Center for Drug Discovery, University of Florida, Gainesville, USA

Recent advances in retrometabolic drug design and targeting approaches

N. BODOR

A number of representative, recent advances achieved within the field of retrometabolic drug design are briefly summarized. For the soft drug approach, some of the results of recent *in vivo* studies of loteprednol etabonate, a soft corticosteroid approved by the FDA in 1998, are reviewed. For the chemical delivery system (CDS) approach, the latest advances achieved in the brain targeting of peptides such as kyotorphin and TRH analogues are described.

1. Introduction

The general principles of retrometabolic drug design approaches were recently reviewed [1–3]; here, we are presenting some of the more important advances, both in the chemical delivery systems (CDS) and the soft drug (SD) fields.

2. Soft drug design

The field of anti-inflammatory corticosteroids provided a good example for a soft drug approach [4–18]. We have previously reported various aspects of the inactive metabolite-based soft corticosteroids, specifically loteprednol etabonate (**1**), which was designed starting from the inactive metabolite of prednisolone, Δ^1-cortienic acid (Fig. 1). Loteprednol etabonate (chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene, 17β-carboxylate), an active corticosteroid that lacks the usual steroidal systemic side effects and, uniquely, the specific ophthalmic side effects, such as elevation of intraocular pressure and formation of cataract, received FDA approval in 1998 as the active ingredient of two ophthalmic preparations, Lotemax™ and Alrex™ [19–33]. Thus, loteprednol etabonate became the only corticosteroid that received FDA approval for use in all inflammatory and allergy-related ophthalmic disorders, including inflammation following post-cataract surgery, uveitis, allergic conjunctivitis, and giant papillary conjunctivitis.

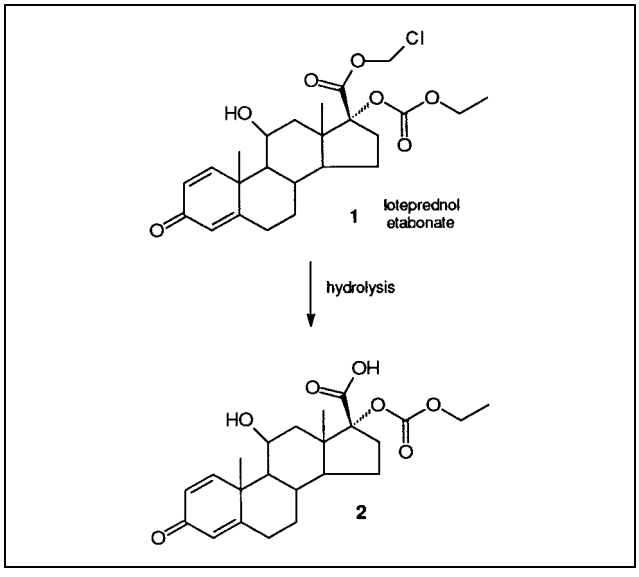


Fig. 1: Loteprednol etabonate (**1**) and its primary inactive metabolite, Δ^1-cortienic acid 17α-ethylcarbonate (**2**)

Recent studies [34, 35] demonstrated, that loteprednol etabonate (LE, **1**) can advantageously be used in many other inflammatory conditions, where separation of activity and side effects is very important. Thus, it was demonstrated that is equipotent with the highly used beclomethasone dipropionate (BD) in various experimental models of allergic diseases, such as ovalbumin (OA) induced rhinorrhea and OA-induced lung eosinophilia in actively sensitized Brown-Norway (BN) rats (Figs. 2, 3). For example, in experiments of OA-induced airway eosinophilia in actively sensitized BN rats, compounds were given 2 h prior to OA challenge intratracheally as a dry powder. The animals were challenged by inhalating OA-aerosol. The number of eosinophils in bronchoalveolar lavage fluid (BALF) were counted for 48 h post challenge. Eosinophilia in BALF was reduced dose dependently by LE (IC_{50} about 10 μg/kg) and BD ($IC_{50} = 10$ μg/kg). In similar studies involving late phase allergic eosinophilia in guinea pigs, eosinophilia in BALF was reduced dose dependently by LE ($IC_{50} = 43$ μg/kg), by dexamethasone ($IC_{50} = 134$ μg/kg), and by BD ($IC_{50} = 19$ μg/kg). In this set of experiments where the OA challenge was applied 2 h after the dose with steroids, fluticasone propionate (FP), a highly potent steroid, showed the highest activity ($IC_{50} = 0.89$ μg/kg). It was found, however, that when LE and FP were compared

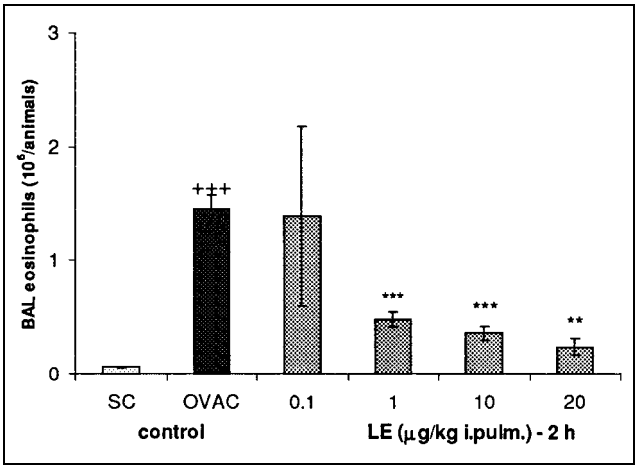
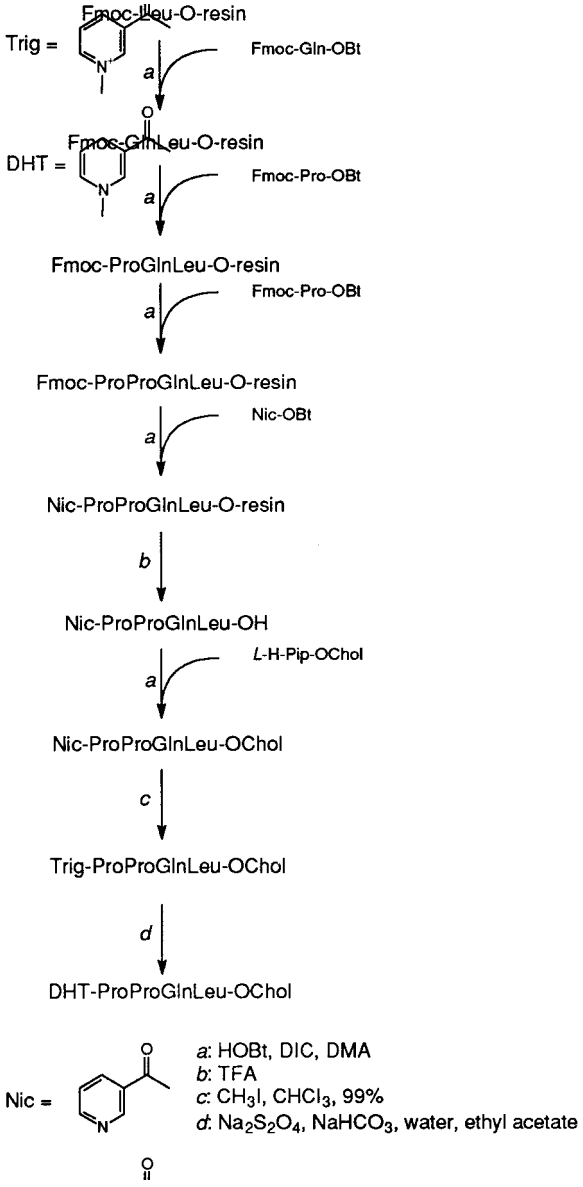


Fig. 2: Effect of loteprednol etabonate LE (0.1–20 μg/kg) on allergen-induced eosinophilia in BALF (bronchoalveolar lavage fluid) 48 h after allergen-challenge in actively sensitized Brown-Norway rats by single intrapulmonar dry powder administration 2 h prior to challenge. SC = lactose treated/saline challenged control group; OVAC = lactose treated/OVA (ovalbumin) challenged control group; $+++$ $p < 0.001$ compared to animals sham-challenged with saline (SC); ** $p < 0.01$ and *** $p < 0.001$ compared to vehicle-treated, allergen challenged animals (OVAC). Data are mean ± SEM

Scheme



Preparation of the CDS for [Leu^2, Pip^3]-TRH

in a study with longer separation (6 h) between administration of steroids and OA challenge, LE still showed a strong activity with $IC_{50} = 77$ μg/kg, but FP showed very weak and not dose-dependent effect. These data indicate that LE produced a strong anti-inflammatory effect *in vivo* after intranasal and intrapulmonary administration. In comparison to FP, LE showed significantly longer lasting action. Accordingly, LE is being now developed for the treatment of allergic conditions, such as rhinitis and asthma.

3. Chemical delivery systems

Significant advances in brain targeting of peptides using the molecular package [36] approach were recently achieved. First, brain targeting of kyotorphin was reported

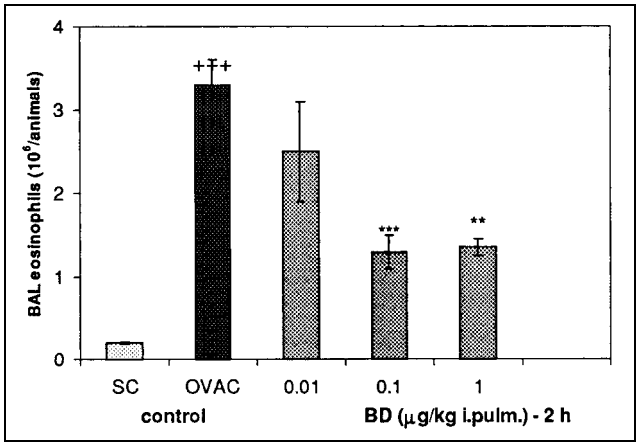


Fig. 3: Effect of beclomethasone dipropionate BD (0.01 – 1 μg/kg) on allergen-induced eosinophilia in BALF 48 h after allergen-challenge in actively sensitized Brown-Norway rats by single intrapulmonar dry powder administration 2 h prior to challenge. See notation for Fig. 2

using a modified version of the molecular packaging approach [37]. The target compound was the kyotorphin analogue (Tyr-Lys, YK). The brain targeted compound contains the active peptide in a packaged, disguised form, flanked between the lipophilic cholesteryl ester on the C terminus, and the 1,4-dihydrotrigonellyl redox targetor, attached to the N-terminus through a strategically selected L-amino acid(s) spacer. However, it was found that for successful brain targeting the ε-amine of Lys also needs to be converted to a lipophilic function. The ε-amine function was made lipophilic by coupling to a Boc moiety. It was assumed, and confirmed, that Boc is biodegradable if the corresponding precursor is locked-in the brain. Accordingly, the structure of the packaged kyotorphin analogue (**3**) is shown on Fig. 4. For the final release, the proline endopeptidase was the target enzyme, and accordingly the spacer used was either proline or alanine, or a combination of these two amino acids. The best results were obtained when two prolines were used as spacer.

A basically different strategy was also tested. Recognizing that highly basic amino acids, such as arginine and lysine, are essentially protonated all the time at biological pHs, it was considered that isoelectronic-isosteric replacement of amino acids could accomplish dual roles. As shown on Fig. 5, a "redox amino acid" pair [38] mimics the protonation process. Accordingly, the oxidized, quaternary pyridinium form might produce an active dipeptide, while the corresponding 1,4-dihydro form could replace the targetor moiety of CDS. In this construct, the use of spacer is not needed, but we assume that the free amino acid of tyrosine needs to be made lipophilic in a similar way by Boc. Thus, replacing Lys by the Nys $\leftrightarrow$ Nys$^+$ redox system, as shown in Figs. 4 and 5, could be an effective way to produce centrally mediated analgesic activity. Fig. 6 provides a composite summary of the two alternate approaches. After administration to the general circulatory system, both the CDS and brain targeted redox analogue (**4**, BTRA), respectively, will penetrate the blood-brain-barrier by passive transport, followed by oxidation of the dihydrotrigonellyl function in the CDS or that of Nys in BTRA.

Tail-flick latency, an index of spinal cord mediated analgesia, was used to evaluate the analgesic effects in rats of both types of kyotorphin analogues. Significant and prolonged analgesic activity was found with both approaches at about 5 mg/kg dose, which represents a dramatic enhancement in activity, as kyotorphin requires a dose of 200 mg/kg to provide brief analgesia. The central media-

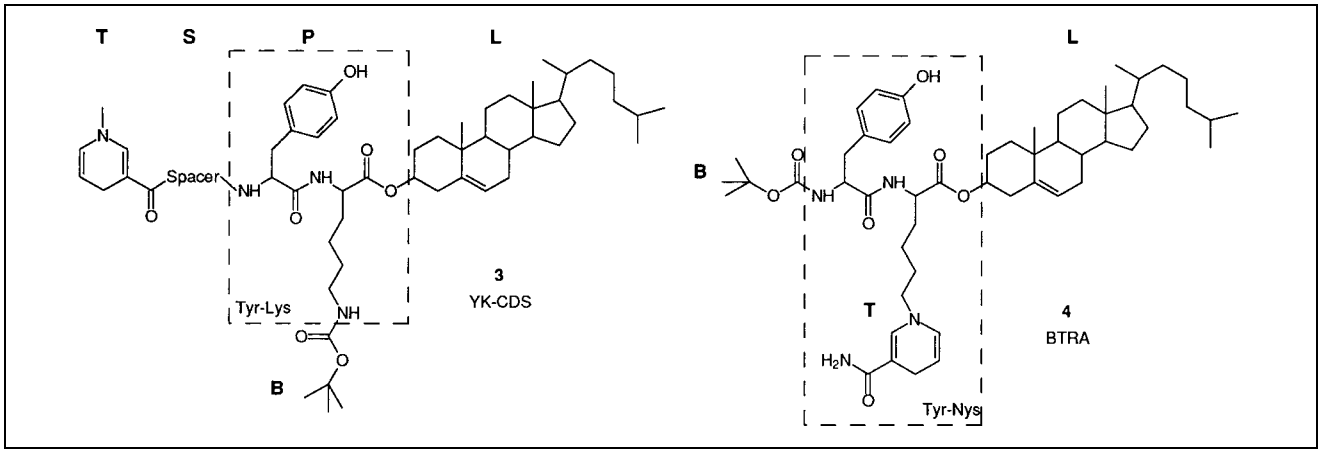


Fig. 4: The chemical delivery system (**3**, CDS) and the brain-targeted redox analog (**4**, BTRA) used for the delivery of the kyotorphine analogue Tyr-Lys (YK) (Spacer: Pro, Pro-Pro, or Pro-Ala)

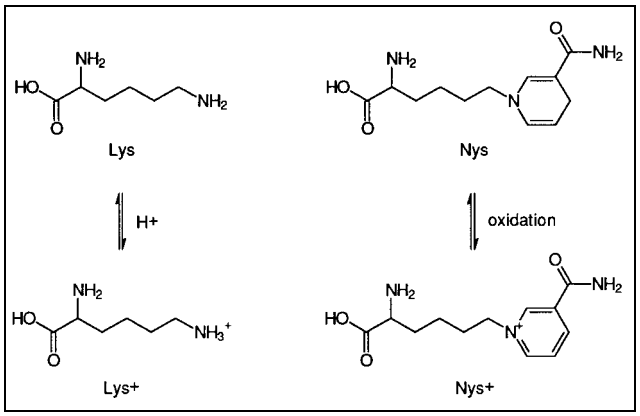


Fig. 5: Isoelectronic/isosteric analogy of Lys and Nys

tion of the observed analgesia was demonstrated in both cases, by administering naloxone at 30 min after administration of BTRA and CDS-PP, respectively, as shown on Fig. 7.

In conclusion, a modified molecular packaging allows basic amino acid containing peptides to be targeted to the brain by converting them into a lipophilic, bioreversible function. On the other hand, it was found that novel "redox amino acids" accomplish both brain targeting and activity functions. This novel approach could be standard for a variety of other basic amino acids containing peptides.

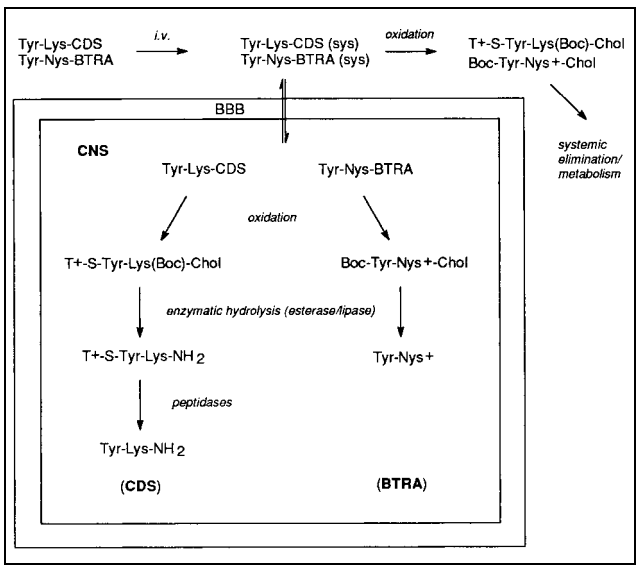


Fig. 6: Sequential metabolism of brain-targeted chemical delivery system of Tyr-Lys and brain targeted redox analogue (Tyr-Nys)

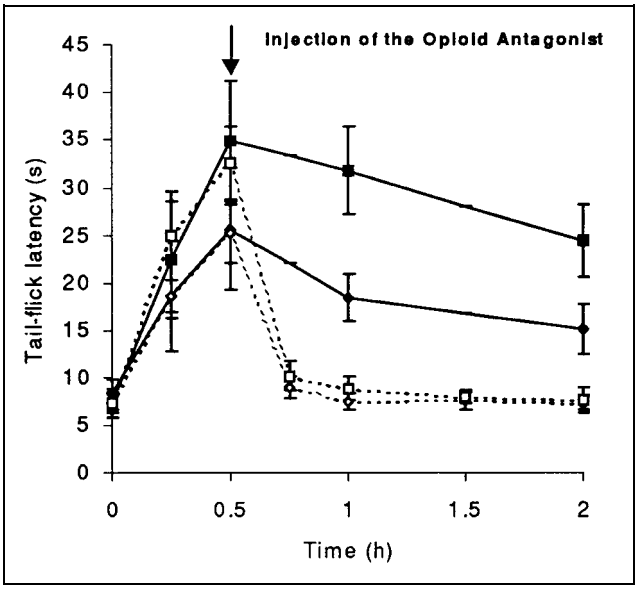


Fig. 7: Reversal of the analgesia produced by YK-CDS and BTRA administered i.v. by naloxone administered 30 min later. Data represents mean ± SE of 6 rats for each group
—◆— BTRA; —■— CDS(PP); ···◇··· BTRA + N;
···□··· CDS(PP) + N

Another recent development in brain targeting of peptides involves a chemical targeting system for TRH analogues, Pyr-Leu-Pip-OH. We have previously reported brain targeting of the Leu[2] analogue of TRH by extending the C-terminal with glycine in order to end up with the C-ter-

Table: Comparison of the pentobarbital-induced sleeping time in mice after the administration of vehicle and test compounds

Compound	Sleeping time (min)
Vehicle	100.5 ± 6.3
Pyr-Leu-Pro-NH_2	78.2 ± 4.7*
DHT-Pro-Pro-Gln-Leu-Pip-O-Chol	58.2 ± 3.4*
DHT-Pro-Pro-Gln-Leu-Pro-Gly-O-Chol	62.0 ± 3.9*

Ten minutes after i.v. injection of the compound (10 μmol/kg)[1], pentobarbital, 60 mg/kg, was i.p. injected in the animal. The sleeping time was recorded as the time elapsed from the onset of loss to regain of the righting reflex.[2,3] Table entries are mean ± SE.
[1] TRH, 10 μmol = 3.7 mg
[2] Mixture of propylene glycol and dimethyl sulfoxide (2:1) was used as vehicle
[3] Six to seven Swiss Webster mice (30 ± 3 g) were used in each group
* $p < 0.05$ when compared to vehicle control using Student's *t*-test

minal proline amide necessary for the activity [39]. Thus, the targeting construct is constituted of DHT-Pro-Pro-Gln-Leu-Pro-Gly-OChol. After delivery to the brain, this compound underwent sequential biotransformation: first, lock-in by oxidation of the dihydrotrigonellyl (DHT) to the corresponding pyridinium salt, then removal of cholesterol (Chol), oxidation of glycine by peptidyl glycine α-amidating monoxegenase to prolineamide, cleavage of the targetor-spacer combination, and, finally, cyclization of glutamine by glutaminyl cyclase to pyroglutamate. It was found, however, that the amide precursor was susceptible to deamination by TRH-deaminase, a side reaction process competitive with the "designed-in" cleavage of the spacer-Gln peptide bond. Therefore, we have selected an analogue of TRH, where proline was replaced by pipocolic acid (Pip), which derivative is active as such having the carboxy C-terminal, thus, there was no need to insert a lysine to produce the amide [40]. The CDS was prepared by a $5 + 1$ segment coupling approach, as shown in the Scheme. The cholesteryl ester of pipecolic acid was prepared using either Fmoc or Boc as protection.

The antagonism of barbiturate-induced sleeping time in mice was used to assess the activation effect on mice CNS cholinergic neurons by the previously published [39] and the current TRH analogues. As the Table indicates, this approach is at least equally effective with the previous packaging; actually, the brain-targeted pipecolic acid analogue of TRH appears to be even somewhat more effective.

4. Computer aided design

Complex computer programs were developed to be able to predict full libraries of soft drugs and chemical delivery systems, starting from various drugs as lead compounds [41, 42]. In addition, most importantly, methods were developed to rank the compounds designed in order of isosteric-isoelectronic closeness to the lead compound and others to predict various physical and chemical properties of the analogues. Most recently, a novel general approach to predict the important hydrolysis rates by human plasma esterases was also developed [43].

5. Conclusions

Significant advancements in retrometabolic drug design approaches were reported from our laboratories, but also a large number of other research groups are using these novel design approaches in a variety of therapeutic classes.

This research paper was presented during the 2nd Conference on Retrometabolism based Drug Design and Targeting, May 11–14, 1999, Amelia Island, Florida, USA

References

1 Bodor, N.; Buchwald, P.: Pharmacol. Ther. **76**, 1 (1997)
2 Bodor, N.; Buchwald, P.: Med. Res. Rev. **20**, 58 (2000)
3 Bodor, N.; Buchwald, P.: Adv. Drug. Del. Rev. **36,** 229 (1999)
4 Bodor, N.: Soft steroids having antiinflammatory activity, Belgian patent, BE889,563 (Cl. CO7J) 1981
5 Bodor, N.; Varga, M.: Exp. Eye Res. **50**, 183 (1990)
6 Druzgala, P.; Hochhaus, G.; Bodor, N.: J. Steroid Biochem. **38**, 149 (1991)
7 Druzgala, P.; Wu, W.-M.; Bodor, N.: Curr. Eye Res. **10**, 933 (1991)
8 Alberth, M.; Wu, W.-M.; Winwood, D.; Bodor, N.: J. Biopharm. Sci. **2**, 115 (1991)
9 Bodor, N. S.; Kiss-Buris, S. T.; Buris, L.: Steroids **56**, 434 (1991)
10 Bodor, N.; Bodor, N.; Wu, W.-M.: Curr. Eye Res. **11**, 525 (1992)
11 Bodor, N.; Loftsson, T.; Wu, W.-M.: Pharm. Res. **9**, 1275 (1992)
12 Hochhaus, G.; Chen, L.-S.; Ratka, A.; Druzgala, P.; Howes, J.; Bodor, N.; Derendorf, H.: J. Pharm. Sci. **81**, 1210 (1992)
13 Loftsson, T.; Bodor, N.: Adv. Drug Deliv. Rev. **14**, 293 (1994)
14 Bodor, N.; Murakami, T.; Wu, W.-M.: Pharm. Res. **12**, 869 (1995)
15 Bodor, N.; Wu, W.-M.; Murakami, T.; Engel, S.: Pharm. Res. **12**, 875 (1995)
16 Reddy, I. K.; Khan, M. A.; Wu, W. M.; Bodor, N. S.: J. Ocul. Pharmacol. Ther. **12**, 159 (1996)
17 Buris, L. F.; Bodor, N.; Buris, L.: Pharmazie **54**, 58 (1999)
18 Little, R. J.; Bodor, N.; Loftsson, T.: Pharm. Res. **16**, 961 (1999)
19 Bartlett, J. D.; Howes, J. F.; Ghormley, N. R.; Amos, J. F.; Laibovitz, R.; Horwitz, B.: Curr. Eye Res. **12**, 313 (1993)
20 Bartlett, J. D.; Horwitz, B.; Laibovitz, R.; Howes, J. F.: J. Ocul. Pharmacol. **9**, 157 (1993)
21 Howes, J. F.; Baru, H.; Vered, M.; Neumann, R.: J. Ocul. Pharmacol. **10**, 289 (1994)
22 Asbell, P.; Howes, J.: CLAO J. **23**, 31 (1997)
23 Friedlaender, M. H.; Howes, J.: Am. J. Ophthalmol. **123**, 455 (1997)
24 Dell, S. J.; Shulman, D. G.; Lowry, G. M.; Howes, J.: Am. J. Ophthalmol. **123**, 791 (1997)
25 Howes, J.; Novack, G. D.: J. Ocul. Pharmacol. Th. **14**, 153 (1998)
26 Dell, S. J.; Lowry, G. M.; Northcutt, J. A.; Howes, J.; Novack, G. D.; Hart, K.: J. Allergy Clin. Immunol. **102**, 251 (1998)
27 Shulman, D. G.; Lothringer, H. L.; Rubin, J. M.: Ophthalmology **106**, 362 (1999)
28 Novack, G. D.; Howes, J.; Crockett, R. S.; Sherwood, M. B.: J. Glaucoma **7**, 266 (1998)
29 Beehler, C.; Bodner, B.; Bowman, B.; Cooke, D.; Crabb, J. L.; DeBarge, L. R.; Donnenfeld, E.; Estopinal, M.; Fox, K.; Kastl, P.; Olander, K.; Sharpe, E.; Shofner, R.; Stahl, J.; Stevenson, D.; Stewart, W.; Walters, T.; Gill, S.; Howes, J.; Lawson, C.; Smerick, M.; Zaccardelli, D.; Fazio, R.; Novack, G. D.; Crockett, R. S.; Hart, K.: Ophthalmology **105**, 1780 (1998)
30 Stewart, R.; Horwitz, B.; Howes, J.: J. Cataract Refr. Surg. **24**, 1480 (1998)
31 Abelson, M.; Howes, G.; George, M.: J. Ocul. Pharmacol. Ther. **14**, 533 (1998)
32 Noble, S.; Goa, K. L.: BioDrugs **10**, 329 (1998)
33 Caldwell, D.; Cohen, G. R.; Davis, J.; DeBarge, L. R.; Foley, J.; Foster, C. S.; Fox, K.; Friedlaender, M.; John, G.; Kaufman, A.; Nozik, R.; Olander, K.; Ostrov, C.; Raizman, M.; Rosenbaum, J.; Sall, K.; Sheppard, J.; Stewart, D.; Stewart, W.; Tauber, J.; Trocme, S.; Zimmerman, P.; Howes, J.; Coultas, S.; Crockett, R. S.; Novack, G. D.; Strahlman, E. R.; Neumann, R.: Am. J. Ophthalmol. **127**, 537 (1999)
34 Poppe, H.; Küsters, S.; Szelenyi, I.: Am. J. Respir. Crit. Care Med. **159/3** (Suppl.): A33 (1999)
35 Poppe, H.; Szelenyi, I.: Presented at XIIIth International Congress of Pharmacology (July 26–31, 1998) Munich, Germany
36 Bodor, N.; Prokai, L.; Wu, W.; Farag, H.; Jonnalagadda, S.; Kawamura, M.; Simpkins, J. W.: Science **257**, 1698 (1992)
37 Chen, P.; Bodor, N.; Wu, W.-M.; Prokai, L.: J. Med. Chem. **41**, 3773 (1998)
38 Bodor, N.: US Patent 5,639,885, June 17, (1997)
39 Prokai, L.; Ouyang, X-D.; Wu, W-M.; Bodor, N.: J. Am. Chem. Soc. **116**, 2643 (1994)
40 Yoon, S-H.; Wu, J.; Wu, W-M.; Prokai, L.; Bodor, N.: Biorg. Med. Chem., in press (2000).
41 Bodor, N.; Buchwald, P.; Huang, M.-J.: SAR QSAR Environ. Res. **8**, 41 (1998)
42 Bodor, N.; Buchwald, P.; Huang, M.-J.; in: Leszczynski, J. (Ed.): Computational Molecular Biology, Theoretical and Computational Chemistry, Vol. 8, p. 569, Elsevier, Amsterdam 1999
43 Buchwald, P.; Bodor, N.: J. Med. Chem. **42**, 5160 (1999)

Prof. Nicholas Bodor
Center for Drug Discovery
University of Florida
Box 100497
Gainesville, FL 32610–0497
USA
bodor@cop.ufl.edu