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Mosquito Larvicidal activity on derivatives of adenine

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Abstract

Derivatives of adenine **(I)** 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid, **(II)** 4-(((9H-purin-6-yl)imino)methyl)phenol, **(III)** 3-(((9H-purin-6-yl)imino) methyl) phenol, **(IV)** 2-(((9H-purin-6-yl)imino)methyl)phenol, **(V)** N-(4-chlorobenzylidene)-9H-purin-6-amine and **(VI)** N-(4-methoxybenzylidene)-9H-purin-6-amine were prepared as reported in the literature[1] and characterized by physical and analytical data, FTIR , ^{1}H NMR, ^{13}C NMR spectra[2,3] and were screened for larvicidal activity against larvae of *Aedes aegypti*.

Keywords: Zika virus, *Flaviviridae*, *Aedes aegypti*, *Juvenile Hormone*, antibacterial, antifungal, larvicidal, antiparasitic, anticancer, 5-formyl-2-hydroxybenzoic acid, 4-Hydroxybenzaldehyde, 3-Hydroxybenzaldehyde, 2-Hydroxybenzaldehyde, 4-Chlorobenzaldehyde and 4-Methoxybenzaldehyde.

Introduction

Zika virus discovered in 1947 belongs to the family of *Flaviviridae* and the genus flavi virus, West Nile virus, *dengue* virus[4]. They are transmitted to humans through a day time bite of an infected female *Aedes aegypti*[5] mosquito such as dengue fever, yellow fever, West Nile fever, chikungunya and eastern equine encephalitis. Zika virus usually remains in the blood of an infected person for a week but it can be found longer in some people. The incubation period for Zika virus fever is not known but is likely to a week.

Dengue virus infected female of *Aedes aegypti* is capable of transmitting the virus for the rest of its lifetime after ten days of virus incubation. It lives near dense population area of human being and breed mostly in a variety of water-holding containers, tree holes and bromeliad leaf axils and bamboo trunks. Artificial containers include a variety of man-made receptacles such as discarded tires, cans, flower pots, bird baths, pet dishes, and many, many others containers. It is a day-time feeder. Its peak biting periods is early in the morning andin the evening before dusk.

The female of mosquitos bites many people during each feeding period. Currently there is no vaccine or treatment for the Zika virus. An obvious method for preventing the spread of these diseases is to control larvae of *Aedes aegypti* population by larvicides which have been

developed and employed in the field with considerable success. In recent years, derivatives of nucleic acid base were found to have potential non-toxic and non-antibiotic resistance of antibacterial, antifungal, mosquito larvicidal, antiparasitic and anticancer properties. They have been prepared by starting from nucleic acid bases like cytosine or adenine and aldehydes[6-17] or ketones. In the present study we have prepared **(I)** 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxy benzoic acid, **(II)** 4-(((9H-purin-6-yl)imino)methyl)phenol, **(III)** 3-(((9H-purin-6-yl)imino) methyl) phenol, **(IV)** 2-(((9H-purin-6-yl)imino)methyl)phenol, **(V)** N-(4-chlorobenzylidene)-9H-purin-6-amine and **(VI)** N-(4-methoxybenzylidene)-9H-purin-6-amine and have been subjected to *in vitro* larvicidal activities against larvae of *Aedes aegypti*.

Materials and Methods

Materials

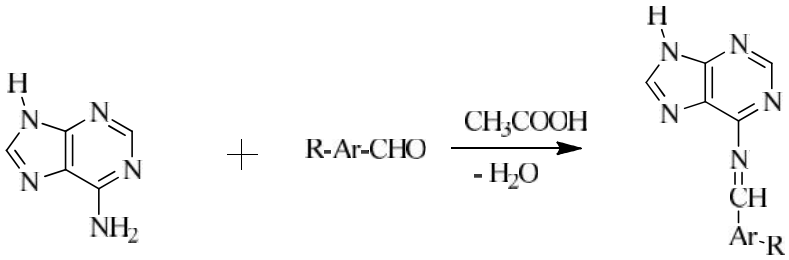
All the reagents used were of AR grade. Commercially available rectified spirit was dried over anhydrous quicklime for 24 hours, filtered and distilled before use (BP 78 ^{o}C). Dimethylsulphoxide (sigma) and N, N dimethylformamide (sigma) were used as such. adenine, 5-formyl-2-hydroxybenzoic acid, 4-Hydroxybenzaldehyde, 3-Hydroxybenzaldehyde, 2-Hydroxybenzaldehyde, 4-Chlorobenzaldehyde and 4-Methoxybenzaldehyde were purchased from Alfa Aesar.

Instruments

Melting points were determined using Elico melting point apparatus. Elemental analyses were performed using ElementarVario EL III. IR spectra of the compounds were recorded with KBr pellets with carry 630 FTIR Spectrometer in the 4000-400 cm-1range. The 1HNMR and 13CNMRspectra were recorded on a Bruker 400 MHz FT- PMR Spectrometer.

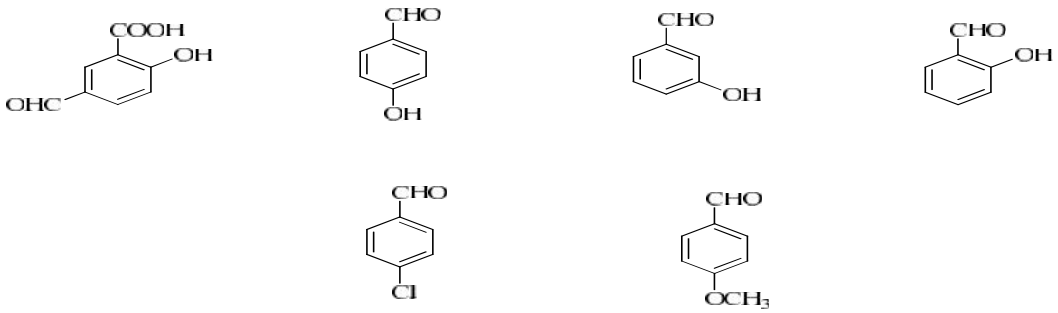
General preparation of derivatives of adenine

All the azomethine compounds of derivatives of **adenine** were prepared as reported in the literature[6-18]by the following scheme – 1



Scheme 1

Where, R-Ar-CHO =



Preparation of (I)5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid[1-3]

25ml of ethanolic solution of adenine(1.36 g 0.01mol) was added to 25ml of ethanolic solution of 5-formyl-2-hydroxybenzoic acid(1.66 g 0.01mol).Then three drops of acetic acid was added to the reaction mixture. The reaction mixture was heated and refluxed for about 5 hours at 90°C. The resulting solution was further concentrated by water bath. The product 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid obtained was precipitated, cooled and collected after filtration. The precipitate was purified by washing with distilled water and then with ethanol. The 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid was again recrystallized in ethanol and then dried over vacuum desiccator.

Preparation of (II)4-(((9H-purin-6-yl)imino)methyl)phenol

2.7 grams of adenine (1.36 g 0.01mol) was mixed with 2.44 g of 4-hydroxybenzaldehyde (1.22 g 0.01mol) and was grained well in acidic acid medium at room temperature. The mixture was transferred into hundred milliliter Round Bottom flask and was refluxed for six hours in oil bath. The solid product 4-(((9H-purin-6-yl)imino)methyl)phenol was filtered and washed with ethanol and recrystallized in DMSO and then dried over vacuum desiccator.

Preparation of (III) 3-(((9H-purin-6-yl)imino) methyl) phenol,

A mixture of 3-hydroxybenzaldehyde (1.22 g 0.01mol) and adenine(1.36 g 0.01mol) was grained in a mortar with a pestle made of porcelain for 10 minutes. The mixture turned pasty after few minutes of grinding. It was grained yellow Colour product appears. The mixture was left overnight. The resultant product 3-(((9H-purin-6-yl)imino) methyl) phenol, was recrystallized using ethanol and then dried over vacuum desiccator.

Preparation of (IV)2-(((9H-purin-6-yl)imino)methyl)phenol

Equimolar quantities of 0.01 mole of adenine (1.36 g 0.01mol) and2-hydroxybenzaldehyde (1.22 g 0.01mol) were dissolved in 20 ml of DMSO and 3 drop of glacial acetic acid was added and refluxed for 3 hours. After completion of the reaction(monitored by TLC), some solvent was distilled out, the reaction mixture was poured on ice cold water and the solid 2-(((9H-purin-6-yl)imino)methyl)phenol came out which was filtered and then recrystallized by DMSO and then dried overvacuum desiccator.

Preparation of (V)N-(4-chlorobenzylidene)-9H-purin-6-amine

N-(4-chlorobenzylidene)-9H-purin-6-amine was prepared from equimolar quantity of adenine(1.36 g 0.01mol) and 4-Chlorobenzaldehyde(1.40 g 0.01mol).in 30 ml of methanol were heated at 70oC on water bath for 4-hrs in presence of few drops of glacial acetic acid. The crude product was obtained after removal of methanol under reduced pressure. The products were recrystallized from methanol and then dried over vacuum desiccator.

Preparation of (VI)N-(4-methoxybenzylidene)-9H-purin-6-amine

A mixture 4-Methoxybanzaldehyde(1.36 g 0.01mol) and adenine (1.36 g 0.01mol) were grained with a pestle in an open mortar at room temperature for 3 minutes. To this reaction mixture sulphuric acid 2 drops and 20ml DMF were added and grained for 5 minutes. On completion of reaction as monitored by TLC, the light greenish-colored solid N-(4-methoxybenzylidene)-9H-purin-6-aminewas was separated out. The obtained solid was isolated by simple Buchner filtration and was recrystallized from DMF and then dried over vacuum desiccator.

Aedes aegypti rearing

The mosquito larvae of *Aedes aegypti* and *Aedes albopictus* werecollected from National Centre for disease control, Government of India ministry of healthand family welfare, Southern India branch, field station, Mettupalayam, Coimbatore district, Tamilnadu, India. The larvae were kept in the plastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

Larvicidal bioassay (Abbott, 1925).

The larvicidal activities of six novel derivatives (I-VI) of adenine were assessed by using the standard method as prescribed by WHO. From the stock solution, five different test concentrations (100, 150, 200 and 250ppm was prepared and tested against the freshly molted (0 – 6 hrs) 4thinstar larvae of *Aedes aegypti* and *Aedes albopictus* DMSO (emulsifier) in water was treated as control. The larvae of these mosquito species (10 larvae) was introduced in 250-ml plastic cups containing 100 ml of aqueous medium (99 ml of dechlorinated water + 1ml of emulsifier) and the required amount of six novel derivatives of adenine was added. The larval mortality was observed and recorded after 24 hrs of post treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula[19] (Abbott, 1925). The LC_{50}, LC_{90}, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), chi-square values and the degrees of freedom was calculated by using Probit analysis with Statistical Package for Social Sciences (SPSS) 16.0 Version in MS-Excel, 2007

Results

The physical and analytical data of the derivatives adenine **(I)** 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid**, (II)** 4-(((9H-purin-6-yl)imino)methyl)phenol, **(III)** 3-(((9H-purin-6-yl)imino) methyl) phenol, **(IV)** 2-(((9H-purin-6-yl)imino)methyl)phenol, **(V)** N-(4-chlorobenzylidene)-9H-purin-6-amine and **(VI)** N-(4-methoxybenzylidene)-9H-purin-6-amine are given in table.1

(I)2-hydroxy-5-(((2-oxo-1,2-dihydropyrimidin-4-yl)imino)methyl)benzoic acid,
(II) 4-(((9H-purin-6-yl)imino)methyl)phenol,
(III)4-((3-hydroxybenzylidene)amino)pyrimidin-2(1H)-one,
(IV)2-(((9H-purin-6-yl)imino)methyl)phenol,
(V)N-(4-chlorobenzylidene)-9H-purin-6-amine and
(VI)N-(4-methoxybenzylidene)-9H-purin-6-amine are given in table.1

[I] 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid

FTIR (cm^{-1}):3640 & 679 cm^{-1} ($-O-H$), 3433 & 751 cm^{-1} ($-\overset{|}{N}-H$), 1687 cm^{-1} ($>C=O$), 1660 cm^{-1} ($-N=\overset{|}{C}H$), 1624 cm^{-1} ($-N=\overset{|}{C}-$), 1300 cm^{-1} ($-\overset{||}{C}-OH$) & 1219 cm^{-1} ($Ar-OH$)

^{1}HNMR δ(ppm): 11.0 (s, 2H) (Purin Ring & Acid), 9.03 (s, 1H), 8.57 (s, 1H), 8.53 (s, 1H), 8.48 (s, 1H), 8.12 (d, 1H), 7.23 (d, 1H) &5.35 (s, 1H)

^{13}CNMR δ(ppm):171.8 (s), 164.5 (s), 160.0 (s), 158.6(s), 153.8 (s), 152.4 (s), 144.5 (s), 135.8 (s), 130.9 (s), 126.2 (s), 125.2 (s), 118.0 (s) &112.1 (s)

[II]4-(((9H-purin-6-yl)imino)methyl)phenol

FTIR (cm^{-1}):3590 & 660 cm^{-1}($-O-H$), 3270 & 880 cm^{-1} ($-\overset{|}{N}-H$), 1680 cm^{-1} ($-N=\overset{|}{C}H$), 1620 cm^{-1} ($-N=\overset{|}{C}-$) & 1150 cm^{-1} ($Ar-OH$)

^{1}HNMR δ(ppm):11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 7.78 (d, 2H), 6.85 (d, 2H)&5.35 (s, 1H)

^{13}CNMR δ(ppm):160.8 (s), 160.0 (s), 158.6 (s), 153.8 (s), 152.4 (s), 144.5 (s), 130.6 (s), 129.0 (s), 125.2 (s), 116.0 (s)

[III] 3-(((9H-purin-6-yl)imino)methyl)phenol

FTIR (cm^{-1}):3580 & 680 cm^{-1} (—O—H), 3270 & 820 cm^{-1} (—N—H), 1120 cm^{-1} (Ar—OH), 1620 cm^{-1} (—N=C—) & 1660 cm^{-1} (—N=CH)

^{1}HNMR δ(ppm):11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48(s, 1H), 7.46 (s, 1H), 7.39 (d, 1H), 7.25 (t, 1H), 7.02 (d, 1H) &5.35 (s, 1H)

^{13}CNMR δ(ppm):160.0 (s), 158.6 (s) (-N=C- &Ar-OH), 153.8 (s), 152.4 (s), 144.5 (s), 138.7 (s), 130.2 (s), 125.2 (s), 121.8 (s), 118.2 (s), 114.9 (s)

[IV]2-(((9H-purin-6-yl)imino)methyl)phenol

FTIR (cm^{-1}): 3586 & 651 cm^{-1}(—O—H), 3289 & 778 cm^{-1} (—N—H), 1678 cm^{-1} (—N=CH), 1615 cm^{-1} (—N=C—) & 1219 cm^{-1} (Ar—OH)

^{1}HNMR δ(ppm): 11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 7.66 (d, 1H), 7.52 (t, 1H), 7.08 (t, 1H), 7.02 (d, 1H) & 5.35 (s, 1H)

^{13}CNMR δ(ppm):161.8 (s), 161.1 (s), 160.0 (s), 153.8 (s), 152.4 (s), 144.5 (s), 132.4 (s), 132.1 (s), 125.2 (s), 121.4 (s), 120.5 (s) & 117.8 (s)

[V] N-(4-chlorobenzylidene)-9H-purin-6-amine

FTIR (cm^{-1}):3289 & 889 cm^{-1} (—N—H), 1678 cm^{-1} (—N=CH), 1606 cm^{-1} (—N=C—) & 778 cm^{-1} (Ar—Cl)

^{1}HNMR δ(ppm):11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 7.77 (d, 2H) &7.52 (d, 2H)

^{13}CNMR δ(ppm):160.0 (s), 158.6 (s), 153.8 (s), 152.4 (s), 144.5 (s), 136.6 (s), 134.5 (s), 130.6 (s), 128.9 (s), 125.2 (s)

[VI] N-(4-methoxybenzylidene)-9H-purin-6-amine

FTIR (cm^{-1}):3270 & 780 cm^{-1}(—N—H), 1640 cm^{-1} (—N=CH), 1620 cm^{-1} (—N=C—),1250 cm^{-1} (Ar—OR), 1110 cm^{-1}(ArO—R) & 1010 cm^{-1} (—N—C—)

^{1}HNMR δ(ppm):11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 7.84 (d, 2H), 7.06 (d, 2H) &3.83 (s, 3H)

^{13}CNMR δ(ppm):162.9 (s), 160.0 (s), 158.6 (s), 153.8 (s), 152.4 (s), 144.5 (s), 130.2 (s), 128.7 (s), 125.2 (s), 114.4 (d) &55.8 (s)

Table 1 The physical and analytical data of derivatives of adenine

Derivatives of Cytosine	Molecular Weight	Nature of Appearance	% of yield	Elemental Analysis (in %)				
				C	H	O	N	Cl
[I] $C_{13}H_9N_5O_3$	283.2423	Yellow Crystalline Solid	85	55.13	3.20	16.95	24.73	-
[II] $C_{12}H_9N_5O$	239.2328	Yellow Crystalline Solid	68	60.25	3.79	6.69	29.27	-
[III] $C_{11}H_9N_3O_2$	215.2081	Yellow Crystalline Solid	75	61.39	4.22	14.87	19.53	-
[IV] $C_{12}H_9N_5O$	239.2328	Yellow Crystalline Solid	77	60.25	3.79	6.69	29.27	-
[V] $C_{12}H_8ClN_5$	257.6784	Yellow Crystalline Solid	81	55.93	313	-	27.18	13.76
[VI] $C_{13}H_{11}N_5O$	253.2593	Yellow Crystalline Solid	59	61.65	4.38	6.32	27.65	-

Larvicidal activity

Larvicidal activity[20-30] of all azomethine compounds are determined as recommended by WHO in150, 200, 250 and 300ppm concentration in dimethyl sulfoxide(DMSO) solvent. The results are compared with derivatives of adenine **(I)** 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid, **(II)** 4-(((9H-purin-6-yl)imino)methyl)phenol, **(III)** 3-(((9H-purin-6-yl)imino) methyl) phenol, **(IV)** 2-(((9H-purin-6-yl)imino)methyl)phenol, **(V)** N-(4-chlorobenzylidene)-9H-purin-6-amine and **(VI)** N-(4-methoxybenzylidene)-9H-purin-6-amine **Table. 2**

Table 2.Larvicidal activity of derivatives of adenine against larvae of *Aedes aegypti*

Compounds	Con .(ppm)	Larval mortality	95% Confidence Limits (ppm)		
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2
I	150	19.22±1.20	218.39 (201.12-237.17)	386.58 (349.62-442.94)	3.793
	200	31.31±3.34			
	250	49.30±1.50			
	300	78.40±4.20			
II	150	20.60±3.40	188.64 (169.21-197.43)	311.79 (289.41-342.39)	0.056
	200	36.40±3.40			
	250	48.30±3.30			
	300	88.30±1.30			
III	150	22.10±2.60	205.97 (187.70-224.82)	383.06 (344.91-442.25)	2.929
	200	36.50±3.20			
	250	56.00±2.40			
	300	79.30±2.44			
IV	150	20.30±2.20	226.59 (206.25-250.18)	428.47 (378.50-511.58)	1.149
	200	33.70±5.40			
	250	52.00±2.30			
	300	70.20±3.30			
V	150	21.20±1.30	185.12 (169.06-200.34)	329.98 (303.61-367.46)	0.401
	200	39.30±2.40			
	250	73.00±1.20			
	300	83.30±1.50			
VI	150	29.30±2.30	170.01 (152.89-185.46)	316.52 (290.72-353.37)	1.984
	200	41.20±3.20			
	250	72.00±2.20			
	300	90.20±1.20			

Values are mean ± S.D of five replication; Number of larvae =10; LC_{50}=Lethal concentration 50 and LC_{90}=Lethal concentration 95; Values with different alphabet in column are statistically significant (p<0.05 level; DMRT).

Larvicidal activity of derivatives of adenine **(I)** 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid, **(II)** 4-(((9H-purin-6-yl)imino)methyl)phenol, **(III)** 3-(((9H-purin-6-yl)imino) methyl) phenol, **(IV)** 2-(((9H-purin-6-yl)imino)methyl)phenol, **(V)** N-(4-chlorobenzylidene)-9H-purin-6-amine and **(VI)** N-(4-methoxybenzylidene)-9H-purin-6-amine are given in the **Table 2.** The Larvicidal activities of azomethine compounds I-VI (Table. 2) clearly indicate that allthe compounds (I-VI) control the growth of larvae. The nature of bonding[6] and structure of azomethine organic compounds are elucidated by the elemental analysis, melting point, FTIR, ^{1}HNMR,^{13}CNMR,spectral analysis, chromatography and molar ratio methods.

In accordance with the data obtained in the present investigation, it is found that the larvicidal activity of **(I)** 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid, **(II)** 4-(((9H-purin-6-yl)imino)methyl)phenol, **(III)** 3-(((9H-purin-6-yl)imino) methyl) phenol, **(IV)** 2-(((9H-purin-6-yl)imino)methyl)phenol, **(V)** N-(4-chlorobenzylidene)-9H-purin-6-amine and **(VI)** N-(4-methoxybenzylidene)-9H-purin-6-amine increases depend upon the functional groups present in the derivatives of adenine Schiff bases (IV<I<III<V<II<VI) **Table 2**.

Discussion

Juvenile Hormone are secreted by a pair of endocrine glands behind the brain called the corpora allata. They are also important for the production of eggs in female *Aedes aegypti* mosquitoes. They were discovered in 1965 by Williams and Slama and the first molecular structure was solved in 1967. They are a group of acyclic sesquiterpenoids that regulate many aspects of mosquitoes physiology[21-23]. They regulate development, reproduction, diapause, and polyphenisms. In mosquitoes, Juvenile hormones which ensure growth of the larva, while preventing metamorphosis. Because of their rigid exoskeleton, mosquitoes grow in their development by successively shedding their exoskeleton. It is a process known as molting. Derivatives of adenine act as a Juvenile Hormone, like methoprene analog against larvae [24-26]the growth regulator when used as an larvicides.

Most insect species contain only juvenile growth hormone III.To date Juvenile hormones, Juvenile hormones I, and Juvenile hormones II have been identified only in butterflies and moths-Lepidoptera. The form Juvenile hormones B_3 appears to be the most important Juvenile hormones in the diptera or flies. Certain species of crustaceans have been shown to produce and secrete methyl farnesoate, which is juvenile hormone III lacking the epoxide group. Methyl farnesoate is believed to play a role similar to that of Juvenile hormones in crustaceans. Being a sesquiterpenoids, Juvenile hormones chemical structure differs significantly from the structure of other animal hormones. Some Juvenile hormones analogs have been found in conifers.

Derivatives of adenine **(I)** 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid, **(II)** 4-(((9H-purin-6-yl)imino)methyl)phenol, **(III)** 3-(((9H-purin-6-yl)imino) methyl) phenol, **(IV)** 2-(((9H-purin-6-yl)imino)methyl)phenol, **(V)** N-(4-chlorobenzylidene)-9H-purin-6-amine and **(VI)** N-(4-methoxybenzylidene)-9H-purin-6-amine are believed to play a role similar to that of Juvenile hormones in crustaceans. Juvenile hormone synthesis is controlled by the rate of flux of isoprenoid precursors, with a complex interplay of changes in precursor pools, enzyme levels and nutritional and developmental modulators, such as 20-hydroxyecdysone 20E, ecdysiast-triggering hormone, insulin and allatostatin-C. Juvenile hormone uses multiple molecular mechanisms to exert its pleiotropic functions at different stages of the mosquito life cycle. Juvenile hormone acts via an unidentified membrane receptor. Here derivatives of adenine treated larvae of *Aedes aegypti* died [27-30] without growth regulator, Juvenile hormone.

Conclusion

The derivatives of adenine **(I)** 5-(((9H-purin-6-yl)imino)methyl)-2-hydroxybenzoic acid, **(II)** 4-(((9H-purin-6-yl)imino)methyl)phenol, **(III)** 3-(((9H-purin-6-yl)imino) methyl) phenol, **(IV)** 2-(((9H-purin-6-yl)imino)methyl)phenol, **(V)** N-(4-chlorobenzylidene)-9H-purin-6-amine and **(VI)** N-(4-methoxybenzylidene)-9H-purin-6-amine were prepared by the condensation of 5-formyl-2-hydroxybenzoic acid, 4-Hydroxybenzaldehyde, 3-Hydroxybenzaldehyde, 2-Hydroxybenzaldehyde, 4-Chlorobenzaldehyde and 4-Methoxybenzaldehyde with a denine and were screened against larvae of *Aedes aegypti*. It was concluded that the increase in the larval mortality of *Aedes aegypti* depend upon the functional groups present in the Schiff bases.

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