INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

STUDY OF IMIDAZOLE DERIVATIVES TO IDENTIFY THEM AS *MYCOBACTERIUM TUBERCULOSIS* 14_{DM} INHIBITORS

Rupinder Pal[1], Naresh Singh[1] and Prabh Simran Singh[2]*

[1]Rayat Institute of Pharmacy, Railmajra, Distt. Nawanshahar, Punjab, India.
[2]S.B.S College of Pharmacy Patti, Tarn-Taran, Punjab, India.

*Corresponding author: Prabh.7750@gmail.com

ABSTRACT

This work describes a theoretical study of series of Bis-imidazole derivatives of N-monosubstituted amides **1(a-u)** as possible inhibitor of the Cytochrome P450 14 α-sterol demethylase inhibitors by Molecular Docking method. Compounds **3** and **4** were used to identify the active sites on this enzyme and compared with Bis-imidazole derivatives of N-monosubstituted amides. The analysis of Docking results show that compounds **1**(**a-u**) could inhibit 14_{DM}, due to the fact that they act in the same region as reference compounds **(3, 4).** These designed inhibitors make several interactions with the amino acid residues that conform the active sites of 14_{DM}. ΔG values for all compounds were in between –7.91 and –5.48. *14_{DM}-**1n*** complex was found to be most stable. The Molinspiration on-line cheminformatics service was used for calculation of important molecular properties (logP, number of hydrogen bond donors and acceptors).

Keywords: Bis-imidazole, AutoDock, Antimycobactrium, Cytochrome P450 14 α-sterol demethylase.

INTRODUCTION

The treatment of tuberculosis (TB) has been the most challenging worldwide medical problem[1]. Many studies have been devoted to discover new antimycobacterium agents[2]. TB is caused by an infection of *Mycobacterium tuberculosis* (Mtb) which is an acid-fast bacilli bacterium from the genus *Mycobacterium*[3]. In *Mycobacterium tuberculosis,* Cytochrome P450 14 α-sterol demethylase enzyme is a key enzyme that constitutes an important target for the development of new anti-mycobacterial agents[4-7]. 14α-Sterol demethylase (14_{DM}) belongs to a cytochrome P450 family (CYP51) catalyzing removal of a methyl group at position C14 in the sterol molecule. In the conversion of lanosterol to cholesterol, the three methyl groups are removed oxidatively. The reaction involves the NADP(H) dependent sequential oxidations of the initial sterol substrate (lanosterol or 24, 25-dihydrolanosterol) to the corresponding l4α-hydroxymethyl, l4α-carboxaldehyde and finally to the conjugated A8,l4-diene product with the release of formic acid[8-10]. Inhibitors of Mtb $P45014_{DM}$ are one of the most important goals in the treatment of tuberculosis. Azoles (imidazoles and triazoles) are the most widely studied and currently used class of antimycobactrial agents. The crystal structure of Mtb $P45014_{DM}$ alone and in complex with different azoles such as fluconazole and phenylimidazole has been solved[11-12]. The crystallographic data indicated that the above compound have binding pocket. Moreover, there are series of bisimidazole derivatives **2(a-m)** (Fig. 2) synthesized and docked into binding pocket of the 14_{DM} of the *M. tuberculosis*[4]. Therefore, there is a greet demand to identify and discover new compounds with high binding

affinity in same binding pocket. The AutoDock4 program was used for molecular docking simulation. AutoDock is available for academic use from the Scripps Research Institute for free of charge[13-15]. In the docking studies, the Lamarckian Genetic algorithm (LGA) parameter was used[16-17]. To validate the docking studies, the fluconazole was docked with the protein structure of Mtb $P45014_{DM}$ as reference[18]. All the newly designed compounds obey the Lipinski rule of 5 (c logP < 5, molecular weight (MW) 6500, number of hydrogen bond acceptors <10 and donors <5)[19-20]. The Molinspiration on-line cheminformatics service was used for calculation of important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors)[21].

MATERIAL AND METHOD

Molecular modeling was performed on an Intel core2 duo dell laptop (CPU at 1.83 GHz) with Windows XP operating system. Atomic coordinates for the three dimensional protein models (complex of fluconazole with Mtb $P45014_{DM}$; PDB code1EA1[22] were obtained from the Brookhaven Protein Data Bank. The ligand and all the water molecules were removed from the protein database file and hydrogens were added to the protein molecule. The resulting 3D structure formed of the 1EA1 was saved in as a new PDB file. The structures of the compounds used in this study are listed in Table 1. All the ligands used for docking were drawn by the online demonstration of CORINA for generating 3D coordinates program[23]. The MOPAC2009 program was used to optimize the structures of designed ligands. The optimization of each ligand is repeated many times.

For docking studies, the latest version of AutoDock (4.0) was chosen because its algorithm allows full flexibility of small ligands. It has been shown that it successfully reproduces many crystal structure complexes and includes an empirical evaluation of the binding free energy. The enzyme structure was first cleaned of its water molecules and cocrystallized ligands. The preparation of protein and ligand input structures and the definition of the binding sites were carried out under a GRID-based procedure. Then, a rectangular grid box was constructed over all proteins (126 X 126 X 126 $Å^3$) with grid points separated by 0.375 Å under blind docking procedure. All docking simulations were carried out by using the hybrid Lamarckian Genetic Algorithm, with an initial population of 300 randomly placed individuals and a maximum number of energy evaluations (1.0×10^7). The resulting docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound was used for further analysis. Consequently a population of 10 docked configurations is produced for each inhibitor. All other parameters were maintained at their default settings. All the docking result visualizations were achieved by using AutoDock[4].

For the molecular properties descriptions, a series of molecules were drawn in java based JME molecule editor in Molinspiration on-line server accessed on 2nd may 2009. No information about the 3D structure of receptor was stipulated. Different molecular properties (c logP, number of hydrogen bond donors and acceptors) of the molecules were then calculated.

RESULTS AND DISCUSSION

In order to propose new inhibitors, we designed a series of different Bisimidazole derivatives. All the Bisimidazole derivatives designed have N-monosubstituted acetamides connected two imidazole moieties with a carbon bridge. The geometrical optimization of all the derivatives has been done with MOPAC2009 software which uses the semiemprical molecular orbital calculation method for geometrical optimization[24]. Geometrical optimization study indicates that all designed compounds have a butterfly like geometry. The general structures of different derivatives (**1a-u**) used for this study is shown in Fig. 1.

Docking analysis

For computational drug design, there are two key requirements for accurate molecular docking:

(i) The generation of optimized conformations of docked ligands, and (ii) the accurate prediction of binding affinity of the ligand with the crystallographic structure of the inhibitor. To check whether our procedure complied with requirement (i), we modeled and docked fluconazole and 4-Phenylimidazole, for which the crystallographic structure (obtained from Protein drug database) in complex with 14_{DM}

of *M. tuberculosis* is available, as a reference system. This was done by removing the inhibitor from the enzyme allosteric site, building a new molecular model for this compound, applying the conformational procedure described in details afterwards. The best docked structure, which is the configuration with the lowest docking energy in a prevailing cluster, was then compared with the corresponding crystal structure. Figures 4(a) and (b) show a comparison between the co-crystallized conformation of fluconazole into the allosteric binding site of the 14_{DM} from *M. tuberculosis* and the docked conformation obtained upon application of the computational strategy adopted in this work. A comparative study between the crystallographic data and the structural data obtained by fluconazole docking revealed that the docking results matched those obtained by crystallography.

According to the procedure adopted for the molecular docking, all designed bisimidazole compounds (**1a-u**) (Figure 1) and fluconazole were characterized by a similar docking mode in the binding pocket of the 14_{DM} from *M. tuberculosis*. The active site of the 1EA1 interacting with inhibitors consist of residues from B′ and I helix, β strand and the loop connecting the K helix. For the analysis of the binding mode of the different inhibitor with 1EA1 residues, the best docked conformation of each molecule was taken. In binding mode of all the inhibitors and fluconazole, one of the two imidazole rings and the corresponding imidazole moiety of fluconazole lies almost perpendicular to the heme group, with a ring nitrogen atom just above and coordinating the iron group of the heme group. Furthermore, the same imidazole ring also shows non-bonded interaction with the side chain residues ALA256 and THR260. The analysis of average dynamic distance between the nitrogen atom (N3) of the inhibitors and the iron atom of the heme group is 2.4±0.5Å which is in good agreement with that found in the crystal structure of P450cam complexed with azole inhibitors. The second imidazole ring of all inhibitors undergoes interaction with PHE83, ARG96, PHE255, HIS259 and MET79. The substituted phenyl group of the various compounds undergoes various types of bonded and non-bonded interaction with various residues depending on the type of group present on designed inhibitors. The molecular docking of different molecules shows that the predicted binding free energies are quite good, without any reparametrization (Table. 1). The Figure 7 describe the compound **1n, 1i, 1o** and **1a** into the active site of the 14_{DM}.

The docking results indicates that the compound **1n** (Fig. 5) has minimum free binding energy (ΔG = -7.9), among the all designed compounds. Along with interaction of two imidazole moiety with the residue described above, the 4-substituted phenyl group of **1n** establishes interaction with MET433, VAL435, ILE322, ILE323, LEU321 and HIS259. The compound **1o** also shows good free binding energy (ΔG = -7.68). Along with other common interactions, the compound **1o** shows interaction with ILE322, ILE323, PHE78 and MET433 residues. Furthermore, the compound **1i** (Fig. 6) establishes a hydrogen bond between OH group of the inhibitor and MET433 residue. Analysis of the data reveals that the investigated Bisimidazoles either show interactions with same amino acids as is shown by the reference compound fluconazole or they show interaction nearby amino acid residues. Consequently Bisimidazoles may prove to be as good inhibitors as fluconazole is.

Calculation of molecular properties

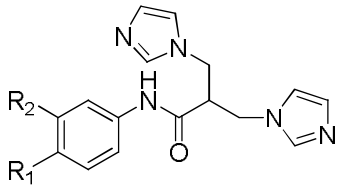
In effort to study the various molecular properties, we initially evaluated their hydrophobic pattern, by calculating c logP, molecular weight (MW) and molecular formula (MF), Number of rule violation, and number of hydrogen bond acceptors and donors (Table 2).

This study indicates that all the designed molecule obey the Lipinski Rule of five. Our results pointed all compounds as sufficiently hydrophobic for penetrating the biological membranes, probably including the cellular wall of the mycobacterium, as determined by Lipinski rule of 5 (c logP < 5, molecular weight (MW) 6500, and number of hydrogen bond acceptors <10 and donors <5).

CONCLUSION

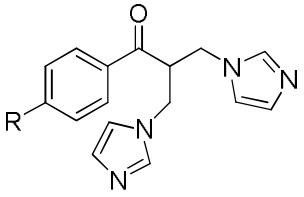
The different Bisimidazole derivatives were designed with the aim of synthesis of compounds having good anti-TB activity. We have described a theoretical study of a series of bisimidazole derivatives (**1a-u**) as inhibitor of 14_{DM} of *M. tuberculosis*. About 21 compounds were docked into the active site of

the 1EA1. All the designed inhibitors show interaction with the residues in the same pocket of 14_{DM} as established by reference drugs (fluconazole and 4-Phenylimidazole). All the compounds show good binding affinity along with acceptable free binding energy. When free binding energy calculation results of different derivatives **1a-u** were compared, the compound **1n** showed best binding affinity with the 14_{DM}. Furthermore, the study of molecular properties described that all the designed inhibitors obey the Lipinski rule of five and thus should be a good drug candidate. Our results might be useful for designing more effective Anti-TB inhibitors targeted to the binding pocket of the 14_{DM}.



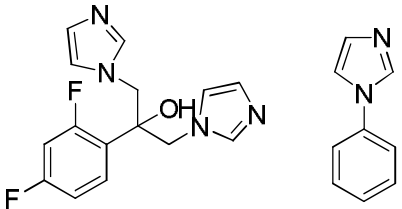
1a-u

Fig. 1: General Structures of Different Compounds used in Molecular Docking.



2a-m

Fig. 2: Bis-imidazole Derivatives Described in Literature



3 4

Fig. 3: Structure of Reference Compounds Fluconazole (3) and 4-Phenylimidazole (4)

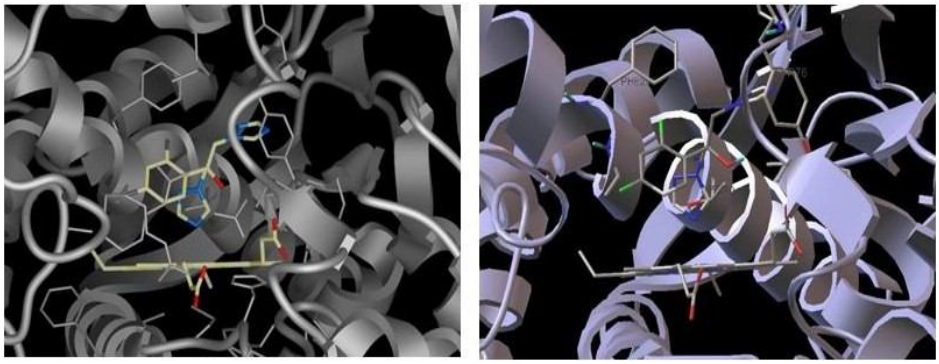
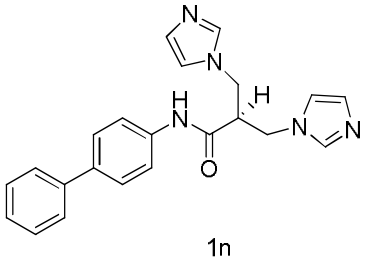


Fig. 4 Comparison between (a) the co-crystallized Conformation of Fluconazole into the active site of 14a-sterol demethylase from M. tuberculosis and (b) the Corresponding Docked Conformation of Fluconazole in the same enzyme pocket obtained upon application of the computational strategy adopted in this work



1n

Fig. 5: Structure of Compound 1n

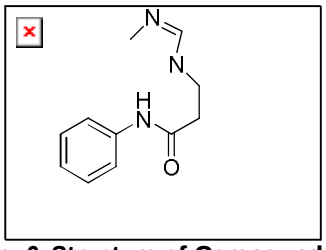


Fig. 6: Structure of Compound 1i

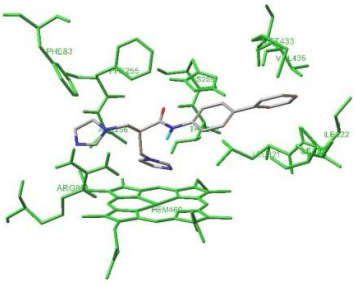


Fig. 7(a): Structure of Compound 1n docked in pocket of 1EA1.

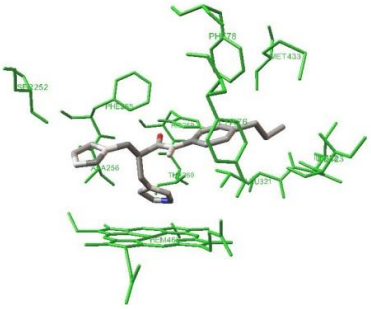


Fig. 7(b): Binding of Compound 1o in active site of 1EA1.

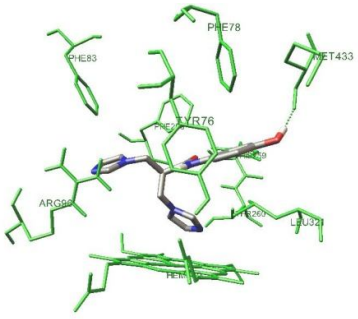


Fig. 7 (c): Interaction of compound compound 1i in active site of 1EA1.

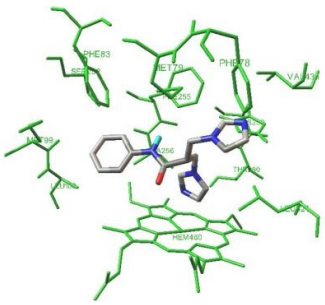


Fig. 7(d): Docked conformation of 1a in binding site of 1EA1.

Table 1: Total Free Energy of Binding (kcal/mol) Between Sterol 14α-Demethylase of M. *Tuberculosis* and Compounds 1a–u with their Docking Rank

S. No.	Structure ID	R1	R2	ΔG_{BIND}	Rank
1	1a	H	H	-6.63	16
2	1b	CH_3COO^-	H	-7.54	3
3	1c	Br	H	-7.30	7
4	1d	CHO	H	-7.02	12
5	1e	Cl	H	-7.05	11
6	1f	C_2H_5O	H	-7.21	9
7	1g	C_2H_5	H	-7.19	10
8	1h	F	H	-6.62	17
9	1i	OH	H	-6.53	18
10	1j	I	H	-7.52	4
11	1k	CH_3O	H	-6.74	13
12	1l	CH_3	H	-6.73	14
13	1m	NO_2	H	-6.41	19
14	1n	C_6H_5	H	-7.91	1
15	1o	C_3H_7	H	-7.68	2
16	1p	CH_3	Cl	-7.50	5
17	1q	H	CH_3	-6.70	15
18	1r	CH_3	CH_3	-7.28	8
19	1s	Br	Br	-5.48	21
20	1t	Cl	Cl	-5.49	20
21	1u	Cl	CH_3	-7.43	6

Table 2: Molecular Descriptors of Designed Compounds. (Predicted with Molinspiration Server)

S. No.	Struc-ture ID	Molecular Formula	Lipinski rule of five				
			Molecular weight	cLog P	No. of Hydrogen acceptor	No. of Hydrogen donor	No. Of rule violation
1	1a	$C_{16}H_{17}N_5O$	295	0.850	6	1	0
2	1b	$C_{18}H_{19}N_5O_2$	337	0.749	7	1	0
3	1c	$C_{16}H_{16}N_5OBr$	374	1.659	6	1	0
4	1d	$C_{17}H_{17}N_5O_2$	323	0.640	7	1	0
5	1e	$C_{16}H_{16}N_5OCl$	330	1.528	6	1	0
6	1f	$C_{17}H_{19}N_5O_2$	325	1.283	7	1	0
7	1g	$C_{18}H_{21}N_5O$	323	1.765	6	1	0
8	1h	$C_{16}H_{16}N_5OF$	313	1.014	6	1	0
9	1i	$C_{16}H_{17}N_5O_2$	311	0.371	7	2	0
10	1j	$C_{16}H_{16}N_5OI$	421	1.933	6	1	0
11	1k	$C_{16}H_{17}N_6O_3$	341	0.907	7	1	0
12	1l	$C_{17}H_{19}N_5O$	309	1.299	6	1	0
13	1m	$C_{17}H_{19}N_5O$	319	0.809	8	1	0
14	1n	$C_{22}H_{21}N_5O$	371	2.645	6	1	0
15	1o	$C_{19}H_{23}N_5O$	337	2.155	6	1	0
16	1p	$C_{17}H_{18}N_5OCl$	344	1.905	6	1	0
17	1q	$C_{17}H_{19}N_5O$	309	1.275	6	1	0
18	1r	$C_{18}H_{21}N_5O$	323	1.675	6	1	0
19	1s	$C_{16}H_{15}N_5OBr_2$	451	2.936	6	1	0
20	1t	$C_{16}H_{15}N_5OCl_2$	364	2.134	6	1	0
21	1u	$C_{17}H_{18}N_5OCl$	343	1.905	6	1	0

REFERENCES

1. Laughon BE, Hurley FL, Losick R, Marks GL, Mitchison D, Narayanan PR, Prokocimer P, Slater EE and Confronting TB. In TB alliance. 2008.
2. Zhang Y and Amzel L M.Tuberculosis Drug Targets. Curr. Drug Tar. 2002;*3:*131-154.
3. Miller N, Cleary T, Kraus G, Young AK, Spruill G and Hnatyshyn HJ. Rapid and Specific Detection of *Mycobacterium tuberculosis* from Acid-Fast Bacillus Smear-Positive Respiratory Specimens and BacT/ALERT MP Culture Bottles by Using Fluorogenic Probes and Real-Time PCR. Jour of Clin Microbio. 2002;43(11): 4143-4147.
4. Zampieri D, Mamolo MG, Vio L, Banfi E, Scialino G, Fermeglia M, Ferrone M and Pricl S. Synthesis, Antifungal and Antimycobacterial activities of New Bis-Imidazole Derivatives, and prediction of their binding to P45014DM by molecular docking and MM/PBSA method. Bioorg. Med. Chem. 2007;15: 7444–7458.
5. Banfi E, Scialino G, Zampieri D, Mamolo MG, Vio L, Ferrone M, Fermeglia M, Paneni MS and Pricl S. Antifungal and antimycobacterial activity of new imidazole and triazole derivatives. A combined experimental and computational approach. J Antimicrob Chemother. 2006;58: 76–84.
6. Mamolo MG, Zampieri D, Falagiani V, Vio L, Fermeglia M, Ferrone M, Pricl S, Banfi E and Scialino G, Antifungal and antimycobacterial activity of new *N*1-[1-aryl-2-(1*H*imidazol-1-yl and 1*H*-1,2,4-triazol-1-yl)-ethylidene]-pyridine-2-carboxamidrazone derivatives: a combined experimental and computational approach. Arkivoc. 2004;231-250.
7. Mamolo MG, Zampieri D, Vio L, Fermeglia M, Ferrone M, Pricl S, Scialino G and Banfi E. Antimycobacterial activity of new 3-substituted 5-(pyridin-4-yl)-3H-1,3,4-oxadiazol-2-one and 2-thione derivatives. Bioorg Med Chem. 2005;13:3797–3809.
8. Trzaskos J, Kawata S and Gaylor JL. Microsomal Enzymes of Cholesterol Biosynthesis. J Bio Chem. 1986;261(31):14651-14657.
9. George J and Schroepfer J. Sterol Biosynthesis. Ann Rev Biochem. 1982;15:555-585.
10. Aoyama Y, Yoshida Y, Sonoda Y and Sato Y. Deformylation of 32-0xo-24,25-dihydrolanosterobly the Purified Cytochrome P-45OI4DM (Lanostero1l4 a-Demethylase) from Yeast Evidence Confirming the IntermediateS tep of Lanosterol 14a-Demethylation. J Bio Chem. 1989;264(31):18502-18505.
11. Podust LM, Poulos TL and Waterman MR. Crystal structure of cytochrome P450 14a-sterol demethylase (CYP51) from Mycobacterium tuberculosis in complex with azole inhibitors. Proc Natl Acad *Sci U.S.A*. 2001;98: 3068.
12. Bellamine A, Lepesheva GL and Waterman MR. Fluconazole binding and sterol demethylation in three CYP51 isoforms indicate differences in active site topology. J Lipid Res. 2004;45:2000-2007.
13. http://autodock.scripps.edu/downloads.
14. Huey R, Morris GM, Olson AJ, Goodsell DS. A semiempirical free energy force field with charge-based desolvation. J Comp Chem. 2007;28(6):1145-1152.
15. Cross JB, Thompson DC, Rai BK, Baber JC, Fan KY, Hu Y and Humblet C.Comparison of Several Molecular Docking Programs: Pose Prediction and Virtual Screening Accuracy. J Chem Inf. 2009.
16. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK and Olson AJ.Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comp Chem. 1999;19(14):1639-1662.
17. Magalhães CS, Barbosa HJC and Dardenne LE. A genetic algorithm for the ligand-protein docking problem. Genet Mol Biol. 2004;27(4):605-610.
18. Matsuura K, Yoshioka S, Tosha S, Hori H, Ishimori K, Kitagawa T, Morishima I, Kagawa N and Waterman MR. Structural diversities of active site in

clinical azole bound forms between sterol 14α-demethylases (CYP51) from human and Mycobacterium tuberculosis. J Bio Chem. 2008;1-35.

19. Duchowicz PR, Talevi A, Bellera C, Bruno-Blanchb LE and Castroa EA. Application of descriptors based on Lipinski's rules in the QSPR study of aqueous solubilities. *Bioorg* Med. Chem. 2007;15:3711–3719.
20. Tetko IV, Bruneau P, Mewes HW, Rohrer DC and Poda GI.Can We Estimate the Accuracy of ADMET Predictions. Drug Discovery Today. 2006; 11(15/16): 700-707.
21. http://www.molinspiration.com/cgi-bin/properties
22. http://www.pdb.org/pdb/explore.do?structureId=1EA1
23. http://www.molecular-networks.com/software/corina/
24. Stewart JJP and Mopac. A Semiemprical Molecular Orbital Program. J Computer-Aided Drug Design. 1990;4:1-105.