

## Pyrrolidine-modified and 6-substituted analogs of nicotine: a structure–affinity investigation

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**Summary** — Because the structural requirements for the binding of nicotine to central nicotine receptors remain largely uninvestigated, we undertook a systematic investigation of pyrrolidine ring-opened analogs. This led to a subsequent investigation of related conformationally restricted derivatives of these analogs. The results are reported relative to the binding of several well-known and widely used nicotine receptor ligands. Although none of the ring-opened analogs binds with higher affinity than (–)nicotine ( $K_i$  = 2.3 nM), 3-(*N*-methyl-*N*-ethylaminomethyl)pyridine (**12a**;  $K_i$  = 28 nM) binds with significant affinity. A conformationally restricted analog of **12a**, *N*-methyl [2,7]naphthyridine **30b** ( $K_i$  = 18 nM), binds with similar affinity. 6-Substitution of **12a** and racemic nicotine seems to be tolerated when the substituent is halogen or methyl. In functional studies (hypolocomotion and antinociception in mice; stimulus generalization in nicotine-trained rats) **30b** retains nicotine-like properties. Several of the 6-substituted compounds were 2 to 20 times more potent than (±)nicotine. Although the intact pyrrolidine ring of nicotine appears important for optimal affinity, its presence is not an absolute requirement for activity, and 6-position substitution of the pyridine nucleus can influence both binding and functional activity.

nicotine / nicotine receptor / drug discrimination / antinociception

### Introduction

Various drugs of abuse have been structurally modified to afford therapeutically useful agents. Nicotine (**1a**) is a widely abused substance that, despite its abuse liability [1], has already found application in smoking cessation therapy [2]. Nicotine receptors have also been implicated as playing potential roles in anxiety, obesity, nociception, and various cognitive and neurological disorders [3–5]. Nicotine itself is a fairly toxic agent [5]; thus, there is a need to identify novel nicotinic agents with reduced toxicity. The development of such agents should be possible on the basis of established structure–activity relationships (SAR) for central nicotinic activity. However, surprisingly little is known about the SAR of nicotine. Early studies focussed on the SAR for the interaction of nicotinic agents at peripheral nicotine receptors (eg, see references [6–8]). However, the exact relationship between peripheral and central nicotine receptors is not fully understood at this time. More recently, radioligand binding studies have furnished information on central nicotine receptors. However, over the past

decade, a broad array of radioligands has been used, and even the reported affinity of (–)nicotine itself has varied over a > 100-fold range [9]. Neuronal nicotinic receptors are members of the ligand-gated ion channel family and are composed of a pentameric combination of subunits; the combination of subunits present in a given pentamer appears to dictate the pharmacology of the receptor [5]. In addition to binding sites with high affinity for (–)nicotine, also present are allosteric sites and channel activator sites [5]. Evidence suggests that different radioligands may label different sites or different combinations of sites. For example, sites labeled by [<sup>125</sup>I]- $\alpha$ -bungarotoxin and by [<sup>3</sup>H]nicotine show a different anatomical distribution [10, 11].

Due to different binding results from the use of different radioligands, and a paucity (yet structural diversity) of agents, it has been difficult to formulate central structure–affinity relationships. Nevertheless, using distance geometry techniques, Sheridan et al [12] developed a common nicotinic pharmacophore on the basis of four different, structurally related nicotinic agents. This pharmacophore seems to account for the activity of several other agents also examined in

the same study [12]. However, the minimal structural requirements for central nicotine receptor binding have yet to be identified. Thus, rather than attempting a detailed structure–affinity study on a wide array of structurally diverse nicotinic ligands, it was thought more prudent to focus on nicotine-related structures, and to determine in a stepwise and systematic manner the contribution to binding of the various structural features of nicotine itself. It was with the intention of developing novel nicotinic ligands that we undertook this structure–affinity study. We proposed to systematically investigate the impact of structural modification of the pyrrolidine and pyridine portions of nicotine on [ $^3\text{H}$ ]nicotine-labeled nicotine receptor binding. In this report, we primarily describe our work on modification of the pyrrolidine ring. It might be noted that while this manuscript was in preparation a report appeared by Lin and coworkers [13] in which they described the effect of pyrrolidine modification on nicotine receptor binding. Because their studies involved elaboration of the pyrrolidine ring (by incorporation of various alkyl groups, with and without diverse polar substituents), whereas this present study is primarily concerned with ring-abbreviated structures, the two studies may be considered as being complementary. In addition, because we have already identified the 6-position of nicotine as possibly being associated with a region of bulk tolerance on the receptor [14], we also attempted to exploit the present findings by preparing several 6-substituted derivatives of ring-opened nicotine analogs and comparing their affinities with the corresponding 6-substituted analogs of nicotine.

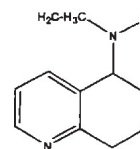
## Chemistry

### Pyrrolidine-modified analogs

Compound **3**, the 5'-methyl derivative of (–)nicotine [(–)**1a**], was prepared as a mixture of 3'(S),5'(S)- and 3'(S),5'(R)-diastereomers following the procedure of Shibagaki [15, 16]. The aminomethylpyridines (table I) were prepared by one of five methods (*Methods A–E*). Reductive amination of pyridine-3-carboxaldehyde with the appropriate amine was accomplished using either catalytic reduction conditions (*Method A*; compounds **6–10** and **12a**) or sodium cyanoborohydride according to the general reaction conditions described by Borch et al [17] (*Method B*; compounds **13** and **22**). Compounds **14**, **16** and **18** were prepared by diborane reduction of the *N,N*-dialkylnicotinamides obtained from reaction of nicotinoyl chloride with the appropriate amine in the presence of triethylamine (*Method C*). Compound **15** was derived from **6** by alkylation with *n*-pentanol tosylate (*Method D*), whereas *N*-methylation of **9** and

**10** under Eschweiler–Clarke conditions (*Method E*) afforded tertiary amines **17** and **19**, respectively. The 2- and 4-methyl analogs of **12a** and **13** (ie, **23–26**) were prepared according to the general procedure of Sattler and Schunack [18] (*Method F*).

5-Amino-5,6,7,8-tetrahydroquinoline was prepared by reductive amination of the corresponding tetrahydroquinolone; acetylation of the amine followed by  $\text{LiAlH}_4$  reduction afforded the *N*-ethyl derivative **28a**; the *N*-methyl derivative of **28a**, **28b**, was prepared by reductive methylation.



**28a**, R = H; **28b**, R =  $\text{CH}_3$

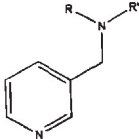
Compound **29** was prepared from 3,4-bis(hydroxymethyl)pyridine by treatment with  $\text{SOCl}_2$  and  $\text{MeNH}_2$  following the procedure of Armarego and coworkers [19]. [2,7]Naphthyridine was synthesized according to a literature procedure [20, 21], and catalytically reduced to **30a** [22]. The *N*-substituted derivatives of **30a** (ie, **30b–e**) were obtained by Eschweiler–Clarke methylation, ethylation using ethanol tosylate, and reductive alkylation using propionaldehyde and acetone, respectively.

Compound **31a** was prepared by ring-expansion of 5,6,7,8-tetrahydroisoquinol-8-one via a Schmidt reaction followed by  $\text{LiAlH}_4$  reduction of the resulting amide. The *N*-methyl derivative **31b** was obtained by Eschweiler–Clarke alkylation of **31a**.

### 6-Substituted derivatives

The 6-halonicotine analogs **1b**, **1e** and **1f**, were prepared by diazotization of 6-aminonicotine [23], followed by treatment of the diazonium salt with HCl gas in concentrated HCl,  $\text{Br}_2$  in 48%  $\text{HBr}/\text{HOAc}$ , or 48%  $\text{HBF}_4$ , respectively. 6-Methylnicotine (**1c**) was prepared following the method of Seeman et al [24] except that *N*-vinylpyrrolidin-2-one and sodium hydride were used in the first step of the preparation of the intermediate 6-methylmyosmine. The procedure used by Leete and Leete [25] for the synthesis of nicotine was modified for the preparation of 6-methoxynicotine (**1d**). In brief (scheme 1), addition of aqueous KCN to the iminium salt formed upon reaction of 6-methoxypyridine-3-carboxaldehyde with morpholine perchlorate yielded **35**; subsequent treatment with acrylonitrile under basic conditions afforded **36**. Acid hydrolysis of **36** followed by reduction of the  $\beta$ -ketonitrile **37** using Raney-Nickel provided 6-methoxynicotine (**38**). Reductive alkylation of **38** was



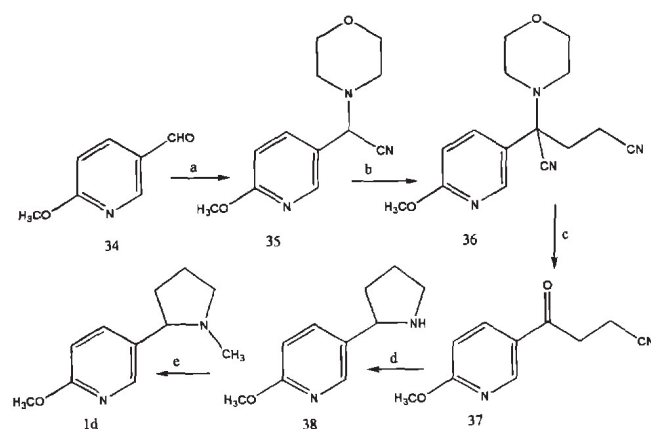
**Table I.** Physicochemical properties and binding data for the aminomethylpyridine derivatives<sup>a</sup>.


| Compound   | R                                  | R'              | Method | Yield (%)       | Mp (°C) <sup>b</sup> | Empirical formula <sup>c</sup>                                                                             | K <sub>i</sub> (nM) (±SEM) |
|------------|------------------------------------|-----------------|--------|-----------------|----------------------|------------------------------------------------------------------------------------------------------------|----------------------------|
| <b>5</b>   | H                                  | H               | —      | 93              | 155–156              | C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> ·C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>                 | > 10 000                   |
| <b>6</b>   | H                                  | Me              | A      | 42              | 196–197              | C <sub>7</sub> H <sub>10</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>               | > 10 000                   |
| <b>7</b>   | H                                  | Et              | A      | 73              | 185–186              | C <sub>8</sub> H <sub>12</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>               | 970 (275)                  |
| <b>8</b>   | H                                  | <i>n</i> -Pr    | A      | 69              | 202–204              | C <sub>9</sub> H <sub>14</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>               | 2470 (300)                 |
| <b>9</b>   | H                                  | CycloPr         | A      | 67              | 168–170              | C <sub>9</sub> H <sub>12</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>               | 5775 (850)                 |
| <b>10</b>  | H                                  | CycloBu         | A      | 79              | 160–164              | C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>              | > 10 000                   |
| <b>11</b>  | Me                                 | Me              | —      | 81              | 163–164              | C <sub>8</sub> H <sub>12</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>               | 540 (40)                   |
| <b>12a</b> | Me                                 | Et              | A      | 37              | 127–129              | C <sub>9</sub> H <sub>14</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>               | 28 (6)                     |
| <b>13</b>  | Me                                 | <i>n</i> -Pr    | B      | 72              | 141–142              | C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> ·C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>               | 1140 (125) <sup>f</sup>    |
| <b>14</b>  | Me                                 | <i>n</i> -Bu    | C      | 92 <sup>d</sup> | 143–146              | C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> <sup>e</sup> | > 10 000                   |
| <b>15</b>  | Me                                 | Pentyl          | D      | 87              | 128–130              | C <sub>12</sub> H <sub>20</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>              | > 10 000                   |
| <b>16</b>  | Me                                 | <i>iso</i> -Pr  | C      | 91 <sup>d</sup> | 160–162              | C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>              | 159 (15)                   |
| <b>17</b>  | Me                                 | CycloPr         | E      | 20              | 161–162              | C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> ·2.5C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>            | 840 (100)                  |
| <b>18</b>  | Me                                 | <i>tert</i> -Bu | C      | 95 <sup>d</sup> | 132–134              | C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>              | 195 (50)                   |
| <b>19</b>  | Me                                 | CycloBu         | E      | 68              | 85–87                | C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> <sup>g</sup> | 2170 (180)                 |
| <b>20</b>  | Et                                 | Et              | —      | 68              | 139–140              | 2C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> ·3C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>             | 122 (2)                    |
| <b>21</b>  | -(CH <sub>2</sub> ) <sub>4</sub> - | — <sup>h</sup>  | —      | —               | —                    | —                                                                                                          | 30 (3)                     |
| <b>22</b>  | -(CH <sub>2</sub> ) <sub>5</sub> - | B               | —      | 47              | 183–184              | C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> ·C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> <sup>i</sup>  | 55 (6)                     |

<sup>a</sup>Certain of these compounds have been previously reported as their free bases and/or as their picrates. For example: **5** [28, 29, 32], **6** [29, 33], **7** [28, 33, 34], **8** [33], **11** [28, 29, 32], **12a** and **13** [24], **20** [29, 32, 35], **21** [30], and **22** [29, 32]. <sup>b</sup>All compounds were recrystallized from absolute EtOH, except for **7** (MeOH/H<sub>2</sub>O), **12a** (2-PrOH/H<sub>2</sub>O), **14–16** and **18** (absolute EtOH/anhydrous Et<sub>2</sub>O), and **19** and **22** (95% EtOH). <sup>c</sup>All compounds analyzed within 0.4% of theory for C, H, N. <sup>d</sup>Yield of reduction step. <sup>e</sup>Crystallized with 0.25 mol of H<sub>2</sub>O. <sup>f</sup>In a preliminary report, we published a K<sub>i</sub> value of **17** nM for **13**; this has been found to be a typographical error. Compound **13** was resynthesized and both the new sample and the original sample give comparable K<sub>i</sub> values. <sup>g</sup>Crystallized with 2 mol of H<sub>2</sub>O. <sup>h</sup>Prepared as previously reported [33]. <sup>i</sup>In a separate reaction, compound **22** was also isolated in 45% yield as its dioxalate salt (mp 155–156 °C) (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>·2C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>).

achieved with sodium cyanoborohydride to afford the desired **1d**.

The 6-substituted aminomethylpyridines (ie, **12b–e**) were prepared either from the corresponding pyridine-3-carboxaldehyde or nicotinic acid. The 6-methyl derivative **12c** was obtained from 6-methylnicotinic acid via a mixed anhydride following the method of Sattler and Schumack [18]; the intermediate nicotinamide was reduced to **12c** using LiAlH<sub>4</sub>. The 6-fluoro derivative **12e** was prepared in a similar manner, except that the amide intermediate was reduced with AlH<sub>3</sub> instead of LiAlH<sub>4</sub> to minimize reductive dehalogenation. Reductive amination of 6-methoxypyridine-3-carboxaldehyde with *N*-ethyl-*N*-methylamine under catalytic hydrogenation conditions provided **12d**. A similar reaction employing 6-chloropyridine-3-carboxaldehyde resulted in dehalogenation; however, the use of sodium cyanoborohydride afforded the desired **12b**.



**Scheme 1.** a) morpholine perchlorate, KCN, morpholine, H<sub>2</sub>O; b) acrylonitrile, *t*-BuOH, KOH, MeOH; c) AcOH, H<sub>2</sub>O, THF, 53 °C; d) NH<sub>3</sub>, EtOH, Ni/Ra, H<sub>2</sub> (40 psi); e) NaOAc, H<sub>2</sub>O, HCOH, NaBH<sub>3</sub>CN.

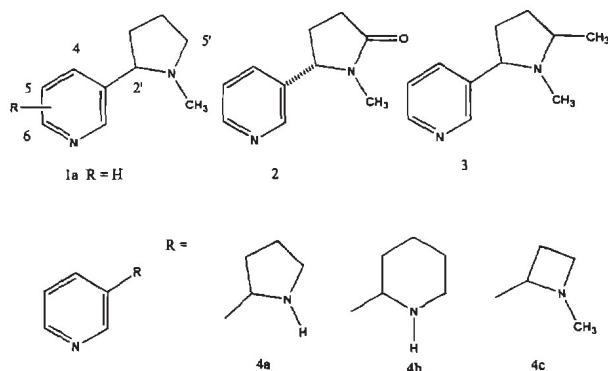
## Results and discussion

### Pyrrolidine-modified analogs

#### Aminomethylpyridine derivatives

The pyrrolidine ring nitrogen atom of nicotine ( $K_i$ ,  $S(-)$ **1a** = 2.3 nM;  $K_i$ ,  $R(+)$ **1a** = 30 nM) [9] seems to contribute to binding. For example, cotinine (**2**;  $K_i$  > 1500 nM), a metabolite of nicotine, binds with > 650-fold lower affinity than  $(-)$ nicotine [9]. The 5'-methyl derivative of nicotine (ie, **3**;  $K_i$  =  $90 \pm 3$  nM) binds with higher affinity than cotinine (**2**). Lin et al [13] recently reported that the affinity of **3** is attributable to the 5'(*S*)-isomer ( $K_i$  = 34.9 nM) as opposed to the 5'(*R*)-isomer ( $K_i$  = 1205 nM), and they suggest that the reduced affinity of 5'-alkyl nicotine analogs is related to either, or a combination of, conformational and steric factors. On the basis of the higher affinity of **3** relative to cotinine (**2**), the low affinity of cotinine may also reflect its reduced basicity. The nature of the amine substituents is also important for binding. For example, nicotine can be quaternized to  $N_1'$ -methyl-nicotine with retention of affinity [9]; however, *N*-demethylation to normicotine (**4a**) reduces affinity by at least tenfold [9], and replacement of the *N*-methyl group of nicotine by larger *N*-alkyl groups results in even further reduced affinity [26, 27].

Ring expansion of normicotine to anabasine (**4b**;  $K_i$  = 210 nM) reduces affinity by about tenfold [9], whereas ring contraction to the azetidines **4c** results in an agent that binds with the same affinity as nicotine [37, 38].



Is the entire or intact pyrrolidine ring required for binding? To explore this in greater detail, we began by abbreviating the pyrrolidine portion of nicotine to the primary amine **5**, and then reconstituted the pyrrolidine ring in a systematic manner. Because some of the results were already included in an earlier QSAR study [39], only the overall conclusions will be discussed here. The primary amine **5** is inactive, secondary amines bind only with low affinity, and tertiary amines appear optimal. With respect to tertiary

amines, small alkyl groups are favored, bulk tolerance is limited, and the *N*-methyl-*N*-ethyl substitution pattern found in **12a** is optimal. Branching is tolerated but results in a reduced affinity. Cyclization of the terminal amine alkyl groups, as with **21**, is also tolerated. Nevertheless, these compounds still bind with lower affinity than nicotine itself.

#### Conformationally restricted analogs

Nicotine is a conformationally flexible molecule. Although the conformation of nicotine has been examined [24, 40, 41], its receptor relevant conformation is unknown. Likewise, compound **12a** is conformationally flexible. In an attempt to force the side chain into different orientations, we prepared the 2-methyl and 4-methyl analogs of **12a** (ie, **23** and **24**) and **13** (ie, **25** and **26**). Both **25** and **26** were inactive ( $K_i$  > 10 000 nM; table II). The affinities of **23** ( $K_i$  = 4614 nM) and **24** ( $K_i$  = 1745 nM) were also low compared to their parent **12a** ( $K_i$  = 28 nM). The reduced affinity of **23** and **24** might reflect a lack of bulk tolerance for the aromatic methyl groups by the receptor. Alternatively, the presence of the methyl groups might prevent the side chain from achieving the proper binding conformation. To examine this further, we prepared several conformationally restricted analogs. Because compound **27** had been previously shown to bind poorly at nicotine receptors ( $K_i$  > 600 nM) [27], we focussed on several analogs that locked the side chain into an 'up' conformation. Specifically, we prepared compound **28a** and **28b**; both lacked affinity for nicotine receptors ( $K_i$  > 10 000 nM; table III). The low affinity of **28b** relative to **12a**, and the lower affinity of **28b** relative to **23**, might reflect a lack of tolerance by the receptor for the added ring structure. Thus, we prepared and evaluated **29–31**. Compound **29** ( $K_i$  = 85 nM; table III) binds with about six times the affinity of the simple *N,N*-dimethyl derivative **11**, whereas the corresponding tetrahydro[2,7]naphthyridine **30b** ( $K_i$  = 18 nM) binds with an affinity 30 times that of **11**. Compound **30b** had been previously reported to exhibit activity in a rabbit jejunum preparation, but not in the frog rectus muscle assay [7]. Other *N*-substituted (ie, **30c–e**) and the unsubstituted (ie, **30a**) tetrahydro[2,7]naphthyridines bind with reduced affinity (table III). The ring expanded derivatives **31a** and **31b** ( $K_i$  = 1100 and 780 nM, respectively) also bind with low affinity.

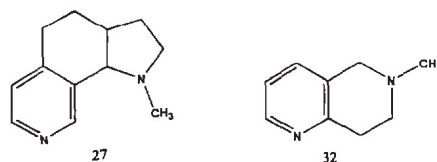
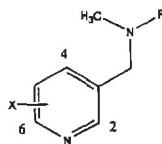




**Table II.** Binding of **12a** and aryl-substituted aminomethylpyridines.

| Compound   | R              | X     | Yield (%) | Mp (°C) <sup>a</sup> | Empirical formula <sup>b</sup>                                                                               | K <sub>i</sub> (nM) (±SEM) |
|------------|----------------|-------|-----------|----------------------|--------------------------------------------------------------------------------------------------------------|----------------------------|
| <b>12a</b> | -Et            | -H    |           |                      |                                                                                                              | 28 (6)                     |
| <b>12b</b> | -Et            | 6-Cl  | 12        | 109–112              | C <sub>9</sub> H <sub>13</sub> ClN <sub>2</sub> ·C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>                | 41 (1)                     |
| <b>12c</b> | -Et            | 6-Me  | 60        | 124–129              | C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>                | 66 (1)                     |
| <b>12d</b> | -Et            | 6-OMe | 22        | 114–116              | C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O·C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>                | 640 (115)                  |
| <b>12e</b> | -Et            | 6-F   | 25        | 122–124              | C <sub>9</sub> H <sub>13</sub> FN <sub>2</sub> ·C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> <sup>c</sup>    | 93 (7)                     |
| <b>23</b>  | -Et            | 2-Me  | 14        | —                    | C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> <sup>d</sup>   | 4614 (110)                 |
| <b>24</b>  | -Et            | 4-Me  | 32        | 137–139              | C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> ·1.5C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> <sup>c</sup> | 1745 (470)                 |
| <b>25</b>  | - <i>n</i> -Pr | 2-Me  | 67        | —                    | C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>                | > 10 000                   |
| <b>26</b>  | - <i>n</i> -Pr | 4-Me  | 30        | 120–124              | C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> ·2C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> <sup>e</sup>   | > 10 000                   |

<sup>a</sup>Compounds **23** and **25** were isolated as oils. <sup>b</sup>Compounds analyzed correctly for C, H, and N. Recrystallization solvents: abs EtOH for **12b,d,e**; 2-PrOH for **12c**; 95% EtOH for **24** and **26**. Synthetic detail is provided for compounds **12b–e** in the *Experimental protocols*. Compounds **23–26** were prepared by *Method F* as exemplified for **25** in the *Experimental protocols*. <sup>c</sup>Crystallized with 0.25 mol of H<sub>2</sub>O. <sup>d</sup>Crystallized with 0.5 mol of H<sub>2</sub>O. <sup>e</sup>Crystallized with 1.5 mol of H<sub>2</sub>O.

Compound **30b**, as described, represents a ring-abbreviated 'up' conformer of nicotine. For purpose of comparison, we prepared **32**, the corresponding 'down' conformer of **30b**. Compound **32** ( $K_i = 165 \pm 10$  nM) binds with about one-tenth the affinity of **30b** ( $K_i = 18$  nM; table III).

#### Functional studies

Because most of the pyrrolidine ring-abbreviated derivatives of nicotine were found to bind at nicotine receptors with substantially lower affinity than nicotine itself, there was little impetus to evaluate these compounds in functional assays. Nevertheless, several of the compounds that displayed interesting affinities were investigated further. Nicotine produces a wide range of behavioral changes in rodents, particularly in its actions on locomotor activity and pain perception [43–47]. Nicotine produces hypolocomotion and antinociception by mechanisms that have been demonstrated to involve nicotine receptors (see [43–47] and references cited therein). The antinociceptive properties of nicotine, as measured using the tail-flick assay in mice, seem to involve spinal nicotine receptors [44–46] and there may be secondary involvement of serotonin-1A (5-HT<sub>1A</sub>) receptors [47]. Accordingly, we examined selected agents in these assays together with (–)nicotine as control. Compounds **12a** and **13** were of interest because, although they differ structurally by only a single methylene group, they display a 40-fold difference in receptor affinity. Although both compounds showed some activity, neither was as

potent as (–)nicotine (table IV). The *N*-ethyl-*N*-methyl derivative **12a** displayed antinociceptive character in mice whereas **13** failed to produce the maximal possible effect (MPE) even at doses as high as 75 mg/kg. The piperidine derivative **22** was about as potent as **12a** in both assays.

**Table III.** Conformationally restricted aminomethylpyridines<sup>a</sup>.

| Compound   | R              | K <sub>i</sub> (nM) | (±SEM) |
|------------|----------------|---------------------|--------|
| <b>28a</b> | H              | >10 000             |        |
| <b>28b</b> | Me             | >10 000             |        |
| <b>29</b>  | Me             | 85                  | (15)   |
| <b>30a</b> | H              | 12 330              | (1850) |
| <b>30b</b> | Me             | 18                  | (1)    |
| <b>30c</b> | Et             | 52                  | (12)   |
| <b>30d</b> | <i>n</i> -Pr   | 1640                | (370)  |
| <b>30e</b> | <i>iso</i> -Pr | 123                 | (5)    |
| <b>31a</b> | H              | 1100                | (30)   |
| <b>31b</b> | Me             | 780                 | (45)   |

<sup>a</sup>SEM not shown where  $K_i > 10\,000$  nM.

With regard to the conformationally restricted analogs, the pyrrolopyridine derivative **29** was not evaluated in the locomotor assay but failed to produce the MPE in the antinociception assay at the highest dose tested (table IV). The [2,7]naphthyridines **30a**, **30b**, and **30e** were active in the locomotor assay. Although **30b** and **30e** were also active in the antinociception assay, the lower-affinity **30d** failed to produce the MPE, and **30a** was essentially inactive (table IV). The [1,6]naphthyridine **32**, the 'down' conformer of **30b**, although it binds with only tenfold lower affinity than **30b**, is conspicuous for its failure to produce the MPE in either the locomotor or tail-flick assay (table IV).

Perhaps the most precise manner of determining whether or not an agent possesses nicotine-like behavioral character is to examine it in tests of stimulus generalization in rats trained to discriminate nicotine from vehicle. Relatively few compounds produce nicotine-like discriminative stimulus responding [48]. Compounds **12a**, **13**, **22**, **30a**, **30b** and **32** were examined in rats trained to discriminate 0.4 mg/kg of (–)nicotine from saline. The *N*-ethyl-*N*-methyl aminomethylpyridine derivative **12a** resulted in partial generalization whereas its *N*-propyl homolog **13** produced saline-like effects at the highest dose tested (table IV); higher doses resulted in disruption of behavior. Known [29–31] compound **21** has been previously reported to produce nicotine-like stimulus effects in rats and to be about sevenfold less potent than nicotine [31]. Its ring-expanded piperidine derivative **22** [30] produced only partial (37%) generalization at the highest dose evaluated (table IV); administration of higher doses resulted in behavioral disruption. The *N*-unsubstituted [2,7]naphthyridine **30a** produced only saline-appropriate responding when administered at

nearly 40 times the training dose of (–)nicotine. It is noteworthy that the *N*-methyl [2,7]naphthyridine **30b** resulted in complete stimulus generalization whereas its [1,6]naphthyridine counterpart **32** did not (ie, **32** produced only 32% nicotine-appropriate responding at 20 mg/kg or 50 times the training dose of (–)nicotine).

These results indicate that several of the pyrroli-dine ring-opened derivatives of nicotine retain the locomotor and antinociceptive properties of nicotine but are rather weak. Only the naphthyridine derivative **30b** produced nicotine-like discriminative stimulus effects. It should be emphasized that the present investigation was not aimed at the development of potent nicotine-like agents; rather, in an effort to better understand the structure–affinity relationships for nicotine receptor binding, several compounds synthesized during the course of these investigations were selected to determine whether they retained any nicotine-like functional character.

#### 6-Substituted analogs

##### Receptor binding

Epibatidine (**39**) is a novel high-affinity ( $K_i = 0.05$  nM) nicotine receptor ligand [49, 50]. Epibatidine binds with more than ten times the affinity of (–)nicotine, and molecular modeling studies suggest that the structures of nicotine and epibatidine are nearly superimposable with regard to their inter-nitrogen distances [14, 39]. Although the specific contribution or necessity of the epibatidine chloro group to binding remains largely unknown, an early study indicated the chloro group is at least tolerated by nicotine receptors [51]. In order to explore the role of 6-position substitution on the pyridine ring, we prepared 6-chloronicotine

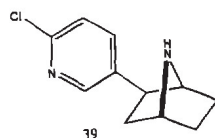
**Table IV.** Functional data for selected aminomethylpyridine derivatives.

| Compound   | Spontaneous activity <sup>a</sup> | Tail-flick <sup>b</sup> | Drug discrimination <sup>c</sup> |
|------------|-----------------------------------|-------------------------|----------------------------------|
| <b>12a</b> | 13 (10–17) mg/kg                  | 27 (14–43) mg/kg        | 49% (±9) at 9.0 mg/kg            |
| <b>13</b>  | 37 (29–48) mg/kg                  | 11% at 75 mg/kg         | 9% (±5) at 0.8 mg/kg             |
| <b>22</b>  | 16 (12–20) mg/kg                  | 18 (12–25) mg/kg        | 37% (±8) at 8.0 mg/kg            |
| <b>29</b>  | NT                                | 17% at 30 mg/kg         | NT                               |
| <b>30a</b> | 37 (21–58) mg/kg                  | 3% at 80 mg/kg          | 16% (±10) at 15 mg/kg            |
| <b>30b</b> | 16 (11–26) mg/kg                  | 21 (11–26) mg/kg        | 5 (2–12) mg/kg                   |
| <b>30d</b> | NT                                | 55% at 40 mg/kg         | NT                               |
| <b>30e</b> | 12 (9–17) mg/kg                   | 20 (14–28) mg/kg        | NT                               |
| <b>32</b>  | 55% at 60 mg/kg                   | 39% at 80 mg/kg         | 32% (±15) at 20 mg/kg            |

<sup>a</sup>Spontaneous activity in mice. ED<sub>50</sub> values (±95% confidence limits) for inhibition of locomotor activity. Compound **32** only reduced activity by 55% at the highest dose examined. For purposes of comparison, the ED<sub>50</sub> value for (–)nicotine = 0.65 mg/kg. NT = Not tested. <sup>b</sup>Tail-flick assay in mice. Results are presented as ED<sub>50</sub> values (±95% confidence limits) or as percent of maximal possible response at the highest dose evaluated. For purposes of comparison, the ED<sub>50</sub> value for (–)nicotine = 1.3 mg/kg. <sup>c</sup>Results of stimulus generalization tests in drug discrimination studies with rats trained to discriminate 0.4 mg/kg of (–)nicotine from 0.9% saline. Where stimulus generalization occurred, ED<sub>50</sub> values are provided; where stimulus generalization failed to occur, the highest non-disruptive dose, and the percentage drug-appropriate responding at that dose, are given.



(**1b**;  $K_i = 0.63$  nM) and found it to bind with an affinity at least comparable to that of nicotine itself [14]. These results encouraged us to examine additional 6-substituted pyridine derivatives. We prepared several such derivatives of **12a** including the 6-chloro (ie, **12b**;  $K_i = 41$  nM), 6-methyl (ie, **12c**;  $K_i = 66$  nM), 6-methoxy (ie, **12d**;  $K_i = 640$  nM) and 6-fluoro (ie, **12e**;  $K_i = 93$  nM) derivatives (table II). Small substituents, such as chloro, methyl, and fluoro, seem to be tolerated, but the methoxy derivative **12d** binds with about 20-fold reduced affinity relative to **12a**.



All of the nicotine analogs (ie, **1b–f**), examined as racemates, were compared with ( $\pm$ )nicotine (**1a**). ( $\pm$ )Nicotine binds at [ $^3$ H]nicotine-labeled rat brain nicotine receptors with high affinity ( $K_i = 1.26$  nM; table V). Introduction of a halogen or a methyl group at the 6-position had relatively little (ie, < fourfold) effect on binding ( $K_i$  values ranging from 0.45 to 1.8 nM). The 6-methoxy derivative **1d** was found to bind with 17-fold lower affinity than nicotine. As with derivatives of **12**, small 6-position substituents seem to be tolerated.

#### Functional studies

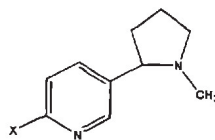
Due to their low affinity, and because of the weak activity of **12a** (table IV), compounds **12b–e** were not examined in the functional assays. Although the span

of binding affinities for the nicotine analogs (with the exception of **1d**) is fourfold, there seems to be more variation in their potencies in the tail-flick assay. For example, although the fluoro and methyl derivatives **1e** and **1c**, respectively, are about equipotent with nicotine (**1a**), the chloro and bromo derivatives **1b** and **1f**, respectively, are about 15 times more potent than their parent (table V). The methoxy derivative **1d**, on the other hand, displays less than one-tenth the activity of **1a**. Likewise, the fluoro and methyl derivatives are about two or three times more potent than nicotine in increasing spontaneous activity in mice whereas the chloro and bromo derivatives are about 7 to 20 times more potent than **1a**. The antinociceptive effects of **1a–c**, **1e**, **1f**, and **2a**, and locomotor actions of **1a–f** and **12a**, were antagonized by 1.0 mg/kg of the nicotine antagonist mecamylamine (data not shown); due to the low activity of **1d** in the tail-flick assay, no attempt was made to evaluate the effect of **1d** in combination with mecamylamine in this assay. The nature of the mecamylamine antagonism was examined in detail for compound **1b** (table VI); antagonism of **1b**-induced antinociception and hypoactivity was found to occur in dose-related manner. Chloro compound **1b** ( $ED_{50} = 0.04$  mg/kg) was examined in rats trained to discriminate (–)nicotine from vehicle and was found to be about twice as potent as racemic nicotine ( $ED_{50} = 0.1$  mg/kg).

#### Conclusion

With regard to nicotine, one of the goals of the present investigation was to determine the minimum structu-

**Table V.** Nicotine receptor affinity and functional data for derivatives of **1**.



| Compound  | X                | $K_i$ (nM) (SEM)    | Tail flick assay  |                      | Spontaneous activity |                      |
|-----------|------------------|---------------------|-------------------|----------------------|----------------------|----------------------|
|           |                  |                     | $ED_{50}$ (mg/kg) | Rel pot <sup>a</sup> | $ED_{50}$ (mg/kg)    | Rel Pot <sup>a</sup> |
| <b>1a</b> | H                | 1.26 ( $\pm 0.02$ ) | 1.64 (1.23–2.38)  | 1.0                  | 0.78 (0.59–1.02)     | 1.0                  |
| <b>1b</b> | Cl               | 0.63 <sup>b</sup>   | 0.13 (0.08–0.24)  | 15.4                 | 0.14 (0.09–0.20)     | 6.8                  |
| <b>1c</b> | CH <sub>3</sub>  | 1.80 ( $\pm 0.15$ ) | 0.89 (0.79–1.52)  | 2.0                  | 0.26 (0.19–0.42)     | 3.3                  |
| <b>1d</b> | OCH <sub>3</sub> | 22 ( $\pm 1$ )      | > 30              | < 0.1                | 8.0 (6.25–10.21)     | 0.1                  |
| <b>1e</b> | F                | 1.03 ( $\pm 0.06$ ) | 1.52 (0.77–3.02)  | 1.2                  | 0.41 (0.23–0.79)     | 2.1                  |
| <b>1f</b> | Br               | 0.45 ( $\pm 0.01$ ) | 0.17 (0.09–0.29)  | 14.4                 | 0.06 (0.03–0.15)     | 19.4                 |

<sup>a</sup> $ED_{50}$  values in mg/kg (followed by 95% confidence limits) are calculated on the basis of the free base. Rel pot = potency relative to ( $\pm$ )nicotine (tail flick:  $ED_{50} = 10.19$   $\mu$ mol/kg; spontaneous activity:  $ED_{50} = 4.84$   $\mu$ mol/kg) = 1, ie,  $ED_{50}$  ( $\mu$ mol/kg) nicotine/ $ED_{50}$  ( $\mu$ mol/kg) test compound. <sup>b</sup>Binding data, but not functional data, previously reported [14]; included for comparison.

**Table VI.** Antagonism of the effects produced by 0.4 mg/kg of compound **1b** by various doses of the nicotine antagonist mecamlamine.

| Mecamylamine dose (mg/kg) | Tail-flick assay                           |              | Spontaneous activity                        |            |
|---------------------------|--------------------------------------------|--------------|---------------------------------------------|------------|
|                           | % MPE ( $\pm$ SEM) <sup>a</sup>            | % Inhibition | Photocell counts <sup>b</sup>               | % Blockade |
| 0                         | 80 ( $\pm$ 14)                             | —            | 195 ( $\pm$ 64)                             | —          |
| 0.01                      | 81 ( $\pm$ 11)                             | 0            | 589 ( $\pm$ 107)                            | 21         |
| 0.05                      | 51 ( $\pm$ 8)                              | 35           | 835 ( $\pm$ 156)                            | 45         |
| 0.1                       | 22 ( $\pm$ 9)                              | 72           | 901 ( $\pm$ 296)                            | 57         |
| 0.5                       | 5 ( $\pm$ 1)                               | 94           | 1260 ( $\pm$ 782)                           | 75         |
| 1.0                       | —                                          | —            | 1960 ( $\pm$ 152)                           | 100        |
|                           | Mecamylamine AD <sub>50</sub> = 0.05 mg/kg |              | Mecamylamine AD <sub>50</sub> = 0.075 mg/kg |            |

<sup>a</sup>MPE = maximal possible effect. <sup>b</sup>Saline control = 1614  $\pm$  95 counts.

ral binding requirements associated with the pyrrolidine ring. Some of our early results were published in communication form [9] and some of the binding data were used to investigate the quantitative structure–affinity relationships (QSAR) for nicotine receptor binding [39]. We report here the details of the synthesis, binding, and functional studies of these and the newer ligands.

Replacement of the pyrrolidine *N*-methyl group with larger alkyl groups results in a progressive and dramatic decrease in receptor affinity [26]. Lin et al [13] have reported that substituents at the 3'-, 4'- or 5'-positions of nicotine also result in reduced affinity. In the present investigation, we found that none of the pyrrolidine-abbreviated nicotine analogs binds with higher affinity than nicotine itself. Although the intact pyrrolidine ring is not an absolute requirement for binding, it certainly appears optimal. The primary amine **5** lacks affinity, and most secondary amine derivatives (eg, **7–9**) bind only with low ( $K_i$  > 500 nM) affinity. Tertiary amines (where one of the amine substituents is a methyl group) bind with higher affinity than their secondary amine counterparts, and the *N*-ethyl-*N*-methyl derivative **12a** ( $K_i$  = 28 nM) was identified as the highest affinity pyrrolidine ring-opened derivative examined. And yet, **12a** still binds with ten-fold lower affinity than (–)nicotine ( $K_i$  = 2.3 nM). Conformational restriction of the *N*-substituted 3-(aminomethyl)pyridines by elaboration to a cyclic structure results in varying effects depending upon ring size. Nevertheless, the high affinity of **30b** ( $K_i$  = 18 nM) is consistent with the concept that nicotine likely binds in a *trans* or 'up-conformation', as suggested by the Sheridan et al [12] pharmacophore, rather than in a cisoid conformation. This finding is further supported by the reduced affinity and lack of functional activity of the 'down' conformer of **30b**, ie, naphthyridine **32**, as well as of **27**. The present results, together with those of Lin et al [13], provide a clearer

picture than previously available about the binding requirements associated with the pyrrolidine portion of nicotine.

We also prepared and evaluated several 6-substituted derivatives of aminomethylpyridine **12a** and (±)nicotine. A comparison of the binding affinities of the 2- and 4-methyl derivatives of **12a**, ie, **23** and **24**, with the 6-methyl derivative **12c** ( $K_i$  = 4614, 1745, and 66 nM, respectively; table II), is consistent with the notion that there may exist a region of bulk tolerance associated with the 6-position binding area of these compounds. However, the reduced affinity of the 6-methoxy derivative **12d** ( $K_i$  = 640 nM) suggests that this region is either limited in size, or that binding is sensitive to the electronic nature of the substituents. Similar results were seen with the nicotine series **1a–f**; with the exception of the 6-methoxy derivative **1d**, all bind with an affinity comparable to (±)nicotine. These same derivatives are at least as active as racemic nicotine in the functional assays. It should be noted, however, that although nicotine receptor mechanisms may be involved in the antinociceptive and locomotor effects of nicotine derivatives, it has already been demonstrated that a direct relationship does not necessarily exist between receptor affinity and these functional effects [26, 27, 43]. For example, certain nicotine derivatives possess functional potencies roughly comparable to that of nicotine, but at the same time bind at nicotine receptors with several hundred-fold lower affinity than nicotine itself [26, 27]. Several other explanations for the observed disparity are possible. For example, the distributional and metabolic characteristics of the various nicotine derivatives may vary. Another possibility is that the different nicotine analogs are binding in a non-parallel fashion to different subpopulations of nicotine receptors that are not readily identified by the radioligand binding methodology used in the present investigation. Additional studies are required to further explore these possibilities.



## Experimental protocols

### Synthesis

Chemicals were purchased from Aldrich Chemicals (Milwaukee, WI). Elemental analysis was performed by Atlantic Micro-lab, Inc (Norcross, GA), and all results are within 0.4% of theory. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a GE QE-300 FT NMR; chemical shifts are reported as ppm. Tetramethylsilane was used as the internal standard. Spectral data are consistent with assigned structures. Thin layer chromatography was performed on pre-coated silica-gel (60F254, Merck) glass plates.

**6-Methyl-3-(1-methyl-2-pyrrolidinyl)pyridine dihydrochloride 1c**  
A solution of methyl 6-methylnicotinate (2.0 g, 13.2 mmol) and *N*-vinylpyrrolidinone (1.8 g, 15.8 mmol) in THF (10 mL) was added in one portion to a refluxing suspension of sodium hydride (0.5 g, 20.8 mmol) in dry THF (50 mL). The reaction mixture was allowed to stir and after several minutes an exothermic reaction with vigorous gas evolution commenced. After the exothermic reaction had subsided, the mixture was heated under reflux for 1 h and then cooled to room temperature. Concentrated HCl (3 mL), diluted with H<sub>2</sub>O (5 mL), was added, and the THF was removed under reduced pressure. Additional concentrated HCl (5 mL) and H<sub>2</sub>O (10 mL) were added, and the mixture was heated under reflux overnight. The solution was made basic with concentrated aqueous NaOH (ice-bath cooling), and was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 50 mL). The pooled extracts were washed with H<sub>2</sub>O (2 × 50 mL), dried (MgSO<sub>4</sub>), and then evaporated under reduced pressure. The crude 6-methylmyosmine (1.67 g, 79%) was used without further purification. Sodium cyanoborohydride (0.6 g, 9.5 mmol) was added to a suspension of the above 6-methylmyosmine (1.67 g, 10.4 mmol) in H<sub>2</sub>O (75 mL). Concentrated HCl was added to the above solution in a drop-wise manner with stirring over 30 min, during which time the pH of the solution dropped to 1.7. The solution was allowed to stir for an additional 15 min, and then enough solid NaOAc was added to adjust the pH to 6. Formaldehyde (1 mL, 40%) was added, followed by NaBH<sub>3</sub>CN (0.6 g, 9.5 mmol). After being allowed to stir for 30 min, the solution was made basic with 2 M NaOH and was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 100 mL). The organic portion was extracted with 1 N H<sub>2</sub>SO<sub>4</sub> (30 mL), and the CH<sub>2</sub>Cl<sub>2</sub> portion was discarded. The acidic aqueous phase containing the product was made basic with NaOH and was extracted with CHCl<sub>3</sub> (3 × 50 mL). The combined organic portions were dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography using silica gel (eluted, EtOAc) to afford a colorless oil (1.1 g, 57%). An anhydrous Et<sub>2</sub>O solution of the free base was treated with a saturated solution of HCl gas in Et<sub>2</sub>O to give a white solid hydrochloride salt. Recrystallization from a mixture of 2-ProH/anhydrous Et<sub>2</sub>O (1:10) gave 0.2 g (14%) of **1c** as white crystals; mp 200 °C (dec). Anal (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>·2HCl·0.5H<sub>2</sub>O) C, H, N.

### 6-Methoxy-3-(1-methyl-2-pyrrolidinyl)pyridine dihydrochloride 1d

6-Methoxy-3-pyridine carboxaldehyde [52] (**34**; 4.4 g, 32.1 mmol) was added to a stirred solution of morpholine perchlorate (8.4 g, 45.3 mmol) in morpholine (40 mL) and the mixture was heated at 80 °C for 1 h. Potassium cyanide (2.9 g, 43.8 mmol) dissolved in a minimum amount of H<sub>2</sub>O (1–2 mL), was added and the mixture was heated at 100 °C for an additional

hour. The cooled reaction mixture was added to aqueous 10% K<sub>2</sub>CO<sub>3</sub> solution (100 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 60 mL). The combined extracts were washed with aqueous NaHSO<sub>3</sub> and then dried (MgSO<sub>4</sub>). Evaporation yielded a yellow oil. Crystallization from hexane gave 3.6 g (48%) of **35** as a solid material; mp 79–81 °C. Acrylonitrile (1.1 g, 21.0 mmol) in *tert*-butyl alcohol (60 mL) was added slowly (during 40 min) under an N<sub>2</sub> atmosphere and at room temperature to a stirred solution of the above  $\alpha$ -morpholino- $\alpha$ -[3-(6-methoxy)pyridyl] acetonitrile (**35**; 3.5 g, 15.0 mmol) in *tert*-butyl alcohol (175 mL) containing 20 drops of a methanolic solution of KOH (30%). After stirring for an additional 5 min the reaction mixture was diluted with an equal volume of H<sub>2</sub>O and extracted with CHCl<sub>3</sub> (4 × 100 mL). The combined organic portions were dried (MgSO<sub>4</sub>), filtered, and evaporated under reduced pressure. The remaining oil was purified by flash chromatography using silica gel (eluted, 6:4, EtOAc/petroleum ether) to give 3.2 g (75%) of  $\gamma$ -cyano- $\gamma$ -morpholino- $\gamma$ -3-[(6-methoxy)pyridyl]butyronitrile as white crystals; mp 103–105 °C. This solid (**36**; 3.2 g, 11.2 mmol), in a mixture of HOAc (18 mL), H<sub>2</sub>O (9 mL), and THF (3 mL), was heated at 53 °C for 18 h. The reaction mixture was made basic with solid K<sub>2</sub>CO<sub>3</sub> and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 50 mL). The combined organic portions were dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. The residue was crystallized from anhydrous Et<sub>2</sub>O, affording the  $\beta$ -ketonitrile **37** as colorless plates (1.6 g, 75%); mp 82–84 °C.

The  $\beta$ -ketonitrile **37** (1.5 g, 7.9 mmol) in 95% EtOH (125 mL), which had previously been saturated with ammonia, was hydrogenated at room temperature in the presence of Raney Nickel (one spoonful) at 40 psi for 24 h. The mixture was filtered, acidified with HCl and evaporated under reduced pressure. The residue was made basic with aqueous K<sub>2</sub>CO<sub>3</sub> (10%) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 50 mL). The combined organic portions were dried (MgSO<sub>4</sub>), filtered, and purified by flash chromatography using silica gel (eluted, 8:2, EtOAc/petroleum ether) to afford 1.0 g (73%) of 6-methoxymyosmine (**38** and 0.3 g (22%) of 6-methoxymyosmine. The 6-methoxymyosmine (0.5 g, 2.8 mmol) was dissolved in H<sub>2</sub>O (5 mL) and NaOAc was added to achieve pH 6. Formaldehyde (40%) (0.5 mL, 6.2 mmol) and NaBH<sub>3</sub>CN (0.2 g, 2.8 mmol) was added to the stirred solution and stirring was allowed to continue for an additional 15 min. The solution was made acidic with concentrated HCl (3 mL). The aqueous solution was washed with Et<sub>2</sub>O (3 × 30 mL) and then made basic with 50% NaOH. The basic solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 50 mL) and the combined organic portions were washed with brine (2 × 50 mL), dried over MgSO<sub>4</sub>, and evaporated under reduced pressure. The remaining oil was purified by flash chromatography using silica gel (eluted, 1:1, EtOAc/petroleum ether) to afford 0.8 g (82%) of the free base of **1d**. An anhydrous Et<sub>2</sub>O solution of the free base was treated with HCl gas to afford the crude salt, and recrystallization from 2-ProH gave colorless crystals; mp 135–138 °C. Anal (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O·2HCl·0.25H<sub>2</sub>O) C, H, N.

### 6-Fluoro-3-(1-methyl-2-pyrrolidinyl)pyridine maleate 1e

A cold solution (below 5 °C) of 6-aminonicotine [23] (1.5 g, 8.47 mmol) in 48% HBF<sub>4</sub> (10 mL) was diazotized by the addition of sodium nitrite (0.6 g, 8.50 mmol), the solid being added slowly in small portions with vigorous stirring. Since the diazonium salt decomposes almost immediately with liberation of heat, it was necessary to add the sodium nitrite slowly to keep the temperature below 10 °C. After an hour of standing in an ice-bath, the solution was warmed to 37 °C to complete the decomposition. The acidic solution was cooled to 5 °C and neutralized with sodium carbonate. The aqueous solution was



extracted with  $\text{CHCl}_3$  ( $4 \times 50$  mL). The combined organic portions were dried ( $\text{MgSO}_4$ ), filtered, and evaporated under reduced pressure. The remaining oil was purified by flash chromatography using silica gel (eluted, 6:4,  $\text{EtOAc}$ /petroleum ether). The free base was converted to the maleate salt in anhydrous  $\text{Et}_2\text{O}$  and recrystallized from 2-PrOH to give 0.5 g (17%) of **1e** as colorless crystals; mp 96–98 °C. Anal ( $\text{C}_{10}\text{H}_{13}\text{FN}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$ ) C, H, N.

**6-Bromo-3-(1-methyl-2-pyrrolidinyl)pyridine maleate 1f**

Bromine (1.5 mL) was added to a solution of 6-aminonicotine [23] (1.50 g, 8.50 mmol) in 48% hydrobromic acid (15 mL). A solution of sodium nitrite (1.46 g, 21.17 mmol) in  $\text{H}_2\text{O}$  (10 mL) was then gradually added, keeping the temperature below 5 °C until near the end of the reaction when it rose spontaneously to 12 °C. A solution of 33% KOH (60 mL) was added while maintaining the temperature below 20 °C. The reaction mixture was extracted with  $\text{CH}_2\text{Cl}_2$  ( $4 \times 50$  mL). The combined organic portions were dried ( $\text{MgSO}_4$ ), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (eluted, 4:6,  $\text{EtOAc}$ /petroleum ether) to give 0.45 g (22%) of a brown oil. The free base was converted to the maleate salt; mp 103–105 °C after recrystallization from 2-PrOH. Anal ( $\text{C}_{10}\text{H}_{13}\text{BrN}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$ ) C, H, N.

**3-(N-n-Propylaminomethyl)pyridine oxalate 8. Method A**

A solution of pyridine-3-carboxaldehyde (0.54 g, 5 mmol) and *n*-propylamine (0.35 g, 6 mmol) in MeOH (20 mL) was, after the addition of Pd on C (5%, 0.05 g), hydrogenated at 50 psi  $\text{H}_2$  in a Parr apparatus. After 2.5 h the reaction mixture was filtered, the residue was washed with MeOH (ca, 10 mL), and the filtrate was evaporated and dried under high vacuum. The remaining oil was redissolved in anhydrous  $\text{Et}_2\text{O}$  (ca, 25 mL) and the solution added to a solution of anhydrous oxalic acid (0.90 g, 10 mmol) in the same solvent (ca, 25 mL) under ultrasonic treatment to ensure complete mixing. The crude oxalate salt, obtained as a highly viscous mass, solidified to give a colorless solid. Recrystallization from  $\text{EtOH}/\text{H}_2\text{O}$  (19:1) gave 1.14 g (69%) of **8** as colorless needles; mp 202–204 °C. Anal ( $\text{C}_9\text{H}_{14}\text{N}_2 \cdot 2\text{C}_2\text{H}_2\text{O}_4$ ) C, H, N.

**6-Chloro-3-(N-ethyl-N-methyl)aminomethylpyridine oxalate 12b**

A mixture of 6-chloropyridine-3-carboxaldehyde (0.35 g, 2.5 mmol), *N*-ethyl-*N*-methylamine (0.18 g, 3 mmol),  $\text{NaBH}_3\text{CN}$  (0.10 g, 15.0 mmol), molecular sieves (4 Å, 1.20 g) and bromocresol green (ca, 2 mg) in absolute  $\text{EtOH}$  (10 mL) was allowed to stir for 15 min. A solution of 5% HCl in MeOH was then added until the initial blue color turned to green. If the color turned black to blue, more HCl in MeOH was added in a dropwise manner until the green color persisted. The solution was allowed to stir for an additional 16 h and concentrated HCl (0.5 mL) was added (caution: HCN evolution!) until pH 2 was reached. The mixture was allowed to stir 1 h and MeOH was removed under reduced pressure, then 40% NaOH was added until pH >10. The aqueous solution was extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 25$  mL). The combined organic portions were dried ( $\text{MgSO}_4$ ), filtered, and evaporated under reduced pressure to give the crude amine. An anhydrous  $\text{Et}_2\text{O}$  solution of the amine was treated with an  $\text{Et}_2\text{O}$  solution of oxalic acid to afford the crude salt; recrystallization from absolute  $\text{EtOH}$  gave 0.08 g (12%) of **12b** as colorless crystals; see table II.

**6-Methyl-3-(N-ethyl-N-methylaminomethyl)pyridine oxalate 12c**

A solution of 6-methylnicotinic acid (1.37 g, 10.0 mmol) and  $\text{Et}_3\text{N}$  (1.5 mL, 11.0 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (10 mL) was added dropwise to a solution of ethyl chloroformate (1.25 g, 11.5 mmol)

in dry  $\text{CH}_2\text{Cl}_2$  (10 mL) at –30 °C under an  $\text{N}_2$  atmosphere for 10 min. The mixture was allowed to stir at 0 °C for 30 min and cooled to –20 °C before adding *N*-ethyl-*N*-methylamine (1.1 mL, 12.0 mmol) in toluene (10 mL). The mixture was heated at reflux for 30 min. A solution of 25% NaOH (5 mL) was carefully added at 0 °C, and the basic solution was extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 10$  mL). The organic portions were dried ( $\text{MgSO}_4$ ), filtered, and evaporated under reduced pressure to give an oily residue. This oil was purified by Kugelrohr distillation to give 1.10 g (62%) of the intermediate amide. A solution of the amide (1.10 g, 6.2 mmol) was added in a dropwise manner to a stirred suspension of  $\text{LiAlH}_4$  (0.5 g, 13.0 mmol) in anhydrous  $\text{Et}_2\text{O}$  (20 mL) at 0 °C under an  $\text{N}_2$  atmosphere. The reaction mixture was heated under reflux for 16 h, and excess  $\text{LiAlH}_4$  was decomposed at 0 °C by the dropwise addition of  $\text{H}_2\text{O}$  (1.5 mL), 15% NaOH (0.5 mL), and  $\text{H}_2\text{O}$  (1.5 mL). The insoluble salts were removed by filtration and washed with  $\text{Et}_2\text{O}$  ( $3 \times 10$  mL). The organic portions were combined, and solvent was removed under reduced pressure. The oxalate salt was prepared to give 1.28 g (60%) of colorless crystals after recrystallization from 2-PrOH; see table II.

**6-Methoxy-3-(N-ethyl-N-methylaminomethyl)pyridine oxalate 12d**

A solution of 6-methoxypyridine-3-carboxaldehyde [52] (0.69 g, 5.0 mmol) and *N*-ethyl-*N*-methylamine (0.39 g, 6.6 mmol) in absolute  $\text{EtOH}$  (15 mL) was allowed to stir for 1 h at 20 °C in the presence of 4 Å molecular sieve (5 g). Then 5% Pd/C (0.10 g) was added and this final mixture was hydrogenated on a Parr hydrogenator at 50 psi for 5 h and filtered over Celite. The Celite pad was rinsed with MeOH ( $2 \times 20$  mL) and the filtrate and combined washings were evaporated under reduced pressure. An anhydrous  $\text{Et}_2\text{O}$  (25 mL) solution of the remaining oil was filtered and treated with a solution of oxalic acid in  $\text{Et}_2\text{O}$  (0.90 g in 5 mL) to afford the crude salt, and recrystallization from absolute  $\text{EtOH}$  (10 mL) gave 0.24 g (22%) of **12d** as colorless crystals; see table II.

**6-Fluoro-3-(N-ethyl-N-methyl)aminomethylpyridine oxalate 12e**

A solution of 6-fluoronicotinic acid (1.44 g, 10 mmol), DMF (two drops) and  $\text{SOCl}_2$  (12.5 mL) was heated under reflux under dry conditions ( $\text{CaCl}_2$  tube) for 4 h. Excess  $\text{SOCl}_2$  was removed under reduced pressure and the crude 6-fluoronicotinyl chloride (1.32 g) was taken up in dry  $\text{CH}_2\text{Cl}_2$  (2 mL). A solution of *N*-ethyl-*N*-methylamine (2.5 mL) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was added in a dropwise manner at –20 °C to a stirred solution of the nicotinyl chloride and the mixture was allowed to stir for 5 min. The reaction mixture was treated with  $\text{H}_2\text{O}$  (5 mL), and the layers were separated; the organic portion was dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure to give the amide as a yellow oil. Kugelrohr distillation gave 1.61 g (88%) of the amide intermediate as a clear oil.  $\text{AlH}_3$  was prepared by the careful addition of  $\text{AlCl}_3$  (1.15 g, 9 mmol) to a suspension of  $\text{LiAlH}_4$  (1.10 g, 29 mmol) in anhydrous  $\text{Et}_2\text{O}$  (75 mL) at 0 °C under an  $\text{N}_2$  atmosphere. The suspension was allowed to stir for 30 min at 20 °C, then a solution of the above amide (0.91 g, 5 mmol) in anhydrous  $\text{Et}_2\text{O}$  (50 mL) was added in a dropwise manner. The reaction mixture was allowed to stir for 5 h at room temperature. Excess  $\text{AlH}_3$  was decomposed by the addition of crushed ice (5 g) and 20% NaOH solution (10 mL) at 0 °C. The mixture was filtered and the organic portion was washed with  $\text{H}_2\text{O}$  (10 mL) and brine (10 mL), and dried ( $\text{Na}_2\text{SO}_4$ ). An anhydrous  $\text{Et}_2\text{O}$  solution of the amine was treated with an  $\text{Et}_2\text{O}$  solution of oxalic acid to afford the crude salt; recrystallization from absolute  $\text{EtOH}$  gave 0.33 g (25%) of **12e** as colorless crystals; see table II.



*3-(N-Methyl-N-n-propylaminomethyl)pyridine oxalate 13. Method B*

A solution of HCl (5%) in MeOH was added to a mixture of pyridine-3-carboxaldehyde (2.7 g, 25 mmol), *N*-methyl-*N*-n-propylamine (2.2 g, 20 mmol), NaBH<sub>3</sub>CN (2.0 g, 30 mmol), and bromocresol green (ca. 5 mg) in MeOH (10 mL) until the initial blue color turned to green. The pH of the reaction mixture was kept at ca 6–7 by the dropwise addition of additional HCl in MeOH until no further color change to blue was observed. After stirring at 20 °C at a final green color for 16 h, concentrated HCl (2 mL) was added (caution: HCN evolution!) and a pH of 2 was reached. After allowing the mixture to stand for 1 h, MeOH was evaporated under reduced pressure, the acidic solution was washed once with Et<sub>2</sub>O (5 mL), basified (KOH), and extracted with CHCl<sub>3</sub> (3 × 15 mL). The dried (Na<sub>2</sub>SO<sub>4</sub>) extracts were evaporated under reduced pressure; the remaining oil was distilled (Kugelrohr) and converted to the oxalate salt as in *Method A*. Recrystallization from absolute EtOH gave **13** (4.6 g, 72%); mp 141–142 °C. Anal (C<sub>10</sub>H<sub>16</sub>N<sub>2</sub>·C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

*3-(N-n-Butyl-N-methylaminomethyl)pyridine oxalate 14. Method C*

Nicotinoyl chloride hydrochloride (0.89 g, 5 mmol) was added slowly with vigorous stirring to a mixture of *N*-methyl-*N*-n-butylamine (0.43 g, 5 mmol) and Et<sub>3</sub>N (1.00 g, 10 mmol) in CHCl<sub>3</sub> (5 mL) in an ice-water bath. Stirring was allowed to continue overnight at room temperature. After the addition of CHCl<sub>3</sub> (10 mL), the reaction mixture was washed with H<sub>2</sub>O (3 × 10 mL). The organic portion was dried (MgSO<sub>4</sub>), and the solvent was evaporated in vacuo to give 0.76 g of crude product. The yellow oil was distilled (Kugelrohr) to give 0.71 g (74%) of *N*-n-butyl-*N*-methylnicotinamide as a colorless oil, which was used in the next step without further characterization. A 1 M solution of BH<sub>3</sub> in THF (10 mL, 10 mmol) was added dropwise to a solution of the carboxamide (0.19 g, 1 mmol) in dry THF (2 mL) at 0 °C under N<sub>2</sub>. The reaction mixture was heated at reflux for 3 h, cooled to room temperature, and 6 M HCl (10 mL) was added slowly. The THF was removed by distillation at atmospheric pressure. Sodium hydroxide pellets were added to saturate the aqueous phase and the mixture was extracted with Et<sub>2</sub>O (3 × 10 mL). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated to dryness, and the residue was distilled (Kugelrohr) to give **14** (0.16 g, 92%) as the free base. A portion of the amine was converted to the oxalate salt; mp 143–146 °C (absolute EtOH/anhydrous Et<sub>2</sub>O). Anal (C<sub>11</sub>H<sub>18</sub>N<sub>2</sub>·2C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>·0.25H<sub>2</sub>O) C, H, N.

*3-(N-Methyl-N-n-pentylaminomethyl)pyridine oxalate 15. Method D*

*p*-Toluenesulfonyl chloride (2.87 g, 15.0 mmol) was added to a solution of 1-pentanol (0.88 g, 10.0 mmol) in dry pyridine (20 mL) and the mixture was allowed to stand at 5 °C for 2 days. Water (400 mL) was added and the reaction mixture was extracted with Et<sub>2</sub>O (3 × 50 mL). The combined Et<sub>2</sub>O extract was washed with 1 N HCl (30 mL), H<sub>2</sub>O (50 mL), and dried (MgSO<sub>4</sub>). The solvent was removed under reduced pressure to give 0.49 g (24%) of *O*-pentyl *p*-toluenesulfonate as a colorless oil which was used in the next step without further characterization. A mixture of **6** (0.16 g, 1.3 mmol), the above sulfonate (0.49 g, 2.0 mmol), and K<sub>2</sub>CO<sub>3</sub> (0.18 g, 1.3 mmol) in dioxane (10 mL) was heated at reflux for 18 h, cooled to room temperature, and solvent was removed under reduced pressure. The residue was dissolved in H<sub>2</sub>O (5 mL), extracted with Et<sub>2</sub>O (3 × 10 mL), and the combined Et<sub>2</sub>O portions were dried (MgSO<sub>4</sub>). The solvent was removed under reduced pressure to

give an oily residue. The free base of **15** (0.22 g, 87%) was isolated by flash chromatography using silica gel (CHCl<sub>3</sub>/MeOH 9:1) and converted to the oxalate salt; mp 128–130 °C. Anal (C<sub>12</sub>H<sub>20</sub>N<sub>2</sub>·2C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

*3-(N-Cyclopropyl-N-methylaminomethyl)pyridine oxalate 17. Method E*

A mixture of **9** (1.10 g, 3.35 mmol), HCHO (40% v/v, 0.11 g, 3.69 mmol), and HCOOH (95%, 1.7 mL) was heated under reflux for 8 h and kept at room temperature for 16 h. After basification of the solution with NaOH (10 M) to ca pH 12, the liberated amine was extracted into Et<sub>2</sub>O (2 × 10 mL). The combined extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and added to a solution of anhydrous oxalic acid (6.70 mmol) in anhydrous Et<sub>2</sub>O (ca 30 mL). Recrystallization of the salt gave 0.24 g (20%) of **17** as colorless crystals; mp 161–162 °C. Anal (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>·2.5C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

*3-(N-Methyl-N-n-propylaminomethyl)-2-methylpyridine oxalate 25. Method F*

Triethylamine (2 mL, 14.5 mmol) was added at 0 °C with stirring to a suspension of 2-methylnicotinic acid (1.37 g, 10.0 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The clear solution was added to a stirred mixture of ethyl chloroformate (1.1 mL, 11.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at –30 °C. After 30 min at 0 °C, the mixture was cooled to –20 °C and a solution of *N*-methyl-*N*-n-propylamine (1 mL, 10.0 mmol) in dry toluene (10 mL) was added. After warming to 35 °C for 30 min the mixture was cooled in an ice-bath, NaOH (25%, 5 mL) was added, the organic layer was separated, dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated to an oil, and the oil was distilled (bp 120 °C, 0.2 mmHg) to give 0.48 g (25%) of *N*,2-dimethyl-*N*-n-propylnicotinamide as a colorless oil. <sup>1</sup>H NMR indicated a mixture of *syn* and *anti* isomers. The amide (2.0 mmol) in anhydrous Et<sub>2</sub>O was added to a suspension of LiAlH<sub>4</sub> (0.19 g, 5.0 mmol) in anhydrous Et<sub>2</sub>O (20 mL); after stirring the suspension for 1 day at room temperature, H<sub>2</sub>O (1 mL) was added to the reaction mixture under ice cooling. The mixture was filtered, the residue was washed with MeOH, and the oily residue obtained after evaporation was converted to the oxalate salt (0.60 g, 67%) which was isolated as an oily substance; see table II.

Compounds **23**, **24** and **26** were prepared using *Method F* and either 2-methylnicotinic acid or 4-methylnicotinic acid [25, 53], and either *N*-methyl-*N*-ethylamine or *N*-methyl-*N*-n-propylamine.

*5-(N-Ethyl)amino-5,6,7,8-tetrahydroquinoline maleate 28a*

A solution of 5,6,7,8-tetrahydroquinolin-5-one [55] (3.50 g, 23.8 mmol), NH<sub>4</sub>OAc (18.35 g, 238.0 mmol) and NaBH<sub>3</sub>CN (1.04 g, 16.6 mmol) in MeOH (75 mL) was allowed to stir for 48 h at room temperature. Concentrated HCl was added until pH < 2, and the MeOH was removed in vacuo. The residue was dissolved in H<sub>2</sub>O (50 mL) and washed with Et<sub>2</sub>O (3 × 20 mL). The aqueous solution was adjusted to pH > 10 with solid KOH, saturated with NaCl, and extracted with Et<sub>2</sub>O (5 × 20 mL). The combined extracts were dried (MgSO<sub>4</sub>) and evaporated in vacuo. The remaining oil was purified by column chromatography using silica gel (eluted, EtOAc/MeOH, 1:1) to afford 1.22 g (35%) of the intermediate quinolinamine, which was used without further characterization.

Acetyl chloride (1.26 g, 20.3 mmol) in dry THF (10 mL) was added in a dropwise manner to a stirred solution of the amine (1.20 g, 8.1 mmol) and Et<sub>3</sub>N (1.64 g, 16.2 mmol) in THF (10 mL) at 0 °C under an N<sub>2</sub> atmosphere. The solution was allowed to stir at 0 °C over 1 h and then for 0.5 h at room temperature. The mixture was filtered and the solid material was



washed with THF (30 mL). The combined filtrate and washings were evaporated under reduced pressure, and the oily residue was dissolved in Et<sub>2</sub>O (60 mL) and was successively washed with saturated NaHCO<sub>3</sub> solution and brine, and dried (MgSO<sub>4</sub>). Solvent was removed under reduced pressure to afford 0.76 g (47%) of 5-(acetamido)-5,6,7,8-tetrahydroquinoline. A solution of the acetamide (0.76 g, 3.8 mmol) in toluene (8 mL) was added carefully to a stirred suspension of LiAlH<sub>4</sub> (0.29 g, 76.0 mmol) in anhydrous Et<sub>2</sub>O (30 mL). The mixture was allowed to stir under reflux for 18 h, cooled, and excess hydride decomposed by the successive addition of wet Et<sub>2</sub>O and then H<sub>2</sub>O (10 mL). The suspension was filtered; the aqueous portion was extracted with Et<sub>2</sub>O (2 × 20 mL). The combined organic portions were washed with H<sub>2</sub>O, dried (MgSO<sub>4</sub>) and purified by column chromatography using silica gel (eluted CHCl<sub>3</sub>/MeOH, 9:1). A solution of the free base in anhydrous Et<sub>2</sub>O was treated with a solution of maleic acid in Et<sub>2</sub>O to give colorless crystals. Recrystallization from 2-PrOH gave 0.28 g (18%) of the title compound; mp 132–134 °C. Anal (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>·2C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) C, H, N.

**5-(*N*-Ethyl-*N*-methyl)amino-5,6,7,8-tetrahydro-5-quinoline hydrochloride **29b****

Solid NaBH<sub>3</sub>CN (0.22 g, 3.45 mmol) was added to a stirred, cooled (0 °C) solution of the free base of **29a** (0.38 g, 2.16 mmol) and 40% aqueous HCOH (1.9 mL, 19.46 mmol) in CH<sub>3</sub>CN (10 mL). AcOH (100 µl) was added after 20 min, and the reaction mixture was stirred for 6 h. Excess HCl in MeOH (2.0 M) was added and the resulting solution was concentrated to a slurry. The crude product was dissolved in H<sub>2</sub>O (20 mL); the solution was made basic with 6.0 M KOH (pH > 9) and washed with Et<sub>2</sub>O (5 × 25 mL) and CH<sub>2</sub>Cl<sub>2</sub> (2 × 20 mL). The combined extracts were dried (MgSO<sub>4</sub>) and the filtrate was concentrated in vacuo to an oil. Column chromatography using silica gel (eluted, EtOAc/MeOH, 80:20) gave the title compound (0.14 g, 34%) as the free base. A solution of the free base in anhydrous Et<sub>2</sub>O was treated with saturated HCl gas in Et<sub>2</sub>O to give a white solid hydrochloride salt. Recrystallization from a mixture 2-PrOH/acetone (1:1) gave 0.05 g (24%) of **29b**; mp 124–125 °C. Anal (C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>·2HCl·0.75H<sub>2</sub>O) C, H, N.

**2-Methyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine oxalate **29****

The free base, prepared according to the procedure of Arma-rego et al [19], was purified by distillation (Kugelrohr, bp 40 °C/0.05 mmHg), isolated as a pale-yellow oil in 67% yield, and converted to the oxalate salt; mp 175–176 °C. Anal (C<sub>8</sub>H<sub>10</sub>N<sub>2</sub>·2C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

**1,2,3,4-Tetrahydro[2,7]naphthyridine oxalate **30a****

Pd (5% on CaCO<sub>3</sub>) (0.20 g) was added to a solution of [2,7]naphthyridine [20, 21] (2.27 g, 17.4 mmol) in MeOH (25 mL) and hydrogenation in a Parr apparatus was performed at 30 psi for 2 h. The catalyst was removed by filtration and the solvent was removed under reduced pressure; the remaining green oil in anhydrous Et<sub>2</sub>O (ca 20 mL) was added to a solution of oxalic acid (2 equiv) in Et<sub>2</sub>O (25 mL) to precipitate the crude oxalate salt. Recrystallization from 95% EtOH afforded **30a** as red crystals (2.91 g, 53%); mp 190 °C. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 8.65 (s, 1H, C8-H), 8.56 (d, 1H, C6-H), 7.87 (d, 1H, C5-H), 4.55 (s, 2H, CH<sub>2</sub>), 3.59 (t, 2H, CH<sub>2</sub>), 3.36 (t, 2H, CH<sub>2</sub>). Anal (C<sub>8</sub>H<sub>10</sub>N<sub>2</sub>·2C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

**2-Methyl-1,2,3,4-tetrahydro[2,7]naphthyridine oxalate **30b****

Compound **30b** was obtained from **30a** in 82% yield, after recrystallization from absolute EtOH, following *Method E*; mp 184–186 °C. Anal (C<sub>9</sub>H<sub>12</sub>N<sub>2</sub>·1.5C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

**2-Ethyl-1,2,3,4-tetrahydro[2,7]naphthyridine oxalate **30c****

A mixture of **30a** (from 315 mg dioxalate, 1.0 mmol + KOH in MeOH), ethyl *p*-tolylsulfonate (240 mg, 1.2 mmol), and NaHCO<sub>3</sub> (200 mg) in CHCl<sub>3</sub> (10 mL) was heated at reflux for 16 h. The cooled reaction mixture was washed with H<sub>2</sub>O (5 mL), the aqueous portion was reextracted with CHCl<sub>3</sub> (10 mL), the combined CHCl<sub>3</sub> solutions were dried (MgSO<sub>4</sub>), evaporated and the crude product was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 19:1) on silica gel. The oxalate salt was prepared and recrystallized from 95% EtOH to give 160 mg (62%) of **30c**; mp 152–154 °C. Anal (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>·1.5C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

**2-Propyl-1,2,3,4-tetrahydro[2,7]naphthyridine oxalate **30d****

A solution of NaOH (1 M in MeOH, 4 mL) was added to a suspension of **30a** (270 mg, 0.7 mmol) in MeOH (20 mL). After filtration, the clear solution was mixed with propionaldehyde (1 M in MeOH, 1.2 mL), and 5% Pd on carbon (50 mg), and hydrogenated at 50 psi in a Parr apparatus for 2.5 h. Filtration and evaporation gave an oily residue which was dissolved in anhydrous Et<sub>2</sub>O (ca, 5 mL) and added dropwise to a solution of oxalic acid (135 mg) in anhydrous Et<sub>2</sub>O (ca, 5 mL). The precipitate was washed with Et<sub>2</sub>O and recrystallized from absolute EtOH to yield 240 mg (84%) of **30d**; mp 194 °C (dec). Anal (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>·1.75C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

**2-Isopropyl-1,2,3,4-tetrahydro[2,7]naphthyridine oxalate **30e****

Compound **30e** was prepared from **30a** and acetone following the method described for **30d** to give a 21% yield of the desired product after recrystallization from MeOH/anhydrous Et<sub>2</sub>O; mp 118–121 °C. Anal (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>·2.75C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

**5,6,7,9-Tetrahydro-8*H*-pyrido[3,4-*c*]azepine oxalate **31a****

A solution of **33** (250 mg, 1.5 mmol) in dry THF (10 mL) was added in a dropwise manner to a stirred suspension of LiAlH<sub>4</sub> (250 mg, 6.6 mmol) in THF (10 mL) under an N<sub>2</sub> atmosphere at 0 °C. After 2 h at this temperature, the ice-bath was removed and the suspension was heated at room temperature for 24 h. At ice-bath temperature, H<sub>2</sub>O (0.25 mL), NaOH (10%, 0.4 mL), and H<sub>2</sub>O (0.5 mL) were added in succession. The suspension was filtered and the filtrate was concentrated by evaporation under reduced pressure and subjected to column chromatography (silica gel, CHCl<sub>3</sub>/MeOH 8:2). A dihydrochloride salt was prepared to afford 243 mg of **31a**·2HCl in an overall 73% yield; mp 249–252 °C. Due to the apparent unstable nature of the HCl salt, the product was converted back to the free base and isolated as its oxalate salt; mp 202–205 °C. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 8.71 (s, 1H, C1-H), 8.65 (d, 1H, C3-H), 7.92 (d, 1H, C4-H), 4.58 (s, 2H, ArCH<sub>2</sub>N), 3.56 (t, 2H, CH<sub>2</sub>), 3.30 (t, 2H, CH<sub>2</sub>), 2.04 (m, 2H, CH<sub>2</sub>). Anal (C<sub>9</sub>H<sub>12</sub>N<sub>2</sub>·2C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

**8-Methyl-5,6,7,9-tetrahydro-8*H*-pyrido[3,4-*c*]azepine oxalate **31b****

Formaldehyde (37%, 0.09 mL) was added to a solution of **31a** (220 mg, 1.0 mmol) in HCOOH (88%, 20 mL) and the reaction mixture was heated at reflux for 16 h. Evaporation under reduced pressure gave an oily residue which was treated with KOH (2 M in MeOH) to adjust the pH to 12. The mixture was filtered and the filtrate was evaporated to an oil. The crude oxalate salt was prepared and recrystallized from 95% EtOH to give 50 mg (17%) of **31b**; mp 168–170 °C. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 8.67 (s, 1H, C1-H), 8.63 (d, 1H, C3-H), 7.82 (d, 1H, C4-H), 4.66 (s, 2H, Ar-CH<sub>2</sub>N), 3.63 (m, 2H, CH<sub>2</sub>), 3.24 (t, 2H, CH<sub>2</sub>), 2.91 (s, 3H, -CH<sub>3</sub>), 2.08 (m, 2H, CH<sub>2</sub>). Anal (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>·1.5C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.



### 6-Methyl-5,6,7,8-tetrahydro[1,6]naphthyridine oxalate **32**

Compound **32** was prepared by the general procedure of Shiozawa et al [54]; the free base, but not a salt, has been previously reported [56]. A mixture of  $\beta$ -aminoacrolein [57] (0.20 g, 3.5 mmol), *N*-methylpiperidone (0.26 g, 2.3 mmol),  $\text{Et}_3\text{N}$  (0.15 mL) and  $\text{NH}_4\text{OAc}$  (0.01 g) was heated at 120 °C for 2 h under an  $\text{N}_2$  atmosphere. The cooled reaction mixture was dissolved in 3 N HCl and washed with  $\text{CHCl}_3$  ( $3 \times 10$  mL). The aqueous portion was made alkaline with saturated  $\text{Na}_2\text{CO}_3$  solution and extracted with  $\text{CHCl}_3$  ( $3 \times 20$  mL). The combined organic portions were washed with brine, dried ( $\text{MgSO}_4$ ), and evaporated under reduced pressure. The oily residue was purified by flash chromatography using silica gel (eluted  $\text{EtOAc}/\text{MeOH}$  8:2) to afford 0.15 g of the free base. The oxalate salt was prepared and recrystallized from 2-PrOH to give 0.19 g (35%) of **32**; mp 172–174 °C. Anal ( $\text{C}_9\text{H}_{12}\text{N}_2 \cdot 1.5\text{C}_2\text{H}_2\text{O}_4$ ) C, H, N.

### 4,5,6,7-Tetrahydro-9H-pyrido[3,4-c]azepin-9-one **33**

Solid  $\text{NaN}_3$  (166 mg, 2.56 mmol) was added in small portions over a 1 h period to a solution of 5,6,7,8-tetrahydroisquinolin-8-one [58] (392 mg, 2.14 mmol) in  $\text{H}_2\text{SO}_4$  (96%, 2.1 mL) at 0–5 °C. Stirring was allowed to continue at room temperature for 4 h, and then the mixture was poured onto crushed ice (10 g) and neutralized with 5 M NaOH solution. The solution was extracted with  $\text{CHCl}_3$  ( $3 \times 10$  mL), solvent was evaporated and the crude product was subjected to column chromatography ( $\text{SiO}_2/\text{EtOAc}$ ) to obtain 230 mg (66%) of product as an oil.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  8.85 (s, 1H, C1-H), 8.65 (d, 1H, C3-H), 7.14 (d, 1H, C4-H), 6.67 (br s, 1H, NH), 3.13 (q, 2H, N- $\text{CH}_2$ ), 2.85 (t, 2H, Ar- $\text{CH}_2$ ), 2.05 (m, 2H,  $\text{CH}_2$ ). Anal ( $\text{C}_9\text{H}_{10}\text{N}_2\text{O} \cdot \text{H}_2\text{O}$ ) C, H, N.

### Radioligand receptor binding

The assay was conducted as previously described [59]. Rat brain without the cerebellum was homogenized in 10 volumes of ice-cold 0.05 M Na–K phosphate buffer (pH 7.4) and centrifuged at 17 500 g (4 °C) for 30 min. The pellet was resuspended in 20 volumes of ice-cold glass-distilled  $\text{H}_2\text{O}$  and allowed to incubate on ice for 60 min before centrifugation as described above. The resulting pellet was then resuspended to a final tissue concentration of 40 mg/mL of buffer. [ $^3\text{H}$ ]Nicotine was incubated with 0.5 mL of tissue homogenate in a final incubation volume of 1 mL for 2 h at 4 °C. The samples were rapidly filtered through Whatman GF/C filters. Specific binding was defined as the difference in the amount of binding in the presence and absence of 100  $\mu\text{M}$  (–)nicotine tartrate. Following three consecutive washes with ice-cold buffer, the filters were allowed to air-dry and were placed in scintillation vials for quantification of radioactivity. Following transformation of the data by the Scatchard method, the  $K_D$  and  $B_{\text{max}}$  values were determined using the program LIGAND [60]. Displacement of [ $^3\text{H}$ ]nicotine binding at 1 nM was determined in the presence of increasing concentrations of the various ligands and converted to percent displacement of specific binding. The  $\text{IC}_{50}$  values were determined from a plot of the log concentration versus percent displacement and converted to  $K_i$  values by the method of Cheng and Prusoff [61].

### Behavioral assays

Male ICR mice (20–25 g) were used for all the assays, and assays were performed as previously described [43]. Animals were housed in groups of six and had free access to food and water. Locomotor activity in mice was measured by placing the animals into individual Omnitech photocell activity cages 5

min after a subcutaneous injection of either 0.9% saline or of drug. Activity was measured for 10 min and data were expressed as percentage of depression, where % depression =  $[1 - (\text{activity level of drug-treated animals}/\text{activity of vehicle-treated animals}) \times 100]$ . Antinociception in mice was measured by the method of D'Amour and Smith as modified by Dewey et al [62]. A control response (2–4 s) was determined for each animal before treatment, and test latencies were assessed 5 min after subcutaneous drug administration. The cut-off time was 10 s. Antinociceptive response was calculated as % MPE (maximal possible effect), where % MPE =  $[(\text{test latency} - \text{control latency})/(\text{10} - \text{control latency})] \times 100$ .

### Drug discrimination studies

The drug discrimination studies were performed as previously reported in detail [48]. Male Sprague–Dawley rats (175–225 g) obtained from Harlan Laboratories (Indianapolis, IN) were used in these studies. Rats were trained on a two-lever operant drug discrimination paradigm using a VI-15s schedule of reinforcement. Eight operant chambers were used (four Lafayette model 80001 and four BRS/LVE model 002). Reinforcement was a Bioserv 45 mg precision dustless food pellet. Rats were trained to respond on one lever after administration of an sc injection of nicotine (0.4 mg/kg) and on another lever after sc administration of 0.9% saline. The animals were placed in the operant chambers 5 min after injection and allowed 15 min to respond. Stimulus generalization was said to have occurred when the animals made  $\geq 80\%$  of their total responses on the drug-designated lever. Where stimulus generalization occurred,  $\text{ED}_{50}$  values (representing the calculated dose at which the animals would make 50% of their responses on the drug-designated lever) were calculated.

### Statistical analysis

Data were analyzed statistically by an analysis of variance followed by the Fisher PLSM multiple comparison test. The null hypothesis was rejected at the 0.05 level.  $\text{ED}_{50}$  (effective dose 50%) values with 95% confidence limits were determined by the method of Litchfield and Wilcoxon.

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