropriate hydrazinopyridazine **2** (2.14 g, 10 mmol) was added ethoxalyl chloride (2.73 g, 20 mmol). The reaction mixture was stirred at room temperature (2 h) and then refluxed (3 h). After cooling, the solution was poured into water (100 mL) and extracted with chloroform ( $3 \times 100 \text{ mL}$ ). The combined extracts were dried (sodium sulfate) and evaporated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate). Yield 18%, mp  $94^\circ \text{C}$ . IR (KBr)  $\nu \text{ cm}^{-1}$ : 1730, 1600, 1570.  $^1\text{H}$  NMR: 1.35 (t, 3H, 7.2 Hz,  $\text{CH}_3$ ), 2.45 (s, 3H,  $\text{CH}_3$ ), 4.15 (s, 2H,  $\text{CH}_2$ ), 4.40 (q, 2H, 7.2 Hz,  $\text{CH}_2$ ), 7.25–7.40 (m, 5H,  $\text{C}_6\text{H}_5$ ), 8.05 (s, 1H,  $\text{CH}=\text{N}$ ).

**7-Benzyl-6-methyl-triazolo[4,3-*b*]pyridazine-3-carboxamide (4a).** To a suspension of **3** in 30% aqueous ammonia (20 mL) was added ammonium chloride (1.06 g, 20 mmol). The reaction mixture was stirred at room temperature for 48 h. The precipitate formed was filtered off, washed with water and recrystallized in an ethanol :water mixture (75:25).

For the synthesis of compounds **7** and **10**, the same procedure was carried out as described above.

**7-Benzyl-2,6-dimethyl-imidazo[1,2-*b*]pyridazine-3-ethyl carboxylate (6a).** To a suspension of the appropriate aminopyridazine **5** (2.68 g, 10 mmol) in anhydrous *n*-butanol (35 mL) was added ethyl-2-chloroacetoacetate (4.94 g, 30 mmol). The reaction mixture was heated at reflux for 30 h. After cooling, the aminopyridazine hydrochloride precipitate formed was eliminated, the filtrate was evaporated in vacuo and the resulting residue was purified by column chromatography on silica gel (ethyl acetate:hexane, 70:30). Yield 10%, mp  $98^\circ \text{C}$ . IR (KBr)  $\nu \text{ cm}^{-1}$ : 3380, 1680, 1600, 1530.  $^1\text{H}$  NMR: 1.35 (t, 3H, 7.2 Hz,  $\text{CH}_3$ ), 2.45 (s, 3H,  $\text{CH}_3$ ), 2.60 (s, 3H,  $\text{CH}_3$ ), 4.10 (s, 2H,  $\text{CH}_2$ ), 4.30 (q, 2H, 7.2 Hz,  $\text{CH}_2$ ), 7.20–7.35 (m, 5H,  $\text{C}_6\text{H}_5$ ), 7.75 (s, 1H,  $\text{CH}=\text{N}$ ).

**7-Benzyl-2,6-dimethyl-imidazo[1,2-*b*]pyridazine-3-carboxylic acid (8a).** To an aqueous ethanol (3 mL water + 10 mL ethanol) suspension of ester **6a** (0.45 g, 1.46 mmol) was added a 10% aqueous solution of sodium hydroxide (5 mL). The reaction mixture was stirred at room temperature (2 h) and then heated to reflux (210 min). After cooling, the solution was acidified to pH 2 with 6 N hydrochloric acid. The precipitate formed was filtered off, washed with water and recrystallized in an ethanol :water mixture (50:50).

**7-Benzyl-6-methyl-imidazo[1,2-*b*]pyridazine-2-ethyl carboxylate (9a).** To a suspension of the appropriate aminopyridazine **5** (1.99 g, 10 mmol) in anhydrous

ethanol (12 mL) was added a solution of ethyl bromopyruvate (1.95 g, 10 mmol) in anhydrous ethanol (12 mL). The reaction mixture was stirred at room temperature for 1 h and then refluxed for 30 h. After filtration, the filtrate was evaporated in vacuo and the resulting residue was purified by column chromatography on silica gel (ethyl acetate). Yield 15%, mp 120 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 3400, 2940, 1730, 1660, 1600, 1490, 1450. <sup>1</sup>H NMR: 1.35 (t, 3H, 7.2 Hz, CH<sub>3</sub>), 2.45 (s, 3H, CH<sub>3</sub>), 4.15 (s, 2H, CH<sub>2</sub>), 4.35 (q, 2H, 7.2 Hz, CH<sub>2</sub>), 7.25–7.40 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 7.80 (s, 1H, CH=), 8.70 (s, 1H, CH=).

The procedure used for the synthesis of acid **11**, was the same as described for compounds **8a**.

### Pharmacology

In the studies described below, all compounds were orally administered in a 0.5% hydroxypropyl methyl cellulose suspension to Swiss male mice purchased from Depre (Saint-Doulchard, France) weighing 18–22 g. Mice were kept in groups of ten in a temperature controlled room with 12 h light/dark cycle. Twenty animals were used in each test. Food and water were available ad libitum until the time of experiment. The allocation of animals in different groups was randomized and experiments were carried out in blind conditions.

**Sedative activity.** This activity was evaluated by a determination of spontaneous motor activity. The test was performed by the method of Boissier and Simon<sup>14</sup> in photoelectric activity cages (Apelex, Massy, France). Test drugs were administered 30 min before evaluation of spontaneous motor activity, and the number of passages was scored during 10 min.

**Neurotoxicity.** The rotorod test<sup>15</sup> was used to evaluate central nervous system toxicity. Test drugs were administered 30 min before assay. Neurologic toxicity was defined as the failure of the dosed animal to remain on a 3 cm diameter wood rod rotating at 6 rpm for 3 min.

**Anticonvulsant activity.** Compounds were tested for their ability to protect mice against electrically and chemically induced seizures.

**Effect on maximal electroshock seizures.**<sup>16</sup> Test drugs and vehicles were orally administered 30 min before subjecting the animals to maximal electroshock through corneal electrodes. Protection against seizures was defined as the abolition of the hind limb tonic extensor component of seizures.

**Effect on seizures induced by pentylenetetrazole.**<sup>16,17</sup> Test compounds were orally administered 30 min before evaluation for their ability to prevent the tonic extensor

component induced by 85 mg kg<sup>-1</sup> sc of pentylenetetrazole. Anticonvulsant activity was judged when the component was blocked.

**Effect on seizures induced by strychnine.**<sup>18</sup> The ability of the test compounds to provide a protection against seizures was measured 30 min after administration. Protection was defined as the abolition of the hind leg tonic extensor component of the seizure induced by 1 mg kg<sup>-1</sup> sc injection of strychnine.

**Effect on seizures induced by bicuculline.**<sup>19</sup> The antagonism of bicuculline-induced seizures was evaluated according to a procedure similar to that described by Heyer. Compounds were administered 30 min before the test. Bicuculline was intravenously administered at a dose of 0.8 mg kg<sup>-1</sup>. Protection against clonic seizures was recorded.

**Effect on seizures induced by yohimbine.**<sup>20</sup> For the antagonism of yohimbine seizure studies, compounds were administered 30 min before the test. Yohimbine was subcutaneously administered at a dose of 45 mg kg<sup>-1</sup>. Animals that did not exhibit at least one clonic seizure within 60 min were considered protected.

**Effect on seizures induced by NMDLA.**<sup>21</sup> Test compounds were given 30 min prior to injection of 345 mg kg<sup>-1</sup> s.c. of *N*-methyl-D,L-aspartate. Protection against full generalized tonic seizures was recorded.

**Data Analysis.** Statistical analysis of the results was performed using the method of Schwartz.<sup>22</sup> The data on the spontaneous motor activity were analyzed by using the Student's *t*-test. All values were expressed as mean  $\pm$  SD. The data of electrically and chemically induced seizures as well as the data of rotorod test were analyzed by means of chi-square test with Yates correction. The ED<sub>50</sub> and TD<sub>50</sub> values were determined using the method of Litchfield and Wilcoxon.<sup>23</sup>

### Molecular modeling studies

The topographical and electrostatic characterization of the studied molecules was performed using the SYBYL 6.3 software package on a Silicon Graphics Personal IRIS 4D 35TG workstation. Structures were built within SYBYL and minimized by MAXIMIN 2 Tripos force field, in vacuo conditions, to provide reasonable standard geometries. Molecules were deemed to be minimized when there was a minimum energy change of less than 0.021 kJ mol<sup>-1</sup> for one iteration. The conjugate gradient method was used for minimization. All AM1 calculations involved the singlet state. Molecules were deemed to be minimized when the gradient fell to less than 0.021 kJ mol<sup>-1</sup>. The conformational space of **PC25**

was explored as previously described.<sup>1</sup> Compounds **4b**, **7a** and **10** were built by modifications of **PC25** and lowest energy conformers were obtained after rotation of the amide or the acid group. These conformers were submitted to AM1 calculations (MOPAC version 5.0)<sup>24</sup> to optimize their geometry and determine atomic charges distribution. The molecular electrostatic potential surfaces were calculated in SYBYL and partitioned into two intervals as follows:  $-5 \text{ kcal mol}^{-1}$  and down, and  $+5 \text{ kcal mol}^{-1}$  and up.

### References and Notes

- Moreau, S.; Coudert, P.; Rubat, C.; Gardette, D.; Vallee-Goyet, D.; Couquelet, J.; Bastide, P.; Tronche, P. *J. Med. Chem.* **1994**, *37*, 2153.
- Louvet, P.; Lallement, G.; Pernot-Marino, I.; Luu-Duc, C.; Blanchet, G. *Eur. J. Med. Chem.* **1993**, *28*, 71.
- Palaty, J.; Abbott, F. S. *J. Med. Chem.* **1995**, *38*, 3398.
- Palmer, G. C.; Stagnitto, M. L.; Garske, G. E.; Napier, J. J.; Harris, E. W.; Kaiser, F. C.; Griffith, R. C.; Woodhead, J. H.; White, H. S.; Wolf, H. H.; Swinyard, E. A. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 1023.
- Bailleux, V.; Vallee, L.; Nuyts, J. P.; Hamoir, G.; Poupaert, J. H.; Stables, J. P.; Vainecq, J. *Eur. J. Med. Chem.* **1995**, *30*, 439.
- Choi, D.; Stables, J. P.; Kohn, H. *J. Med. Chem.* **1996**, *39*, 1907.
- Taouflk, J.; Couquelet, J. M.; Couquelet, J. D.; Tronche, P. *J. Heterocycl. Chem.* **1985**, *22*, 1615.
- Porter, R. J. *Jpn. J. Psychiatr. Neurol.* **1993**, *47*, 145.
- Löscher, W.; Schmidt, B. *Curr. Opin. Invest. Drugs* **1993**, *2*, 1067.
- Kupferberg, H. J. *Epilepsia* **1989**, *30*, 551.
- Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* **1978**, *19*, 409.
- Kelley, J. L.; Davis, R. G.; Mc Lean, E. W.; Glen, R. C.; Soroko, F. E.; Cooper, B. R. *J. Med. Chem.* **1995**, *38*, 3884.
- Tripos Associates, Inc., 1699 S. Harley Rd., Suite 303, St Louis, MO 63144.
- Boissier J. R.; Simon, P. *Arch. Int. Pharmacodyn. Ther.* **1965**, *158*, 212.
- Kinnard, W. J.; Carr, C. J. *J. Pharmacol. Exp. Ther.* **1957**, *121*, 354.
- Brodie, J. M. *Lancet* **1992**, *339*, 1397.
- Swinyard, E. A.; Brown, W. C.; Goodman, L. S. *J. Pharmacol. Exp. Ther.* **1952**, *106*, 319.
- Gouret, C.; Linee, P. *J. Pharmacol. (Paris)* **1972**, *3*, 57.
- Heyer, E. J.; Nowak, L. M.; McDonald, R. L. *Neurology* **1981**, *31*, 1381.
- Dunn, R. W.; Corbett, R. *Neuropharmacology* **1992**, *31*, 389.
- Czuczwar, S. J.; Frey, H. H.; Löscher, W. *Eur. J. Pharmacol.* **1985**, *108*, 273.
- Schwartz, D. In *Méthodes Statistiques à L'usage des Médecins et des Biologistes*; Editions médicales Flammarion: Paris, 1984; pp 263–307.
- Litchfield, J. T.; Wilcoxon, E. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.
- Merz, K. M.; Besler, B. H. MOPAC version 5.0 ESC, QCPE 589, **1990**, with AM1 force field parameters.