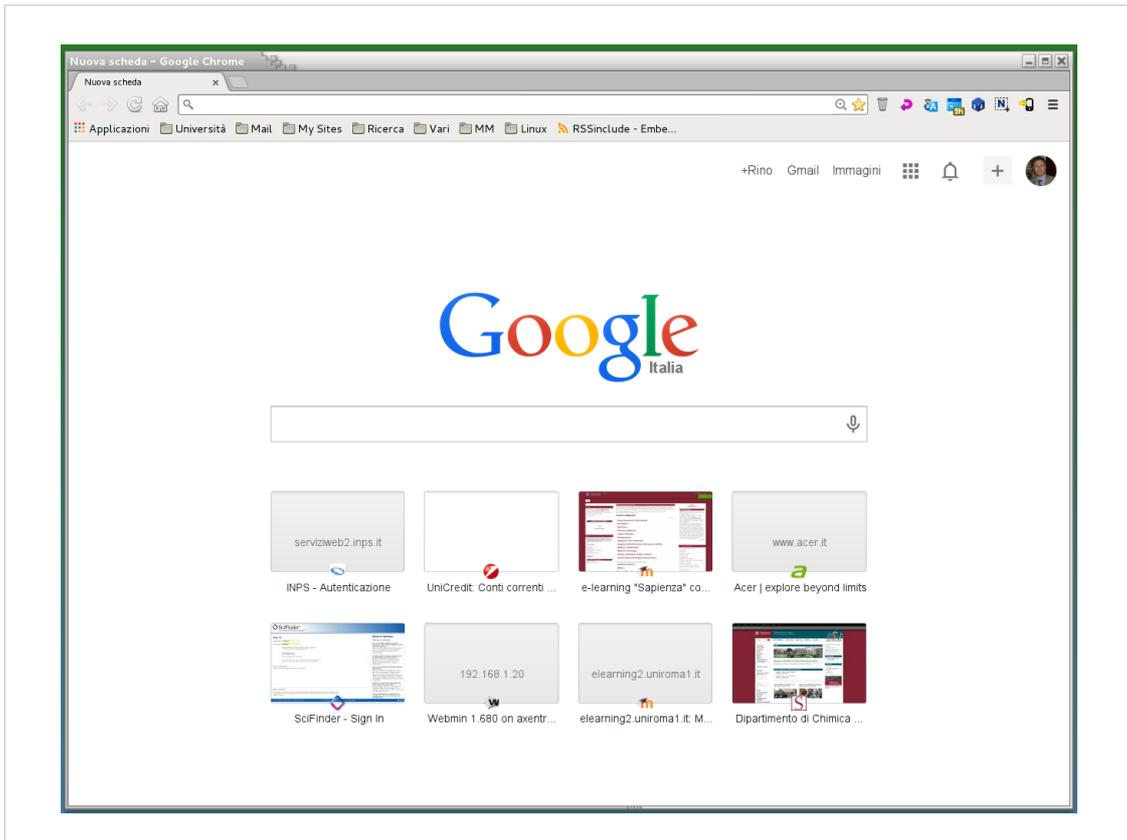
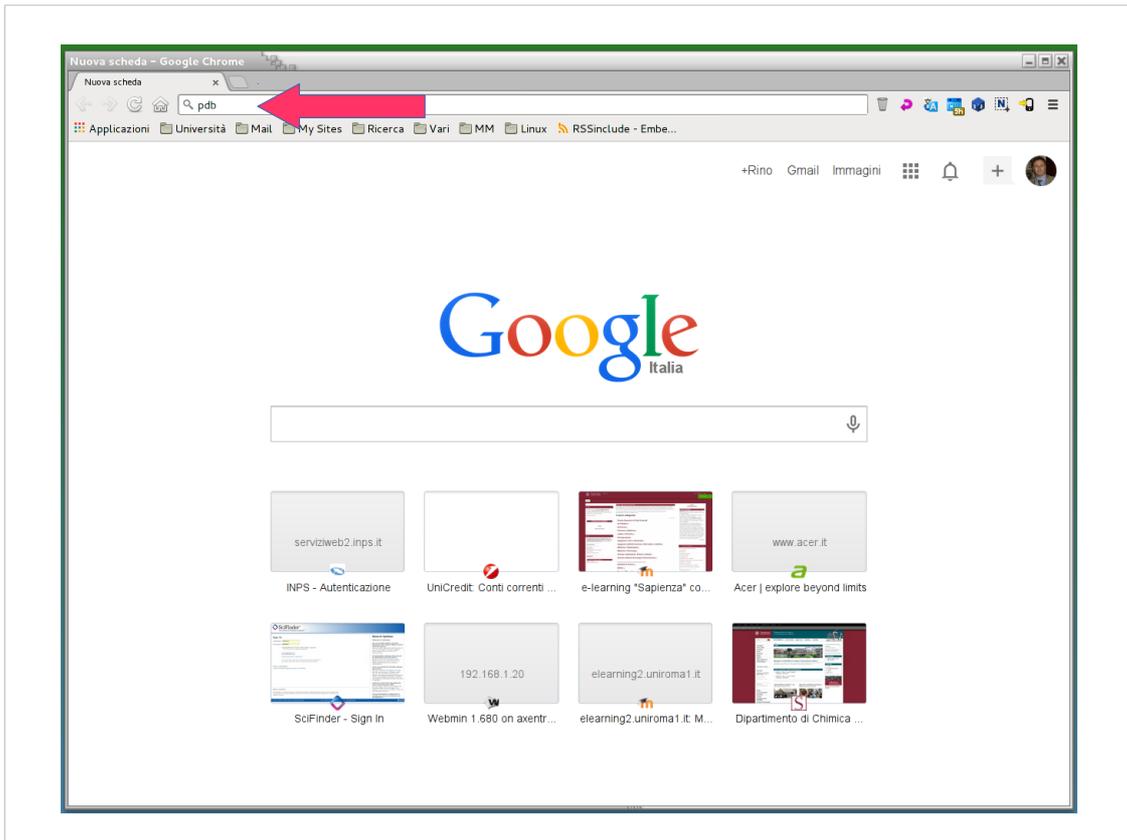


Chimera Usage

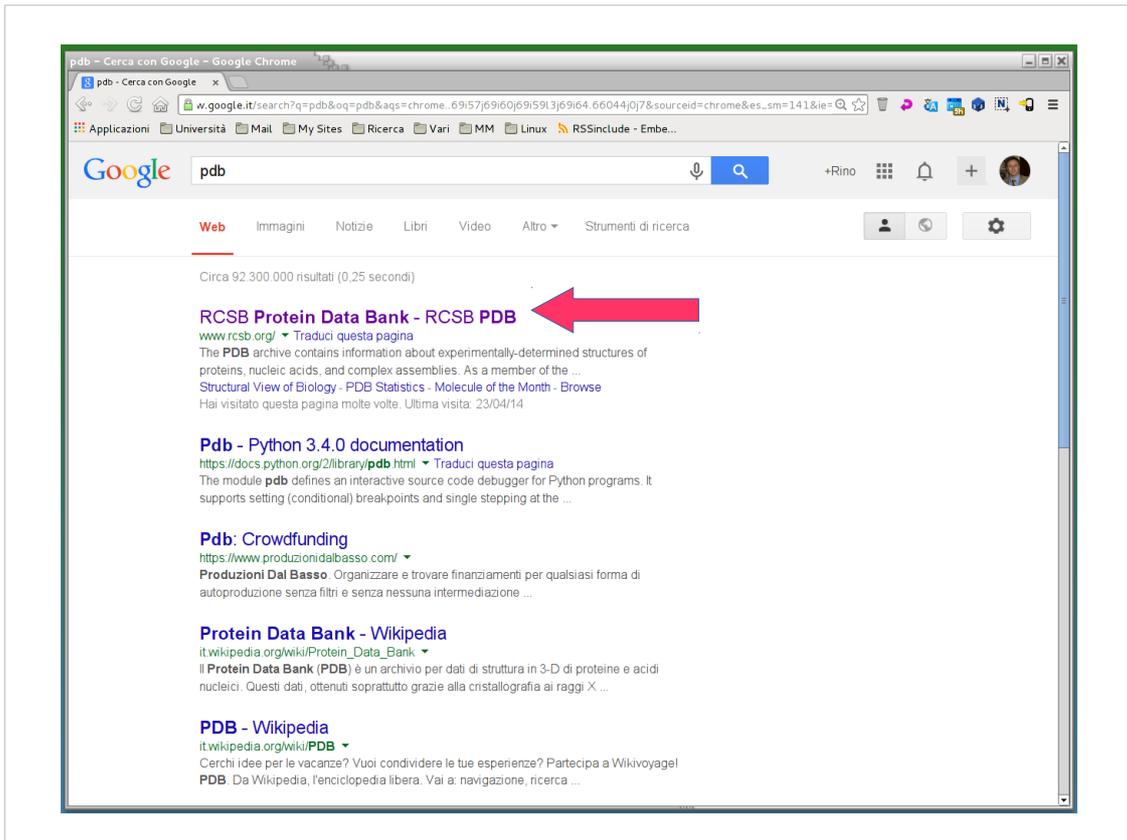
In questa serie di immagini sono illustrate le varie operazioni per usare Chimera nella visualizzazione di strutture disponibili dal PDB



Si deve aprire un browser (internet explore, firefox, chrome, etc. etc.)

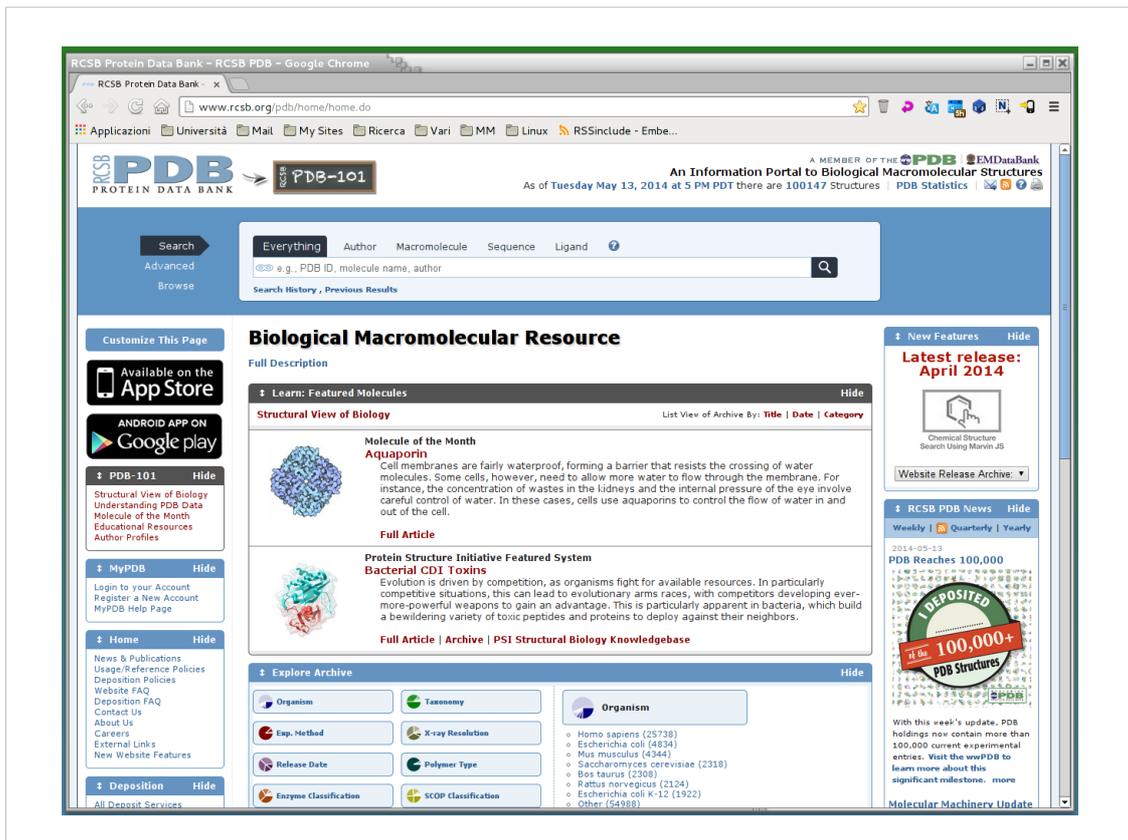


Quindi nella barra degli indirizzi digitare “pdb” e dare invio

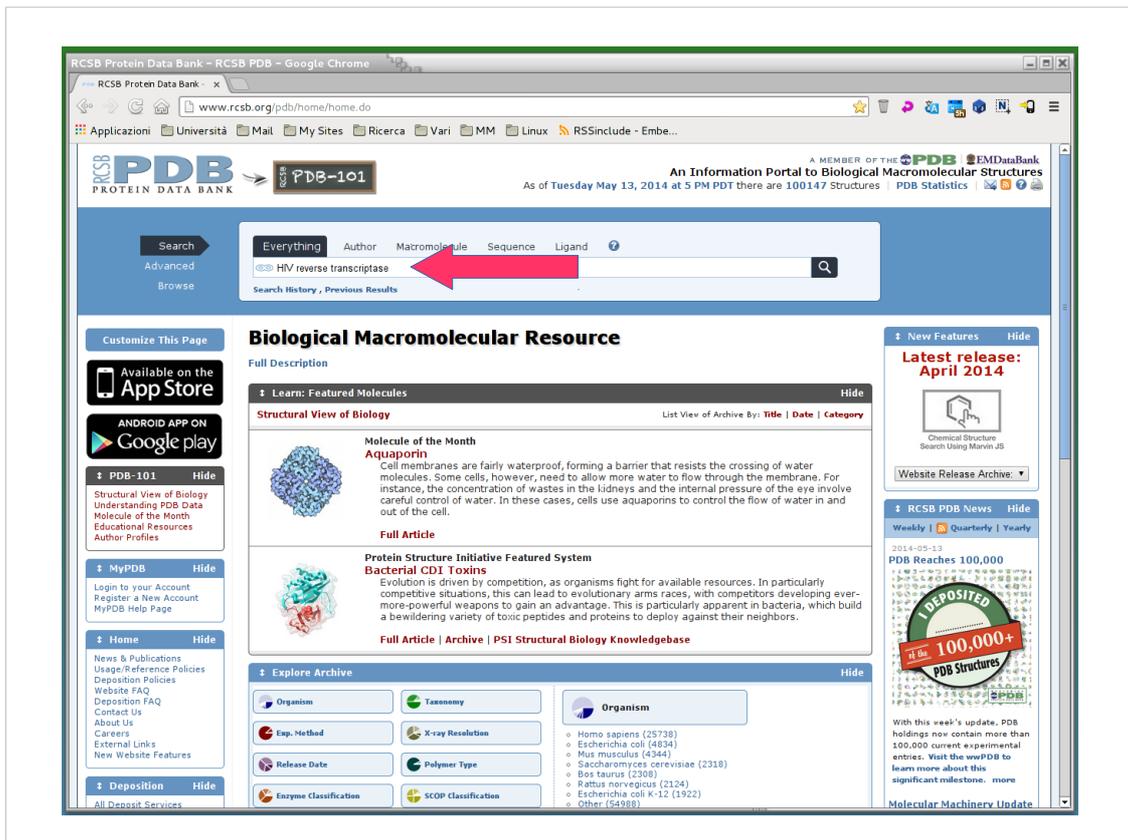


Di solito la prima voce risultante e' il link al Protein Data Bank (PDB).

Si clicca sul link ...



... e quindi si raggiunge il portale del PDB



Nel box search si scrive “HIV reverse transcriptase wild type”

The screenshot shows the RCSB PDB website search results for the query "hiv reverse transcriptase". The search bar at the top contains the text "e.g., PDB ID, molecule name, author" and a search icon. Below the search bar, the results are categorized into "299 Structure Hits", "187 Ligand Hits", and "236 Web Page Hits". A red arrow points to the "299 Structure Hits" tab. The "Query Parameters" section shows the text search for "hiv reverse transcriptase". The "Other search suggestions" section includes "Molecule Name", "Structural Domains", and "Molecule of the Month". The "Ontology Terms" section shows "D08.811 ... :187: HIV Reverse Transcriptase [MeSH ... (218)". The "Query Refinements" section includes "Organism", "Taxonomy", "Experimental Method", "X-ray Resolution", and "Release Date".

RCSB PDB - Query Results - Google Chrome
www.rcsb.org/pdb/results/results.do?grid=DAD43DC&tabtoShow=Current

Applicationi Università Mail My Sites Ricerca Vari MM Linux RSSinclude - Embe...

RCSB PDB PROTEIN DATA BANK

SEARCH
Advanced
Browse

Everything Author Macromolecule Sequence Ligand ?

e.g., PDB ID, molecule name, author

Search History (1), Previous Results (299)

PDB-101 Hide
Structural View of Biology
Understanding PDB Data
Molecule of the Month
Educational Resources
Author Profiles

MyPDB Hide
Login to your Account
Register a New Account
MyPDB Help Page
Query Results (299)
Query History (1)

Home Hide
News & Publications
Usage/Reference Policies
Deposition Policies
Website FAQ
Deposition FAQ
Contact Us
About Us
Careers
External Links
New Website Features

Deposition Hide
All Deposit Services
Electron Microscopy
X-ray I NMR
Validation Server
BioSync Beamlines/Facilities
Related Tools

Tools Hide
Download Files
Compare Structures

299 Structure Hits 187 Ligand Hits 236 Web Page Hits

Query Parameters: Query Details | Save Query to MyPDB

Text Search for: hiv reverse transcriptase

Other search suggestions:

Molecule Name Structural Domains Molecule of the Month

- HIV-1 REVERSE TRANSCRIPTASE [Gag-Po ... (216)
- Anti-HIV-1 reverse transcriptase si ... (6)
- HIV-1 reverse transcriptase [SCOP] (112)
- HIV Type 1 Reverse Transcriptase, S ... (180)
- HIV RNase H (Domain of reverse tran ... (86)
- HIV-1 Reverse Transcriptase; Chain ... (6)
- HIV-1 Reverse Transcriptase; Chain ... (6)
- HIV Type 1 Reverse Transcriptase; C ... (180)
- Reverse Transcriptase [HIV]

Ontology Terms

- D08.811 ... :187: HIV Reverse Transcriptase [MeSH ... (218)

Query Refinements: Select an item or pie chart ? Hide

Organism Taxonomy Experimental Method X-ray Resolution Release Date

- Human immunodeficiency virus 1 (110)
- HIV-1 M.B. HXB2R (67)
- Homo sapiens (48)
- Human immunodeficiency virus ty ... (45)
- Viruses (272)
- Eukaryota (59)
- Unassigned (6)
- Other (2)
- X-ray (290)
- Solution NMR (9)
- less than 1.5 Å (8)
- 1.5 - 2.0 Å (45)
- 2.0 - 2.5 Å (61)
- 2.5 - 3.0 Å (127)
- before 2000 (41)
- 2000 - 2005 (78)
- 2005 - 2010 (75)
- 2010 - today (105)

E si ottengono 299 voci che soddisfano la richiesta.

The screenshot shows the RCSB PDB Query Results page in Google Chrome. The browser address bar displays the URL: www.rcsb.org/pdb/results/results.do?grid=6100A0A7&tabtoshow=Current. The page title is "RCSB PDB - Query Results".

On the left side, there is a navigation menu with options like "Compare structures", "Drug & Drug Target Mapping", "File Formats", "RESTful Web Services", "Widgets", and "Help".

The main content area is titled "Refine Query with Advanced Search" and shows "Showing 1 - 25 of 63 Results". Below this, there are filters for "Filter: Check All", "View: Detailed", "Reports: Select one...", and "Sort: Relevance".

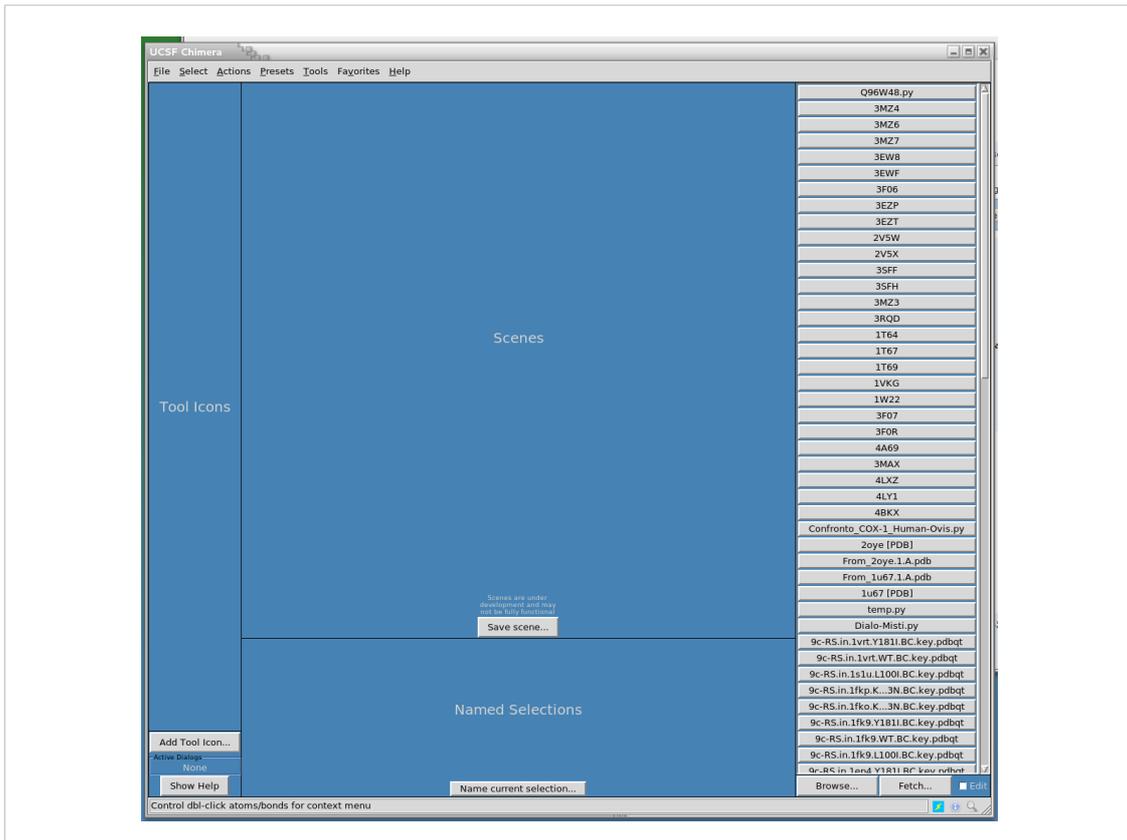
The search results are listed in a table-like format. The first result is highlighted with a red arrow pointing to its ID, "2WQO". The details for this entry are:

- ID:** 2WQO
- Title:** CRYSTAL STRUCTURE OF UK-453061 BOUND TO HIV-1 REVERSE TRANSCRIPTASE (WILD-TYPE).
- Authors:** Phillips, C., Irving, S.L., Knoechel, T., Ringrose, H.
- Release:** 2010-08-11
- Experiment:** X-RAY DIFFRACTION with resolution of 2.80 Å. Residue Count: 1000
- Compound:** 2 Polymers [[Display Full Polymer Details](#) | [Display for All Results](#)], 1 Ligand [[Display Full Ligand Details](#) | [Display for All Results](#)]
- Citation:** Lersivirine: A Non-Nucleoside Reverse Transcriptase Inhibitor with Activity Against Drug-Resistant Human Immunodeficiency Virus-1. (2010) *Antimicrob. Agents Chemother.* 54: 4451-4463 [[Display Full Abstract](#) | [Display for All Results](#)]
- Molecule of the Month:** Reverse Transcriptase
- Search Hit:** Title: CRYSTAL STRUCTURE OF UK-453061 BOUND TO HIV-1 REVERSE TRANSCRIPTASE (WILD-TYPE).

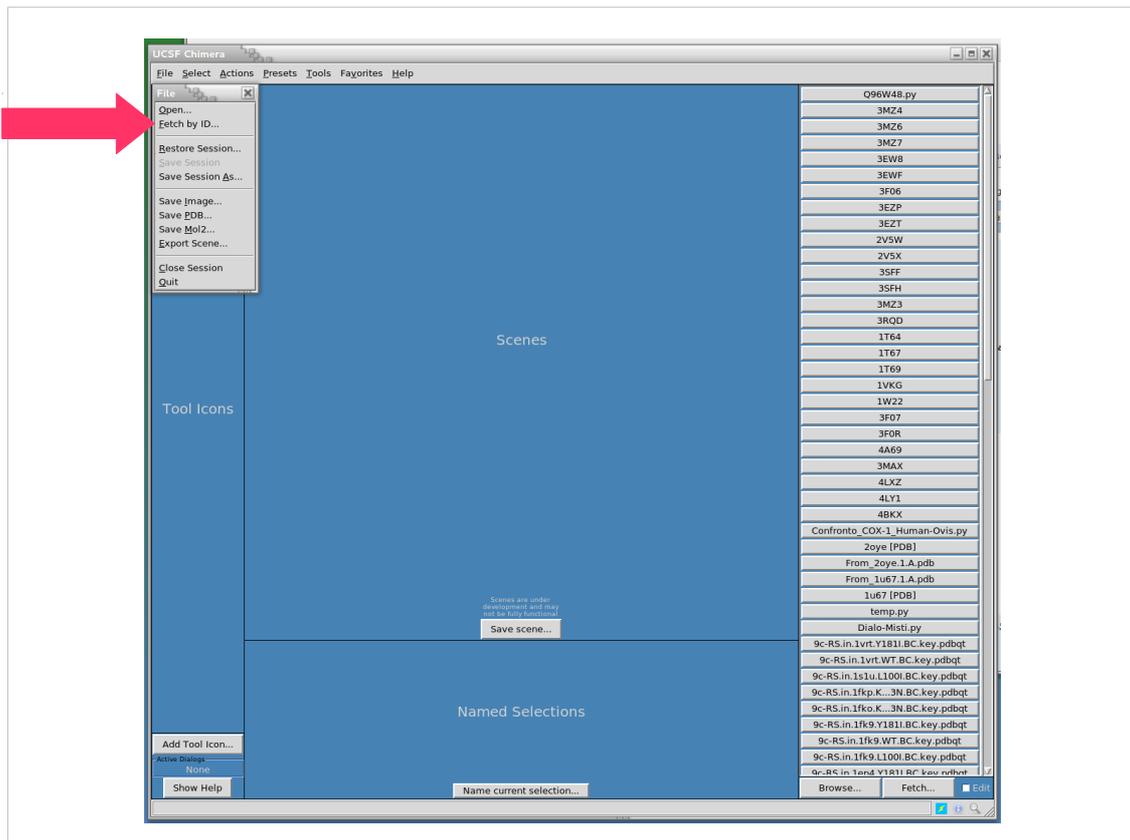
The second result is "3T19" with the title "Crystal structure of HIV-1 reverse transcriptase (wild type) in complex with inhibitor M05".

The third result is "1SUQ" with the title "CRYSTAL STRUCTURE OF HIV-1 REVERSE TRANSCRIPTASE (RT) IN COMPLEX WITH JANSSEN-R185545".

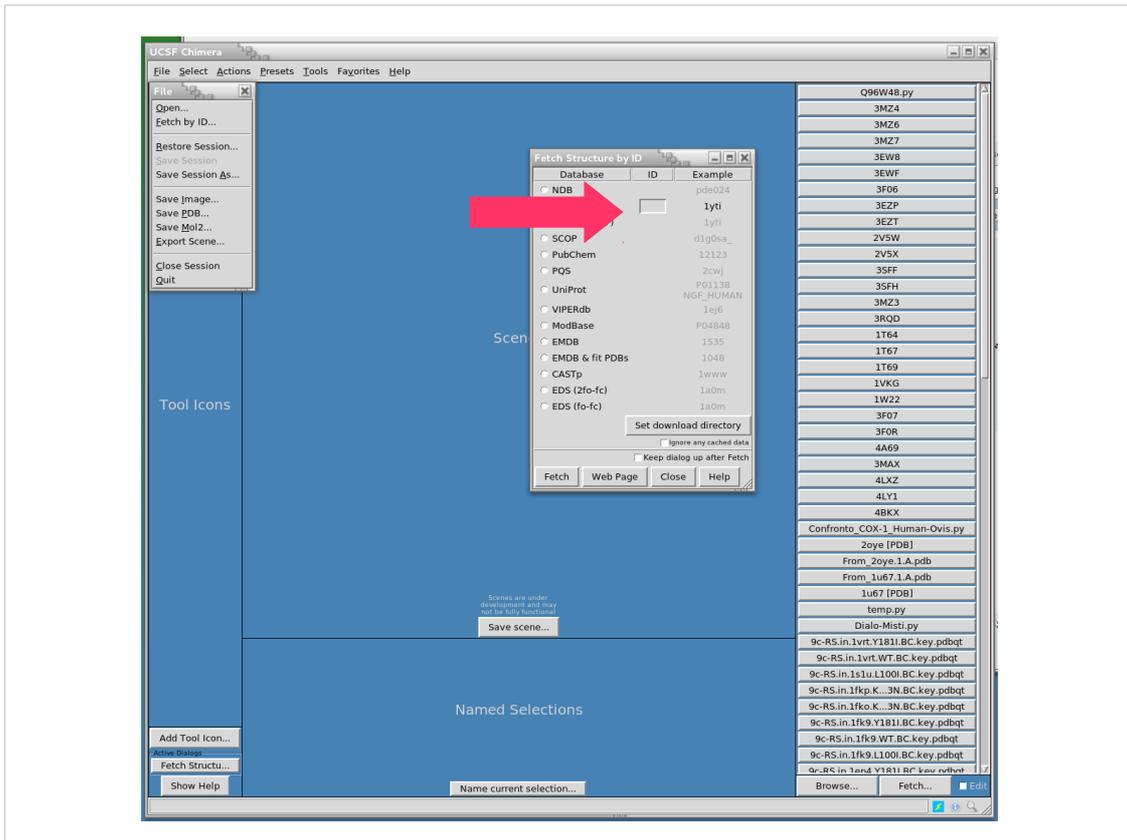
Si scorre in basso e la prima voce (in questo esempio) è una struttura di HIV-RT wild type co-cristallizzata con un inibitore. Si prende nota e ...



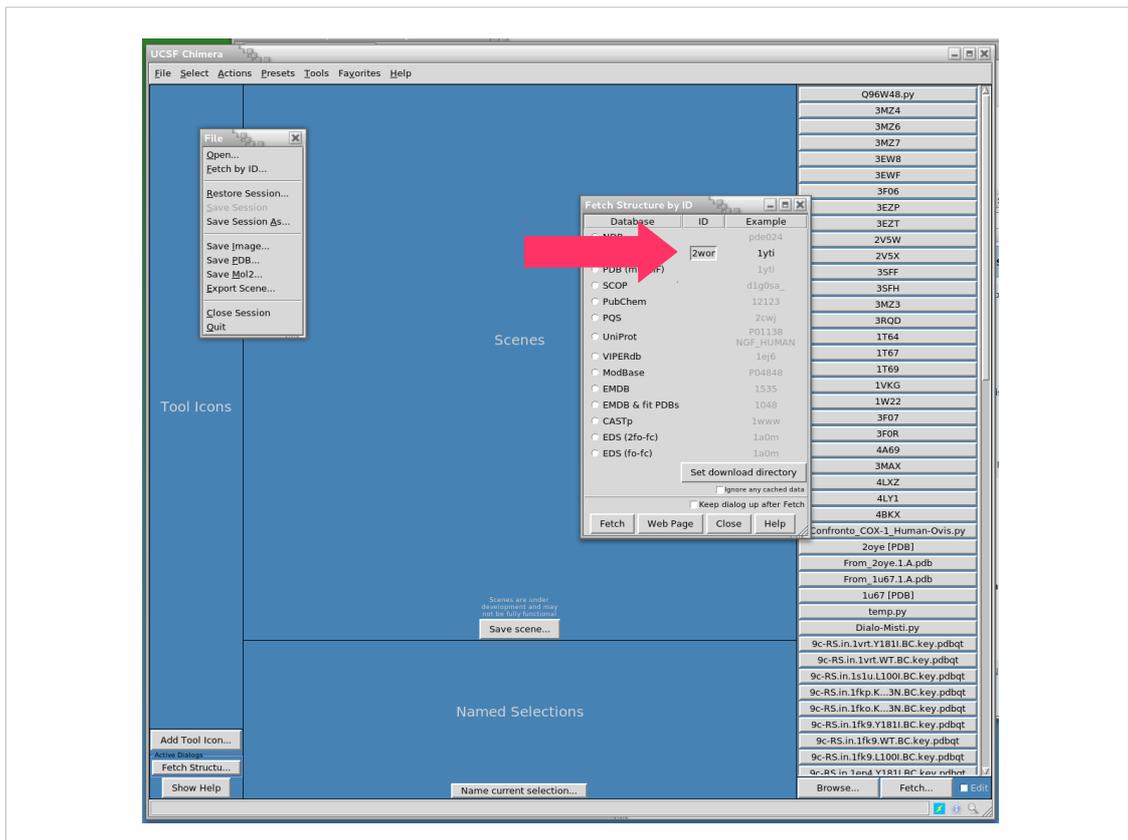
... quindi si lancia Chimera (doppio click sull'icona sulla scrivania)



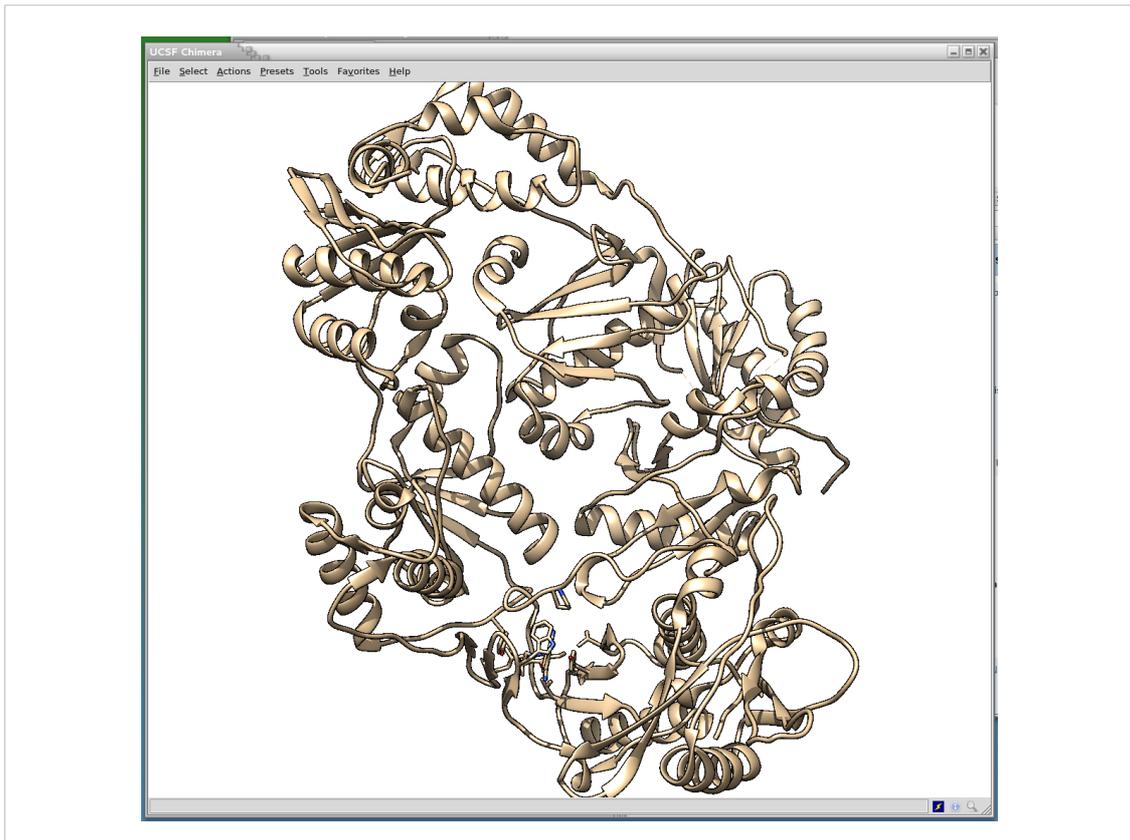
Andando sul menù File si sceglie la voce “Fetch by ID...”



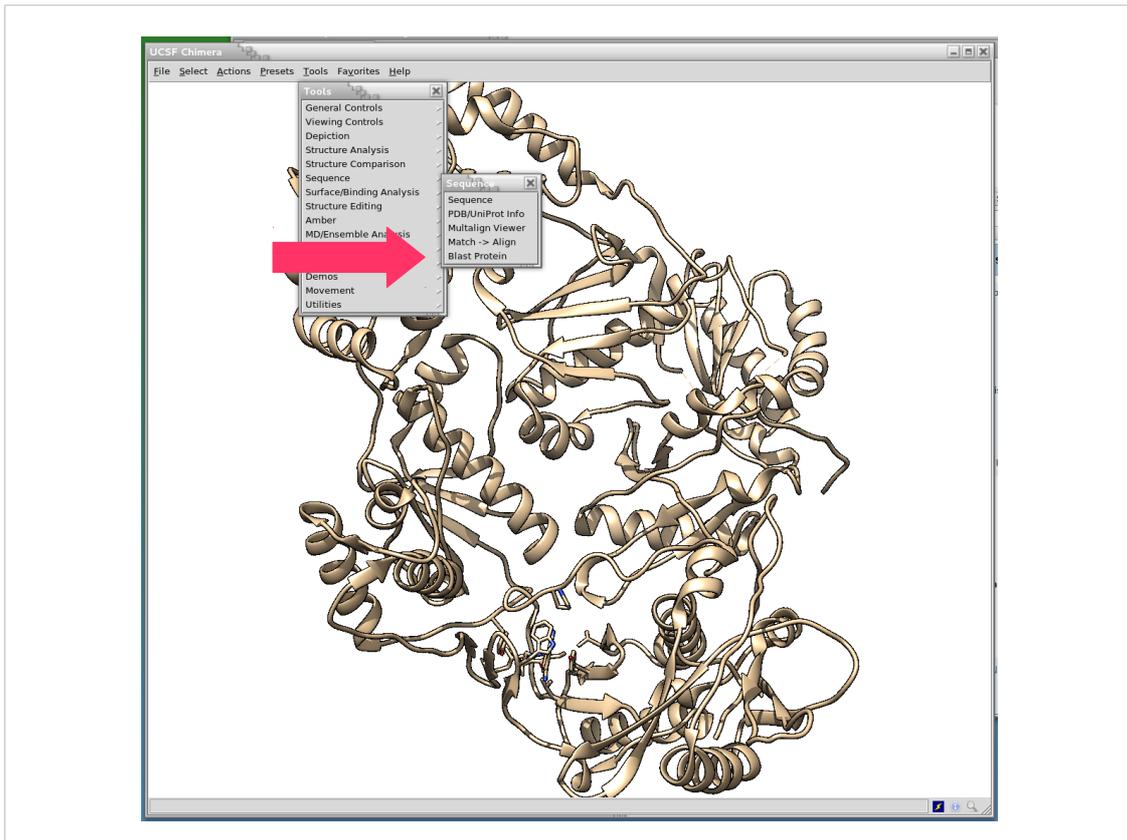
Nella finestra che appare si scrive il codice prima registrato



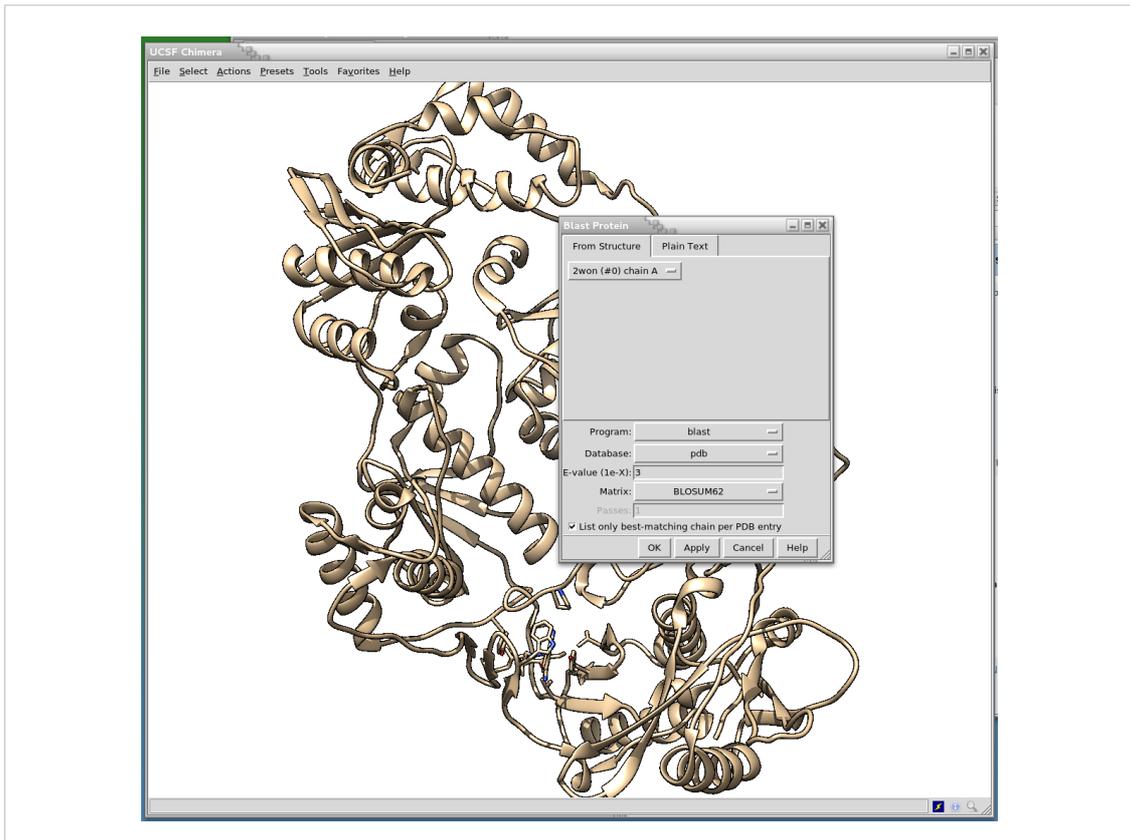
... come mostrato qui.
Quindi si clicca su pulsante "Fetch" e ...



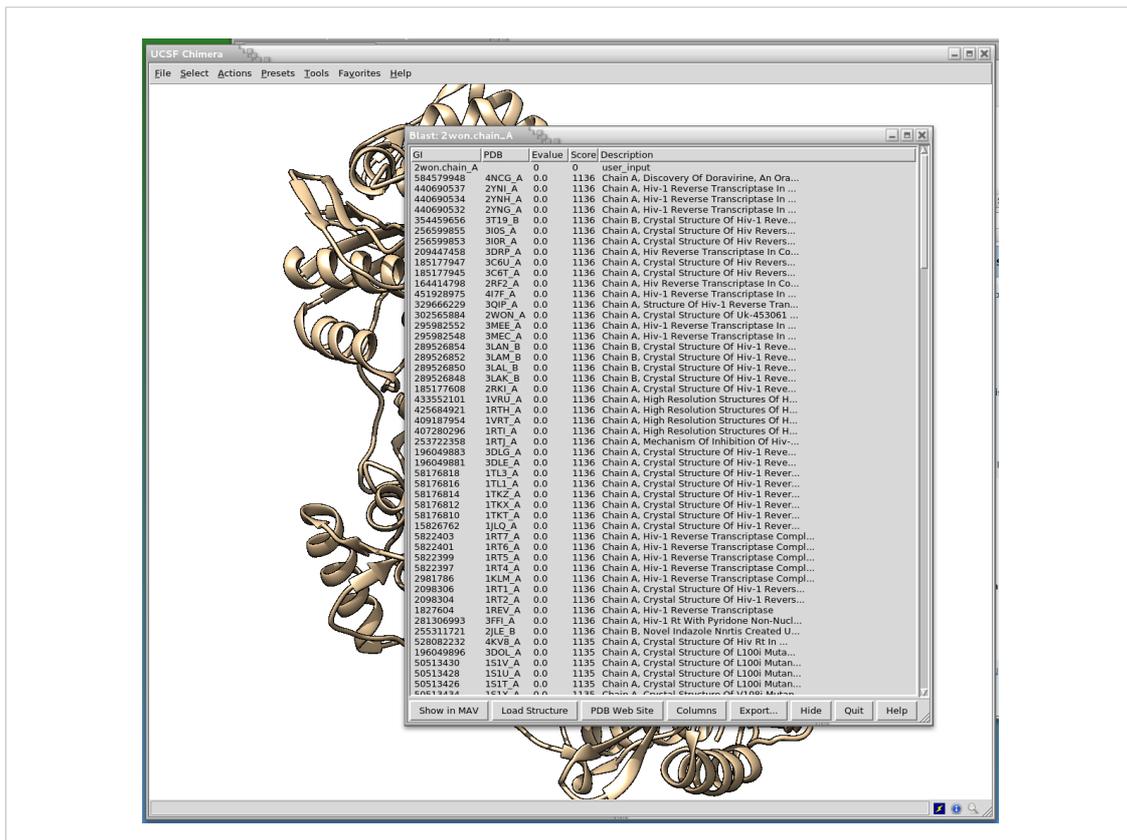
Viene scaricata la struttura scelta e visualizzata a schermo



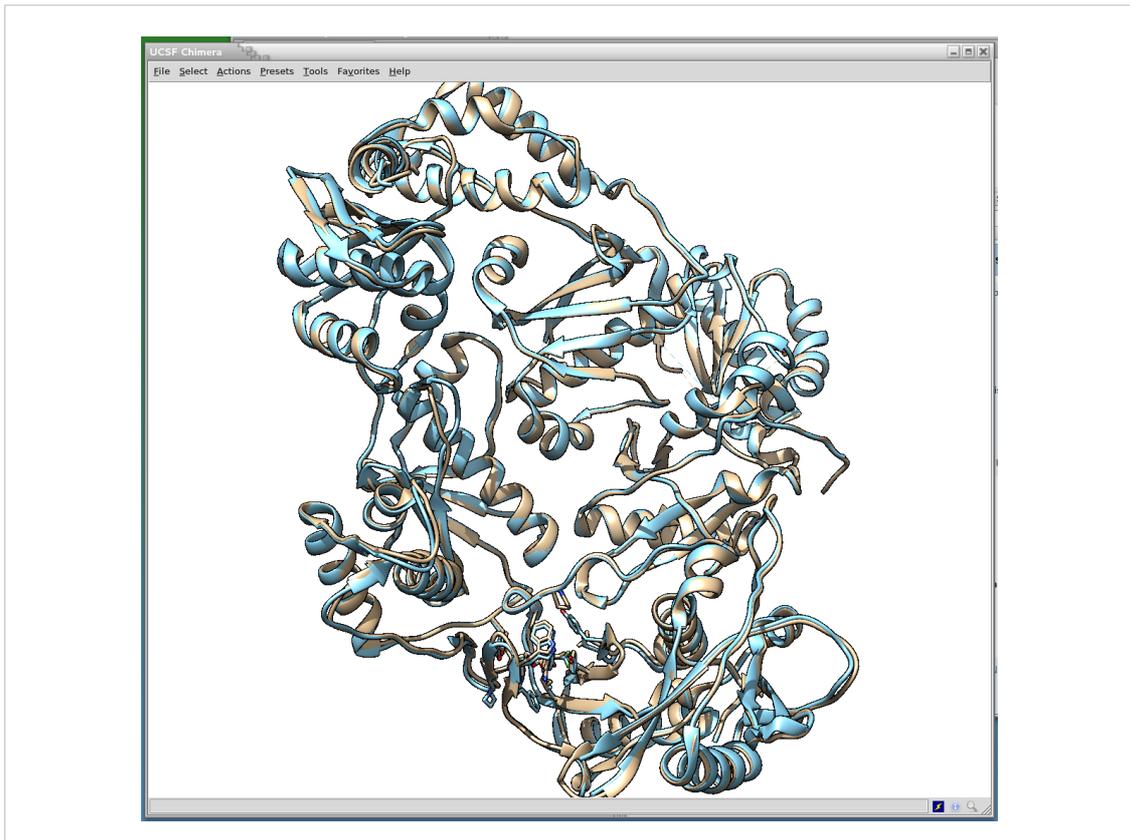
Quindi mediante il modulo Blast si effettua una ricerca per strutture omologhe scegliendo il menù “Tools”, selezionando “Sequenze” e quindi “Blast Protein” (Attenzione Blast non funziona nell'aula di matematica)



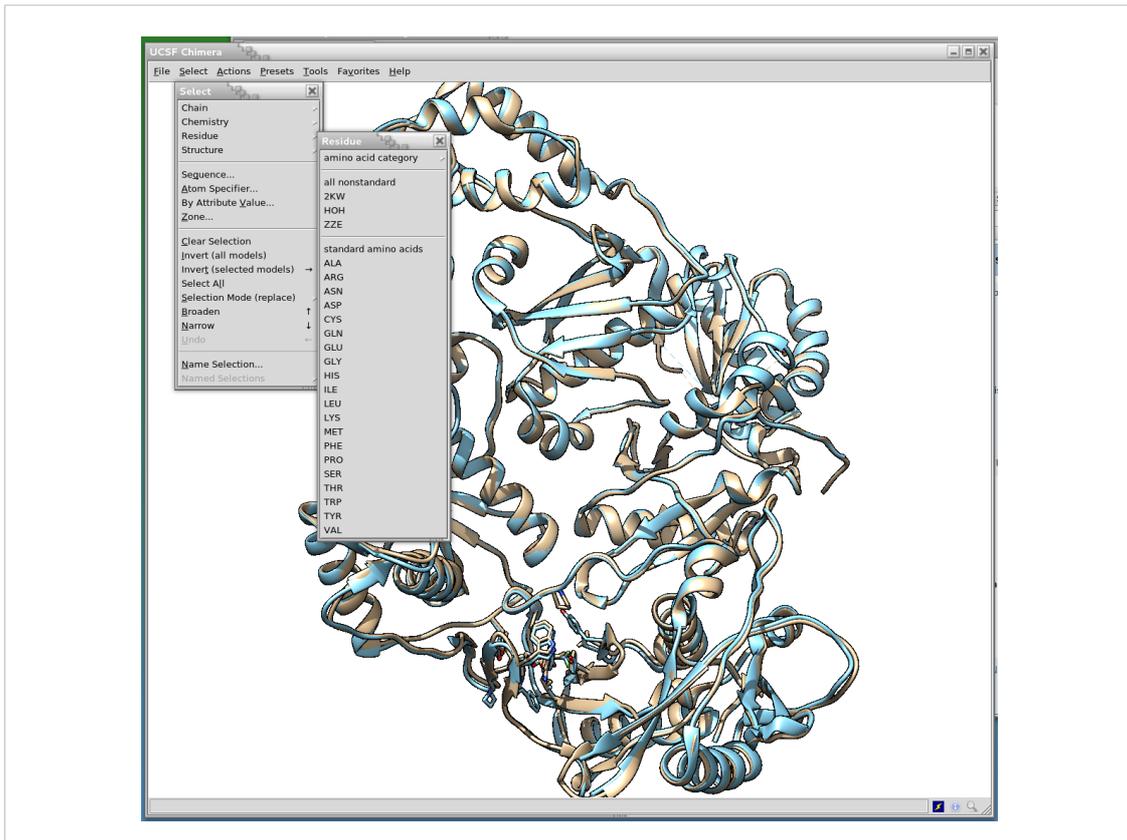
Semplicemente cliccando sul pulsante “OK”



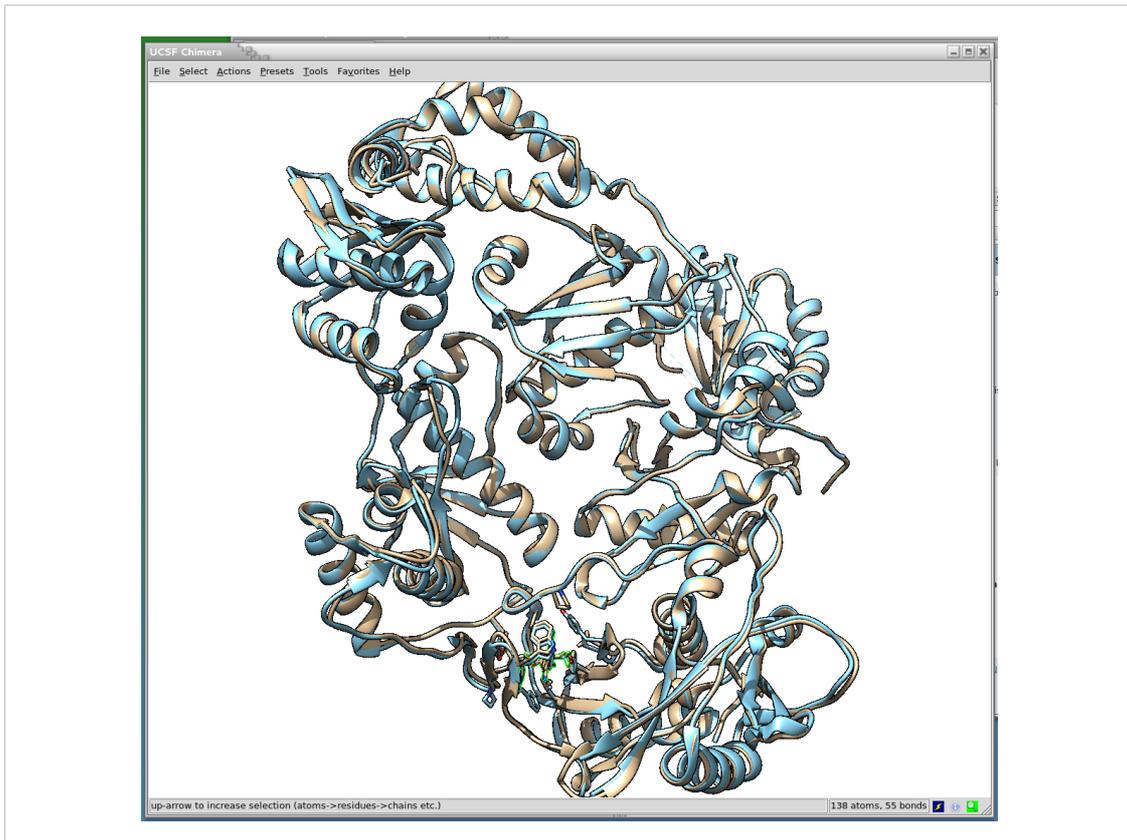
Dopo qualche secondo appare una finestra che viene riempita di linee indicanti le strutture in ordine decrescente di omologia. Tutte le strutture con “score” 1136 sono identiche alla struttura prima caricata. Si seleziona la prima e con il pulsante “load structure” ...



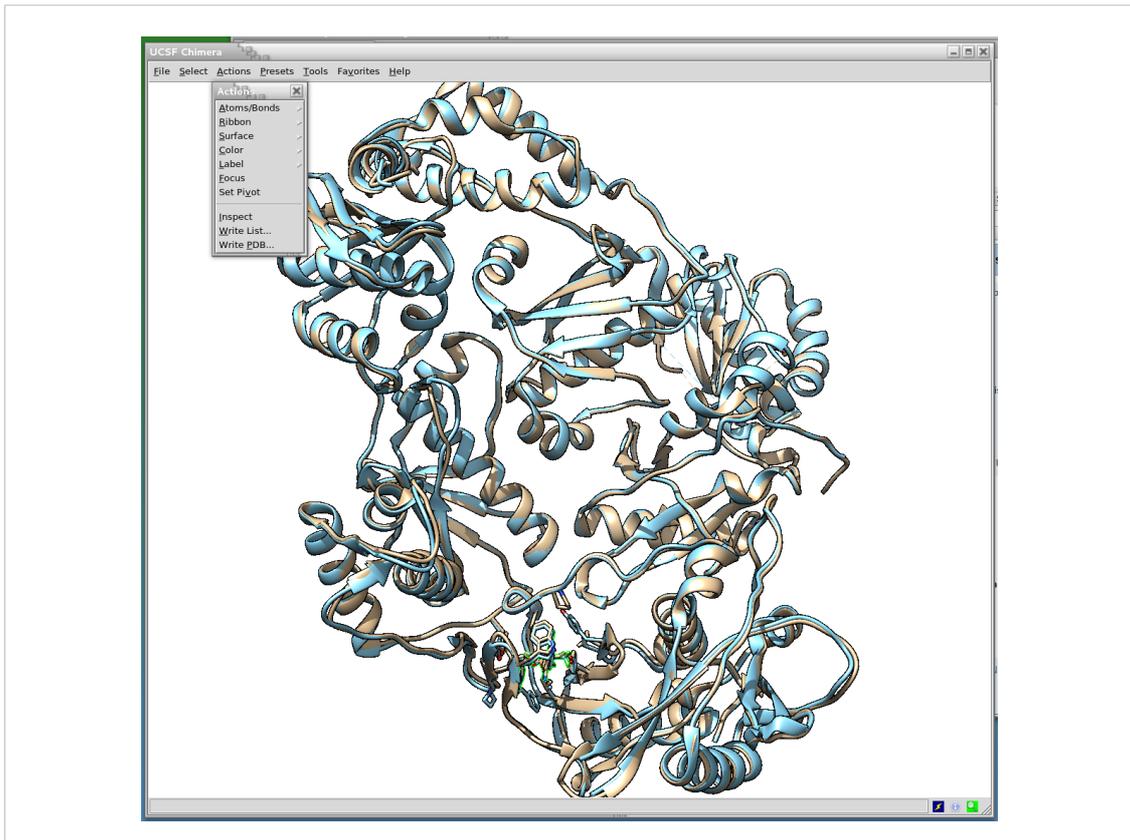
Viene caricata una seconda HIV-RT direttamente allineata sulla precedente.



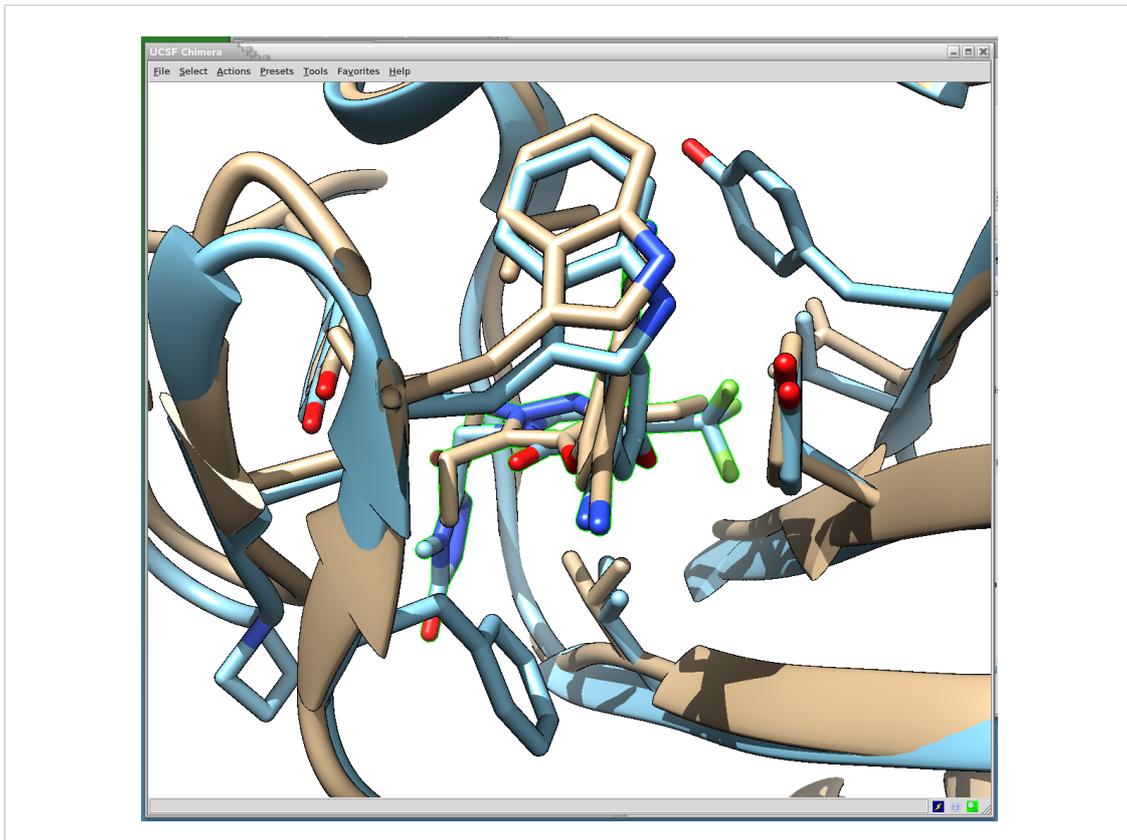
Sul menu “Select” si seleziona la voce “Residue” e quindi “all nonstandard”



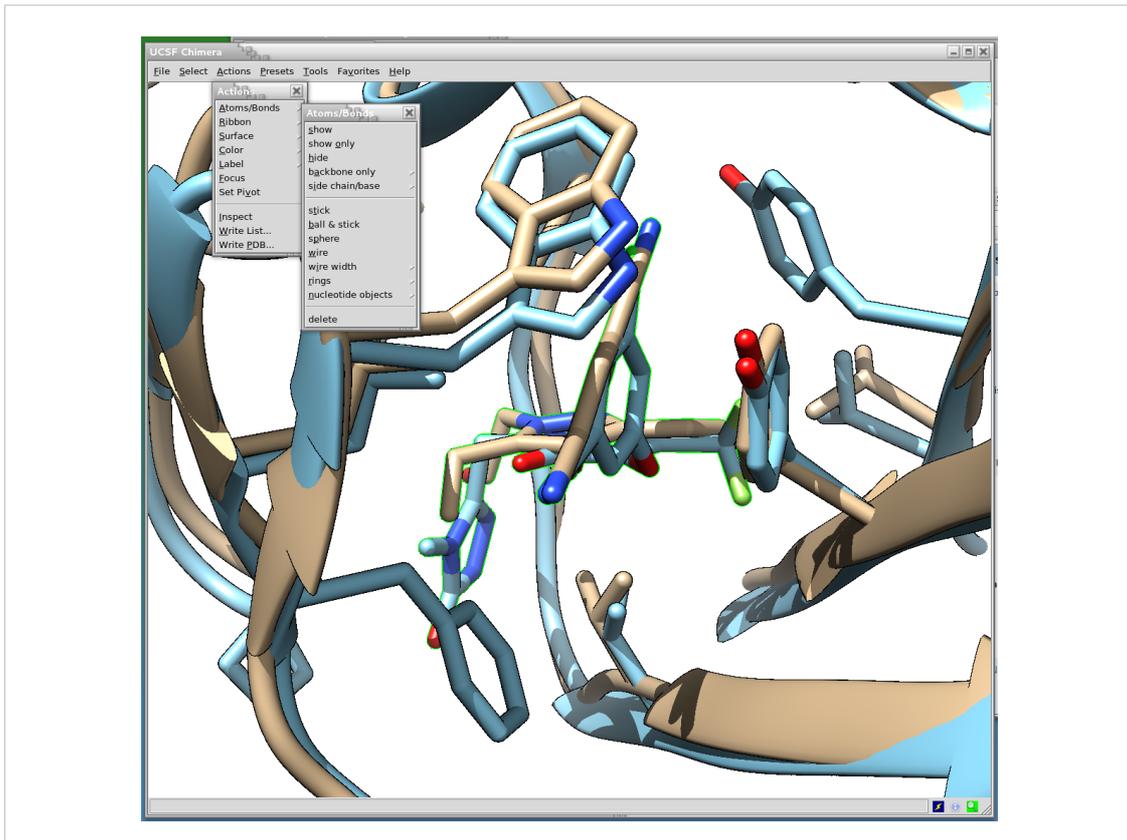
Vengono così selezionati in verde i due inibitori co-cristallizzati (in basso).



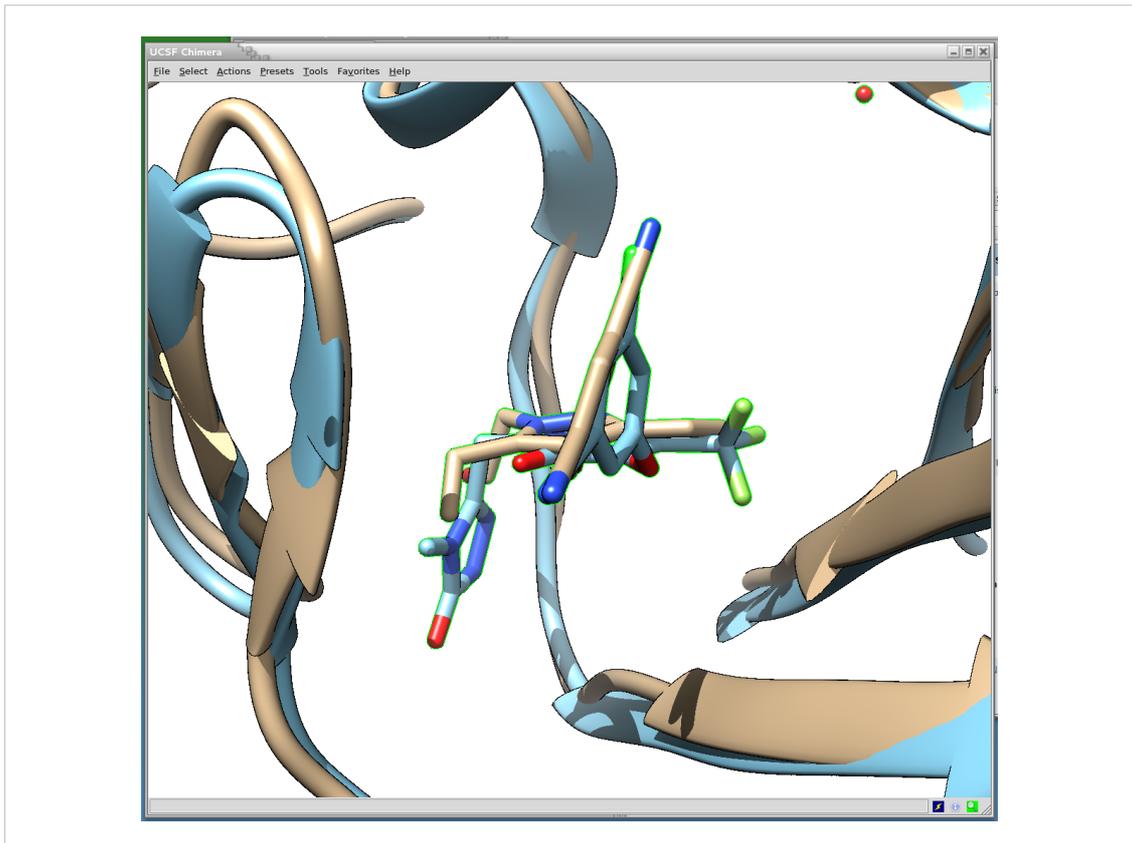
Usando il menù “Action” e selezionando la voce “focus”



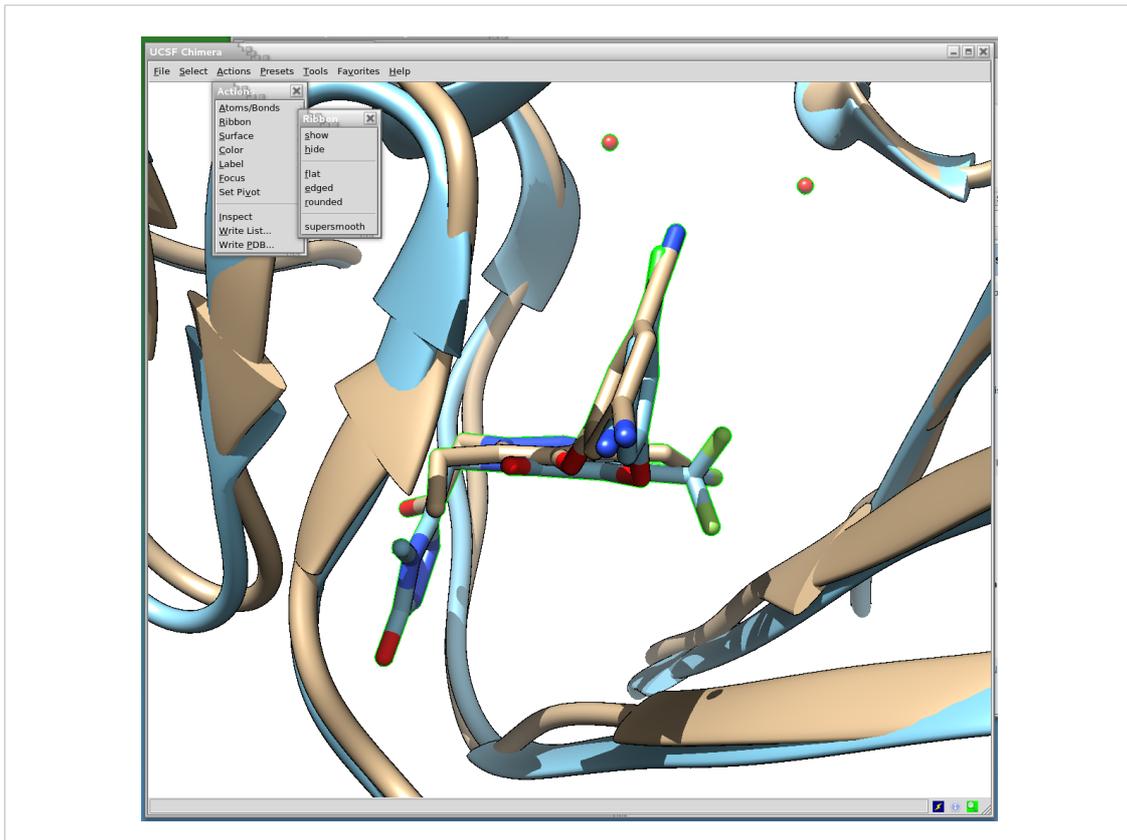
Le due strutture selezionate sono automaticamente “zoommate”



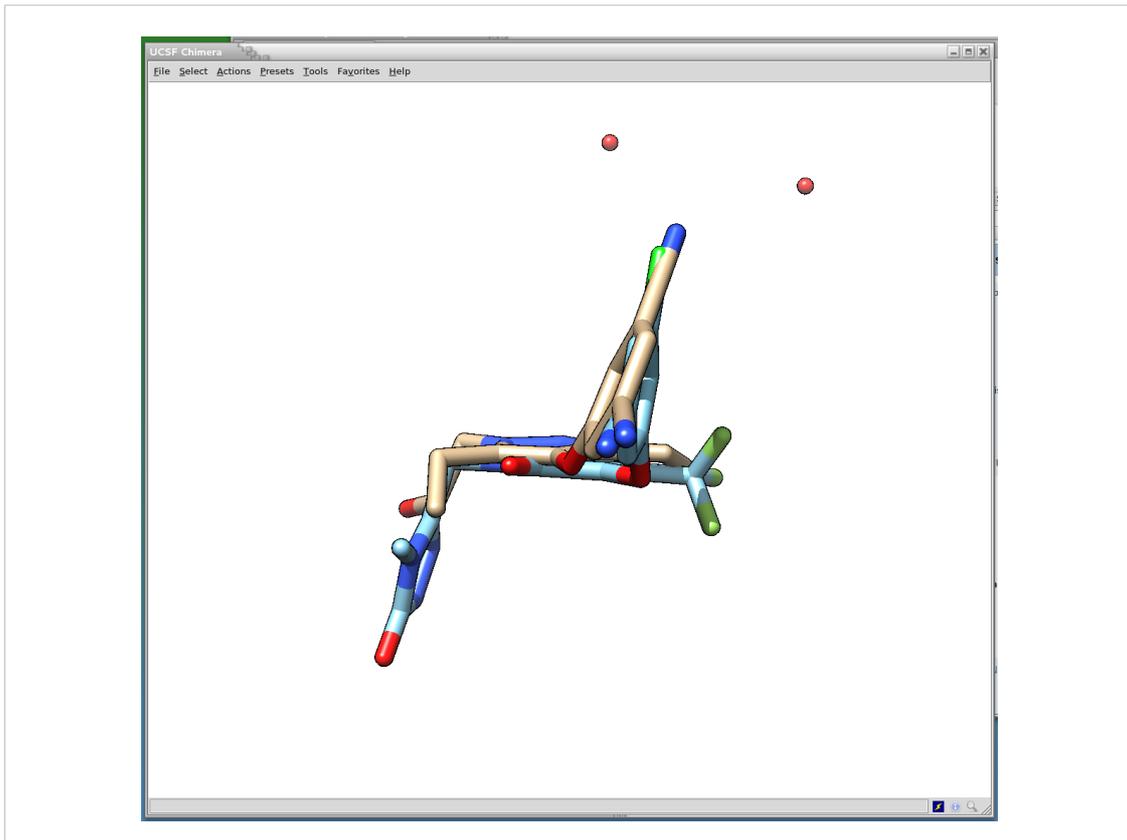
Quindi andando sul menù “Action”, “Atoms/Bonds”
e poi “show only”



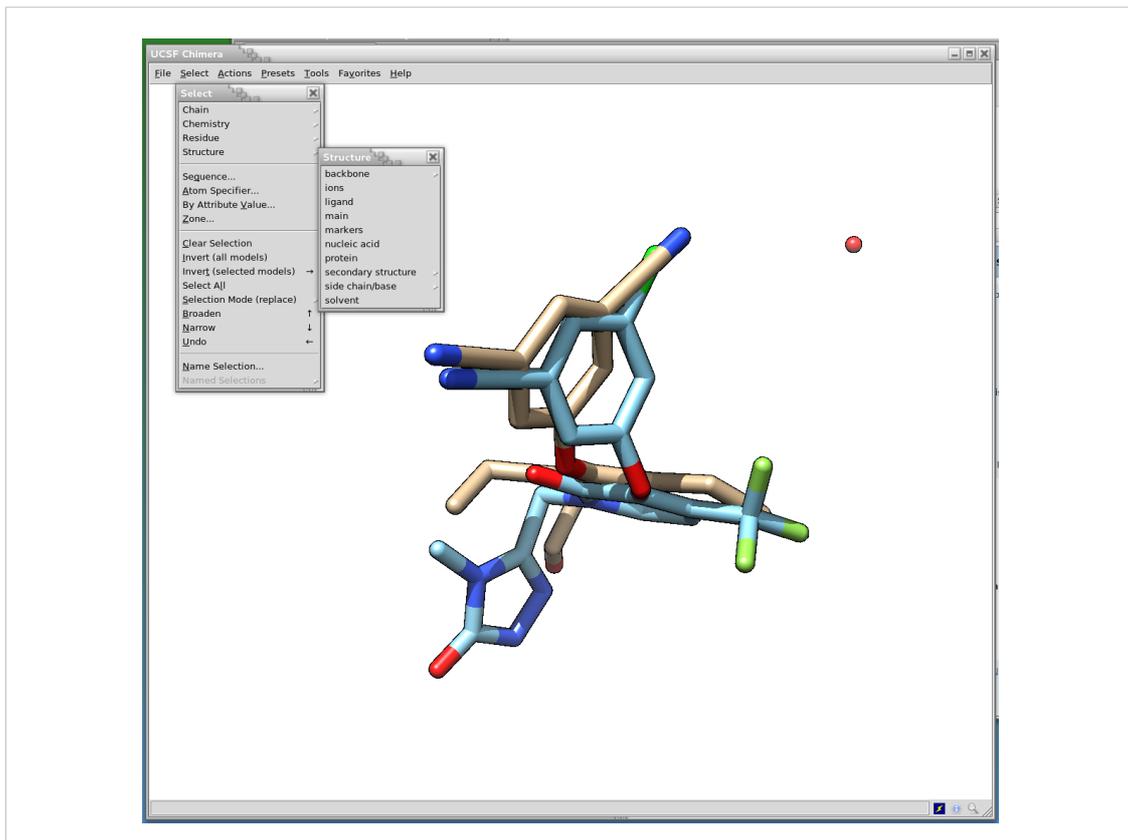
Solo gli atomi selezionati saranno visualizzati



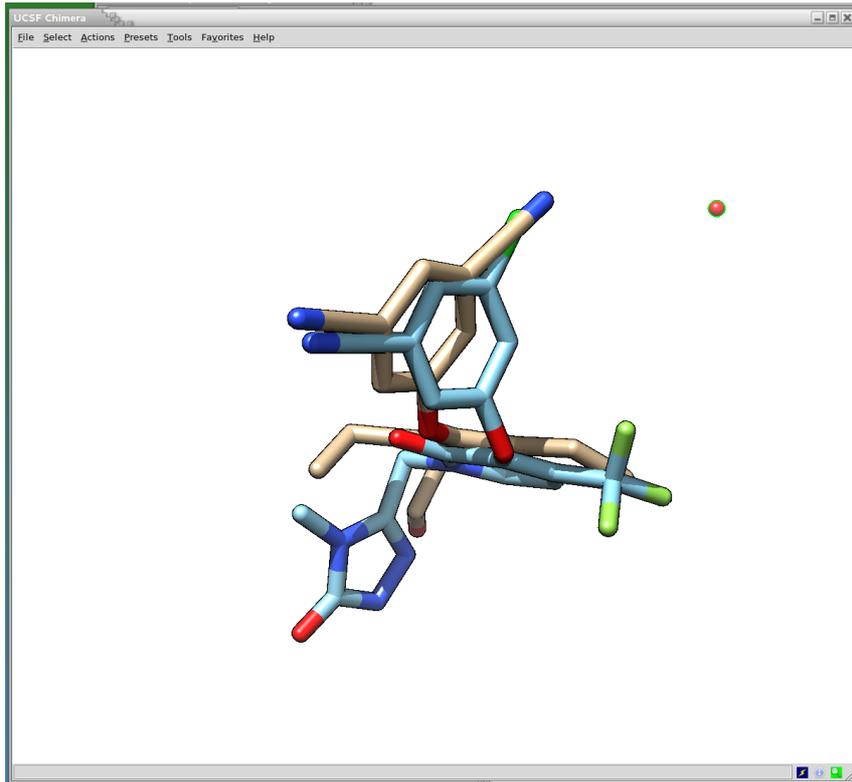
Si deseleziona tutto semplicemente cliccando nel in una porzione vuota della finestra di Chimera, mantenendo premuto il tasto “Ctrl” della tastiera. Quindi si toglie la visualizzazione del “ribbon” mediante il menù “Action”, poi “Ribbon” ed infine “hide”

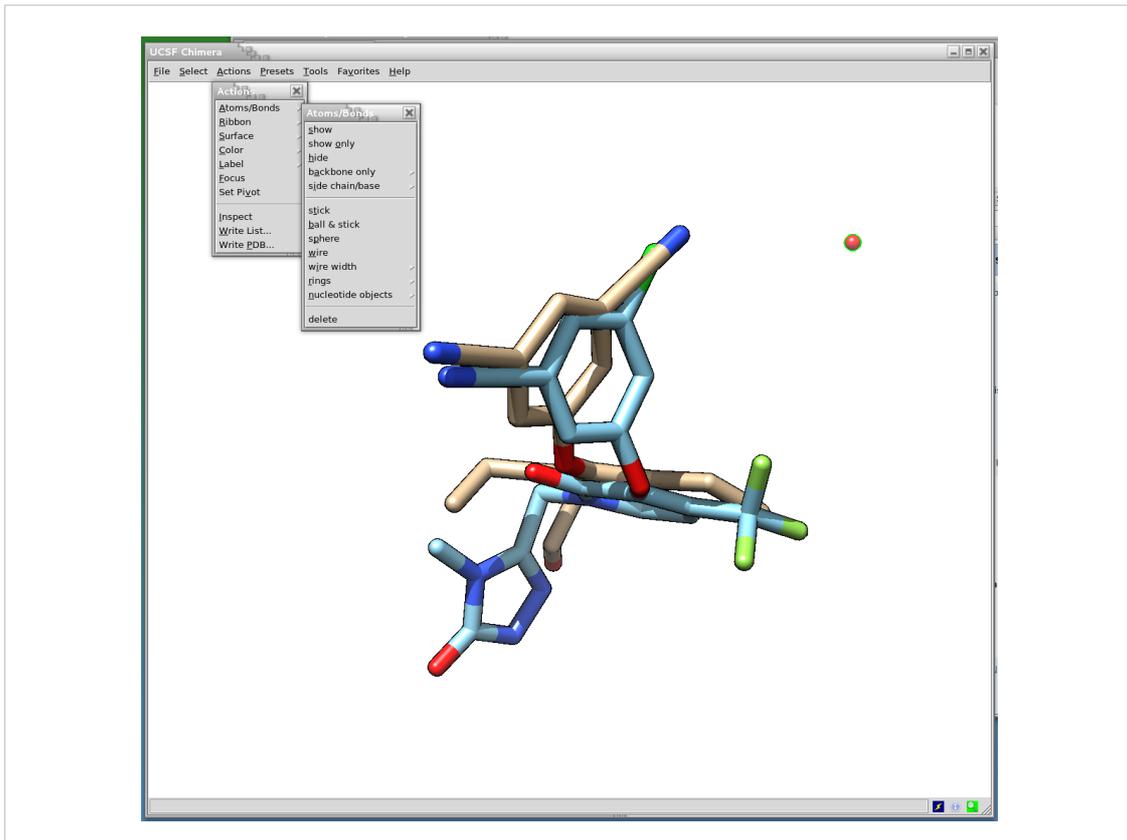


E rimangono visualizzate solo le molecole degli inibitori (nulla e' stato cancellato!)

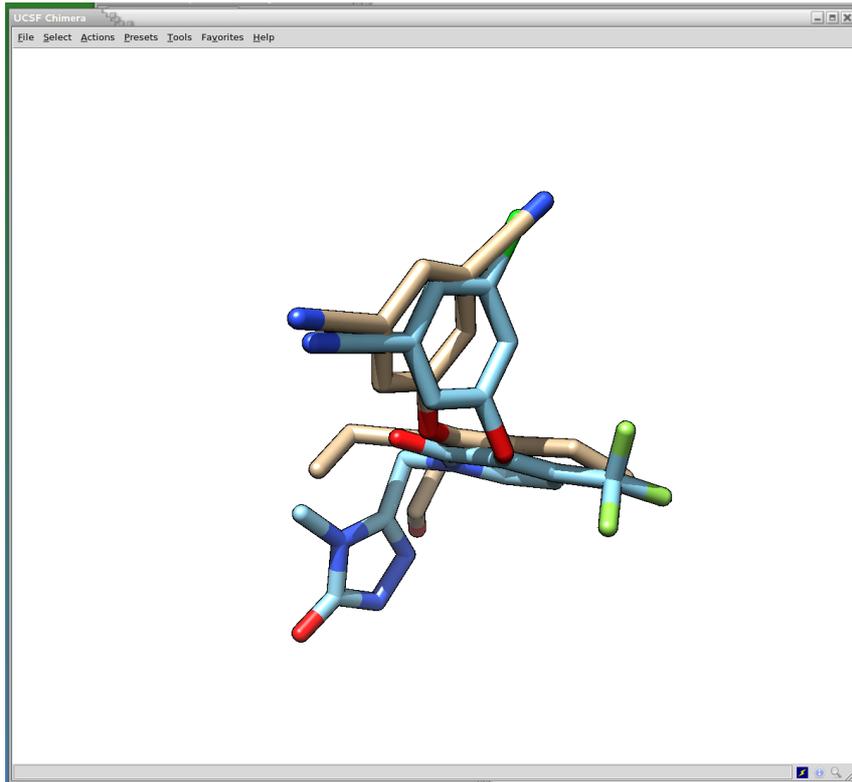


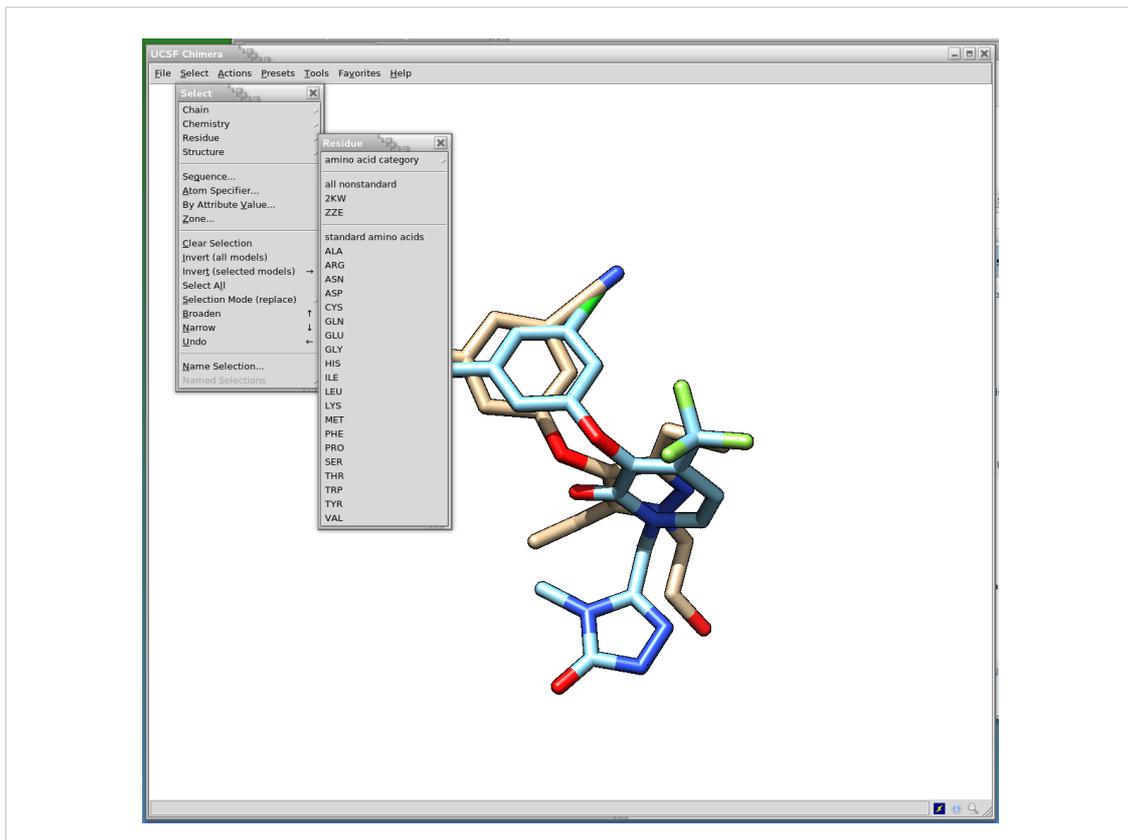
Se ci sono molecole di acqua (pallini rossi isolati) si possono selezionare andando nel menù “Select”, poi “Structure” e infine “Solvent”





E quindi cancellarle con la sequenza:
“Action” > “Atoms/Bonds” > “delete”

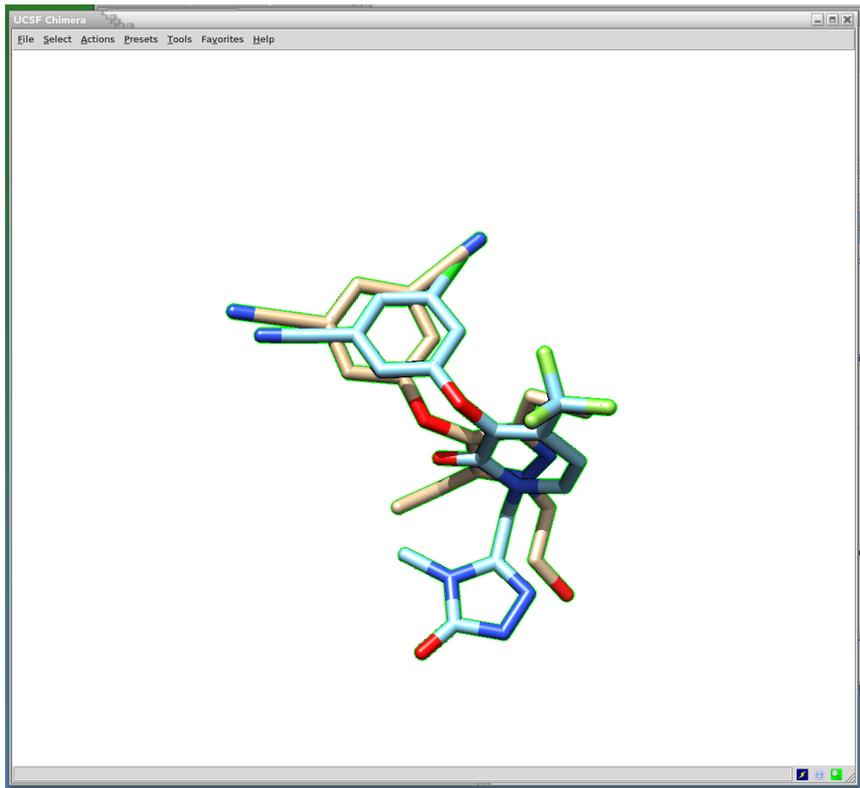


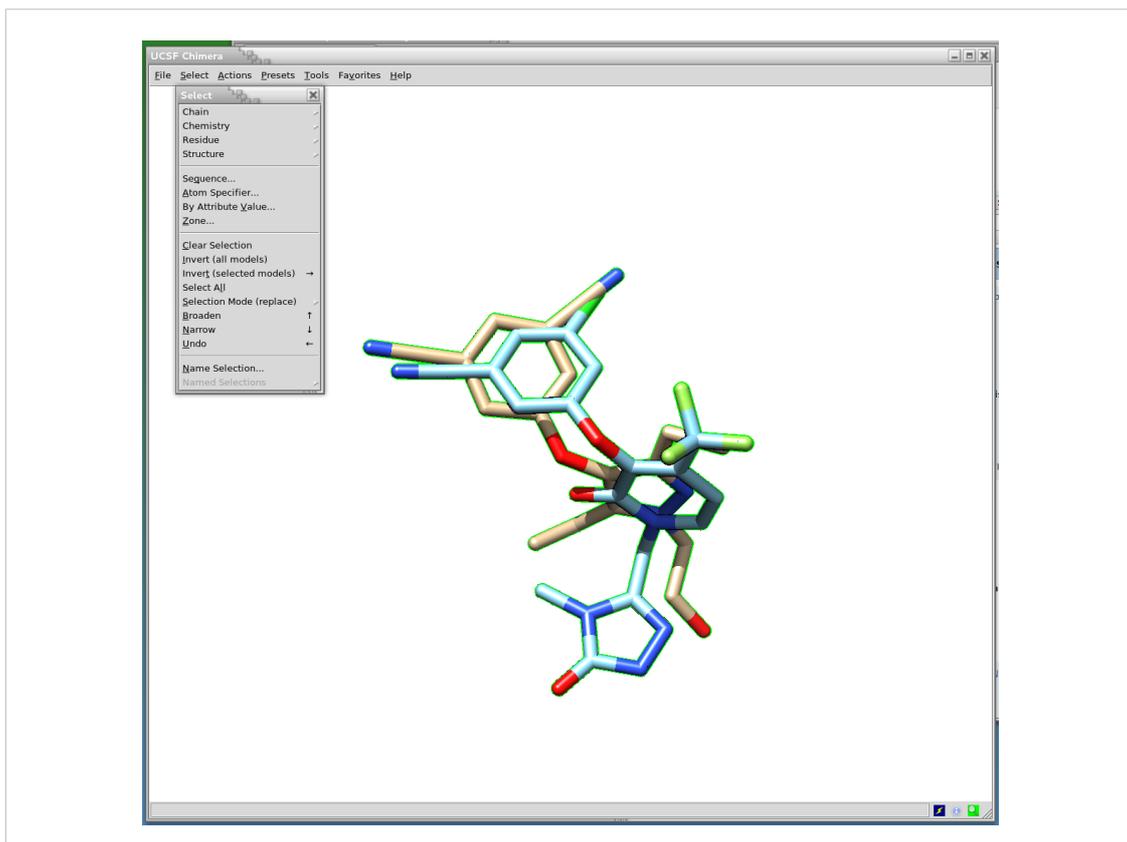


Quindi con una serie di operazioni possiamo visualizzare una parte del sito di legame allosterico.

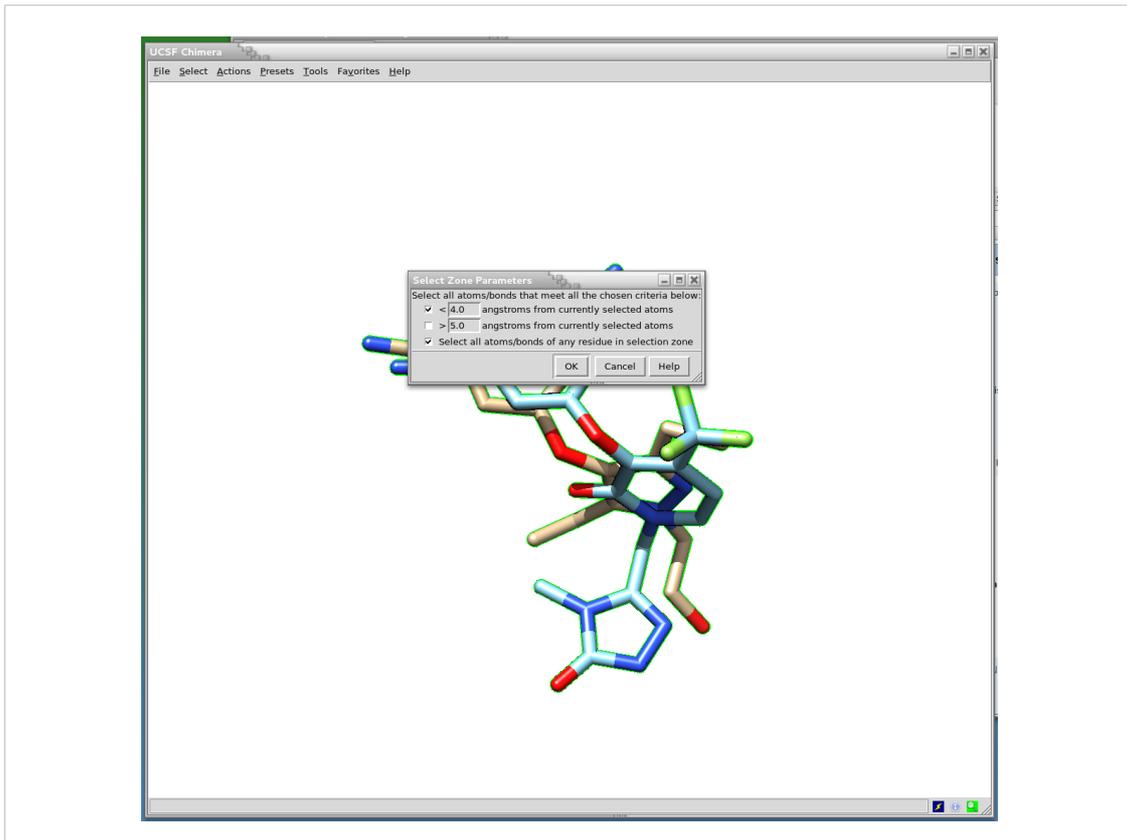
Per fare questo si usa la sequenza:

“Select” > “Residue” > “all nonstandard”

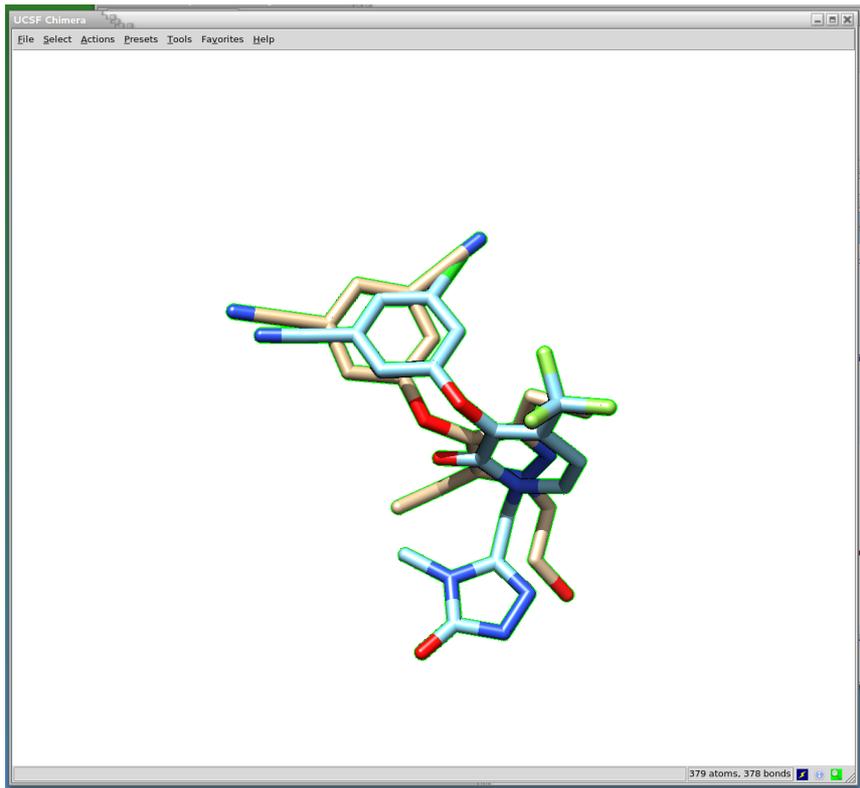


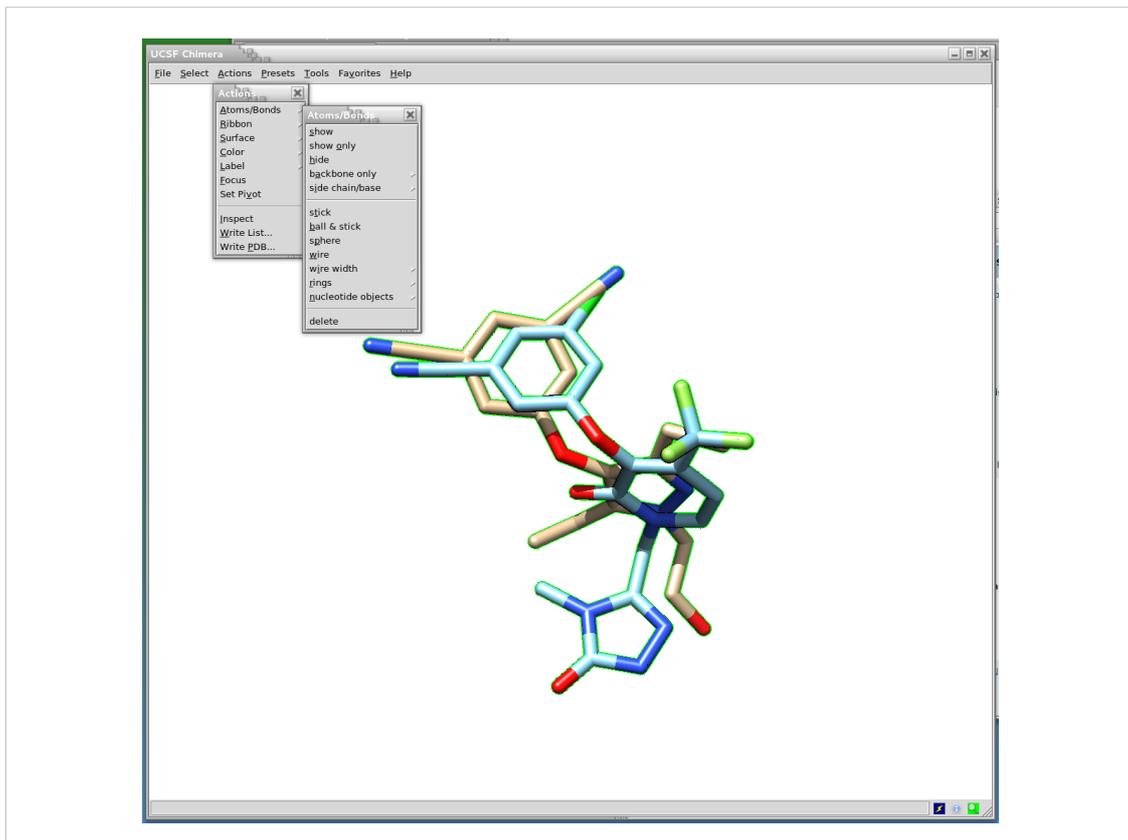


Quindi:
“Select” > “Zone ...” >

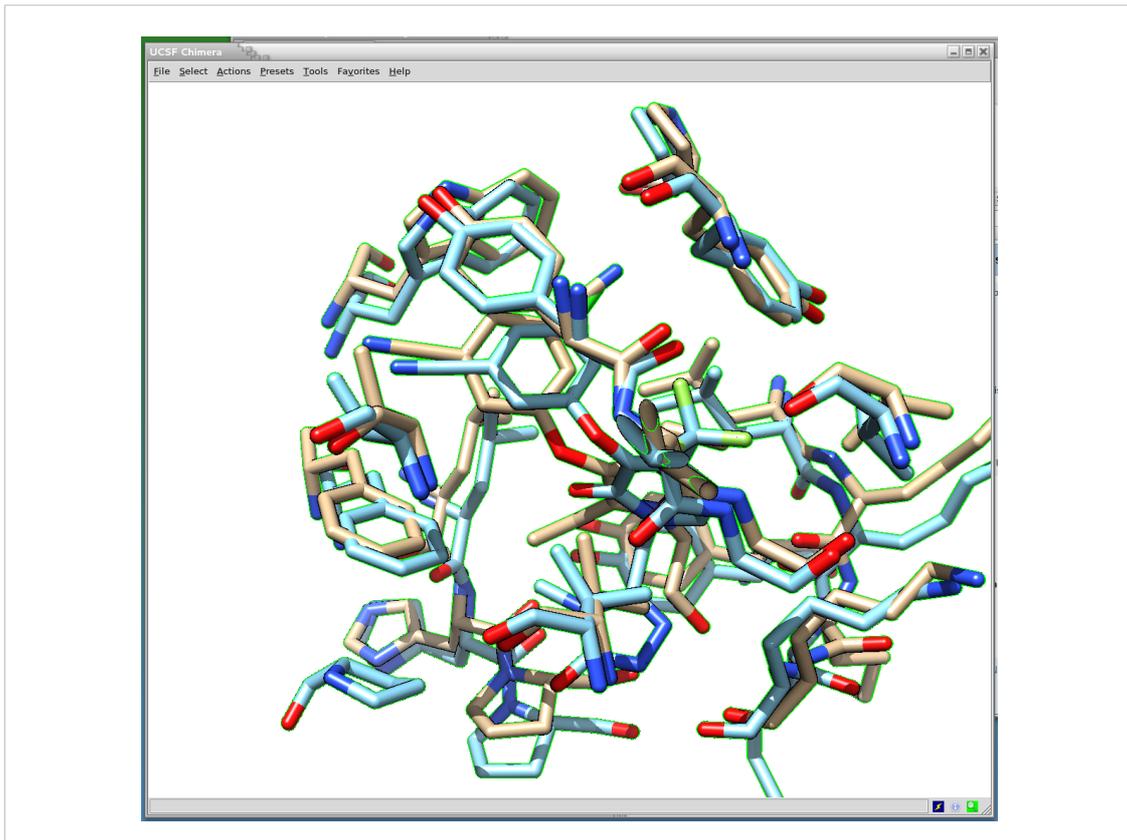


E nella fine star che appare si indica di selezionare tutti i residui che sono entro 4 angstrom dalle molecole selezionate.

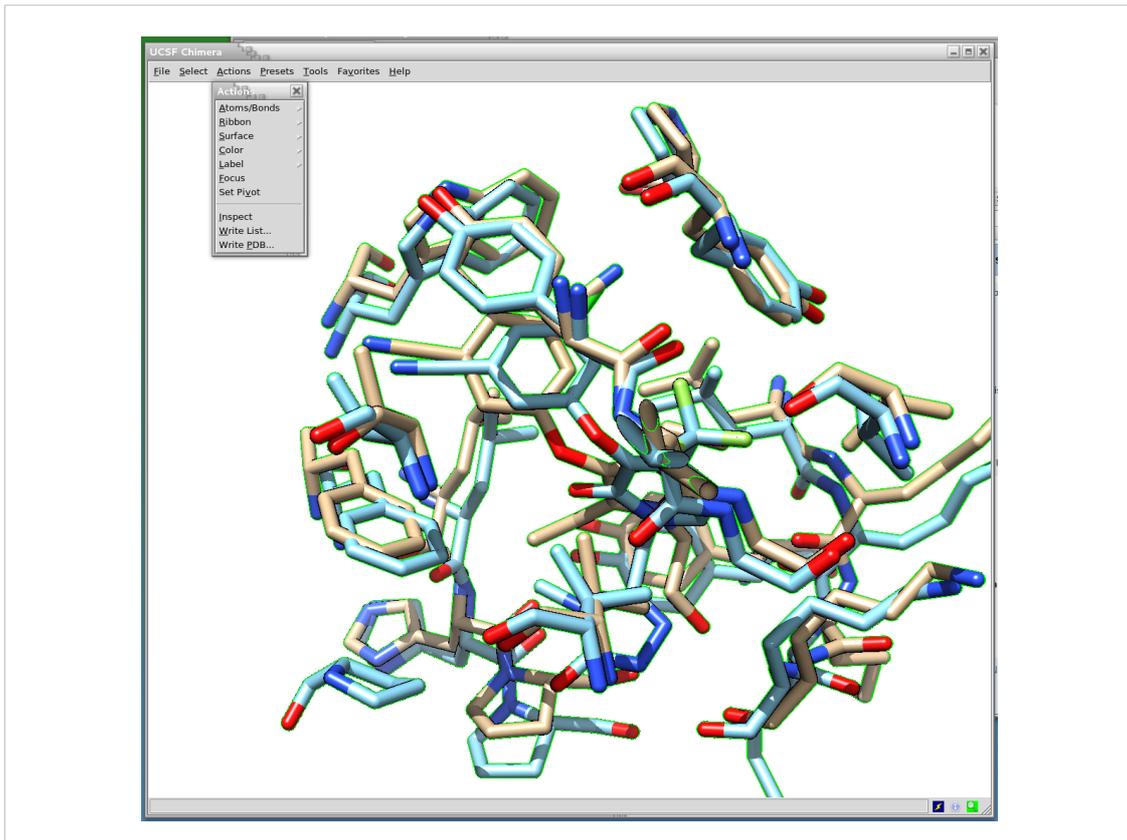




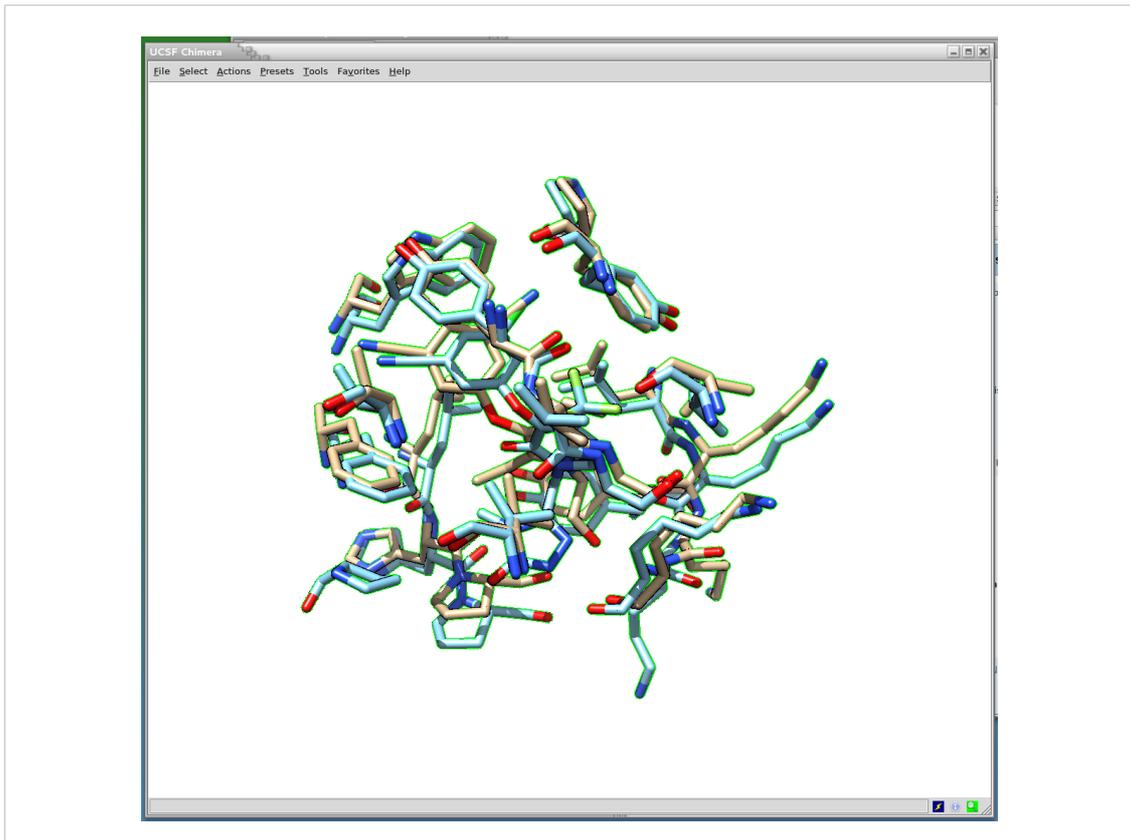
Quindi con il menu “Action”, poi “Atoms/Bonds” e quindi “show”



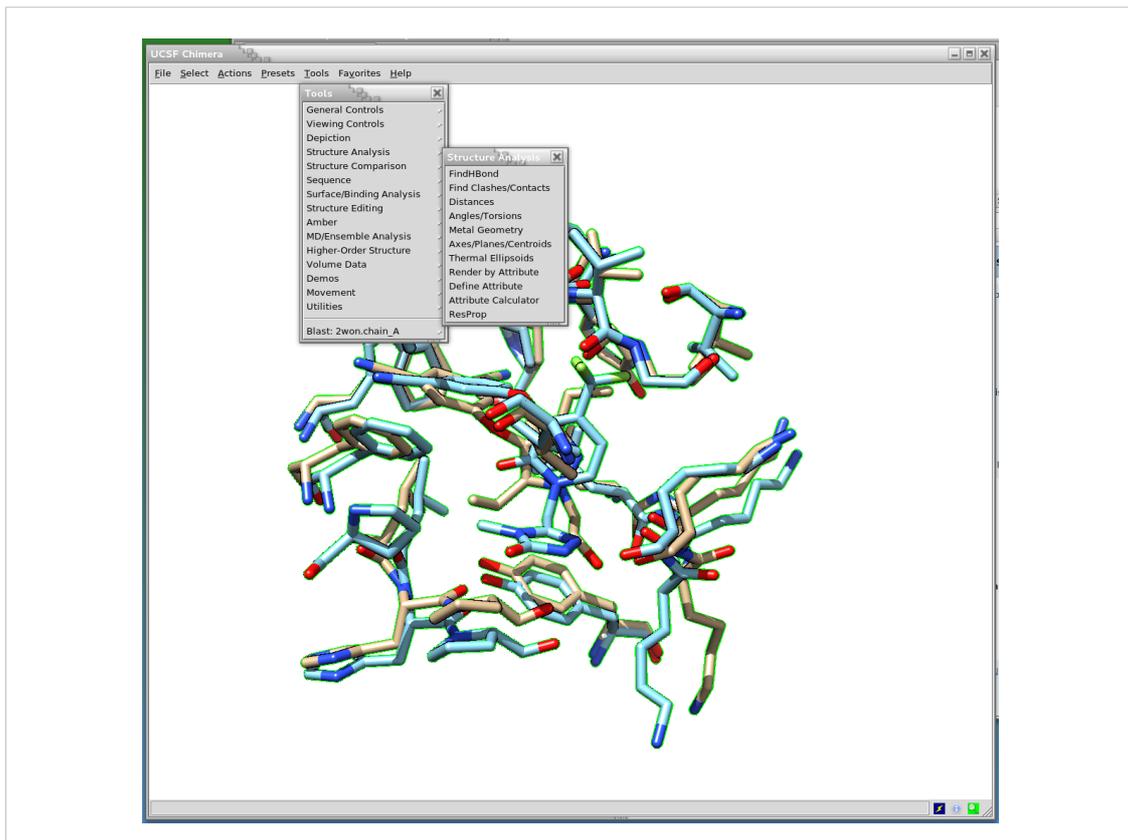
Vengono visualizzati i residui di cui almeno un atomo si trova ad una distanza minore o uguale a 4 angstrom.



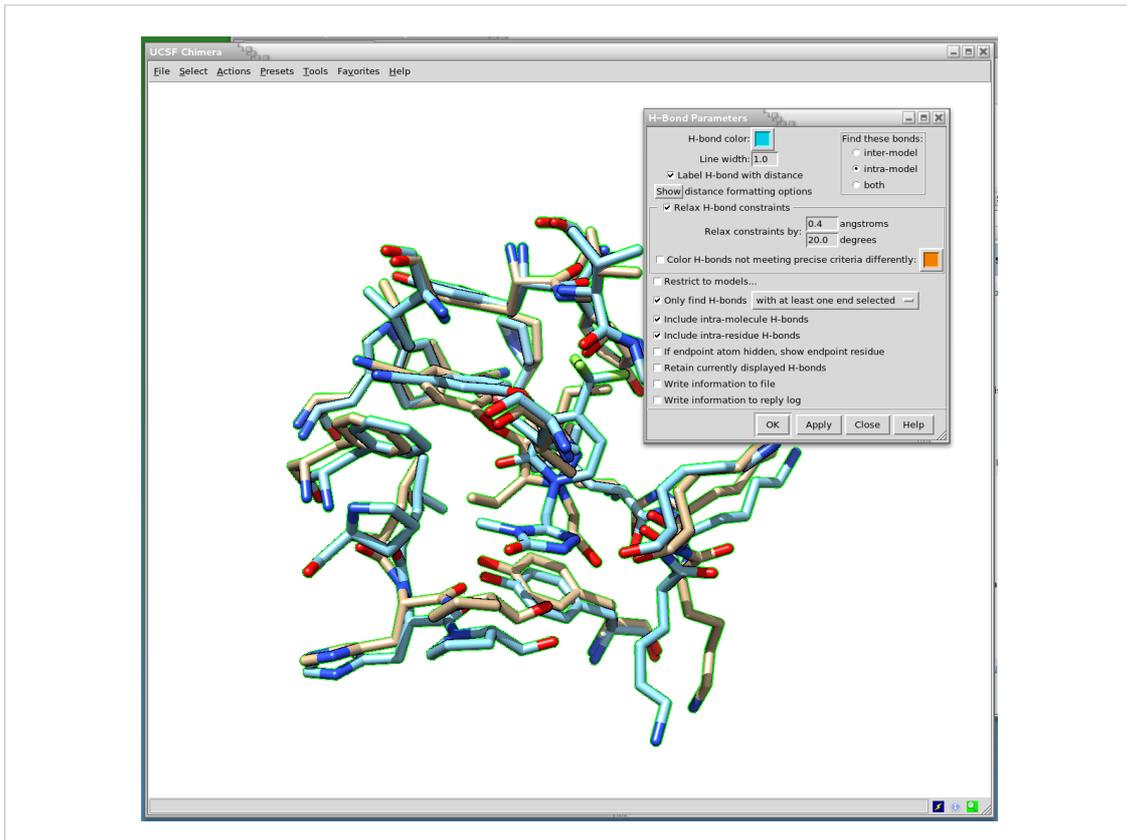
Poi con la sequenza “Action” > “focus”



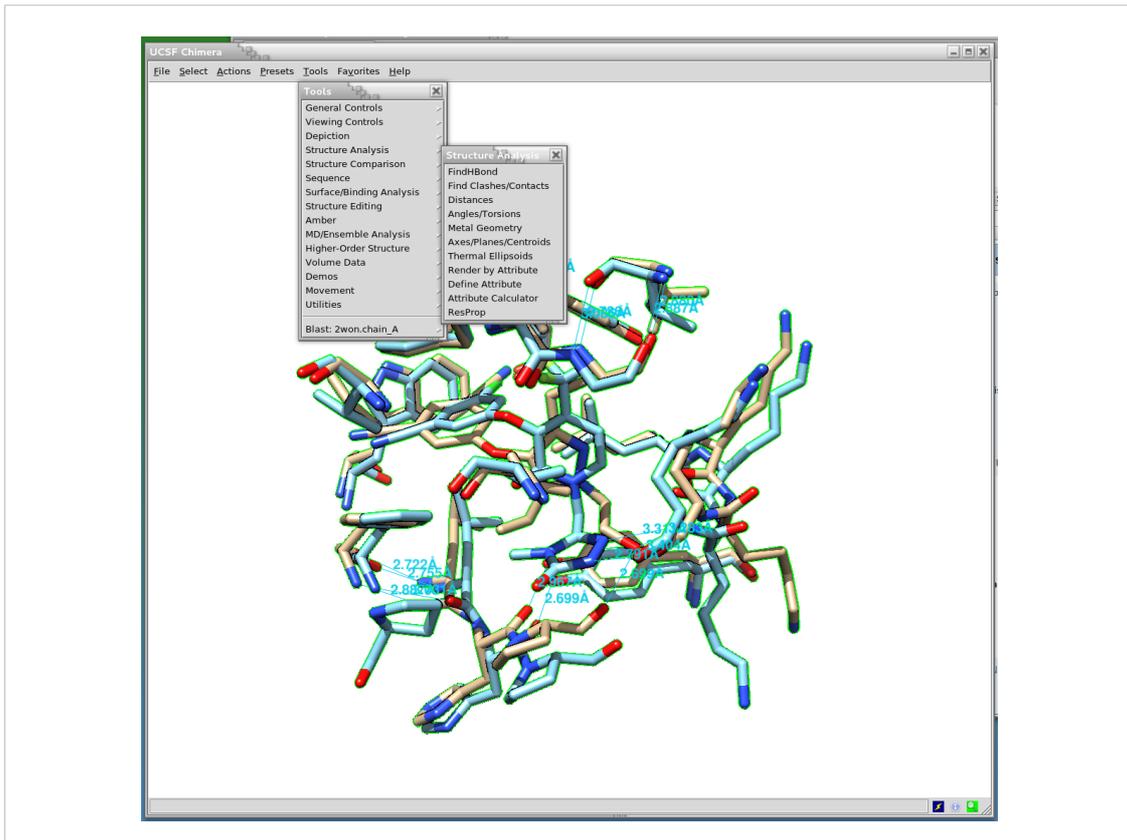
Gli atomi visualizzati sono centrati nella finestra di Chimera



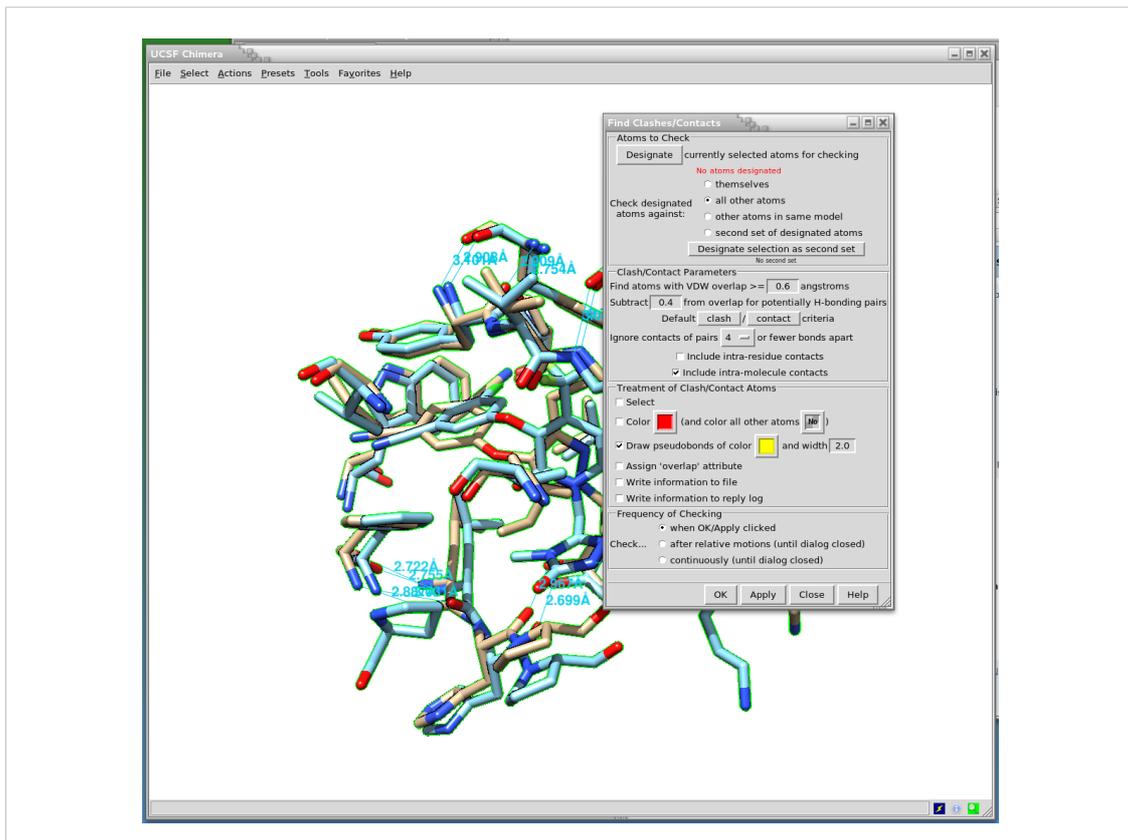
Tra le azioni che si possono fare vediamo che si possono visualizzare i legami idrogeno utilizzando “Tools” > “Structure Analysis” > “FindHBond”



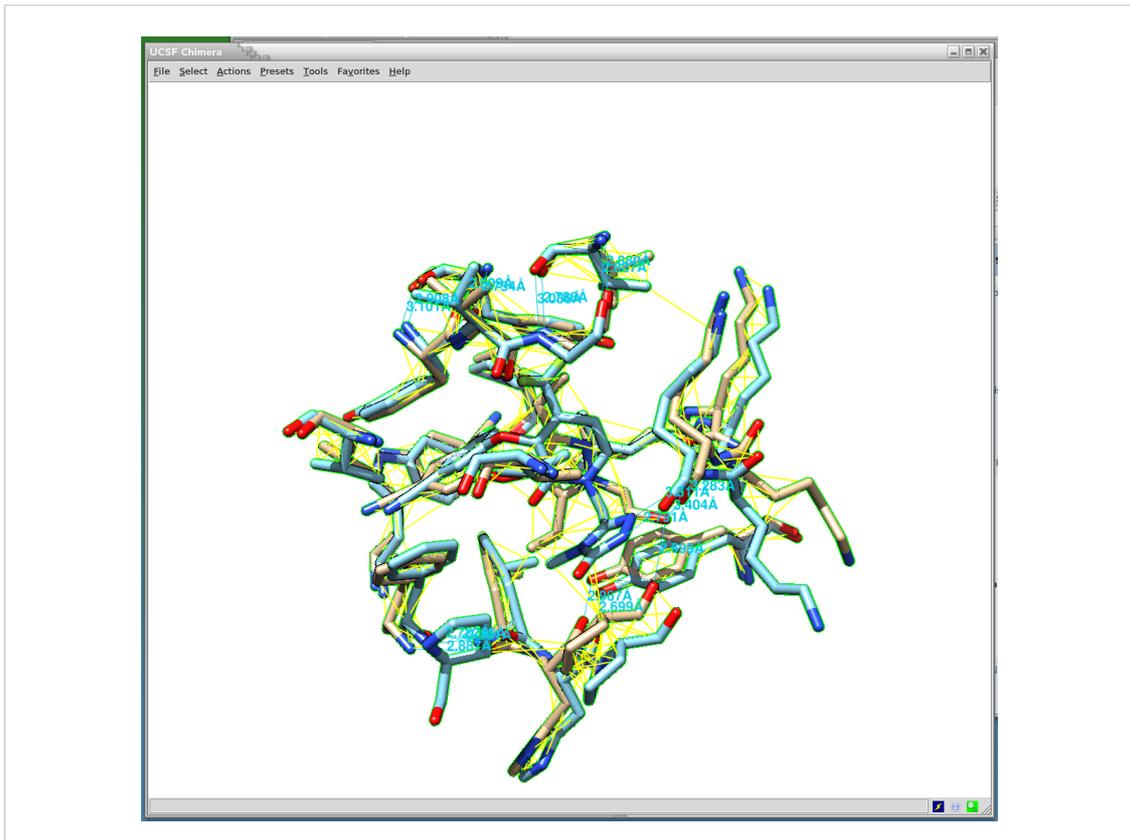
Nella finestra che appare fare le modifiche come mostrato e poi cliccare su “OK”



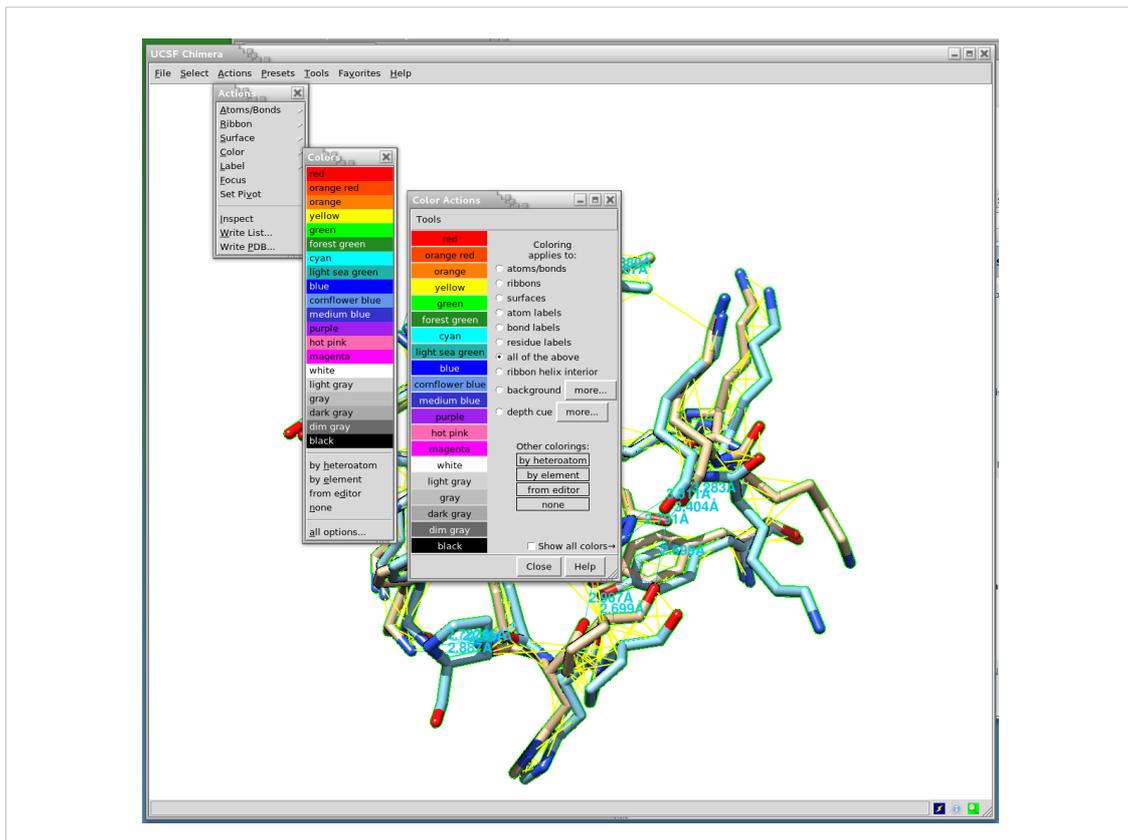
In modo analogo si possono evidenziare le zone che possono dare interazioni steriche:
“Tools” > “Find Clashes/Contacts”



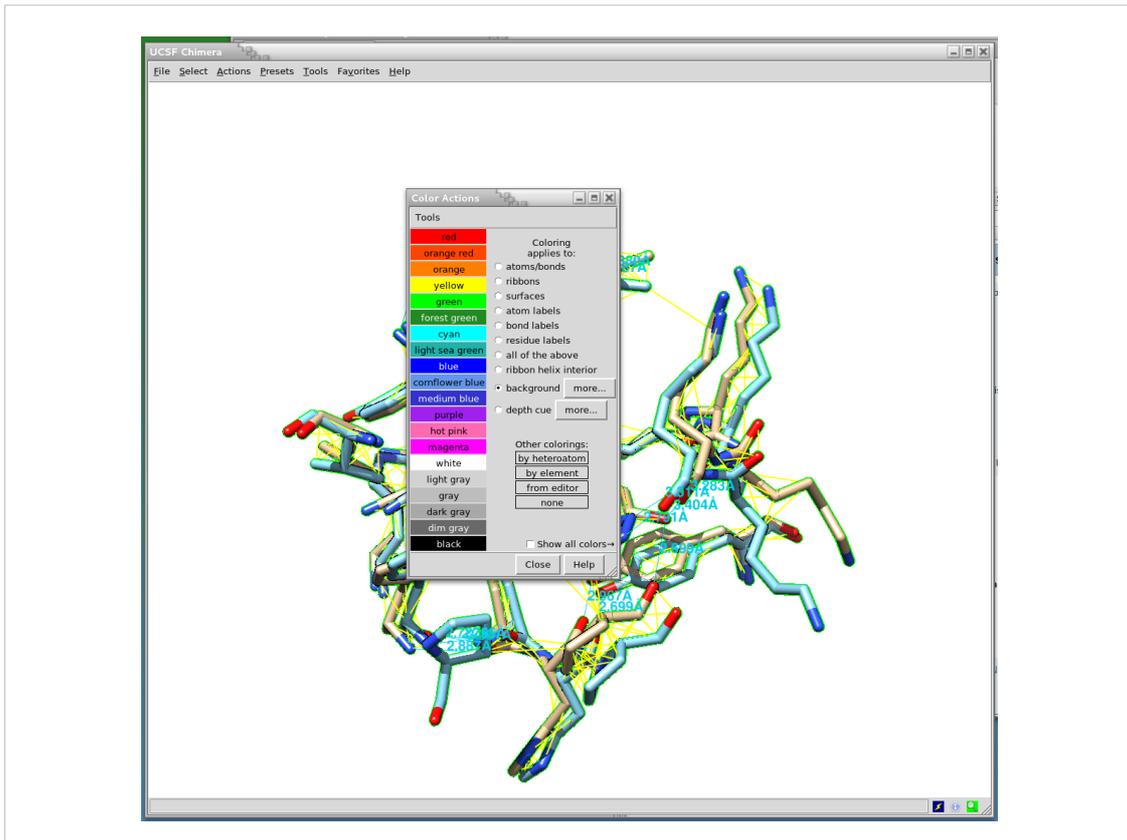
Si clicca sul pulsante “Designate” e quindi si “OK”



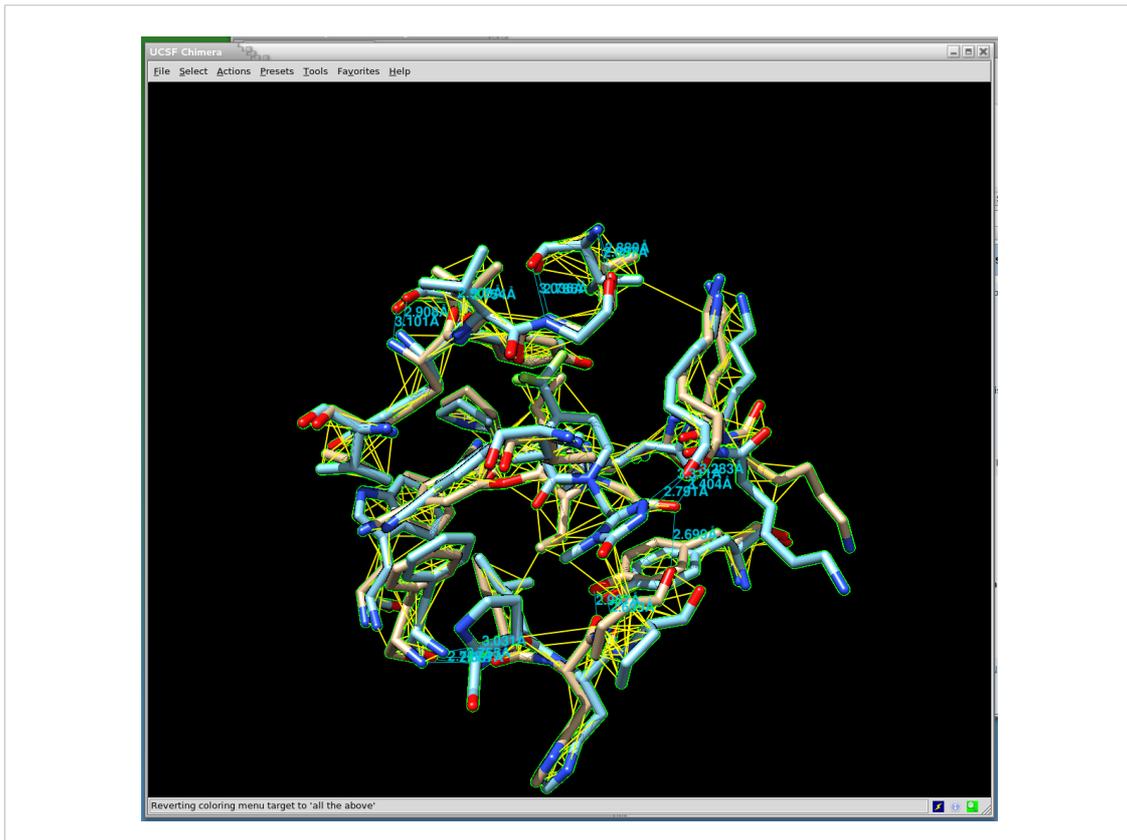
Le interazioni steriche saranno indicate da linee gialle



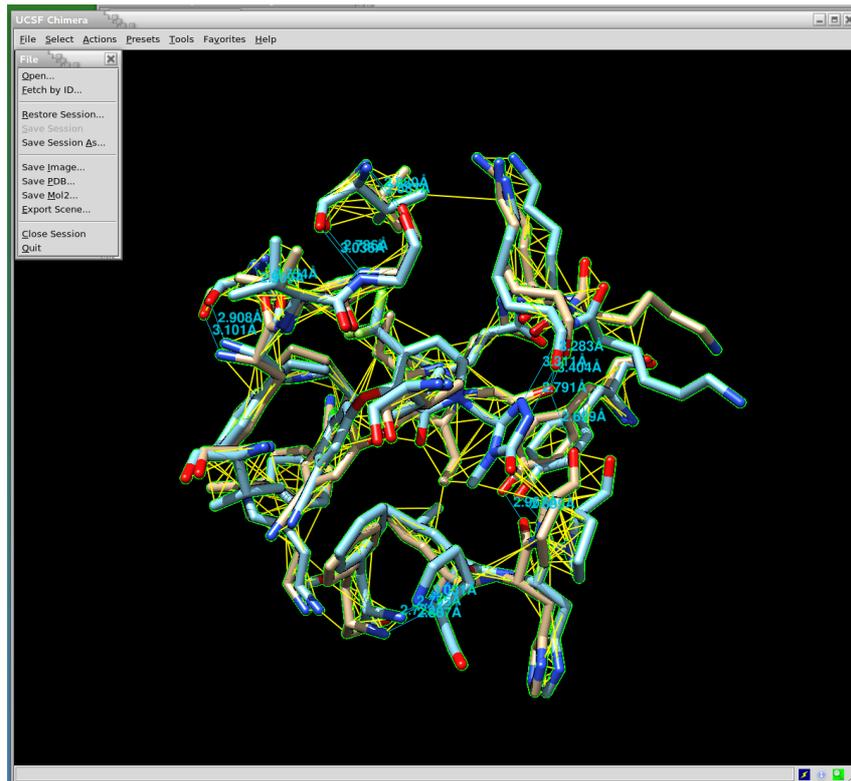
Per migliorare la visualizzazione si puo' cambiare lo sfondo in nero con la sequenza di comandi:
"Actions" > "Color" > "all option"



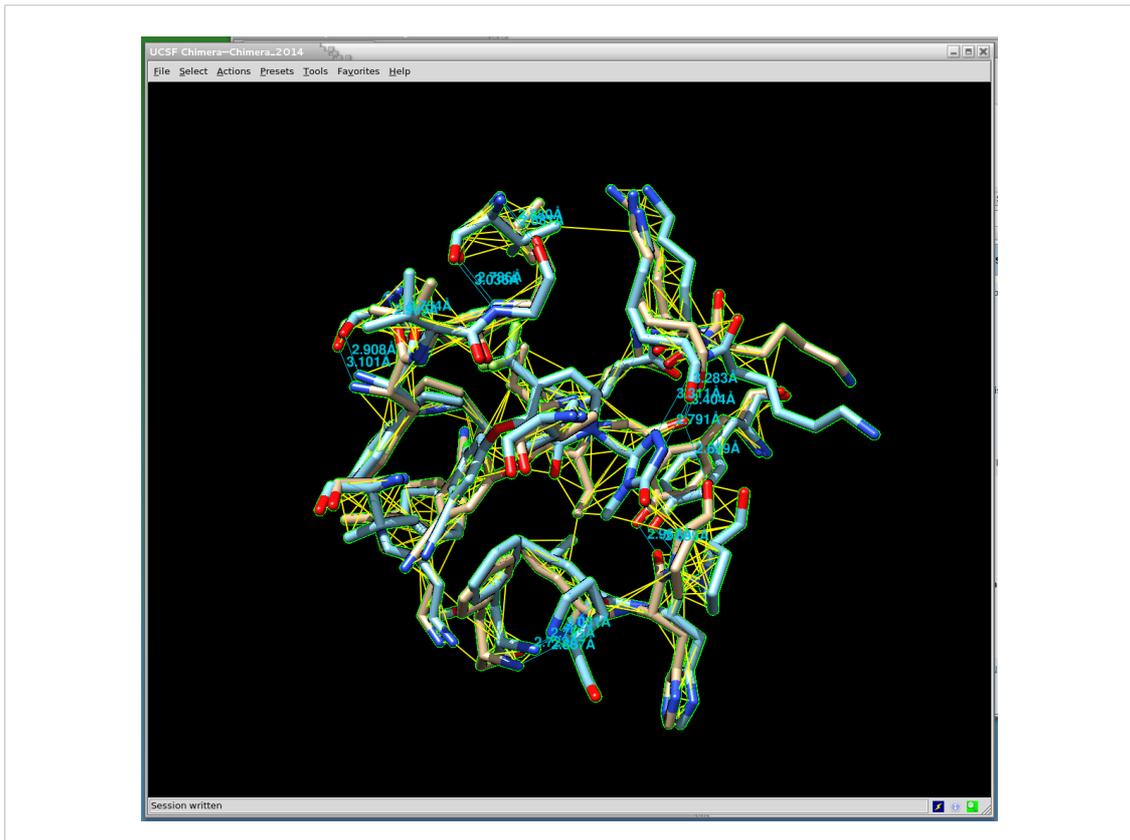
Nella finestra dei colori, selezionare “background” e poi si clicca sul nero



In questo modo lo sfondo e' diventato nero.
E si possono notare le interazioni steriche tra i
ligandi e i residui della tasca di legame.



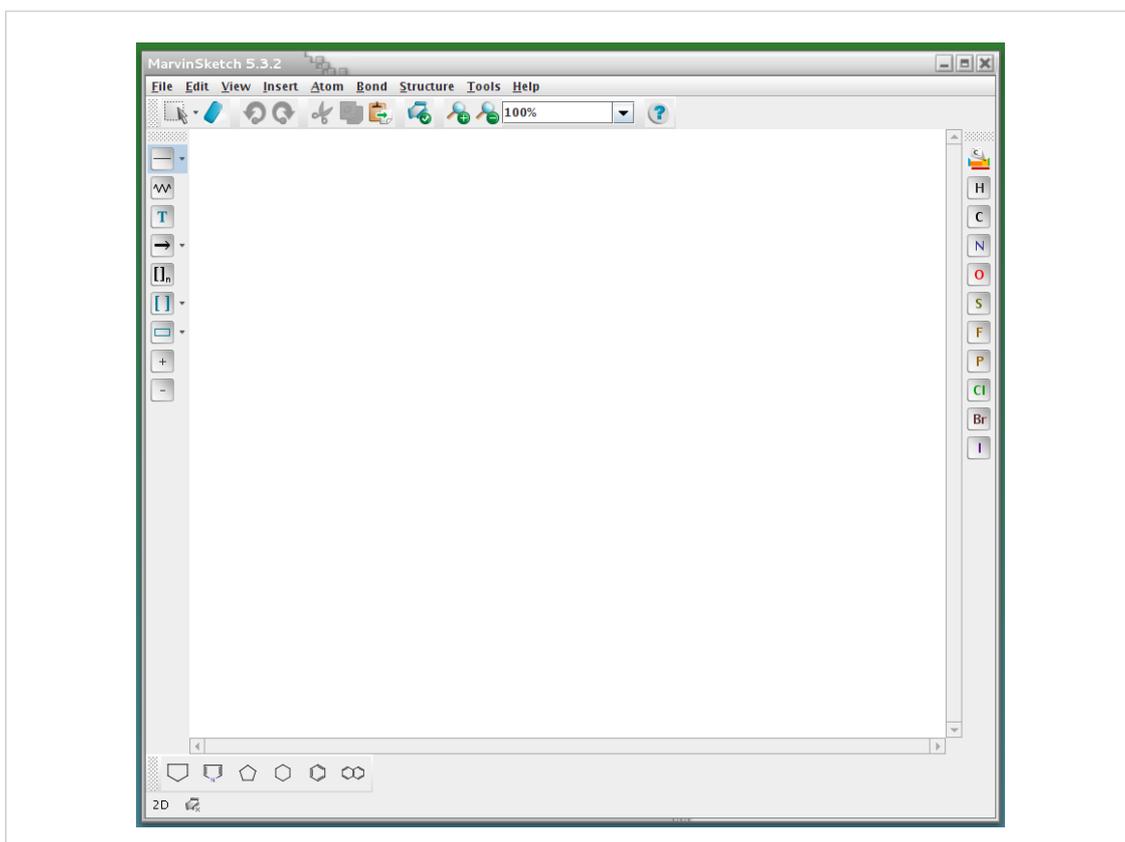
A questo punto e' saggio salvare il lavoro fatto fin qui. Mediante ilmenù "File" > "Save Session As ..."



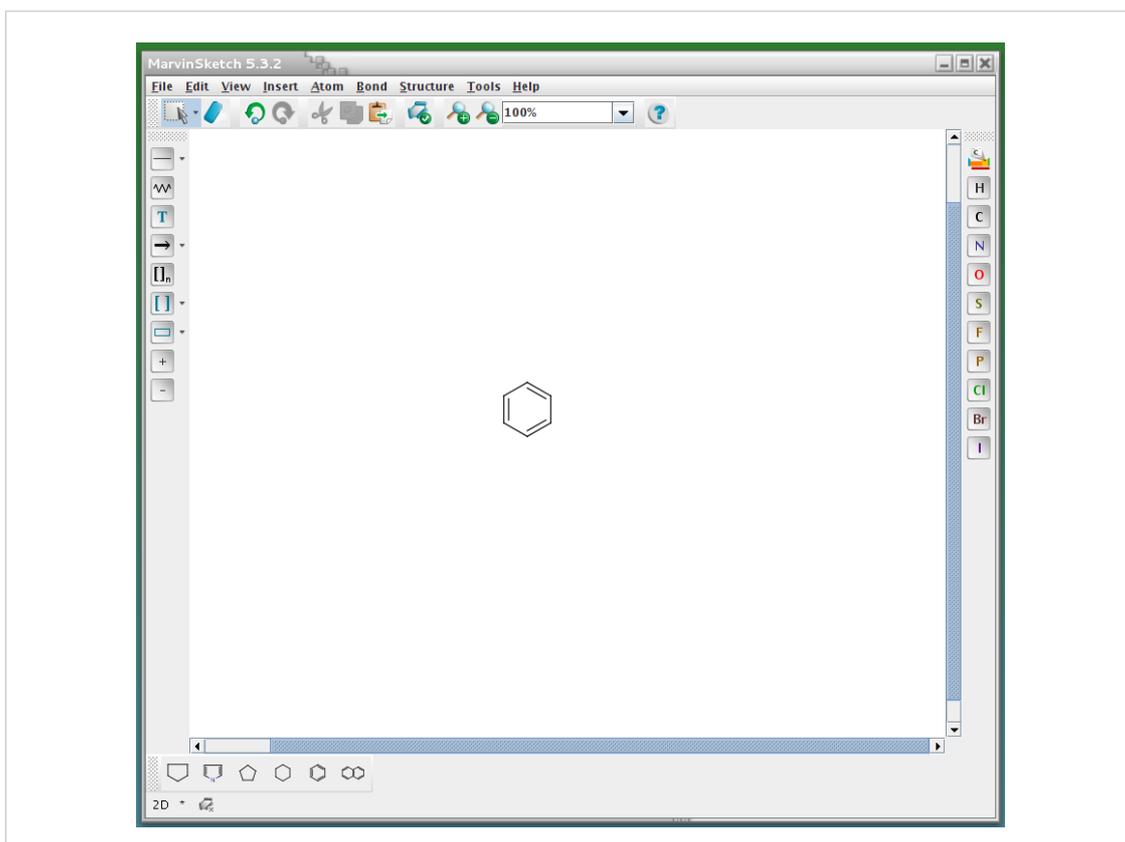
Appare la scritta “Session written”

Marvin Sketch Usage

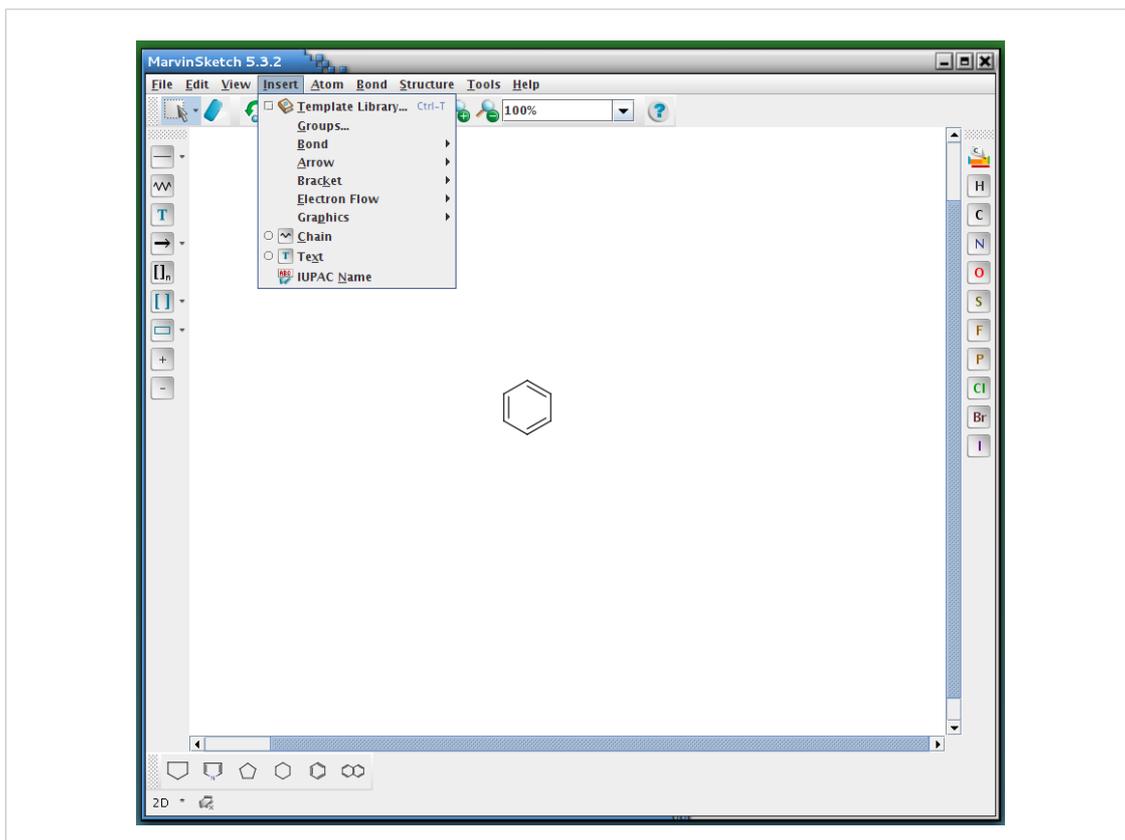
Si indicheranno le azioni per iniziare ad usare questo programma per generare strutture molecolari 2D e 3D.



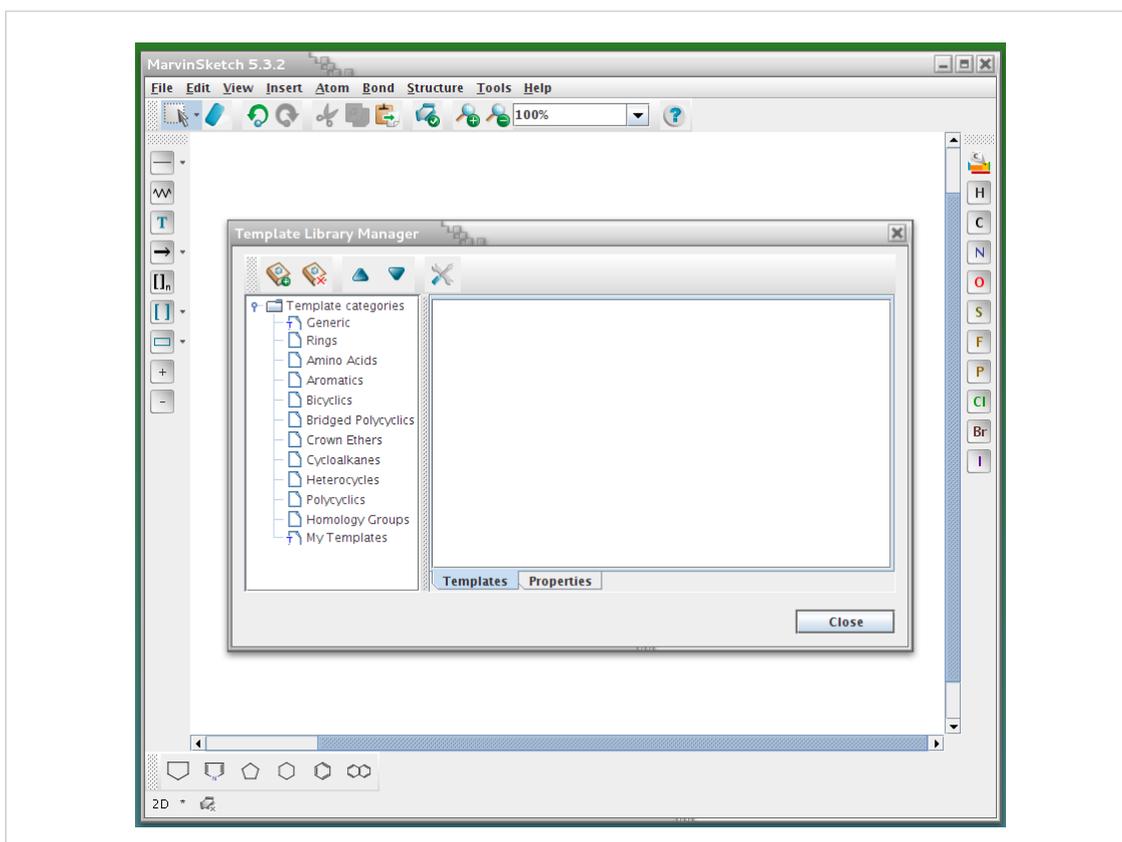
Si apre il programma marvin sketch (icona sulla scrivania)



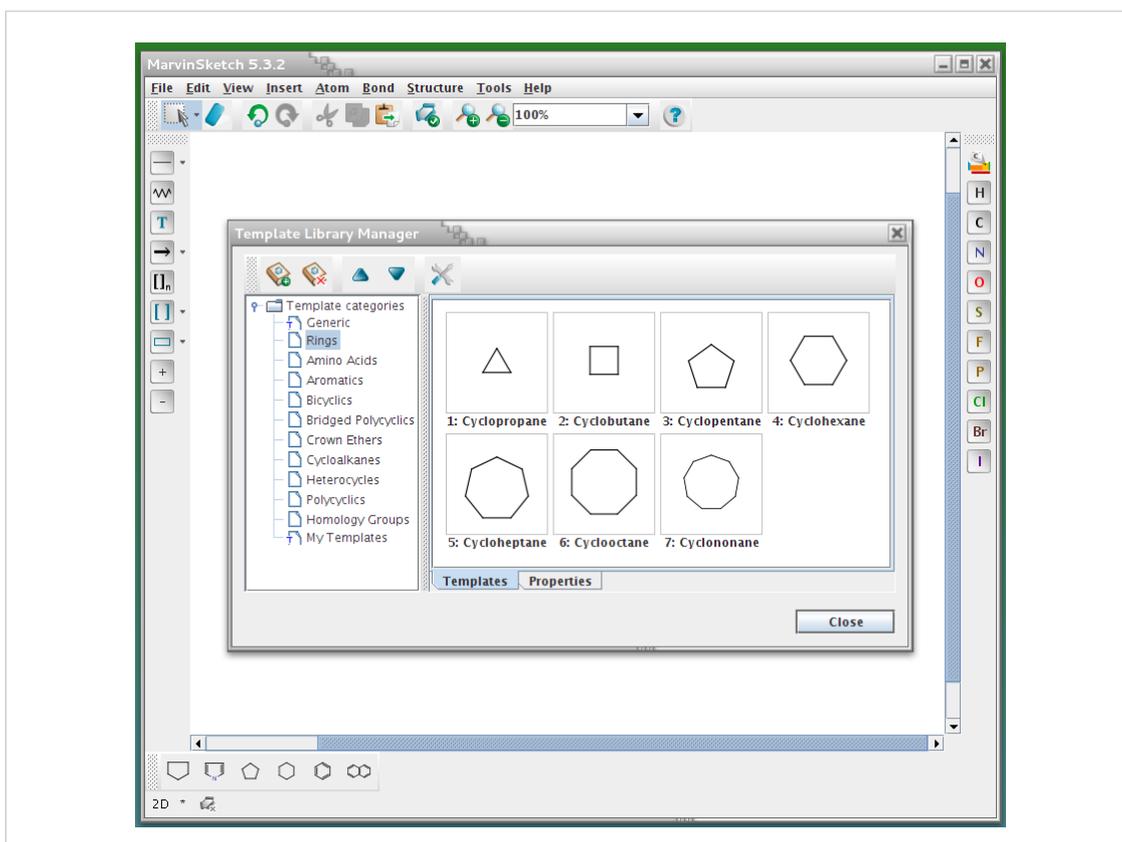
Iniziamo a disegnare un benzene nel centro della finestra



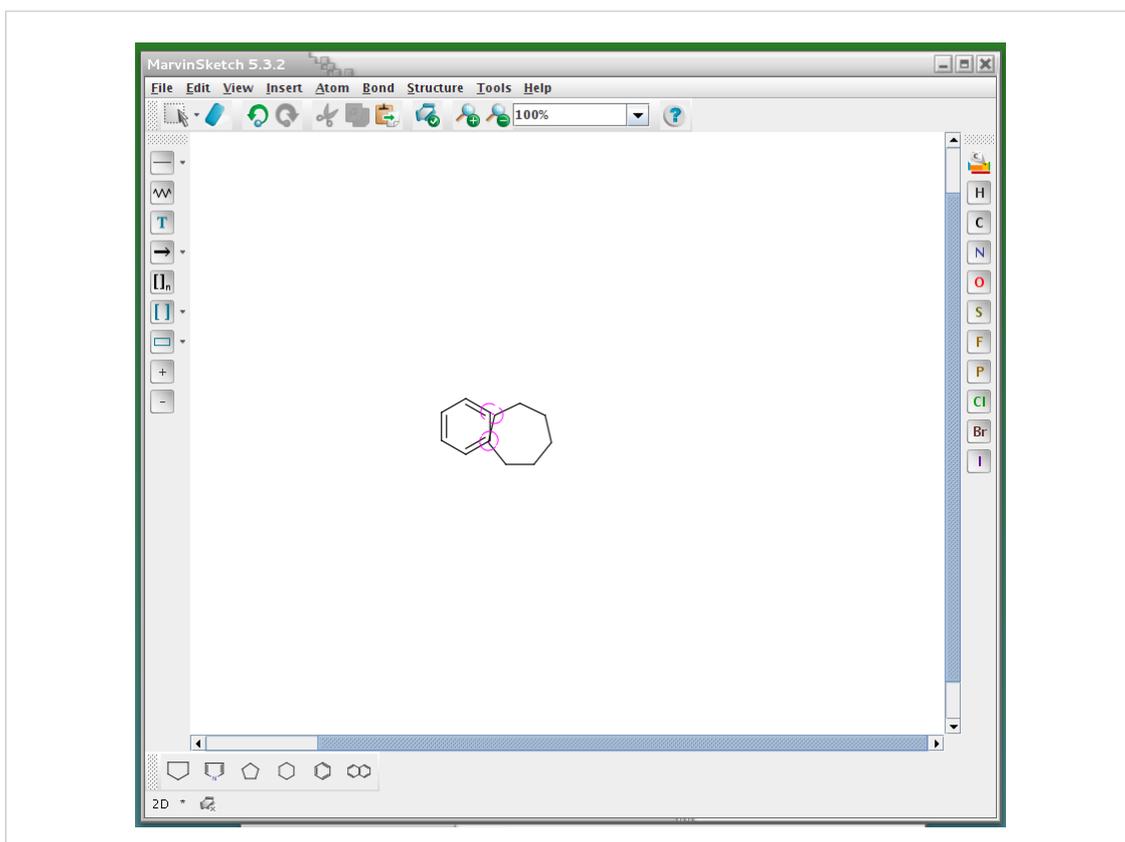
Quindi utilizzando il menu “insert” selezioniamo “Template Library ...”



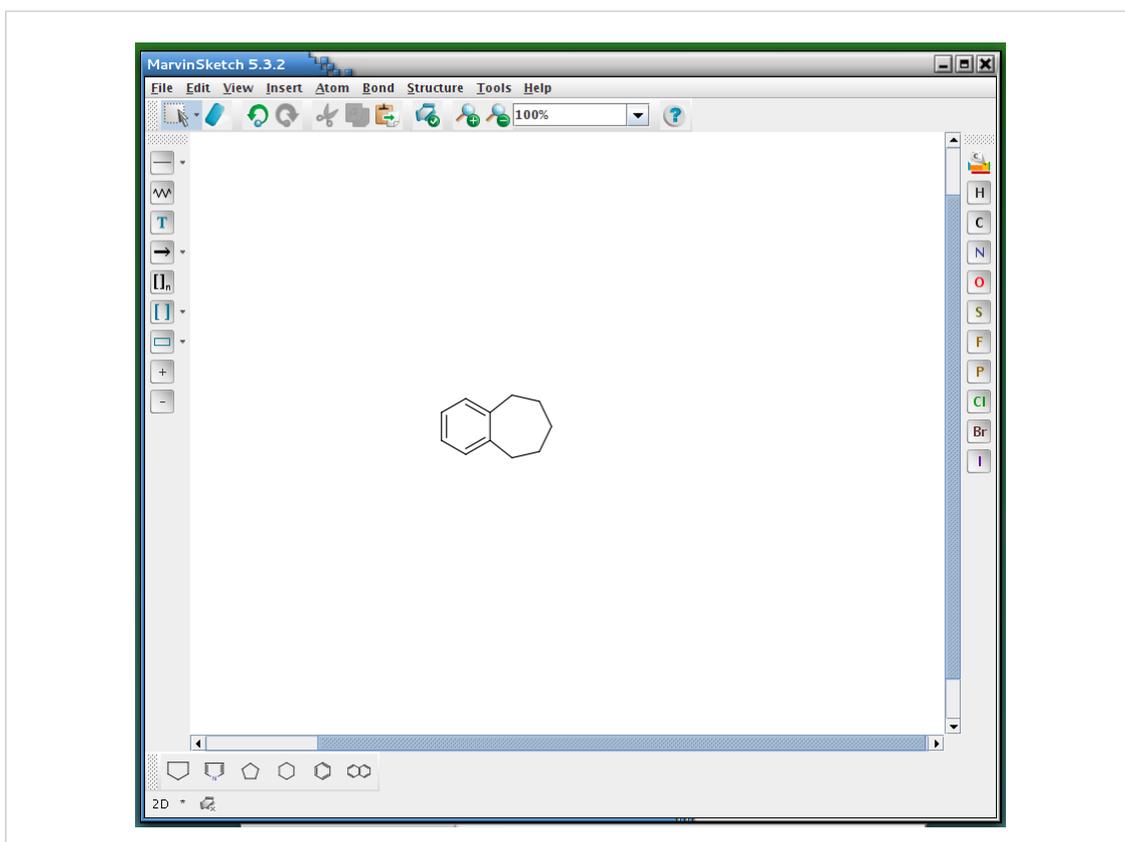
E nella finestra che appare selezioniamo “Rings”



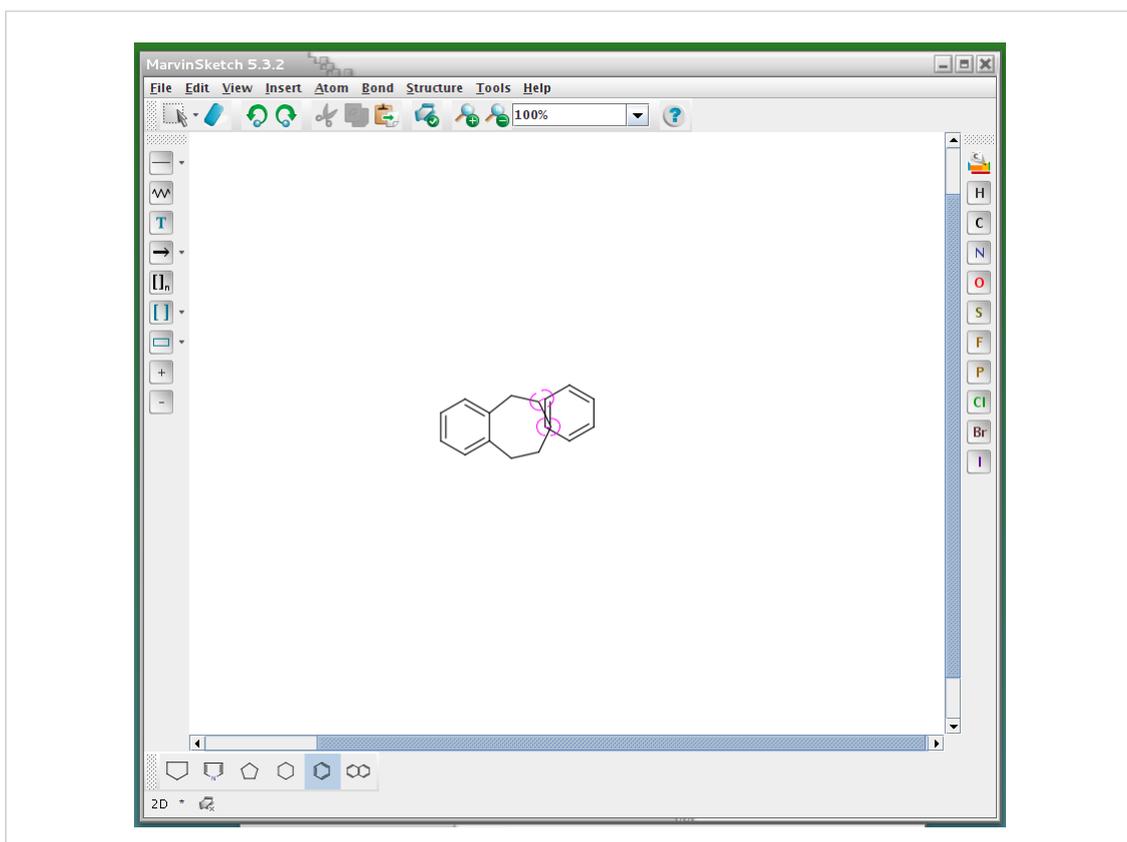
Scegliamo il ciclo a sette termini "Cycloheptane"



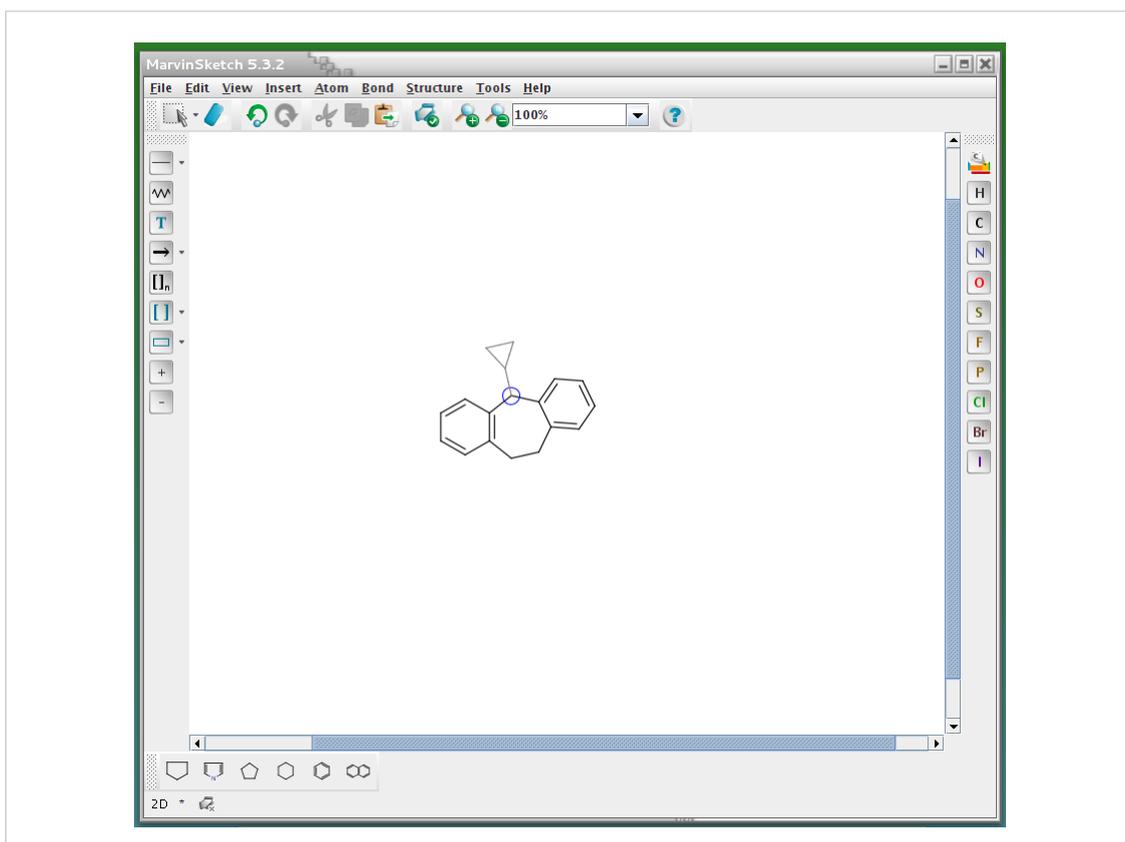
E nella finestra principale spostiamo il cursore fino a far comparire la formazione della fusione tra i due cicli. Quindi si clicca.



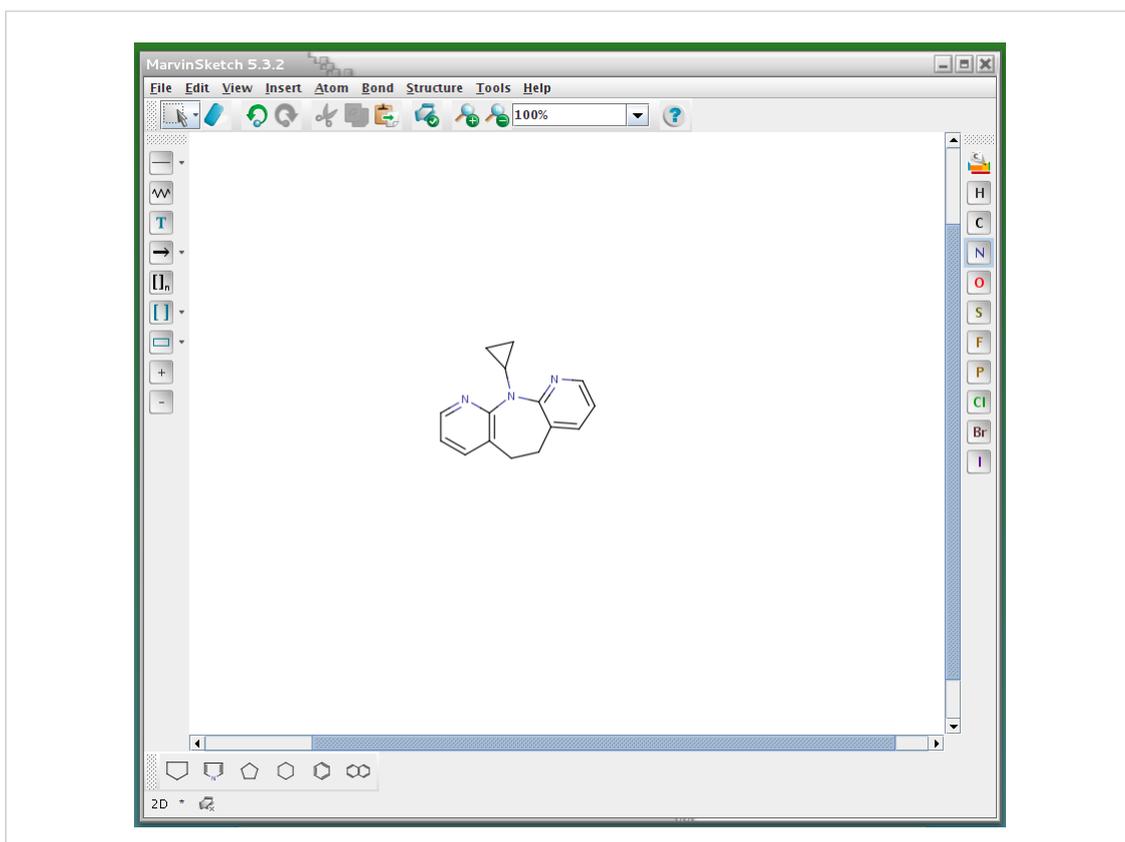
Si ottiene un diciclo



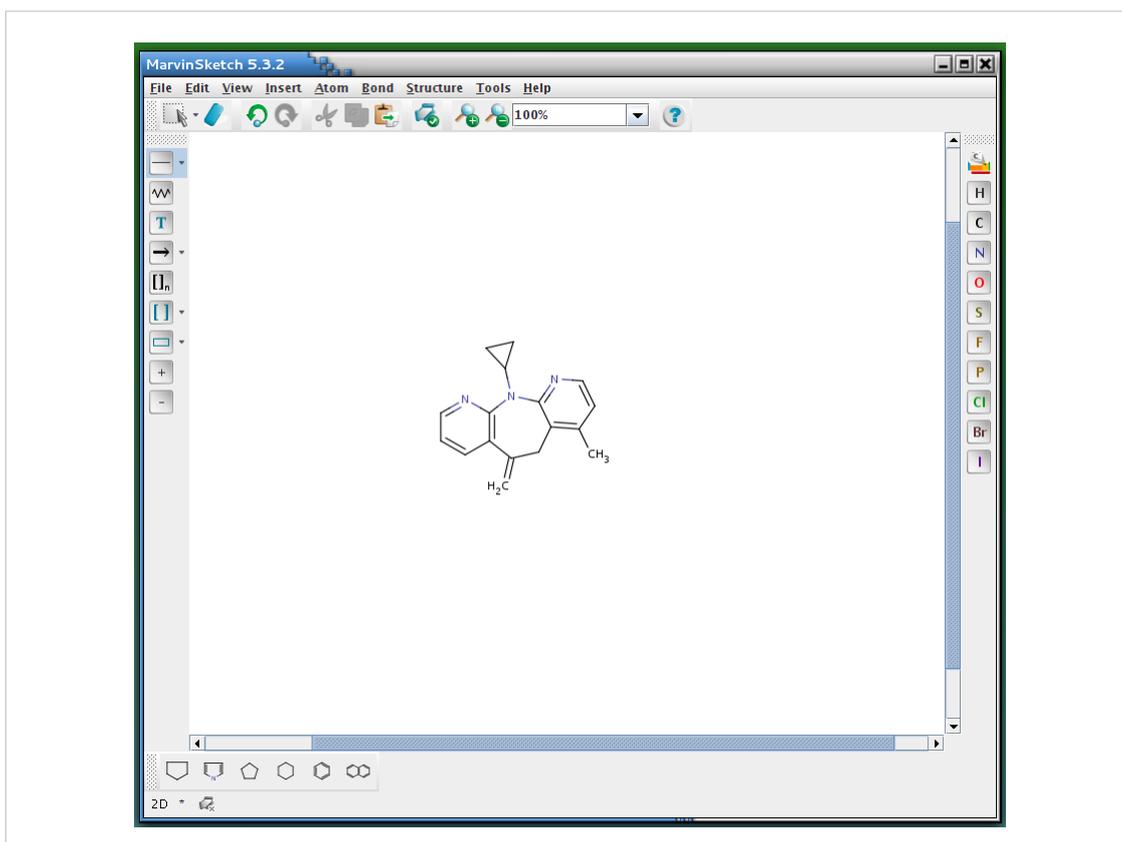
Poi si seleziona il benzene nuovamente e si fonde con il biciclo.



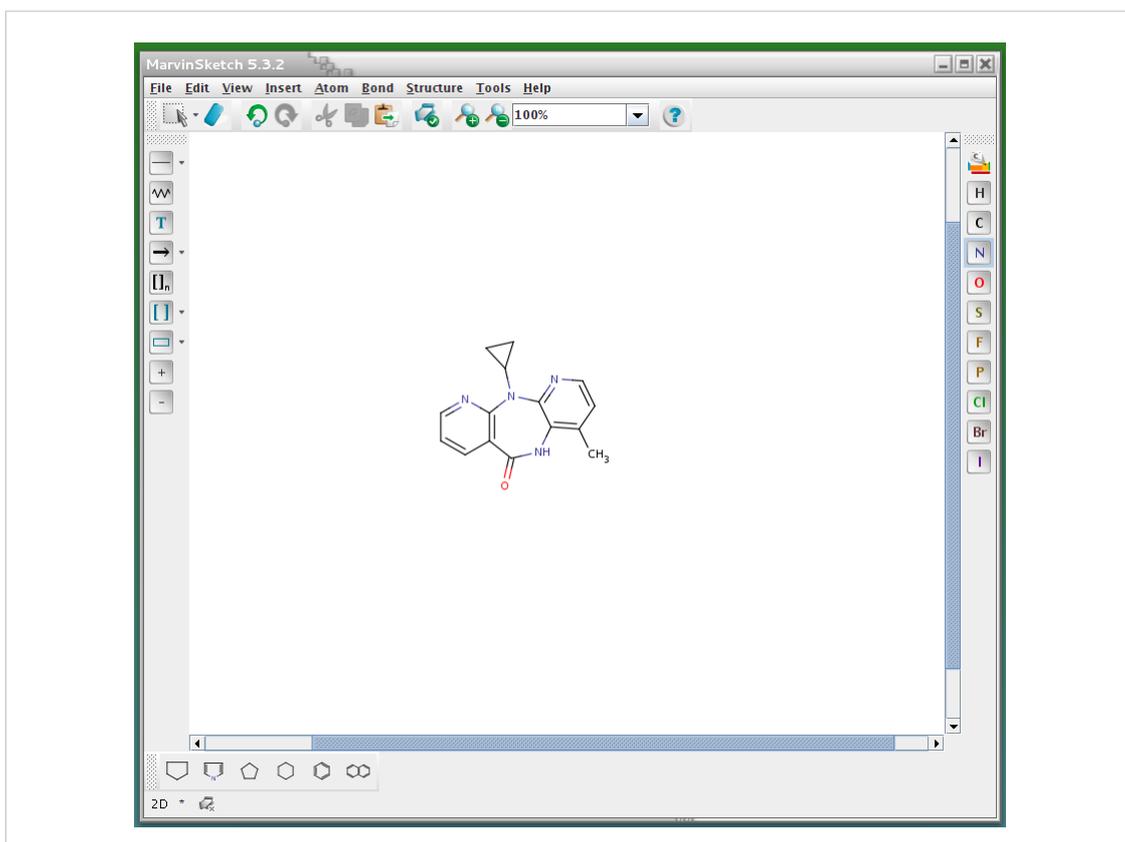
Dalla finestra della libreria dei “Rings” si seleziona il ciclopropano e si aggiunge al tricyclo precedentemente costruito



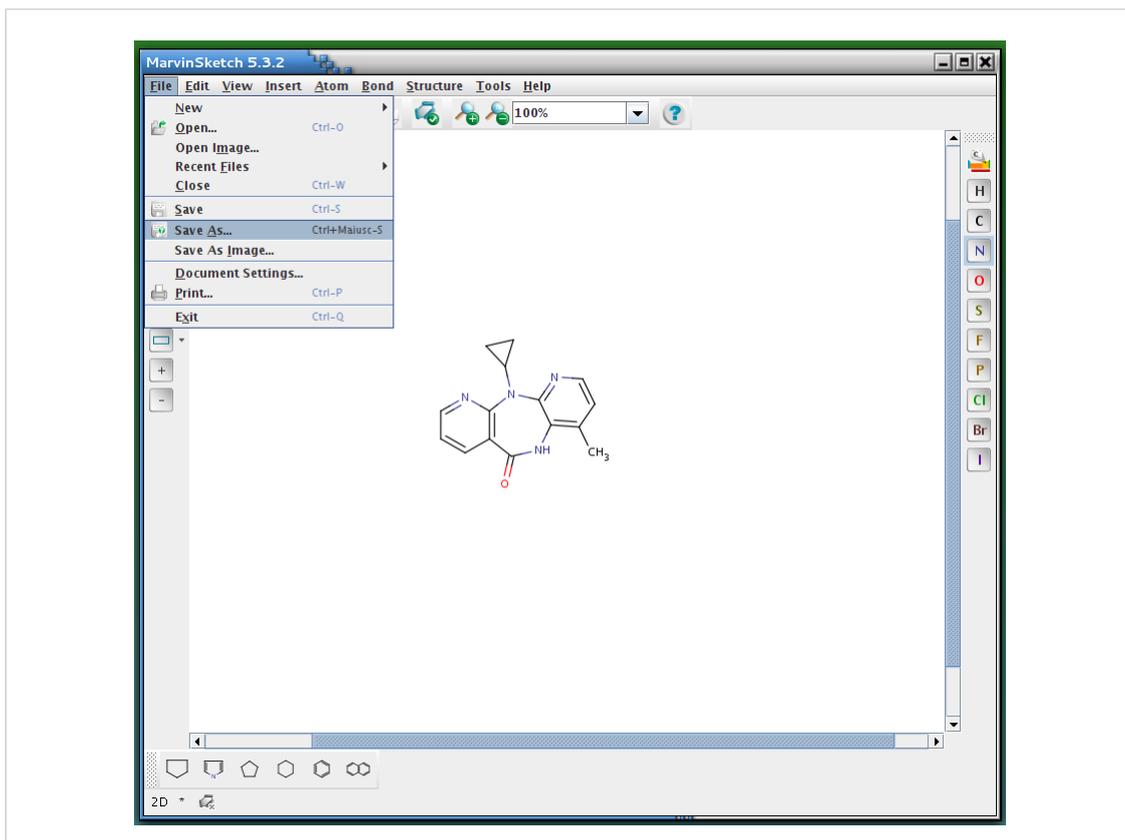
Utilizzando l'atomo di azoto "N" sulla barra di destra, semplicemente cliccando sulle posizioni degli atomi si convertono i carboni in azoti



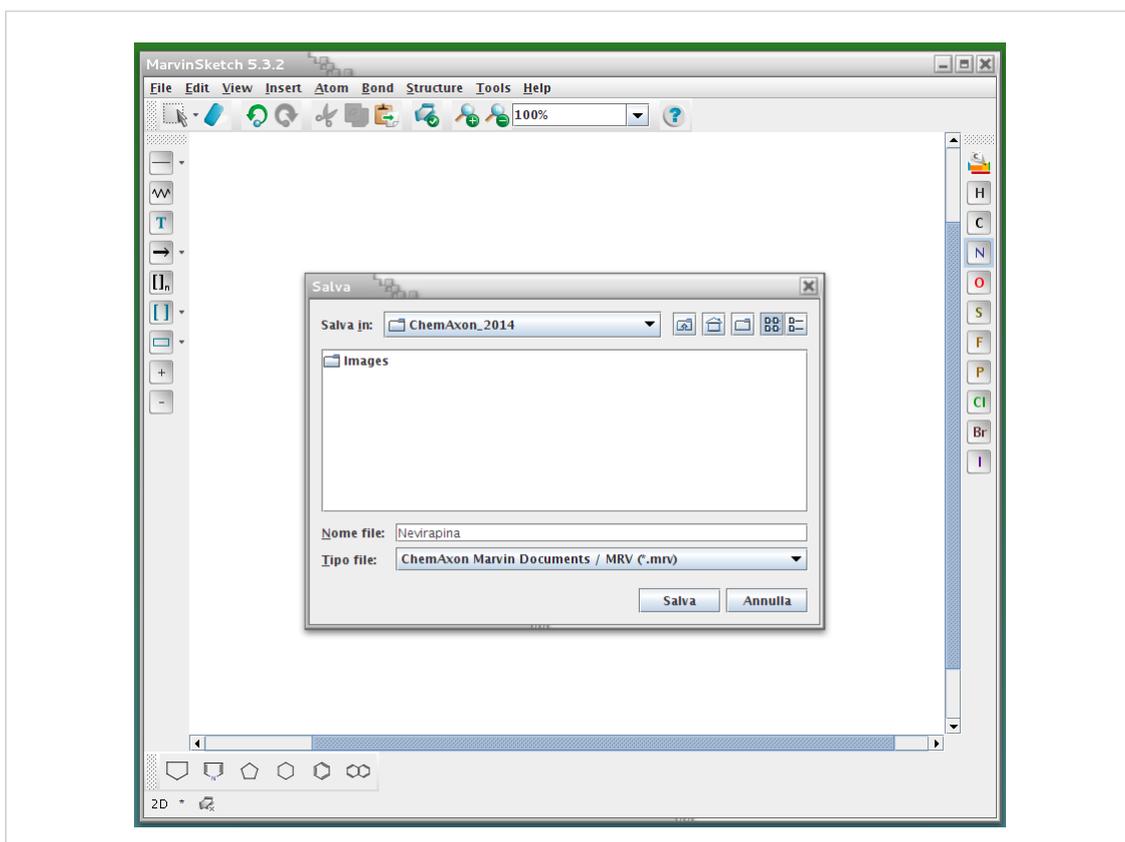
Con lo strumento “legame” (primo pulsante in alto sul menu' di sinistra) si costruiscono un metilene sul ciclo a sette e un metile sull'anello benzenico di destra come mostrato in figura.



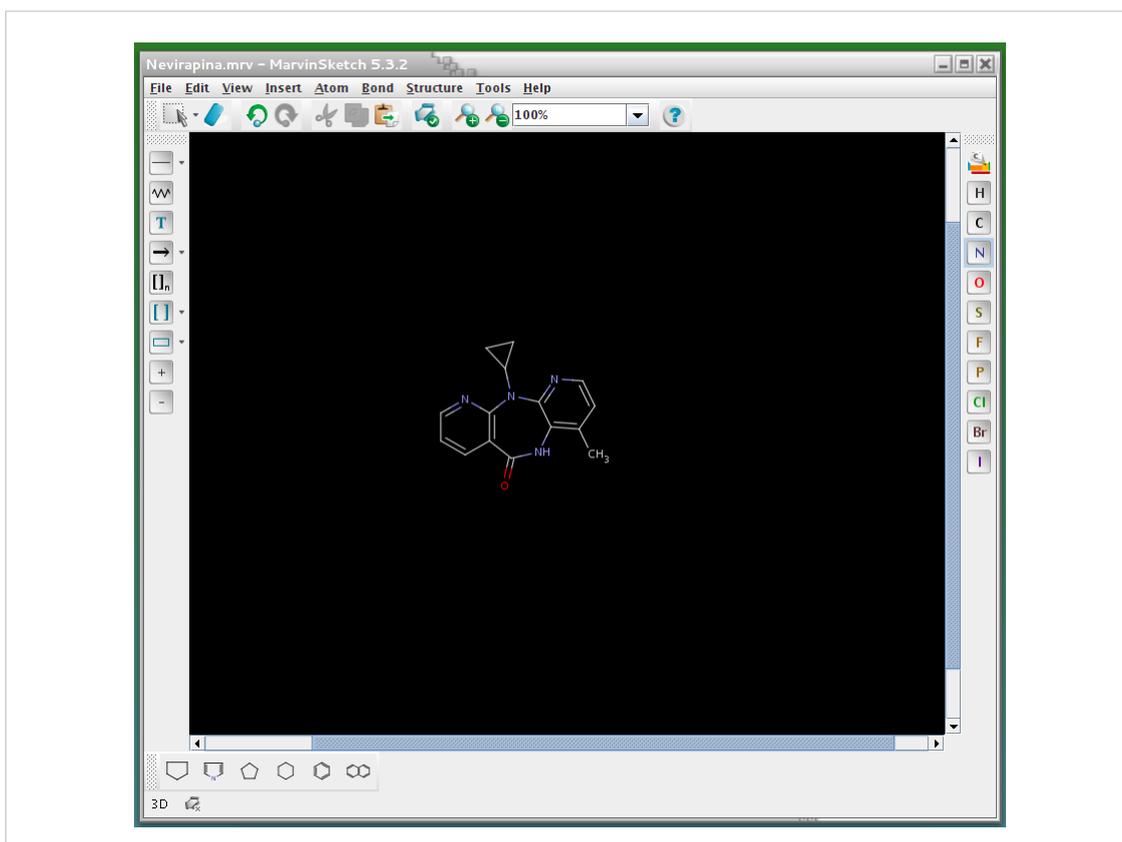
Quindi si completa la struttura con gli eteroatomi e otteniamo la NEVIRAPINA.



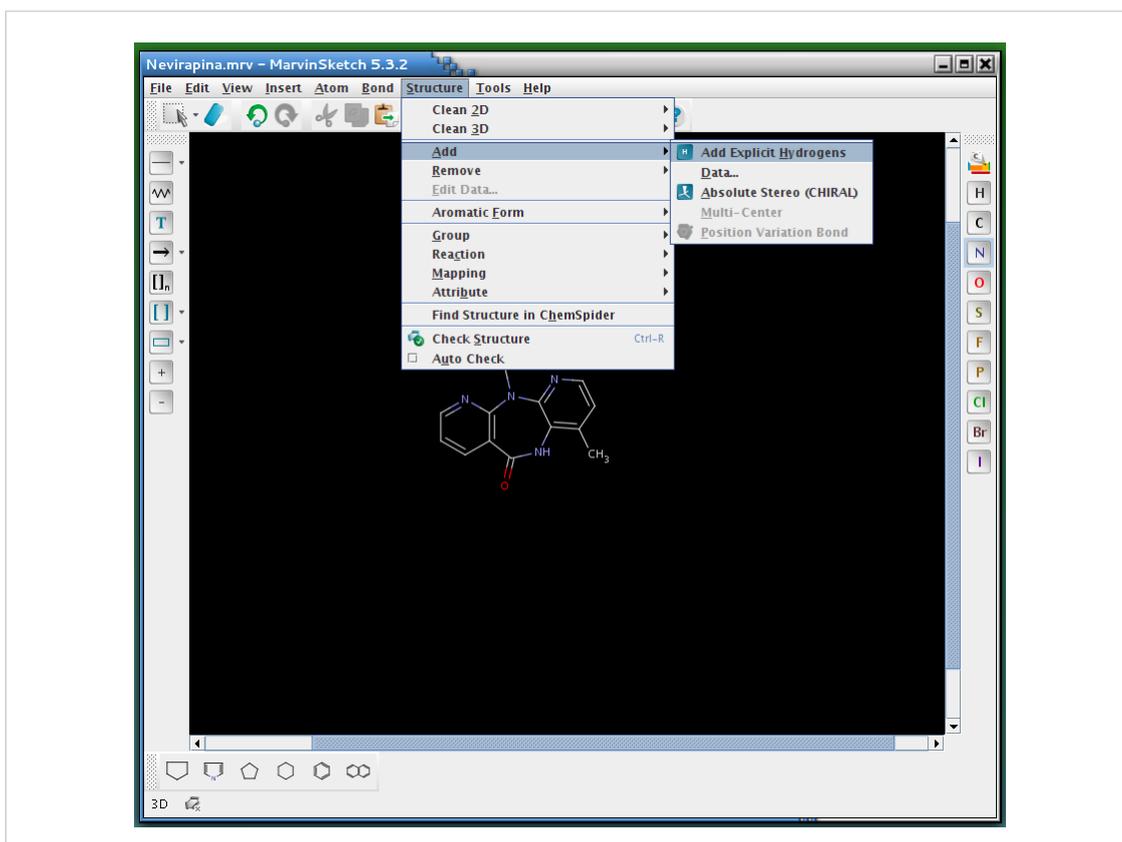
Salviamo il lavoro



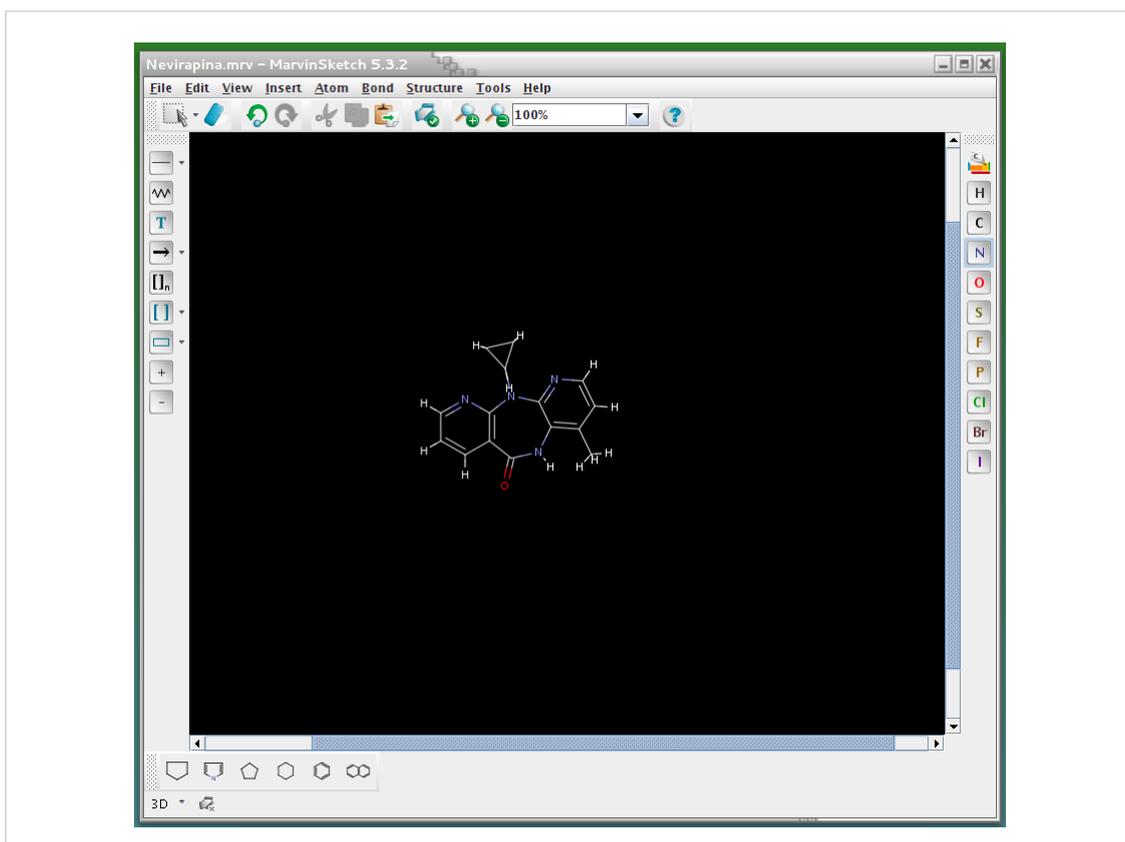
Per ora nel formato “ChemAxon”



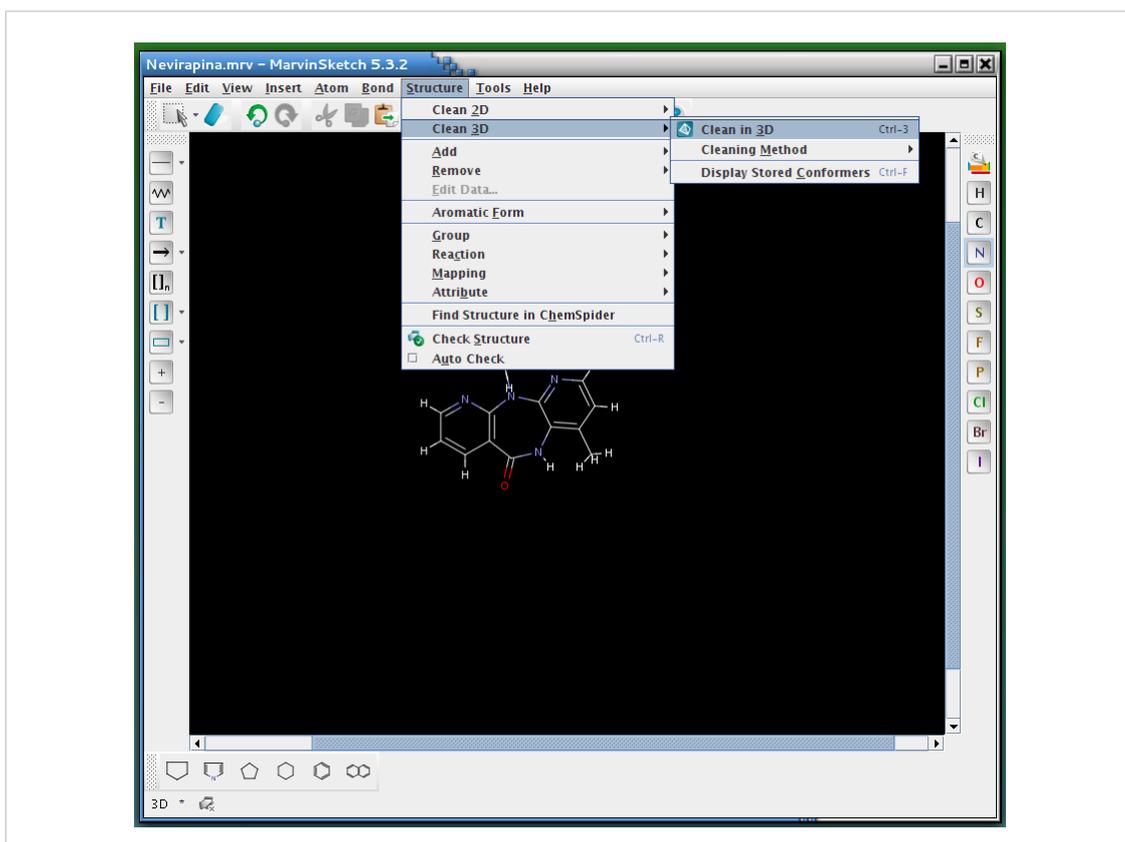
Quindi si “gira” nella modalita' “3D” cliccando sul bottone in basso a sinistra.



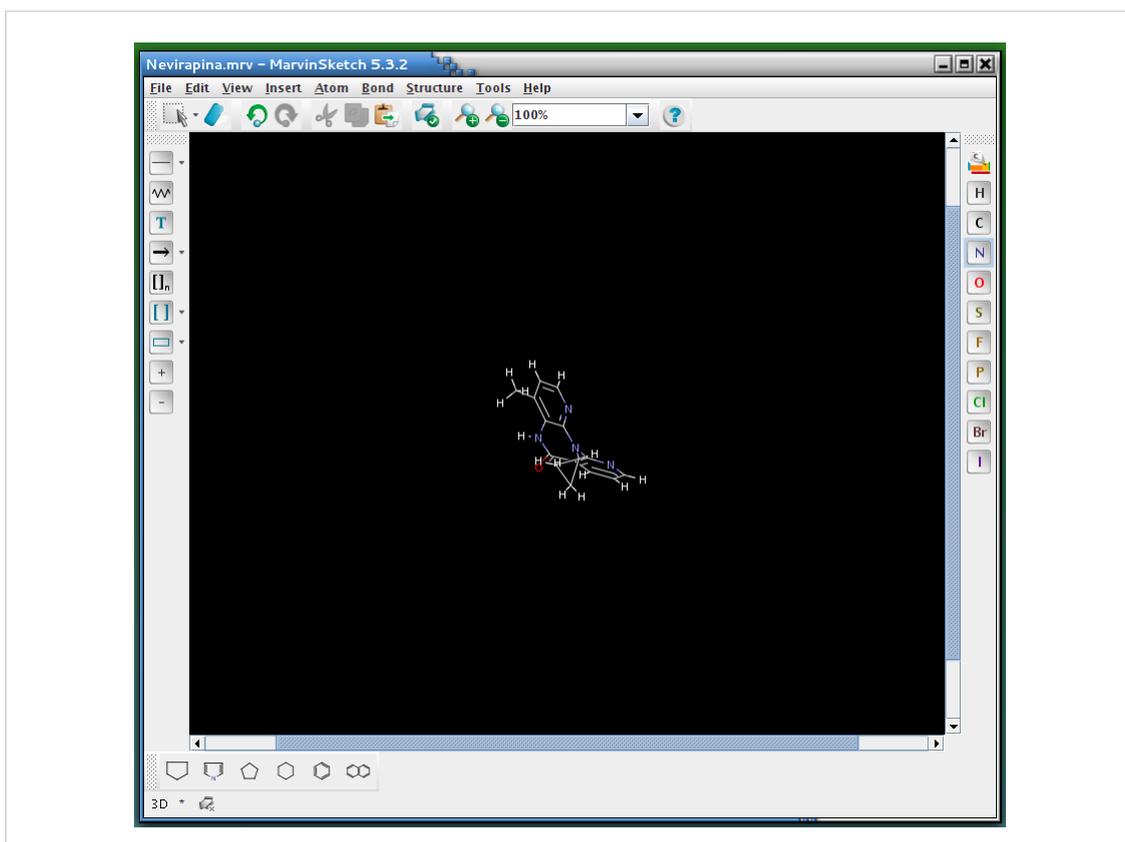
Mediante il menu 'Structure' si aggiungono gli idrogeni alla struttura



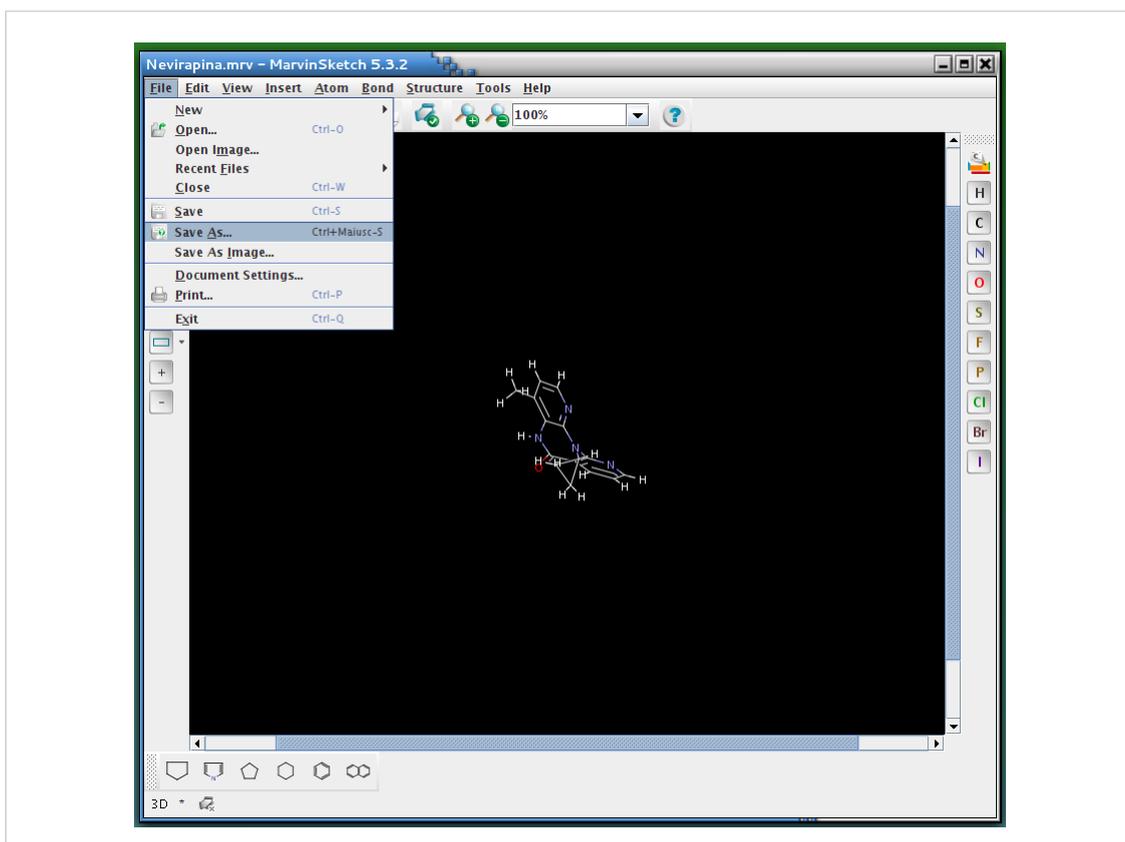
Si controlla che sia corretto



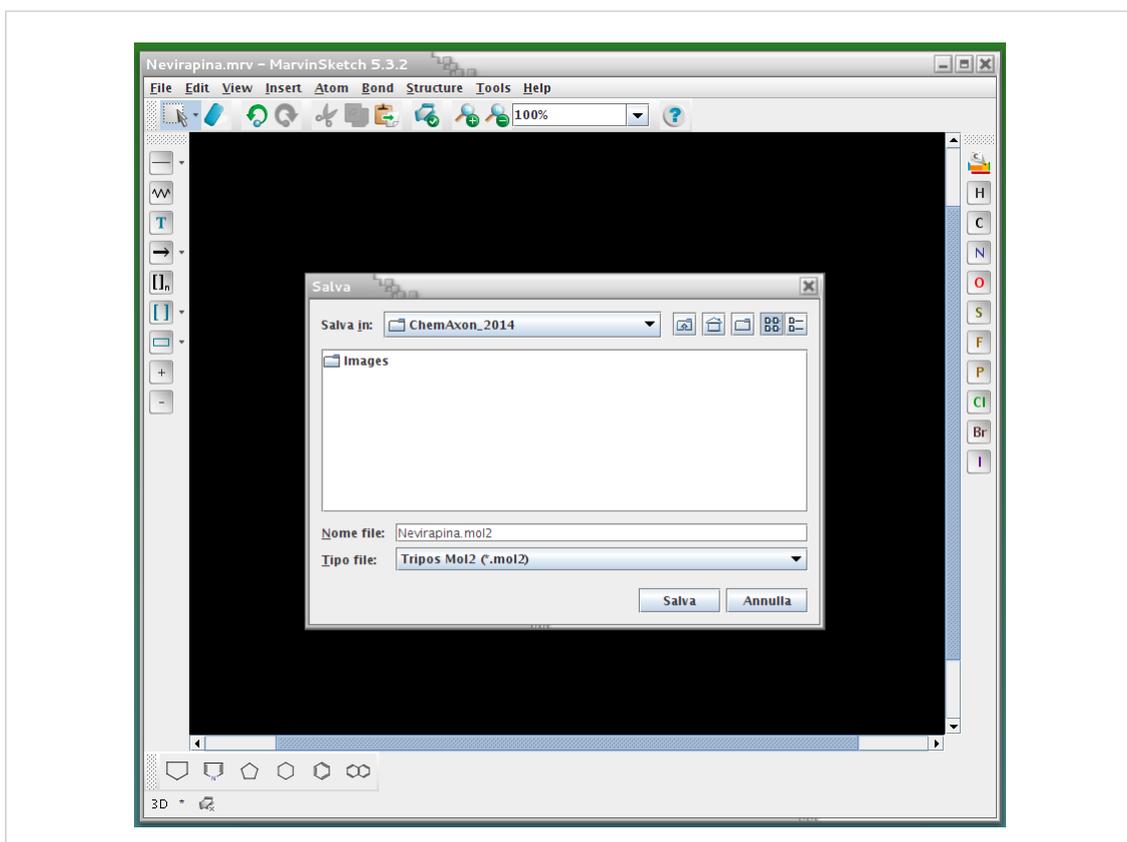
E quindi si chiede al programma di ottimizzare la struttura in 3D (Structure > Clean 3D > Clean in 3D)



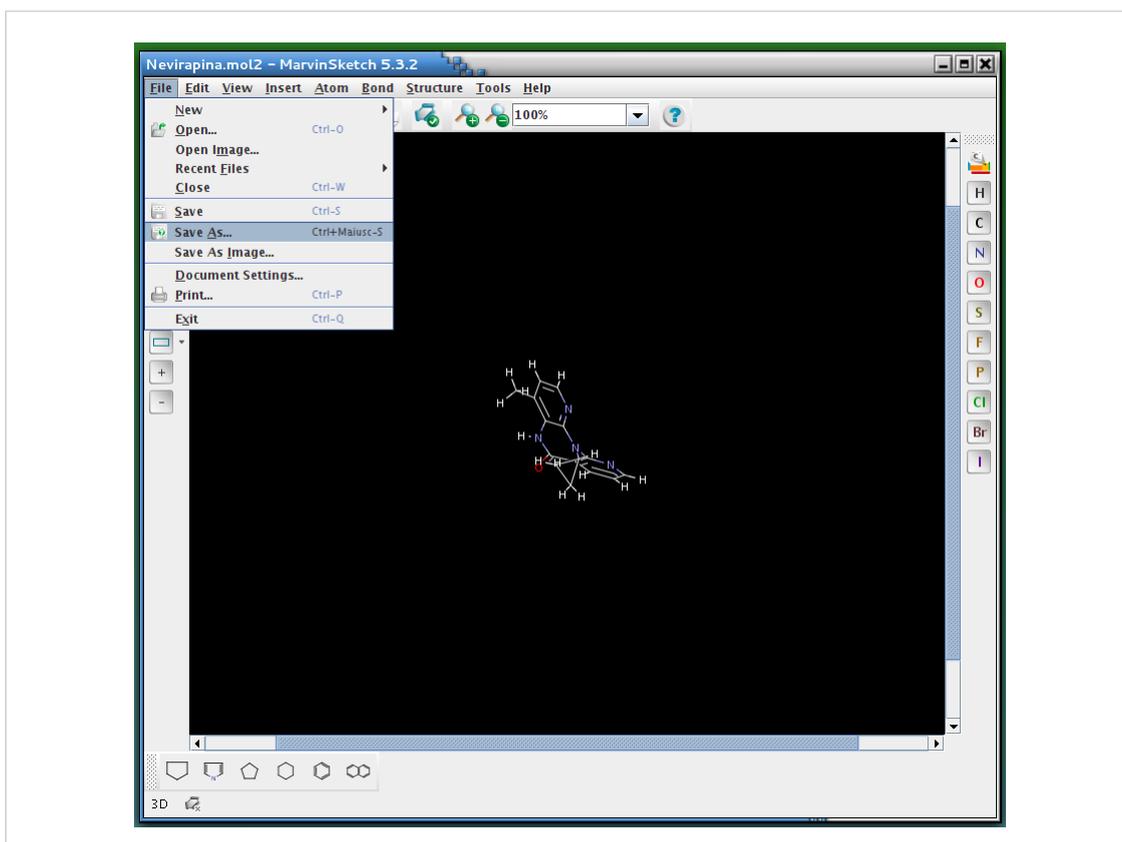
E si ottiene la NEVIRAPINA in 3D



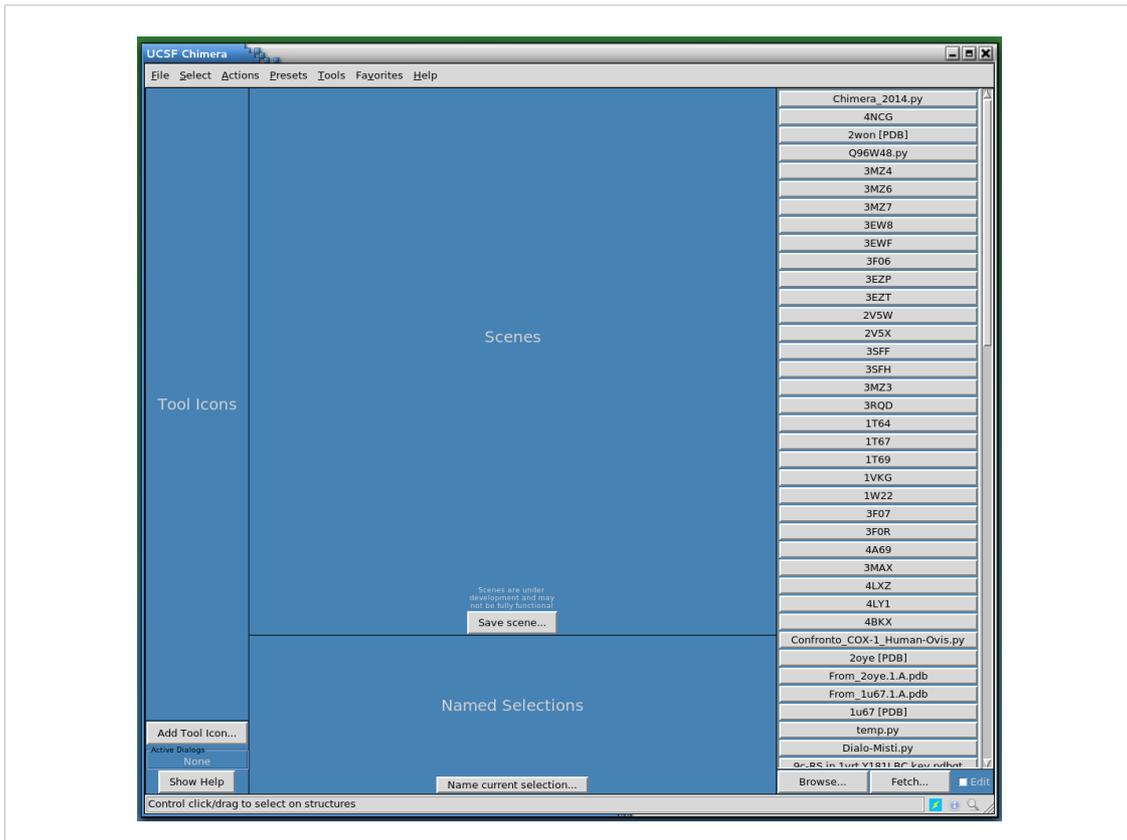
Si salva la struttura creata



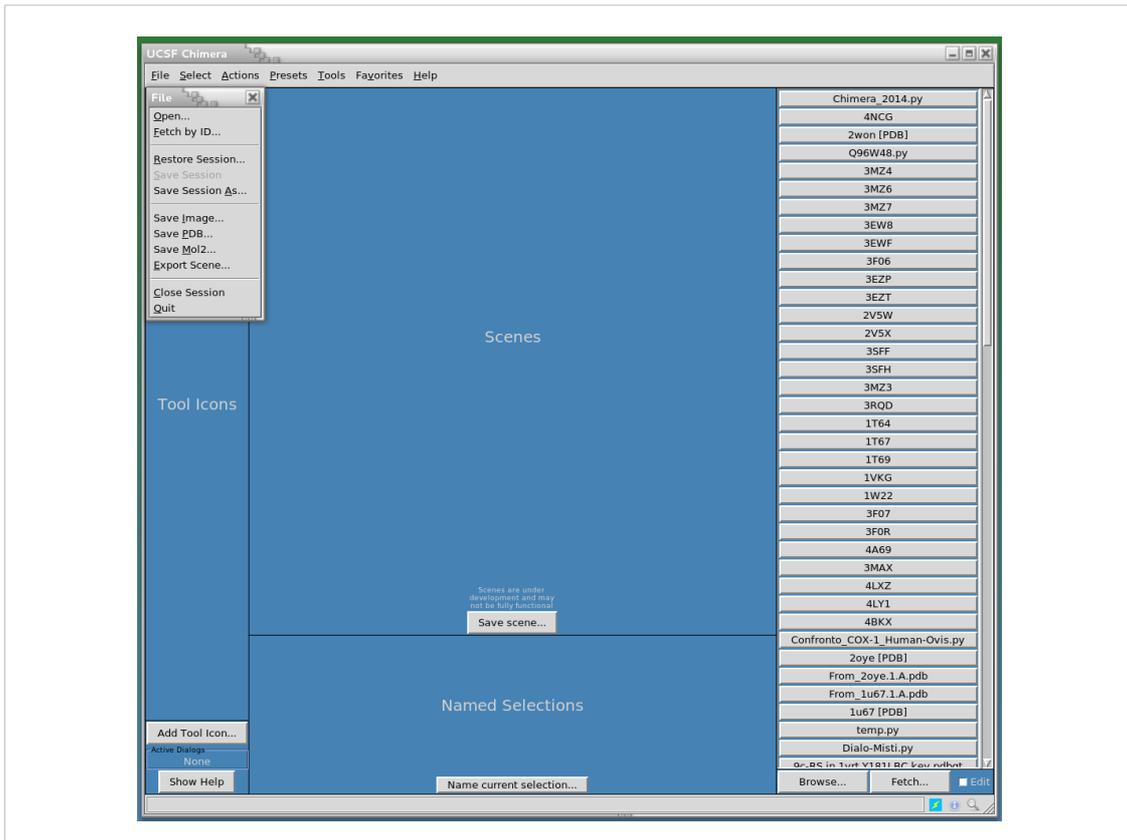
Questa volta in formato Tripos mol2



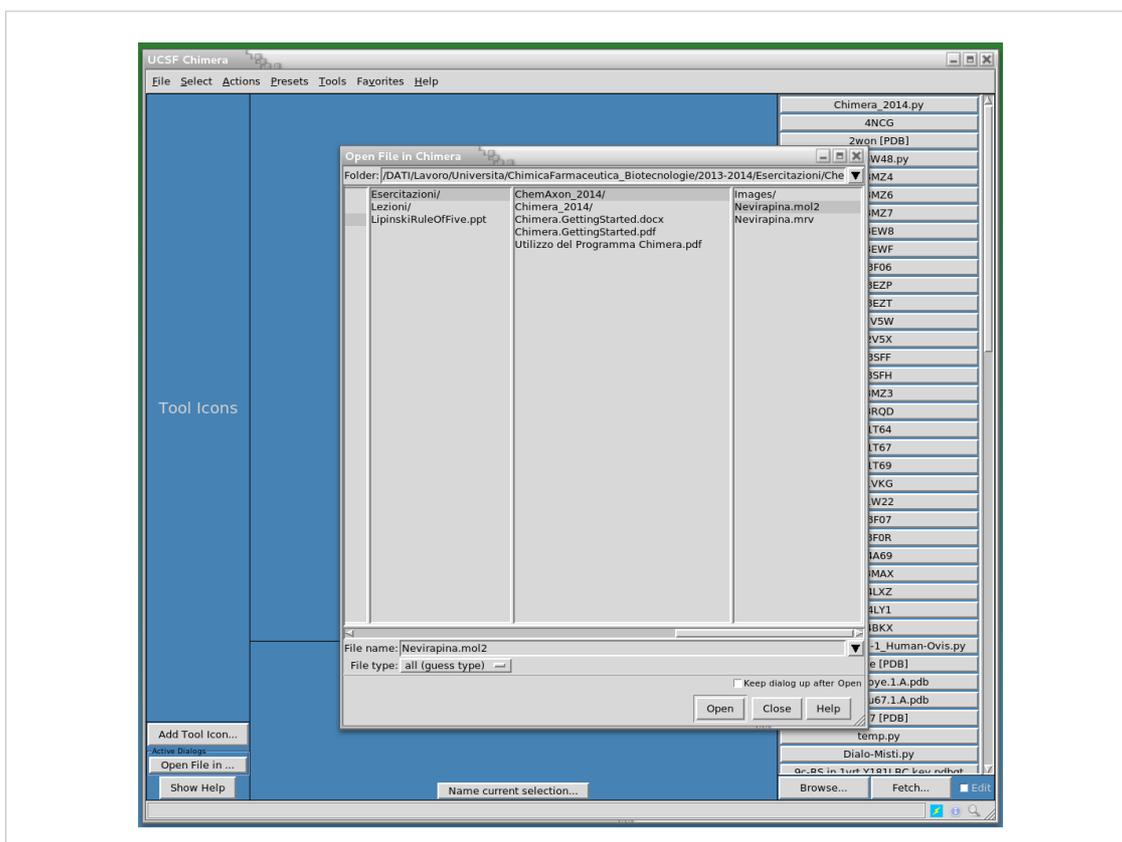
Si chiude il programma (File > Exit)



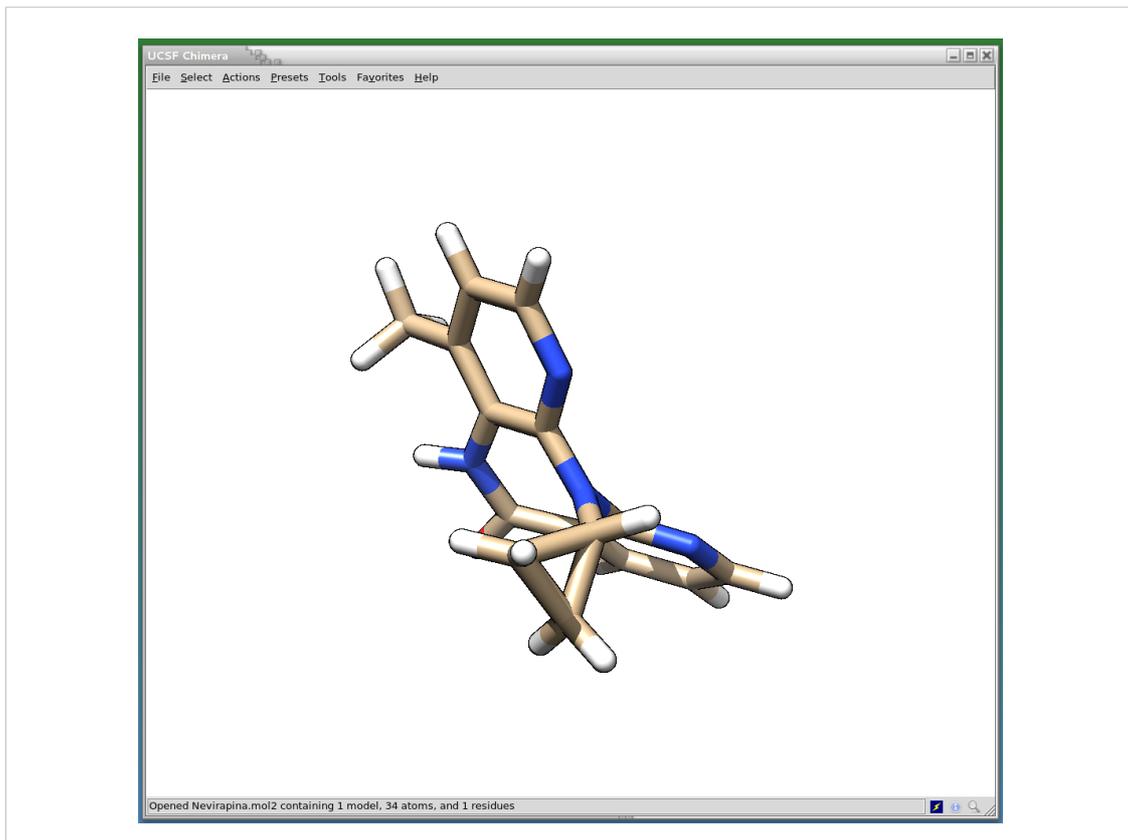
Si apre Chimera



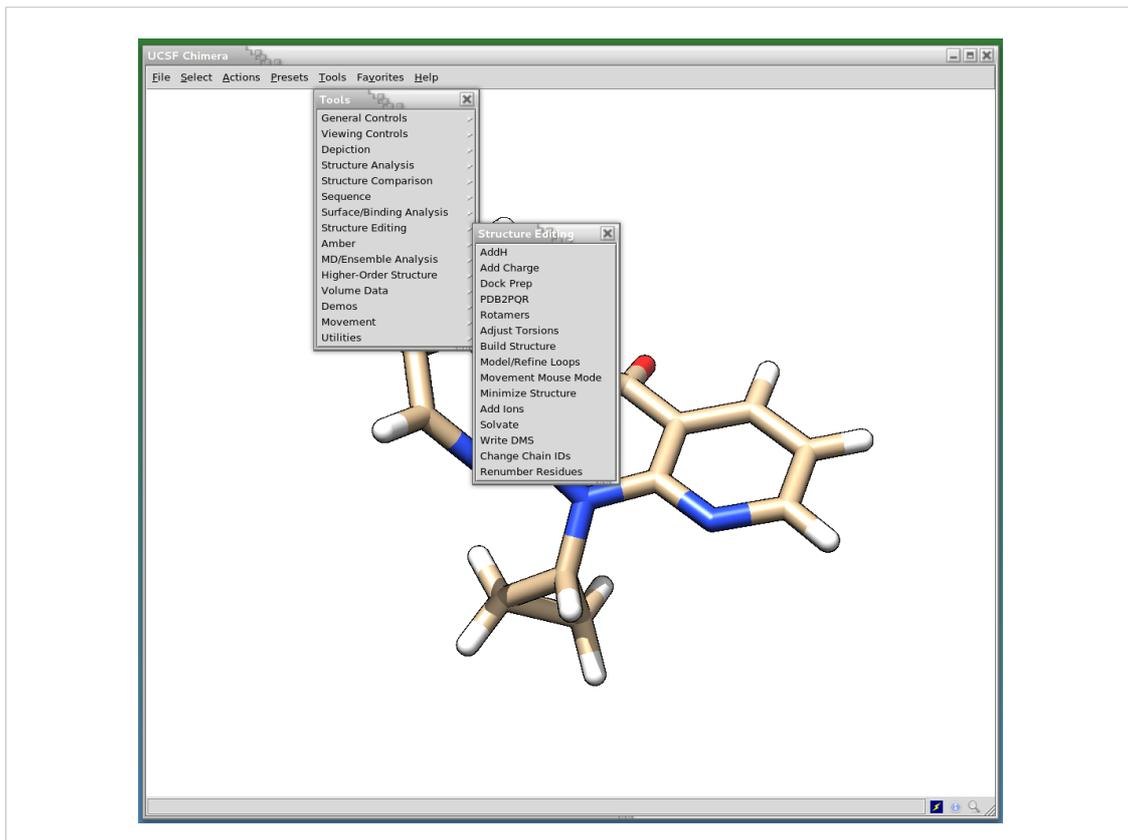
E tramite il menu “File” > “Open”



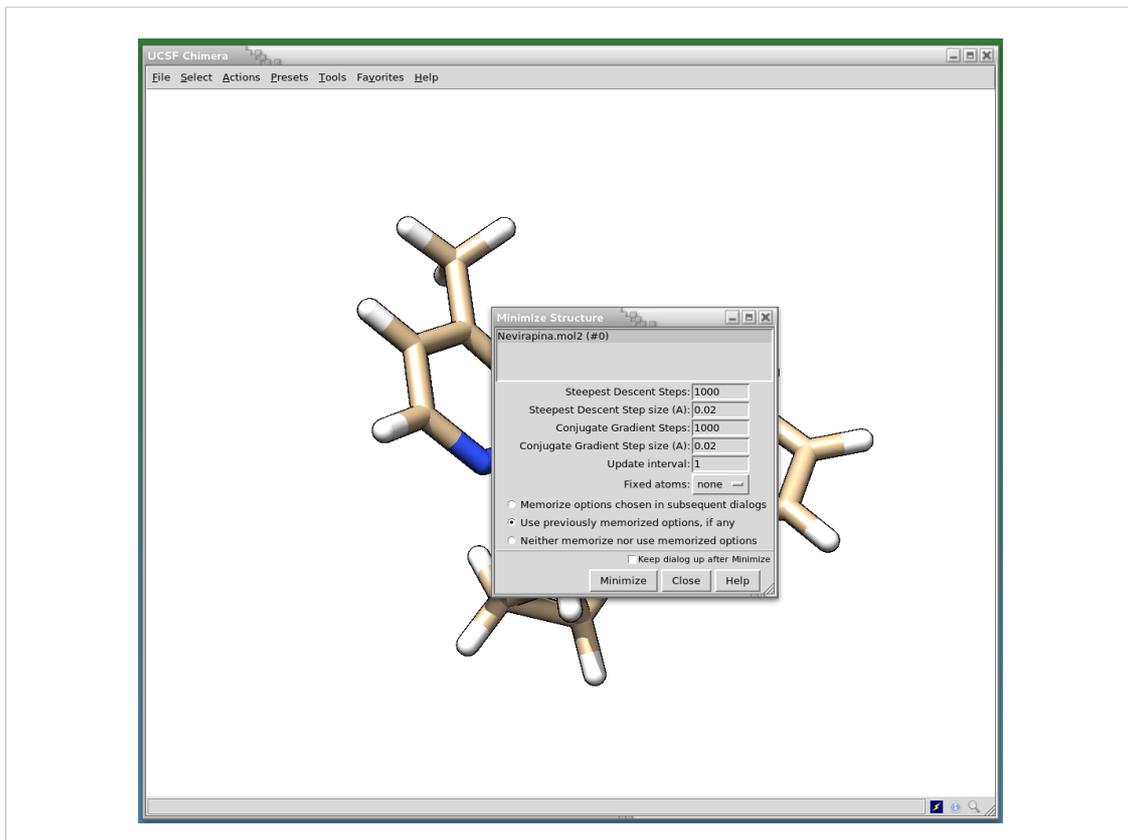
Nella finestra che appare si cerca il file mol2 appena creato con Marvin Sketch



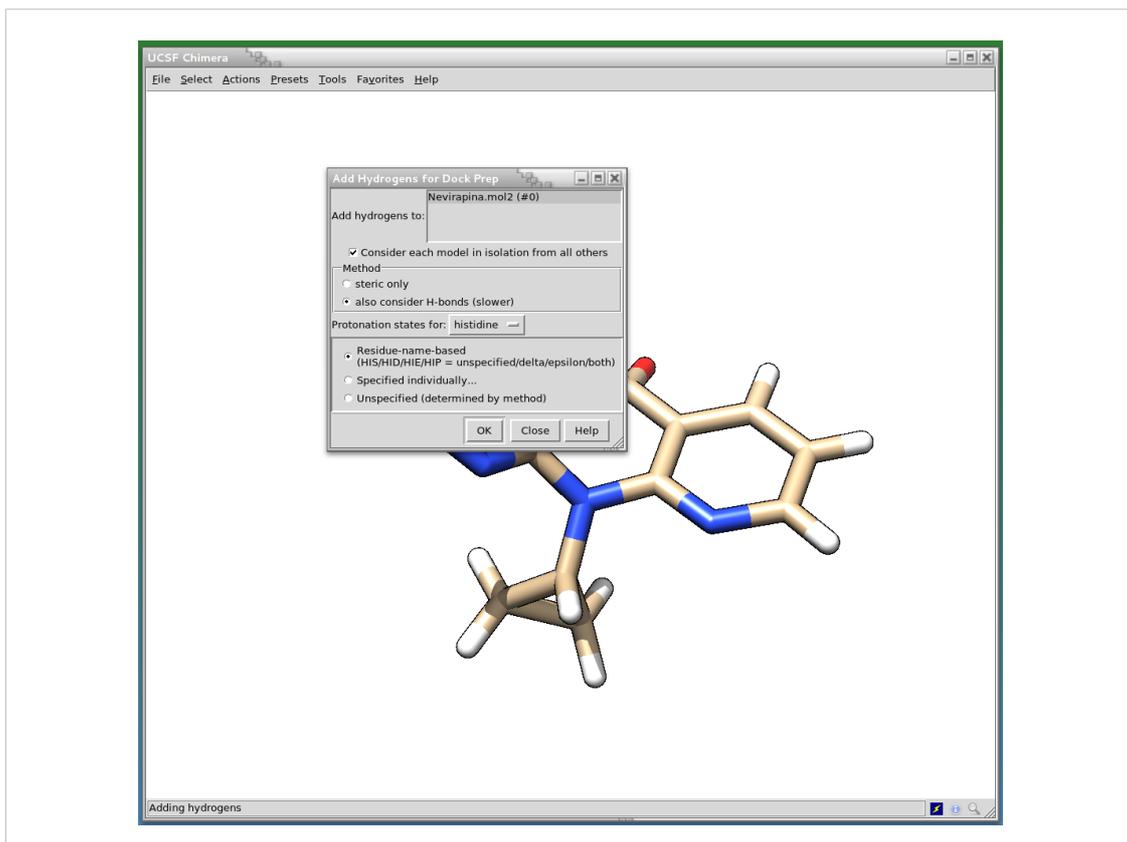
E abbiamo la NEVIRAPINA visibile anche in Chimera



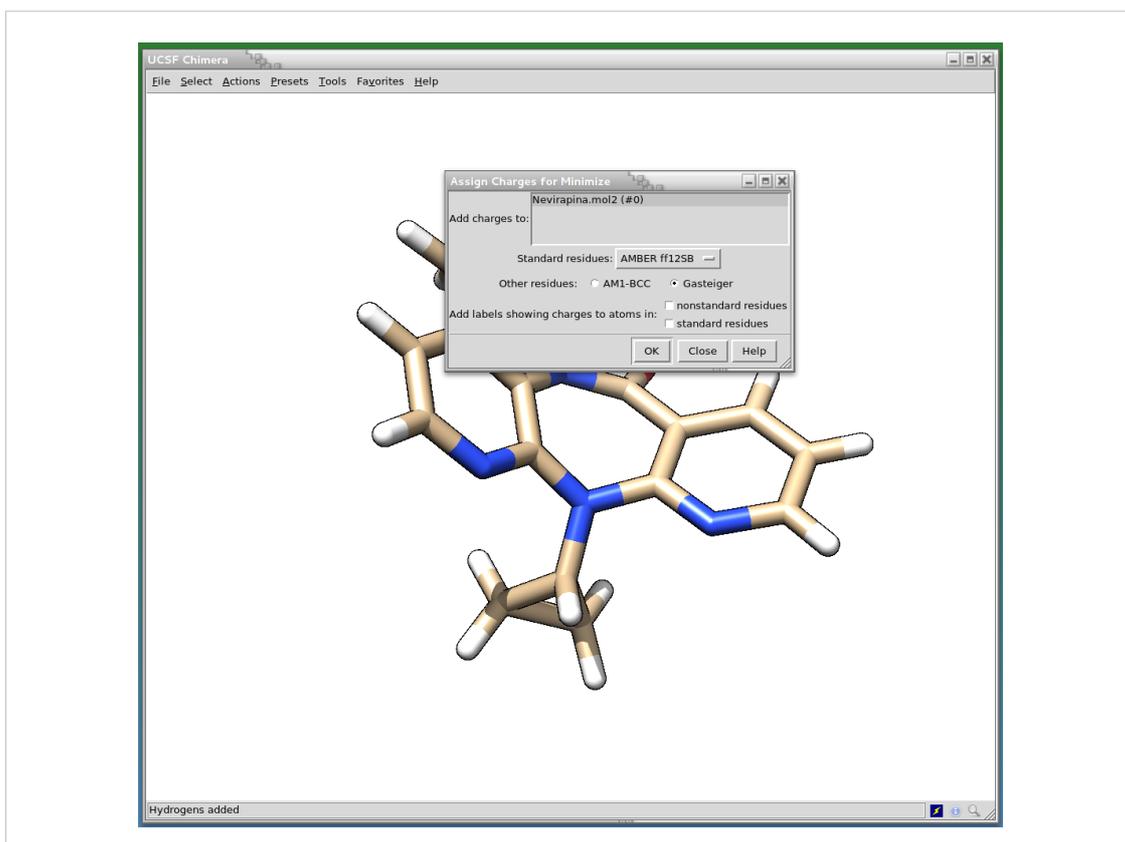
Possiamo ottimizzare la struttura con la sequenza:
“Tools” > “Minimize Structure”



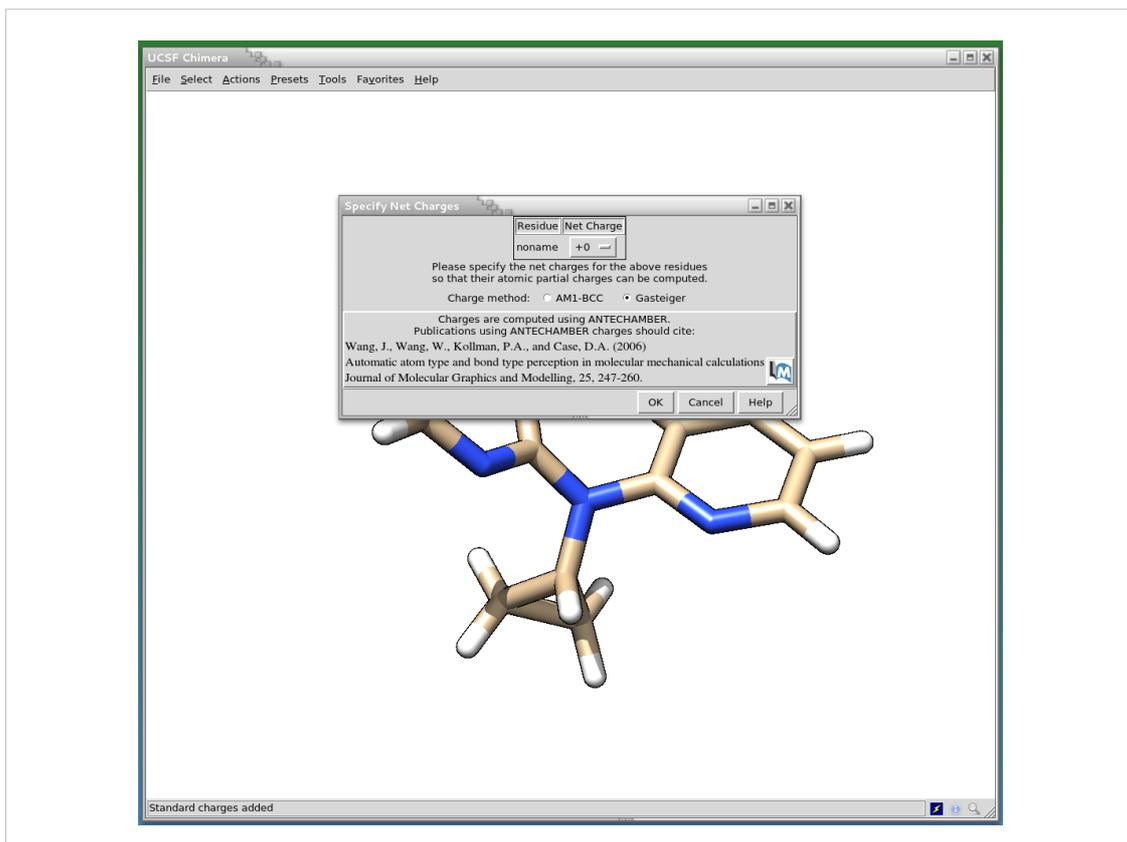
Nella finestra chge appare modifichiamo i numeri come mostrato



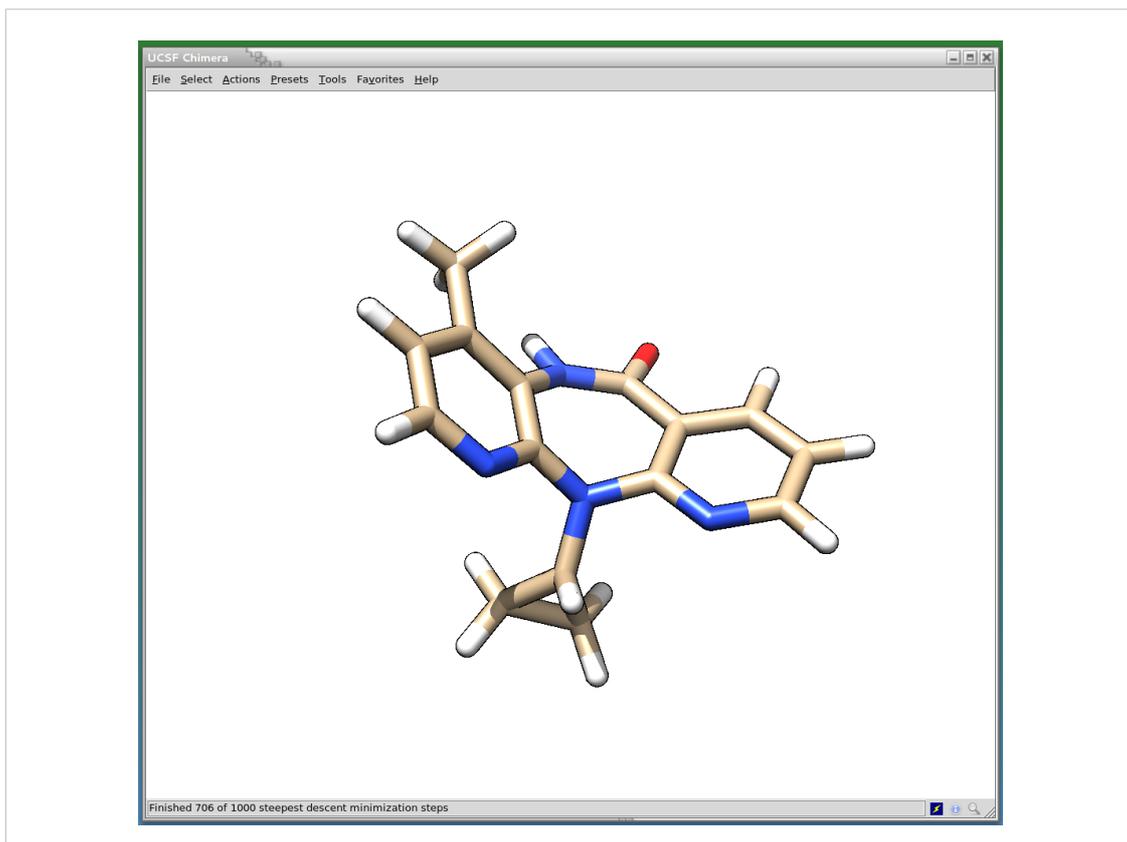
Si da OK alla seconda finestra



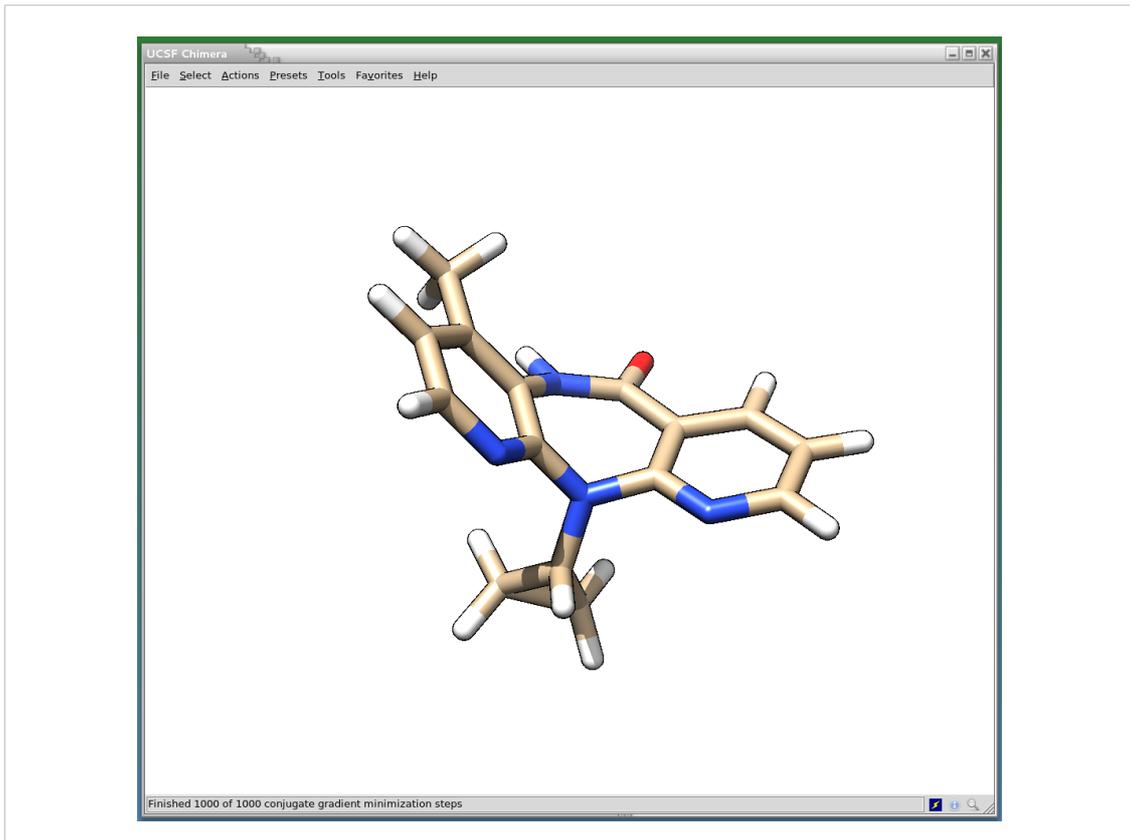
Anche alla terza



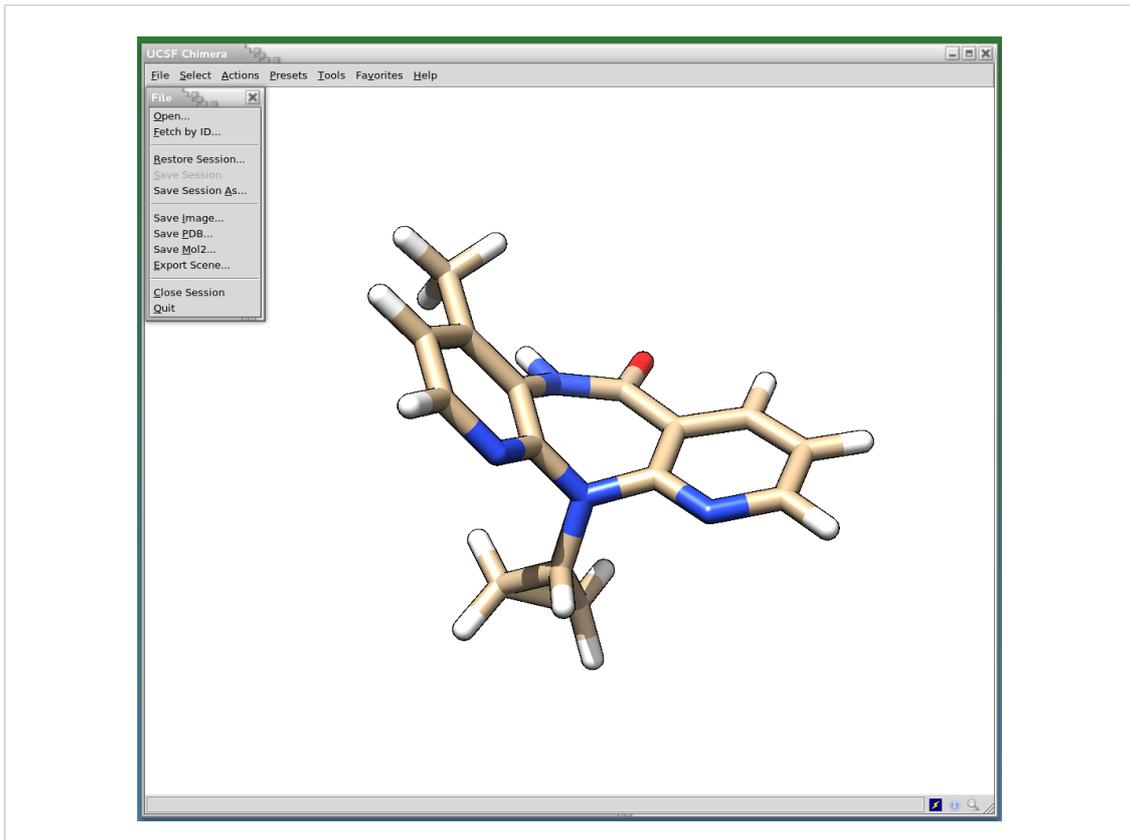
E alla quarta



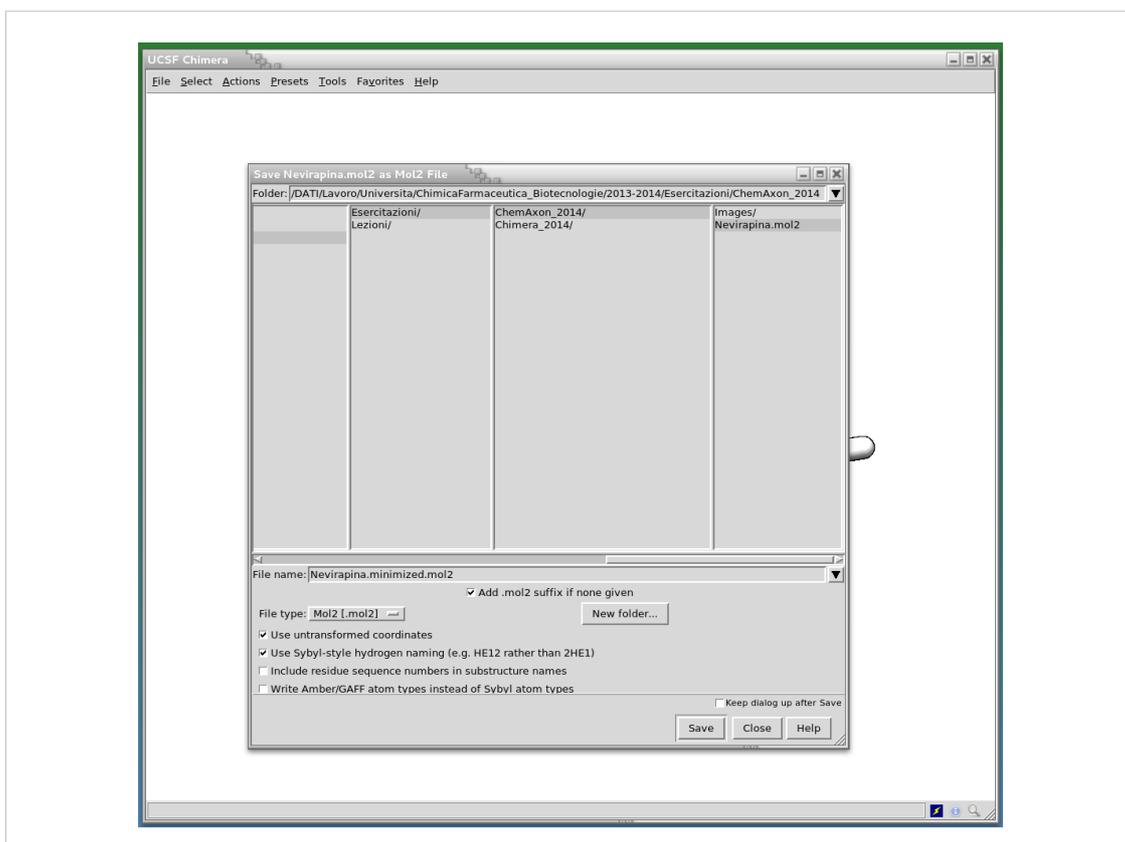
E parte l'ottimizzazione strutturale della
NEVIRAPINA



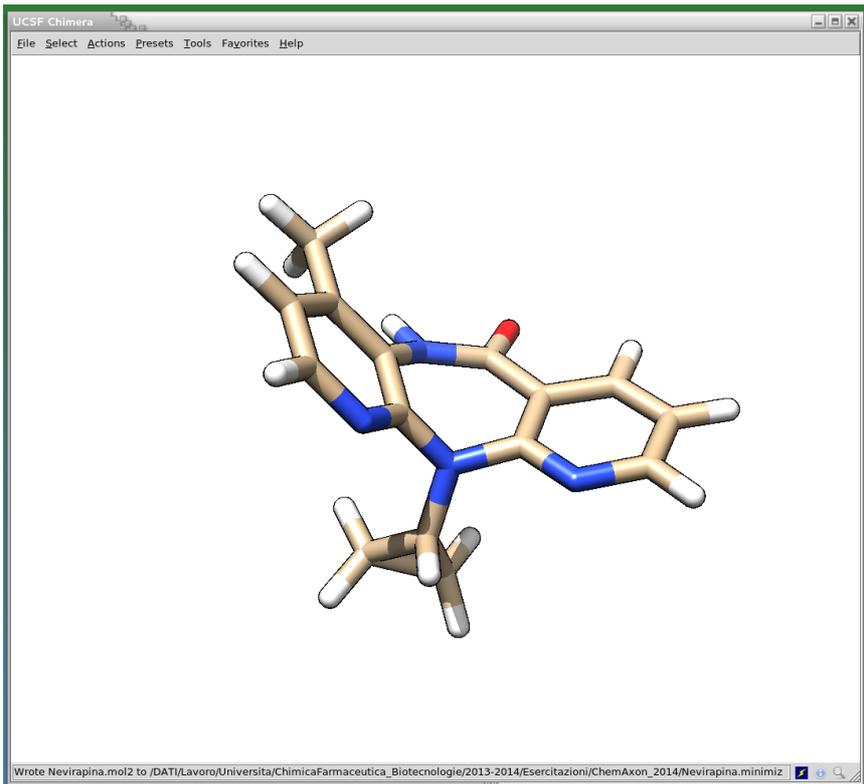
Fino a completare il calcolo come indicato precedentemente

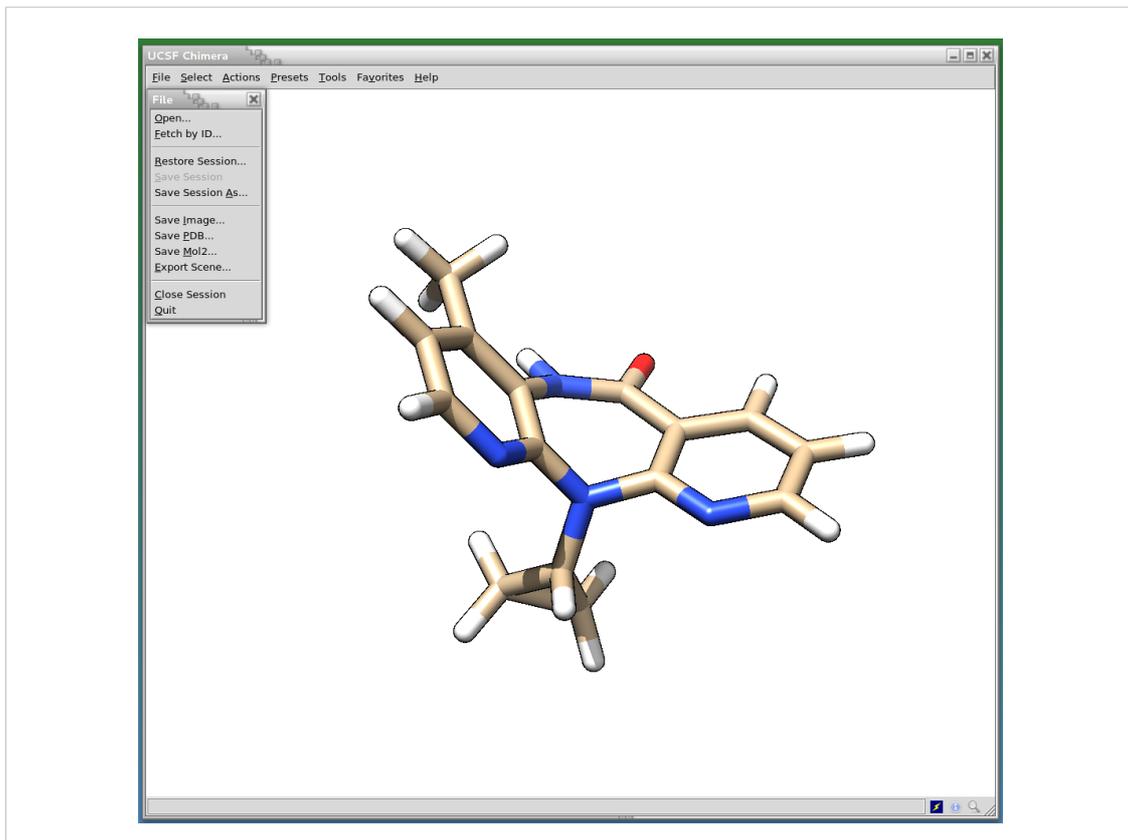


Si salva il lavoro



Indicando un nome diverso





Quindi si chiude il programma.

In questo modo si possono preparare una serie di molecole che possono essere studiate.