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Pharmacological effects of some newly developed soft anticholinergics and a receptor-binding QSAR study

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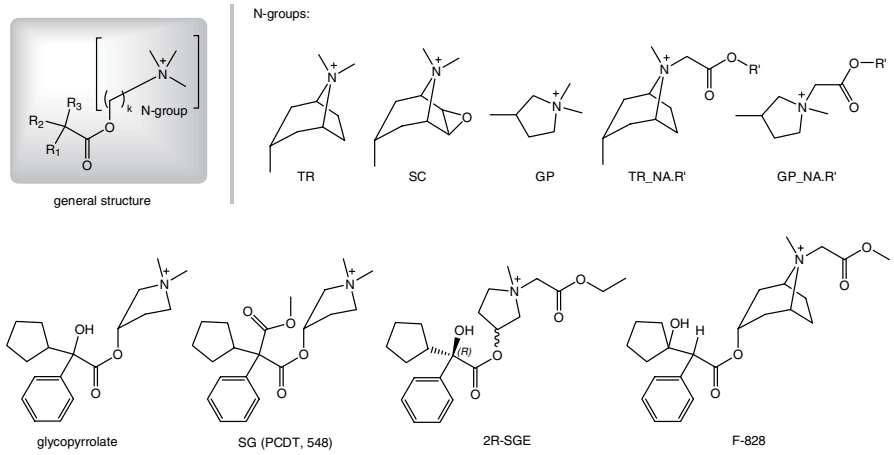
Receptor-binding studies using cloned human muscarinic receptors (M_1-M_4 subtypes) were performed on newly synthesized soft anticholinergics (F-828, F-838, SGM, SGE, SA-A) that are isosteric/isoelectronic analogs of glycopyrrolate. The receptor binding pK_i values of the new soft drugs were in the 5.5–9.5 range; with the majority being in the 7.0–8.5 range. As previously observed for similar structures, the pK_i values tended to decrease with increasing molecular size, and with the introduction of three structural indicator variables, a QSAR equation accounting for close to 75% of the variability could be established. Confirming the known stereospecificity of these receptors, pure 2*R* isomers were found more active than the corresponding isomeric mixtures. In agreement with soft drug design principles, acid metabolites (SA-A) were found considerably less active than their parent esters. The more active, 2*R* isomer of SA-A showed some muscarinic subtype selectivity (M_3/M_2), which was not observed for the parent compounds of this zwitterionic metabolite. Guinea pig ileum assay pA_2 values have also been determined, and they were found to be in good agreement with the pK_i values obtained from the binding study ($r^2 = 0.72$). SGM and SGE caused pupil-dilation in rabbit eyes, but their mydriatic effects lasted considerably shorter than that of glycopyrrolate, and they did not induce dilation of the pupil in the contralateral, water-treated eyes, indicating that they are locally active and safe, with a low potential to cause systemic side effects.

1. Introduction

Anticholinergics (muscarinic receptor antagonists) are frequently used therapeutic agents that inhibit the effects of acetylcholine by blocking its binding to muscarinic cholinergic receptors (Brown and Taylor 1996). They, however, can have numerous side effects including blurred vision, constipation, decreased sweating, disorientation, dizziness, drowsiness, dry mouth, hallucinations, irritability, nausea, photophobia, restlessness, urinary hesitancy and retention, tachycardia and cardiac arrhythmias, and severe allergic reactions (Ali-Melkillä et al. 1993; Brown and Taylor 1996), which limit their clinical use. Even topical anticholinergics can cause the same unwanted side effects (Birkhimer et al. 1984; Hamborg-Petersen et al. 1984; Osterholm and Camoriano 1982). Glycopyrrolate, one of the quaternary ammonium anticholinergics that have reduced CNS-related side effects as they cannot cross the blood-brain barrier, is eliminated mainly as unchanged drug or active metabolite in the urine; therefore, its administration is problematic in young or elderly patients and especially in uraemic patients (Ali-Melkillä et al. 1993; Franko et al. 1970; Franko et al. 1971; Kirvela et al. 1993; Mirakhur and Dundee 1983).

The soft drug approach (Bodor 1984; Bodor and Buchwald 2000) is a possible strategy to increase the therapeutic index of anticholinergics, and it has been applied in a number of different designs starting from various lead compounds (Brouillette et al. 1996; Hammer et al. 1988, 1991; Kumar and Bodor 1996; Kumar et al. 1993). Soft drugs are biologically active, therapeutically useful compounds (drugs) that are characterized by a predictable and controllable metabolism into nontoxic moieties after achieving their therapeutic role (Bodor 1984; Bodor and Buchwald 2000). By incorporating an adequate metabolically labile moiety into their structure, they can still act as potent and locally active agents (e.g., anticholinergics), but they will have only minimal systemic effects due to their rapid metabolism in the systemic circulation. Thus, the overall therapeutic index is greatly improved. Soft glycopyrrolate analogs have also been previously reported (Ji et al. 2000, 2002, 2005). The aims of the present study were (1) to examine the potency and subtype selectivity of existing and newly synthesized anticholinergics, (2) to examine the correlation of pK_i values with pA_2 values determined by the *in vitro* guinea pig ileum contraction method, (3) to investigate the quantitative structure-activity relationship (QSAR) of these soft anticholinergic agents, and (4) to examine the mydriatic effect of soft analogs in rabbit eyes.

Table 1: Structures of the soft glycopyrrolate analogs 4–13



No.	Name	Formula	R_1	R_2	R_3	N-group
1	Glycopyrrolate	$C_{19}H_{28}N_1O_3^+$	Ph	cPe	OH	GP
2	Methylatropine	$C_{18}H_{26}N_1O_3^+$	Ph	H	CH_2OH	TR
3	*N*-Methylscopolamine	$C_{18}H_{24}N_1O_4^+$	Ph	H	CH_2OH	SC
4	SG (PCDT, 548)	$C_{21}H_{30}N_1O_4^+$	Ph	cPe	COO−Me	GP
5	SGA (PCTM, 544)	$C_{20}H_{30}N_1O_4^+$	Ph	cPe	COO−Me	CH_2−TMA
6	F-828	$C_{24}H_{34}N_1O_5^+$	Ph	cPe, 1-OH	H	TR, N−CH_2COO−Me
7	F-838	$C_{21}H_{30}N_1O_5^+$	Ph	cPe, 1-OH	H	GP, N−CH_2COO−Me
8	SGM	$C_{21}H_{30}N_1O_5^+$	Ph	cPe	OH	GP, N−CH_2COO−Me
9	2*R*-(+)SGM	$C_{21}H_{30}N_1O_5^+$	Ph	cPe	OH	GP 2*R*, N-CH_2COO−Me
10	SGE	$C_{22}H_{32}N_1O_5^+$	Ph	cPe	OH	GP, N−CH_2COO−Et
11	2*R*-(+)SGE	$C_{22}H_{32}N_1O_5^+$	Ph	cPe	OH	GP 2*R*, N-CH_2COO−Et
12	SA-A	$C_{20}H_{28}N_1O_5^+$	Ph	cPe	OH	GP, N−CH_2COOH
13	2*R*-(+)SA-A	$C_{20}H_{28}N_1O_5^+$	Ph	cPe	OH	GP 2*R*, N−CH_2COOH

2. Investigations, results and discussion

2.1. Receptor binding studies

Receptor binding affinities determined by radioligand binding assays using human cloned muscarinic receptor subtypes M_1−M_4 are presented in Table 2. pK_i Values of the new soft drugs were in the 5.5−9.5 range, with the majority being in the 7.0−8.5 range. As expected, the racemic forms, SGM and SGE, showed lower receptor binding affinities than their corresponding 2*R* isomers, confirming that stereospecificity is important at these receptors (Barlow et al. 1973; Pauling and Datta 1980). In agreement with soft drug design principles ("inactive metabolite-based approach"), acid metabolites (SA-A) were found considerably less active than their parent esters. The more active 2*R* isomer of SA-A showed some muscarinic subtype selectivity (M_3/M_2), which was not observed for the parent compounds of this zwitterionic metabolite.

2.2. pA_2 Studies

The pA_2 values determined from guinea pig ileum contraction assays are a classical functional study of anticholinergic affinity (at M_3 muscarinic receptors) (Cheng and Prusoff 1973). They represent the negative logarithm of the molar concentration of the antagonist that produces a two-

Table 2: Receptor binding affinities and pA_2 values

Compd.	Subtypes of cloned human muscarinic receptors[a]				pA_2[b]
	M_1	M_2	M_3	M_4	
1	9.76 ± 0.05 (1.37 ± 0.20)	9.19 ± 0.18 (0.99 ± 0.11)	8.73 ± 0.05 (1.14 ± 0.25)	9.90 ± 0.08 (1.02 ± 0.01)	8.57 ± 0.12
3	9.69 ± 0.01 (0.92 ± 0.10)	9.18 ± 0.21 (1.02 ± 0.02)	9.29 ± 0.12 (1.07 ± 0.01)	9.92 ± 0.21 (0.90 ± 0.04)	9.16 ± 0.19
4	7.54 ± 0.05 (0.98 ± 0.11)	6.95 ± 0.02 (1.02 ± 0.11)	7.81 ± 0.01 (1.03 ± 0.04)	8.02 ± 0.02 (0.87 ± 0.06)	7.37 ± 0.11
5	6.62 ± 0.12 (0.93 ± 0.03)	6.54 ± 0.20 (1.04 ±0.08)	6.46 ± 0.29 (0.90 ± 0.03)	6.84 ± 0.21 (0.88 ± 0.14)	6.72 ± 0.14
6	7.10 ± 0.13 (0.78 ± 0.11)	6.81 ± 0.09 (0.88 ± 0.07)	6.89 ± 0.18 (1.05 ± 0.10)	7.45 ± 0.01 (0.96 ± 0.03)	7.12 ± 0.11
7	7.52 ± 0.03 (1.09 ± 0.16)	7.63 ± 0.02 (1.09 ± 0.03)	6.98 ± 0.06 (1.02 ± 0.01)	8.18 ± 0.07 (1.15 ± 0.07)	7.69 ± 0.16
8	7.91 ± 0.05 (1.02 ± 0.12)	7.79 ± 0.11 (1.25 ± 0.08)	7.80 ± 0.10 (1.17 ± 0.18)	8.29 ± 0.19 (1.12 ± 0.05)	7.90 ± 0.13
9	8.89 ± 0.04 (0.83 ± 0.11)	8.87 ± 0.05 (1.10 ± 0.11)	9.00 ± 0.06 (0.83 ± 0.01)	9.52 ± 0.01 (0.83 ± 0.01)	8.31 ± 0.05
10	7.51 ± 0.17 (0.91 ± 0.09)	7.32 ± 0.07 (1.23 ± 0.06)	7.54 ± 0.15 (1.18 ± 0.08)	7.94 ± 0.09 (1.18 ± 0.09)	7.36 ± 0.34
11	8.67 ± 0.16 (0.86 ± 0.08)	8.84 ± 0.34 (0.92 ± 0.01)	8.74 ± 0.02 (1.09 ± 0.15)	8.85 ± 0.13 (0.89 ± 0.02)	8.55 ± 0.16
12	6.19 ± 0.06 (1.11 ± 0.06)	5.48 ± 0.13 (1.02 ± 0.20)	5.84 ± 0.07 (1.01 ± 0.07)	6.44 ± 0.06 (0.84 ± 0.06)	6.42 ± 0.30
13	8.11 ± 0.16 (1.12 ± 0.25)	7.48 ± 0.12 (0.95 ± 0.11)	8.12 ± 0.10 (0.80 ± 0.01)	8.23 ± 0.12 (1.02 ± 0.10)	7.20 ± 0.19

[a] The affinity estimates, the negative logarithm of K_i, were derived from [^{3}H]NMS displacement experiments. Data represent mean ± SD of 3 experiments. The numbers in parentheses denote Hill slopes. [b] pA_2 values were determined on 4–6 ileum strips obtained from different animals. Data represent mean ± SD

Table 3: Anticholinergic structures included in the present receptor-binding QSAR study

No.	Name	Formula	R_1	R_2	R_3	N-group	Vol	ac.	2R	PcH	pA_2	M_3	Ref.
1	Glycopyrrolate	$C_{19}H_{28}N_1O_3^+$	Ph	cPe	OH	GP	263.41	0	0	0	8.57	8.73	
2	Methylatropine	$C_{18}H_{26}N_1O_3^+$	Ph	H	CH_2OH	TR	249.30	0	0	0	8.95	9.15	(Kumar et al. 1993)
3	*N*-Methylscopolamine	$C_{18}H_{24}N_1O_4^+$	Ph	H	CH_2OH	SC	249.64	0	0	0	9.16	9.29	
4	SG (PCDT, 548)	$C_{21}H_{30}N_1O_4^+$	Ph	cPe	COO−Me	GP	293.07	0	0	0	7.37	7.81	(Ji et al. 2000)
5	SGA (PCTM, 544)	$C_{20}H_{30}N_1O_4^+$	Ph	cPe	COO−Me	CH_2-TMA	287.17	0	0	0	6.72	6.46	(Ji et al. 2000)
6	F-828	$C_{24}H_{34}N_1O_5^+$	Ph	cPe, 1-OH	H	TR, N−CH_2COO−Me	333.71	0	0	0	7.12	6.89	
7	F-838	$C_{21}H_{30}N_1O_5^+$	Ph	cPe, 1-OH	H	GP, N−CH_2COO−Me	299.72	0	0	0	7.69	6.98	
8	SGM	$C_{21}H_{30}N_1O_5^+$	Ph	cPe	OH	GP, N−CH_2COO−Me	299.77	0	0	0	7.90	7.80	
9	2R-SGM	$C_{21}H_{30}N_1O_5^+$	Ph	cPe	OH	GP 2R, N−CH_2COO−Me	299.77	0	1	0	8.31	9.00	
10	SGE	$C_{22}H_{32}N_1O_5^+$	Ph	cPe	OH	GP, N−CH_2COO−Et	313.85	0	0	0	7.36	7.54	
11	2R-SGE	$C_{22}H_{32}N_1O_5^+$	Ph	cPe	OH	GP 2R, N−CH_2COO−Et	313.85	0	1	0	8.55	8.74	
12	SA-A	$C_{20}H_{28}N_1O_5^+$	Ph	cPe	OH	GP, N−CH_2COOH	285.30	1	0	0	6.42	5.84	
13	2R-SA-A	$C_{20}H_{28}N_1O_5^+$	Ph	cPe	OH	GP 2R, N−CH_2COOH	285.30	1	1	0	7.20	8.12	
14	PMTR.Et (tematropium)	$C_{20}H_{28}N_1O_4^+$	Ph	H	COO−Et	TR	279.25	0	0	0	7.85	8.16	(Hammer et al. 1988)
15	PMTR.Hx	$C_{24}H_{36}N_1O_4^+$	Ph	H	COO−Hx	TR	335.37	0	0	0	6.40	6.82	(Kumar et al. 1993)
16	PMTR.cHx	$C_{24}H_{34}N_1O_4^+$	Ph	H	COO−cHx	TR	327.06	0	0	0	7.35	7.39	(Hammer et al. 1988)
17	PMTR.MeSOMe	$C_{20}H_{28}N_1O_5S_1^+$	Ph	H	COO−MeSOMe	TR	301.04	0	0	0	7.20	7.02	(Huang et al. 2001)
18	TMTR.Et (52−21)	$C_{18}H_{26}N_1O_4S_1^+$	Th	H	COO−Et	TR	272.79	0	0	0	n/a	7.73	(Huang et al. 2001)
19	TMTR.iPr (52−19)	$C_{19}H_{28}N_1O_4S_1^+$	Th	H	COO−iPr	TR	286.88	0	0	0	n/a	8.05	(Huang et al. 2001)
20	PcPMTR.Me (PCMS-2)	$C_{24}H_{34}N_1O_4^+$	Ph	cPe	COO−Me	TR	327.06	0	0	0	7.04	7.32	(Juhász et al. 1998)
21	PcPMTR.Et (PCMS-1)	$C_{25}H_{36}N_1O_4^+$	Ph	cPe	COO−Et	TR	341.14	0	0	0	7.19	8.18	(Juhász et al. 1998)
22	PCPA.Me	$C_{24}H_{34}N_1O_4^+$	Ph	cPe	H	TR, N−CH_2COO−Me	327.26	0	0	0	n/a	7.51	(Huang et al. 2001; Huang et al. 2002)
23	PCPB.Me	$C_{24}H_{34}N_1O_4^+$	Ph	cPe	H	TR, N−CH_2COO−Me	327.26	0	0	0	6.68	7.75	(Huang et al. 2001; Huang et al. 2002)
24	PCPA.Et	$C_{25}H_{36}N_1O_4^+$	Ph	cPe	H	TR, N−CH_2COO−Et	341.36	0	0	0	n/a	7.21	(Huang et al. 2001; Huang et al. 2002)
25	PCPB.Et	$C_{25}H_{36}N_1O_4^+$	Ph	cPe	H	TR, N−CH_2COO−Et	341.36	0	0	0	n/a	7.57	(Huang et al. 2001; Huang et al. 2002)
26	PCHA.Me (SASS13a)	$C_{24}H_{32}N_1O_4^+$	cHx=, Ph		H	TR, N−CH_2COO−Me	321.79	0	0	1	n/a	8.49	(Huang et al. 2003)
27	PCHB.Me (SASS9a)	$C_{24}H_{32}N_1O_4^+$	cHx=, Ph		H	TR, N−CH_2COO−Me	321.79	0	0	1	n/a	8.99	(Huang et al. 2003)
28	PCHA.Et (SASS13b)	$C_{25}H_{34}N_1O_4^+$	cHx=, Ph		H	TR, N−CH_2COO−Me	335.90	0	0	1	n/a	8.62	(Huang et al. 2003)
29	PCHB.Et (SASS9b)	$C_{25}H_{34}N_1O_4^+$	cHx=, Ph		H	TR, N−CH_2COO−Me	335.90	0	0	1	n/a	8.64	(Huang et al. 2003)

Abbreviations. Me: methyl, Et: ethyl, Pr: propyl, iPr: isopropyl, Bu: butyl, Pe: pentyl, cPe: cyclopentyl, Hx: hexyl, cHx: cyclohexyl, cHx=: cyclohexenyl, Ph: phenyl, Th: thienyl, TMA: trimethylamine

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fold shift to the right in an agonist's concentration-response curve. For the soft anticholinergics of the present study, the pA_2 values obtained from ileum longitudinal contractions by using carbachol as agonist with the method of van Rossum (1963) (Table 2) were, in general, comparable to the pK_i values obtained in the M_3 receptor binding studies.

2.3. Receptor binding QSAR

QSAR results are presented here for the receptor binding affinity pK_i data at the M_3 subtype (Table 3), as this was expected to be 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. Of course, pA_2 and pK_i values are expected to be closely correlated, and this was indeed confirmed by the present data as well ($r^2 = 0.724$, slope = 0.966, n = 19, Fig. 1).

To obtain meaningful QSAR equations it is desirable to have as many data points as possible; therefore, previously published pK_i values of related soft anticholinergics designed based on the inactive metabolite approach were also included (Table 3). This way, a total of 28 pK_i data were available. Data from two structures that contained not one, but two quaternary *N*-heads (PMTR.TR, PSTR.TR) and one that contained a non-cyclic quaternary *N*-head (SGA) have not been included in the analysis.

Previous studies (Juhász et al. 1998; Kumar et al. 1994), already indicated molecular size, characterized by a computed effective van der Waals molecular volume (V), as having an important activity-determining role: size alone accounted for approximately 70% of the variance in the pA_2 data of 28 structures (Juhász et al. 1998). With addition of newer structures, including pure 2*R* enantiomers, 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). We have recently successfully used such approaches to characterize corticosteroid receptor binding (Buchwald and Bodor 2004) and anticonvulsant activity of allosteric AMPA antagonists (Buchwald et al. 2005), and extend it now to the anticholinergic and receptor-binding data of the present set of compounds.

Following a detailed analysis, for the present set of compounds (Table 3), the following indicator variables have been found as having statistically significant effect on receptor-binding activity: I_acid for the presence of a carboxylic acid (*−COOH*), I_2R for enantiomerically pure 2*R* isomers, and I_PcH for phenylcyclohexenecarbonic atropine analogs that showed slight subtype selectivity toward m_3 receptors (Huang et al. 2003) (and, hence, increased pK_i values). With these descriptors, multiple linear regression yielded the following equation:

$$\begin{aligned} pK_i(M_3) = {} & 12.858_{(\pm 1.037)} \\ & - 0.0168_{(\pm 0.0034)}\, V - 1.778_{(\pm 0.365)}\, I\_acid \\ & + 1.391_{(\pm 0.299)}\, I\_2R + 1.354_{(\pm 0.261)}\, I\_PcH \\ & n = 28, \quad r^2 = 0.742, \quad \sigma = 0.455, \\ & F = 16.5 \end{aligned} \tag{1}$$

In this equation, all terms are statistically highly significant ($p < 0.01$), and as indicated by the value of the correlation coefficient ($r^2 = 0.742$), they account for close to 75% of the variability in the pK_i data of the n = 28 compounds with a standard error of $\sigma = 0.455$. In agreement with previous observations (Juhász et al. 1998), activity clearly tends to decrease with increasing size, and the relationship seems linear to a very good extent (Fig. 2). As the correlation with lipophilicity descriptors (calculated log octanol-water parti-

Table 4: Maximum response (R_{max},% change in pupil size at 1 h after administration) and area under the response-time curve (AUC)

Compound, conc.	R_{max},%	$AUC_{0-144\,h}$
6 0.5%	23.83 ± 4.33	60 ± 14
6 1%	47.64 ± 5.00	163 ± 21
7 0.5%	34.67 ± 10.01	93 ± 57
7 1%	54.17 ± 4.33	232 ± 39
8 0.5%	45.83 ± 4.81	185 ± 35
8 1%	59.58 ± 15.72	467 ± 114
9 0.5%	52.92 ± 13.41	677 ± 215
9 1%	57.08 ± 11.66	745 ± 171
10 0.5%	58.33 ± 12.27	645 ± 409
10 1%	54.65 ± 13.99	596 ± 274
11 0.5%	53.96 ± 13.27	1170 ± 308
11 1%	56.04 ± 11.69	1532 ± 526
1 0.05%	51.46 ± 7.71	2779 ± 443
1 0.1%	55.83 ± 6.42	4074 ± 459
1 0.2%	56.04 ± 10.10	5047 ± 1631

Data indicate mean ± SD of four trials

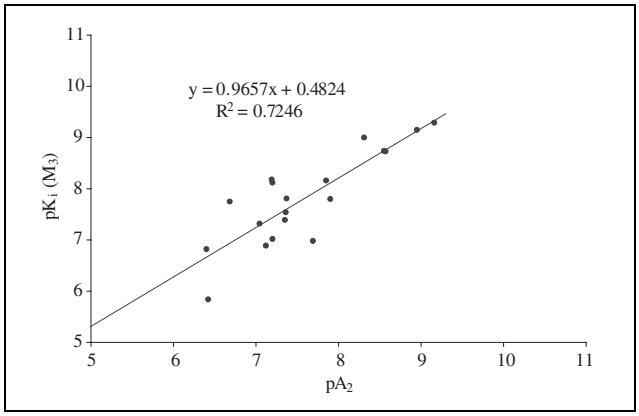

Fig. 1: Correlation between guinea pig ileum assay pA_2 and receptor binding pK_i (M_3) data for the soft anticholinergics of the present study

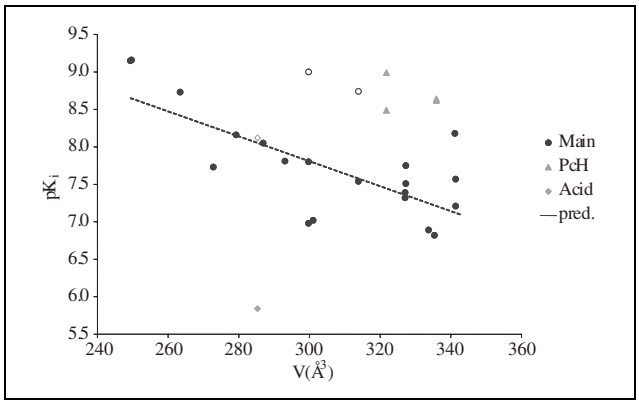

Fig. 2: Correlation between pK_i (M_3) values and calculated molecular volumes. Open symbols denote pure 2*R* isomers. Calculated values showing the size-dependence for the main series (pred.) are also shown as a dashed line

tion coefficients such as CLOGP or QLogP) is much weaker, this is most likely due to size-limitations at the receptor site indicating that these analogs are already somewhat larger than the ideal ligand size for these receptors.

The presence of an acid moiety strongly diminishes (essentially eliminates) activity: pA_2 decreases with close to two orders of magnitude. This is in excellent agreement with the soft drug hypothesis as 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 the soft drugs, and they are expected to be inactive ("inactive metabolite-based approach") (Bodor and Buchwald 2000, 2003).

Most of the activity seems to reside with the 2*R* isomer; only a relatively limited number of pure 2*R* enantiomers have been tested yet (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 (Ji et al. 2005). The obtained about ten-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 two-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. 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*) (Barlow et al. 1973; Pauling and Datta 1980), and this is nicely confirmed by the present data.

2.4. Mydriatic activity

The mydriatic effects of the soft analogs were compared to those of glycopyrrolate in a rabbit model. Mydriatic responses were recorded at appropriate time-intervals after the administration of the drugs as% changes in pupil size. Maximum response (R_{max},% change in pupil size at 1 h after administration) and area under the response-time curve (AUC^{eff}) are shown in Table 4. Compound **6** (F-828) showed the lowest mydriasis potency followed by **7** (F-838) and **8** (SGM). Soft ethyl analogs seem somewhat more potent than corresponding methyl analogs, and 2*R* isomers seem more potent than corresponding isomeric mixtures. As expected for such soft drugs, their durations of action are much shorter than that of the "hard" glycopyrrolate as illustrated for pharmacodynamically equipotent doses in Fig. 3. The mydriatic activity of 2*R*-SGM, 2*R*-SGE, and glycopyrrolate lasted for 24, <48, and 144 h, respectively, indicating that the soft analogs are easily hydrolyzed and rapidly eliminated from the body after the desired pharmacological effect is achieved. In agreement with this and unlike other traditional anticholinergics, these soft drugs did not induce dilation of the pupil in the contralateral (water-treated) eye, indicating no or just low systemic side-effects. Therefore, these compounds are safe, promising short acting anticholinergics with the possibility of largely reduced unwanted side effects.

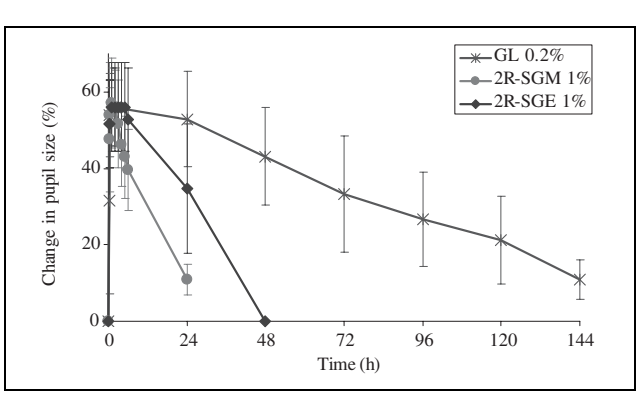

Fig. 3: Mydriatic activities of glycopyrrolate and soft analogs at pharmacodynamically equipotent doses

2.5. Conclusions

In conclusion, a QSAR equation that allowed us to assign quantitative values to the effect of structural substitutions known to have activity-influencing roles has been established. For this series of soft anticholinergics, size is a major determinant of activity, and activity decreases with increasing size possibly indicating that these compounds are already somewhat larger than the ideal ligand size for this receptor site, which seems to be around that of methatropine or glycopyrrolate. In agreement with soft drug design principles, acid metabolites were found essentially inactive. The importance of stereo-specificity at muscarinic receptors was also confirmed as 2*R* analogs were considerably more active than the corresponding isomeric mixtures. Mydriatic studies in rabbit eyes suggest that these soft analogs are somewhat less potent than glycopyrrolate and that their durations of actions are much shorter, indicating that these compounds could provide safe and effective solutions for clinical conditions that require short-lasting anticholinergic effects.

3. Experimental

3.1. Materials

The soft glycopyrrolate analogs of these studies (**6**–**11**) have been synthesized in our laboratories and characterized by NMR and elemental analysis as reported previously (Ji et al. 2005). The zwitterionic (acid) metabolites (**12**, **13**) were obtained by chemical hydrolysis of their parent esters as reported previously (Wu et al. 2005).

3.2. Receptor binding affinity

Receptor binding studies on the present soft drugs, glycopyrrolate, and *N*-methylscopolamine were performed with *N*-[^{3}H]-methyl-scopolamine (NMS) in assay buffer (phosphate-buffered saline, PBS, without Ca^{++} or Mg^{++}, pH 7.4), following the protocol from Applied Cell Science Inc. (Rockville, MD). A 10 mM NaF solution was added to the buffer as an esterase inhibitor. The assay mixture (0.2 ml) contained 20 µl diluted receptor membranes (receptor proteins: M_1, 38 µg/ml; M_2, 55 µg/ml; M_3, 27 µg/ml; M_4, 84 µg/ml). The final concentration of NMS for the binding studies was 0.5 nM. Specific binding was defined as the difference in [^{3}H]NMS binding in the absence and presence of 5 µM atropine for M_1 and M_2 or 1 µM atropine for M_3 and M_4. Incubation was carried out at room temperature for 120 min. The assay was terminated by filtration through a Whatman GF/C filter (presoaked overnight with 0.5% polyethyleneimine). The filter was then washed six times with 1 ml ice cold buffer (50 mM Tris-HCl, pH 7.8, 0.9% NaCl), transferred to vials, and 5 ml of Scintiverse was added. Detection was performed on a Packard 31800 liquid scintillation analyzer (Packard Instrument Inc., Downer Grove, IL). Data obtained from the binding experiments were fitted to the

$$\%[^3H]NMS\ bound = 100 - [100x^n/k/(1 + x^n/k)]$$

equation, to obtain the Hill coefficient n, and then to

$$\%[^3H]NMS\ bound = 100 - [100x^n/IC_{50}/(1 + x^n/IC_{50})]$$

to obtain the IC_{50} values (x being the concentration of the tested compound). Based on the method of Cheng and Prusoff (1973), K_i was derived from the equation $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration

of the radioligand. IC_{50} represents the concentration of the drug causing 50% inhibition of specific radioligand binding, and K_d represents the dissociation constant of the radioligand receptor complex. Data were analyzed by a non-linear least-square curve-fitting procedure using Scientist software (MicroMath Inc., Salt Lake City, UT).

3.3. Determination of pA_2 values

Male guinea pigs obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN) and weighing about 400 g were used after overnight fasting. Animals were sacrificed by decapitation, and the ileum (the region of 5 cm upward of the cecum) was isolated and removed. The ileum was cut into 2.5 cm pieces and suspended in an organ bath containing 30 ml of mixture of Tyrode's solution and 0.1 mM hexamethonium bromide. The organ bath was constantly aerated with oxygen and kept at 37 °C. One end of the ileum strip was attached to a fixed support at the bottom of the organ bath, and the other end to an isometric force transducer (Model TRN001, Kent Scientific Corp., Conn.) operated at 2–10 g range. The ileum strip was kept at a 2 g tension, and carbachol was used as antagonist. The ileum contracted cumulatively upon the addition of consecutive doses of carbachol (10–20 µl of $2 \times 10^{-4} - 2 \times 10^{-3}$ M in water solution). Contractions were recorded on a physiograph (Kipp & Zonen Flarbed Recorder, Holland). After the maximum response was achieved, the ileum was washed three times, and a fresh Tyrode's solution containing appropriate concentration of the antagonist was replaced. An equilibration time of 10 min was allowed for the antagonists before the addition of carbachol. In each experiment, 5 to 6 different concentrations were used, and a Schild plot was used to obtain the pA_2 values. Four to six trials were performed for each antagonist.

3.4. In vivo mydriatic studies

The mydriatic effects of the soft analogs following topical administration have been compared to those of glycopyrrolate in rabbit eyes. Four healthy, male New-Zealand white rabbits weighing about 3.5 kg were used. To investigate the dose-mydriatic-response relationships, 100 µl of various concentrations of the compounds (0–1%) were administered in the eyes to determine the pharmacodynamically equivalent doses, the lowest doses that induce the maximum pupil dilations. Drug solutions were applied to one eye; only water was applied to the other eye that served as control. Experiments were carried out in a light- and temperature-controlled room. At appropriate time intervals, the pupil diameters of both eyes were recorded. Difference in pupil diameters between each time-point and zero time-point were calculated for both treated and control eyes and reported as mydriatic responses [(treated-control)/control in%]. Control eye dilations were monitored to determine whether systemic absorption had occurred or not. The area under the mydriatic response-time curve (AUC^{eff}) was calculated by the trapezoidal rule, and it was used to compare the activity and duration of action of the tested compounds.

3.5. QSAR

Molecular structures were built and optimized in ChemDraw (ChemOffice Ultra 7.0; CambridgeSoft, Cambridge, MA). Molecular volumes and other descriptors were calculated and compiled with an extension of the QLogP program as described previously (Buchwald 2002; Buchwald and Bodor 1999). Statistical analyses were performed using a standard spreadsheet program (Microsoft Excel 2000).

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