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# Discovery of new small molecules that influence neuroblast cell migration from the subventricular zone

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**Abstract**—Using SVZ (subventricular zone) tissue explants from one-day-old mice, we investigated the activity of new amino aromatic disulfide analogues and polyazamacrocycles on the migration of SVZ cells (neuroblasts). We found that among the tested analogues, non-peptidic disulfide derivative **8** significantly decreases the migration of neuroblasts from SVZ cells, and antagonized the stimulating activity of disulfide cyclic peptide **1**. Discovery of compounds **1** and **8** constitutes new chemical tools which could be used to understand the mechanism of neuroblast migration during neurogenesis and eventually to identify specific genes involved in the neurogenesis.
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New neurons are constantly added to the olfactory bulb of rodents from birth to adulthood.[1] This accretion is not only dependent on sustained neurogenesis, but also on the migration of neuroblasts and immature neurons from the cortical and striatal subventricular zone (SVZ) to the olfactory bulb. Migration along this long tangential pathway, known as the rostral migratory stream, is a quite fast process, spans long distance, does not require radial glial guidance and is not limited to postmitotic neurons.[2] In recent years only very few small molecules have been described to directly affect the migration of those neuroblasts.

Two aspects for neuroblast migration could be envisaged: on the one hand discovery of new small molecules that could enhance or stimulate migration and on the other hand new small molecules that could inhibit migration. Interest in searching for both types of drugs that could influence neuroblast migration is double. Drugs which could enhance or stimulate migration present therapeutic potential in neurodegenerative disorders such as ischaemia or stroke as well as in case of brain injury, whereas drugs which could inhibit migration could find a therapeutical use in case of disregulated neurogenesis. Both types of drugs also could represent useful research tools for the understanding of how newly generated neurons must leave the germinal layers and move, sometimes a great distance, to reach their final destination.[3]

To our knowledge, only the dodecapeptide **1** known as PR21 (Fig. 1a) containing a disulfide bond has been reported as a small molecule which stimulates neuroblast migration in SVZ explants of new born rodents.[4]

Among the drugs which have been reported as neuroblast migration inhibitors, compound **2** known as LY 294002 (Fig. 1b), a metalloprotease inhibitor[5] known as PI3 kinase pathway inhibitor, has been described as neuroblast migration inhibitor after treatment of rodent adult SVZ explants.

Compound GM6001 (Fig. 1c), a broad spectrum matrix metalloproteinase inhibitor, was shown to act as a neuroblast migration inhibitor from SVZ to striatum after stroke.[6]

In this paper, we report our efforts for the discovery of new agents which can act as stimulators or inhibitors

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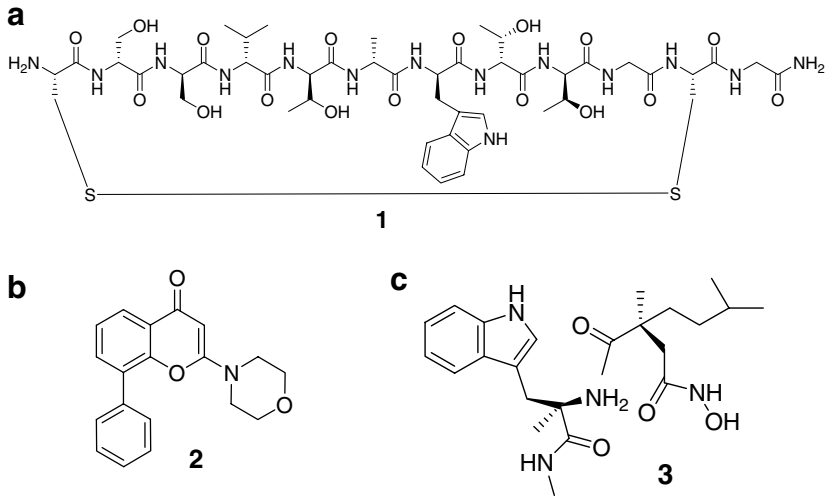



**Figure 1.** (a) PR21, dodecapeptide neuroblast migration stimulator. (b) LY 294002. (c) GM6001.

of neuroblast migration from the SVZ, using a specific adapted explant technique. The rationale we followed in order to identify such small molecules that could influence neuroblast migration of the SVZ from post natal rodents was the following:

Starting from analogue **1**, whose cyclic peptidic structure includes a disulfide bridge, we have synthesized two series of compounds:

— The first series is based on linear non-peptidic analogues which include in their structure a disulfide bridge linked to various N-substituted amide or amine groups in order to mimic **1** structure.
— The second series is composed of cyclic polyamine analogues, known as CXCR4 chemokine receptor antagonists, which have been included in this study since they are known to be involved in development and regeneration of the nervous system.[7,8]

In order to evaluate neuroblast migratory activity of both series of compounds we have developed a screening methodology described under reference.[9]

The synthesis of the first series of symmetric analogues (compounds **4** and **5**), which contain in their structure a disulfide bridge moiety linked to various substituted benzyl amino side arms, has been achieved according to the following procedure: analogue **4** was obtained by *N*-acylation of cystamine by benzoyl chloride in $CH_2Cl_2$ in the presence of $Et_3N$. Compound **5** was obtained by reducing the amide **4** using $BH_3$/THF reagent. Analogues **6** and **7** in which the disulfide bridge has been replaced by an ethylene moiety have been obtained by direct condensation of benzoyl chloride on 1,6-diaminohexane in $CH_2Cl_2$ in the presence of $Et_3N$. Compound **6**, after reduction using $BH_3$/THF, led to the corresponding diamino derivative **7** (these latter compounds were synthesized in order to compare their SVZ cell migratory activity versus their disulfide analogues). Compounds **8** and **9** were synthesized following the procedures described in references.[10,11]

Polyazamacrocycle analogues **10**–**15** were synthesized according to already published procedures.[12,13]

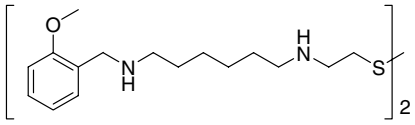
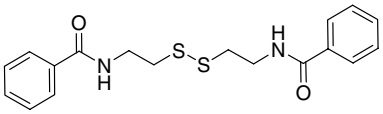
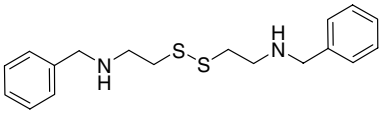
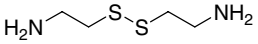
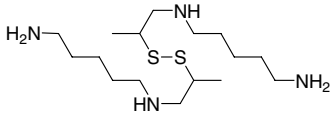
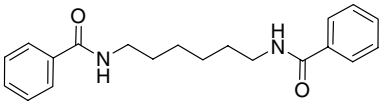
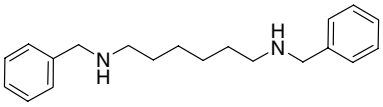
Cystamine **16**, cyclam **17** and cyclen **18** are commercially available compounds. Chemical structures are depicted in Tables 1 and 2. $^1$H NMR and MS data for these new analogues are given under references.[14]

As shown in Tables 1 and 2, disulfide analogues (compounds **4**, **5**, **8** and **16**) and ethylenyl analogues (compounds **6** and **7**) exhibit a neuroblast migration inhibitory effect on SVZ cells at concentration ranging from 30 to 200 μM. Among the polyazamacrocycles series only **10** displays a weak effect at 63 μM, while cyclam **17** and cyclen **18**, show a weakly effect around 200 μM. As it could be seen in Figure 2, disulfide analogue **8**, whose structure includes four amine functions and aromatic rings, appears to be the most active inhibitor (45% inhibition at 34 μM) since this compound elicits the best neuroblast migration inhibition at the lowest concentration, compared to untreated explants.

Disulfide analogue **9**, bearing four amino groups but no benzylic moiety, is totally inactive at 100 μM, indicating that the presence of aromatic moieties favoured neuroblast migration inhibition. In terms of structure/activity relationship, these results suggest that the following structural moieties favour neuroblast migration inhibition:

Presence of a disulfide bridge, several amino groups (four amino groups appear to be optimal) and aromatic moieties.

**Table 1.** Neuroblast migration inhibitory activity of polyamino disulfide analogues

| No. | Structure | Neuroblast migration inhibitory activity[a] | | $c\log P$[c] | Reference |
|---|---|---|---|---|---|
| | | Concentration[b] (μM) | Inhibition[b] (%) | | |
| **8** |  | 68<br>34<br>6.8 | 100<br>45<br>Inactive | 5.64 | 10 |
| **4** |  | 139<br>13.9 | 100<br>Inactive | 3.67 | 14 |
| **5** |  | 123<br>12.3 | 100<br>Inactive | 5.18 | 14 |
| **16** |  | 223 | 33 | 0.62 | 15 |
| **9** |  | 100 | Inactive | 2.14 | 11 |
| **6** |  | 154 | 50 | 3.09 | 14 |
| **7** |  | 135 | 100 | 4.41 | 14 |

a,b,c meanings are given in Table 2.

The most potent inhibitor **8** is a quite lipophilic analogue ($c\log P = 5.64$), indicating that transcellular permeability could be a biophysical factor which should be taken into account in the design of SVZ neuroblast migration at least in cell-based assays. Note that LY 294002 (**2**) reported as neuroblast migration inhibitor is also a lipophilic analogue ($c\log P$ value = 3.63). In contrast to the disulfide series, polyazamacrocycles which are highly hydrophilic compounds ($c\log P$ values ranging from −2.34 to 2.19) are the less potent analogues suggesting that lipophilicity is a factor which should be considered in the design of migration inhibitor. This point could be of interest for future compound optimization since a subtle balance between solubility and permeation should be found in order to attenuate in vivo absorption problems.

The obtained results raise the question of why disulfide cyclic peptide **1** elicits a neuroblast migration stimulating effect (Fig. 2), while polyamine aromatic disulfide **8** displays an inhibitory effect?

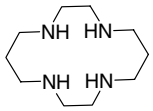
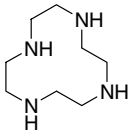
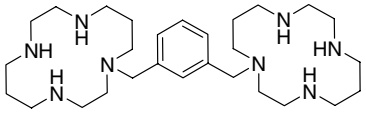
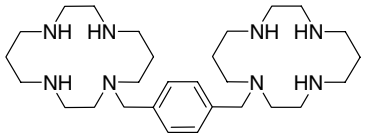
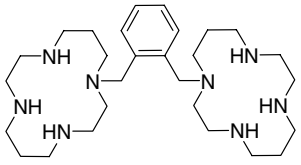
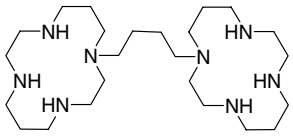
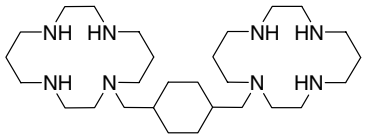
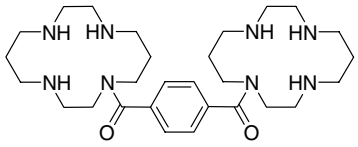
In order to partially answer this question, we have run two competitive experiments.

First experiment: the explants were incubated with stimulating compound **1** for 24 h (Fig. 3A) and then, after washing, the explants were again incubated with analogue **8** for 24 h (Fig. 3B). A blank assay in which the explants were treated for 48 h only with **1** was run simultaneously (Fig. 3C).

Second experiment: we first incubated explants in the presence of inhibitor **8** for 24 h (Fig. 3D), and then, after washing, stimulator **1** was added for additional 24 h (Fig. 3E). A blank assay corresponding to 48 h incubation of the explants with compound **8** was performed simultaneously (Fig. 3F).

As it can be seen in Figure 3, in the first experiment compound **8** antagonized neuroblast migration stimulation effect induced by compound **1** since in these experimental conditions neuroblast migration stimulation due to compound **1** was interrupted by the presence of compound **8**. In the second experiment, when explants are incubated with analogue **8** for 24 h, neuroblast migration is totally inhibited, but if, after washing, analogue **1** was added to the incubation media, neuroblast migration is again observed after 24 h, indicating that compound **8** antagonizes the stimulating activity elicited by analogue **1**.

**Table 2.** Neuroblast migration inhibitory activity of polyazamacrocyclic analogues

| No. | Structure | Neuroblast migration inhibitory activity[a] | | $c\log P$[c] | Reference |
|---|---|---|---|---|---|
| | | Concentration[b] (μM) | Inhibition[b] (%) | | |
| **17** |  | 250<br>25 | 20<br>Inactive | −0.97 | 16 |
| **18** |  | 224<br>22 | 19<br>Inactive | −2.34 | 17 |
| **10** |  | 63 | 10 | 0.20 | 12 |
| **11** |  | 63 | Inactive | 0.20 | 12 |
| **12** |  | 63 | Inactive | 0.20 | 14 |
| **13** |  | 67 | Inactive | 0.06 | 14 |
| **14** |  | 63 | Inactive | 1.24 | 14 |
| **15** |  | 67 | Inactive | 2.19 | 13 |

[a] The activity is expressed as a percent of the migration of explants cultured in the presence of tested compounds versus migration of the explants cultured in media incubation conditions.
[b] Concentrations and inhibitions are expressed with ±10% of standard error.
[c] $c\log P$ determinations were performed using ACD (Advanced Chemistry Development, Inc.)/$\log P$ 1.0 base calculations.

These preliminary results are of interest since for the first time, we have in hands two compounds which present competitive effects on neuroblast migration: compound **1** could stimulate migration while analogue **8** inhibits neuroblast migration or antagonizes the stimulating effect induced by **1**.

Structures of compound **8** as well as compound **1** have to be optimized in order to improve their neuroblast migratory effects. Nevertheless, although quite large secreted proteins like Slit (1 and 2) were known to be repellents for SVZ cells,[18] to our knowledge only very few small molecules were reported to be attractant or repulsive on these neuroblast SVZ cells. These preliminary results have to be refined in order to improve the understanding of how these new analogues act as stimulators[14] or inhibitors of neuroblast migration. Moreover, these new derivatives represents new chemical tools in order to study the biological mechanisms by which SVZ-derived cells migrate and to better understand how this region sustains continued neurogenesis throughout adulthood.

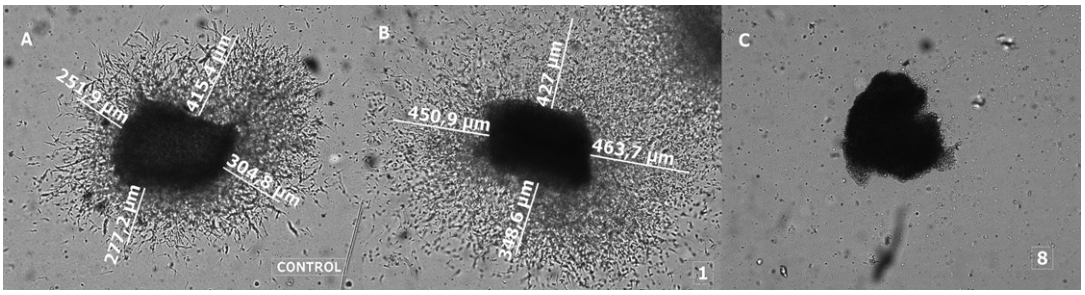



**Figure 2.** (A) Neuroblast migration control assay. (B) Neuroblast migratory effect induced by analogue **1** observed at 40 µM. (C) Neuroblast migration inhibitory effect induced by analogue **8** observed at 68 µM. Four migration distances (in µm) are measured for each explant using AxioVision LE, and the average value gives the final neuroblast migration distance which is compared with the distance obtained for neuroblast in the control assay.

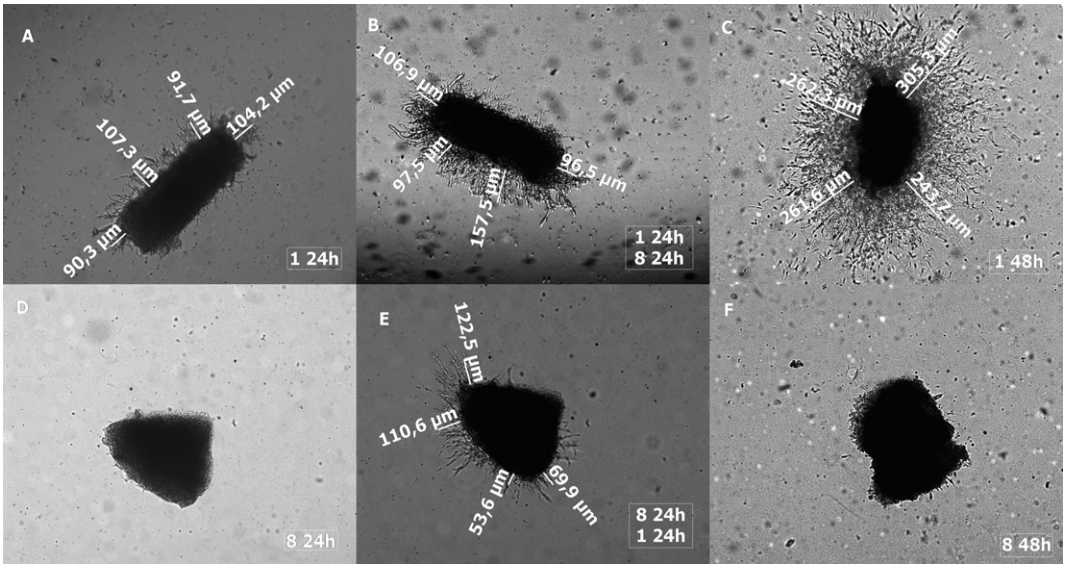



**Figure 3.** (A) Neuroblast migration after 24-h incubation with **1**. (B) Migration after first 24-h incubation with **1** and then 24-h incubation with **8**. (C) Neuroblast migration control assay: 48-h incubation with **1**. (D) Neuroblast migration after 24-h incubation with **8**. (E) Migration after first 24-h incubation with **8** and then 24-h incubation with **1**. (F) Neuroblast migration control assay: 48-h incubation with **8**. Neuroblast migrations are appreciated as already described in Figure 2.

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9. One-day-old mice are killed by decapitation. Brains are dissected and then sectioned in 400 µm slices by vibratome (Leica). Slices are stored in Hanks' Balance Salt Solution (HBSS) while the Sub-Ventricular Zone (SVZ) is dissected and sectioned in about 300 or 400 µm explants.These latter are mixed with Matrigel (BD Biosciences), and distributed in four-well dishes. After the Matrigel polymerization, 400 µL of neurobasal culture medium containing glutamine, penicillin–streptomycin and B27 supplement and the tested compound are added over the Matrigel. Explants are placed in the incubator for 48 h and then observed with Axioplan (5× Zeiss). Pictures are collected after 48 h incubation time by video camera with AxioVision RE and analysed with AxioVision LE software. Analysis consists in measuring the migration distance of the neuroblasts in different experimental conditions. The inhibitory activity is calculated by a ratio between values obtained for explants treated with a compound and for control explants.
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14. *N*,*N*′-bis(benzoyl)cystamine **4**: 80%, NMR $^1$H (250 MHz, $CDCl_3$): $\delta$ 2.99 (t, 4H, $^3J$ = 6.5 Hz), 3.81 (q, 4H, $^3J$ = 6 Hz), 7.45 (m, 6H), 7.83 (d, 4H, $^3J$ = 7 Hz). ESI-MS *m*/*z* $(M+H)^+$ = 361. *N*,*N*′-bis(benzyl)cystamine **5**: 10%, NMR $^1$H (250 MHz, MeOD): $\delta$ 3.04 (t, 4H, $^3J$ = 7.75 Hz), 3.41 (t, 4H, $^3J$ = 7.75 Hz), 4.26 (s, 2H), 7.47 (m, 10H). ESI-MS *m*/*z* $(M+H)^+$ = 333. *N*,*N*′-bis(benzoyl)hexylamine **6**: 16%, NMR $^1$H (250 MHz, $CDCl_3$): $\delta$ 1.45–1.66 (m, 8H), 3.47 (q, 4H, $^3J$ = 6 Hz), 7.45 (m, 6H), 7.79 (d, $^3J$ = 7 Hz). ESI-MS *m*/*z* $(M+H)^+$ = 324. *N*,*N*′-bis(benzyl)hexylamine **7**: 15%, NMR $^1$H (250 MHz, DMSO): $\delta$ 1.28–1.62 (m, 8H), 2.84 (t, 4H, $^3J$ = 7.5 Hz), 4.09 (s, 4H), 7.41–7.52 (m, 10H). ESI-MS *m*/*z* $(M+H)^+$ = 297. 1,1′-(*o*-xylyl)-bis(1,4,8,11-tetraazacyclotetradecane) **12**: 67% NMR $^1$H (250 MHz, DMSO) $\delta$ 2.13 (m, 8H), 3.50 (m, 36H), 7.54 (sl, 2H), 7.90 (sl, 2H). ESI-MS *m*/*z* $(M+H)^+$ = 504. 1,1′-butane-bis(1,4,8,11-tetraazacyclotetradecane) **13**: 93%, NMR $^1$H (250 MHz, $CDCl_3$) $\delta$: 0.90 (m, 4H), 1.77 (m, 8H), 2.70–3.31 (m, 28H), 3.62 (t, 4H), 3.74 (t, 4H). ESI-MS *m*/*z* $(M+H)^+$ = 456. 1,1′-(1,4-dimethylcyclohexane)-bis[1,4,8,11-tetraazacyclotetradecane] **14**: 61% NMR $^1$H (250 MHz, DMSO) $\delta$: 0.85–1.55 (m, 10H), 2.00 (m, 8H), 3.37 (m, 36H), 9.45 (sl, 6H). ESI-MS *m*/*z* $(M+H)^+$ = 510.
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