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MOLECULAR DOCKING STUDY OF FLAVONOIDS DERIVATIVES WITH PDB ID: 3EQM

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ABSTRACT

Flavonoids are the natural phytoconstituents recognized as secondary metabolites of plant, with marked biological significance such as Anti-inflammatory, Antioxidant, Anticancer and Antimicrobial activity. After the cardiovascular diseases, cancer is the second most common cause of death. Aromatase enzyme is mainly involved in the conversion of androgens to estrogen. In post-menopausal female, Aromatase enzyme is mostly localized in breast tumor. After skin cancer, breast cancer is most common in women. This work deals with a molecular docking study of novel flavones derivatives was performed by using Schrodinger (Maestro 11.5v) with *PDB Id: 3EQM* for drug protein interaction study. The main objective of molecular docking is to predict the biological activity of given ligand. On the basis of docking study the ligand protein interaction diagram shows that the flavones interacted with ARG115, LEC477 and MET374 the most active binding site of an aromatase enzyme. Compounds (c, f, h, k) derivatives were most active with (-8.286, -7.923, -8.056, -8.000) docking score and (-8.286, -7.943, -8.078, -8.112) Glide score respectively comparatively higher than standard (Fadrozole) Docking Score (-7.564) and standard Glide Score (-7.725). It was demonstrated that the docking practice could reliably reproduce the interaction of aromatase with its substrate. The insights gained from the study herein have great potential for the design of novel flavones as Aromatase Inhibitors.

KEYWORDS

Aromatase enzyme, Molecular docking, Flavone, Amines and Breast cancer.

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INTRODUCTON

Aromatase enzyme is an essential in estrogen biosynthesis converting the aliphatic androgens testosterone and androstenedione to the aromatic estrogens estradiol and estrone, respectively. Estrogens play a key role in normal cell proliferation by binding to the nuclear estrogen receptor (ER) and triggering a sequence of reactions

leading to cell division[1]. Estrogens are also a key factor in hormone-dependent (ER-positive) tumor development[2]. One approach to treat and/or prevent hormone-dependent tumor development is to decrease the level of circulating estrogens and local tumor estrogen production by inhibiting estrogen producing enzymes[3]. Flavonoids are the natural phytoconstituents widely distributed in plants originate in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine[4,5]. Flavonoids have been recognized as secondary metabolites of plant, with marked biological significance such as Anti-inflammatory[6], Antioxidant[7], Anticancer[2,4] and Antimicrobial activity[8-11]. Hence, flavonoids are considered as an essential component in a variety of nutraceuticals, pharmaceuticals, medicinal and cosmetic applications with versatile health benefits. It is observed that the Flavone moiety possess specific pharmacophore pattern Figure No.1 which is necessary for binding to aromatase enzyme and their by its inhibition. In these study, non-steroidal aromatase inhibitors[12] possess aromatic/aliphatic amines at side chain for suitable position acts as H bond acceptor which bound with MET 374 present in active site of aromatase enzyme. Basic nucleus plays role as hydrophobic spacer moiety which maintained distance between Heme coordinating group and hydrogen bond acceptor moiety[13].

In this research molecular docking we provide an atomic level explanation for the binding of novel flavone derivative to the aromatase active site. In these study to report the binding mode of phytoestrogens to the aromatase enzyme using molecular dynamics simulations (MDS) and ligand-protein docking.

MATERIAL AND METHODS

Molecular modeling simulations (MDS) is a very much investigated technique for recognizing the potent compound without putting excessively exertion and investment in research[14]. Maestro 11.5 Schrodinger software[15] is used by us to investigate the activity in terms of binding affinity (Kcal/mol), and there after the outcomes are compared in binding affinity score for best-docked conformation. For the molecular dockings, the all novel flavone derivatives (ligand) and protein structures were generated within software. Ligands were sketched and minimized with Maestro 11.5 Schrodinger and further converted to the 3D structure. All the designed structures were optimized by energy minimization using MM2 method. To identify the potential or aromatase enzyme, a protein 3EQM[13] was selected and which was downloaded from protein data bank. The outcomes of results were analyzed by Docking score, Glide score, and Binding energy with various bonding interactions. For the molecular dockings, the all novel flavone derivatives (ligand) and protein structures were generated within software. Ligands were sketched and minimized with Maestro 11.5 Schrodinger.

Experimental method

Docking protocol

The molecular docking study of amino substituted novel flavone derivatives was carried out using Maestro 11.5 Schrodinger software .The ligands were docked on the aromatase enzyme with PDB ID (3EQM) taken from the Protein Data Bank (www.rscb.org).

Ligand preparation

The Schrödinger ligand preparation was done by using LigPrep panel application which consists of series of steps that perform conversion of 2D structures to 3D structure, apply correction to the structure by minimizing the proper bond angles and distances and optimize the structure by minimizing its energy through force-field OPLS3.

Protein Preparation and its Refinement

The crystal structure of human placental microsomal aromatase with its bound natural substrate androstenedione was taken from the Protein Data Bank (PDB ID: 3EQM) for protein preparation. The multistep Schrodinger's protein preparation wizard tool (PPrep) has been used for protein preparation, which was minimized using OPLS-3 force field with polack-ribiere conjugate gradient (PRCG) algorithm. As protein is the essential component for molecular docking study it is necessary to minimize the energy of protein molecule prior to docking studies with ligands. These protein for ligand docking study was prepared by using protein preparation wizard tool in

which was used to import proteins for the protein data bank (PDB). Proteins obtained from the PDB, vendors and other sources frequently have missing hydrogen, partial charges, side chain and whole loops region. So, to overcome all these barriers in docking study the proteins to undergone through pre-processing and it was done by selecting following parameters,

- Add hydrogen
- Create zero order bonds to metals
- Create disulfide bonds
- Filling missing side chains using prime
- Fill in missing loops using prime
- Delete water beyond 5.00 Å From het group
- Generate het state using Epik: PH 7.0+/- 2.0

Receptor grid generation

Grid generation must be performed in order to run a virtual screen with glide. The shape and properties of the receptor are represented in a grid by field that provides progressively more accurate scoring of the ligand poses. For receptors that adopt more than one conformation on binding, Glide prepares grids for each conformation, to ensure that possible actives are not missed.

To open the Receptor Grid Generation panel, Receptor Grid Generation sub-menu of Glide was selected from the Application menu. The Receptor Grid Generation panel has three tabs, which are used to specify settings for the receptor grid generation job.

Protein ligand docking

For the ligand protein molecular docking twenty six (a-z) amino substituted flavones derivative were designed and docked on of aromatase enzyme with (PDB: 3EQM).

The ligand docking process helps to predict ligand conformation and orientation (posing) within a targeted binding site and thus helps to interpret interactions of ligand atoms with amino acids of proteins, and to understand the binding affinity. The ligand-protein docking was carried out in the extra precision (XP) mode[15-20].

RESULTS AND DISCUSSION

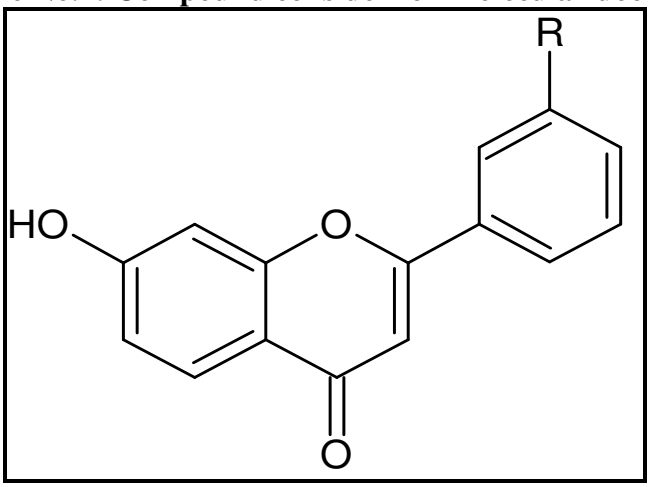
Molecular docking study was done to elucidate the binding mode of novel flavone derivatives with aromatase inhibitors. In these research total twenty six derivatives, were docked to the aromatase binding site. Maestro 11.5 Schrodinger software was used to prepare the molecules and to show the interaction of the compound in binding pocket of an aromatase enzyme results indicated that the X-ray crystallography conformer was almost identical to the docked conformer by giving the result of docking in the form of docking score, glide score and free binding energy. Binding position with the lowest docked energy belonging to the top-ranked cluster was selected as the final model for post-docking analysis. Docking results for the newly designed flavone derivatives along with reference inhibitor (Fadrazole) are summarized for the comparative purpose.

In result it was found that few compounds have docking and glide score more than standard Fadrazole, so the preferences is given according to number of hydrogen bonding while short listing the molecules. Compounds (c, f, h, k) derivatives were most active with (-8.286, -7.923, -8.056, -8.000) docking score and (-8.286, -7.943, -8.078, -8.112) Glide score respectively comparatively higher than standard (Fadrazole) Docking Score (-7.564) and standard Glide Score (-7.725) Table No.2. Therefore from the ligand protein interaction (i.e. H-Bonding, Pi-Pi stacking) and docking score the compound c, f, h, k are having good ligand protein interaction therefore these compounds having good binding capacity thus shows good anti-cancer activity through aromatase inhibition. The docking and ligand protein interaction diagram of top ranked compounds (c, f, h, k) and Fadrozole are given in Figure No.2 and 3.

On the basis of docking study we concluded that all the novel flavonoid are having higher docking and glide score than Fadrazole and compound namely c, f, h, k due to hydroxyl substitution is more effective against 3EQM receptor. The docking study exposed that the top- ranked flavonoids c, f, h, k forms H-bond interaction between the hydroxy group and MET 374, ALA 306, LEU 477 and HEM 600

amino acid residue of 3EQM receptor. Pi-Pi interaction is formed between aromatic ring and ARG 115 amino acid residue of 3EQM.

Table No.1: Compound consider for Molecular docking



S.No	Compound consider for molecular docking			
	Compound Code	**-R**	**Compound Code**	**-R**
1	a	Methylamine	N	Anilineamine
2	b	Ethylamine	O	Benzalamine
3	c	n-propylamine	P	*o*-chloroaniline
4	d	iso-propylamine	Q	*o*-nitroaniline
5	e	n-butylamine	R	3,4-dimethylaniline
6	f	iso-butylamine	S	*p*-bromoaniline
7	g	n- pentylamine	T	*p*-methylaniline
8	h	iso-pentylamine	U	*p*-aminophenol
9	i	neo-pentylamine	V	*p*-chloroaniline
10	j	di-methylamine	W	*p*-nitroaniline
11	k	di-ethylamine	X	2-chloro, 4-bromoaniline
12	l	Ethylmethylamine	Y	4-methoxyaniline
13	m	Ethylpropylamine	Z	Fadrozole (standard)

Table No.2: Docking Score, Glide score and number of H-bonds for the flavones and Fadrazole

S.No	Code	-R	Docking Score (Kcal/mole)	Glide Score (Kcal/mole)	H-Bonds	Amino acids involved in interaction with ligands
1	a	Methylamine	-6.757	-6.770	1	MET 374, ARG 115
2	b	Ethylamine	-7.173	-7.186	1	ARG 115, MET 374
3	c	n-propylamine	-8.286	-8.286	1	MET 374, ARG 115
4	d	iso-propylamine	-6.827	-6.850	2	ASH 309, HEM 600, ARG 115, LEU 477
5	e	n-butylamine	-7.200	-7.213	1	ASH 309, PHE 134, ARG 115
6	f	iso-butylamine	-7.923	-7.943	2	ARG 115, ASH 309, HEM 600, LEU 477
7	g	n- pentylamine	-7.498	-7.511	1	MET 374, ARG 115
8	h	iso-pentylamine	-8.056	-8.078	2	ALA 306, ARG 115, LEU 477
9	i	neo-pentylamine	-6.419	-6.431	1	LEU 477
10	j	di-methylamine	-6.819	-6.832	0	---
11	k	di-ethylamine	-8.000	-8.112	1	ARG 115, MET 374
12	l	Ethylmethylamine	-6.491	-6.511	1	MET 374, HEM 600
13	m	Ethylpropylamine	-6.096	-6.223	0	---
14	n	Aniline amine	-7.748	-7.761	1	LEU 372, ARG 115, TRP 224
15	o	Benzalamine	-5.817	-5.830	1	MET 374
16	p	*o*-chloroaniline	-5.739	-5.752	1	LEU 477, ARG 192, HIE 480
17	q	*o*-nitro aniline	-5.316	-5.329	0	PHE 221, TRP 224, ARG 115, HEM 600
18	r	3, 4-dimethylaniline	-6.391	-6.404	1	LEU 477, ARG 192, HIE 480
19	s	*p*-bromoaniline	-6.524	-6.537	0	TRP 224, HIE 480, ARG 192
20	t	*p*-methylaniline	-6.658	-6.671	1	ARG 192, HIE 480, TRP 224, LEU 477
21	u	*p*-aminophenol	-7.168	-7.181	1	LEU 477, ARG 192, HIE 480
22	v	*p*-chloroaniline	-4.464	-6.476	0	ARG 192, HIE 480, TRP 224
23	w	*p*-nitroaniline	-7.684	-7.696	1	GLU 480, LEU 477, HIE 480, ARG 192, ASP 222
24	x	2-chloro,4-bromoaniline	-2.871	-2.884	1	---
25	y	4-methoxyaniline	-5.152	-5.165	1	LEU 477
26	z	Fadrazole (standard)	-7.564	-7.725	1	HEM 600, MET 374

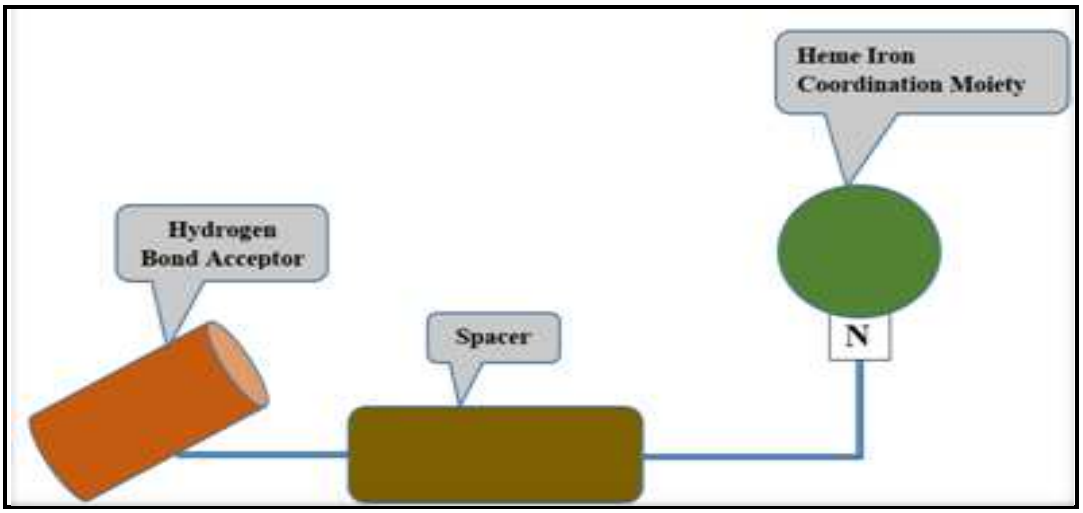


Figure No.1: Pharmacophore pattern of molecule

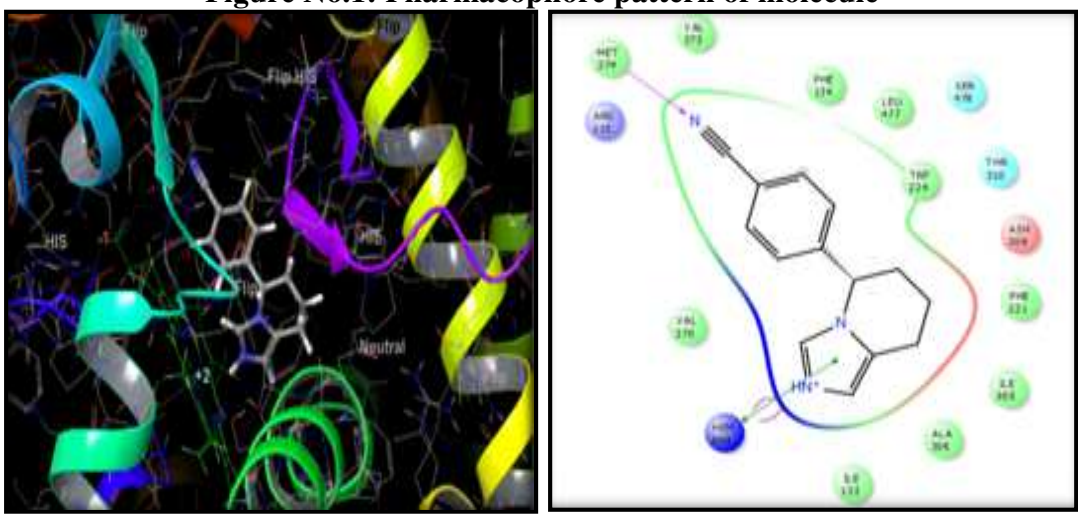


Figure No.2: Ribbon structure and Amino acid interaction of 3EQM with Fadrazole

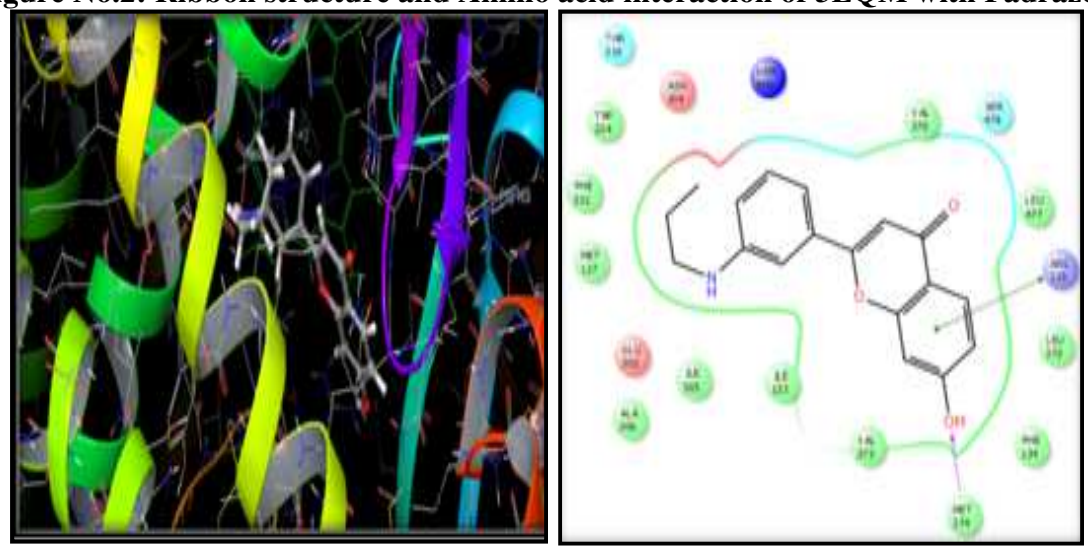


Figure No.3: Ribbon structure and Amino acid interaction of 3EQM with (c)

CONCLUSION

In the present study, the binding and interactions of novel flavone derivatives with aromatase enzyme have been studied using Maestro 11.5 Schrodinger software. Most of the compounds have shown significant binding interactions with the aromatase enzyme. An increase in Docking and Glide score was observed when there is amino substitution at C-3 position of B-ring of the flavanone. Compounds with aliphatic substitution at C-3 in B-ring showed higher Docking and Glide score than aromatic substitution. The hydrogen bond interactions and π–π interactions also contributed to the strong binding of these compounds to the binding site of aromatase compare to reference.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

COMPETING INTERESTS

The authors' declare that they have no competing interests.

BIBILIOGRAPHY

1. Graham L. Patrick. An introduction to medicinal chemistry, *Oxford University press,* 3rd Edition, 2006, 161-162.
2. Akama T, Shida Y, Sugaya T, Ishida H, Gomi K, Kasai M. Novel 5-Amino Derivative as Specific Antitumor Agents in Breast Cancer, *J. Med. Chem,* 39(18), 1996, 3461-3469.
3. Lili Ma, Bonfield K, Amato E, Bankemper T, Agard H, Steller J, Keeler J S, Roy D, Mccallum A, Paula S. Development of a new class of aromatase inhibitors: Design, synthesis and inhibitory activity of 3-phenylchroman-4-one(isoflavanone) derivatives, *Bioorg. Med. Chem,* 20(8), 2012, 2603-2613.
4. Chulia A J, Pouget C, Fagnere C, Basly J P, Leveque H. Synthesis and structure of flavan-4-ols and 4-methoxyflavans as New potential Anticancer Drugs, *Tetrahedron,* 56(33), 2000, 6047-6052.
5. Brueggemeier R W, Hackett J C, Kim Y W, Su B. Synthesis and Characterisation of azole isoflavone inhibitor of aromatase, *Bioorg. Med. Chem,* 13(12), 2005, 4063-4070.
6. Tran Thanh Dao, Yeon Sook Chi, Jeong Kim, Hyun Pyo Kim, Sanghee Kim and Haeil Park. Synthesis and inhibitory activity against COX-2 catalyzed prostaglandin production of chrysin derivatives, *Bioorganic Med. Chem. Lett,* 14(5), 2004, 1165-1167.
7. Cotelle N, Bernier J, Catteau J, Pommery J, Wallet J C, Gaydou E M. Antioxidant properties of hydroxy-flavones, *Free. Rad. Bio. Med,* 20(1), 1996, 35-43.
8. Khan M S Y and Hasan S M. Synthesis, anti-inflammatory and antibacterial activity of some new flavoinoidal derivatives, *IJC,* 2003, 1970-1974.
9. Patil S G, Utale P S, Gholse S B, Thakur S D, Pande S V. Synthesis, Characterization and antimicrobial activity of 6-bromo-4-methoxy-4-(substituted phenyl) iminoflavone, *Journal of Chemical and Pharmaceutical Research,* 4(1), 2012, 501-507.
10. Nitin G. Ghodile, Rajput P R, Banewar V W and Raut A R. Synthesis and antimicrobial activity of some chalcones and flavones having 2-hydroxy acetophenone moiety, *Int. J. Pharm. Bio. Sci,* 3(3), 2102, 389-395.
11. Hakan Goker, David W. Boykin and Sulhiye Yildiz. Synthesis and potent antimicrobial activity of some novel 2- phenyl or methyl -4H-1-benzopyran-ones carrying amidinobenzimidazole, *Bioorg. Med. Chem,* 13(5), 2005, 1704-1714.
12. Kim Y W, Hackett J C, Brueggemeier R W. Synthesis and Aromatase Inhibitory Activity of Novel Pyridine –Containing Isoflavones, *J. Med. Chem,* 47(16), 2004, 4032-4040.
13. Yadav M R, Sabale P M, Geridhar R. Synthesis of some novel androstanes as

potential aromatase inhibitors, *Steroids,* 76(5), 2011, 464-470.
14. Van Drie J H. Computer-aided drug design: the next 20 years, *J. Comput. Aided Mol. Des,* 21(10-11), 2007, 591-601.
15. Vijayakrishnan R. Structure-based drug design and modern medicine, *J. Postgrad. Med,* 55(4), 2009, 301-304.
16. Tanaji T. Talele, Santosh A. Khedkar and Alan C. Rigby. Successful Applications of Computer Aided Drug Discovery: Moving Drugs from Concept to the Clinic, *Current topics in medicinal chemistry,* 10(1), 2010, 127-141.
17. Grinter S Z, Zou X. Challenges, applications, and recent advances of protein-ligand docking in structure-based drug design, *Molecules,* 19(7), 2014, 10150-10176.
18. Lavecchia A, Di Giovanni C. Virtual screening strategies in drug discovery: a critical review, *Curr Med Chem,* 20(13), 2013, 2839-2860.
19. Evanthia Lionta, George Spyrou, Demetrios K. Vassilatis and Zoe Cournia. Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances, *Curr Top Med Chem,* 14(16), 2014, 1923-1938.
20. Philip Prathipati, Anshuman Dixit and Anil K. Saxena. Computer-Aided Drug Design: Integration of Structure-Based and Ligand-Based Approaches in Drug Design, *Current Computer-Aided Drug Design,* 3(2), 2007, 133-148.

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