ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (111-118), Month: April - June 2019, Available at: www.researchpublish.com

ROLE OF TROLOX IN MUSCARINIC ACETYLCHOLINE RECEPTOR ACTIVITY- An *In-silico* approach

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Abstract: **Trolox, chemically 6-hydroxy-2,5,7,8-tetramethylchroman-2-Carboxylic Acid, is a water-soluble derivative of vitamin E known for its potent antioxidant properties.**

Our goal was to understand the best muscarinic acetylcholine enzyme (M3) inhibitors using Trolox derivative. Protein-Ligand interaction has a significant role in structure-based drug design. Totally 314 Trolox derivatives were retrieved with 90% similarity from pubchem database. ADMET screened compounds showed good solubility and absorption (3 level and 0 level) and medium level of BBB penetration. Also, screened compounds are free from noxious effect of hepatotoxicity, followed by no more evidence of interacting with liver enzymes. Consequently, few compounds are out casted during RO5 violation. Finally, compounds after docking showed significant dock score and interactions. Among all 133098(108.912) was consider as a best compound and it is very similar in structure to Trolox. Our studies therefore reveal that these Trolox derivatives could be potential aspirants for further evaluation using in-vivo and in-vitro approaches.

Keywords: **Docking, Trolox derivatives, Intermolecular energy.**

I. INTRODUCTION

Trolox, chemically 6-hydroxy 2,5,7,8-tetramethyl chroman-2-carboxylic acid, is a water-soluble analogue of α-tocopherol and is a known radical scavenger [1] (Fig. 1). It exhibits antioxidant property and has application in reducing oxidative stress (OS) in biological systems and prevents cellular damages [2]. Trolox reacts with singlet oxygen in the presence of riboflavin under light protecting riboflavin in milk [3]. Trolox has a pro-oxidant effect in case of Cu^{2+} dependent low-density lipid (LDL) oxidation, the advantage of Trolox over α-tocopherol is that it can be incorporated both in water and lipid compartments of cells [4]. The intra cellular scavenging is the mechanism by which Trolox provides protection against OS induced by H_2O_2 [5]. Similarly, OS induced damage was reduced in oligodendrocytes by decreasing myelin basic protein suggesting Vit E analog protection in vitro [6]. Studies showed that the Trolox could inhibit lipid peroxidation, cytotoxicity, and apoptosis triggered by cytochrome P450 in Hep G2 cells [7]. Other study indicated its oxygen quenching ability in C6 glial cells induced by methylmercury [8].

Muscarinic receptors are known for their role in Alzheimer's disease and diabetes. Therefore, they can be a significant target to understand the mechanisms of type 2 diabetes mellitus [9]. Trolox decreases M3 muscarinic receptor binding in Alzheimer's disease. [10, 11]. A similar activity was observed in smooth muscle contraction in the lungs reducing OS caused by smoking [12]. In hyperglycemic H9c2 cells treated with Trolox the reduction in OS were evident through decrease in reactive oxygen species [13]. Hence, this study is aimed to understand the role of Trolox and its analog on muscarine receptors using an *in-silico* approach.

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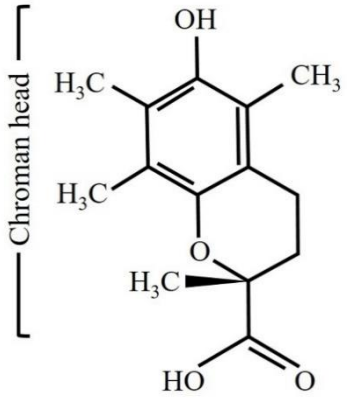

Fig. 1: Structure of Trolox

II. METHODOLOGY

Retrieval of target enzyme structures

Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) is the most commonly database for used for obtaining the 3D the structure of proteins [14]. In the current study M3 Muscarinic Acetylcholine of *Homo sapiens* origin, which is recognized as the target of Trolox was retrieved from PDB (4DAJ). The criteria for selection of the indicated structure was based on Resolution and R value [15].

Ligand retrieval

The Trolox (CID: 70684) and its 374 derivatives were retrieved from the PubChem database. The ligand molecules were subjected to initial screening of drug likeness using Lipinski filter (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). The rule of 5 complies majorly on: molecular mass, cLogP, hydrogen acceptor and donor [16, 17]. The ligands accepting the rule of 5 were subjected to further analysis.

Prominent binding site prediction

The active site of the M3 Muscarinic Acetylcholine was identified using cavity-based method from receptor cavities and coordinates were retrieved using binding pockets calculation using Discovery studio 3.5 software [16].

Docking:

To initiate docking study, selected 3D structure of the retrieved ligands from PubChem database were in SDF format, first converted to PDB format, then optimization was continued using Discovery Studio 3.5. The receptor or target protein (4DAJ: Structure of the M3 Muscarinic Acetylcholine Receptor) was prepared by removing he atoms, water molecules and CHARMm force field was applied using simulation tool/DS 3.5 [18]. Virtually Screened compounds after ADMET were subjected to docking process, top 20 poses were generated for each ligand based on the docking score. LibDock is a kind of docking algorithm which finds hotspots (A group of polar and apolar probes) using grid placed into (-13.119X, -7.776Y, -49.41 Z) 3D direction of the receptor active site. Significantly, it uses hotspot to map and align the ligand conformations for favorable interactions, any poses of ligand which results bad clashes gets removed. Finally, pose optimization was done using Broyden-Fletcher-Goldfarb-Shanno (BFGS) and top scoring ligand poses are ranked and retained [19].

III. RESULTS AND DISCUSSION

A member of G-protein coupled receptor family, Muscarinic acetylcholine receptors plays major role in modulating variety of physiological function [20,21]. This receptor is known as major drug target for several diseases such as Alzheimer's, Parkinson's and chronic obstructive pulmonary disease [20,21]. Muscarinic acetylcholine receptors has five subtypes, viz;M1, M2, M3, M4 and M5 [22-24]. Based on their coupling to G-proteins, the family is divided into two major groups and all subtypes in these groups are known as auto-receptors and experimentally proved or their neuro modulatory functions [25]. Earlier reports have reported the structure of Muscarinic acetylcholine M3 receptors and

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indicated their presence in smooth muscles, endocrine glands, exocrine glands, lungs, pancreas and the brain [26]. Neuronal cells in CNS have high levels of M3 receptors, largely overlapping with that of M1 and M4 receptor subtypes. M3 receptors are known for their role in contraction of smooth muscle, pancreatic β-cells and in different regions of the brain where they majorly persuade insulin discharge, signifying that M3 receptors might be a significant target to understand the mechanisms of type 2 diabetes mellitus [9].

Totally 314 Trolox derivatives were retrieved with 90% similarity form pubchem database.

Only five compounds passed the Lipinski filter and shown as Drug likeness screened compounds in Table I.

TABLE I: Drug likeness screened compounds

Pubchem ID	AlogP	HBA	HBD	Mol_Wt
133098	3.359	4	2	264.317
17994186	3.177	4	2	236.264
21930269	3.359	4	2	264.317
23149416	3.359	4	2	264.317
101214866	3.177	4	2	236.264

TABLE II: ADMET screened compounds

Pubchem ID	Solubility	BBB	CPY2D6	Hepatotoxic	Absorption
133098	3	2	FALSE	FALSE	0
9881728	3	2	FALSE	FALSE	0
12051781	3	2	FALSE	FALSE	0
16097787	3	2	FALSE	FALSE	0
16098185	3	2	FALSE	FALSE	0
16098186	3	2	FALSE	FALSE	0
17994186	3	2	FALSE	FALSE	0
20086587	3	2	FALSE	FALSE	0
21930269	3	2	FALSE	FALSE	0
23149416	3	2	FALSE	FALSE	0
67914444	3	2	FALSE	FALSE	0
101214866	3	2	FALSE	FALSE	0

Table II indicated the ADMET values of screened compounds. All five compounds showed good solubility and absorption (3 level and 0 level) and medium level of BBB penetration. Also, screened compounds are free from noxious effect of hepatotoxicity, followed by no more evidence of interacting with liver enzymes (Figure 2). Consequently, few compounds are casted out during RO5 violation.

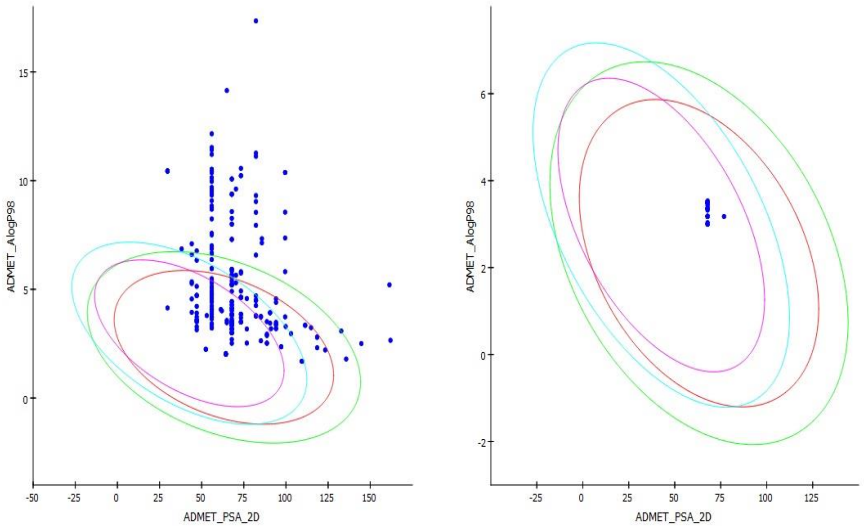

Figure 2: ADMET plot for before and after screening.

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These five compounds were further simulated using DS 3.5 and results are shown in table III.

The best docking result was seen with CID 133098. The forty fifth conformation poses were seen as the best poses and Lib dock score was found to be 108.91. The major interacting amino acid were found to be TRP, TYR, SER at chain A (Table IV, Figure 3).

The next best compound was CID 17994186 and TYR was found to be the major interacting amino acid (Table V, Figure 4). Figure 5,6 and 7 indicated the interaction between M3 receptor and 21930269, 23149416 respectively (Table VI, VII).

TABLE III: Docking score and conformation number for final hits

Pubchem ID	Conf_no	Libdockscore
133098	45	108.912
17994186	4	74.659
21930269	29	101.009
23149416	12	106.16
1.01E+08	4	74.659

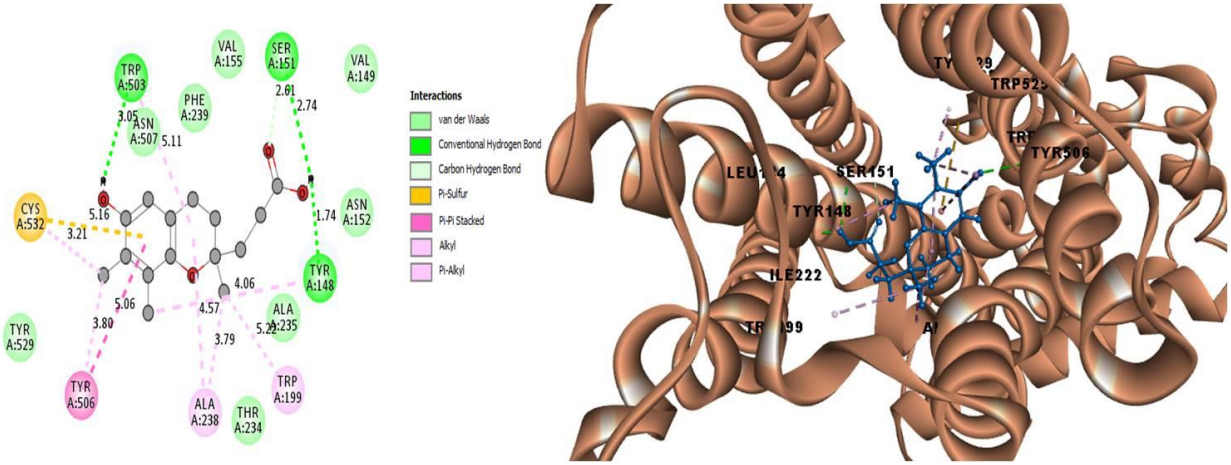

Figure 3: Interaction between M3 receptor and compound 133098

TABLE IV: Interacting amino acid and bonding pattern between M3 receptor and compound 133098

Atom-Atom Interaction	Distance In Angstrom	Types of bond
133098:H20 - A:TRP503:O	3.04725	Conventional Hydrogen Bond
133098:H21 - A:TYR148:O	1.73919	Conventional Hydrogen Bond
133098:H21 - A:SER151:OG	2.73508	Conventional Hydrogen Bond
A:SER151:HB1 - 133098:O4	2.61001	Carbon Hydrogen Bond
A:CYS532:SG - 133098	5.15637	Pi-Sulfur
A:TYR506 - 133098	5.05723	Pi-Pi Stacked
A:ALA238 - 133098	4.56734	Alkyl
A:ALA238 - 133098:C11	3.79185	Alkyl
133098:C19 - A:CYS532	3.21096	Alkyl
A:TYR148 - 133098:C17	4.05857	Pi-Alkyl
A:TRP199 - 133098:C11	5.21865	Pi-Alkyl
A:TRP503 - 133098	5.11292	Pi-Alkyl
A:TYR506 - 133098:C19	3.80302	Pi-Alkyl

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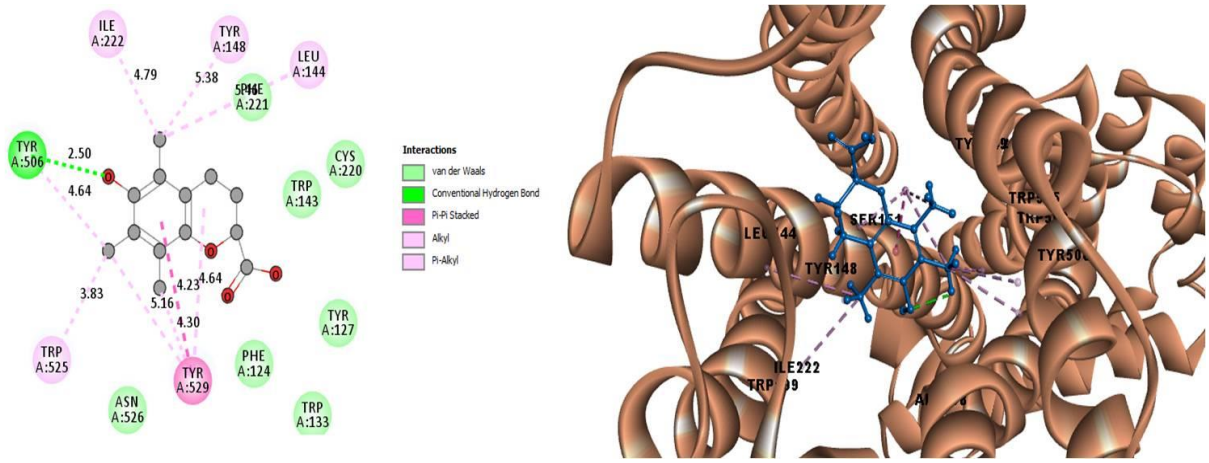

Figure 4: Interaction between M3 receptor and compound 17994186

TABLE V: Interacting amino acid and bonding pattern between M3 receptor and compound 17994186

Atom-Atom Interaction	Distance In Angstrom	Types of bond
A:TYR506:HH - 17994186:O2	2.49846	Conventional Hydrogen Bond
A:TYR529 – 17994186	4.22614	Pi-Pi Stacked
17994186:C14 - A:LEU144	5.45772	Alkyl
17994186:C14 - A:ILE222	4.7922	Alkyl
A:TYR148 - 17994186:C14	5.37779	Pi-Alkyl
A:TYR506 - 17994186:C17	4.64379	Pi-Alkyl
A:TRP525 - 17994186:C17	4.52278	Pi-Alkyl
A:TRP525 - 17994186:C17	4.33315	Pi-Alkyl
A:TYR529 – 17994186	4.63732	Pi-Alkyl
A:TYR529 - 17994186:C15	4.29869	Pi-Alkyl
A:TYR529 - 17994186:C17	5.15981	Pi-Alkyl

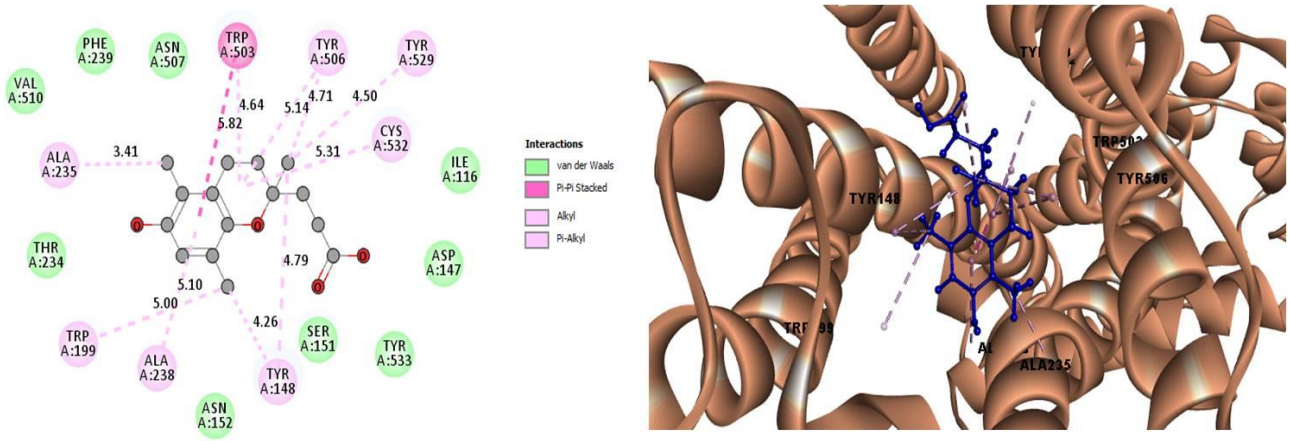

Figure 5: Interaction between M3 receptor and compound 21930269

TABLE VI: Interacting amino acid and bonding pattern between M3 receptor and compound 21930269

Atom-Atom Interaction	Distance In Angstrom	Types of bond
A:TRP503 – 21930269	5.81564	Pi-Pi Stacked
A:ALA235 - 21930269:C17	3.41157	Alkyl
A:CYS532 – 21930269	5.31235	Alkyl
A:TYR148 - 21930269:C11	4.78742	Pi-Alkyl
A:TYR148 - 21930269:C18	4.25807	Pi-Alkyl
A:TRP199 - 21930269:C18	5.00103	Pi-Alkyl

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A:TRP503 – 21930269	4.63861	Pi-Alkyl
A:TYR506 – 21930269	5.13653	Pi-Alkyl
A:TYR506 - 21930269:C11	4.70961	Pi-Alkyl
A:TYR529 - 21930269:C11	4.50464	Pi-Alkyl
21930269 - A:ALA238	5.10251	Pi-Alkyl

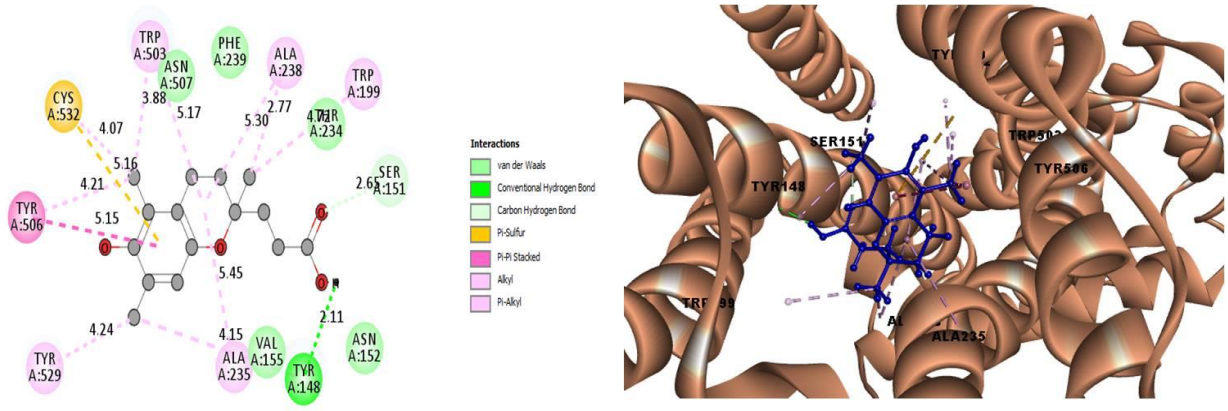

Figure 6: Interaction between M3 receptor and compound 23149416

TABLE VII: Interacting amino acid and bonding pattern between M3 receptor and compound 23149416

Atom-Atom Interaction	Distance In Angstrom	Types of bond
23149416:H21 - A:TYR148:O	2.10912	Conventional Hydrogen Bond
A:SER151:HB1 - 23149416:O4	2.64984	Carbon Hydrogen Bond
A:CYS532:SG - 23149416	5.16305	Pi-Sulfur
A:TYR506 - 23149416	5.14565	Pi-Pi Stacked
A:ALA235 - 23149416	5.44902	Alkyl
A:ALA238 - 23149416	5.2954	Alkyl
A:ALA238 - 23149416:C10	2.77074	Alkyl
23149416:C17 - A:CYS532	4.06652	Alkyl
A:TYR148 - 23149416:C19	4.15491	Pi-Alkyl
A:TRP199 - 23149416:C10	4.72116	Pi-Alkyl
A:TRP503 - 23149416:C17	4.60455	Pi-Alkyl
A:TRP503 - 23149416	5.17133	Pi-Alkyl
A:TRP503 - 23149416:C17	4.38468	Pi-Alkyl
A:TYR506 - 23149416:C17	4.211	Pi-Alkyl
A:TYR529 - 23149416:C19	4.23522	Pi-Alkyl

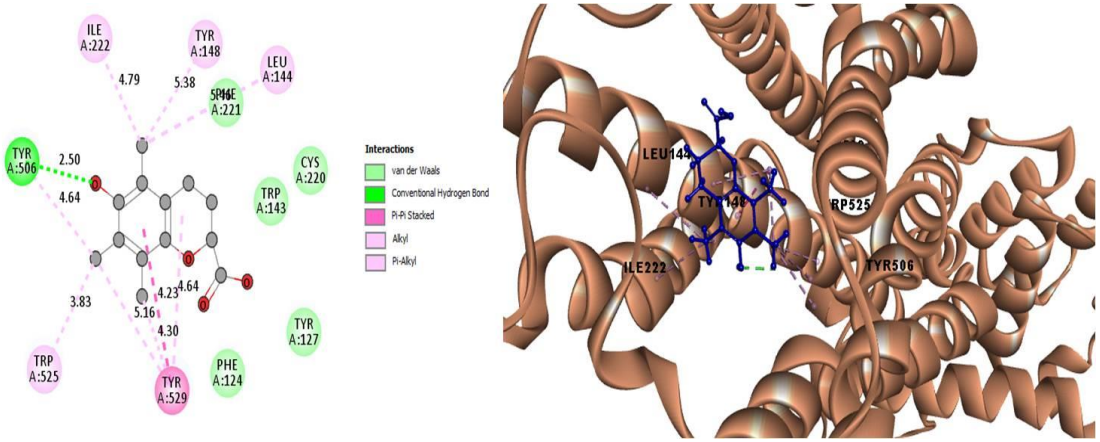

Figure 7: Interaction between M3 receptor and compound 101214866

TABLE VIII: Interacting amino acid and bonding pattern between M3 receptor and compound 101214866

Atom-Atom Interaction	Distance In Angstrom	Types of bond
A:TYR506:HH - 101214866:O2	2.49846	Conventional Hydrogen Bond
A:TYR529 - 101214866	4.22614	Pi-Pi Stacked
101214866:C14 - A:LEU144	5.45772	Alkyl
101214866:C14 - A:ILE222	4.7922	Alkyl
A:TYR148 - 101214866:C14	5.37779	Pi-Alkyl
A:TYR506 - 101214866:C17	4.64379	Pi-Alkyl
A:TRP525 - 101214866:C17	4.52278	Pi-Alkyl
A:TRP525 - 101214866:C17	4.33315	Pi-Alkyl
A:TYR529 - 101214866	4.63732	Pi-Alkyl
A:TYR529 - 101214866:C15	4.29869	Pi-Alkyl
A:TYR529 - 101214866:C17	5.15981	Pi-Alkyl

VI. CONCLUSION

The present study gives an insight into the mechanism involved in radical scavenging ability of Trolox. Trolox is non-toxic and metabolized in cells and its degradation products include Trolox quinine. Trolox show effective interaction with M3 receptor and can be used for future in-vitro and in-vivo investigations.

ACKNOWLEDGEMENT

This work was supported by grants from the DST-PURSE Program [SR/59/z-23/2010/38 (C) dt.27.6.2011], New Delhi; and UGC-CPEPA Scheme [F.No.8-2/2008 (NS/PE) dt.14/12/2011], New Delhi. Authors also acknowledge DBT-BIF facility, *Maharani Lakshmi Ammanni College for Women* for providing Discovery studio software for the study.

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