Bioorganic & Medicinal Chemistry Letters 23 (2013) 2624–2627

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of PDZ ligands by docking-based virtual screening for the development of novel analgesic agents

Naoual Bouzidi [a], Hemantkumar Deokar [a], Alexandre Vogrig [a], Benjamin Boucherle [a], Isabelle Ripoche [b], Isabelle Abrunhosa-Thomas [b], Liam Dorr [c], Anne-Sophie Wattiez [d], Lu-Yun Lian [c], Philippe Marin [e], Christine Courteix [d], Sylvie Ducki [b,*]

[a] Clermont Université, UBP, CNRS UMR 6296, Institut de Chimie de Clermont-Ferrand, BP 10448, F-63000 Clermont-Ferrand, France
[b] Clermont Université, ENSCCF, CNRS UMR 6296, Institut de Chimie de Clermont-Ferrand, BP 10187, F-63174 Aubière, France
[c] NMR Centre for Structural Biology, University of Liverpool, UK-L69 3BX Liverpool, UK
[d] Clermont Université, Université d'Auvergne, Institut NeuroDol, BP 10448, F-63000 Clermont-Ferrand, INSERM, UMR 1107, F-63001 Clermont-Ferrand, France
[e] IGF, CNRS UMR 5203, INSERM U661, Universités Montpellier 1&2, F-34094 Montpellier, France

ARTICLE INFO

Article history:
Received 7 February 2013
Revised 19 February 2013
Accepted 23 February 2013
Available online 14 March 2013

Keywords:
Docking-based virtual screening
PDZ proteins
Pain
Analgesic activity

ABSTRACT

Disrupting the interaction between the PDZ protein, PSD-95, and its target ligands (such as the glutamate NMDA receptor or the serotonin 5-HT_{2A} receptor) was found to reduce hyperalgesia in various models of neuropathic pain. Here, we set out to identify lead molecules which would interact with PSD-95, and hence, would potentially display analgesic activity. We describe the virtual screening of the Asinex and Cambridge databases which together contain almost one million molecules. Using three successive docking filters and visual inspection, we identified three structural classes of molecules and synthesized a potential lead compound from each class. The binding of the molecules with the PDZ domains of PSD-95 was assessed by ^{1}H–^{15}N HSQC NMR experiments. The analgesic activity of the best ligand, quinoline **2**, was evaluated in vivo in a model of neuropathic pain and showed promising results.

Since their discovery two decades ago, about 267 PDZ domains (Post-synaptic density-95 PSD-95, Disc large Dlg, Zonula occludens ZO-1) have been identified in the human genome. PDZ domains regulate multiple biological processes such as transporter and ionic channel activity and membrane-bound receptor-operated signalling. These domains consist of 100 amino acid residues usually composed of 6 β-strands (β_A to β_F), and two α-helices (α_A and α_B) (Fig. 1). PDZ-mediated protein–protein interactions (PPIs) involve the binding of the extreme C-terminus of target proteins (PDZ ligand) to a hydrophobic groove between α_B and β_B, delimited by the GLGF loop of the PDZ domain.[1]

Several PDZ proteins have been associated with pathological states (cancer, cystic fibrosis, schizophrenia, Parkinson's and Alzheimer's diseases, chronic inflammatory and neuropathic pain) and the therapeutic usefulness of inhibiting PDZ-mediated PPIs has been clearly demonstrated by using interfering peptides.[2]

The PSD-95 protein contains three PDZ domains and has been identified as a major partner of various membrane receptors including the glutamate NMDA and the serotonin 5-HT_{2A} receptors.[3] Disrupting the interaction between PSD-95 and its target proteins was found to reduce hyperalgesia in various models of neuropathic pain.[4–6] Thus, identification of PSD-95 ligands could potentially lead to the development of a novel class of analgesics.

Virtual screening provides a valuable alternative to high-throughput screening (HTS) by bioassays,[7] which is a useful but expensive and time-consuming method to identify new lead compounds. In the past decade, structure-based design methods for virtual ligand screening have been successful in lead discovery due to the increased structural information on drug target, the availability of large commercial databases, advancements in computational power, and improvements in the efficiency of docking algorithms.[8–10]

In the present study, we report the docking-based screening of the Asinex and Cambridge databases to identify lead molecules which could potentially interact with the PDZ1 or PDZ2 domains of the PSD-95 protein.

We selected the high-resolution structure of the PDZ1 domain of PSD-95 complexed with cypin (PDB 2KA9) as the starting point of our computational approach. The docking was performed on the active site using the docking module in MOE (Molecular Operating Environment).[11] We developed a protocol for the docking-based screening and searched the Asinex and Cambridge commercial databases (respectively 514,957 and 432,000 molecules)[12,13] using three successive hierarchical docking filters (Fig. 2).

(I) The Alpha Triangle method[12] was used to identify molecules with good affinity towards the target protein. Here the poses are generated by superimposition of ligand atom triplets and

* Corresponding author.
E-mail address: sylvie.ducki@ensccf.fr (S. Ducki).

0960-894X/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmcl.2013.02.100

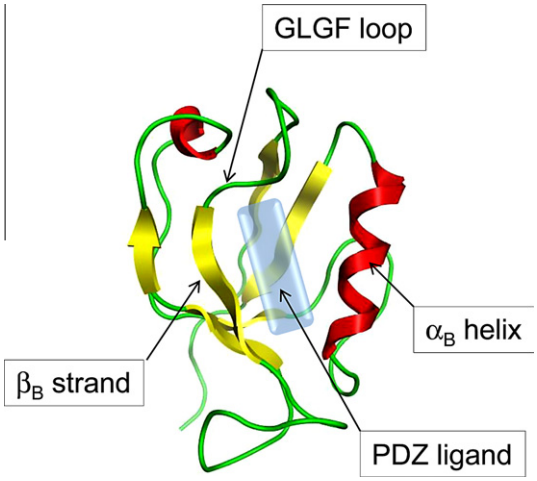


Figure 1. 3D representation of PSD-95 PDZ1 (PDB 2KA9). Helices in red, strands in yellow, loops in green.

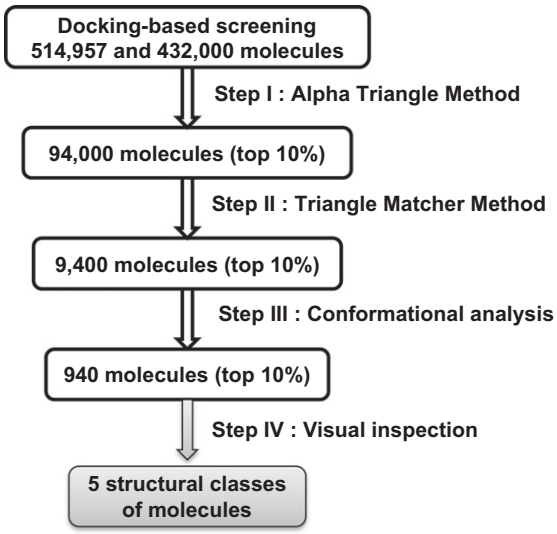


Figure 2. Docking-based screening protocol.

triplets of receptor site points. The receptor site points were alpha sphere centres which represent locations of tight packing. A random conformation was selected at each iteration; a random triplet of ligand atoms and a random triplet of alpha sphere centres were used to determine the pose. At the end of this step, molecules were sorted on the basis of their interaction energy and the top 10% (around 94,000 molecules) was kept.

(II) Triangle Matcher method[14] generated poses by aligning ligand triplets of atoms on triplets of alpha spheres in a more systematic way than in the Alpha Triangle method. As for step 1, the top 10% (9400 molecules) was kept.

(III) A conformational analysis for the 9400 molecules was carried out and all output molecules were kept as input for this step. In the forcefield-based refinement of protein–ligand complex, the energy minimization of the system was carried out using the conventional molecular mechanics setup. At the end of this step the molecules were examined for poses and binding energy. The top 10% (940 molecules) were visually inspected (Step IV) and classified on the basis of their chemical structures.

We hence identified five classes of molecules: indoles, nucleotides, quinolines, *N*-acyl hydrazones and carbohydrates derivatives linked to a phenyl group with polar substituents. Since we have previously described indole derivatives as potential PDZ inhibitors[15,16] and because nucleotides interact with too many biological targets, we focused our attention on the other three classes.

We defined a lead compound from each class which satisfied three criteria: (i) the best docking pose should show interactions with the GLGF loop of the PDZ domain, (ii) molecules should be obtained from commercially available and affordable starting material with easy synthesis, and (iii) they must be easily functionalizable on various positions. In the quinoline family, we chose molecule **2** which has similar substituents as indole **1** (Fig. 3), previously reported to interact with the PDZ1 domain of PSD-95. The best choice in the carbohydrate-linked-to-phenyl-ring class was the glucose analogue **3**. In the *N*-acyl hydrazones family, we selected triol **4** which displayed the best docking energy. The three potential ligands **2–4** displayed interactions with the GLGF loop of PSD-95 PDZ1 (Fig. 3, Table 1).

Quinoline **2** was prepared from aniline **5**, ethyl glyoxylate and propanal using the method described by Shimizu et al.[17] The best results for the cyclization were obtained with Yterbium triflate as a Lewis acid with 20% yield. Saponification of compound **6** gave the desired quinoline **2** in 35% yield (Scheme 1, steps a and b). The glucosylamine derivative **3** was obtained in 50% yield, from D-glucose **8** by reaction with the commercially available 4-amino benzoic acid **7** (Scheme 1, step c), using the method described by Satorelli et al.[18]

The *N*-acyl hydrazone **4** was prepared in two steps (Scheme 1, steps d and e) from the commercially available ethyl 4-hydroxybenzoate **9**, in 33% yield.[19]

Compounds **2–4** were evaluated for their interactions with the PDZ1 and PDZ2 domains of PSD-95.[15]^{15}N-labelled PDZ proteins were produced and purified after subcloning and expression in *Escherichia coli* BL21 (see Supplementary data). ^{1}H–^{15}N heteronuclear single-quantum coherence (HSQC) NMR experiments were recorded in the absence and presence of compounds **2–4**.[16]

Ligand-induced chemical shift perturbations (CSP) in the ^{1}H–^{15}N HSQC of the PDZ domains verified the presence of binding and identified the amino acids whose chemical environments were affected by the bound compound, hence, locating its binding site(s). The reference spectra of free PDZ domains in the buffer and temperature condition used here were assigned during the course of this work and compared to literature.[20]

Unfortunately neither compounds **3** nor **4** had any effect on the NMR spectra of the PDZ domains and only quinoline **2** displayed some binding ability (Fig. 4A). While weak CSP were observed on the spectrum of PSD-95 PDZ1 (see Supplementary data), stronger CSP were observed on the PSD-95 PDZ2 spectrum (Gly18, Ser20, Ile21, Gly23, Lys 53, Ala47, Glu3, Ala96, Asn63 and Asn27, Fig. 4B). These residues are located in the peptide binding groove, hence suggesting that quinoline **2** is binding in the same region as the natural peptide ligands.

The anti-hyperalgesic effect of quinoline **2** was evaluated on chronic constriction nerve injury-induced (CCI) neuropathic pain in rats. This model consists in a unilateral peripheral mononeuropathy induced by four loose ligatures of the sciatic nerve.[21] Intrathecal (i.t.) injections of quinoline **2** at 12.3, 24.5 and 122.6 ng/rat induced a dose-dependent anti-hyperalgesic effect in CCI rats. The highest dose abolished mechanical hyperalgesia 15 min after injection (Fig. 5a). This anti-hyperalgesic effect was similar to that induced by an interfering peptide corresponding to the 10 C-terminal amino acids of the 5-HT$_{2A}$ receptor (TAT-2A peptide, 100 ng/rat, Fig. 5b), which disrupts interactions between the receptor and its PDZ partners.[4]

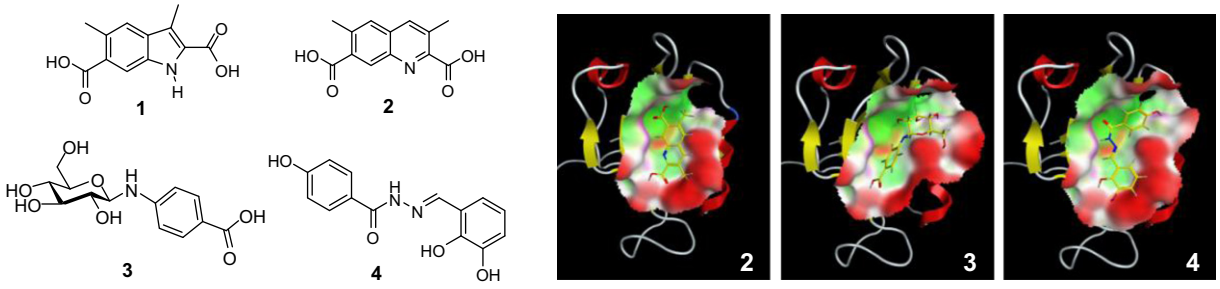
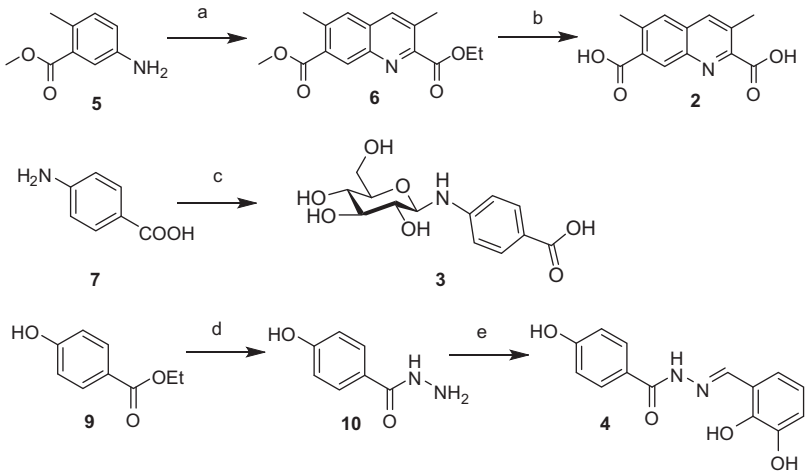


Figure 3. Indole **1** and the best docked poses for the top ranked molecules: quinoline **2**, glycosylamine **3**, *N*-acyl hydrazone **4**.

Table 1
Docking of compounds **2**–**4** into PSD-95 PDZ1

Compounds	Interaction energy (kcal/mol)	Amino acid interactions (H-Bonds)	Amino acid interactions (other interactions)
2	−7.52	Leu17, Gly18, Phe19, Ile21	Leu17, Gly18, Phe19, Ile21, His72, Leu79
3	−8.52	Gly18, Phe19, Ile21, Leu79	Gly18, Phe19, Ile21, His72, Val76, Leu79
4	−7.32	Ile21, Leu79	Gly16, Leu17, Gly18, Phe19, Ser20, Ile21, Leu79

Energy of interaction (in kcal/mol). Localization of amino acids showing interactions with compounds **2**–**4** (from docking). Numbering uses the generic system ^{18}Gly-^{19}Leu-^{20}Gly-^{21}Phe for the PDZ domain.



Scheme 1. Synthesis of compounds **2–4**. Reagents and conditions: (a) $Yb(OTf)_3$, ethyl glyoxylate, propanal, $CHCl_3$, O_2, 90 °C, 16 h, 20%. (b) NaOH, MeOH/H_2O, 70 °C, 12 h, 35%. (c) D-Glucose 8, EtOH, rt, 12 h, 50%. (d) Hydrazine, EtOH, reflux, 48 h, 50%. (e) 2,3-Dihydroxybenzaldehyde, EtOH, AcOH, reflux, 24 h, 66%.

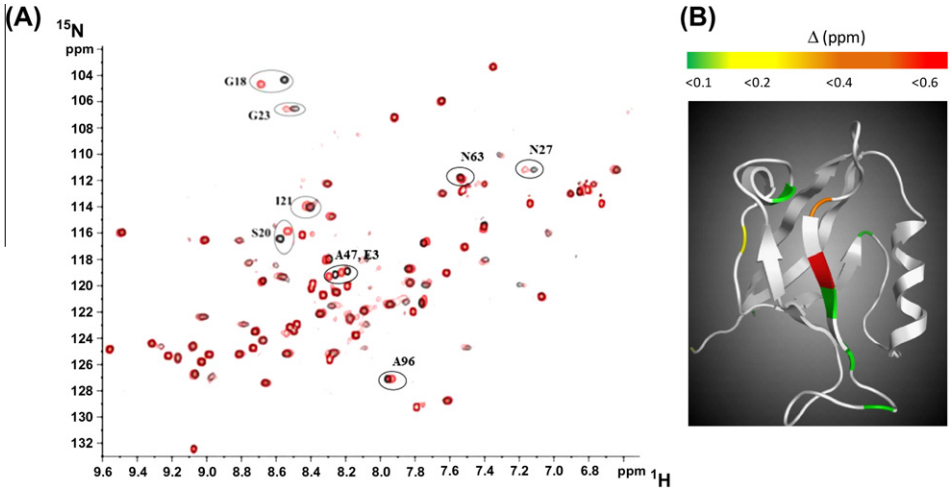


Figure 4. (A) Overlay plot of ^{1}H–^{15}N HSQC spectra of free PSD-95 PDZ2 (black) and in the presence of compound 2 (red) (protein/ligand 1:10). (B) Chemical shift perturbation (CSP) of PSD-95 PDZ2 resulting from compound **2**. D**d** (ppm) = $\sqrt{(DH)^2 + (0.15DN)^2}$. Ie., the nitrogen difference should be scaled by 0.15.

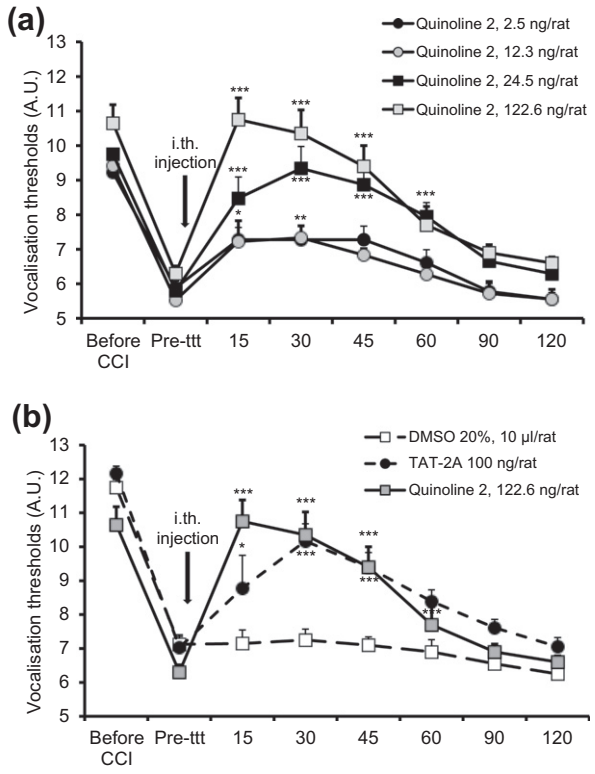


Figure 5. Anti-hyperalgesic effect of quinoline **2** in CCI rats (n = 8–18 rats per dose). (a) CCI rats were i.t. injected with the indicated dose of quinoline **2**. (b) CCI rats were i.t. injected with either quinoline **2** (122.6 ng/rat), or TAT-2A (100 ng/rat) or vehicle (20% DMSO 10 µl/rat). The vocalisation thresholds to paw pressure (Randall and Selitto test[22]) were measured up to 120 min after injection. $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001 compared with values measured before drug or vehicle injection (Pre-treatment values).

We have performed a docking-based screening of the Asinex and Cambridge databases to identify new lead compounds able to interact with the PSD-95 protein. We identified in silico three classes of molecules, quinoline, *N*-acyl hydrazone and substituted carbohydrate. One molecule of each class was synthesized and evaluated for interactions with two PDZ domains of PSD-95 (PDZ1 and PDZ2) using $^{1}H–^{15}N$ HSQC NMR experiments which allowed us to identify quinoline **2** as the best ligand of the PDZ domains of PSD-95. We also showed that this compound displayed a significant anti-hyperalgesic activity in neuropathic rats (CCI), similar to that induced by a natural peptide ligand of PSD-95 PDZ domains. Quinoline **2** will be used as a lead compound for further optimisation in order to develop anti-hyperalgesic agents.

Acknowledgments

We would like to thank the French National Research Agency (ANR) for funding of the project PDZ-CANPAIN and a postdoctoral fellowship for B.B. and N.B.; the Regional Council of Auvergne (Conseil Régional d'Auvergne) and the European Fund for Regional Economic Development (FEDER) for a doctoral scholarship for A.V. and A.S.W.; the PRES Clermont Université for a postdoctoral fellowship for H.D.; the G.E. Healthcare/BBSRC for a CASE studentship for L.D. The authors wish to thank Eric Chapuy (Inserm U1107 Neuro-Dol, Clermont-Ferrand) for his technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.100.

References and notes

1. Doyle, D. A.; Lee, A.; Lewis, J.; Kim, E.; Sheng, M.; MacKinnon, R. *Cell* **1996**, *1067*, 85.
2. Ducki, S.; Bennett, E. *Curr. Chem. Biol.* **2009**, *3*, 146.
3. Bécamel, C.; Gavarini, S.; Chanrion, B.; Alonso, G.; Galéotti, N.; Dumuis, A.; Bockaert, J.; Marin, P. *J. Biol. Chem.* **2004**, *279*, 20257.
4. Pichon, X.; Wattiez, A.-S.; Becamel, C.; Ehrlich, I.; Bockaert, J.; Eschalier, A.; Marin, P.; Courteix, C. *Mol. Ther.* **2010**, *18*, 1462.
5. LeBlanc, B. W.; Iwata, M.; Mallon, A. P.; Rupasinghe, C. N.; Goebel, D. J.; Marshall, J.; Spaller, M. R.; Saab, C. Y. *Neuroscience* **2010**, *167*, 490.
6. Garry, E. M.; Moss, A.; Delaney, A.; O'Neill, F.; Blakemore, J.; Bowen, J.; Husi, H.; Mitchell, R.; Grant, S.; Fleetwood-Walker, S. *Curr. Biol.* **2003**, *13*, 323.
7. Tiziano, T. *Comb. Chem. High Throughput Screening* **2009**, *12*, 303.
8. Caporucio, F.; Rastelli, G.; Imbirano, C.; Del Rio, A. *J. Med. Chem.* **2011**, *54*, 4006.
9. Wang, W.; Wenig, J.; Zhang, X.; Liu, M.; Zhang, M. *J. Am. Chem. Soc.* **2009**, *131*, 787.
10. Lengauer, T.; Rarey, M. *Curr. Opin. Struct. Biol.* **1996**, *6*, 402.
11. MOE 2010 Manual, 1997–2010 Chemical Computing Group Inc.
12. ASINEX Ltd, Moscow, Russia, http://www.asinex.com.
13. Computing Group Cambridge Structural Database System, Daresbury, UK, http://cds.dl.ac.uk/Inc.
14. Idrus, S.; Sumo, U.; Tambunan, F.; Zubaidi, A. A. *Bioinformation* **2012**, *8*, 348.
15. Boucherle, B.; Vogrig, A.; Deokar, H.; Bouzidi, N.; Ripoche, I.; Thomas, I.; Marin, P.; Ducki, S. *Bioorg. Med. Chem.* **2011**, *19*, 4346.
16. Vogrig, A.; Boucherle, B.; Deokar, H.; Thomas, I.; Ripoche, I.; Lian, L.-Y.; Ducki, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3349.
17. Inada, T.; Nakajima, T.; Shimizu, I. *Heterocycles* **2005**, *66*, 611.
18. Wang, L.; Maniglia, C. A.; Mella, S. L.; Sartorelli, A. *J. Med. Chem.* **1983**, *29*, 1323.
19. Forman, D.; Yu, D. U.S. Patent 20,060,189,825, 2008.
20. Piserchio, A.; Pellegrini, M.; Mehta, S.; Blackman, S. M.; Garcia, E. P.; Marshall, J.; Mierke, D. F. *J. Biol. Chem.* **2002**, *277*, 6967.
21. Wang, L.; Piserchio, A.; Mierke, D. F. *J. Biol. Chem.* **2005**, *280*, 26992.
22. Randall, L. O.; Selitto, J. J. *Arch. Int. Pharmacodyn. Ther.* **1957**, *111*, 409.