

Computational and pharmacological investigation of novel 1,5-diaryl-1,4-pentadien-3-one derivatives for analgesic, anti-inflammatory and anticancer potential

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ABSTRACT

Objective(s): The novel 1,5-diaryl-1,4-pentadien-3-one derivatives were studied for analgesic, anti-inflammatory and anticancer potential to establish their role in pain, inflammatory disorders and cancer.

Materials and Methods: Two 1,5-diaryl-1,4-pentadien-3-one derivatives: (1E,4E)-5-(4-fluoro phenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17) and (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) were synthesized and characterized via ¹H NMR and ¹³C NMR techniques. Molecular docking, anti-inflammatory, analgesic and anticancer activities were performed using Auto Doc Vina, carrageenan mediated paw edema and formalin induced chronic inflammation, acetic acid induced writhings and hotplate assay and brine-shrimp lethality assay.

Results: A2K2A17 and A11K3A11 showed high computational affinities (binding energy > -9.0 Kcal/mol) against COX-1, kappa receptor and braf kinase domain. A2K2A17 and A11K3A11 exhibited moderate docking affinities (binding energy > -8.0 Kcal/mol) against COX-2, human capsaicin receptor, tumor necrosis factor, lipoxygenase, colony stimulating factor, delta receptor, cyclin dependent protein kinase-2, mitogen activated kinase, mu receptor and kit kinase domain. A2K2A17 and A11K3A11 possess low docking affinities (binding energy > -7.0 Kcal/mol) against purinoceptor, platelets-derived growth Factor-1 and vascular-endothelial growth factor. In analgesic activity, A2K2A17 (1-30 mg/kg) and A11K3A11 (1-10 mg/kg) decreased acetic acid induced writhes and prolonged the latency time ($P < 0.01$, $P < 0.001$ vs saline group) respectively. A2K2A17 (10-30 mg/kg) and A11K3A11 (1-10 mg/kg) reduced carrageenan as well as formalin mediated edema ($P < 0.01$, $P < 0.001$). A2K2A17 found effective for cytotoxicity assay with LC₅₀ value 1.5 µg/ml.

Conclusion: The *in silico*, *in vitro* and *in vivo* studies on A2K2A17 and A11K3A11 reports their computational binding affinities against targets as well as the analgesic, anti-inflammatory and the anticancer effects.

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Introduction

Pain is an unpleasant sensation which is associated with tissue damage (1). Noxious effects such as ulceration, gastrointestinal bleeding by nonsteroidal anti-inflammatory drugs and drowsiness, nausea and tolerance by opioid analgesic limits their use in pain management (2).

Inflammation is the reaction of living tissues to damage. It includes different events such as activation of enzyme, release of inflammatory mediator and fluid extravasation, migration of cell, tissue breakdown and repair (3, 4). Inflammatory ailments remains one of the major health concerns (5, 6). The adverse effects with nonsteroidal anti-inflammatory drugs (NSAID's) such as gastric lesions, dependence and tolerance produced by opiates, use of NSAID's and opiates has not been effective in all cases (7, 8).

Cancer is diverse group of progressive disorders, characterized by the abnormal and rapid proliferation

and is a major problem worldwide. To cope with this problem new site selective drug discovery and development is required (9).

The 1,5-diarylpentanoid dibenzylidene acetone is the parent structure having an acyclic di-enone attached with aryl groups at b-position. These structures are similar to those of the curcuminoid (1, 7-diaryl heptanes) and the chalcone (1, 3-diaryl propanes). The synthetic chalcone have shown different pharmacological activities; antitumor (10), antioxidant (11) and anti-inflammatory (12, 13). Two 1,5-diaryl-1,4-pentadien-3-one derivatives are: (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17) and (1E, 4E)-5-(4-nitro phenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) were synthesized. A2K2A17 and A11K3A11 were studied for analgesic, anti-inflammatory and anticancer effects, using different computational and pharmacological assays. The structures of A2K2A17 and A11K3A11 are shown in Figure 1.

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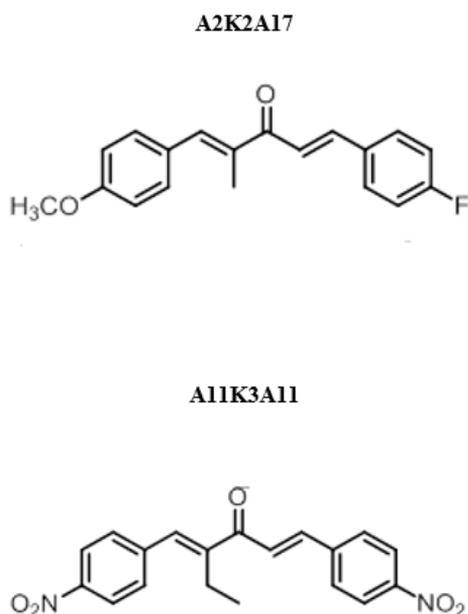


Figure 1. Chemical structure of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17) and (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11)

Materials and Methods

Chemicals

Acetic acid (DAEJUNG Reagents Chemicals), carrageenan (Sigma-Chemicals Co, St-Louis, USA), dimethyl sulphoxide (DMSO), diclofenac (Olive Labs National industrial zone, Islamabad, Pakistan), Formalin (BDH Laboratory supplies, Poole, England), tramadol (Searle company limited F-319, Karachi, Pakistan), methotrexate and ethanol.

Test animals

Mice 25-30 g (Balb-C, n=5 in each group) were kept according to standard protocols ($25 \pm 2^\circ\text{C}$), with natural duration of Light/Dark cycle, each of 12 hrs. Healthy diet was given to mice and water *ad libitum*. The whole study was performed according to the protocols of Animal Resources Institute, Life Science University,

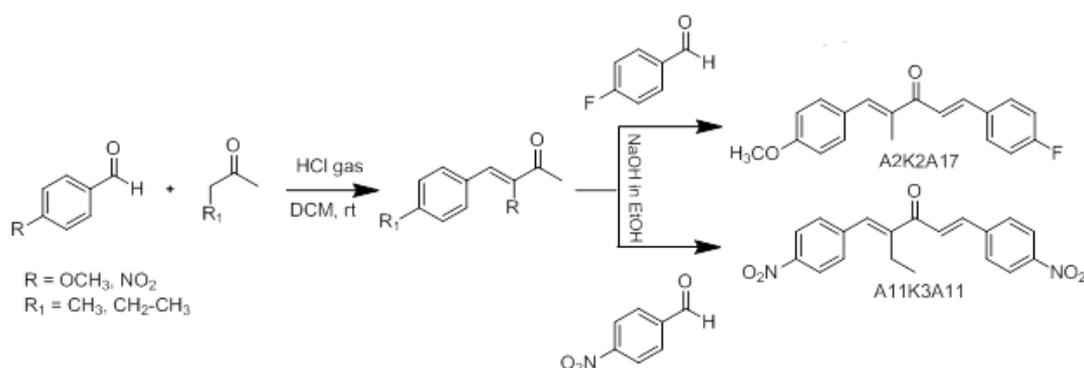
National Research Council (NRC 1996), with prior approval by Ethics Committee of RIPS (Riphah Institute of Pharmaceutical Sciences) with Reference no; REC/RIPS-2016/0012.

Synthesis

The reaction of *p*-methoxy benzaldehyde with 2-butanone in the presence of HCl gas in dichloromethane produces intermediate compounds. The intermediate is further reacted with *p*-Flouro benzaldehyde in ethanol yielding compound A2K2A17. The synthesis of A2K2A17 is reported (14). A11K3A11 is synthesized by first reacting *p*-nitro benzaldehyde with 2-pentanone producing intermediate A11K3, which on further reaction with *p*-nitrobenzaldehyde produces compound A11K3A11 as shown in Scheme 1. Chemical characterization of A11K3A11 was carried out based on the analysis of spectroscopic and crystallographic data.

Computational analysis

Docking is a tool for computational analysis, which is used to investigate affinity and interaction between target protein and ligand (15). We used Auto Dock Vina program for docking study through PyRx. Affinity was determined using interactions of ligand with receptor complex and expressed in the form of binding-energy (E value, Kcal/mol). The 3D-structures of A2K2A17 and A11K3A11 were prepared through Bioviadiscovery Studio Visualizer (DSV) and saved as PDB format. The 3D-structures of target proteins were taken from <http://www.rcsb.org/pdb./home.do>. The proteins target involved in pain, inflammation and cancer pathways are cyclo-oxygenase-1 (COX-1, PDB-ID: 3N8X), cyclo-oxygenase-2 (COX-2, PDB-ID: IPXX), mu receptor (PDB-ID: 5CIM), kappa receptor (PDB-ID: 4DJH), delta receptor (PDB-ID: 4EJ4), human capsaicin receptor (HCR, PDB-ID: 3J9J) and purinoceptor-3 (P2X3, PDB-ID: 5SVL), C-4 synthetase (PDB-ID: 2UUH), tumor necrosis factor (TNF, PDB-ID: 1TNF), lipooxygenase (5-LOX, PDB-ID: 3O8Y), colony stimulating factor (CSF, PDB-ID: 3UF2), cyclin dependent protein kinase-2 (CDPK-2, PDB-ID: 1HCL), mitogen activated kinase (MAK-ERK-1, PDB-ID: 2ZQO), insulin like growth factor-1 (ILGF-1, PDB-ID: 1B9G), platelets derived growth factor-1 (PDGF-1, PDB-ID: 1PDG), braf kinase domain (PDB-ID, 4R5Y), vascular



Scheme 1. Synthesis of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17) and (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11)

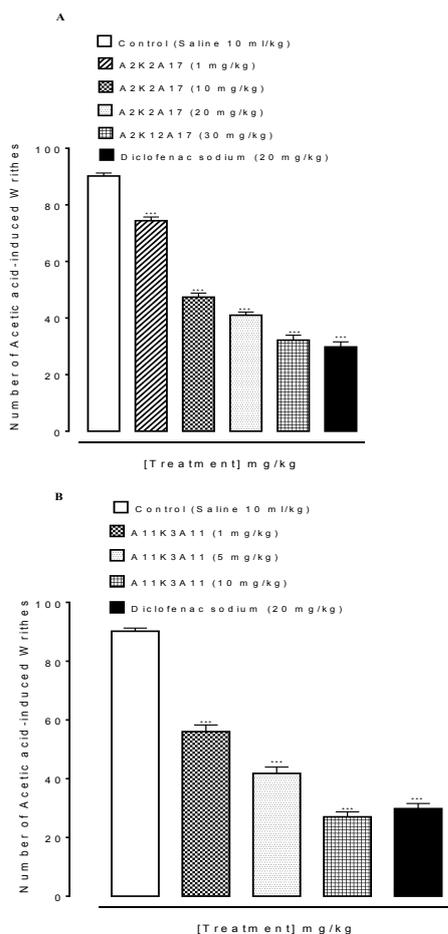


Figure 2. A and B represents the effect of (1E, 4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17) and (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) respectively, on acetic acid-induced writhes in mice. Data expressed as mean \pm SEM, n=5. *** P <0.001 vs. saline group, one way analysis of variance with *post hoc* Tukey's test

endothelial growth factor (VEGF, PDB-ID: 1VPF), nuclear factor kappa (NFK, PDB-ID: 1NFK) and kit kinase domain (PDB-ID: 3G0E). All target proteins were purified by Biovia Discovery Studio Client 2016. The 3D-structures of standard drug molecules were downloaded from the data base (<https://pubchem.ncbi.nlm.nih.gov/search/>). Standard analgesic, anti-inflammatory and anti-cancer drugs are aspirin (Pubchem-CID: 2244), apszepine (Pubchem-CID: 2733484), morphine (Pubchem-CID: 5288826), thymoquinone (Pubchem-CID: 10281), vemurafenib (PubChem-CID: 42611257), sunitinib (Pubchem-CID: 5329102), curcumin (PubCem-CID: 969516) and itraconazole (PubChem-CID: 5793). All these structures were taken in form of .xml and converted to PDB-Format using Open-Babel JUI software. PDB form of both ligand and standard as well as target proteins are converted to PDBQT via Auto Dock Tools (Version1.5.6 Sep_17_14). Both test compounds along with protein targets were loaded in software named as PyRx and then docked against respective targets binding affinity was calculated shown in Kcal/mol. For post docking interaction Discovery studio visualizer was used for number of hydrogen bond (classical/non-classical) and binding residues of

amino-acid: Alanine (ALA), Asparagine (ASN), Arginine (ARG), Cysteine (CYS), Aspartic acid (ASP), Glutamine (GLN), Serine (SER), Proline (PRO), Glutamic acid (GLU), Glycine (GLY), Histidine (HIS), Tryptophan (TRP), Threonine (THR), Tyrosine (TYR), Valine (VAL), Leucine (LEU), Lysine (LYS) and Phenylalanine (PHE).

Analgesic activity

Acetic acid induced writhings

The analgesic potential of test compounds was determined by acetic acid induced writhings in mice (16). The animals after 12 hr fasting were divided into 5 different groups (5 mice in each group). After 30 min of the administration of A2K2A17 (1, 10, 20 and 30 mg/kg, IP) and A11K3A11 (1, 5 and 10 mg/kg), writhings were induced by intraperitoneal injection (IP) of acetic acid (0.1 ml, 0.7% v/v) to induce pain. Pain perception was recorded in the form of stretch of hind limb and abdominal constriction called as writhe. Some mice showed half writhe. Two half writhes were considered as equal to one full writhe. The writhings were recorded for 20 min. Normal saline (10 ml/kg) was given to saline treated group-negative control while diclofenac (20 mg/kg) was administered to positive control group.

Hot plate assay

The mice were distributed into 5 different groups (n=5 in each group). The mice were placed on hot assay plate individually (55 ± 2 °C) and observations (licking paws or jumping) at 30, 60, 90 and 120 min were measured. The latency period of test compounds: A2K2A17 (1, 10, 20 and 30 mg/kg) and A11K3A11 (1, 5 and 10 mg/kg), were evaluated via hot plate assay according to the protocols with little modifications (17). Normal saline (10 ml/kg) was given to control group, tramadol 30 mg/kg (centrally acting opioid analgesic) was used as positive control.

Anti-inflammatory models

Carrageenan mediated paw edema

The mice were fasted overnight. The displacement of paw was determined using Plethysmometer, Ugo-Basile, before administering any drug (18). The animals were placed into 5 groups (5 mice in each group). Acute inflammation was induced in mice by carrageenan subplantar injection (0.1 ml, 1 % w/v). Saline (10 ml/kg) was given to negative control group. Half an hr, prior to carrageenan injection, the animals were administered with test compound: A2K2A17 (10, 20 and 30 mg/kg) and A11K3A11 (1, 5 and 10 mg/kg) IP. The standard drug diclofenac sodium (20 mg/kg), was administered to the positive control group. The paw volume was measured at 0 to 5 hr, with 1 hr interval following carrageenan injection (19).

Formalin induced edema

The anti-inflammatory potential against chronic inflammation was determined using formaldehyde mediated edema (20). The animals were divided into 5 groups (n=5). The base line paw volume displacement was determined using Plethysmometer. Inflammation induced by sub-aponeurotic administration of formalin (0.1 ml, 2% v/v formaldehyde) in the left hind paw on first day and third day. Normal saline (10 ml/kg)

Table 1. E-value (Kcal/mol) and post-docking analysis of best pose of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one(A2K2A17), (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one(A11K3A11) and standard drugs with cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), mu receptor, kappa receptor, delta receptor, human capsaicin receptor (HCR) and purinoceptor-3 (P2X3)

Target	PDB-ID's	A2K2A17			A11K3A11			Standard drugs			
		Kcal/mol	No of H-Bonds	Binding Residues	Kcal/mol	No of H-Bond	Binding residues	Standard	Kcal/mol	No of H-Bonds	Binding residues
COX-1	3N8X	-9.1	2	CYS-47 ASP-135	-9.8	7	ARG-49 TRP-323 GLN-461,44 HIS-43 VAL-48 ARG-469	Aspirin	-6.6	3	GLN-461 PRO-153 ARG-469
COX-2	1PXX	-8.5	3	ALA-156 GLN-1543 ARG-44 HIS-297 LYS-233	-8.9	4	ARG-2376 ASN-2537, 3375 PRO-2538	Aspirin	-7.0	3	TRP-1387 HIS-1388, 1207
Mu opioid	5C1M	-8.3	2	SER-116 THR-321	-7.9	1	HIS-297	Morphine	-7.2	2	HIS-297 ASP-147
Kappa opioid	4DJH	-9.1	2	SER-116 THR-321	-9.7	0	NIL	Morphine	-8.0	0	NIL
Delta opioid	1EJ4	-8.4	0	NIL	-8.4	1	LYS-81	Morphine	-7.2	0	NIL
HCR	3J9J	-8.5	1	TRP-294	-8.7	1	ARG-177	Capsazepine	-8.2	3	ASN-57 SER-103 TYR-107
P2X3	5SVL	-7.2	1	PHE-43	-7.7	2	GLY-40 TYR-49	Capsazepine	-5.4	2	ASP-266 ASN-279

Glutamine (GLN), cysteine (CYS), arginine (ARG), tyrosine (TYR), serine (SER), tryptophan (TRP), alanine (ALA), threonine (THR), histidine (HIS), asparagine (ASN), valine (VAL), lysine (LYS), proline (PRO), glycine (GLY), phenylalanine (PHE) and aspartic acid (ASP)

was given to negative control group. The paw volume displacement of A2K2A17 (10, 20 and 30 mg/kg) and A11K3A11 (1, 5 and 10 mg/kg) administered IP, were measured up to 10 days. Diclofenac sodium (20 mg/kg) was used as the positive control.

Cytotoxicity assay

Brine-shrimp lethality test

The cytotoxicity of test compound was measured using brine shrimp cytotoxicity assay (21). This method requires the preparation of simulated sea water for the hatching of brine shrimp eggs. The newly hatched larvae (nauplii) were observed in the lighter compartment because of their photosensitive nature. Living nauplii (n=10) were collected by Pasteur's pipette and added to the vials of 96 well plate then simulated sea water (5 ml) was added to each vial. These nauplii were subjected to serial dilution of the each of the test compound (1-1000 ug/ml) in 5% DMSO and the number of larvae killed were counted after 24 hr of incubation using 3×magnifying glass. The lethal concentrations (LC₅₀) were then calculated for all of test compounds.

Statistical evaluation

Data expressed as the mean±SEM (Standard Error of Mean). The results were evaluated by GraphPadprism (Graphpad, San-Diego/CA, USA) with one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test. *P*<0.05 was considered different significantly.

Results

Spectral analysis of A11K3A11

¹H NMR (400 MHz, CDCl₃) δ 8.32 – 8.24 (m, 4H), 7.75 (t, *J* = 12.1 Hz, 3H), 7.57 (d, *J* = 8.6 Hz, 2 H), 7.52 – 7.41 (m, 2H), 2.65 (q, *J* = 7.5 Hz, 2 H), 1.16 (t, *J* = 7.5 Hz, 3H). The ¹³C NMR (101 MHz, CDCl₃) δ 191.46 (1C), 148.72 (1C), 147.59 (1C), 142.22 (1C), 141.40 (1C), 141.07 (1C), 135.87 (1C), 129.98 (2C), 129.03 (3C), 125.85

(1C), 124.38 (2C), 124.03 (2C), 20.77 (1C), 13.65 (1C).

Docking evaluation

Table 1 summarized the E-value (Kcal/mol), hydrogen bond and binding residues of A2K2A17 and A11K3A11 with target proteins (COX-1, COX-2, mu receptor, kappa receptor, delta receptor, HCR and P2X3) involved in pain pathways along with standard drugs. The E-value (Kcal/mol), hydrogen bonds and binding residues of A2K2A17 and A11K3A11 with target proteins: C-4 synthetase, TNF, 5-LOX and CSF involved in inflammation along with standard drugs are shown in Table 2. The E-value, hydrogen bonds and binding residues of A2K2A17 and A11K3A11 with target proteins: braf kinase domain, CDPK-2, MAK-ERK-1, ILGF-1, PDGF-1, VEGF, NFKB and kit kinase domain involved in cancer pathway along with standard drugs are shown in Table 3.

Effect on acetic acid-induced writhings

Saline (10 ml/kg) treated group showed 90.20 ± 1.068 numbers of writhes. The writhes count of A2K2A17 (1, 10, 20 and 30 mg/kg) treated groups decreased to 74.40 ± 1.32, 47.40 ± 1.43, 41.00 ± 1.140 and 32.200 ± 1.77 (*P*<0.001 vs saline group) (Figure 2A). The writhes count of A11K3A11 (1, 5 and 10 mg/kg) decreased to 56 ± 2.280, 41.80 ± 2.154, and 27 ± 1.70 (*P*<0.001 vs. saline group) respectively (B). Diclofenac sodium (20 mg/kg) decreased numbers of writhes to 28.80 ± 1.77 (*P*<0.001 vs. saline group).

Effect on latency time in hot plate assay

The latency time of saline (10 ml/kg) treated group at 0, 30, 60, 90, 120 min was 7.35 ± 0.12, 8.33 ± 0.13, 8.56 ± 0.10, 8.71 ± 0.10 and 8.70 ± 0.03 sec respectively. A2K2A17 dose dependently (1, 10, 20 and 30 mg/kg) prolonged latency time (*P*<0.01 vs. saline group) against thermal pain generation (Figure 3A). A11K3A11 dose dependently (1, 5 and 10 mg/kg) prolonged latency

Table 2. E-value (Kcal/mol) and post-docking analysis of best pose of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17), (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) and standard drugs with C4-synthetase, tumor necrosis factor (TNF), lipooxygenase (5-LOX) and colony stimulating factor (CSF)

Target	PDB-ID's	A2K2A17			A11K3A11			Standard drugs			
		Kcal/mol	No of H-Bond	Binding residues	Kcal/mol	No of H-Bond	Binding residues	Standard	Kcal/mol	No of H-bonds	Binding residues
C4-Synthetase	2UUH	-7.2	0	NIL	-6.8	0	NIL	Thymoquinone	-3.0	6	SER-57 GLN-19 SER-23
TNF	1TNF	-8.1	1	GLU-104	-8.5	2	TYR-115 GLN-102				
5-LOX	308Y	-8.0	1	ASN-328	-8.4	2	PHE-67 ARG-384	Thymoquinone	-4.0	3	ASN-425 LEU-420 ALA-424
CSF	3UF2	-7.7	2	LYS-100 GLU-62	-8.7	1	PHE-67	Vemurafenib	-11.6	3	TYR-6 LEU-85 CYS-93

Glutamine (GLN), cysteine (CYS), arginine (ARG), tyrosine (TYR), serine (SER), alanine (ALA), asparagine (ASN), lysine (LYS), leucine (LEU), phenylalanine (PHE) and aspartic acid (ASP)

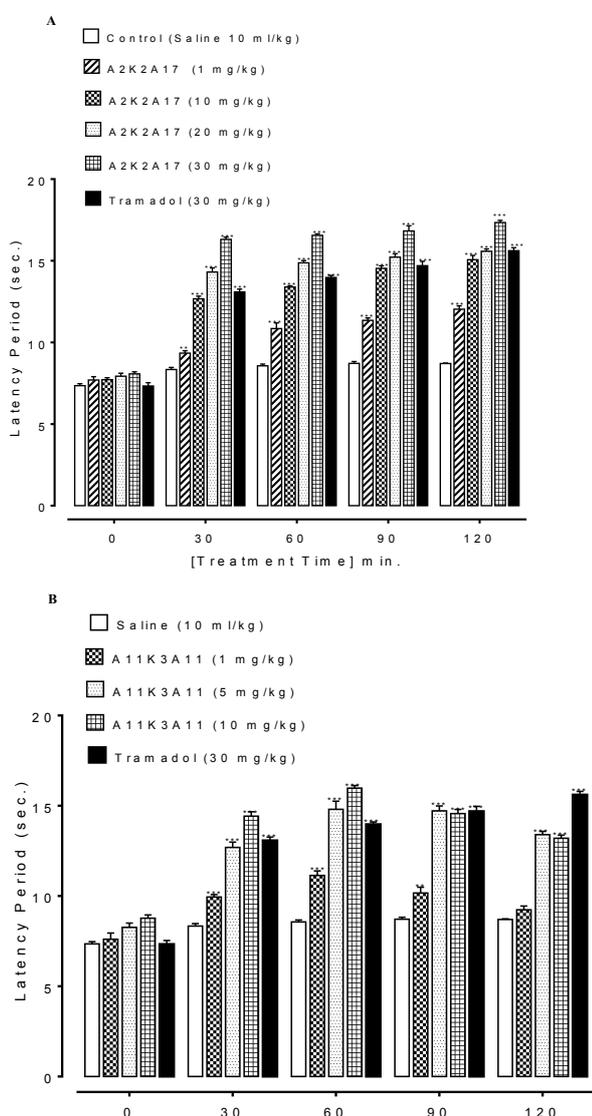


Figure 3. A and B represents the effect of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17) and (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) respectively on latency time in hotplate assay. Data expressed as mean \pm SEM, n=5. ** P <0.01, *** P <0.001 vs. saline group, one way analysis of variance with *post hoc* Tukey's test

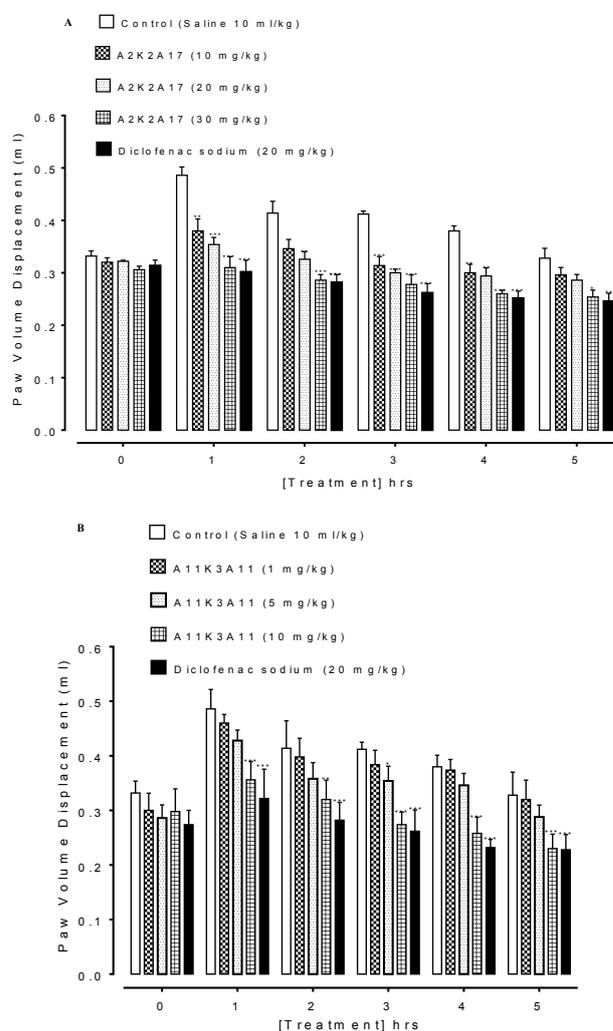


Figure 4. A and B represents the effect of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17) and (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) respectively on carrageenan induced paw edema in mice. Values shown are mean \pm SEM, n=5. ** P <0.01, *** P <0.001 vs. saline group, one way analysis of variance with *post hoc* Tukey's test

Table 3. E-value (Kcal/mol) and post-docking analysis of best pose of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17), (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) and standard drugs with braf kinase domain, cyclin dependent protein kinase-2 (CDPK-2), mitogen activated kinase (MAK-ERK-1), insulin like growth factor-1 (ILGF-1), platelet derived growth factor-1 (PDGF-1), vascular endothelial growth factor (VEGF), nuclear factor kappa b (NFkB) and kit kinase domain

Target	PDB-ID's	Kcal/mol	A2K2A17		Kcal/mol	A11K3A11		Standard	Standard Drugs		Binding residues
			No of H-Bonds	Binding residues		No of H-Bonds	Binding residues		Kcal/mol	No of H-bonds	
Braf kinase domain	4R5Y	-8.1	1	ASP 594	-9.3	3	THR-529, SER-536, 535	Vemurafenib	-13.7	8	CYS-532 GLN-530 THR-529 GLU-501 ASP-594 HIS-574 ARG-36 THR-41,47
CDPK-2	1HCL	-8.3	2	LYS-129 GLY-131	-7.8	1	LYS-33	Sunitinib	-7.1	3	ASP-594 HIS-574 ARG-36 THR-41,47
MAK-ERK-1	2Z0Q	-8.6	3	ARG-87, 165, 189	-8.4	3	ARG-87, 165, 189	Sunitinib	-7.4	5	ASN-316 THR-223 SER-225, 219 ASP-196
ILGF-1	1B9G	-5.8	1	THR-4	-6.0	2	LYS-55 THR-4				
PDGF-1	1PDG	-6.3	2	VAL-39 SER-50	-7.1	2	VAL-39 ARG-56 LYS-48	Sunitinib	-6.9	2	VAL-22 SER-50 ILE-43 CYS-68
VEGF	1VPF	-7.0	3	CYS-68 GLY-59 LYS-48	-7.8	1	SER-110 LYS-146 THR-143	Itraconazole	-8.0	2	ARG-154 THR-143
NFKB	1NFK	-6.6	3	SER-72 ASN-136 SER-63	-6.8	3	ARG-815 CYS-809 GLY-676	Curcumin	-7.1	2	GLY-556,812 ARG-815
Kit kinase domain	3G0E	-8.8	2	THR-670 ASP-810	-8.5	3		Sunitinib	-8.4	4	

Glutamine (GLN), cysteine (CYS), arginine (ARG), tyrosine (TYR), serine (SER), glutamic acid (GLU), threonine (THR), histidine (HIS), asparagine (ASN), valine (VAL), lysine (LYS), isoleucine (ILE), glycine (GLY) and aspartic acid (ASP)

time ($P < 0.01$ vs. saline group) against thermal pain generation (Figure 3B). Tramadol (30 mg/kg) reduced the latency ($P < 0.001$ vs. saline group).

Effect of A2K2A17 and A11K3A11 on Carrageenan mediated paw edema

A2K2A17 (10-30 mg/kg) reduced the carrageenan mediated inflammation in a dose dependent way (Figure 4A). The subplantar injection of carrageenan produce edema which progressively increases with time in the saline treated control group. Treatment of animal with A2K2A17 (10-30 mg/kg) and A11K3A11 (1-10 mg/kg) decreased carrageenan mediated paw inflammation ($P < 0.05$, $P < 0.01$, $P < 0.001$ vs saline group) as presented in Figure 4 (A and B) respectively. Similarly, diclofenac (20 mg/kg) decreased the carrageenan induced inflammation in paw.

Effect on formalin mediated inflammation

A significant increase in the left hind paw thickness was observed in the saline treated control group after formalin injection. Continuous treatment with A2K2A17 (10-30 mg/kg) and A11K3A11 (1-10 mg/kg) remarkably reduces paw edema ($P < 0.05$, $P < 0.01$, $P < 0.001$ vs saline). The reduction in paw thickness was observed from the day 1st and throughout the time period of study (10 days), compared with saline treated group shown in Figure 5 (A and B) respectively. Diclofenac at dose of 20 mg/kg reduces the paw edema ($P < 0.001$ vs saline group).

Effect on brine shrimp lethality

A2K2A17, A11K3A11 and methotrexate exhibited concentration-dependent (1, 3, 5, 10, 100, 300 and 1000 µg/ml) cytotoxic effect against brine shrimps (Table 4). The larvae killed by A2K2A17 and A11K3A11, with LC_{50} value of 1.50 µg/ml and 107.29 µg/ml respectively. The cytotoxic effect by methotrexate occurs at LC_{50} value of 3.39 µg/ml.

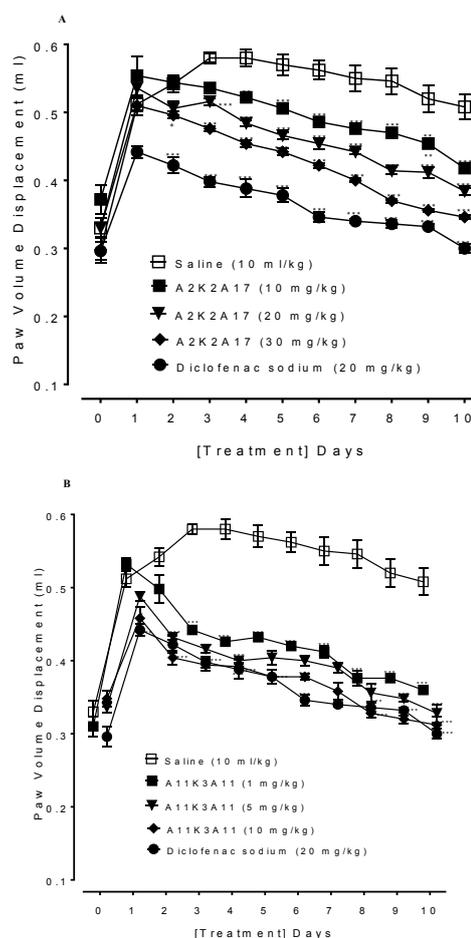


Figure 5. A and B represents the effect of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17) and (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) respectively on formalin induced inflammation in hind paw of mice. Values shown are mean \pm SEM, $n=5$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. saline group, one way analysis of variance with *post hoc* Tukey's test

Table 4. Concentration-dependent ($\mu\text{g/ml}$) cytotoxic effect of (1E,4E)-5-(4-fluorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-dien-3-one (A2K2A17), (1E,4E)-5-(4-nitrophenyl)-1-(4-nitrophenyl)-2-ethylhexa-1,4-dien-3-one (A11K3A11) and methotrexate against brine shrimps

Test samples	Concentration ($\mu\text{g/ml}$)							LC ₅₀ Values ($\mu\text{g/ml}$)
	1	3	5	10	100	300	1000	
A2K2A17	33.3 \pm 0.33	73.3 \pm 0.33	90.0 \pm 0.00	96.6 \pm 0.33	100 \pm 0.00	100 \pm 0.00	100 \pm 0.00	1.50
A11K3A11	20 \pm 0.00	20.66 \pm 0.33	30.33 \pm 0.33	43.3 \pm 0.33	46.6 \pm 0.33	66.6 \pm 0.33	76.6 \pm 0.33	107.29
Methotrexate	15.4 \pm 5.1	48.3 \pm 4.5	59.7 \pm 3.5	81.3 \pm 3.50	91.7 \pm 6.7	100 \pm 0.0	100 \pm 0.0	3.39

Values shown as % mean \pm SEM, n=5

Discussion

Molecular docking has gained valuable importance in the field of drug development. The main purpose of docking is to get the preliminary information about affinity of any compound before the start of *in vivo* experiment. The docking of novel 1, 5-diaryl-1, 4-pentadien-3-one derivative i.e, A2K2A17 and A11K3A11 were carried out using Autodock Vina program through PyRx (22). Docking tool was preliminary tool used to check the affinity of ligands to their respective protein targets. These interactions may be in the form of hydrogen bonds, hydrophobic interaction and Van der Waal forces (23). Hydrogen bonding has significant role in the formation of ligand protein complex. In this study we assessed affinity of ligand using three parameters: E-value, number of hydrogen bond and amino acid residue against the protein targets involve in pain, inflammation and cancer. A2K2A17 order of affinity against target protein was found as: COX-1 > kappa receptor > braf kinase domain > COX-2 > HCR = CSF > kit kinase domain > TNF > MAK-ERK-1 > lipooxygenase > delta receptor > mu receptor > CDPK > VEGF > P2X3 > PDGF-1 > NFK > C-4 synthetase > ILGF-1. A11K3A11 order of affinity against target proteins was found as: COX-1 > kappa receptor > kit kinase domain > MAK-ERK-1 > COX-2 > HCR > delta > mu receptor > CDPK > braf kinase domain = TNF > lipooxygenase > CSF > P2X3 > C4 synthetase > VEGF > NFK > PDGF-1 > ILGF-1.

The analgesic activity was tested with two protocols; Acetic acid induce writhes method and hot plate assay, to evaluate the peripheral as well as central effects of analgesia (24). A2K2A17 and A11K3A11 showed dose-dependent analgesic effect. A2K2A17 is more effective in increasing the latency period compared to A11K3A11, while both compounds were equally effective against acetic acid induce writhing. Acetic acid produces nociception by release of chemical mediator such as histamine, serotonin, bradykinins, prostaglandin and substance-P due to induction of COX-2 that results in increased pain sensitivity after acetic acid injection (25). Generally used nonsteroidal anti-inflammatory drug (NSAID's) such as diclofenac sodium and indomethacin have shown anti-nociceptive effect, by decreasing the production of prostaglandin through blockage of COX-2.

Carrageenan induced inflammation is a well-known experimental model to determine the anti-inflammatory activity (26). Carrageenan produces inflammation via release of several mediator of inflammation (such as

histamine, serotonin, prostaglandins and bradykinin) in early and late phases (27). Several studies show that substances which decreases the carrageenan-induced edema, produces the prostaglandins synthesis inhibition by the cyclo-oxygenase (COX) enzyme inhibition (28). A2K2A17 and A11K3A11 caused carrageenan produced paw edema inhibition in a dose dependent fashion similar to the effect caused by a standard NSAID i.e diclofenac sodium. The NSAID's reduces pain, swelling and inflammation through inhibition of COX-enzyme in the arachidonic acid pathway (29). A2K2A17 and A11K3A11 were effective in inhibiting carrageenan mediated paw edema. On the basis of these results, it can be said that the anti-inflammatory action of A2K2A17 and A11K3A11 occurs through the inhibition of prostaglandin synthesis. A2K2A17 and A11K3A11 were further investigated against chronic inflammation using formalin induced edema (30). Two injections sub-aponeurotically were used to induce the chronic inflammation, the inflammation is characterized by increase in paw-thickness and increase volume, responses of chronic inflammation (20). The test compounds were effective in reduction of the formalin induced cellular damage. The test compounds were investigated for *in vitro* cytotoxicity using brine shrimps assay. The lethality of brine shrimp is because of less developed membrane susceptibility to cytotoxic chemical (15). A2K2A17 was found to be more effective with 1.5 $\mu\text{g/ml}$ LC₅₀ value as compared to A11K3A11, with 107.29 $\mu\text{g/ml}$ LC₅₀ value. Moreover the anticancer potential needs further investigation by screening the test compounds through cell line assays using human cancer cells.

Conclusion

Computational studies reveal binding affinities of 1,5-diaryl-1,4-pentadien-3-one derivatives: A2K2A17 and A11K3A11 against proteins targets involved in the pathogenesis of pain, inflammation and cancer as well as exhibit analgesic, anti-inflammatory and anticancer activities which explore their therapeutic effectiveness in pain, inflammatory disorders and tumor. Further studies are warranted to determine safety profile, pharmacokinetics and pharmacodynamics of test compounds to establish them as lead molecules.

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Conflicts of Interest

The authors declare no conflicts of interest.

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