

Appendix

A. Preparation of the Screening Set of DHFR: Steps and Tools

- a. Tools: MDL ISIS/Base and ISIS/Draw
- b. Databases: the MDDR and PDB
- c. Steps:

ISIS/Base - [MDDR3D.DB/&structure]

File Edit Options Object Database Search List Window Help

Forms Query Browse Update

<MOL> 1 of 132726
Search Domain: All

Structure Model Identification Biology Lit & Patent

Structure

MDL MDDR 3D 2002.2

Extreg 090109

Preferred no. 090109

Preview no.

Active investigation?

Development phase Year
Launched 1988

CAS registry no. Derivative
77671-31-9

Index Activity

28000	Cardiotonic
37240	Phosphodiesterase Inhibitor

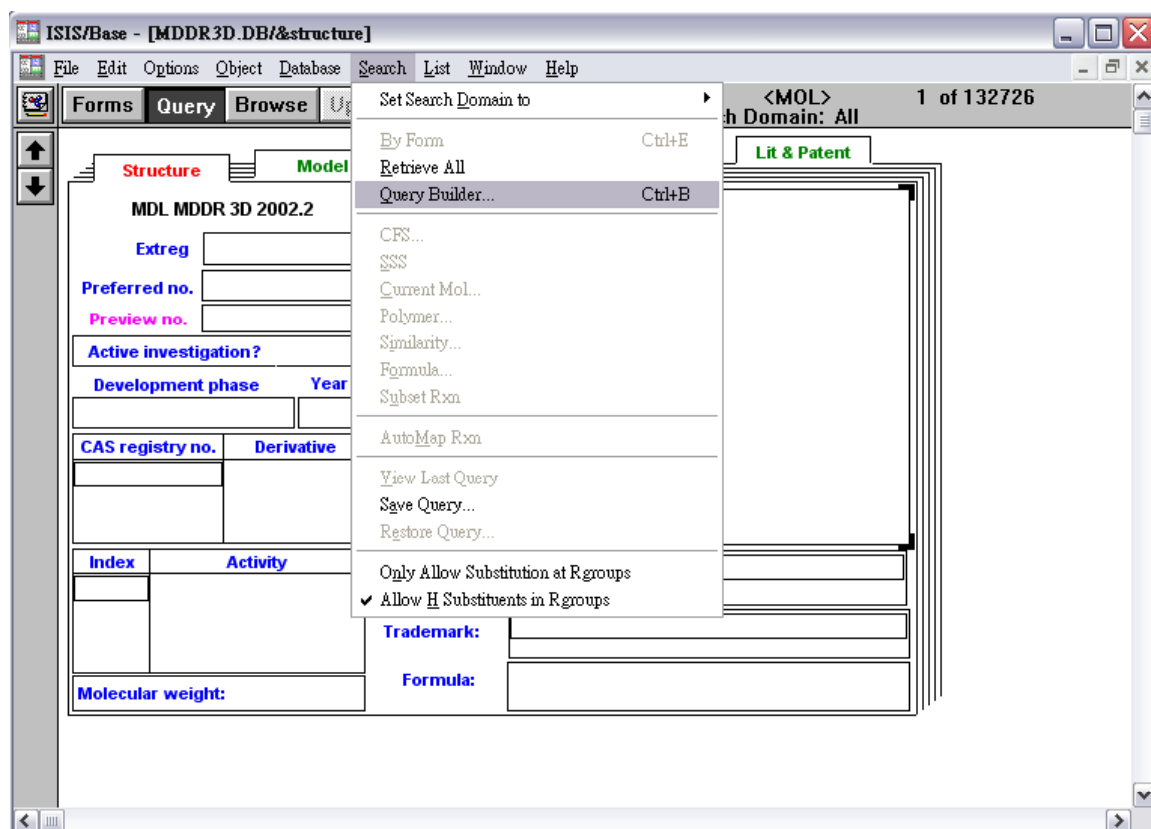
Molecular weight: 248.30

Generic name: Enoximone < Rec INN; BAN; USAN >
Fenoximone

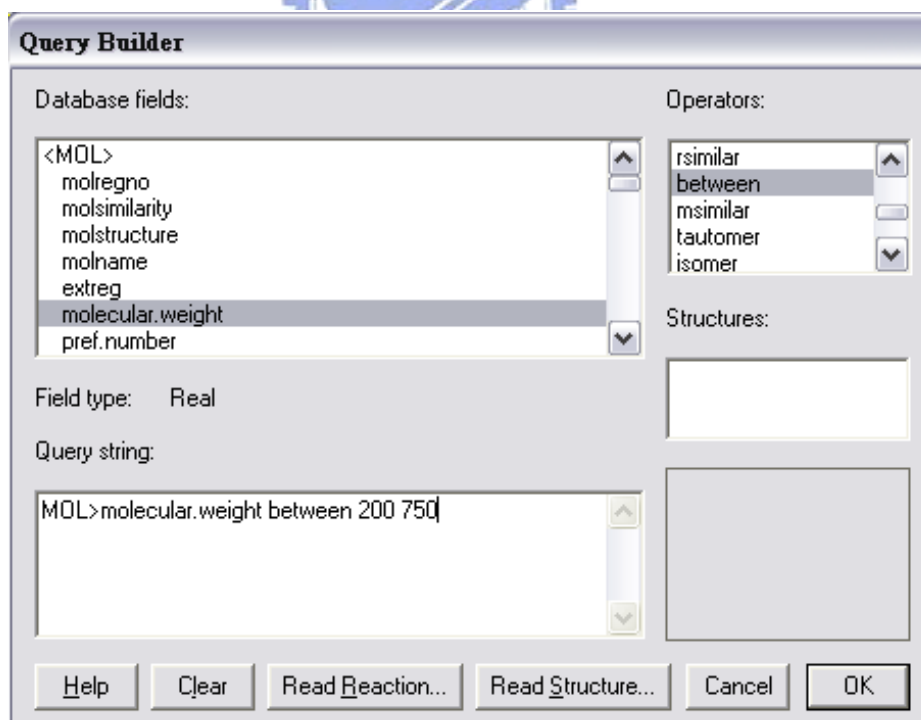
Trademark: Perfane (Aventis Pharma, FR; Lepetit, IT)
Perfan (Aventis Pharma, DE, GR)

Formula: C₁₂ H₁₂ N₂ O₂ S

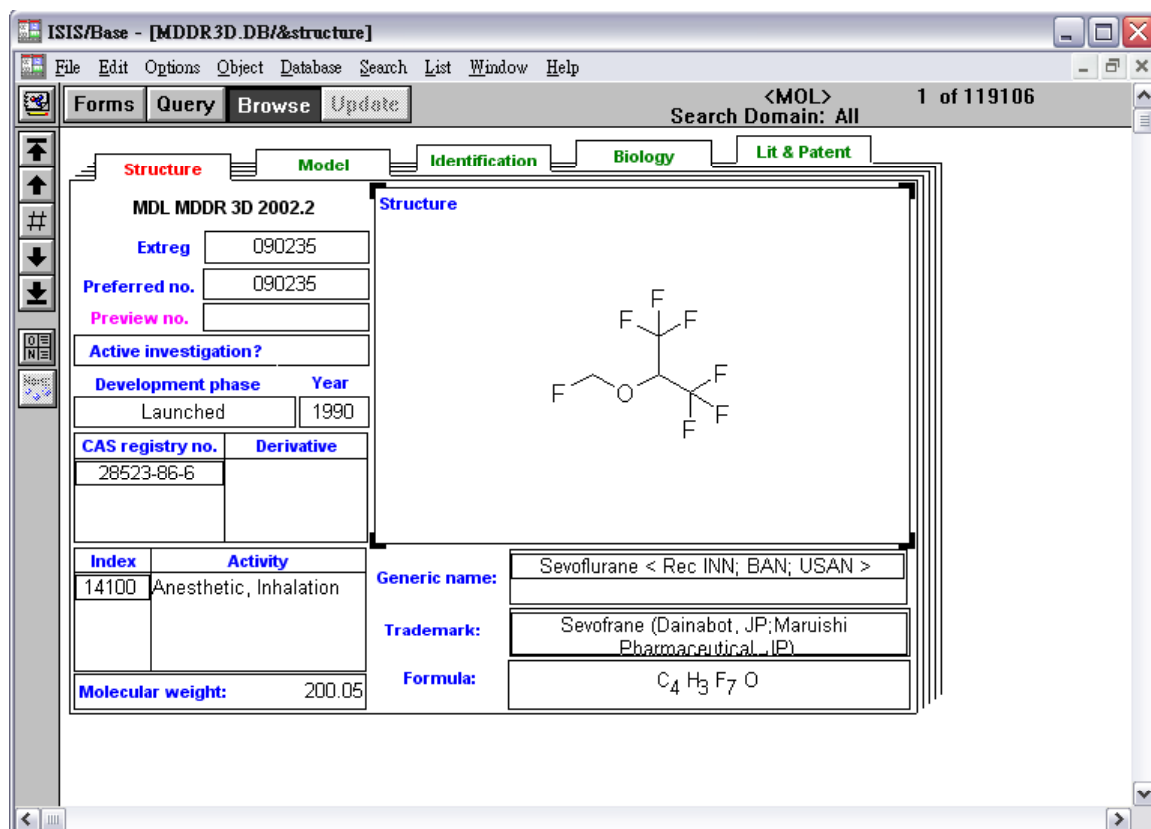
Step 1. Connect to the MDDR with ISIS/Base



Step 2. Build a query with the query builder



Step3. Select “molecular.weight” between 200 and 750



Step 4. 119106 molecules with molecular weights between 200 and 750

ISIS/Base - [MDDR3D.DB/&structure]

File Edit Options Object Database Search List Window Help

Forms Query Browse Update <MOL> 1 of 119106 Search Domain: All

Structure Model Identification Biology Lit & Patent

MDL MDDR 3D 2002.2

Extreg: 090235

Preferred no.: 090235

Preview no.:

Active investigation?:

Development phase: Launched Year: 1990

CAS registry no.	Derivative
28523-86-6	

Index: 14100 Activity: Anesthetic, Inhalation

Molecular weight: 200.05

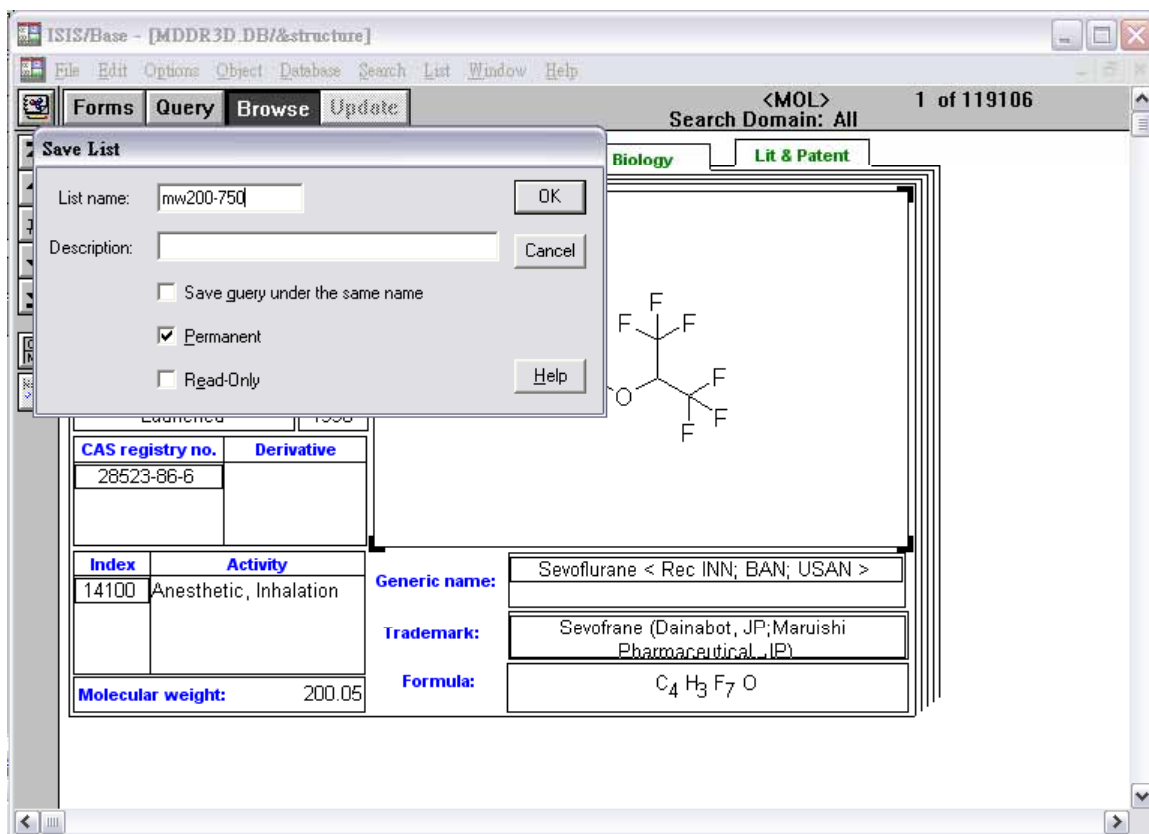
Generic name: Sevoflurane < Rec INN; BAN; USAN >

Trademark: Sevofrane (Dainabot, JP; Maruishi Pharmaceutical, JP)

Formula: C₄ H₃ F₇ O

- Save List...
- Open List...
- Discard Item Ctrl+L
- Move Item to
- Copy Item to
- Repeat Move or Copy to Ctrl+T
- Logic...
- Edit...
- Sort...
- Sort on Search
- Delete Temporary Lists
- Import-Export Local Lists

Step 5. Save the list of molecules with molecular weights between 200 and 750



ISIS/Base - [MDDR3D.DB/&structure]

File Edit Options Object Database Search List Window Help

Forms Query Browse Update

<MOL> 1 of 119106
Search Domain: All

Biology Lit & Patent

Save List

List name: mw200-750 OK

Description: Cancel

Save query under the same name

Permanent

Read-Only Help

Chemical structure: CC(F)(F)C(F)F

CAS registry no.	Derivative
28523-86-6	

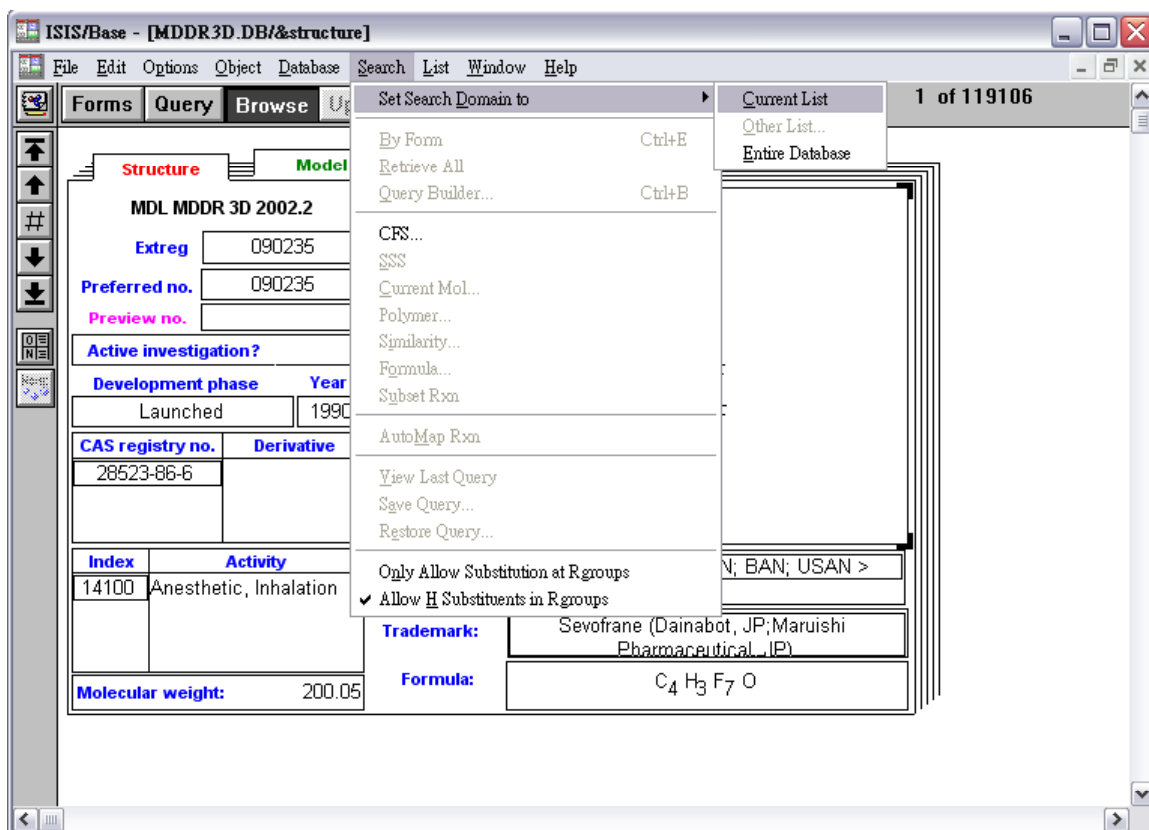
Index	Activity
14100	Anesthetic, Inhalation

Generic name: Sevoflurane < Rec INN; BAN; USAN >

Trademark: Sevofrane (Dainabot, JP; Maruishi Pharmaceutical, JP)

Formula: C₄ H₃ F₇ O

Step 6. Input the list name

ISIS/Base - [MDDR3D.DB/&structure]

File Edit Options Object Database Search List Window Help

Forms Query Browse Update

Set Search Domain to: Current List 1 of 119106

Other List...
Entire Database

By Form Ctrl+E

Retrieve All

Query Builder... Ctrl+B

CFS...

SSS

Current Mol...

Polymer...

Similarity...

Formula...

Subset Rxn

AutoMap Rxn

View Last Query

Save Query...

Restore Query...

Only Allow Substitution at Rgroups

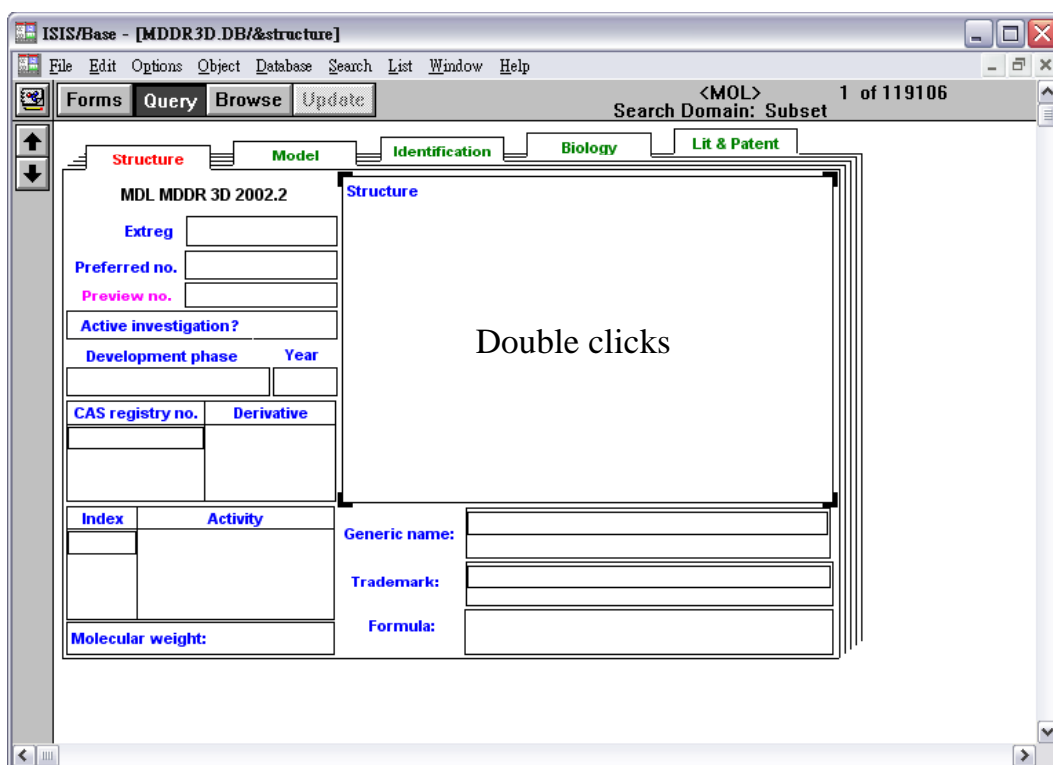
Allow H Substituents in Rgroups

Generic name: Sevoflurane < Rec INN; BAN; USAN >

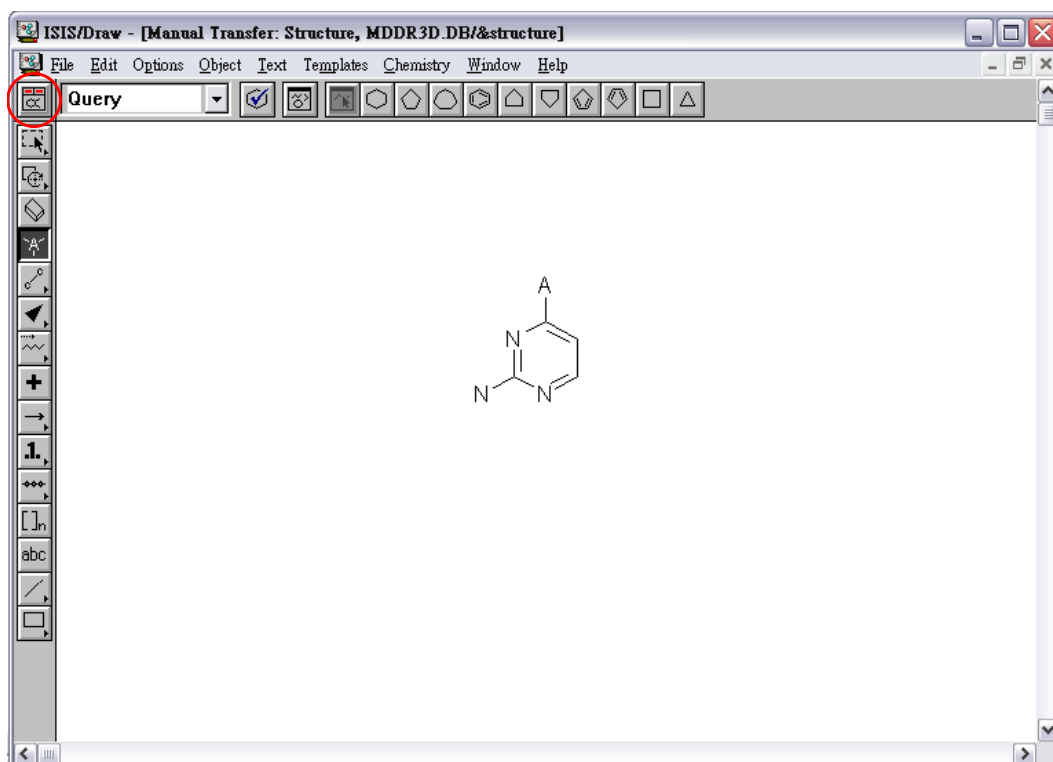
Trademark: Sevofrane (Dainabot, JP; Maruishi Pharmaceutical, JP)

Formula: C₄ H₃ F₇ O

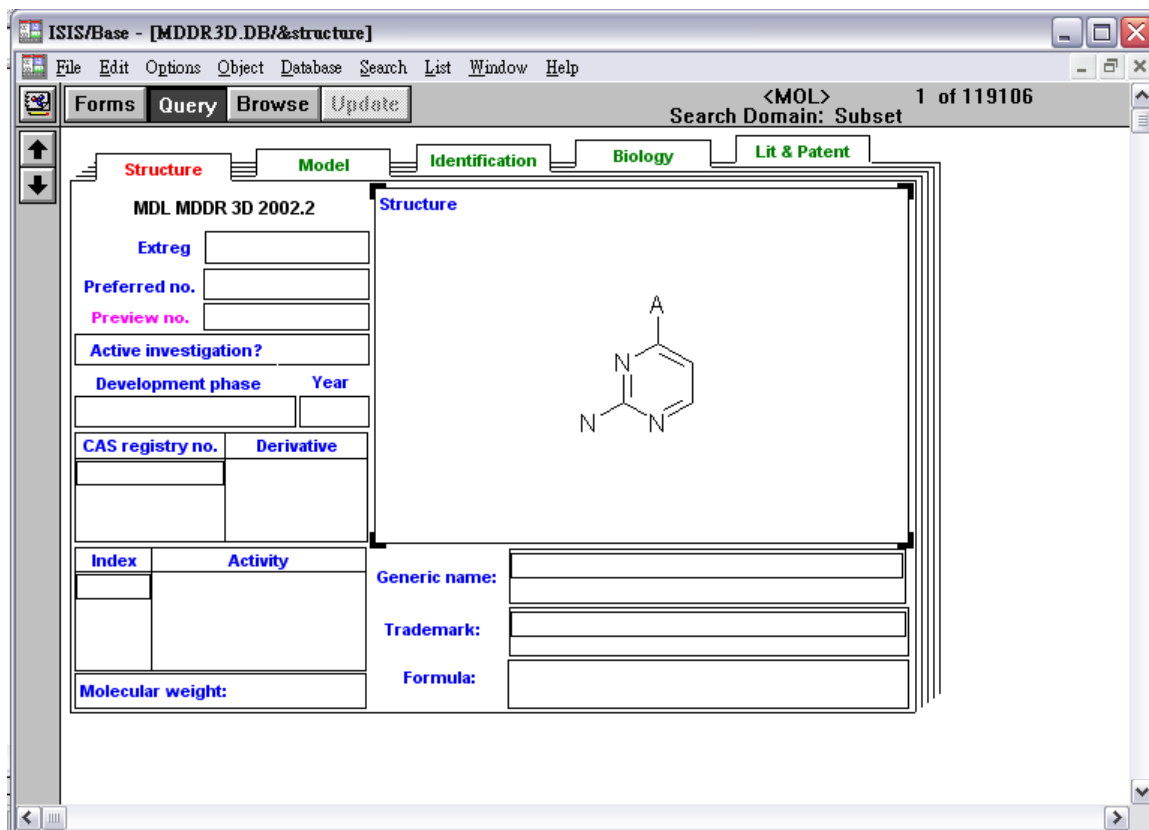
Step 7. Set search domain to the current list



Step 8. Query the similar structures in the current list. Double clicks on the structure block



Step 9. Draw the structure that you want to query with the ISIS/Draw. Click the up-left icon (red circle) to transfer the query structure to the ISIS/Base



Step 10. Transfer completely



ISIS/Base - [MDDR3D.DB/&structure]

File Edit Options Object Database Search List Window Help

Forms Query Browse Update

<MOL> 1 of 119106
Search Domain: mddr200-750

Structure Model Identification Biology Lit & Patent

MDL MDDR 3D 2002.2

Extreg:

Preferred no.:

Preview no.:

Active investigation?:

Development phase: Year:

CAS registry no.: Derivative:

Index: Activity:

Molecular weight:

Generic name:

Trademark:

Formula:

Context Menu:

- Set Search Domain to
- By Form Ctrl+E
- Retrieve All
- Query Builder... Ctrl+B
- CFS...
- SSS
- Current Mol...
- Polymer...
- Similarity...
- Formula...
- Subset Rxn
- AutoMap Rxn
- View Last Query
- Save Query...
- Restore Query...
- Only Allow Substitution at Rgroups
- ✓ Allow H Substituents in Rgroups

Step 11. Search by the form

ISIS/Base - [MDDR3D.DB/&structure]

File Edit Options Object Database Search List Window Help

Forms Query Browse Update

<MOL> 1 of 2197
Search Domain: mddr200-750

Structure Model Identification Biology Lit & Patent

MDL MDDR 3D 2002.2

Extreg: 141315

Preferred no.: 141315

Preview no.:

Active investigation?

Development phase Year

Biological Testing

CAS registry no. Derivative

Index Activity

75000	Antineoplastic
78389	Dihydrofolate Reductase Inhibitor

Molecular weight: 467.49

Generic name:

Trademark:

Formula: C₂₂H₂₅N₇O₅

Chemical structure: CN(C)Cc1ccc(cc1)C(=O)N(C)C(=O)CC(=O)O

Step 12. Search result



ISIS/Base - [MDDR3D.DB/&structure]

File Edit Options Object Database Search List Window Help

Forms Query Browse Update

<MOL> 1 of 2197
Search Domain: mddr200-750

Structure Model Identification Biology Lit & Patent

MDL MDDR 3D 2002.2

Extreg: 141315

Preferred no.: 141315

Preview no.:

Active investigation?

Development phase Year

Biological Testing

CAS registry no. Derivative

Index Activity

75000	Antineoplastic
78389	Dihydrofolate Reductase Inhibitor

Molecular weight: 467.49

Generic name:

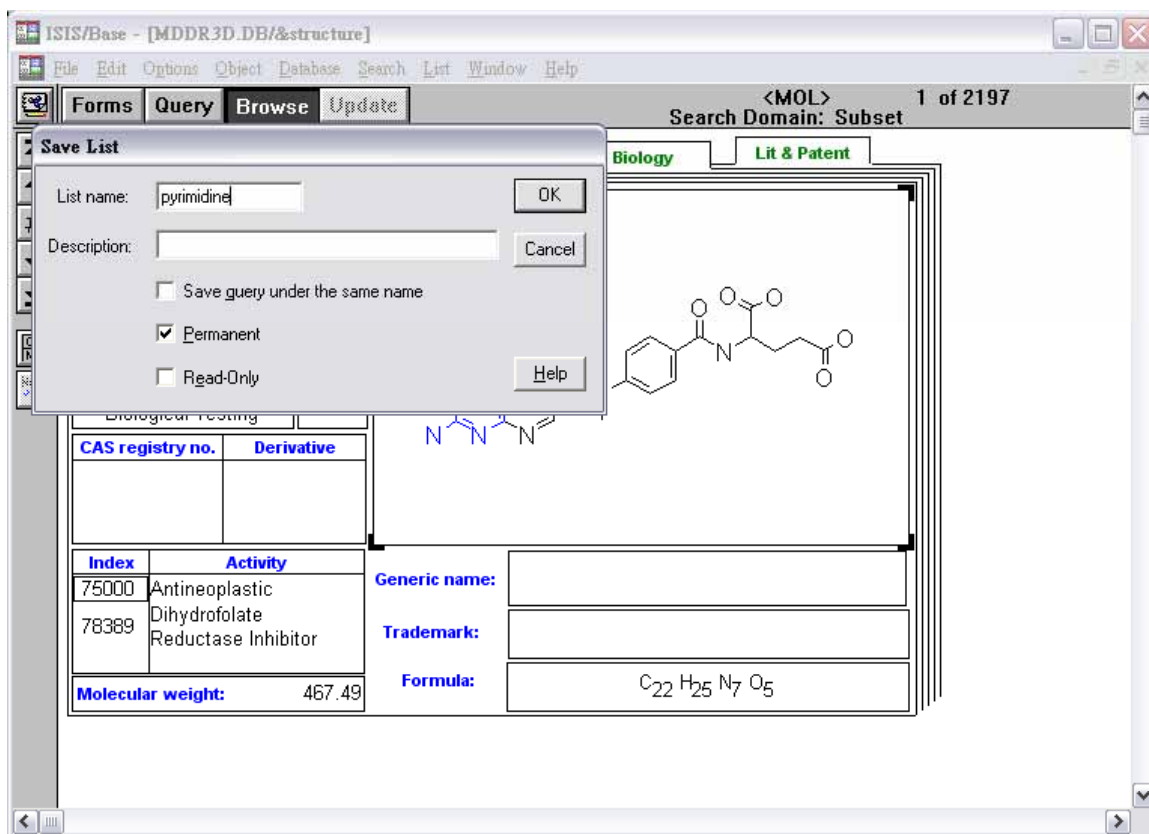
Trademark:

Formula: C₂₂H₂₅N₇O₅

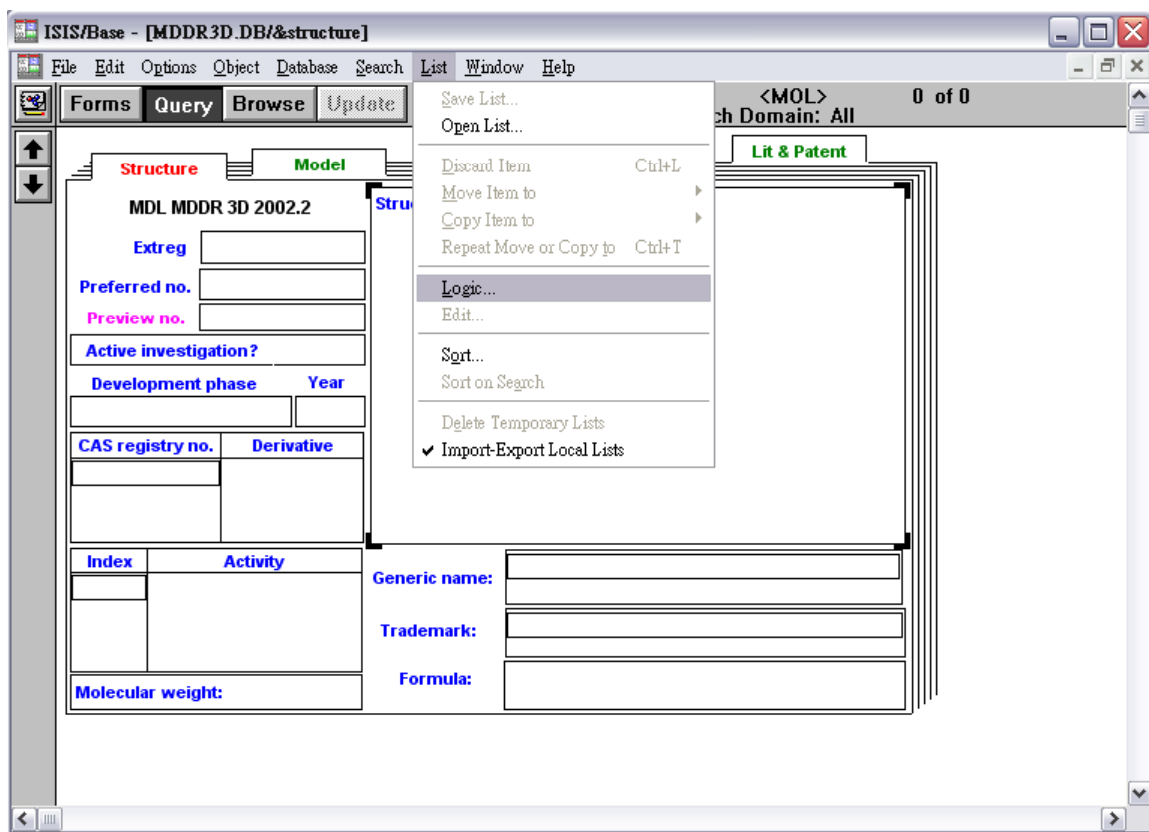
Chemical structure: CN(C)Cc1ccc(cc1)C(=O)N(C)C(=O)CC(=O)O

- Save List...
- Open List...
- Discard Item Ctrl+L
- Move Item to
- Copy Item to
- Repeat Move or Copy to Ctrl+T
- Logic...
- Edit...
- Sort...
- Sort on Search
- Delete Temporary Lists
- Import-Export Local Lists

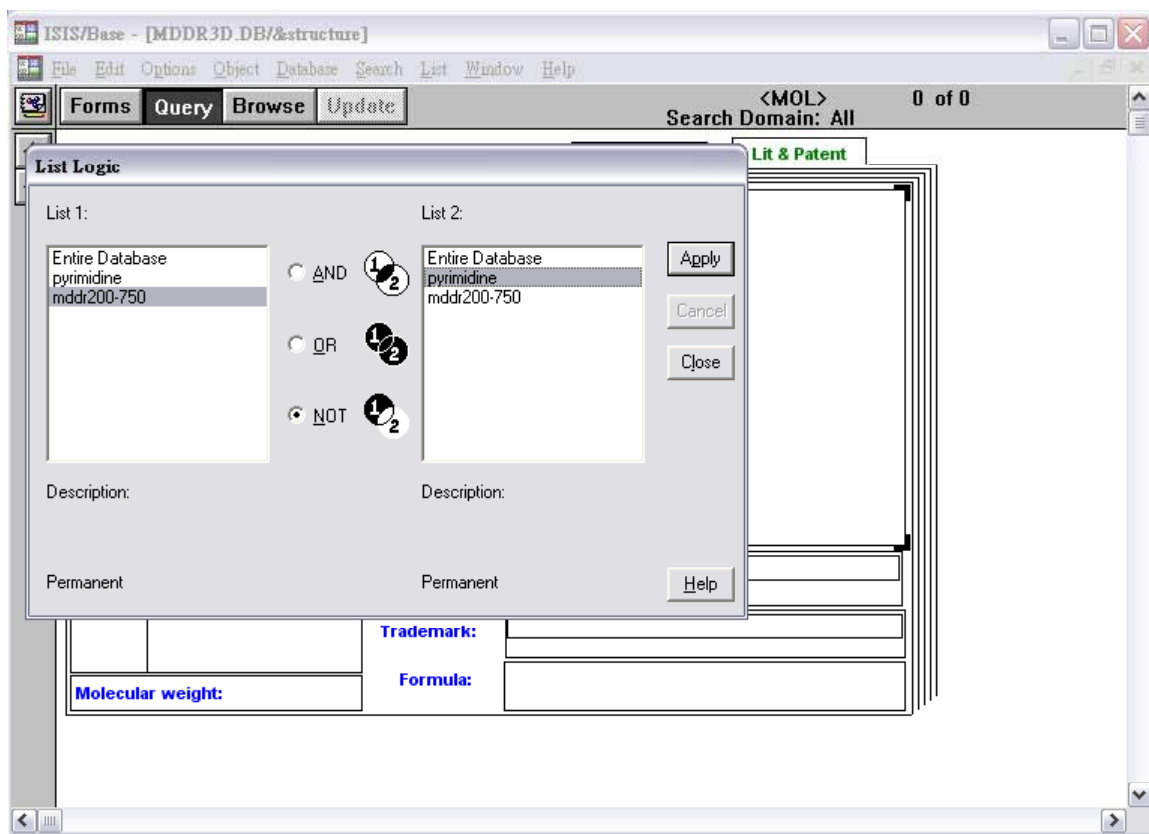
Step 13. Save the list of the search result



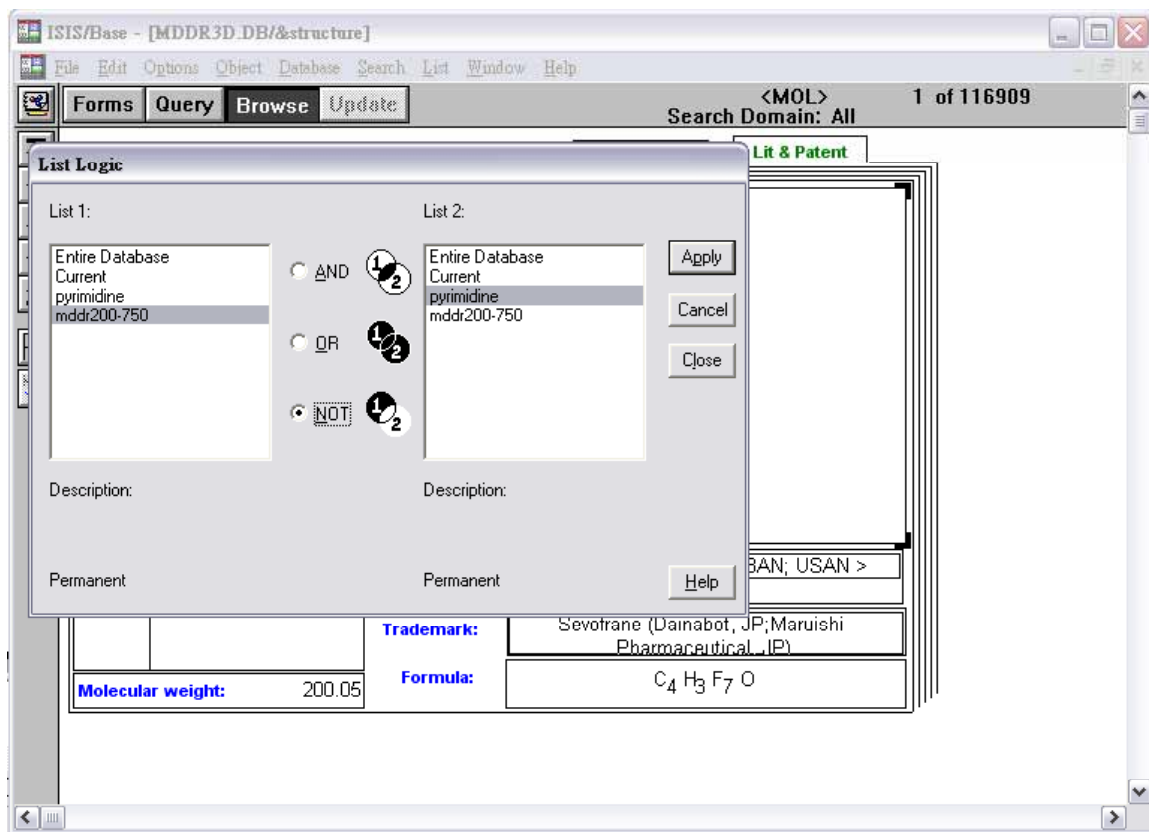
Step 14. Input the list name



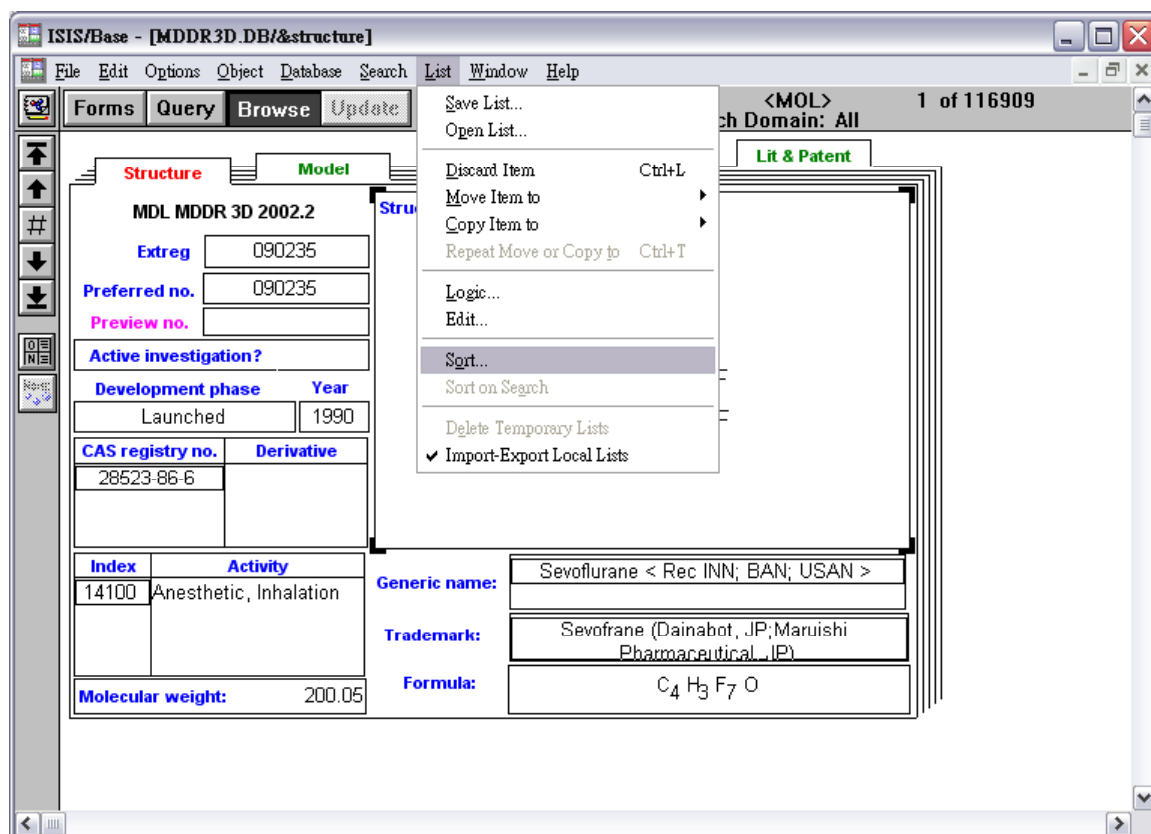
Step 15. Start logic combination of the two lists



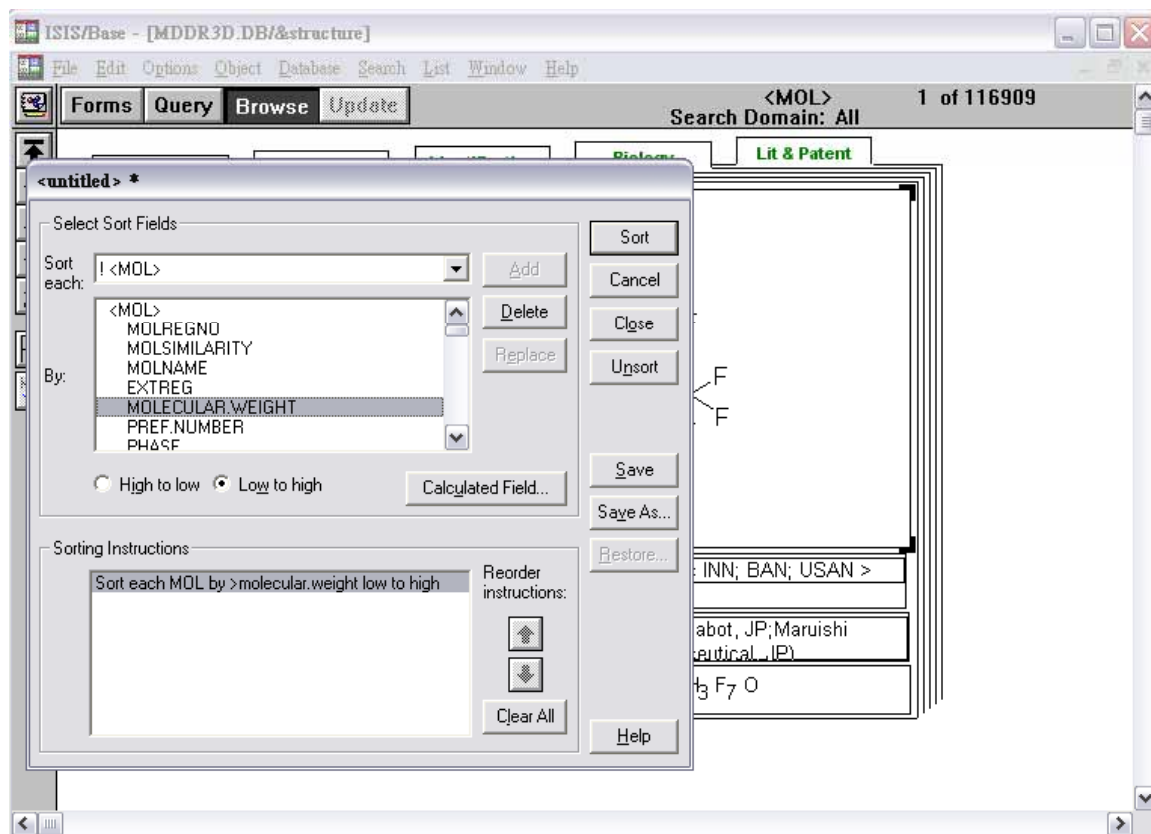
Step 16. The logic combination is “mw200-750” not “pyrimidine”



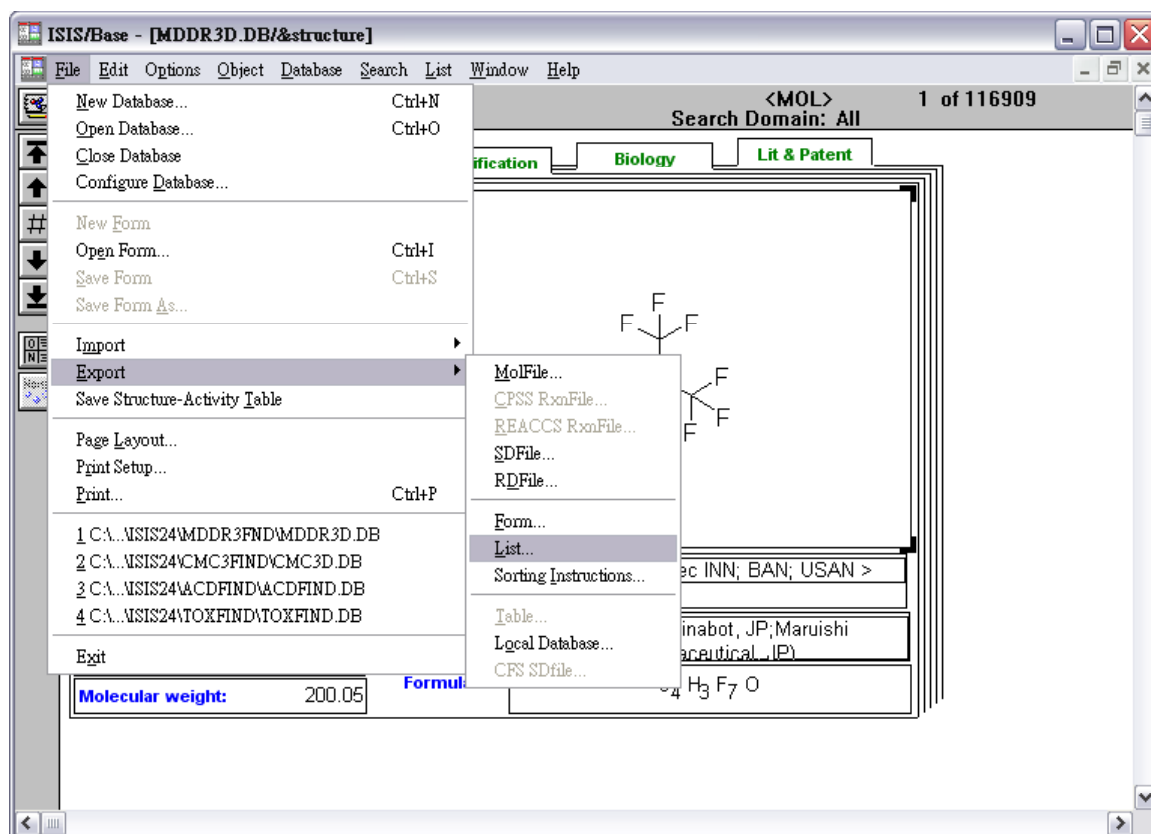
Step 17. Apply the logic combination and the result contains 1169090 molecules



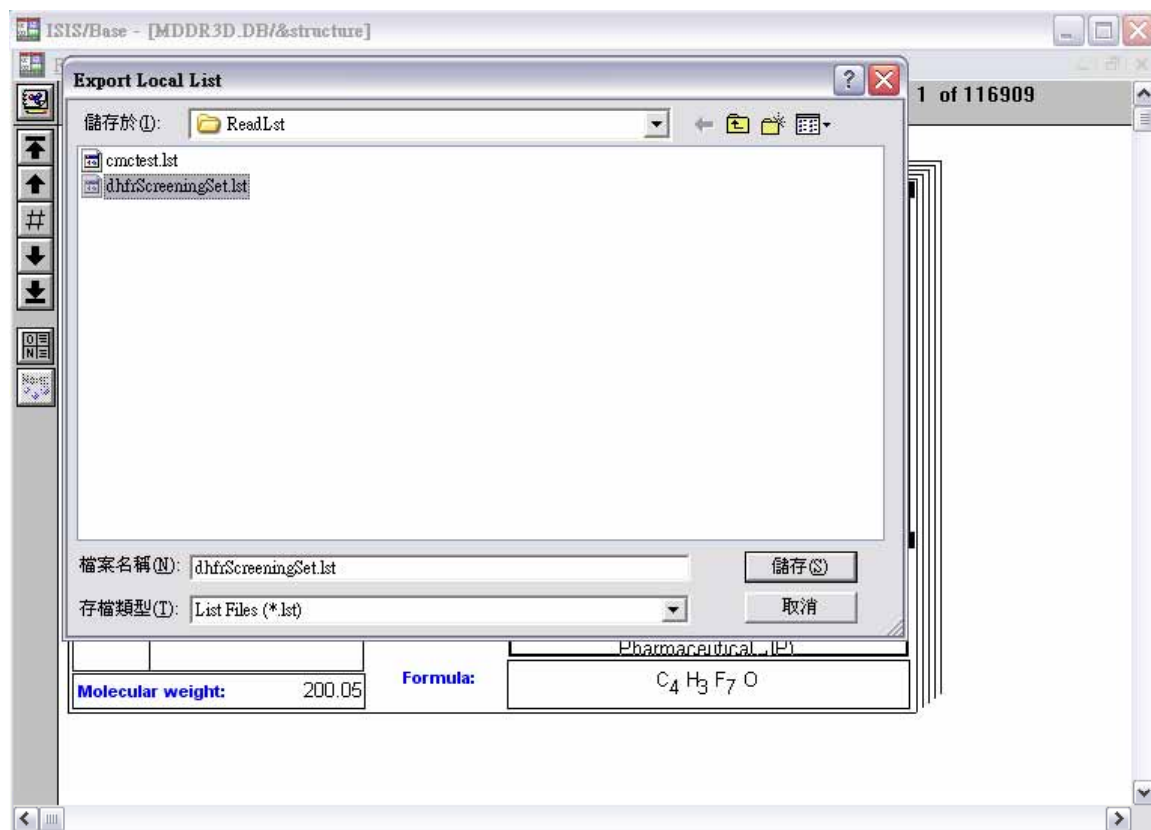
Step 18. Sort the list by the molecular weight



Step 19. Select the sort fields “molecular.weight” and “low to high”



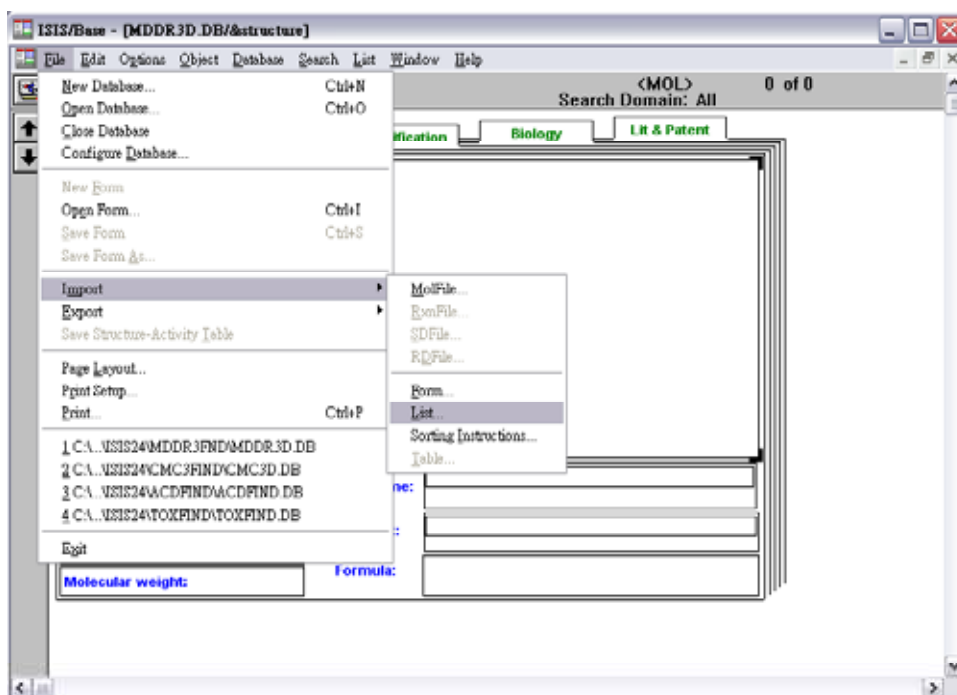
Step 20. Export the current list



Step 21. Input the local path and the list name

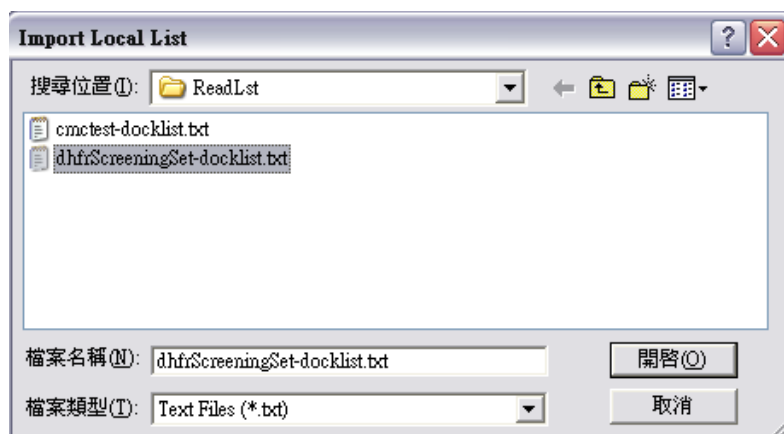
Step 22. Read the list and output the list (InputFile-dockinglist.txt) for docking:

ReadLst dhfrScreeningSet.lst [the number of molecules in the docking list]

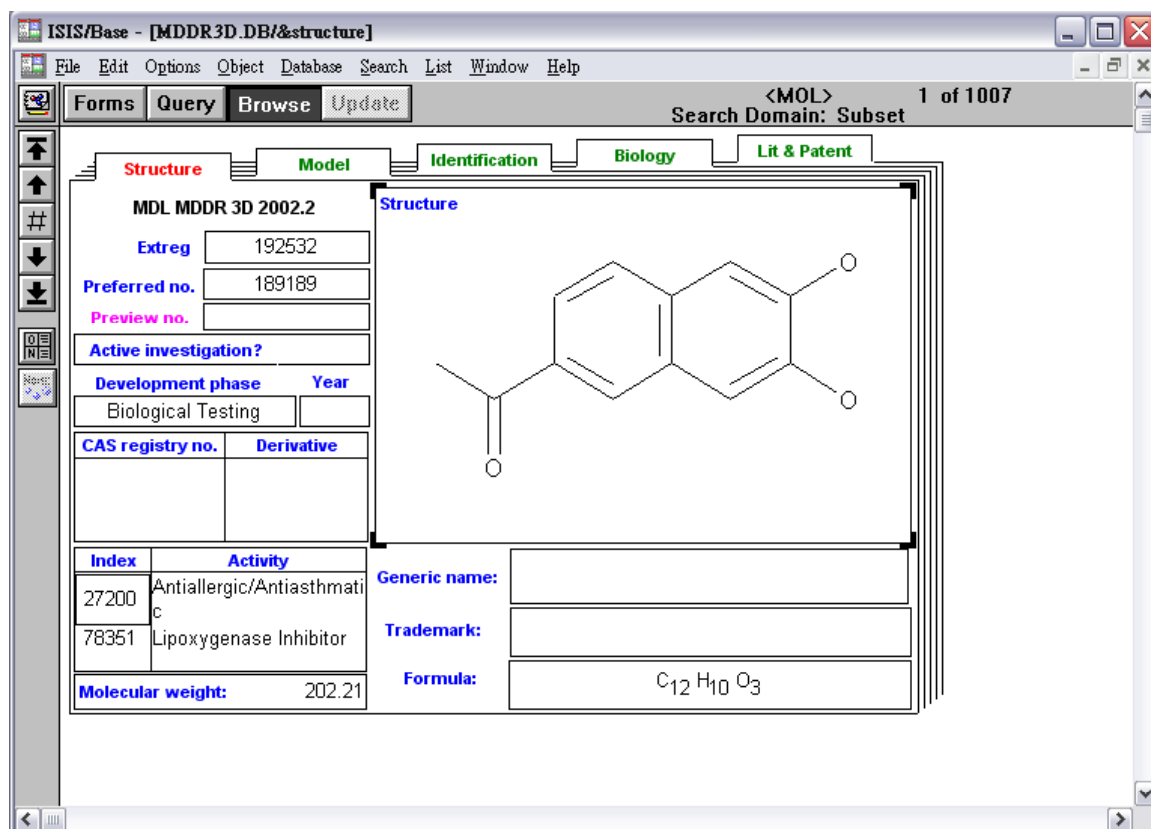


Step 23. Import the docking list (dhfrScreeningSet-dockinglist.txt) to download the coordinates of compounds

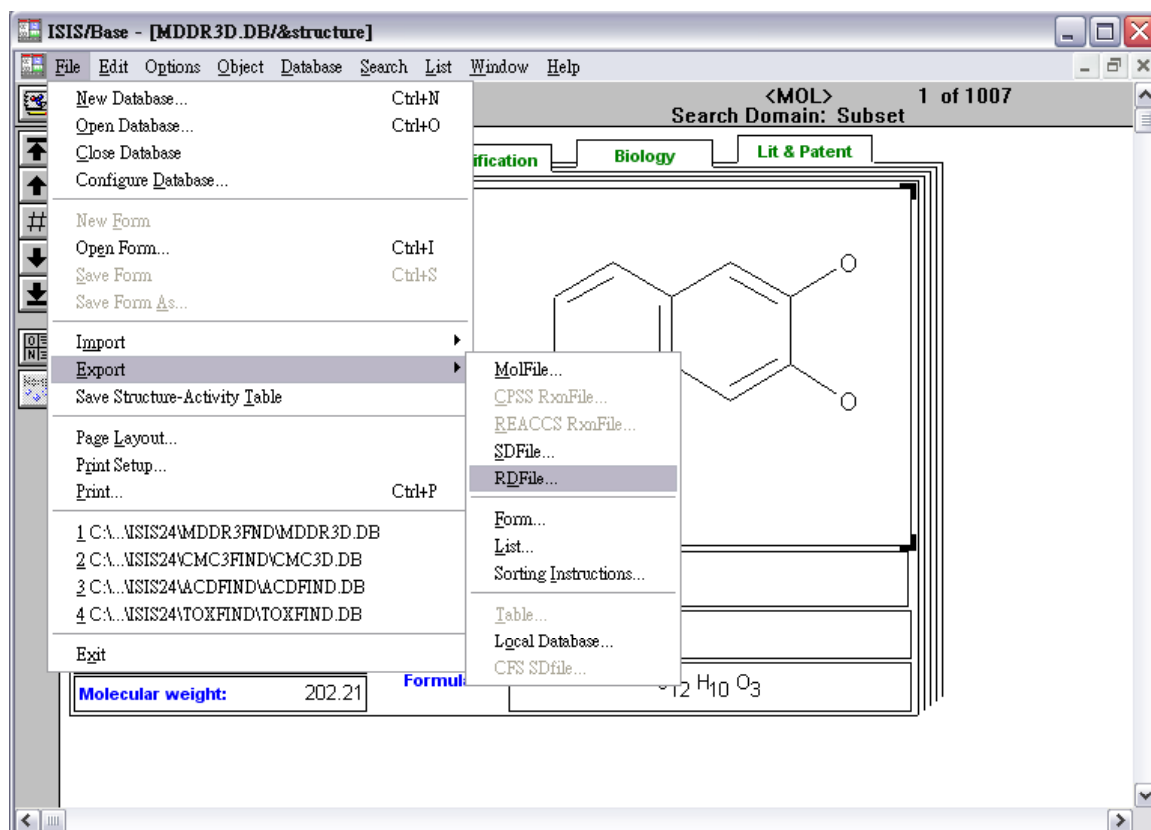
192532
174954
157326
238569
166852
265807
281533
143883
161734
291319
206782
257992
156109
163510
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269548
281920
277952
147140
185588
202814
160825
194713



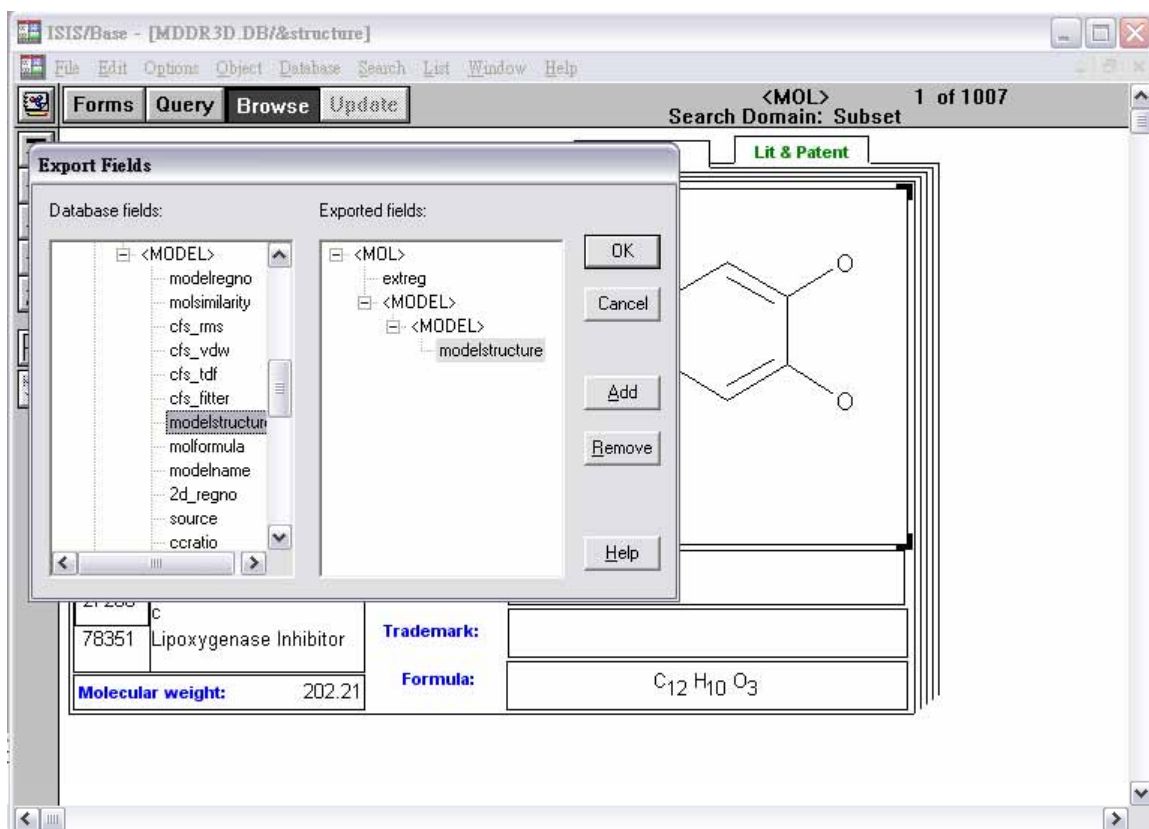
Step 24. Select the docking list



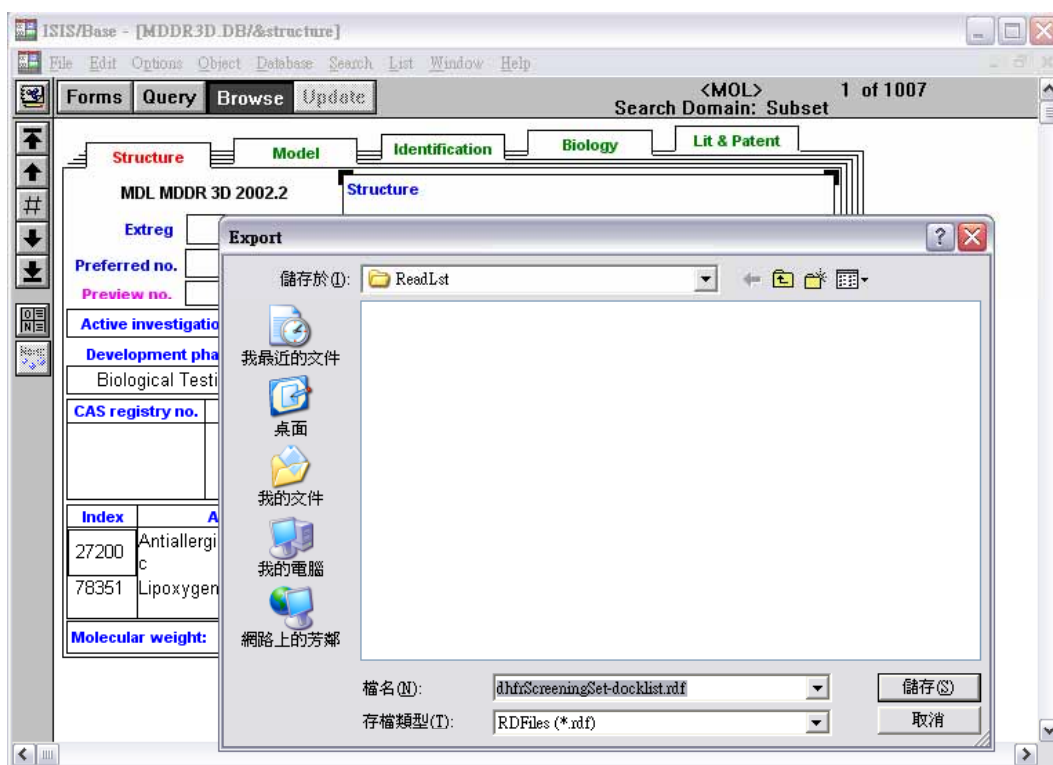
Step 25. The compounds in the docking list



Step 26. Export the reaction data file (RDFFile) of the docking list



Step 27. Specify the items included in the RDFFile. It have to contain two items “extreg” and “modelstructure”



Step 28. Input the RDFFile name

Step 29. Copy corina.exe to same directory

Step 30. Remove small fragments from the RDFfile with CORINA3.0:

```
corina -i t=rdf -o t=rdf -d rs dhfrScreeningSet-docklist.rdf mdhfrScreeningSet-docklist.rdf
```

Step 31. Split the RDFfile to separated single-structure files:

```
splitRDF mdhfrScreeningSet-docklist.rdf
```

Step 32. The docking list is finished and each structure of the molecule in the docking list is prepared.

