Mapping Drug-Target Interaction and Synergy in Multi-molecular Therapeutics for Pressure Overload Cardiac Hypertrophy

Aparna Rai¹, Vikas Kumar², Gaurav Jerath¹,³, CC Kartha⁴,* and Vibin Ramakrishnan¹,*

¹ Molecular Informatics and Design Laboratory, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam 781039, India.

² Laboratory of Cardiovascular Science, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA.

³ PepThera Laboratories Private Limited, Guwahati, Assam 781014, India.

⁴ Society for Continuing Medical Education & Research, Kerala Institute of Medical Sciences, Thiruvananthapuram 695029, India.

* To whom correspondence should be addressed

Email: cckartha@gmail.com, vibin@iitg.ac.in

Supplementary Information

The supplementary information file includes:

- Supplementary Information text
- Supplementary Figure. S1. Gallic and Ellagic acid in AR
- Supplementary Figure S2. Biophore Fingerprints for ACADM
- Supplementary Figure. S3. Biophore Fingerprints for COX4I1
- Supplementary Figure. S4. Biophore Fingerprints for COX6B1
- Supplementary Figure. S5. Biophore Fingerprints for HBB with Gallic acid
- Supplementary Figure. S6. Biophore Fingerprints for MYH14
- Supplementary Figure. S7. Biophore Fingerprints for SLC25A4
- Supplementary Figure. S8. Biophore Fingerprints for HBB with Cholic acid
- Supplementary Table S1. AR Metabolites
- Supplementary Table S2. Peak Intensities
- Supplementary Table S3. Topological Characteristics of PPI network
- Supplementary Table S4. Pathway Analysis for L4 proteins
- Supplementary Table S5. Similarity Search Data
- Supplementary Table S6. Plasma Absorbance of AR metabolites
- Supplementary Table S7. Binding Complexes of Protein-Drug/Metabolite
- Supplementary Table S8. Biophore Fingerprint Matrix
- Legend for extra file (Supplementary Dataset S1)
- Supplementary References

Other supplementary materials for this manuscript include the following:

- Supplementary Dataset S1. In-vivo data, Similarity search, Proteins lists and PPI interactions
I. **Drug Repositioning for Cardiac Hypertrophy**

- **Molecular Docking: Binding complexes**

The similar structure-function theory described in the above sections relies on the binding of ligands (drugs and metabolites) to their respective receptors (proteins/drug-targets). For a drug to exert similar or identical action of its structurally similar metabolite or drug, should bind to the same site on the receptor protein/drug target. The combination of the drug pharmacophore and the target binding site residues is referred to as a biophore. Therefore, we used molecular docking to identify the similar biophores for determining the drug molecules, which can be repositioned for use in cardiovascular diseases. The set of important target proteins from the topological analysis of the protein-protein interaction network were used in this study.

We performed blind docking for the metabolites and their structurally similar drugs by incorporating the entire protein in the docking grid, using AutoDock Vina. The docked complexes were analyzed for similarity (recurring residues) of their respective biophores and further, investigated for known interactions and biological activity of the protein targets used for molecular docking. Such information is crucial for ascertaining the utility of metabolites of AR as potential alternates to currently used therapeutic drugs targeted against identical protein targets. Conversely, the drugs similar to metabolites can also be repositioned for their combinatorial use in the treatment of cardiac hypertrophy. The list of metabolites, their similar drugs and associated target proteins used for molecular docking are given in Table 1 of the main manuscript. The biophores obtained for each of the protein-drug and their corresponding protein-metabolite complexes had similar binding pockets except for HBB-Cholic acid and HBB-Sebacic acid complexes.

The drug-receptor and metabolite-receptor complexes obtained through a blind docking protocol are given in Figures. S2-S8. The complete biophore fingerprint of recurring and non-recurring residues for each of the protein complexes are enlisted in (Table S8).
Supplementary Figures

1. Gallic and Ellagic acid in AR:

Supplementary Figure S1: Gallic and Ellagic acid in AR. a) The HPTLC chromatogram shows the peaks identified as Gallic acid and Ellagic acid found in the AR concoction. b) The table shows the concentration of Gallic and Ellagic acid in AR as a weight by weight percentage.
2. **ACADM-Guanylic Acid-FAD:**

Supplementary Figure S2: Biophore Fingerprints for ACADM: ACADM protein was docked with Flavin Adenine Dinucleotide (FAD) and guanylic acid (AR metabolite). The docking of the two ligands with ACADM resulted in identical biophoric signatures. The ligands interact with 13 identical amino acid residues of ACADM. The common residues have been encircled in the two complexes for comprehension. This suggests the common biological effects of the two ligands (FAD and guanylic acid).
3. **COX4I1-L-methionine-L-formyl Methionine:**

**Supplementary Figure S3: Biophore Fingerprints for COX4I1:** COX4I1 protein was docked with N-Formyl Methionine and L-methionine (AR metabolite). The docking of the two ligands with COX4I1 resulted in identical biophoric signatures. The ligands interact with 8 identical amino acid residues of COX4I1. The common residues have been encircled in the two complexes for comprehension. This suggests the common biological effects of the two ligands (N-Formyl Methionine and L-methionine).
4. **COX6B1- L-methionine-L-formyl Methionine:**

**Supplementary Figure S4: Biophore Fingerprints for COX6B1:** COX6B1 protein was docked with N-Formyl Methionine and L-methionine (AR metabolite). The docking of the two ligands with COX6B1 resulted in identical biophoric signatures. The ligands interact with 8 identical amino acid residues of COX6B1. The common residues have been encircled in the two complexes for comprehension. This suggests the common biological effects of the two ligands (N-Formyl Methionine and L-methionine).
5. HBB-Gallic Acid-2,6-Dicarboxy Napthalene:

Supplementary Figure S5: Biophore Fingerprints for HBB with Gallic Acid: HBB protein was docked with 2,6-Dicarboxy naphthalene and gallic acid (AR metabolite). The docking of the two ligands with HBB resulted in identical biophoric signatures. The ligands interact with 7 identical amino acid residues of HBB. The common residues have been encircled in the two complexes for comprehension. This suggests the common biological effects of the two ligands.
Supplementary Figure S6: Biophore Fingerprints for MYH14: MYH14 protein was docked with Mant-Adenosine di-phosphate and guanylic acid (AR metabolite). The docking of the two ligands with MYH14 resulted in identical biophoric signatures. The ligands interact with 10 identical amino acid residues of MYH14. The common residues have been encircled in the two complexes for comprehension. This suggests the common biological effects of the two ligands.
Supplementary Figure S7: Biophore Fingerprints for SLC25A4: SLC25A4 protein was docked with Adenosine tri-phosphate and guanylic acid (AR metabolite). The docking of the two ligands with SLC25A4 resulted in identical biophoric signatures. The ligands interact with 9 identical amino acid residues of SLC25A4. The common residues have been encircled in the two complexes for comprehension. This suggests the common biological effects of the two ligands.
8. **HBB-Cholic Acid-Sebacic Acid:**

Supplementary Figure S8: Biophore Fingerprints for HBB with Cholic Acid: HBB protein was docked with Sebacic acid and Cholic acid (AR metabolites). The docking of the two ligands with HBB resulted in non-identical biophores. No identical amino acids were identified in the two biophores, suggesting different binding sites and action for the two metabolites.
Supplementary Tables

1. Supplementary Table S1: AR Metabolites (other than primary constituent’s Gallic acid and Ellagic acid). The table lists metabolites (in addition to Gallic acid and Ellagic acid) that are present in the AR concoction. These metabolites were obtained from the LC-MS analysis.

| S. No. | Metabolite                                                                                   | m/z value   | Identifier Peak |
|-------|--------------------------------------------------------------------------------------------|-------------|-----------------|
| 1.    | Biocytin                                                                                     | 373.192     | M + H[1+]       |
| 2.    | Methylcobalamin                                                                             | 1382.5472   | M + K[1+]       |
| 3.    | L-Methionine                                                                                | 150.0575    | M + H[1+]       |
| 4.    | Pyridoxamine Phosphate                                                                      | 249.0617    | M + H[1+]       |
| 5.    | Prostaglandin B1                                                                           | 359.2163    | M + Na[1+]      |
| 6.    | 4-Hydroxy-all-trans-retinyl acetate                                                         | 328.218     | M-NH3 + H[1+]   |
| 7.    | 13’-carboxy-alpha-tocotrienol                                                               | 454.3103    | M + H[1+]       |
| 8.    | Cholic Acid                                                                                 | 409.2919    | M + H[1+]       |
| 9.    | 1alpha,24R,25-trihydroxyvitamin D3 (Calcitrol)                                             | 455.3133    | M + Na[1+]      |
| 10.   | Guanylic Acid                                                                              | 402.0196    | M + K[1+]       |
| 11.   | Carbamoyl Phosphate                                                                         | 141.9899    | M + H[1+]       |
| 12.   | 5-formimino tetrahydrofolate                                                                | 473.1939    | M + H[1+]       |
| 13.   | Nicotinate D-ribonucleoside                                                                  | 279.0692    | M + Na[1+]      |
| 14.   | Sulfate derivative of norepinephrine (Noradrenaline Sulfate)                                | 250.0401    | M + H[1+]       |
| 15.   | 12-oxo-20-dihydroxy-leukotriene B4                                                          | 388.1884    | M + Na[1+]      |
| 16.   | 5S,6S-epoxy-15S-hydroxy-7E,9E,11Z,13E-eicosatetraenoic acid                                 | 334.2144    | M + H[1+]       |
2. **Supplementary Table S2: Peak Intensities.** The table details the peak intensities of other metabolites present in AR concoction and retention time.

| S. No. | Metabolite                                                                 | Retention Time (mins) | Maximum Peak intensity  |
|--------|-----------------------------------------------------------------------------|-----------------------|-------------------------|
| 1.     | Biocytin                                                                    | 14.3                  | 5.38E + 03              |
| 2.     | Methylcobalamin                                                             | 0.68                  | 3.93E + 03              |
| 3.     | L-Methionine                                                                | 15.54                 | 2.93E + 03              |
| 4.     | Pyridoxamine Phosphate                                                      | 8.72                  | 1.22E + 03              |
| 5.     | Prostaglandin B1                                                            | 15.44                 | 1.83E + 03              |
| 6.     | 4-Hydroxy-all-trans-retinyl_acetate                                         | 6.31                  | 6.74E + 02              |
| 7.     | 13'-carboxy-alpha-tocotrienol                                               | 15.63                 | 1.54E + 04              |
| 8.     | Cholic Acid                                                                 | 15.51                 | 4.96E + 03              |
| 9.     | 1alpha,24R,25-trihydroxyvitamin D3 (Calcitrotol)                            | 15.63                 | 4.43E + 03              |
| 10.    | Guanylic Acid                                                               | 7.98                  | 1.04E + 03              |
| 11.    | Carbamoyl Phosphate                                                         | 18.29                 | 2.09E + 03              |
| 12.    | 5-formiminotetrahydrofolate                                                  | 13.61                 | 9.78E + 02              |
| 13.    | Nicotinate D-ribonucleoside                                                 | 0.79                  | 1.71E + 03              |
| 14.    | Sulfate derivative of norepinephrine (Noradrenaline Sulfate)                | 14.72                 | 7.82E + 02              |
| 15.    | 12-oxo-20-dihydroxy-leukotriene_B4                                          | 14.33                 | 1.09E + 04              |
| 16.    | 5S,6S-epoxy-15S-hydroxy-7E,9E,11Z,13E-eicosatetraenoic acid                 | 13.64                 | 2.40E + 03              |
3. **Supplementary Table S3: Topological Characteristics of PPI network.** The PPI network for list of proteins (L4) identified from the intersection of lists L1, L2 and L3, was analyzed for different topological properties such as betweenness centrality, clustering coefficient and degree given below.

| Protein/Target | Degree | Clustering Coefficient | Betweenness Centrality |
|----------------|--------|-------------------------|------------------------|
| PRKAR1A        | 0      | 0                       | 0                      |
| NNT            | 0      | 0                       | 0                      |
| HBB            | 2      | 0                       | 0.05555556             |
| HBA1           | 1      | 0                       | 0                      |
| PGK1           | 17     | 0.32352941              | 0.10014426             |
| GAPDH          | 21     | 0.24285714              | 0.34199597             |
| DLAT           | 6      | 0.66666667              | 0.03527007             |
| OGDH           | 7      | 0.80952381              | 0.00333168             |
| COX4I1         | 6      | 0.33333333              | 0.11557687             |
| COX6B1         | 2      | 1                       | 0                      |
| MDH1           | 14     | 0.37362637              | 0.05803491             |
| GOT1           | 7      | 0.52380952              | 0.00922991             |
| PYGB           | 6      | 0.86666667              | 0.00111413             |
| PYGM           | 7      | 0.76190476              | 0.00322977             |
| GOT2           | 10     | 0.42222222              | 0.0504836              |
| PYGL           | 6      | 0.86666667              | 0.00111413             |
| LDHA           | 15     | 0.39047619              | 0.09450452             |
| SOD1           | 12     | 0.36363636              | 0.07175438             |
| EEF2           | 7      | 0.42857143              | 0.06280045             |
| EIF2S3         | 1      | 0                       | 0                      |
| ACSS1          | 2      | 0                       | 0.00171958             |
| ACADM          | 7      | 0.14285714              | 0.08419521             |
| NME2           | 9      | 0.33333333              | 0.02873425             |
| AK2            | 5      | 0.5                     | 0.00531935             |
| HINT1          | 5      | 0.6                     | 0.00272404             |
| FKBP1A         | 4      | 0.5                     | 0.00655695             |
| HPRT1          | 5      | 0.8                     | 0.00305922             |
| IVD            | 2      | 0                       | 0.004355               |
| ACTA1          | 7      | 0.38095238              | 0.05667962             |
| CALM1          | 10     | 0.26666667              | 0.07244107             |
| ACADS          | 1      | 0                       | 0                      |
| AK1            | 4      | 0.5                     | 0.00101023             |
| YWHAE          | 5      | 0.7                     | 0.00265419             |
| DBI            | 4      | 0.16666667              | 0.01619864             |
| GC             | 2      | 0                       | 0.00147977             |
| CRP            | 4      | 0.33333333              | 0.02350986             |
| AKR1B1         | 5      | 0.7                     | 0.00427041             |
| MYH14          | 2      | 1                       | 0                      |
| SLC25A4        | 2      | 1                       | 0                      |
| CA             | 1      | 0.1                     | 0                      |
4. **Supplementary Table S4: Pathway Analysis for L4 proteins.** The pathways associated with L4 list of proteins shown as nodes in the PPI network (Figure 2 of manuscript). The ontology done using STRING database having false discovery rate (FDR) < 0.05.

| Pathway                                           | Proteins involved                                                                 | FDR       |
|---------------------------------------------------|-----------------------------------------------------------------------------------|-----------|
| Metabolic pathways                                | ACADM, ACADS, ACSS1, AK1, AK2, AKR1B1, COX411, COX6B1, DLAT, GAPDH, GOT1, GOT2, HPRT1, IVD, LDHA, MDH1, NME2, NNT, OGDH, PGK1, PYGB, PYGL, PYGM | 1.28E-15 |
| Carbon metabolism                                | ACADM, ACADS, ACSS1, DLAT, GAPDH, GOT1, GOT2, MDH1, OGDH, PGK1                    | 3.16E-12 |
| Glycolysis / Gluconeogenesis                      | ACSS1, DLAT, GAPDH, LDHA, PGK1                                                   | 1.30E-05 |
| Pyruvate metabolism                              | ACSS1, DLAT, LDHA, MDH1                                                          | 4.54E-05 |
| Glucagon signaling pathway                       | CALM1, LDHA, PYGB, PYGL, PYGM                                                   | 4.76E-05 |
| Cysteine and methionine metabolism               | GOT1, GOT2, LDHA, MDH1                                                          | 4.76E-05 |
| Insulin signaling pathway                        | CALM1, PRKAR1A, PYGB, PYGL, PYGM                                                 | 1E-04    |
| Biosynthesis of amino acids                      | GAPDH, GOT1, GOT2, MDH1                                                          | 2E-04    |
| Citrate cycle (TCA cycle)                        | DLAT, MDH1, OGDH                                                                 | 5E-04    |
| Starch and sucrose metabolism                    | PYGB, PYGL, PYGM                                                                 | 5E-04    |
| Propanoate metabolism                            | ACADM, ACSS1, LDHA                                                              | 5E-04    |
| Phenylalanine, tyrosine and tryptophan biosynthesis | GOT1, GOT2                                                                      | 7E-04    |
| Valine, leucine and isoleucine degradation        | ACADM, ACADS, IVD                                                               | 0.0012   |
| Necroptosis                                       | PYGB, PYGL, PYGM, SLC25A4                                                        | 0.0023   |
| Alzheimer's disease                               | CALM1, COX411, COX6B1, GAPDH                                                     | 0.0029   |
| Purine metabolism                                | AK1, AK2, HPRT1, NME2                                                           | 0.003    |
| Thiamine metabolism                              | AK1, AK2                                                                       | 0.003    |
| Phenylalanine metabolism                         | GOT1, GOT2                                                                     | 0.004    |
| 2-Oxocarboxylic acid metabolism                  | GOT1, GOT2                                                                     | 0.004    |
| Huntington's disease                              | COX411, COX6B1, SLC25A4, SOD1                                                   | 0.004    |
| Arginine biosynthesis                             | GOT1, GOT2                                                                     | 0.0046   |
| HIF-1 signaling pathway                          | GAPDH, LDHA, PGK1                                                               | 0.0054   |
| Insulin resistance                                | PYGB, PYGL, PYGM                                                               | 0.0064   |
| African trypanosomiasis                          | HBA1, HBB                                                                      | 0.0103   |
| Alanine, aspartate and glutamate metabolism      | GOT1, GOT2                                                                     | 0.0104   |
| Tyrosine metabolism                              | GOT1, GOT2                                                                     | 0.0106   |
| Parkinson's disease                               | COX411, COX6B1, SLC25A4                                                        | 0.012    |
| Fatty acid degradation                            | ACADM, ACADS                                                                   | 0.0144   |
| Malaria                                           | HBA1, HBB                                                                      | 0.0157   |
| Arginine and proline metabolism                  | GOT1, GOT2                                                                     | 0.0158   |
| Fatty acid metabolism                             | ACADM, ACADS                                                                   | 0.0158   |
| PPAR signaling pathway                            | ACADM, DBI                                                                     | 0.0318   |
| Drug metabolism - other enzymes                   | HPRT1, NME2                                                                    | 0.0332   |
| Cardiac muscle contraction                        | COX411, COX6B1                                                                 | 0.0332   |
5. **Supplementary Table S5: Similarity Search Data.** Associated Drug Targets (Proteins), AR constituents and Structurally Similar Drugs with their similarity scores.

| S.No. | PROTEIN   | METABOLITE                  | SIMILAR DRUGS              | TANIMOTO COEFFICIENT (Tc) |
|-------|-----------|-----------------------------|----------------------------|---------------------------|
| 1.    | ACADM     | Guanylic Acid               | DB03147                    | 0.72                      |
| 2.    | ACADS     | Guanylic Acid               | DB03147                    | 0.72                      |
| 3.    | ACSS1     | Guanylic Acid               | DB00131, DB00171           | 0.79, 0.72                |
| 4.    | ACTA1     | Guanylic Acid               | DB04395                    | 0.66                      |
| 5.    | AK1       | Guanylic Acid               | DB01717                    | 0.717                     |
| 6.    | AK2       | Guanylic Acid               | DB01717                    | 0.717                     |
| 7.    | AKR1B1    | Guanylic Acid               | DB03461                    | 0.66                      |
| 8.    | CA2 (CAR2)| Ellagic acid                | DB08846                    | 1                         |
| 9.    | CALM1     | Cholic Acid                 | DB08231                    | 0.86                      |
| 10.   | COX4I1    | Cholic Acid, L-Methionine   | DB02659, DB04464           | 1, 0.697                  |
| 11.   | COX6B1    | Cholic Acid, L-Methionine   | DB02659, DB04464           | 1, 0.697                  |
| 12.   | CRP       | Guanylic Acid               | DB03945                    | 0.6, 0.6                  |
| 13.   | DBI       | Guanylic Acid               | DB01992                    | 0.64                      |
| 14.   | DLAT      | Cholic Acid                 | DB03461                    | 0.66                      |
| 15.   | EEF2      | Guanylic Acid               | DB04315, DB02059           | 0.94, 0.73                |
| 16.   | EIF2S3    | Guanylic Acid               | DB04315                    | 0.94                      |
| 17.   | FKBP1A    | Cholic Acid                 | DB08231                    | 0.86                      |
| 18.   | GAPDH     | Guanylic Acid               | DB02059, DB03893           | 0.73, 0.65                |
| 19.   | GC        | Calcitetol                 | DB00136                    | 0.98                      |
| 20.   | GOT1      | Pyridoxamine Phosphate      | DB00114                    | 0.84                      |
| 21.   | GOT2      | Pyridoxamine Phosphate      | DB00114, DB02783           | 0.84, 0.79                |
| 22.   | HBA1      | Gallic Acid, Cholic Acid    | DB08262, DB07645           | 0.61, 0.86                |
| 23.   | HBB       | Gallic Acid, Cholic Acid    | DB08262, DB07645           | 0.61, 0.86                |
| 24.   | HINT1     | Guanylic Acid               | DB01972, DB00131           | 1, 0.79                   |
| 25.   | HPRT1     | Guanylic Acid               | DB02309                    | 0.68                      |
| 26.   | IVD       | Guanylic Acid               | DB04036                    | 0.6                       |
| 27.   | LDHA      | Guanylic Acid               | DB02483                    | 0.67                      |
| 28.   | MDH1      | Guanylic Acid               | DB03461                    | 0.66                      |
| 29.   | MYH14     | Guanylic Acid               | DB03412                   | 0.63                      |
| 30.   | NME2      | Guanylic Acid               | DB040315                   | 0.94                      |
| 31.   | NNT       | Guanylic Acid               | DB03461, DB01763           | 0.66, 0.63                |
| 32.   | OGDH      | Cholic Acid                 | DB00313                    | 0.77                      |
| 33.   | PKP1      | Guanylic Acid               | DB03909                    | 0.67                      |
| 34.   | PRKAR1A   | Guanylic Acid               | DB02315, DB02527           | 0.94, 0.75                |
| 35.   | PYGB      | Pyridoxamine Phosphate      | DB00114                    | 0.84                      |
| 36.   | PYGL      | Guanylic Acid, Pyridoxamine Phosphate | DB00131, DB00114 | 0.79, 0.84 |
| 37.   | PYGM      | Guanylic Acid, Pyridoxamine Phosphate | DB00131, DB00114 | 0.79, 0.84 |
| 38.   | SLC25A4   | Guanylic Acid               | DB00171                    | 0.72                      |
| 39.   | SOD1      | Noradrenaline Sulfate       | DB00668, DB01064           | 0.62, 0.60                |
| 40.   | YWHAE     | Carbamoyl Phosphate         | DB01780                    | 0.66                      |
6. **Supplementary Table S6: Plasma Absorbance of AR metabolites.** The table reports previous studies available on the candidate metabolites on absorbance in plasma upon oral administration. These candidate metabolites correspond to the proteins resultant from the network analysis of L4 proteins and have potential therapeutic importance in the treatment of Left Ventricular Cardiac Hypertrophy (LVCH).

| S. No. | Candidate metabolite | Absorbance in Plasma                                                                                                                                                                                                 | Reference |
|--------|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 1.     | Guanylic Acid        | As per the ADMET predictions of the DrugBank database, guanylic acid has the probability of 71% of absorbance from intestines and a 95% probability of crossing the blood brain barrier.                                               | 4         |
| 2.     | Cholic Acid          | Cholic Acid is an approved drug and orally administered for the treatment of bile acid synthesis disorders and peroxisomal disorders. This suggests it is absorbed in the plasma.                                 | 5         |
| 3.     | L-Methionine         | The supplementation of L-methionine increases the plasma absorbance of other metabolites.                                                                                                                             | 6         |
| 4.     | Gallic Acid          | Pharmacokinetic analysis revealed 4-O-methylgallic acid (4OMGA) as the major metabolite in human plasma.                                                                                                              | 7         |
7. **Supplementary Table S7: Binding Complexes of Protein-Drug/Metabolite.**
The binding complexes studied using molecular docking for analyzing the biophore associated for each set of AR metabolite and similar drug molecule with a common target protein are given. The PDB ID for the target protein is also provided. All AR metabolites have been categorized as Experimental molecules while their similar drugs have been categorized as approved/investigational as per their clinical trial outcome/process.

| S No. | Complexes       | Drug name                              | PDB ID | Category                  |
|-------|----------------|----------------------------------------|--------|---------------------------|
| 1     | ACADM-DB03147  | FAD (Flavin Adenine Dinucleotide)      | 2A1T   | Approved                  |
| 2     | ACADM-GUAN     | Guanylic acid                          | 2A1T   | Experimental              |
| 3     | COX4I1-CHOLIC  | Cholic Acid                            | 5Z62   | Approved                  |
| 4     | COX4I1-DB04464_L | N-formyl methionine                | 5Z62   | Experimental              |
| 5     | COX4I1-LMETH   | L-methionine                           | 5Z62   | Approved, Nutraceutical    |
| 6     | COX6B1-CHOLIC  | Cholic Acid                            | 5Z62   | Approved                  |
| 7     | COX6B1-DB04464_L | N-formyl methionine                | 5Z62   | Experimental              |
| 8     | COX6B1-LMETH   | L-methionine                           | 5Z62   | Approved, Nutraceutical    |
| 9     | HBB-CHOLIC     | Cholic Acid                            | 2DN2   | Approved                  |
| 10    | HBB-DB07645_C  | Sebacic Acid                           | 2DN2   | Experimental              |
| 11    | HBB-DB08262_GA | 2,6-dicarboxy naphthalene            | 2DN2   | Experimental              |
| 12    | HBB-GALLIC     | Gallic acid                            | 2DN2   | Approved                  |
| 13    | MYH14-DB03126  | Mant-Adp                               | 5I4E   | Experimental              |
| 14    | MYH14-GUAN     | Guanylic acid                          | 5I4E   | Experimental              |
| 15    | SLC25A4-DB00171 | ATP (Adenosine triphosphate)        | 1OKC   | Investigational, Nutraceutical |
| 16    | SLC25A4-GUAN   | Guanylic acid                          | 1OKC   | Experimental              |
8. **Supplementary Table S8: Biophore Fingerprint Matrix.** The Table showing similarity among the binding pockets of protein-drug and protein-metabolite complexes. Each of the column represents the residues that comprise the biophore followed by rows representing the complexes. The table is shown in a binary format, 0 indicating the non-recurring residues and 1 indicating the recurring ones.

| ACADM Residues | Arg 281 | Asn 354 | Asn 214 | Gln 292 | Gln 349 | Gln 380 | Gly 141 | Gly 352 | Gly 353 | His 291 | Ile 294 | Ile 350 | Ile 371 | Ile 374 | Leu 103 | Leu 288 | Leu 351 | Leu 384 | Phe 284 | Phe 356 | Ser 142 | Thr 136 | Thr 168 | Thr 222 | Thr 283 | Thr 378 | Trp 166 |
|----------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Metabolite     | 1       | 1       | 0       | 0       | 1       | 0       | 1       | 1       | 0       | 1       | 0       | 0       | 0       | 1       | 0       | 0       | 1       | 1       | 0       | 0       | 0       | 1       | 1       | 1       |
| Similar Drug   | 1       | 0       | 1       | 1       | 1       | 1       | 1       | 1       | 0       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       |
| COX4I1 Residues| Ser 89  | Tyr 82  | Phe 86  | Pro 45  | Met 93  | Asp 40  | Arg 41  | Glu 88  | Phe 90  | Tyr 38  |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Metabolite     | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 0       | 1       | 1       | 1       |         |         |         |         |         |         |         |         |         |         |         |
| Similar Drug   | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 0       | 1       | 1       | 1       |         |         |         |         |         |         |         |         |         |         |         |
| COX6B1 Residues| Asp 18  | Phe 21  | Asn 33  | Asn 29  | Ala 15  | Trp 56  | Pro 16  | Val 60  | Leu 64  | Arg 20  | Phe 17  |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Metabolite     | 1       | 1       | 1       | 1       | 0       | 1       | 1       | 0       | 1       | 0       | 1       | 1       |         |         |         |         |         |         |         |         |         |         |         |         |
| Similar Drug   | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 0       | 1       | 1       |         |         |         |         |         |         |         |         |         |         |         |         |
| HBB Residues   | Ala 70  | His 92  | Leu 141 | Phe 103 | Ala 138 | Phe 71  | Gly 107 | Val 137 | Leu 106 | Val 67  |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Metabolite     | 1       | 0       | 1       | 1       | 0       | 0       | 1       | 0       | 1       | 1       | 1       |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Similar Drug   | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       |         |         |         |         |         |         |         |         |         |         |         |         |         |
| MYH14 Residues | Ser 377 | His 302 | Leu 344 | Ala 247 | Gln 306 | Thr 348 | Pro 243 | Gla 246 | Asn 258 | Gln 340 | Cys 299 | Ser 335 | Asp 257 | Glu 206 | Ser 336 |         |         |         |         |         |         |         |         |
| Metabolite     | 0       | 0       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 0       | 0       | 0       | 0       |         |         |         |         |         |         |         |         |
| Similar Drug   | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       |         |         |         |         |         |         |         |         |
| SLC25A4 Residues| Arg 235 | Arg 80  | Ser 180 | Arg 280 | Arg 236 | Asp 232 | Lys 33  | Tyr 187 | Gly 225 | Asn 277 | Tyr 229 | Ser 228 | Phe 231 | Gly 183 | Ile 184 | Lys 23  | Glu 30  |         |         |         |         |         |         |
| Metabolite     | 1       | 1       | 1       | 0       | 1       | 0       | 1       | 0       | 0       | 0       | 1       | 1       | 1       | 1       | 1       |         |         |         |         |         |         |         |         |
| Similar Drug   | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 0       | 0       | 0       |         |         |         |         |         |         |         |         |
Legend for extra files (Dataset S1)

1. **Supplementary Dataset S1: In-vivo data, Similarity search, Proteins lists and PPI interactions.** The dataset tabulates the lists of proteins with their expression profiles from *in-vivo* experiments for Aorta Constricted and Biologically Aged rat models. Additionally, the dataset S1 enlists L1 (AC) and L2 (BA), targets associated with metabolites and their similar drugs (L3) and the intersection of L1, L2 and L3 proteins as common proteins (L4). It also gives information on the similar drugs associated with all the 18 AR metabolites and targets corresponding to them. The metabolites associated with common proteins (L4), their similar drugs and other related details such as Tc score, the status of drugs (approved, experimental, investigational) etc. are also found. The dataset S1 also shows of the network connections of the L4 proteins which were further analyzed for various network topologies.
Supplementary References

1 Berger, S. I. & Iyengar, R. Role of systems pharmacology in understanding drug adverse events. *Wiley Interdiscip Rev Syst Biol Med* **3**, 129-135, doi:10.1002/wsbm.114 (2011).

2 Mehra, A., Jerath, G., Ramakrishnan, V. & Trivedi, V. Characterization of ICAM-1 biophore to design cytoadherence blocking peptides. *Journal of molecular graphics & modelling* **57**, 27-35, doi:10.1016/j.jmgm.2015.01.004 (2015).

3 Trott, O. & Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* **31**, 455-461, doi:10.1002/jcc.21334 (2010).

4 Wishart, D. S. *et al.* DrugBank: a knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Res* **36**, D901-D906, doi:10.1093/nar/gkm958 (2008).

5 McRae, M. *et al.* Plasma Bile Acid Concentrations in Patients with Human Immunodeficiency Virus Infection Receiving Protease Inhibitor Therapy: Possible Implications for Hepatotoxicity. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* **30**, 17-24, doi:10.1592/phco.30.1.17 (2010).

6 Mertens-Talcott, S. U., Jilma-Stohlawetz, P., Rios, J., Hingorani, L. & Derendorf, H. Absorption, Metabolism, and Antioxidant Effects of Pomegranate (Punica granatum L.) Polyphenols after Ingestion of a Standardized Extract in Healthy Human Volunteers. *Journal of Agricultural and Food Chemistry* **54**, 8956-8961, doi:10.1021/jf061674h (2006).

7 Ditscheid, B. *et al.* Effect of L-methionine supplementation on plasma homocysteine and other free amino acids: a placebo-controlled double-blind cross-over study. *European Journal of Clinical Nutrition* **59**, 768-775, doi:10.1038/sj.ejcn.1602138 (2005).