Pakistan Journal of Pharmaceutical Sciences
Vol. 18, No.4, October 2005, pp.35-40

SYNTHESIS AND CHARACTERIZATION OF GLICLAZIDE COMPLEXES OF MAGNESIUM, CALCIUM, CHROMIUM, MANGANESE, IRON, NICKEL, COPPER, ZINC AND CADMIUM SALTS

M. SAEED ARAYNE, NAJMA SULTANA*, M. KAMRAN ZAMAN AND AFGAN FAROOQ**
Department of Chemistry, University of Karachi, Karachi-75270, Pakistan - E-mail: arayne@gawab.com
**Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan.*
***HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*

Gliclazide is a well known agent used for NIDDM. Present paper reports the synthesis and characterization of its metal complexes with magnesium, calcium, chromium, manganese, iron, nickel, copper, zinc and cadmium. These complexes were characterized through physical characteristics, IR, H^1-NMR, and atomic absorption spectroscopic studies.

Keywords: Antidiabetics; gliclazide; coordination complexes; transition metals; NMR; IR; atomic absorption; magnesium; chromium; manganese; iron; nickel; copper; zinc; cadmium.

INTRODUCTION

Gliclazide N-[[(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-amino]-carbonyl]-4-methyl benzene sulfonamide, or 1-(hexahydrocyclopenta[c] pyrrol-2-(1H)-yl)-3-(p-tolyl-sulfonyl) urea or N-(4-methylbenzene sulfonyl) -N'-(3-azabicyclo [3.3.0] oct-3-yl) urea or 1-(3-azabicyclo[3.3.0] oct-3-yl) -3-p-tolylsulfonyl urea $C_{15}H_{21}N_3O_3S$, molecular weight 323.4 is a white or almost white crystalline powder, odorless, tasteless, m.p., 165-170°C (figure 1) and is stable under normal conditions (British Pharmacopoeia, 1998; Martindale, 1999 and Parvez *et al*., 1999).

Fig. 1: Gliclazide

Gliclazide is an acceptable first-line agent for treating patients with NIDDM who have failed to respond adequately to diet and exercise. Numerous studies have demonstrated that gliclazide is effective for controlling NIDDM (Melander *et al*., 1989; Holmes *et al*., 1984; Akanuma *et al*., 1991; Harrower, 1991 and Akanuma *et al*.,

1988) and was also effective in patients poorly controlled by first-generation sulfonylureas or biguanides (Akanuma *et al*., 1991; Harrower, 1991; Shaw *et al*., 1985; Guillausseau, 1991; Hernandez and Lavielle, 1986; Kilo *et al*., 1991 and Krall, 1991). The hypoglycemic efficacy of gliclazide has been maintained for up to 5 years (Akanuma *et al*., 1991; Harrower, 1991; Guillausseau, 1991; Hernandez and Lavielle, 1986) in one 5-year study, the secondary failure rate was less than 10% (Harrower, 1991). In several studies, adequate control of blood glucose has been achieved with average daily doses of 160 to 200 milligrams (Shaw *et al*., 1985; Guillausseau, 1991; Hernandez and Lavielle, 1986). In general, lower doses are required in patients previously untreated with sulfonylureas and/or biguanides (Akanuma *et al*., 1991 and Shaw *et al*., 1985).

Time to peak concentration after oral administration of gliclazide is 2 to 4 hours (Akanuma *et al*., 1991 and Davis *et al*., 2000). For an 80 mg dose in normal volunteers the average time to reach maximum concentration 3.9 mg / liter is four hours (Duhault *et al*., 1980). The mean plasma half-life is ten hours (6-14h) (Courtois *et al*., 1999 and (Courtois *et al*., 1999 and Kobayashi *et al*., 1984). The increased proportion of free drug may cause hypoglycemia but the effect will be short-lived as the free drug is excreted and a new equilibrium is established (Tsuckiya *et al*., 1984). Gliclazide is extensively metabolized and less than 20% is excreted in urine as unchanged (Campbell *et al*., 1991 and Hatao *et al*., 1985). About 20% of the radioactivity appears in bile 8 hours after intravenous administration to rats (Benakis and Glasson, 1980).

There are number of drug interactions of gliclazide reported in the literature with other drugs like NSAID'S H_2-receptor antagonists, sulfonamides, barbiturates, diuretics, gluco-carticords, estrogens or sympathomimetic drugs (Archambeaud-Mouveroux *et al.*, 1987; Colin, 1999; Koda, 1992; O'Byrne and Feely, 1990; Shorr *et al*., 1997 and Fu *et al*., 1992). However, there are relatively few interactions of gliclazide reported with essential and trace elements.

The hypoglycemic effect of gliclazide is mainly due to its action on ATP stimulated K^+ channels, but the calcium ionophoretic effect of this drug may also be involved in its physiological properties. Using mitochondria, it was demonstrated that gliclazide exhibits a calcium ionophoretic activity. Indeed, gliclazide induces a decrease in the respiratory control of mitochondria; this effect is increased by addition of Ca^{2+} and corrected by ruthenium red, all characteristics of a calcium ionophoretic compound (Ubeaud, 1991). In a clinical trial study of interaction of gliclazide with deltiazem and other drugs showed no *in vivo* interactions (Monges *et al*., 1991), while verapamil inhibited gliclazide-induced insulin release. The inhibitory effect of verapamil was enhanced at a subnormal calcium concentration and reduced at a high calcium concentration (Devis *et al*., 1975). Gliclazide also provoke the translocation of calcium from an aqueous medium into or across a hydrophobic region. The magnitude of translocation of calcium was more marked in the presence, rather than absence of the antibiotic A23187 (Couturier and Malaisse, 1980). Gliclazide has been shown to have free radical-scavenging activity *in vitro*. Its effects on LDL oxidation and role of copper ion in oxidation has been investigated by many workers (Nomura *et al*., 2000; O'Brien & Luo, 1997 and Desfaits *et al*., 1997 and Zbronska *et al*., 1995). Similarly, activity of superoxide dismutase in erythrocytes and leukocytes in relation to levels of zinc and copper in blood of patients with diabetes and its effect on diabetic treatment has been investigated (Zbronska *et al*., 1995 and Ozawa *et al*., 2003).

There are reported interactions of not only calcium, copper and zinc but also the interaction of magnesium (Ubeaud *et*

Table 1
Physical characteristics of complexes of gliclazide with essential and trace elements

S. No.	Compound	Color	State	M.P °C	Yield	pH
1	Gliclazide Mg complex	Yellow	Crystalline	158	13.66%	-
2	Gliclazide Ca complex	Off white	Crystalline	159	14.94%	-
3	Gliclazide Cr complex	Green	Crystalline	90	16.94%	-
4	Gliclazide Mn complex	Light yellow	Crystalline	157	20.24%	-
5	Gliclazide Fe complex	Brown	Crystalline	100	16.23%	2-3
6	Gliclazide Ni complex	Green	Crystalline	100	29.41%	2-3
7	Gliclazide Cu complex	Dark brown	Crystalline	100	20.24%	2-3
8	Gliclazide Zn complex	Off white	Crystalline	95	33.26%	2-3
9	Gliclazide Cd complex	White	Crystalline	120	38.09%	2-3

al., 1991 and Bayes *et al*., 2002) and manganese (Onozato *et al*., 2004) with gliclazide are evident in the literature. On the contrary, there are no interactions reported in the literature for chromium, iron, nickel and cadmium with this drug. However, there are chances of coadministration of various essential and trace elements with gliclazide as they are present in many multivitamin preparations and food etc, it was thought worthwhile to synthesize the metal complexes of gliclazide with various essential and trace elements, so that stoichiometry of these complexes be studied to understand the role of these elements towards NIDDM drugs.

In present study gliclazide was reacted with magnesium, calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc and cadmium, which were in the form of their water soluble salts. The structures of the synthesized complexes were established on the basis of their physical properties and spectroscopic data like IR, UV, 1H-NMR, and AA studies.

Materials

Gliclazide standard was a gift from M/s Ali Gohar Pharmaceuticals (Pvt) Ltd. Solvents and the metal salts used were of the analytical grade (E. Merck). Melting points were determined on a Buchi 531 m.p. apparatus and are uncorrected. pH values were determined on Gallenkamp pH stick meter. The H^1-NMR spectra were recorded on a Bruker AMX 500 MHz spectrometer in $CDCl_3$ using TMS as an internal standard. IR studies were carried out by Shimadzu Model IR 470 infrared spectrophotometer. Atomic absorption studies were carried out on a Pye-Unicam atomic absorption spectrometer.

Reaction between gliclazide and various essential and trace elements

Solution of metal chlorides (1mM) in methanol were individually added to a solution of gliclazide (1mM:0.323gm) in methanol and some in methanolic HCl (0.1N) to maintain the pH of solution between 2-3 and refluxed for 2-3 hours. The colored solutions were then

Table 2
IR absorption bands of gliclazide metal complexes

Compound	Main IR absorptions in cm^{-1}
Gliclazide (reference)	460 sm, 520-580 trp, s, 625 -660 db, 700 sm, 740 sm, 800 s, 880 - 910 trp, s, 990 m, 1080 m, 1160 s, 1350 s, 1425 s, 1590 sm, 1700 s, 2900 s, 3100 -3300 db,s.
Gliclazide-Mg complex	421 sm, 440 sm, 460 sm, 530-570 trp, 630 sm, 660 s, 700 m, 750 m, 810 m, 870 - 920 trp,s, 990-1020 trp, s, 1040 sm, 1090 s, 1120 sm, 1350 s, 1430-1460 dp, s, 1590 s, 1720 s, 2900 s, 3100-3200 trp, s.
Gliclazide-Ca complex	440 sm, 450 sm, 530 -570 trp,s, 630 sm, 670 s, 700 m, 750m, 810 s, 880 - 910 trp,s, 990 - 1020 trp, s,1040 sm, 1080 s, 1120 sm, 1160 s, 1240 sm, 1420 s, 1530-1590 qdp,s, 1600 s, 1710 db,s, 1920 sm, 2900 m, 3100-3200 db,sm.
Gliclazide-Cr complex	550 sm, 570 sm, 660 s, 700 sm, 770 sm, 810 sm, 850 s, 950 sm, 1020 sm, 1090 m, 1120 sm, 1160-1190 db,m, 1230 sm, 1350 sm, 1420sm, 1450-1470 trp, sm, 1600 sm, 1730 sm, 3250 sm.
Gliclazide-Mn complex	460 sm, 530 - 580 trp,s, 670 m, 700sm, 750 sm, 810 m, 890 sm, 920 m, 990 sm, 1080 m, 1160 s, 1350 db, m, 1440 m, 1600 sm, 1710 db,s, 2900 sm, 3210 sm.
Gliclazide-Fe complex	480 sm, 550 - 580 db,m, 680 s, 700 sm, 730 sm, 770 m, 810 sm, 850 m, 950 sm, 1020 sm, 1090 m, 1180-1190db,m, 1230 m, 1350 m, 1420 m, 1450-1470 db,m, 1600 sm, 1730 s, 2900 sm, 3250 m.
Gliclazide-Ni complex	480 sm, 540 - 580 db,s, 660 s, 700 sm, 725 sm, 770 m, 810 m,850 s, 940 sm, 1020 sm, 1090 s, 1120 sm, 1160s, 1190 sm, 1230 s, 1350 s, 1420 s, 1450 - 1490 trp,s,1600m, 1730 sm, 2900 sm, 3240 s.
Gliclazide-Cu complex	490 sm, 550 m, 580 m, 660 s, 700 sm, 730 sm, 770 m, 810 sm, 850 s, 940 sm, 1020 sm, 1090 m, 1160 - 1190 db, 1230 m, 1350 s, 1420 s, 1450-1490 db,1600 sm, 1730 db,s, 2450 - 2550 trp, s, 3230 sm.
Gliclazide-Zn complex	480 sm , 540 - 580 db,m, 660 m, 725 sm, 760 sm, 800 sm, 860 s, 920 sm, 1010 sm, 1080 m, 1120 - 1160 db,m,1220 sm, 1350 m, 1420 sm, 1450 s, 1590 sm, 1760 s, 3200 sm.
Gliclazide-Cd complex	480 sm, 550 - 580 db, s, 660 s, 700 sm, 730 sm, 770 m, 810 m, 850 s, 950 sm, 1020 sm, 1090 s, 1120 sm, 1160 -1180 db, s, 1230s, 1350s, 1420 s, 1450-1460 db,s, 1600 m, 1730 s, 3225 m.

s = sharp, m = medium, sm = small, db =doublet.

filtered and left for crystallization at room temperature for 24 hours. Crystals of different colors for different metal ions were obtained which were filtered, washed, dried and their melting points determined. The physical characteristics are shown in table 1.

RESULTS AND DISCUSSION

Gliclazide on treatment with different metals i.e., Mg, Ca, Cr, Mn, Fe, Ni, Cu, Zn, and Cd yielded crystalline complexes of different colors, which were characterized by their physical characteristics (table 1), IR studies (table 2), NMR studies (table 3) and AA (table 4). All the metals coordinated with both the nitrogens of urea functionality.

Infrared absorption studies

The infrared spectrum of gliclazide and its metal complexes were recorded as a potassium bromide disc method on a Shimadzu Model IR 470 infrared spectrophotometer. The major absorption bands for the infrared frequencies and the corresponding assignments are listed in table 2.

The prominent IR absorption bands of gliclazide were in the range of 3100-3300 cm^{-1} due to urea NH stretching. A very sharp peak observed at 2900 cm^{-1} due to CH stretching. At 1700 cm^{-1}, a strong absorption due to C=O stretching. Strong absorption bands appeared in the range of 1350 – 1590 due to C=C, at 1160 cm^{-1} peaks were due to SO_2, while C-O peak was at 1080 cm^{-1}. The carbonyl band appeared at 1700 cm^{-1} in reference standard, but in transition metal complexes this stretching frequency increased towards 1720–1760 cm^{-1}. For magnesium complex the band was observed at 1720 cm^{-1} in the form of sharp peak. In case of calcium complex this band appears at 1710 cm^{-1} in the form of sharp-doublet, whereas in chromium complex it occurs at 1730 cm^{-1} as a weak band. In case of manganese complex the frequency of the carbonyl is observed at 1710 cm^{-1} as a sharp-doublet band. In addition, iron complex it occurs at 1730 cm^{-1} as a sharp band. Nickel complex shows a weak band in the region of 1730 cm^{-1}. In case of copper complex the carbonyl band appears at 1730 cm^{-1} as a sharp doublet, whereas in zinc complex it is observed at 1760 cm^{-1} as a sharp band. In case

Table 3
Proton NMR of gliclazide metal complexes

Compound	δ and Multiplicity
Gliclazide (reference)	1.42-1.63 CH_2, 1.86-2.18 CH_2, 2.39 CH_2, 2.48 CH_2, 2.53 CH_2, 2.74 CH_3, 6.40 aromatic, 7.25 aromatic, 7.92 aromatic
Gliclazide-Mg complex	1.43-1.93 CH_2, 2.41 CH_2, 2.81 CH, 3.29 $(HN)_2Mg$, 7.28 aromatic, 7.93 aromatic
Gliclazide-Ca complex	1.55 CH_2, 2.42 CH_3, 2.84 CH_2, 3.32 $(HN)_2Ca$, 7.29 aromatic, 7.95 aromatic
Gliclazide-Cr complex	2.44 CH_3, 2.50 CH_2, 3.72 $(HN)_2Cr$, 7.32 aromatic, 7.35 aromatic, 7.90 aromatic
Gliclazide-Mn complex	1.41 CH_2, 1.58 CH_2, 2.41-2.54 CH_3, 3.50 $(HN)_2Mn$, 7.29 aromatic, 7.93 aromatic
Gliclazide-Fe complex	1.62 CH_2, 1.64 CH_2, 2.44 CH_3, 3.66 $(HN)_2Fe$, 7.24 aromatic, 7.91 aromatic
Gliclazide-Ni complex	1.61 CH_2, 2.44 CH_3, 3.70 $(HN)_2Ni$, 7.31 aromatic, 7.33 aromatic, 7.90 aromatic
Gliclazide-Cu complex	1.61 CH_2, 2.44 CH_3, 3.69 $(HN)_2Cu$, 7.25 aromatic, 7.32 aromatic, 7.93 aromatic
Gliclazide-Zn complex	1.71 CH_2, 2.39 CH_2, 2.44 CH_3, 3.68 $(HN)_2Zn$, 7.20 aromatic, 7.90 aromatic
Gliclazide-Cd complex	1.22 CH_2, 1.63-1.64 CH_2, 2.03-2.07 CH_2, 3.64 $(HN)_2Cd$, 7.08 aromatic

Table 4
Estimation of metals from gliclazide metal complexes by AA spectroscopy

Compound	<—— Absorption ——>			<—— Concentration ——>			Mean	Metal (%)	S.D. (±)
	1	2	3	1	2	3			
Gliclazide-Mg complex	0.169	0.171	0.171	0.2770	0.2803	0.2803	0.2792	6.98	0.002
Gliclazide-Ca complex	0.021	0.022	0.021	0.4565	0.4783	0.4565	0.4638	11.59	0.013
Gliclazide-Cr complex	0.029	0.029	0.030	0.5918	0.5918	0.6122	0.5986	14.97	0.012
Gliclazide-Mn complex	0.041	0.041	0.041	0.6308	0.6308	0.6308	0.6308	15.77	0.000
Gliclazide-Fe complex	0.019	0.019	0.019	0.6333	0.6333	0.6333	0.6333	15.83	0.000
Gliclazide-Ni complex	0.014	0.013	0.013	0.7000	0.6500	0.6500	0.6667	16.67	0.029
Gliclazide-Cu complex	0.043	0.042	0.043	0.7544	0.7368	0.7544	0.7485	18.71	0.010
Gliclazide-Zn complex	0.155	0.155	0.155	0.7277	0.7277	0.7277	0.7277	18.19	0.000
Gliclazide-Cd complex	0.077	0.077	0.078	1.2833	1.2833	1.3000	1.2889	32.22	0.010

Sample weight = 0.100 gm; Final sample concentration (ppm) = 4

of cadmium complex the carbonyl peak appears at 1730 cm^{-1} in the form of a sharp band.

All these shifting of bands clearly indicate that coordination is possible at the site through the –NH-CO- group.

Other substitutions

The absorption peak between 600-800 cm-1 are due to the deformation of adjacent hydrogen, in metal complexes this is also present with slight changes, but these changes are negligible. Gliclazide shows band in the region 3100-3300 cm^{-1} as sharp triplet, which is due to the urea NH stretch. In metal complexes such as magnesium, this occurs at 3100-3300 cm^{-1} as a sharp doublet, in calcium complex it occurs in the region 3100-3200 cm^{-1} as a weak band in the form of doublet. In case of chromium complex amide appears at 3250 cm^{-1} as a weak band, whereas in case of manganese complex this occurs at 3210 cm^{-1} as a smallest band. In iron complex it exists at 3250 cm^{-1} as a medium peak, in case of nickel complex it appears at 3240 cm^{-1} as a sharp band. In case of copper complex it is present at 3230 cm^{-1} as a smallest band. Urea NH stretch appears in zinc complex at 3200 cm^{-1} as a weak band and in case of cadmium it exists at 3225 cm^{-1} in the form of a medium band.

NMR studies

NMR data of all the complexes are summarized in table 3. The ^{1}H–NMR of Gliclazide-metal complex displayed nitrogen – metal signals in the range of δ 3.29 – 3.72 due to the deshielding of N-bearing protons. The ^{1}H–NMR of gliclazide-Mg(II) complex displayed nitrogen – Mg signal at δ 3.29, gliclazide - Ca(II) complex at δ 3.32, gliclazide - Cr(II) complex at δ 3.72, gliclazide - Mn(II) complex at δ 3.50, gliclazide - Fe (II) complex at δ 3.66, gliclazide - Ni (II) complex at δ 3.70, gliclazide - Cu (II) complex at δ 3.69, gliclazide - Zn (II) complex at δ 3.68 and gliclazide - Cd(II) complex at δ 3.64 due to deshielding of N-. bearing protons. In all these cases both the nitrogens of the amide took part in bonding to metals (figure 2).

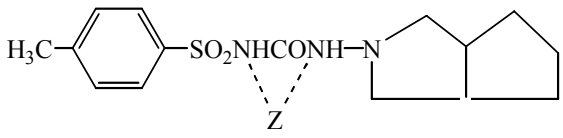

Fig. 2: Gliclazide metal complex; Z = metal

AA studies

This analysis was carried out by direct method which gave total metal content. A number of reference standard solutions of each metal were also prepared having various concentration ranges. Absorbances of these solutions were measured at the specific wavelength of each metal using background correction technique (Smith *et al*., 1982). A graph was plotted between absorbance and concentration of each metal solution, which showed a straight line in each case. The concentrations of unknown solutions were calculated from the absorbance of unknown solutions by using the standard values. Results of analysis are given in table 4.

CONCLUSION

The change in color of the synthesized complexes as well as differences in melting point of all of these complexes as compared to gliclazide suggested that a new product was formed. The shifts of peaks in IR region as well as new signals around at δ 3 due to the de shielding of N-bearing protons in H^{1}-NMR further confirmed the drug metal complexation. The final proof of metal incorporation in gliclazide was obtained by the estimation of the metals from these complexes by atomic absorption spectroscopy.

ACKNOWLEDGMENT

We are thankful to M/s Ali Gohar Pharmaceuticals (Pvt) Limited, Pakistan for a gift of Gliclazide.

REFERENCES

Akanuma Y, Kosaka K and Kanazawa Y (1991). Long-term comparison of oral hypoglycemic agents in diabetic retinopathy. Gliclazide vs. other sulfonylureas. *Am. J. Med*., **90**(Suppl 6A): 74S-76S.

Akanuma Y, Kosaka K, Kanazawa Y, Kasug M, Fukuda and Aoki S (1988). Long term comparison of oral hypoglycemic agents in diabetic retinopathy- gliclazide vs other sulphonylureas. *Diabetes Res.Clin. Pract*.,**5**(1): 81-90.

Archambeaud-Mouveroux F, Nouaille Y, Nadalon S, Treves R and Merle L (1987). Interaction between gliclazide and cimetidine. *European Journal of Clinical Pharmacology*, **31**: 631.

Bayes M, Rabasseda X and Prous JR (2002). Gateways to Clinical Trials. *Methods Find Exp. Clin. Pharmacol*., **24**(7): 431-55.

Benakis A and Glasson B (1980). Metabolic study of ^{14}C-labelled gliclazide in normal rats and in rats with streptozotocin induced diabetes, Royal Society of Medicine International Congress and Symposium Series, **20**: 57-69.

British Pharmacopoeia (1998). The Stationery Office, United Kingdom, pp.637-638.

Campbell DB, Lavielle R and Nathan C (1991). The mode of action and clinical pharmacology of gliclazide: a review. *Diabetes Res. Clin. Pract.* **14**(suppl 2): S21-S36.

Colin D (1999). Therapeutic drugs 2nd Edition, United Kingdom, Churchill Livingstone G53-G56 & G64-G69.

Courtois P, Sener A, Herbaut C, Turc A and Malaisse WJ (1999). Pharmacokinetics of gliquidone, glibenclamide, gliclazide and glipizide in middle aged and aged subjects. *Res. Commun. Mol. Pathol. Pharmacol*., **103**(2): 211-222.

Couturier E and Malaisse WJ (1980). Synergistic effects of hypoglycaemic sulphonylureas and antibiotic ionophores upon calcium translocation. *Br. J. Pharmacol.,* **71**(1): 315-20.

Davis TME, Daly F and Walsh JP (2000). Pharmacokinetics and pharmacodynamics of gliclazide in Caucasians and Australian Aborigines with type-2 diabetes. *Br. J. Clin. Pharmacol*., **49**: 223-230.

Desfaits AC, Serri O and Renier G (1997). Gliclazide decreases cell-mediated low-density lipoprotein (LDL) oxidation and reduces monocyte adhesion to endothelial cells induced by oxidatively modified LDL. *Metabolism.* **46**(10): 1150-6.

Devis G, Somers G, Van Obberghen E and Malaisse WJ (1975). Calcium antagonists and islet function. I. Inhibition of insulin release by verapamil. *Diabetes.* **24**(6): 247-51.

Duhault J, Boulanger M and Lonchampt M (1980). Royal Society of Medicine International Congress and Symposium Series, **20**: 9-18.

Fu ZZ, Yan T, Chen YJ and Sang JQ (1992). Thromboxane/ prostacycline balance in type II diabetes: gliclazide effects. *Metabolism.* **41**(5)(Suppl 1): 33-35.

Guillausseau PJ (1991). An evaluation of long-term glycemic control in non-insulin-dependent diabetes mellitus: the relevance of glycated hemoglobin. *Am. J. Med.,* **90**(Suppl 6A): 46S-49S.

Harrower ADB (1991). Efficiency of gliclazide in comparison with other sulphonylureas in the treatment of NIDDM. *Diabetes Res.Clin. Pract.*, **14**(Suppl 2): S65-S68.

Hatao K, Kaku K, Matsuda M, Tsuchiya M and Kancko T (1985). Sulphonylurea stimulates liver fructose 2,6-bipholsphate formation in proportion to its hypoglycaemic action. *Diabetes Research and Clinical Practice*, **1**: 49-53.

Hernandez JZ and Lavielle R (1986). Is sulphonylurea therapy effective long term? A 3-year study with gliclazide. *Curr. Med. Res. Opin.,* **10**: 351-358.

Holmes B, Heel RC and Brogden RN (1984). Gliclazide, *Drugs*. **27**: 301-327.

Kilo C, Dudley J and Kalb B (1991). Evaluation of the efficacy and safety of Diamicron(R) in non-insulin-dependent diabetic patients. *Diabetes Res. Clin. Pract*., **14**: S79-S82.

Kobayashi K, Kimura M, Sakoguchi TP, Hase A, Matsuoka A and Kaneko S (1984). Pharmacokinetics of gliclazide in healthy and diabetic subjects. *J. Pharm. Sciences*, **73**(12): 1684-1687.

Koda Kimble MA (1992). Diabetes mellitus. *In:* Koda-Kimble MA & Young LY (eds): Applied Therapeutics - The Clinical Use of Drugs, 5th ed. Applied Therapeutics, Vancouver, WA.

Krall LP (1991). The treatment of NIDDM in the decade of the 90s. *Diabetes Res. Clin. Pract*., **14**: S15-S20.

Martindale (1999). The Complete Drug Reference, Pharmaceutical Press, London 32nd Ed, p.320.

Melander A, Bitzen PO and Faber O (1989). Sulphonylurea antidiabetic drugs: an update of their clinical pharmacology and rational therapeutic use. *Drugs.* **37**: 58-72.

Monges AM, Pisano P, Aujoulat P, Salducci D, Durand A, Panagides D, Bory M and Crevat A (1991). ^{1}H nuclear magnetic resonance and clinical studies of interaction of calcium antagonists and hypoglycemic sulfonylureas. *Fundam. Clin. Pharmacol.,* **5**(6): 527-38.

Nomura H, Naito M, Kodama M, Kato Y and Iguchi A (2000). Effects of gliclazide on low-density lipoprotein oxidizability and atherosclerosis in cholesterol-fed rabbits. *J. Atheroscler. Thromb.,* **7**(2): 104-9.

O'Brien RC and Luo M (1997). The effects of gliclazide and other sulfonylureas on low-density lipoprotein oxidation *in vitro. Metabolism.* **46**(12 Suppl 1): 22-5.

O'Byrne S and Feely J (1990). Effects of drugs on glucose tolerance in non-insulin-dependent diabetes (Part I). *Drugs*, **40**: 6-18.

Onozato ML, Tojo A, Goto A and Fujita T (2004). Radical scavenging effect of gliclazide in diabetic rats fed with a high cholesterol diet. *Kidney Int*., **65**(3): 951-60.

Ozawa H, Murai Y and Ozawa T (2003). A 50-year history of new drugs in Japan-the development and progress of anti-diabetic drugs and the epidemiological aspects of diabetes mellitus. *Yakushigaku Zasshi*. **38**(1): 11-27.

Parvez M, Arayne MS, Zaman MK and Sultana N (1999). Acta Crystallographica-Section C -Crystal Structure Communication **55**(1): 74-5.

Shaw KM, Wheeley MSG and Campbell DB (1985). Home blood glucose monitoring in non-insulin-dependent diabetics: the effect of gliclazide on blood glucose and weight control, a multicentre trial. *Diabet. Med*., **2**: 484-490.

Shorr RI, Ray WA and Daugherty JR (1997). Antihypertensives and the risk of serious hypoglycemia in older persons using insulin or sulfonylureas. *JAMA*, **278**: 40-43.

Smith S, Schleicher RG and Hieftje GM (1982). Paper no. 442, 33rd Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Atlantic City, USA.

Tsuckiya S, Sakurai T and Sokiguchi S (1984). Non enzymatic glucosylation of human serum albumin and its influence on binding capacity of sulfonylureas. *Biochemical Pharmacology*, **33**: 2967-2971.

Ubeaud G, Chauvet-Monges AM and Crevat A (1991). Counteraction by nicardipine of the ionophoretic effect of gliclazide: a mitochondrial study. *Pharmacol. Res.,* **24**(1): 33-40.

Zbronska H, Grzeszczak W, Jendryczko A, Zbronski R and Kuzniewicz R (1995). Activity of superoxide dismutase in erythrocytes and leukocytes and levels of zinc and copper in blood of patients with diabetes. Effect of diabetic treatment on examined parameters. *Pol. Arch. Med. Wewn.,* **94**(3): 228-34.