ISSN: 2321-676X

STAR
Research Journal

Available online at www.starresearchjournal.com (Star International Journal)

Bioinformatics

Star. Bio. Info. 01 (2013)

MOLECULAR DOCKING STUDIES ON INHIBITION OF HUMAN KYNURENINE AMINOTRANSFERASE-1 FOR TREATING SCHIZOPHRENIA DISORDER

T. Dhinesh Kumar[1],Dr. D. Velmurugan[2].
[1]M.Sc Student, Department of Biotechnology, University of Madras, Chennai-600 025, Tamilnadu, India.
[2]Professor, Coordinator, Bioinformatics Infrastructure Facility (DBT), University Of Madras, Chennai-600 025, Tamilnadu, India.

ARTICLE INFO

Article history:
Received: 7 June 2013;
Received in revised form:
11 June 2013;
Accepted: 13 June 2013;

Keywords
Schizophrenia,
Neuropsychiatric
Disorder

ABSTRACT
Schizophrenia is a complex neuropsychiatric disorder which is caused by a combination of numerous genetic and environmental factors leading to neurochemical disturbances. A japp-klingmann condensation of the diazonium salts with the enolate anion of ethyl 4-oxocyclo pentane carboxylate subsequent hydrolysis affords the phenylhydrazonohexanoic acid derivatives in moderate to good yields. The Induced Fit Docking process was performed using the software called GLIDE and the compound with the best docking score, Glide energy and interactions were identified. Among these compounds, (5Z)-6-ethoxy-5-[2-(4-nitrophenyl) hydrazinylidene]-6-oxohexanoic acid (A3) appeared to the most potent. These results have important implications in optimizing the metabolic stability of Human Kynurenine aminotransferase to improve therapeutic value.

1. INTRODUCTION:

Schizophrenia, also sometimes colloquially called split personality disorder, is a chronic, severe, debilitating mental illness that affects about 1% of the population, corresponding to more than million people in the United States alone. Schizophrenia is a complex neuropsychiatric disorder which is caused by a combination of numerous genetic and environmental factors leading to neurochemical disturbances (Procopio, M. Br. J. Psychiatry 2001). For epochs, the familial-genetic relationship between schizophrenia and other disorders has been disputed (Gershon et al., 1988; Taylor, 1992; Maier et al., 1993).Additional statistics about schizophrenia include that it affects men about one and half times more commonly than women. A recent review of family studies exploring the familial relationship between schizophrenia and other disorders demonstrated that even the studies most frequently cited in favor of a nosological dichotomy also revealed an excess of affective disorders in families of probands with schizophrenia (Kendler and Gardner, 1997);the excess rate (odds ratio) ranged between1.6 and 2.2 either for bipolar disorder or for unipolar depression among 1st degree relatives of probands with schizophrenia compared to control families. It is one of the mental disorders and is characterized by symptoms of thought, behavior, and social problems. The problems associated with schizophrenia are described as psychosis, in that the person's thinking is completely out of touch with reality at times. For example, the sufferer may hear voices or see people that are in no way present or feel like bugs are crawling on their skin when there is none. The human with this disorder may also have disorganized speech, disorganized behavior, physically rigid or lax behavior (catatonia), significantly decreased behaviors or feelings, as well as delusions, which are ideas about themselves or others that have no basis in reality (for example, the individual might experience paranoia, in that he or she thinks others are plotting against them when they are not). The term schizophrenia has only been in use since 1911. Shortly before that, it was deemed a separate mental illness in 1887 by Emil Kraepelin. Despite that relatively recent history, it has been described throughout written history. Ancient Egyptian, Hindu, Chinese, Greek, and Roman writings described symptoms similar to the positive symptoms of schizophrenia. During medieval times, schizophrenia, like other illnesses, was often viewed as evidence of the sufferer being possessed by spirits or evil powers. A number of accomplished individuals suffer from schizophrenia. The film A Beautiful Mind depicts the life of John Nash, a noted scientist, and his struggles with paranoid schizophrenia. The film The Soloist explores the challenges faced by Juilliard-trained musician Nathaniel Ayers as a result of schizophrenia. There are five types of schizophrenia, each based on the kind of symptoms the person has at the time of assessment (i.e.) paranoid schizophrenia, disorganized schizophrenia, catatonic schizophrenia, undifferentiated schizophrenia, residual schizophrenia. Conversely, more number of patients with schizophrenia have symptoms of depression or mania. Indeed,

depression is common among patients presenting with their first schizophrenic episode, being reported in up to 75% of cases (Koreen et al., 1993; Hafner et al., 1999). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM), symptoms of schizophrenia is beliefs that have no basis in reality (delusions), Hearing, seeing, feeling, smelling, or tasting things that have no basis in reality (hallucinations), Disorganized speech, Disorganized behaviors, Catatonic behaviors. Besides, schizophrenia and mania share a number of characteristics such as their typical onset in young adults, slightly earlier in males (Frangou et al., 2002; Kennedy et al., 2004), and the frequent occurrence of life events prior to the onset, or relapse, of illness (Ventura et al., 1989; Bebbington et al., 1993).Human Kynurenine aminotransferase I (Hkat I) possesses broad amino acid specificity as an aminotransferase. Abnormal concentrations of Kynurenine observed in patients with multiple neurodegenerative diseases and syndrome, include Huntington's disease, Alzheimer's disease, schizophrenia, and acquired immunodeficiency syndrome dementia. These data suggest that Kynurenine, acting as an endogenous modulator of glutamatergic and cholinergic neurotransmission, may be functionally significant. In addition to its role as an excitatory amino acid antagonist, Kynurenine is also involved in the control of cardiovascular function by acting at the rostral ventrolateral medulla of the central nervous system. Glutamine transaminase K, which is a freely reversible glutamine aromatic amino acid aminotransferases, is present in most mammalian tissues, including brain. Quantitatively, the most important donor in vivo is glutamine. The Human Kynurenine aminotransferase length is 422 amino acid. It's otherwise called as Kynureine-oxoglutarate transaminase 1, Glutamine Transaminase k, Kynurenine Aminotransferase 1, KAT1 or KAT. The Enzyme Commission Number of Human Kynurenine Aminotransferase is EC: 2.6.1.7. The crystal structure of Hkat-I revealed was in complex with L-phenylalanine (PDB ID: 1W7L) (Rossi, F.; Han, Q.; Li, J.; Rizzi, M. J. Biol. Chem. 2004). Later, the crystal structure of Hkat-I in complex with indole- 3-acetic acid (IAC) was reported (PDB ID: 3FVU) (Han, Q.; Robinson, H.; Cai, T.; Tagle, D. A.; Li, J. J. Med. Chem. 2009) and the binding interactions of IAC inside the substrate binding site of Hkat-I were identified. The indole ring of IAC is inserted into a hydrophobic pocket defined by several residues, including Tyr63, His279, Phe278, Tyr101, and Phe125, while the carboxylic end forms a salt bridge with the guanido group Arg398.

2.-MATERIALS AND METHODS

2.1-Docking Analysis Using Maestro:

Maestro is the graphical user interface for all of Schrodinger's products like CombiGlideTM, EpikTM, GlideTM, ImpactTM, LiaisonTM, LigprepTM,MacroModelTM,PhaseTM, PrimeTM, QikPropTM, QsiteTM, and StrikeTM. It contains tools for building, displaying, and manipulating chemical structures; for organizing, loading and storing these structures and associated data; and for setting up, monitoring, and visualizing the results of calculations on these structures.

2.2-Protein Preparation:

The protein preparation facility performs the final stages of the preparation of proteins for use in Glide. A typical PDB structure file consists only of heavy atoms. Therefore, hydrogen does have to be added prior to use in Glide calculations, which use an all-atom force field. The charge state of protein residues is also important to the results generated by Glide. Before running a protein preparation job, one must perform some preliminary preparation tasks that are not automated. The protein preparation facility consists of two components, preparation and refinement. After ensuring chemical correctness, the preparation component adds hydrogen and neutralizes side chains that are not close to the binding cavity and do not participate in salt bridges. The refinement component performs a restrained impact minimization of the co-crystallized complex, which reorients side-chain hydroxyl groups and alleviates potential steric clashes. The protein preparation panel is used to set up jobs that perform these tasks.

2.3-Ligand Preparation:

The structure of hkat1 was taken to the docking studies. The crystallographically solved structure is taken in the form of a PDB format and it was converted into Maestro format using the Amber force field. Glide also allows importing the lead molecule in SDF, mol or mol2 format which is drawn using chembasic software. These determined structures are minimized using two methods
1. Ligprep Minimization and 2. Impact Minimization.

Ligprep generate tautomer and conformers for single ligand and impact minimization uses two algorithms for minimization steepest descent and conjugate gradient which run for 500 to 1000 cycles. Both methods use the OPLS force field for minimizing the structures.

2.4-Docking Method – Glide (Grid Based Ligand Docking With Energetics):

Glide searches for favorable interactions between one or more typical small ligand molecules and a typically larger receptor molecule usually a protein. Each ligand must be a single molecule, while the receptor may include more than one molecule such as a protein and a cofactor. GLIDE can be run in rigid or flexible docking modes and at the later automatically generates conformation for each input ligand. The combination of positions and orientation of the ligand relative to the receptor along with its conformation in flexible docking is referred to as a ligand pose. Ligand poses that GLIDE generates pass through a series of hierarchical filters that evaluate the ligand interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complementarity of ligand–receptor interactions using the GRID based method patterned after the empirical ChemScore function. Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non bonded ligand–receptor interaction energy. Final scoring is then carried out on the energy-minimized poses. Schrödinger's proprietary GLIDE score multi ligand scoring function is used to score the poses. If Glide score was selected as the scoring function, a composite Emodel score is then used to rank the poses of each ligand and to select the poses to report to the user. Emodel combines Glide score non-bonded interaction energy, and for flexible docking, the excess internal energy of the generated energy conformation. Two types of Docking Algorithms are used:

1) Induced fit docking
2) High throughput virtual screening

2.5-High Throughput Virtual Screening Using Grid:

1. The grid files produced by a single receptor grid generation task can be used for any number of jobs docking ligands to that receptor.

2. After correcting formal charges and bond orders in the ligand, set up and start the automated preparation and refinement portions of the protein preparation procedure using the protein preparation panel.

3. Ensure that the ligands to be docked are in the right form.

4. To the prepared receptor- ligand complex in the workforce, use the receptor grid generation panel to specify setting, and start the receptor grid generation job.

5. Specify the base name for the receptor grid files you want to use in the ligand docking panel, and use the other setting and option in the panel to set up and start a ligand docking job. As many docking jobs as you want can be set up in the panel, using the current receptor grids or specifying a different set of grids to use.

2.6-Grid Generation:

Choose receptor grid generation from the glide submenu of the applications menu. The receptor grid generation panel has three tabled folders, to specify settings for the receptor grid generation job,

- Receptor
- Site
- Constraints

2.7-Specifying the Receptor Grid:

To specify the receptor grid for the docking job, click Browse in the receptor grid section of the settings folder to open a file selector and choose a grid file. The file name, without the extension, is displayed in the receptor grid base name text box. You can also enter the base name directly into the text box. HTVS (High Throughput Virtual screening) docking is intended for the rapid screening of very large numbers of ligands. HTVS has much more restricted sampling than SP docking and cannot be used with constraints, score-in-place, or rigid docking. Advanced setting is not available for HTVS, but is fixed at predetermined values.

2.8-Induced Fit Docking Protocol:

Prepared protein and phenylhydrazonohexanoic acid derivatives are docked using induced fit protocol using the following system:

1. Constrained minimization of the receptor with a RMSD cutoff of 0.18Å
2. Initial Glide docking of the ligand using a softened potential that is by van der Waals radii scaling. By default, a maximum 20 poses for ligand is retained and by default poses to be retained, it must have a Coulombic-vdW score less than 100 and a H-bond scoreless than –0.05.
3. One round of prime side-chain prediction for each protein or ligand complex, on residues within a given distance of ligand poses default distance is 5 Å.
4. Prime minimization of the same set of residues and the ligand for each protein or ligand complex pose. The receptor structure in each pose now reflects an induced fit to the ligand structure and conformation.
5. Glide redocking of each protein or ligand complex structure within a specified energy of the lowest-energy structure. The default energy value is 30kcal/mol. Ligand is now rigorously docked, using default Glide settings, into the induced-fit receptor structure.
6. Minimization of redocked ligand within the protein.
7. Estimation of binding energy or glide energy for each output poses.
8. Schrödinger has developed a Python script that automates the induced fit docking process. This Python script has an interface in Maestro, in which one can specify the structures and enter settings for various options, and then the job running. The script then completes the protocol without further intervention. The structures used for induced fit docking must be prepared in the same manner as for glide. The protein and ligand preparation must precede the use of the protocol.

2.9-Output Job Files:

Jobname_lig.mae: The input ligand structure file

Jobname_lig_prep.mae: The post preparation ligand structure file

Jobname_lig_prep.mae: The post refinement ligand structure file if present, the receptor structure file contains only the Receptor.

Jobname_prot.mae: The input receptor structure file.

Jobname_prot_prep.mae: The post preparation receptor structure file.

Jobname_prot_prep.mae: The post refinement receptor structure file contains the Receptor and Ligand structure unless there is a separate ligand structure file.

Jobname.log: The log files for the complete preparation and Refinement job.

2.10-Chemsketch:

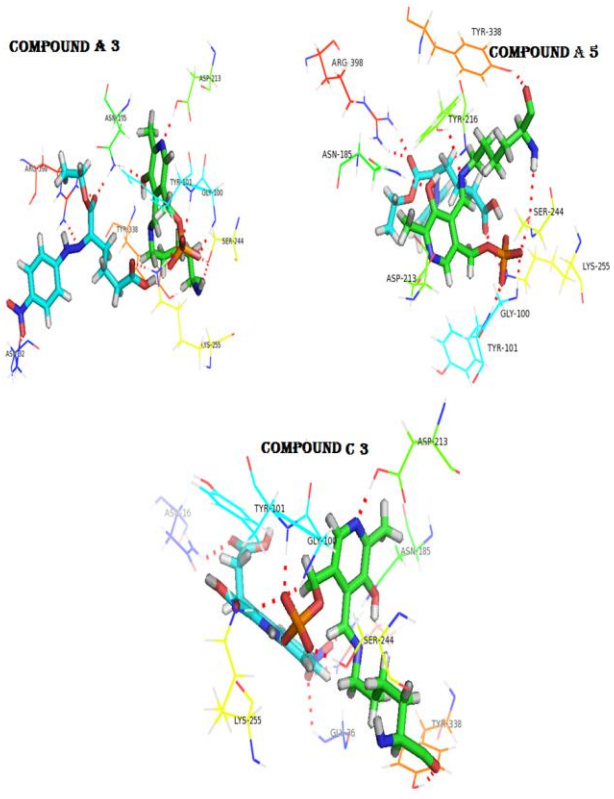
ChemSketch is designed to be used on its own for drawing chemical structures, reactions, schematic diagrams or integrated with other ACD applications and as the front end to our software. Able to import Windows Metafile, MDL MOL, CS ChemDraw, or ISIS/Sketch BIN file. Export Bitmap, TIFF, Metafile, MOL, Paintbrush, ISIS/Sketch, GIF, and ChemDraw. The Chembasic language, which is integrated with ACD/ChemSketch chemistry drawing software, is intended to be a tool for

- Manipulating chemical structures, both 2D- and 3D;
- Customizing ACD/Labs software, through direct access to its embedded functionality;
- Developing add-ons to ACD/Labs chemistry software.

3. RESULTS:

The Human Kynurenine aminotransferase is response for schizophrenia disorder. Totally, thirty one ligands have been selected. Among these ligands (5Z)-6-ethoxy-5-[2-(4-nitrophenyl) hydrazinylidene]-6-oxohexanoic acid (A3) and (5Z)-6-ethoxy-5-[2-(4-methoxyphenyl) hydrazinylidene]-6-oxohexanoic acid (A5) showed best Interaction, Docking score and Glide energy when compared to other ligands.

PYMOL RESULTS:



INDUCE FIT:

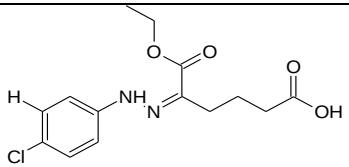
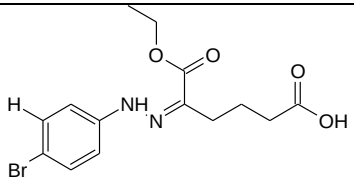
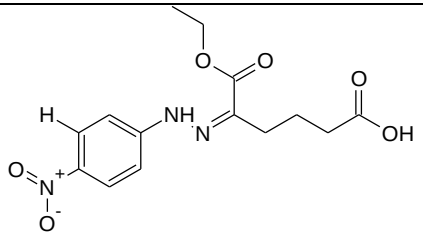
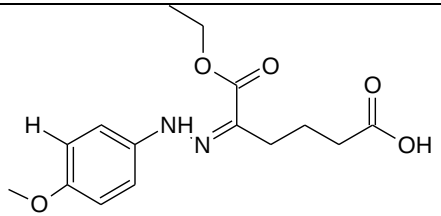
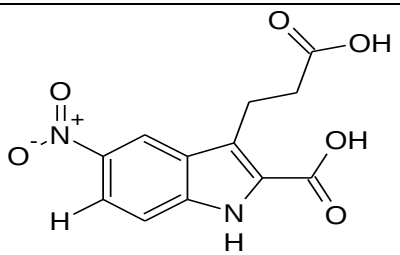
COMPOUND	POSES	HB	D	DS	GE
A3	1	ASN32 N-H…O	3.001	-6.847	-51.635
		LYS255 N-H…O	3.259		
		ARG398 N-H…O	2.896		
	2	ARG398 N-H…O	3.061	-6.687	-48.482
		ARG398 N-H…O	2.932		
		O-H…O LLP247	3.824		
	3	LYS255 N-H…O	3.155	-6.281	-46.727
		ARG398 N-H…O	2.833		
		ASN185 N-H…O	2.999		
A5	1	ARG398 N-H…O	2.915	-6.673	-49.260
		ARG398 N-H…O	2.973		
		O-H…O LLP247	3.768		
	2	GLY 36 N-H…O	3.042	-5.753	-47.443
		O-H…O LLP247	2.516		
	3	ASN185 N-H…O	3.415	-5.560	-46.673
		ARG398 N-H…O	2.933		
		O-H…OLLP247	2.616		
C3	1	LYS255 N-H…O	3.110	-6.871	-48.657
		ARG398 N-H…O	2.916		
		ARG398 N-H…O	3.342		
	2	ARG398 N-H…O	3.314	-6.096	-47.635
		O-H…O ASP126	2.693		
		N-H…O ASP126	2.750		
	3	ARG398 N-H…O	3.065	-6.713	-45.622
		ARG398 N-H…O			
		O-H…O LLP247			

(SP – STANDARD PRECISION, HTVS - HIGH THROUGHPUT VIRTUAL SCREENING,
XP – EXTRA PREECISION, IF – INDUCE FIT, DS – DOCKING SCORE,
GE – GLIDE ENERGY, D – DISTANCE, HB – HYDROGEN BOND)
[A3: (5*Z*)-6-ethoxy-5-[2-(4-nitrophenyl) hydrazinylidene]-6-oxohexanoic acid.
A5: (5*Z*)-6-ethoxy-5-[2-(4-methoxyphenyl) hydrazinylidene]-6-oxohexanoic acid.
C3: 3-(2-carboxyethyl)-5-nitro-1*H*-indole-2-carboxylic acid.
A2: (5*Z*)-5-[2-(4-bromophenyl) hydrazinylidene]-6-ethoxy-6-oxohexanoic acid.
A1: (5*Z*)-5-[2-(4-chlorophenyl) hydrazinylidene]-6-ethoxy-6-oxohexanoic acid.

GLIDE DOCKING:

COMPOUND	SP		HTVS		XP		IF	
	DS	GE	DS	GE	DS	GE	DS	GE
A3	-6.695	-45.839	-5.837	-41.809	-7.224	-42.107	-6.847	-51.635
A5	-6.235	-41.832	-6.037	-37.297	-6.961	-36.270	-6.673	-49.260
C3	-6.880	-40.222	-6.287	-36.746	-8.752	-36.800	-6.871	-48.657
A2	-6.884	-39.221	-5.472	-37.476	-7.200	-39.309	-6.578	-47.815
A1	-7.071	-39.221	-5.572	-36.403	-8.230	-38.184	-6.423	-45.815

SYNTHETIC LIGANDS BY CHEMSKETCH:

COMPOUND	LIGANDS	NAME
A1		(5*Z*)-5-[2-(4- chlorophenyl) hydrazinylidene]-6-ethoxy-6-oxohexanoic acid
A2		(5*Z*)-5-[2-(4-bromophenyl)hydrazinylidene]-6-ethoxy-6-oxohexanoic acid
A3		(5*Z*)-6-ethoxy-5-[2-(4-nitrophenyl)hydrazinylidene]-6-oxohexanoic acid
A5		(5*Z*)-6-ethoxy-5-[2-(4-methoxyphenyl)hydrazinylidene]-6-oxohexanoic acid
C3		3-(2-carboxyethyl)-5-nitro-1*H*-indole-2-carboxylic acid

4. DISCUSSION:

Fady N. Akladios et.al, reported 6-ethoxy-6-oxo-5-(2-phenylhydrazono) hexanoic acid and 3-(2-carboxyethyl)-1Hindole-2-carboxylic acid derivatives helpful for control the production of human kynurenine aminotransferase-1, especially 5-(2-(4 chlorophenyl) hydrazono)-6-ethoxy-6-oxohexanoic acid (9a). Rigid docking have been done for the protein with seventeen Ligands(A1-7, B1-5, C1-5)(Fady N. Akladios, Naveed A. Nadvi, Joohong Park, Jane R. Hanrahan, Vimal Kapoor, Mark D. Gorrell, W. Bret Church., 2012) in XP modes, SP modes using Glide Software, then Docking score hydrogen bond interactions were noted down. Then Induced Fit Docking studies have been carried out and the results were compared. On my report, among these Ligands, (5Z)-6-ethoxy-5-[2-(4-nitrophenyl) hydrazinylidene]-6-oxohexanoic acid (A3) has good Docking Score of–6.847 and best Glide Energy of -51.635 compared to the existing original native ligand IAC. (5Z)-6-ethoxy-5-[2-(4-methoxyphenyl)hydrazinylidene]-6 oxohexanoic acid (A5) is the second best compound with the Docking score of -6.673 and Glide energy of -49.260. In the compound of 9c has better results of Glide docking (i.e. HTVS, XP and SP) and Induce Fit Docking.

REFERENCE:

Bebbington, P., Wilkins, S., Jones, P., Foerster, A., Murray, R., Toone, B., Lewis, S., 1993. Life events and psychosis. Initial results from the Camberwell Collaborative Psychosis Study. Br. J. Psychiatry 162, 72– 79.

Fady N. Akladios, Naveed A. Nadvi, Joohong Park, Jane R. Hanrahan, Vimal Kapoor, Mark D. Gorrell, W. Bret Church. Design and Synthesis of novel inhibitors of human kynurenine Aminotransferase-1. Bioorganic & Medicinal Chemistry Letters 22, 1579–1581.

Frangou, S., Hadjulis, M., Chitnis, X., Baxter, D., Donaldson, S., Raymont, V., 2002. The Maudsley bipolar disorder project: brain structural changes in bipolar I disorder. Bipolar Disord. 4, 123–124.

Gershon, E.S., DeLisi, L.E., Hamovit, J., Nurnberger, J.O., Maxwell, M.E., Schreiber, J., Dauphinais, D., Dingman, C.W., Guroff, J.J., 1988. A controlled family study of chronic psychoses. Arch. Gen. Psychiatry 45, 328–336.

Hafner, H., Loffler, W., Maurer, K., Hambrecht, M., an der Heiden, W., 1999. Depression, negative symptoms, social stagnation and social decline in the early course of schizophrenia. ActaPsychiatr. Scand. 100, 105– 118.

Han, Q.; Robinson, H.; Cai, T.; Tagle, D. A.; Li, J. J. Med. Chem. 2009, 52, 2786.

Kendler, K.S., Gardner, C.O., 1997. The risk for psychiatric disorders in relatives of schizophrenic and control probands: a comparison of three independent studies. Psychol. Med. 27, 411 – 419.

Kennedy, N., Boydell, J., Kalidindi, S., Fearon, P., Jones, P.B., van Os, J., Murray, R.M.,, 2004. Gender differences in incidence and age at onset of mania and bipolar disorder: results from a 35-year study. Am. J. Psychiatry (in press).

Koreen, A.R., Siris, S.G., Chakos, M., Alvir, J., Mayerhoff, D., Lieberman, J., 1993. Depression in first-episode schizophrenia. Am. J. Psychiatry 150, 1643– 1648.

Procopio, M. Br. J. Psychiatry 2001, 179, 75.

Rossi, F.; Han, Q.; Li, J.; Rizzi, M. J. Biol. Chem. 2004, 279, 50214.

Ventura, J., Nuechterlein, K.H., Lukoff, D., Hardesty, J.P., 1989. A prospective study of stressful life events and schizophrenic relapse. J. Abnorm. Psychol. 98, 407–411.