Soft Quaternary Anticholinergics: Comprehensive Quantitative Structure−Activity Relationship (QSAR) with a Linearized Biexponential (LinBiExp) Model

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A comprehensive quantitative structure−activity relationship (QSAR) study is presented for quaternary soft anticholinergics including two distinctly different classes designed on the basis of the soft analogue and the inactive metabolite approaches. Because of the clear biphasic (bilinear) nature of the activity data when all structures ($n = 76$) were considered as a function of molecular size (volume), a nonlinear model had to be used, and a linearized biexponential (LinBiExp) model proved very adequate. LinBiExp can fit activity data that show a maximum (or a minimum) around a given parameter value but tend to show linearity away from this turning point. Contrary to Hansch-type parabolic models, LinBiExp represents a natural extension of linear models, and a direct correspondence between its parameters and those obtained earlier by linear regression on compound subsets covering more limited parameter ranges could be easily established. Stereospecificity was confirmed as important, and the presence of an acid moiety was found to essentially eliminate activity. The consideration of bilinear behavior, which most likely results from size limitations at the binding site, can also explain the embarrassingly low activity found for a relatively large compound predicted as highly active by Lien, Ariëns, and co-workers based on their QSAR study.

Introduction

Muscarinic acetylcholine receptors (mAChRs) are a major class of metabotropic receptors that mediate the actions of acetylcholine (together with the ionotropic nicotinic receptors). Five (pharmacological[1,2] and molecular[3−6]) receptor subtypes, M_1−M_5, have been identified,[7] but the lack of selective agonists and highly selective antagonists has hampered the delineation of the physiological roles of these subtypes. However, with the availability of knockout mice[8] and newer generations of more specific antagonists,[9,10] this is likely to change rapidly, and there is already sufficient correlation among these molecular subtypes and pharmacological subtypes to warrant use of a unified M_1−M_5 notation.[7] Mutagenesis and docking studies using homology models are also beginning to unravel the binding requirements at these receptors, which are members of the rhodopsin-like family of G-protein-coupled seven-transmembrane receptors.[11,12] Muscarinic receptors are involved in the regulation of smooth muscle contraction, glandular secretion, modulation of heart rate and force, and CNS effects such as motor control, temperature and cardiovascular regulation, and memory. Smooth muscle contraction, gland secretion, pupil dilation, and food intake seem mainly mediated by M_3, whereas heart rate effects seem mainly mediated by M_2 subtypes.[7,8]

Muscarinic receptor antagonists inhibit the effects of acetylcholine by blocking its binding to muscarinic cholinergic receptors at neuroeffector sites on smooth muscle, cardiac muscle, and gland cells, as well as in peripherial ganglia and in the central nervous system (CNS); therefore, they are used or are of therapeutic interest for a variety of applications including treatment of asthma/COPD, prevention of motion sickness, mydriasis/cycloplegia, Alzheimer's and Parkinson's disease, and disorders of intestinal motility, cardiac function, and urinary bladder function.[13,14] Obviously, subtype selectivity, if achievable, can provide increased therapeutic advantage.[15] Because of their ability to inhibit local antisecretory activity, anticholinergics have even been explored as antiperspirants for excessive sweating.[16−20] There is renewed interest in these agents due to their applicability as inhaled bronchodilators for the treatment of bronchospasm associated with COPD and other diseases (tiotropium (**3**), ipratropium).[21] However, in many cases, their use is still limited by the possibility of a number of side effects, such as cardiac arrhythmias, tachycardia, dry mouth, difficulty in urination, constipation, photophobia, irritability, restlessness, disorientation, dementia, and hallucinations.[14,22] Even topically applied anticholinergics can cause unwanted side effects[23−31] because they are absorbed into the systemic circulatory system and are eliminated only relatively slowly. Muscarinic antagonists include the naturally occurring alkaloids of the belladonna plants such as atropine and scopolamine. The *Atropa belladona* (deadly nightshade) plant has been long, at least since Ancient Rome, used for poisoning (hence, its name Atropos, from the oldest of the three Fates, who cuts the thread of life) and by women to dilate their pupil (hence, its name belladona); the *Datura stramonium* (jimsonweed) plant has been smoked to treat asthma in India. Quaternary derivatives are usually more active, and they cannot cross the blood-brain barrier (BBB) and reach the CNS (usually an advantage because they are less likely to cause CNS-related side effects), but they also tend to be more poorly absorbed, which might cause problems in ensuring adequate bioavailability.

Soft anticholinergic agents designed (Figure 1) using the soft drug approach of the general retrometabolism-based drug design and targeting concept[32−34] can provide viable solutions for the problems faced during the development of local anticholinergics. Soft drugs are biologically active molecules, often isosteric/isoelectronic analogues of a lead compound, specifically designed to allow predictable metabolism into inactive metabolites after exerting the desired therapeutic effect. In most cases, soft drugs are expected to produce pharmacological activity

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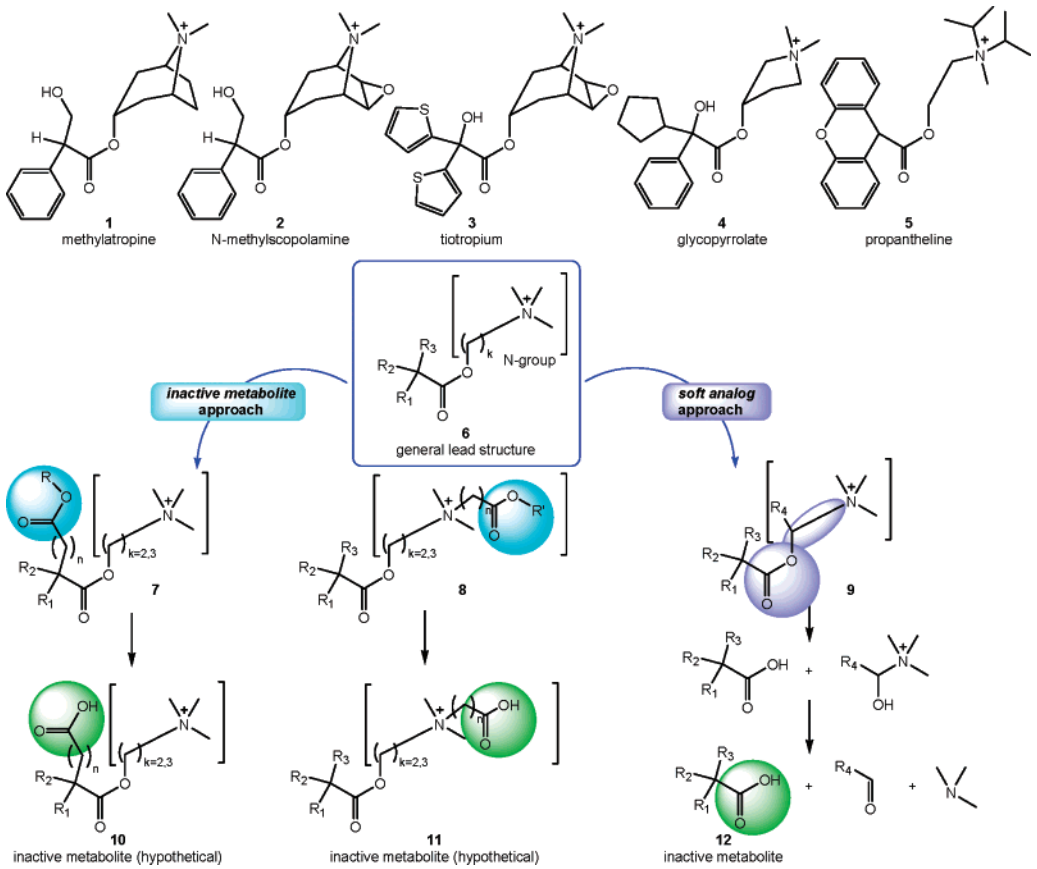

Figure 1. The general design concept of soft anticholinergics agents. One compound series (**9**) was designed using a soft analogue approach; two different compound series (**7** and **8**; note the differently positioned esters) were designed using the inactive metabolite approach, but they can all be traced back to a common, generalized lead (**6**), and they are all inactivated in a single hydrolytic step.

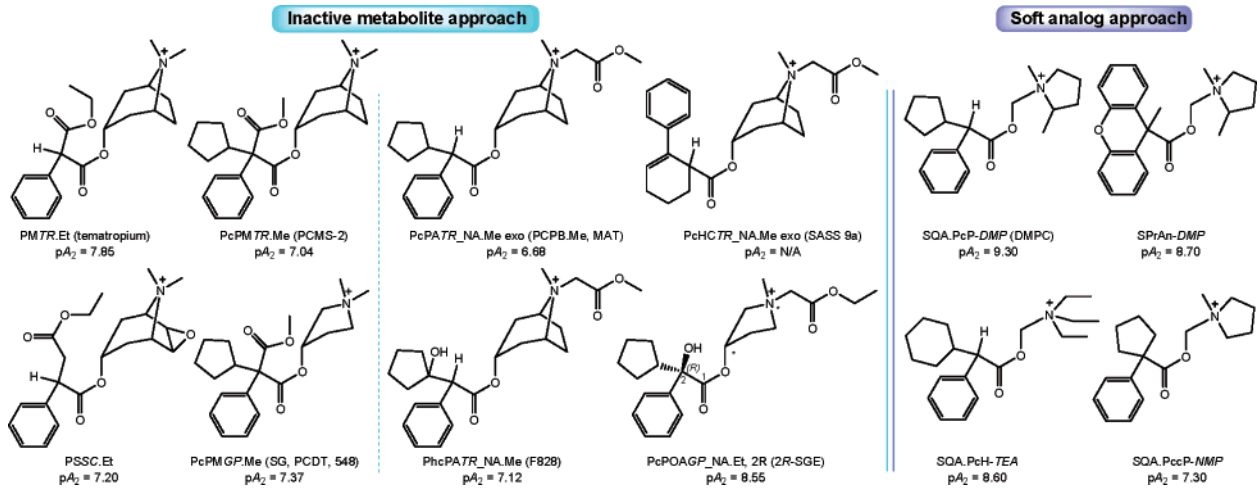

Figure 2. Representative structures for soft quaternary anticholinergics designed based on the inactive metabolite and the soft analogue approaches.

locally but be rapidly deactivated by metabolic (preferably hydrolytic) processes as they distribute away from their site of action to prevent any kind of undesired pharmacological activity or toxicity. Because a locally active soft anticholinergic, which has high local but practically no systemic activity, can provide a viable solution to many of the problems hindering the development of anticholinergic drugs, different series of soft anticholinergics based on methylatropine (**1**), *N*-methylscopolamine (**2**), glycopyrrolate (**4**), propantheline (**5**), or other lead structures were designed and tested during the last two-and-a-half decades (see refs 33 and 34 for detailed reviews).

Soft anticholinergics include two distinctly different classes designed on the basis of the soft analogue (**9**) and the inactive metabolite approaches (**7**) (Figures 1 and 2). Therefore, they include structures designed using either the soft analogue approach (soft quaternary anticholinergics,[35] soft propantheline analogues[36]) or the inactive metabolite-based approach (phenylmalonic analogues,[37−40] phenylsuccinic analogues,[41,42] phenylcyclopentyl atropine and glycopyrrolate analogues[40,43,44]). More recently, within the inactive metabolite-based class a set of different structures (**8**) with the hydrolytically labile ester moiety attached to the quaternary nitrogen head of the atropine- or glycopyrrolate-type ring structure have been synthesized and tested.[45−50]

Here, a comprehensive quantitative structure−activity relationship (QSAR) study based on activity data that include pA_2

values (the negative logarithm of the molar concentration of the antagonist that produces a 2-fold right shift in the concentration−response curve of the agonist) measured using the in vitro guinea pig ileum assay and receptor binding pK_i values for cloned muscarinic receptors (M_3 subtype) measured using experimental procedures described earlier[40,43] is presented. First, a classical multiple linear regression-based (MLR) approach is described for the larger inactive metabolite-based structures—an extension of our previous similar work that found activity to decrease linearly with molecular size. In a second step, this is extended into a more comprehensive relationship for a total of $n = 76$ structures including all quaternary soft anticholinergics; however, because of the clear biphasic (bilinear) nature of the activity data, this required the use of a nonlinear model. A recently introduced linearized biexponential (LinBiExp) model[51] proved very adequate for this purpose. LinBiExp can fit bilinear-type activity data, that is, data that show a maximum (or a minimum) around a given parameter value but tend to show good linearity away from this turning point. Contrary to Hansch-type parabolic models, LinBiExp represents a natural extension of linear models, and a direct correspondence between its parameters and those obtained by linear regression on subsets of the whole data can be easily established. Therefore, LinBiExp[51] was specifically selected from the many modeling possibilities not only because it provided a good description of the present extended dataset but also because, contrary to any other nonlinear model, it could be considered a direct extension of our previous linear models.

Results and Discussion

The general structures of the compounds included in the present comprehensive QSAR study are presented in Figure 1 with a few illustrative examples included in Figure 2; individual structures, activity data with the corresponding references, and structural descriptors/parameters used in the QSAR equation are summarized in detail in Table 1 of the Supporting Information. Because a large number of structures based on various design concepts have been synthesized, it is relatively difficult to summarize all of them using a single general structure even if all were designed starting from acid-containing inactive (hypothetical) metabolites of methylatropine (**1**), *N*-methylscopolamine (**2**), or glycopyrrolate (**4**). Nevertheless, structure **6** provides a good starting point with quaternary N-groups designated as TR, SC, and GP corresponding to those of methylatropine (**1**), *N*-methylscopolamine (**2**), and glycopyrrolate (**4**), respectively.

Linear Regression-Based Approach. A total of 55 quaternary soft anticholinergic structures designed using the inactive metabolite approach for which some experimental activity data were available are included in the MLR-based studies that cover two closely related anticholinergic activity data: pA_2 values determined using the in vitro guinea pig ileum assay and M_3 receptor binding pK_i values.

For these structures, a total of 46 pA_2 activity values for the guinea pig ileum assay were available; all but three have been used in deriving the final QSAR equation. Two structures that contained not one but two quaternary N-heads (PMTR·TR, PSTR·TR) and one that contained a noncyclic quaternary N-head (PcPMCH$_2$TMA, TMA = N(CH$_2$)$_3$) have been excluded; therefore, a total of 43 data points were used for the regression. Previous studies[43,52] already indicated molecular size, characterized by a computed effective van der Waals molecular volume (*V*), as having an important activity-determining role and showing good linearity: size alone accounted for approximately 70% of the variance in the pA_2 data of 28 structures.[43] With addition of newer structures, additional descriptors were needed to maintain the quality of the predictions, but size provided a good starting point. Analysis of the activity data revealed a number of structural features that influence activity, and corresponding indicator variables, I_*str*, were introduced into the model to account for their presence (I_*str* = 1 if *str* is present, 0 otherwise). Ultimately, the resulting model represents a sort of mixed approach that combines elements from both the extrathermodynamic (linear free energy relationship, LFER, or Hansch) approach and the additivity model (Free−Wilson) approach.[53] We preferred this approach, which relies on descriptors carefully created and selected after detailed comparisons and based on intimate knowledge of all involved chemical structures and their activity, even if it is more time-consuming than approaches using hundreds or thousands of software-generated, often physicochemically completely meaningless descriptors and automated selection processes. By this process, one can ensure that results (i) are mechanistically relevant, (ii) allow the formulation of quantitative hypotheses in terms familiar to medicinal chemists, (iii) can easily be applied in further structural designs, and (iv) are usually more suitable for a graphical presentation that allows visual conceptualization. We have recently successfully used such approaches to characterize corticosteroid receptor binding[54] and anticonvulsant activity of allosteric AMPA antagonists[55] and extend it now to the anticholinergic and receptor-binding data of the present set of compounds.

For the present set of compounds (Figure 2, Table 1 of Supporting Information), the following indicator variables have been introduced: I_acid for the presence of a carboxylic acid (−COOH), I_2R for enantiomerically pure 2*R* isomers, I_PS for succinic analogues where the carboxylic ester is one position away from the substitution center (R_3 = −CH$_2$COOR vs R_3 = −COOR in the malonic series), and I_cPe for the presence of a cyclopentyl substitution at the 2-position (as in glycopyrrolate). With these, we obtained the following equation by using an MLR approach that gives a good description of the data (Figure 3a):

$$\begin{aligned} pA_2 = {} & 15.115_{(\pm 0.878)} - 0.0260_{(\pm 0.0029)}V - \\ & 2.413_{(\pm 0.249)}\text{I\_acid} + 0.980_{(\pm 0.297)}\text{I\_2R} - \\ & 0.760_{(\pm 0.182)}\text{I\_PS} + 0.529_{(\pm 0.169)}\text{I\_cPe} \end{aligned}$$

$$n = 43, \quad r^2 = 0.833, \quad q^2_{\text{LO4GO}} = 0.755, \quad \sigma = 0.449, \quad F = 37.0 \quad (1)$$

In this equation, all terms are statistically highly significant, and as indicated by the value of the correlation coefficient (r^2 = 0.833), they account for more than 80% of the variability in the pA_2 data of the $n = 43$ compounds with a standard error of $\sigma = 0.449$. Activity clearly tends to decrease with increasing size, and the relationship seems linear to a very good extent. The obtained slope (-0.026 ± 0.003) is in excellent agreement with that obtained earlier[43] on a considerably smaller set and using no other structural descriptors (-0.030 ± 0.004), proving the consistency of this approach and the important, activity-determining role of molecular size. Because the correlation with lipophilicity descriptors (calculated log octanol−water partition coefficients, log *P*, such as CLOGP or QLogP) is much weaker (r^2 of 0.68 or 0.63 if used instead of *V* in eq 1), this is most likely due to size limitations at the receptor site indicating that these analogues are already somewhat larger than the ideal ligand size for these receptors.

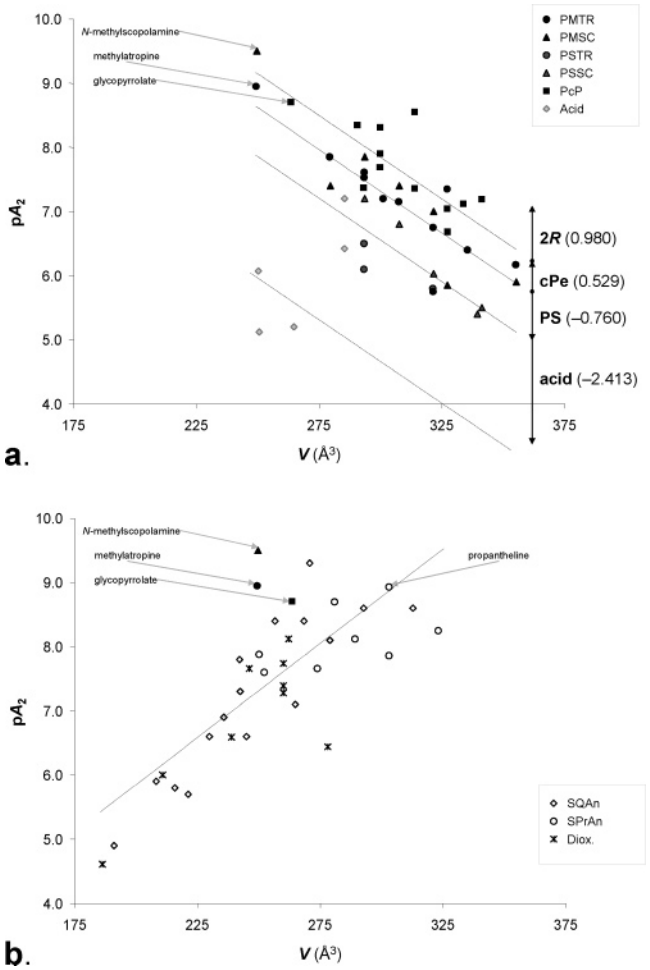

Figure 3. The pA_2 data for quaternary soft anticholinergics designed using the inactive metabolite (a) and the soft quaternary analogue approaches (b) shown as a function of calculated molecular volume *V*, a measure of three-dimensional molecular size. Both are well-described by linear trendlines, but they show opposing size dependencies. In panel a, parallel trendlines showing the effect of various structural indicator variables found to have statistically significant effects in the QSAR analysis (eq 2) are shown as different dashed lines.

The presence of an acid moiety strongly diminishes (essentially eliminates) activity: pA_2 decreases by about 3 orders of magnitude (maybe slightly less in glycopyrrolate-type structures). This is in excellent agreement with the main soft drug hypothesis used for their design because the acids are the designed-in metabolites formed by hydrolysis of the ester moieties that are the metabolically labile functionalities built into the structure of these soft drugs, and they are expected to be inactive ("inactive metabolite-based approach") to allow a facile, one-step deactivation. This has been experimentally confirmed in a total of five cases (PMTR·H, PMSC·H, PSTR·H, PcPOAGP_NA·H, and 2*R*-PcPOAGP_NA·H) where the acids were found to be, indeed, much (more than 1−2 orders of magnitude) less active than the corresponding esters.

Most of the activity seems to reside with the 2*R* isomer; only a relatively limited number of pure 2*R* enantiomers have been synthesized and tested until now ($n = 3$, including an acid), but they are clearly more active than the corresponding enantiomeric mixtures (these structures include a total of three chiral centers; hence, the separated 2*R* isomers have one resolved and two unresolved chiral centers and are in fact a mixture of four diastereoisomers; Figure 2).[48] The obtained almost 10-fold increase is, obviously, a result of an overemphasis resulting from the limited number of data; for a pure enantiomer, one would expect something around a 2-fold increase compared to the isomeric mixture (hence, a corresponding coefficient of only about $\log 2 = 0.3$) as long as the composition of the enantiomeric mixture is not heavily unbalanced and the isomers do not have opposing effects. It is well-known that stereospecificity is important at these receptors: improved anticholinergic activity is obtained if the absolute configuration of the $R_{1,2,3}$-substituted carbon is *R* for most substances (e.g., glycopyrrolate), which because of changes in the priority assignments corresponds to *S* for atropine and related structures (where $R_3 = CH_2OH$ replaces OH),[56,57] and this is nicely confirmed by the present data.

Introduction of a cyclopentyl moiety (I_cPe), which among the lead compounds of these designs is present in glycopyrrolate but absent in methatropine and *N*-methylscopolamine, seems to increase activity. However, because the overall unfavorable effect due to the increase in size produced by introduction of the cyclopentyl moiety (approximately -0.026×60 Å$^3 = -1.56$) is larger than the specific increase associated with the presence of a cyclopentyl ring, I_cPe (+0.53), cyclopentyl analogues tend to be less active than their unsubstituted analogues (e.g., PcPMTR·Et = PCMS1 vs PMTR·Et). Distancing the ester from the substitution center (succinic analogues) seems to decrease activity (I_PS). With the present quantitative approach, one can estimate these effects (after accounting for the size-related changes) as an approximate 3−4-fold increase and 5−6-fold decrease, respectively. Scopolamine analogues also seem slightly more active (Figure 3a) than corresponding atropine analogues, but the corresponding term was statistically not significant with the existing data.

Receptor Binding pK_i (M_3) Values. Compared to the pA_2 values, only a more limited number of receptor binding data is available ($n = 28$). QSAR results are presented here for the M_3 subtype, because this was expected to be most closely related to the pA_2 values and most relevant for the desired pharmacological activity of these compounds. The corresponding M_3 muscarinic receptor subtype is mainly responsible for smooth muscle contraction, and M_3/M_2 muscarinic receptor selectivity is usually a desired goal to prevent the M_2-mediated cardiac effects. As expected, pA_2 and pK_i values were found to be closely correlated ($r^2 = 0.722$, $n = 19$), and the corresponding slope has a value very close to unity (1.030 ± 0.155); therefore, one would expect to obtain a similar relationship here as well. Indeed, the same descriptors used earlier (with a slight modification) also give a somewhat less good, but reasonable description of the pK_i data:

$$\mathrm{p}K_i\,(M_3) = 13.946_{(\pm 1.260)} - 0.0201_{(\pm 0.0041)}V - 1.889_{(\pm 0.443)}\mathrm{I\_acid} + 1.340_{(\pm 0.363)}\mathrm{I\_2R} + 1.364_{(\pm 0.317)}\mathrm{I\_PcHC}$$

$$n = 28, \quad r^2 = 0.687, \quad q^2_{\mathrm{LO4GO}} = 0.493, \quad \sigma = 0.553, \quad F = 12.6 \quad (2)$$

For these data, the presence of a cyclopentyl substitution may still be slightly favorable, but it is no longer statistically significant, and therefore, the corresponding descriptor, I_cPe, was omitted. Another descriptor, I_PcHC, which denotes PcHCTR_NA·R′-type phenylcyclohexenecarbonic atropine analogues that showed slight subtype selectivity toward M_3 receptors[47] (and, hence, increased pK_i values), had to be included (pA_2 values were not available for these compounds). Otherwise, the correlation is slightly weaker (it accounts for close to 70%

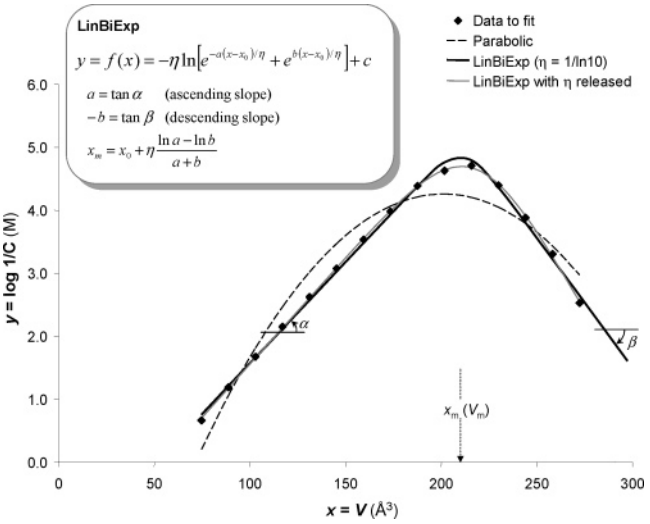

Figure 4. Illustration of the linearized biexponential (LinBiExp) model[51] as fitting the antimicrobial activity of straight-chain aliphatic amines (1-butanamine to 1-octadecanamine) against *Rhinocladium beurmanni*[71,72] as a function of calculated molecular volume *V*. In addition to the default LinBiExp model ($\eta = 1/(\ln 10) = 0.434$) for activity data on decimal logarithmic scale (e.g., $y = \log 1/C$), the model with η considered as an additional optimizable parameter is also shown (η released; thinner, gray line), together with the best parabolic model fitting the same data (dashed line), which is included to emphasize the clear bilinear nature of these data.

of the variability, compared to the slightly more than 80% of the previous equation) but is qualitatively about the same considering that fewer data are available, and all coefficients have qualitatively similar values indicating the consistency of the model. The size dependence is about the same: -0.020 ± 0.004 vs the -0.026 ± 0.003 slope obtained for the pA_2 data. The effects caused by the presence of an acid moiety and by having enantiomerically pure 2*R* isomers are also about the same as indicated by the corresponding coefficients in the two different equations.

Another isomerism also seems to have some effect on activity: among N-substituted atropine analogues, such as PcPATR_NA·Me[46] or PcHCTR_NA·Et,[47] exo isomers, which have the bridged ester substituent closer to the shorter, unsubstituted ring (and, hence, in equatorial position; Figure 2), seem slightly more active than the corresponding endo isomers, at least in receptor-binding studies. This is somewhat contrary to a previous observation that found better activity when the larger substituent was in the endo (axial) position.[58] Nevertheless, the corresponding effect is relatively small, and even if it is present in four compound pairs, it was not found as statistically significant and, therefore, was not included in the model.

LinBiExp. Extension of this study into a more comprehensive relationship covering all quaternary soft anticholinergics with available activity data was no longer possible within an MLR-based framework because of the clear nonlinear nature of the activity data. Only additional pA_2 data were available for the structurally smaller and historically earlier synthesized soft quaternary analogue structures (SQA, Table 1 of Supporting Information), but they showed a different type of behavior because here activity clearly seemed to increase with size (Figure 3b). Linear regression (with an imposed zero intercept because it was not significantly different from zero) results in

$$pA_2 = +0.029_{(\pm 0.001)} V$$
$$n = 36, \quad r^2 = 0.562, \quad \sigma = 0.796, \quad F = 45.0 \qquad (3)$$

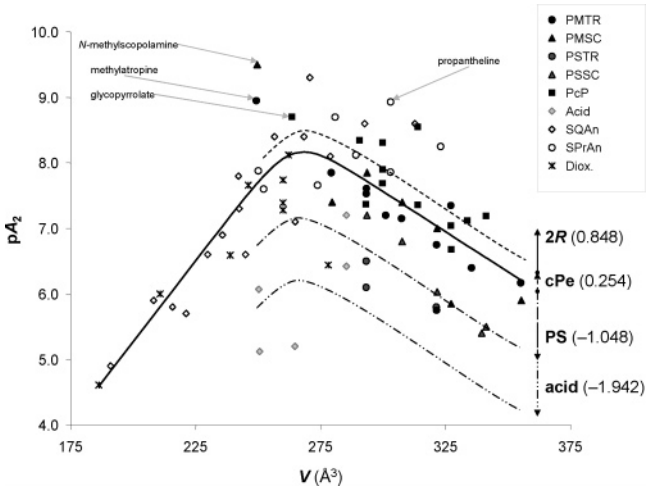

Figure 5. The pA_2 data of all quaternary soft anticholinergics shown as a function of *V* in a single graphic with the common trendline obtained from the linearized biexponential LinBiExp model, which allows a comprehensive description (eq 5).

Therefore, the combination of all data can be described only by a nonlinear model that allows the existence of a maximum. Excellent comprehensive description of all data could be achieved with the recently introduced linearized biexponential (LinBiExp) model, which allows the fit of activity data that show a maximum (or a minimum) around a given parameter value and essentially linear-type behavior away from this turning point.[51] The form of the model

$$f(x) = -\eta \ln[e^{-a(x-x_0)/\eta} + e^{b(x-x_0)/\eta}] + c \qquad (4)$$

[where e (e = 2.718...) denotes the base of the natural logarithm ($\ln x = \log_e x$), $\eta = 1/(\ln 10) = 0.4343$, and *a*, *b*, *c*, and x_0 are adjustable parameters; see Experimental Section and ref 51 for further details] is somewhat more complex than those of the linear models because it contains the logarithm of the sum of two exponentials, but it allows a very convenient extension of linear models *a* and *b* representing the ascending and descending slopes, respectively, and x_0 (here V_0) the turning point (essentially, the location of the extreme value, V_m) (Figure 4).

The indicator variables that were found as having statistically significant effect on activity in the MLR study (eq 1) were maintained within this approach as well. This way, for a total of 76 compounds with available experimental pA_2 data (Table 1), the LinBiExp model results in (Figure 5)

$$pA_2 = -0.434 \ln[e^{-a(V-V_0)/0.434} + e^{b(V-V_0)/0.434}] + c + d_{acid}\cdot I\_acid + d_{2R}\cdot I\_2R + d_{PS}\cdot I\_PS + d_{cPe}\cdot I\_cPe$$
$$a = 0.050_{(\pm 0.007)}, \quad b = 0.025_{(\pm 0.004)}, \quad c = 8.443_{(\pm 0.145)}, \quad V_0 = 264.1_{(\pm 5.2)}$$
$$d_{acid} = -1.942_{(\pm 0.317)}, \quad d_{2R} = 0.848_{(\pm 0.420)}, \quad d_{PS} = -1.048_{(\pm 0.244)}, \quad d_{cPe} = 0.254_{(\pm 0.225)}$$
$$n = 76, \quad r^2 = 0.698, \quad q^2_{LO4GO} = 0.620, \quad \sigma = 0.631 \qquad (5)$$

Hence, this equation, which uses size as the main descriptor, accounts for 70% of the variability in this large set of various anticholinergic structures. Note that maximum activity is predicted to occur with ligands having a size somewhere around $V_m = 268.2 \pm 4.1$ Å^3, a value very close to the size of the well-known anticholinergics methylatropine (249.3), *N*-methyl-

scopolamine (249.6), and glycopyrrolate (263.4), which, in fact, served as the lead structures of these designs. Because the correlation with lipophilicity descriptors is again much weaker (e.g., r^2 of 0.27 or 0.29 if CLOGP or QLogP is used instead of V in eq 5) and even a clear maximum is lacking, this is most likely due to size-related effects at the ligand binding site of the receptor: when the compound deviates too much from an ideal size, the fit is obviously either too loose or too tight. Unfortunately, currently no crystal structures of ligand-bound muscarinic acetylcholine receptors are yet available publicly to confirm this hypothesis; only a homology model has been published.[11]

This type of size dependence (Figure 5) is very much what one would expect. On one hand, for smaller ligands, size is a major determinant of activity, and activity (receptor binding) tends to increase linearly with size as nonspecific interactions (van der Waals forces) increase as the binding pocket is being better filled. On the other hand, for larger ligands, where the binding pocket is beginning to be filled up, activity decreases with size within a given congener series, but the series themselves tend to be somewhat scattered depending on how well they each fit. Hence, one trendline alone on the ascending portion gives adequate description, but a number of parallel trendlines are needed on the descending portion. Here, additional descriptors related to the presence of specific substituents, overall shape, and so on will play a more important role, and corresponding indicator variables are needed in the QSAR equation to account for the corresponding shifts among the trendlines. Obviously, size is only a nonspecific descriptor; with certain other structures, specific interactions such as additional hydrogen bonds may exist within the ligand-binding domain that cause significantly stronger (or weaker) activity. It is also very much possible that at some specific location(s), the ligand-binding domain has a side pocket(s) flexible enough to accommodate larger substituents, and therefore, some specific sets of larger structures may still exist for which activity does not decrease with size.

The ascending ($a = 0.050 \pm 0.007$) and descending ($-b = -0.025 \pm 0.004$) slopes of the overall LinBiExp model (eq 4) are in good agreement with the corresponding ascending and descending slopes obtained on the corresponding subsets of compounds of different sizes: 0.029 ± 0.001 for the smaller compounds consisting mainly of soft analogues (eq 3) and -0.026 ± 0.003 for the larger compounds consisting mainly of the inactive-metabolite-based analogues (eq 1). The ascending slopes are somewhat different, but this is mainly due to the existence of only relatively few truly small compounds, most being in the turning region where they are more scattered and, obviously, somewhat differently fit by the linear and the nonlinear model. Nevertheless, even this and especially the excellent agreement in the descending slopes provide a convenient consistency check and a nice confirmation of the validity of the approach, a main advantage of the LinBiExp model being that, under suitable conditions, it represents a natural extension of the linear approaches applicable only on more restricted parameter ranges on the left or right side of the turning point (maximum or minimum). Obviously, no such correspondences can be established with Hansch-type parabolic models, which are also entirely unsuitable to describe activity data that show unsymmetrical behavior (different ascending and descending slopes). For the present data, a parabolic model with the same four indicator variables gives a less adequate fit than the bilinear LinBiExp model (eq 5) according to model selection criteria such as the Akaike information criteria[59,60] (AIC, which increases to 275.0 for the parabolic model from 266.7 for the LinBiExp model) or the Schwarz Bayesian information criteria[61] (SBIC, which increases to 293.6 from 285.4), and the F test indicates the difference in the quality of fit to be statistically significant ($p = 0.007$).

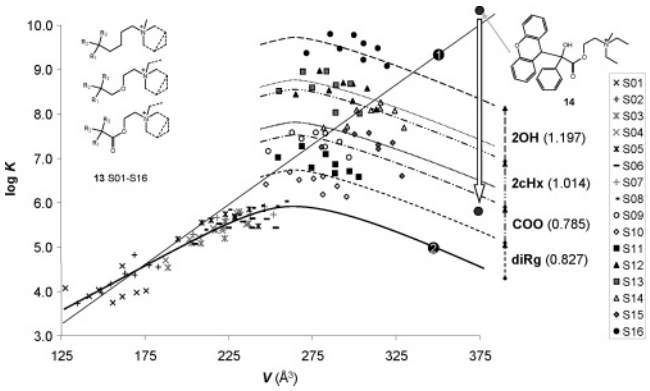

Figure 6. The log receptor binding affinity of the anticholinergic compounds **13** as determined by Abramson and co-workers[62] as a function of molecular volume. Structure **14** was predicted as highly active by Lien and co-workers[63] on the basis of their QSAR work as highly active, but turned out to be essentially inactive when synthesized by Lambrecht and co-workers[64] because of the biphasic size-dependence that is well-described by the LinBiExp model (eq 6).

Bilinear-Type Behavior—Additional Evidence. Further evidence for a bilinear behavior of the anticholinergics comes from activity data (log affinity constant data, log K = pK, for postganglionic muscarinic receptors from guinea pig ileum) of a large set of quaternary anticholinergics (**13**, Figure 6) determined by Abramson and co-workers[62] and used in a QSAR study by Lien, Ariëns, and Beld.[63] At first sight, activity seems to increase essentially linearly with size (trendline 1, Figure 6); however, a more detailed look clearly reveals that within each of the larger, double-ring substituted compounds (series S09−S16) a descending tendency is clearly present (each series having the same structure except for an increasing substituent at the quaternary N head). The largest of all these compounds (S16−8, 330 Å^3) is still just somewhat larger than propantheline (303.0 Å^3); nevertheless, a descending tendency is clearly present, even if not at very significant levels. In clear contrast, all smaller compounds show an increase in activity with increasing size. With a set of carefully selected medicinal chemical descriptors (indicator variables), an excellent description is obtained with LinBiExp (trendline 2, Figure 6):

$$\log K = -0.434 \ln[e^{-a(V-V_0)/0.434} + e^{b(V-V_0)/0.434}] + c + d_{2OH}\cdot I\_2OH + d_{2cHx}\cdot I\_2cHx + d_{COO}\cdot I\_COO + d_{diRng}\cdot I\_diRng$$

$$a = 0.020_{(\pm 0.001)},\quad b = 0.012_{(\pm 0.003)},\quad c = 6.177_{(\pm 0.113)},\quad V_0 = 255.0_{(\pm 8.8)}$$

$$d_{2OH} = 1.197_{(\pm 0.083)},\quad d_{2cHx} = 1.014_{(\pm 0.084)},\quad d_{COO} = 0.785_{(\pm 0.077)},\quad d_{diRng} = 0.827_{(\pm 0.140)}$$

$$n = 128,\quad r^2 = 0.975,\quad q^2_{LO4GO} = 0.967,\quad \sigma = 0.256 \quad (6)$$

Here, the I indicator variables denote the presence (1) or absence (0) of the following structural moieties: I_2OH a 2-hydroxy substituent, I_2cHx a 2-cyclohexyl substituent, I_COO an ester moiety, I_diRng two ring substituents (see the original publication[62] and Table 2 of Supporting Information

for detailed structural data and Figure 6 for representative structures). A 2-hydroxy substituent, which in addition to size was found to have the largest influence on activity (d_{2OH}), is a known requirement for anticholinergic activity in such structures. The structural rigidity provided by inclusion of an ester moiety or two ring substituents also seems beneficial for activity, and a cycloalkyl ring substituent at the 2 position also seemed beneficial in the previous study (I_cPe). Note the very close similarity of the V_0 (or more importantly the $V_{max} = 262.3 \pm 6.4$ Å^3) obtained here with that obtained in the soft anticholinergics series ($V_{max} = 268.2 \pm 4.1$ Å^3); clear evidence that "size matters" for receptor binding at these receptors.

The best evidence for bilinear behavior comes from the fact that a structure predicted by Lien and co-workers[63] based on their QSAR equations to be highly active turned out to be in fact very much inactive when actually synthesized a few years later by Lambrecht and co-workers.[64] The equations used by them, such as

$$\log K = 0.784_{(\pm 0.06)}\pi_R - 0.353_{(\pm 0.26)}(\pi_{-N^{\oplus}\equiv})^2 - 0.171_{(\pm 0.24)}(\pi_{-N^{\oplus}\equiv}) + 0.736_{(\pm 0.11)}\mu_R + 2.309_{(\pm 0.27)}n_{OH} + 2.173_{(\pm 0.29)}$$

$$n = 128, \quad r^2 = 0.924, \quad \sigma = 0.441, \quad F = 7.0 \qquad (7)$$

incorporated some nonlinearity (the π^2 term of the above equation), but their predictions were still highly size-correlated ($r^2 = 0.795$ with V). Therefore, they predicted a large compound (**14**, Figure 6, $V = 372$ Å^3), which was clearly outside their original parameter range and should have not been used for predictions, as highly active, expecting a pA_2 value in the 10−11 range. However, when actually synthesized a few years later,[64] the compound turned out to be essentially inactive (pA_2 of 5.5), in agreement with the hypothesis of bilinear behavior and the predictions of the LinBiExp model (Figure 6).

Finally, one needs to mention that for a set of nonquaternary anticholinergics with similar structural elements, size was also found as the main structural element determining activity, the activity increasing with increasing size ($n = 16$, $r^2 = 0.80$),[65] but this was a set whose largest member (TCPG, 293.8 Å^3) was just slightly larger than the V_m value obtained here (around 270 Å^3) or, for example, tiotropium (282.8 Å^3) and still smaller than propantheline (303.0 Å^3).

Conclusions

The present QSAR approach allowed us to assign quantitative values to the effect of structural substitutions that were known to have activity-influencing roles. For quaternary anticholinergics, size seems a major determinant of activity, and best activity is achieved with ligands not considerably smaller or larger than the known highly active anticholinergics such as *N*-methylscopolamine, glycopyrrolate, or tiotropium, which seem to be close to the ideal ligand size at these receptors. This behavior could be well described by the LinBiExp model employed. In agreement with soft drug design principles, acid metabolites are indeed essentially inactive: their activities being around 2 orders of magnitude less than those of the corresponding esters. The importance of stereospecificity at muscarinic receptors was also confirmed on this series of compounds: 2*R* analogues are considerably more active than the corresponding isomeric mixtures. The effect of other substitutions, such as inclusion of a scopolamine-type oxygen or a cycloalkyl ring, endo/exo isomerism, or distancing of the metabolically labile ester from the substitution center (phenylsuccinate vs phenylmalonate esters), could also be quantified by the use of the present QSAR approach.

Experimental Section

Data Collection and Molecular Modeling. In vitro activity data (guinea pig ileum assay pA_2 and M_3 receptor binding pK_i) were collected from our previous publications;[35−48,50] corresponding references are indicated for each compound in Table 1 of Supporting Information. In an attempt at systematization, a set of more-or-less descriptive names has also been assigned using this framework to all these quaternary soft anticholinergics; representative ones are as follows:

- •PMTR·R: phenylmalonic atropine analogues, *R* ester ($R_3 = -COOR$)
- •PSTR·R: phenylsuccinic atropine analogues, *R* ester ($R_3 = -CH_2COOR$)
- •PMSC·R: phenylmalonic scopolamine analogues, *R* ester ($R_3 = -COOR$)
- •PSSC·R: phenylsuccinic scopolamine analogues, *R* ester ($R_3 = -CH_2COOR$)
- •TMTR·R: thienylmalonic atropine analogues, *R* ester ($R_3 = -COOR$)
- •PcPMTR·R′: phenylcyclopentylmalonic atropine analogues, *R* ester ($R_3 = -COOR$)
- •PcPATR_NA·R′: phenylcyclopentylacetic atropine analogues, N−R′acetate ($R_3 = H$)
- •PhcPATR_NA·R′: phenyl(hydroxyl)cyclopentylacetic atropine analogues, N−R′acetate ($R_3 = H$)
- •PcPOAGP_NA·R′: phenylcyclopentylhydroxyacetic glycopyrrolate analogues, N−R′acetate ($R_3 = OH$)
- •PcHCTR_NA·R′: phenylcyclohexenecarbonic atropine analogues, N−R′acetate
- •SQA·$R_1R_2R_3$-Ngrp: soft quaternary analogs with Ngrp quaternary head
- •SPrAn-Ngrp: soft propantheline analogs with Ngrp quaternary head

A completely unrelated set of 1,3-dioxolane analogues[66] with similarly determined pA_2 values has also been included to show the generality of the present approach; they are denoted as DioxR_1R_2. For the additional, second set of compounds (Table 2 of Supporting Information), log affinity constants (log K = pK) were determined by Abramson and co-workers[62] and used here from the QSAR study of Lien and co-workers.[63] Molecular structures were built and optimized in ChemDraw/Chem3D (ChemOffice Ultra 7.0; CambridgeSoft, Cambridge, MA), and molecular weight, volumes, surface areas, and other descriptors of interest were calculated and compiled with an extension of the QLogP program as described previously.[55,67,68] All statistical analyses and multiple linear regressions were carried out using a standard spreadsheet program (Microsoft Excel 2000), and $p < 0.05$ was considered as statistically significant.

Linearized Biexponential Model. Fitting of bilinear-type data (i.e., data that have a maximum or minimum but tend to show linear behavior away from this extreme with the ascending and descending slopes not necessarily equal) was done with a general linearized biexponential (LinBiExp) model (Figure 4) based on eq 4 as its main equation.[51] In this equation, e (e = 2.718...) denotes the base of the natural logarithm ($\ln x = \log_e x$), and a, b, c, and x_0 are adjustable parameters. Natural logarithm and the corresponding canonical form of the exponential function, e^x, are used because they are the physically correct and mathematically most convenient forms. The a and b parameters characterize the ascending and descending slopes, respectively, and x_0 corresponds approximately to the position of the extreme (maximum) (Figure 4). Because the data to be characterized here, as most QSAR-type data, are on the decimal ($\log x = \log_{10} x$) and not the natural log scale, an $\eta = 1/(\ln 10) = 0.4343$ coefficient is used in the LinBiExp model to correctly scale the logarithm. Mathematical aspects of the model have been described elsewhere,[51] together with the main assumptions of the model, which was obtained by a differential equation-

based approach starting from very general assumptions that cover both static equilibria and first-order kinetic processes. Contrary to Hansch-type parabolic models, LinBiExp allows the natural extension of linear models and fitting of asymmetrical data. LinBiExp is also more general, more intuitive, and easier to implement for descriptors other than log *P* than Kubinyi's[69,70] bilinear model, which in fact corresponds to a special case of LinBiExp [$\eta = (a + b)/(\ln 10)$].[51] Because these models rely on different numbers of adjustable parameters (e.g., n_{par} equals 2, 3, and 4 for the linear, parabolic, and bilinear models, respectively), quantitative model selection criteria, such as the partial (sequential) *F*-test, the Akaike information criteria (AIC),[59,60] or the Schwarz Bayesian information criteria (SBIC),[61] have to be used to select the best-suited model; corresponding details have been described previously.[51] Fitting of the data, which requires nonlinear regression, was performed using custom-built models in WinNonlin Professional 4.0.1 (Pharsight Corp., Mountain View, CA).[60] The Gauss−Newton (Levenberg and Hartley) minimization algorithm was used with the convergence criteria set to 0.000 01, the increment for partial derivatives set to 0.001, and the number of iterations set to 50. User-provided initial parameter estimates and software-provided bounds were used. All fittings were done with unweighted data. All nonlinear fittings were also built and optimized in Microsoft Excel 2000 using a worksheet-based implementation and the Solver data analysis tool.[51] Numerical results were essentially identical; final values presented here are from the more detailed and more specific WinNonlin output.

QSAR models have also been validated with a leave-one-group-out (LOGO) cross-validation approach, as described previously.[51] Data sets were divided into four equal groups by using a random number generator with values between 1 and 4 and accepting the first distribution that gave groups with as close to equal sizes as possible (e.g., 11 + 11 + 11 + 10 for $n = 43$). Each one of these groups has been used alternatively as the test set to obtain predicted values with a model fitted on the training set formed by the remaining three groups (leave-one-of-four-groups-out, LO4GO). The q^2_{LO4GO} values used for cross-validation were calculated on the whole set of combined predicted values with the usual formula derived by analogy with the correlation coefficient, $q^2 = 1 - \sum_i (y_i - y_{i,pred})^2 / \sum_i (y_i - y_{mean})^2$.

Supporting Information Available: Tables with detailed structural and activity data for the QSAR studies discussed. This material is available free of charge via the Internet at http://pubs.acs.org.

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