Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Synthesis of novel thiol-reactive clenbuterol analogues

A. Preželj and S. Pečar

β_2-Agonists originally developed and used as bronchospasmolytics or tocolytics in humans and animals have been used illegally at a higher dosage as a repartitioning agent in cattle feed, because they improve growth rate, reduce fat depositioning and increase protein accretion. Considering the potential risk for human health, the use of β_2-agonists as growth promoters in farm animals is now banned within the European community (Directive 86/469/EEC). Multi-residue methods should be able to detect as many compounds as possible to control their misuse via enzyme immunoassay (EIA) screening and GC-MS confirmatory techniques [1–7].

The overall aim is to develop a general diagnostic assay for quantitating a wide range of β_2-agonists found in farm animals. Considering the structure of various β_2-agonists, thiol-reactive ligands have been designed for preparation of the immunogen, that seems to be suitable for obtaining antibodies with broad group specificity. 2-(*tert*-butylamino)-1-phenyl-1-ethanol was recognised as the common feature of many β_2-agonists.

The aromatic amino group was identified as possible site for the attachment of new acyl residues. To prepare antibodies with broad group specificity any substituent on the aromatic ring of thiol-reactive hapten should be omitted. 4-Aminoacetophenone had to be N-acylated in order to avoid the simultaneous introduction of a bromo residue into the aromatic nucleus during α-bromination (Scheme) [8, 9].

However, the main problem that emerged, was the amination of the α-bromo derivative **2** following the published procedure for the synthesis of clenbuterol and its analogues [10–13]. All attempts to prepare expected N^1-4-[2-(*tert*-butylamino)acetyl]phenyl-2,2,2-trifluoroacetamide using a small excess of *tert*-butylamin under reflux conditions in chloroform were unsuccessful. Applying an excess of *tert*-butylamin without any solvent, immediately followed by reduction with sodium borohydride, we discovered a novel approach to amination of the bromoketone **2**. The reaction is completed in 5 min at room temperature instead of 4 h refluxing in chloroform. The aminoketone **3** was proved by preparative TLC (eluent: chloroform/methanol 9/1) followed by MS characterisation and was found to be acid labile. The yield of compound **4** after reduction, deprotection and isolation with column chromatography is reasonably high and the reaction can be consistently performed.

Experimental

1. Apparatus

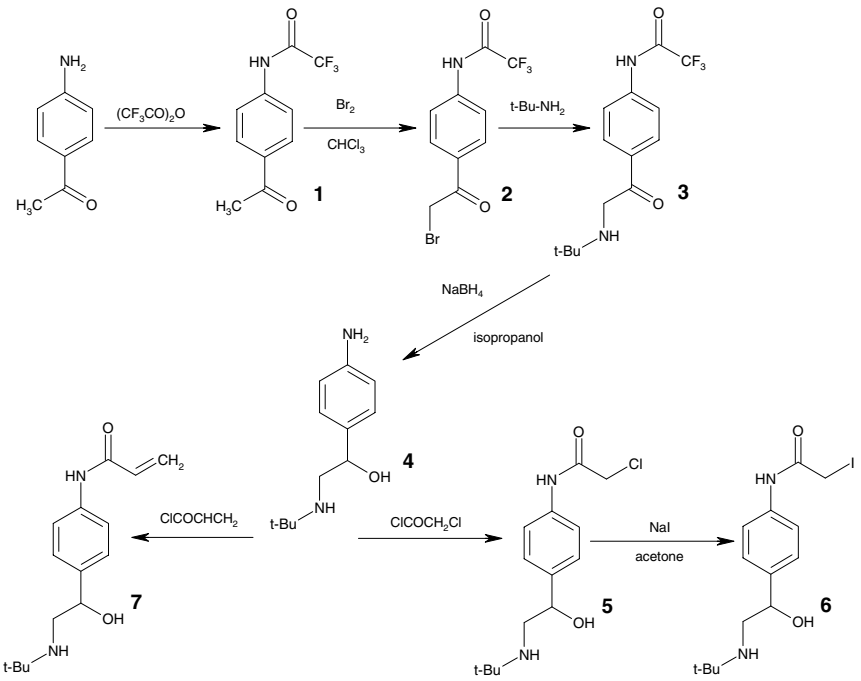
All compounds have been fully characterized by m.p.'s IR, ^{1}H NMR and mass spectroscopy. Elemental analyses were performed using Perkin-Elmer 240 C microanalyzer, all compounds gave results in an acceptable range.

2. Chemistry

2.1. N^1-(4-Acetylphenyl)-2,2,2-trifluoroacetamide (**1**)

4-Aminoacetophenone (13.5 g, 0.10 mol) in CH_2Cl_2 (60 ml) and Et_3N (10.5 g, 0.10 mol) was cooled in an ice bath. The solution of trifluoroacetic anhydride (14.8 ml, 1.05 equival.) in CH_2Cl_2 (10 ml) was added dropwise over a period of 15 min. Stirring was continued at 10 °C until no more starting compound was detected by TLC. CH_2Cl_2 was evaporated under reduced pressure and the reaction mixture poured on the mixture of ice and H_2O (300 ml). The resulting solid was collected by filtration and successively washed with H_2O (3 × 50 ml) and 2M HCl (3 × 50 ml). The crude product was re-crystallised from EtOH and dried (in vacuo, P_2O_5). Yield: 22.8 g (99%), white needles, m.p. 131–134 °C. IR (KBr): ν = 3304, 1751, 1675, 1607, 1547, 1278, 1153 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 2.63 (s, 3 H, C<u>H</u>$_3$), 7.72 (d, 2 H, J = 9.04 Hz, Ar$_{2,6}$-<u>H</u>), 8.03 (d, 2 H, J = 9.04 Hz, Ar$_{3,5}$-<u>H</u>), 8.10 (s, 1 H, N<u>H</u>). MS (EI): m/z (%) = 231 (M^+, 41), 216 (100).

Scheme



2.2. N^1-[4-(2-Bromoacetyl)phenyl]-2,2,2-trifluoroacetamide (**2**)

Bromine (10.4 g, 65.0 mmol) in $CHCl_3$ (10 ml) was added over half an hour to a refluxing solution of **1** (15.0 g, 64.9 mmol) in $CHCl_3$ (200 ml). A vigorous exotermic reaction started after a few minutes. After the bromine colour had disappeared the suspension was refluxed for 15 min. The product which separated on cooling was collected, re-crystallised from EtOH and dried (in vacuo, P_2O_5). The remaining mother liquide was evaporated under reduced pressure and the crude product containing also unreacted compound **1** and α,α′-dibromo product was worked up with several successive re-crystallisation steps from EtOH to increase the yield. Yield: 14.8g (74%), white needles, m.p. 165–169 °C. IR (KBr): ν = 3307, 1736, 1695, 1604, 1551, 1417, 1297, 1202 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 4.44 (s, 2 H, $C\underline{H}_2Br$), 7.76 (d, 2 H, J = 9.04 Hz, $Ar_{2,6}$-$\underline{H}$), 8.07 (d, 2 H, J = 9.04 Hz, $Ar_{3,5}$-$\underline{H}$), 8.10 (s, 1 H, N$\underline{H}$). MS (FAB): m/z (%) = 310 (MH^+, 100).

2.3. (±) 1-(4-Aminophenyl)-2-(tert-butylamino)-1-ethanol (**4**)

An excess of *t*-butylamine (59.3 g, 0.81 mol) was quickly added to the powdered compound **2** (14.6 g, 47.2 mmol) and vigorously mixed for 5 min. Exotermic reaction started immediately. The excess of *tert*-butylamin was removed under reduced pressure and the solid residue dissolved in 2-propanol (150 ml). The solution was treated with $NaBH_4$ (2.62 g, 70.8 mmol) and mixed overnight at room temperature. After complete reduction (TLC monitoring, the overall time can be reduced to 3 h), the pH of the solution was carefully lowered to pH = 1 with 4 M HCl. After 15 minutes it was made alkaline with 10 M NaOH (pH = 12) and refluxed for 2 h. 2-Propanol was evaporated under reduced pressure, than H_2O was added (100 ml) and the mixture was extracted with ethylacetate. The combined organic phases were successively washed with brine (3 × 50 ml). The organic layer was dried using anh. Mg_2SO_4 and after filtration, the solvent was removed under reduced pressure. The crude product was washed with ether, re-crystallised from ethylacetate and n-hexane and dried (in vacuo, P_2O_5). Yield: 8.0 g (81%), yellow solid, m.p. 124–126 °C. IR (KBr): ν = 3342, 2965, 1615, 1516, 1364, 1267, 1220, 1074 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.12 (s, 9 H, t-$\underline{Bu}$), 2.61 (dd, 1 H, J_1 = 8.67 Hz, J_2 = 11.38 Hz, $C\underline{H}_AH_B$), 2.86 (dd, 1 H, J_1 = 3.77 Hz, J_2 = 11.68 Hz, $CH_A\underline{H}_B$), 3.65 (s, 2 H, N$\underline{H}_2$), 4.51 (dd, 1 H, J_1 = 3.76 Hz, C$\underline{H}$), 6.69 (d, 2 H, J = 8.29 Hz, $Ar_{2,6}$-$\underline{H}$), 7.18 (d, 2 H, J = 8.29 Hz, $Ar_{3,5}$-$\underline{H}$). MS (FAB): m/z (%) = 209 (MH^+, 100).

2.4. (±) N^1-4-[2-(tert-Butylamino)-1-hydroxyethyl]phenyl-2-chloroacetamide (**5**)

To a stirred solution of **4** (333 mg, 1.60 mmol) in 350 mg of DMF and 96.1 µl of anhydrous acetic acid at 0 °C, 1.05 equivalent of chloroacetylchloride (132.7 µl, 1.68 mmol) was added. Stirring was continued for 30 min at 0 °C and then for 20 min at room temperature. To the reaction mixture H_2O (15 ml) was added, made alkaline with anh. K_2CO_3 and extracted with ethylacetate. The organic extract was washed with brine, dried over anh. Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was purified by precipitation from ethylacetate with hexane and dried (in vacuo, P_2O_5). Yield: 332 mg (73%), white solid, m.p. 159–164 °C. IR (KBr): ν = 3270, 2970, 1668, 1605, 1553, 1413, 1341, 1223, 1088 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.12 (s, 9 H, t-$\underline{Bu}$), 2.58 (dd, 1 H, J_1 = 8.67 Hz, J_2 = 12.06 Hz, $CH_A\underline{H}_B$), 2.91 (dd, 1 H, J_1 = 3.77 Hz, J_2 = 12.06 Hz $CH_A\underline{H}_B$), 4.21 (s, 2 H, CH_2Cl), 4.59 (dd, 1 H, J_1 = 3.39 Hz, J_2 = 8.67 Hz, C$\underline{H}$), 7.39 (d, 2 H, J = 8.67 Hz, $Ar_{2,6}$-$\underline{H}$), 7.54 (d, 2 H, J = 8.67 Hz, $Ar_{3,5}$-$\underline{H}$), 8.25 (s, 1 H, NH). MS (FAB): m/z (%) = 285 (MH^+, 84), 85 (100).

2.5. (±) N^1-4-[2-(tert-Butylamino)-1-hydroxyethyl]phenyl-2-iodoacetamide (**6**)

To a stirred solution of **4** in the form of salt with maleic acid (247 mg, 0.62 mmol) in acetone (10 ml) 1.2 equivalent of NaI (111.2 mg, 0.74 mmol) was added. Stirring was continued for 6 h at 45 °C, protected from light. After NaCl was filtered off the solvent was evaporated under reduced pressure and the residue dried (in vacuo, P_2O_5). Yield: 272 mg (90.0%), white solid in the form of salt with maleic acid **6***, m.p. 187–188 °C (for compound **6**). IR (KBr): ν = 3586, 3302, 3136, 2820, 1692, 1609, 1546, 1472, 1356, 1212, 1081 cm^{-1}. ^1H NMR (DMSO-d_6, for compound **6***): δ = 1.27 (s, 9 H, t-Bu), 2.87 (dd, 1 H, J_1 = 8.67 Hz, J_2 = 12.06 Hz, $CH_A\underline{H}_B$), 3.00 (d, 1 H, J_1 = 12.43 Hz , $CH_A\underline{H}_B$), 4.26 (s, 2 H, $C\underline{H}_2J$), 4.78 (d, 1 H, J_1 = 7.16 Hz, C$\underline{H}$), 7.40 (d, 2 H, J = 8.67 Hz, $Ar_{2,6}$-$\underline{H}$), 7.61 (d, 2 H, J = 8.67 Hz, $Ar_{3,5}$-$\underline{H}$), 10.34 (s, 1 H, N$\underline{H}$). MS (FAB): m/z (%) = 377 (MH^+, 25), 57 (100).

2.6. (±) N^1-{4-[2-(tert-Butylamino)-1-hydroxyethyl]phenyl}acrylamide (**7**)

To a stirred solution of **4** (245 mg, 1.18 mmol) in 900 mg of DMF and 72.7 µl of anhydrous acetic acid at 0 °C, 1.05 equivalent of acryloylchloride (101.5 µl, 1.68 mmol) was added. Stirring was continued for 30 min at 0 °C and then for 20 min at room temperature. To the reaction mixture H_2O (15 ml) was added, made alkaline with anh. K_2CO_3 and extracted with ethylacetate. The organic extract was washed with brine, dried over anh. Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was purified by precipitation from ethylacetate with hexane and dried (in vacuo, P_2O_5). Yield: 193 mg (63%), white solid, m.p. 165–166.5 °C. IR (KBr): ν = 3289, 2968, 1662, 1602, 1551, 1418, 1330, 1223 cm^{-1}. ^1H NMR (DMSO-d_6): δ = 1.05 (s, 9 H, t-$\underline{Bu}$), 1.36 (s, broad, 1 H, N$\underline{H}$), 2.61 (d, 2 H, J_1 = 6.78 Hz, $C\underline{H}_2$), 4.51 (t, 1 H, J_1 = 6.40 Hz, C$\underline{H}$), 5.16 (s, 1 H, O$\underline{H}$), 5.78 (dd, 2 H, J_1 = 2.26 Hz, J_2 = 9.79 Hz, $CH_A\underline{H}_B$), 6.29 (dd, 2 H, J_1 = 1.88 Hz, J_2 = 16.59 Hz, $CH_A\underline{H}_B$), 6.48 (dd, 1 H, J_1 = 10.17 Hz, J_2 = 16.96 Hz, C$\underline{H}$–CH_2), 7.33 (d, 2 H, J = 8.66 Hz, $Ar_{2,6}$-$\underline{H}$), 7.64 (d, 2 H, J = 8.66 Hz, $Ar_{3,5}$-$\underline{H}$), 10.12 (s, 1 H, N$\underline{H}$). MS (FAB): m/z (%) = 263 (MH^+, 100).

Acknowledgement: The authors are greatful to The European Community (Brussels, Belgium) and Ministry of education, science and sport (Ljubljana, Slovenia) for financial assistance.

References

1 Blum, J. W.; Flueckiger, N.: Eur. J. Pharm. **151**, 177 (1988)
2 Butery, P. J.; Sweet, A.: Anim. Feed Sci. Tech. **45**, 97 (1993)
3 Elliott, C. T.; Mc Caughey, W. J.; Shortt, H. D.: Food Addit. Contam. **10**, (2), 231 (1993)
4 Moloney, A, P.; Beermann, D. H.: Residues of veterinary drugs and mycotoxins in animal products: new methods for risk assessment and quality control. Proceedings of the teleconference held on internet 1996, p. 124
5 Witkamp, R. F.: Residues of veterinary drugs and mycotoxins in animal products: new methods for risk assessment and quality control. Proceedings of the teleconference held on internet 1996, p. 113
6 Kuiper, H. A.; Noordam, M. Y.; van Dooren-Flipsen, M. M. H.; Schilt, R.; Roos, A. H.: Amer. Soc. Anim. Sci. **76**, 195 (1998)
7 Andre, F.; Montrade, M. P.; Le Bizac, B.: Residues of veterinary drugs and mycotoxins in animal products: new methods for risk assessment and quality control. Proceedings of the teleconference held on internet 1996, p. 139
8 Green, T. W.: Protective groups in organic synthesis, p. 254, A Wiley-Interscience Publication, New York 1980
9 March, J.: Advanced organic chemistry, Reactions, mechanisms, and structure, 4. Ed.; p. 587, A Wiley-Interscience Publication, New York 1992
10 Krüger, G.; Keck, J.; Noll, K.; Pieper, H.: Arzneim.-Forsch. **34**, 1612 (1984)
11 Finnerty, J.: A study of some clenbuterol analogues, A thesis submitted in fulfilment of the requirements for the degree of Master of Science, Department of pharmacology, University of Sydney 1995
12 Kaiser, C.; Colella, D. F.; Schwartz, M. S.; Garvey, E.; Wardell, J. R. Jr.: J. Med. Chem. **17**, 49 (1974)
13 Keck, J.; Krüger, G.; Noll, K.; Machleidt, H.: Arzneim. Forsch. **22**, 861 (1972)

Received June 5, 2001
Accepted August 15, 2001

Preželj Andrej, mag. pharm.
Faculty of Pharmacy
University of Ljubljana
Aškerčeva 7
1000 Ljubljana
Slovenia
prezelj.andrej@ffa.uni-lj-si