Syntheses and Tissue Distribution of ^{99m}Tc-Sulfonylureas

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Abstract □ Four new tolbutamide analogs were prepared and evaluated as hypoglycemics. Hypoglycemic action was observed in two members of the class, and ^{99m}Tc-chelates were tested as potential radiopharmaceutical imaging agents.

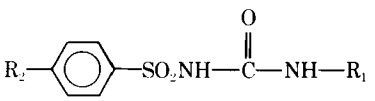
Keyphrases □ Sulfonylureas—synthesized and screened as hypoglycemics, tissue distribution of ^{99m}Tc-chelates, potential pancreatic imaging agents □ Hypoglycemic agents—synthesis and screening of sulfonylureas, tissue distribution of ^{99m}Tc-chelates □ Radiolabeled sulfonylureas—tissue distribution of ^{99m}Tc-chelates, potential pancreatic imaging agents □ Pancreatic imaging agents, potential—synthesis and tissue distribution of ^{99m}Tc-sulfonylureas

As part of a study on the development of γ-emitting radiopharmaceuticals for imaging the pancreas, several chelates of ^{99m}Tc were synthesized which were structural analogs of the pancreatic hypoglycemic, tolbutamide (I). The *para*-substituted phenylsulfonylurea moiety (I–VII) has been identified as a characteristic structural feature in numerous potent hypoglycemics such as tolbutamide (I), chlorpropamide (II), and carbutamide (III).

The structure–activity studies in this class have been reviewed (1, 2); while some active agents have been discovered in which the *para*-position (R_2) is varied, the most potent therapeutic agents appear to have a methyl at this locus. Wide variations in the nature of R_1 have been investigated, with R_2 being held constant as methyl, and agents of comparable or enhanced potency compared to tolbutamide have been uncovered (3). All of these active sulfonylureas effect a marked elevation in blood insulin levels in both nondiabetic human subjects and other individuals with noninsulin-dependent diabetes (4).

The fact that depancreatized humans are not susceptible to the hypoglycemic activity of tolbutamide is interpreted to imply a direct action on the pancreas (5). This view is further supported by the observed degranulations of the pancreatic β-cells of test animals postdosing with sulfonylureas (6), and a general pancreatotrophic behavior has been presumed for the tolylsulfonylurea moiety (1, 2, 7).

In a search for potential pancreatic delineating radiopharmaceuticals, Boyd *et al.* (8) investigated a radioiodinated analog of chlorpropamide (*i.e.*, II but with R_2 = ^{125}I). No hypoglycemic testing data were provided to indicate that the iodopropamide still retained the activity of its parent model, chlorpropamide, but β-cell degranulation was observed. The substitution of iodine (covalent radius = 1.33 Å) for chlorine (covalent radius = 0.99 Å) represents a rather significant steric modification. As more precise models of the *p*-tolylsulfonylurea type, synthetic analogs modified only at the less important locus R_1 with ligand groups capable of chelating the γ-emitting nuclide ^{99m}Tc have been prepared.

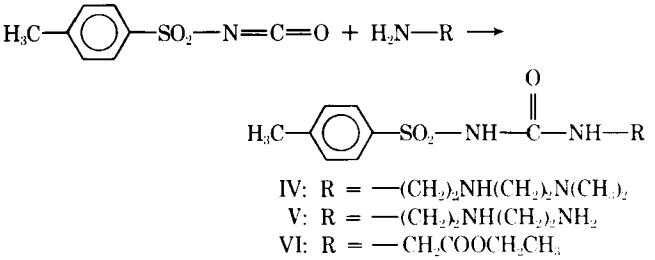


I: R_1 = *n*-butyl, R_2 = CH_3
II: R_1 = *n*-propyl, R_2 = Cl
III: R_1 = *n*-butyl, R_2 = NH_2
IV: R_1 = $—(CH_2)_2NH(CH_2)_2N(CH_3)_2$, R_2 = CH_3
V: R_1 = $—(CH_2)_2NH(CH_2)_2NH_2$, R_2 = CH_3
VI: R_1 = $—CH_2COOCH_2CH_3$, R_2 = CH_3
VII: R_1 = $—CH_2COOH$, R_2 = CH_3

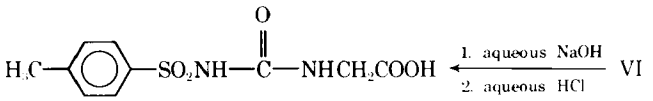
EXPERIMENTAL[1]

These candidate radiopharmaceuticals, IV–VI, were prepared by the controlled addition of the amino component to *p*-toluenesulfonyl isocyanate (Scheme I), while saponification of VI yielded VII.

1-*p*-Toluenesulfonyl-3-(*N,N*-dimethylaminoethylamino-ethyl)urea (IV)—A solution of *p*-toluenesulfonyl isocyanate (2.0 g, 0.0101 mole) in dioxane (10 ml) was added slowly (30 min) to a solution of *N,N*-dimethyldiethylenetriamine (1.32 g, 0.01 mole) in dioxane (30 ml) at room temperature. The resulting solution was stirred at room temperature for 1 hr, during which time a white precipitate formed. The precipitate was collected by filtration, washed with ether, and air dried. Recrystallization from anhydrous alcohol yielded 2.53 g (76.2%) of white hygroscopic crystals, mp 179–180°: IR (mineral oil): 3490, 3390, 3270 (NH), and 1650 (C=O) cm^{-1}; NMR (D_2O): δ 2.30 (s, 3H, $ArCH_3$), 2.52 [s, 6H, $N(CH_3)_2$], 2.86 (m, 8H, NCH_2), and 7.46 (m, 4H, ArH).



IV: R = $—(CH_2)_2NH(CH_2)_2N(CH_3)_2$
V: R = $—(CH_2)_2NH(CH_2)_2NH_2$
VI: R = $—CH_2COOCH_2CH_3$



VII

Scheme I

[1] Proton magnetic resonance (PMR) spectra were obtained on a Perkin-Elmer Hitachi R20A spectrometer, and chemical shifts are reported relative to tetramethylsilane. IR spectra were obtained on a Perkin-Elmer 257 spectrometer. Microanalyses were performed by Robertson Microanalytical Laboratory, Florham Park, N.J.

Anal.—Calc. for $C_{14}H_{24}N_4O_3S$: C, 51.19; H, 7.36; N, 17.05. Found: C, 51.18; H, 7.61; N, 16.82.

TLC on silica gel[2] 1B-F, using methanol–water (3:1), gave a single spot, R_f 0.29.

1-*p*-Toluenesulfonyl-3-(aminoethylaminoethyl)urea (V)—*p*-Toluenesulfonyl isocyanate (3.0 g, 0.0152 mole) in tetrahydrofuran (15 ml) was added slowly (30 min) to a solution of diethylenetriamine (1.58 g, 0.0156 mole) in tetrahydrofuran (70 ml) at room temperature. The resulting solution was stirred at room temperature for 30 min, during which time a white precipitate formed. The precipitate was collected by filtration, washed with ether, and dried at 65° *in vacuo*. Recrystallization from a dioxane–water mixture yielded 3.32 g (73.1%) of white hygroscopic crystals, mp 135–138°; IR (mineral oil): 3350, 3280 (NH), and 1640 (sh) (C=O) cm^{-1}; NMR (D_2O): δ 2.24 (s, 3H, $ArCH_3$), 2.88 (m, 8H, NCH_2), and 7.37 (m, 4H, ArH).

Anal.—Calc. for $C_{12}H_{20}N_4O_3S$: C, 47.97; H, 6.71. Found: C, 47.82; H, 6.86.

TLC on silica gel[2] 1B-F, using methanol–water (19:1), gave a single spot, R_f 0.86. Structure assignment was further supported by formation, in 52% yield, of a dihydro-2-imidazolone, mp 196–198°, from ethanol–water (1:1), generated by treatment of V with phosgene in dry benzene; IR (mineral oil): 1700 and 1655 (C=O) cm^{-1}.

Anal.—Calc. for $C_{13}H_{18}N_4O_4S$: C, 47.84; H, 5.56. Found: C, 47.78; H, 5.54.

1-*p*-Toluenesulfonyl-3-carbethoxymethylurea (VI)—Into 20 ml of anhydrous tetrahydrofuran was dissolved 6.3 g (0.032 mole) of *p*-toluenesulfonyl isocyanate. This mixture was added dropwise over 5 min to a solution of ethyl glycinate (3.54 g, 0.0343 mole) in 50 ml of tetrahydrofuran, during which time the temperature rose to 55°. The solution was agitated for 0.5 hr and allowed to stand at room temperature for 12 hr. Evaporation to dryness left a white powder, which was recrystallized from ethanol–water to yield 7.2 g (75%) of white needles, mp 170–171°; IR (mineral oil): 3110, 3170, 3315 (NH), 1742 (ester C=O), and 1660 (urea C=O) cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 1.15 (t, 3H, CH_3), 2.40 (s, 3H, $ArCH_3$), 3.75 (d, 2H, CH_2), 4.05 (q, 2H, CH_2), 6.80 (t, 1H, NH), and 7.61 (m, 4H, ArH).

Anal.—Calc. for $C_{12}H_{16}N_2O_5S$: C, 47.98; H, 5.37; N, 9.32. Found: C, 48.17; H, 5.62; N, 9.17.

1-*p*-Toluenesulfonyl-3-carboxymethylurea (VIII)—Compound VI (2.0 g, 6.6 mmoles) was refluxed for 1 hr in 10% aqueous sodium hydroxide solution. The solution was cooled to room temperature and acidified with concentrated hydrochloric acid, and a white solid separated from solution. This solid was redissolved by heating the mixture to 80° with sufficient 95% ethanol added to achieve homogeneity. Upon slow cooling to 0°, white needles (1.7 g or 88% of theoretical) were deposited. Upon drying *in vacuo*, these needles melted at 190° with decomposition; IR (mineral oil): 3350, 3170, 3120 (NH and OH), 1730 (acid C=O), and 1680 (urea C=O) cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 2.40 (s, 3H, $ArCH_3$), 3.68 (d, 2H, CH_2), 6.88 (t, 1H, NH), and 7.62 (m, 4H, ArH). The product was isolated as a monohydrate.

Anal.—Calc. for $C_{10}H_{12}N_2O_5S \cdot H_2O$: C, 41.37; H, 4.86; N, 9.65. Found: C, 41.40; H, 4.63; N, 9.68.

BIOLOGICAL EVALUATION[3]

For hypoglycemic testing, the candidate agents were administered as single intraperitoneal injections at 50 mg/kg in methylcellulose to three Sprague–Dawley rats; the rats were fasted for 24 hr prior to dosing. The test animals and four controls were preloaded with 1 g/kg of glucose given orally. Blood aliquots were withdrawn at 30-min intervals and analyzed for mg of glucose/100 ml of blood. Compound IV displayed hypoglycemic effects and dropped blood glucose levels by 34 ± 6 mg % at 30 min and 15 ± 1 mg % at 60 min compared to control standards. Compound V gave evidence of modest hypoglycemia at 90 min, but both VI and VII were inactive. On the basis of these results, IV was selected for technetium labeling and for detailed tissue distribution studies. Several exploratory runs were also carried out with the ^{99m}Tc-chelate of V.

[2] Baker-flex.

[3] Rat glucose tolerance studies were performed in the Biochemistry Department of McNeil Laboratories, Fort Washington, Pa., by Dr. G. F. Tutiler.

Table I—Tissue Distribution of the Sulfonylureas, Percent Dose per Gram and Standard Deviation

Tissue	15 min	30 min	1 hr
	Chelate of ^{99m}Tc and IV		
Lung	0.954 ± 0.111	0.664 ± 0.070	0.474 ± 0.009
Liver	0.915 ± 0.009	0.797 ± 0.081	0.665 ± 0.028
Spleen	0.425 ± 0.023	0.323 ± 0.023	0.237 ± 0.010
Kidney	5.402 ± 0.067	6.986 ± 0.879	6.779 ± 0.017
Pancreas	0.309 ± 0.012	0.265 ± 0.034	0.173 ± 0.007
Blood	1.165 ± 0.060	0.859 ± 0.037	0.600 ± 0.074
	Chelate of ^{99m}Tc and V		
Lung	0.759 ± 0.025	0.560 ± 0.057	0.358 ± 0.013
Liver	0.837 ± 0.003	0.690 ± 0.001	0.602 ± 0.001
Spleen	0.304 ± 0.006	0.232 ± 0.003	0.154 ± 0.001
Kidney	6.654 ± 0.202	5.907 ± 0.482	6.142 ± 0.013
Pancreas	0.312 ± 0.011	0.199 ± 0.003	0.156 ± 0.010
Blood	0.965 ± 0.002	0.636 ± 0.103	0.409 ± 0.010
	Blank Run[a]		
Lung	1.191 ± 0.142	1.072 ± 0.008	0.910 ± 0.192
Liver	6.595 ± 0.964	6.078 ± 1.600	6.114 ± 0.805
Spleen	2.438 ± 0.144	3.988 ± 0.841	2.202 ± 1.194
Kidney	6.694 ± 1.402	6.397 ± 0.815	7.179 ± 0.240
Pancreas	0.484 ± 0.368	0.497 ± 0.121	0.410 ± 0.143
Blood	2.639 ± 0.004	1.907 ± 0.073	1.569 ± 0.235

[a] Injection dose prepared by stannous chloride reduction of $Na^{99m}TcO_4$ with no sulfonylurea ligand present.

A solution of sodium pertechnetate was eluted from a technetium generator and reduced by stannous chloride to cationic technetium and a chelate prepared by the standard method published for diamine-type ligands (9). All of the chelates were purified before injection by gel filtration chromatography with saline elution. In all cases, two radioactive fractions could be eluted from the column, the first being the ^{99m}Tc-chelate of the tolbutamide analog and the second being the unreduced pertechnetate. The latter fraction was normally insignificant, reflecting nearly quantitative reduction and binding to the candidate radiopharmaceutical ligand. These observations are in accord with those reported by Eckelman *et al.* (9).

Purity of the chelate fraction was further verified by paper chromatography[4] using saline eluant (10). The R_f values obtained for the reduced hydrolyzed technetium, pertechnetate, and the ^{99m}Tc-chelate were 0.0, 0.54, and 0.77, respectively. The purity assays for the injected doses employed in the tissue distribution studies always indicated a minimum of 95% chelated material by the Eckelman–Richards method (10) of counting the separated spots on the paper chromatogram.

Tissue distribution studies were performed on pure inbred Wistar rats injected *via* the tail vein with a precalibrated dose of the labeled tolbutamide analogs IV or V. A minimum of two and a maximum of four animals were dosed and were sacrificed at 15, 30, and 60 min postinjection. Immediately after sacrificing, an autopsy was performed and the indicated tissues (Table I) were removed, dissected from fat and connective tissue, weighed, and placed in 20.3-cm (8-in.) test tubes. These tissues were counted[5], and a percent dose per gram was calculated by comparison with a standard that was counted at the same time as the tissues to correct for radioactive decay. Table I indicates the range of values obtained in each run. In an effort to optimize pancreatic uptake, several other physiologically active agents were administered immediately in advance of the labeled candidate radiopharmaceutical.

DISCUSSION

As has been observed for several other chelates, the concentration in the kidney, even at the shortest assay time, was in excess of concentrations in other target organs for ^{99m}Tc-chelates of IV and V. Some reports have suggested that chelates are not specific for any particular organ but are cleared rapidly from the blood and into the kidney by glomerular filtration (11, 12). Since ^{99m}Tc-chelates recently were developed for imaging the gallbladder (13), the

[4] Whatman No.1.

[5] Baird Atomic automatic well counter.

liver (14), amebic abscesses (15), and bone structures (16), this suggestion is obviously not a general truism because these chelates displayed the tissue specificity of the parent ligand.

Anatomical considerations dictate that for development of a satisfactory pancreatic scanning radiopharmaceutical, a suitable dose ratio of pancreas to liver be obtained. In the ^{99m}Tc-chelates of IV and V, pancreas to liver ratios of 0.35 were observed at 15 min and fell to 0.26 at the end of 1 hr. If *in vivo* dissociation of the metal ion–ligand bond, presumably by exchange of the label onto a more strongly complexing native blood protein such as transferrin *or* by exchange of the tolbutamide-like ligand onto a native blood metal ion, were occurring, tissue distribution values should be altered by preadministration of either another metal ion or another ligating group. However, predosing with ion-dextran[6] or ethylenediaminetetraacetic acid calcium salt did not appreciably alter the tissue distribution values. Further support for the conclusion that the ^{99m}Tc-chelates were remaining intact *in vivo* was obtained from a blank run (Table I) in which reduced, unchelated technetium was administered. As reported previously (17), a different distribution favoring liver uptake, by hydrolyzed, reduced technetium, was observed.

Coadministration of the injectable diuretic furosemide[7] did not alter the rapid kidney uptake nor the pancreas to liver ratio, but the anticholinergic, morphine sulfate (0.2 mg/kg iv), often employed in such studies (18, 19), did slightly enhance the ratio to 0.61 for ^{99m}Tc-V after 30 min. The low percent dose values in the pancreas and the diminished pancreas to liver ratio may reflect the fact that the β-cells, the site of action of sulfonylurea hypoglycemics (1, 2), constitute only 1–2% of the pancreas weight. In islet cell neoplasms, the β-cells represent a much higher fraction of pancreas weight (20); studies are now underway to visualize such tumors in hamsters.

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