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TOC

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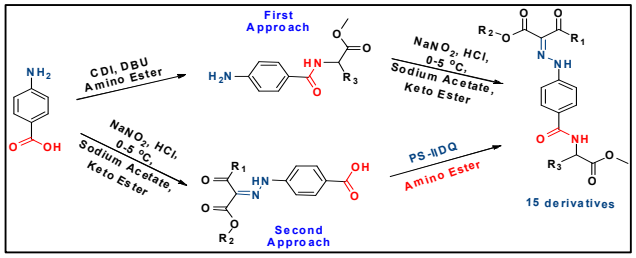

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New Journal of Chemistry RSCPublishing

ARTICLE

The effect of aryl hydrazono ester containing dipeptides (AHED) on mosquito egg-laying behaviour†

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Sumit K. Agrawal,[a] Sachin Tikar,[b] Ruchi Yadav,[b] Anand K. Halve[c] and Manisha Sathe[a,*]

A series of novel aryl hydrazono esters containing dipeptides (AHED) were synthesized by using polystyrene-supported 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydro- quinoline (PS-IIDQ) to study the oviposition responses in *Aedes albopictus* mosquitoes at 1 ppm and 10 ppm concentrations. Two different synthetic routes have been optimized successfully for the synthesis of target compounds. Pheromone and semiochemical mediated oviposition activity in mosquitoes is a well known aspect in mosquito behavioral ecology. Structural elucidation of synthesized AHED was achieved by spectral analysis. In dual choice experiment; the oviposition responses of gravid *Ae. albopictus* was evaluated against AHED-1 to AHED-15 at two different concentrations. Among all the compounds; AHED-6 showed maximum oviposition attractancy with an oviposition activity index (OAI) of +0.538 at 10 ppm. While in contrast to this, AHED-13 exhibited highest oviposition deterrent activity with OAI of –0.774 at 1 ppm.

Introduction

Aedes albopictus also called the Asian tiger mosquito is a day biting mosquito that transmits the viruses that causes dengue and chikungunya. The *Ae. albopictus* population is on rise and it has out competed *Ae. aegypti* in some of its habitats. Some of the possible reasons may be sterility of offspring from interspecific mating; reduced fitness of *Ae. aegypti* from parasites brought in with *Ae. albopictus* and; superiority of *Ae. albopictus* in larval resource competition.[1] Researchers now-a-days are more focused on eco-friendly approaches using semiochemicals of natural and synthetic route to control vectors of medical importance.[2] Identification of suitable oviposition sites by mosquitoes is a critical feature for their life history, because it ultimately influence the survivorship of their progeny.[3] Oviposition behavior in mosquitoes is influenced by visual, tactile and olfactory factors, with the latter considered being of primary importance. These cues include color and optical density of the water, texture and moisture, temperature and reflectance of the oviposition substrate.[4] The female mosquito lays eggs in water holding containers around or further away from homes, trees holes and bamboo internodes etc. Most of the behaviors associated with reproduction/oviposition are mediated by chemical cues of different origin and therefore such chemical identities can determine the ultimate survival of mosquito population.[5] The most important aspect in any organism's life is to ensure successors after them. Therefore, in order to abolish a vector like mosquito, killing of the adult as well as its progeny is of the prime remedial importance. The chemical factors involved in oviposition site selection by mosquitoes have gained much interest in recent years and considerable attention has been paid to the chemical cues influencing mosquito oviposition.[6] Earlier efforts were made to identify a potential synthetic attractant or repellant for mosquitoes using short-chain fatty acid esters against *Ae. aegypti*.[7] The role of larval water and pre-existing eggs in oviposition by *Ae. aegypti* and *Ae. albopictus* were described by allen et al.[8] In addition to this certain fatty acids and esters were identified from egg extracts of *Ae. aegypti* as oviposition attractant.[9] Oviposition response of *Ae. aegypti* and *Ae. albopictus* to certain fatty acid esters[10] laid the foundation to explore synthetic compounds of newer origin. To the best of our knowledge, limited report are available regarding oviposition responses of *Ae. albopictus* to chemical compounds.[10,11]

In view of these we recently reported the synthesis of thirteen derivatives of aryl hydrazono esters (AHE) and their oviposition responses against *Ae. albopictus*, the series of compound showed oviposition attractant as well as deterrent responses against *Ae. albopictus*.[12] From the reported study it was also anticipated that the presence of one or more ester group or other side chain may alter the oviposition responses or may have potential application in mosquito trapping for identification, surveillance and control. Therefore, encouraged by this study[12] and in continuation of an ongoing research program aiming at finding the new synthetic route to the bioactive compounds[13] new series of aryl hydrazono esters containing dipeptides (AHED) have been designed and synthesized using polymer-supported 2-isobutoxycarbonyl-1,2-dihydroquinol-one (PS-IIDQ). PS-IIDQ was easily regenerated; intensive washing followed by reaction with isobutyl

chloroformate yielded a recycled polymer-supported IIDQ with efficiency similar to the original material.[14] In dual choice experiment; the oviposition responses of gravid *Ae. albopictus* was evaluated against AHED-1 to AHED-15 at 1 ppm and 10 ppm concentrations.

Results and discussion

At this stage; we have selected three methyl amino esters (isolecucine, phenylalanine and aspartate methyl ester) and five different keto-esters (ethyl acetoacetate, methyl acetoacetate, isobutyl acetoacetate, ethyl, trifluoro acetoacetate and ethyl, benzoyl acetate) to check the effect of side chains of amino acid and keto-esters on oviposition responses. Hence, we designed and focused our studies only for the synthesis and bio-evaluation of fifteen derivatives of AHED. It has been well documented that the AHE exists in mainly two tautomeric forms (Figure 1).[15] We envisioned two pathways to AHED 1 (Figure 1): disconnecting the diazo linkage reterosynthetically to give aromatic amine containing dipeptides **2** and disconnecting amide linkage reterosynthetically to give aromatic acid containing diazo compound **3**. Conveniently, both the precursors disconnect back to 4-amino benzoic acid **4**.

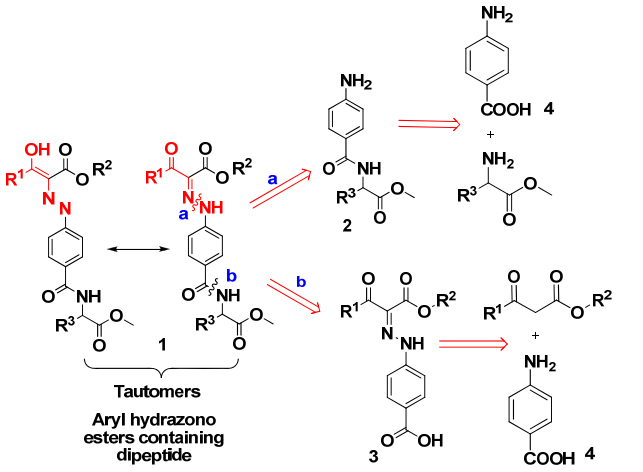
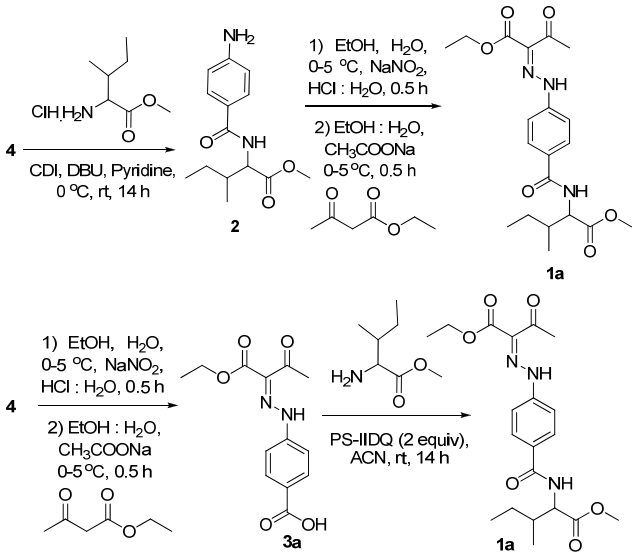


Fig. 1 Tautomeric forms and reterosynthetic analysis of aryl hydrazono esters containing dipeptide (AHED)

Initially, the synthetic optimization has been done for the synthesis of **1** *via* route 'a' (Scheme 1, top). Title compound **1a** was chosen as a model substrate for the optimization of reaction conditions (scheme 1). In the route 'a', synthesis of **2** was achieved *via* 1,1'-carbonyl diimidazole (CDI), 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) and pyridine mediated peptide coupling of **4** with $HCl.NH_2$-Ile-OCH_3. The crude was purified by silica gel column chromatography to afford 60% of desired product. The resulted dipeptides **2** was taken in ethanol : water (1 : 1) and reacted with aqueous sodium nitrite solution in acidic medium (aq HCl) under ice-cold condition (0-5 ^oC) to form corresponding diazonium chloride salt intermediate, which was further condensed with ethanolic solution of ethyl acetoacetate in presence of sodium acetate under ice-cold condition to form desire compound[16] with other undesired products (based on TLC), the desired compound **1a** was purified and isolated with 20% yield.

In order to establish a better synthetic strategy for the synthesis of title compound **1**, we then turned our attention to the route 'b' (Scheme 1, bottom). In the route 'b', **4** were first diazotized by as earlier described method to give **3a** cleanly in 90% yield. Although initial attempts at coupling of **3a** with NH_2-Ile-OCH_3 utilizing CDI, that start product formation after 20-22 h at room temperature (Table 1, entry 1), coupling with *N*-(3-dimethyl-aminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC.HCl) (Table 1, entry 2) and di-isopropyl carbodiimides (DIC) (Table 1, entry 3) showed desired product formation with other undesired products (based on TLC) after 26 h at room temperature. The reaction conditions were further optimized for the coupling step of route 'b' *i.e.* formation of **1a** *via* **3a** by using solid supported coupling reagents.



Scheme 1. Synthesis of AHED via route 'a' (top) and 'b' (bottom)

Table 1. Optimization of coupling reaction for the synthesis of **1a** *via* route 'b'

Entry	Coupling Reagent	Equiv	Time (h)	Yield (%)[c]
1	CDI	1	24[a]	20
2	EDC.HCl	1	26[a]	25
3	DIC	1	26[a]	25
4	PS-IIDQ	2	14[b]	65

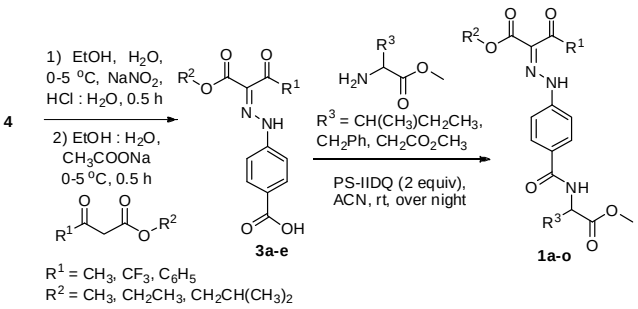
[a]Reaction was incomplete. [b]Reaction completed. [c]Isolated yield (only for second step).

Over the past decade, interest in the development of new polymer-supported reagents has increased,[17] predominantly because these reagents combine the traditional advantages of solution phase chemistry with the convenience of solid-phase handling. Thus using polymer-supported materials, un-reacted reagents and by-products remain on the resin and can be easily removed by filtration at the end of the reaction. In order to standardize the reaction condition and from our earlier experimental protocol,[17c] 2 equiv of PS-IIDQ was used in acetonitrile for the coupling of **3a** with NH_2-Ile-OCH_3 at room temperature (Table 1, entry 4). To our surprise, the desired product **1a** starts forming within 20 minutes. The reaction mixture stirred overnight and resulted into the desired product without giving any other spot on TLC. PS-IIDQ was easily regenerated; intensive washing followed by reaction with isobutyl chloroformate yielded a recycled polymer-supported IIDQ with efficiency similar to the original material.[14] By examining closely the optimized reaction condition for both the routes, we noticed that the route 'b' was preferred over route 'a'. The obtained product after work-up was analytically pure in case of route 'b' while purification was needed for both the steps in case of route 'a'. Furthermore, the obtained overall yield was better in case of route 'b'. With the optimized reaction condition in hand, the scope of the protocol was further explored for the synthesis of various derivatives of AHED (Table 2).

The existence of tautomers of **1** was confirmed by the analysis of spectral data. For example in case of **1a**; the two singlets appearing at 14.56 ppm and 12.62 ppm of the ^{1}H-NMR gives a combined integration of unity for -OH and -NH respectively. In addition to that, two sharp singlets of the methyl group of tautomers collectively for three protons appeared at 2.52 ppm and 2.43 ppm for enol and keto form respectively (See SI). These observations were also reflected in ^{13}C-NMR spectra (refer to experimental section and supporting information). The ratio of the tautomers present in the sample mixture was estimated using quantitative ^{1}H-NMR spectroscopy (against internal standard dimethyl sulfone) (Table 2). Using this protocol, **3a-e** was coupled with different amino ester to give the corresponding AHED (Table 2) in 55-67% yield. Furthermore, the AHEDs containing R^1 = CF_3 group (Table 2, entries **1d**, **1i** and **1n**) exist only in enol form it may be because of the stability of enol form due to the presence of strong intramolecular H-bonding. Additionally, AHEDs containing R^1 = Ph group (Table 2, entries **1e**, **1j** and **1o**) exist in excess of enol form and in rest of the cases keto-enol forms exist in almost equal ratio or slightly excess of keto form. In most of the cases, the reactions were clean and the products were obtained with simple work-up in good yield, but in some cases column chromatography was performed to get the clean NMR spectra. The structures of all the synthesized compounds were deduced by ^{1}H-NMR, ^{13}C-NMR, FT-IR and ESI-MS analysis.

Furthermore, the exact orientation of **1a** was confirmed by ROESY analysis. The ^{1}H-NMR and ROESY spectra of compound **1a** reveals the existence of combination of two isomers (See SI), in which keto form **1a-l** is the major isomer and enol form **1a-ll** is the minor isomer (Figure 2). The ROESY NMR spectrum of **1a** in $CDCl_3$ revealed that, the –NH (**1a-l**) and –OH (**1a-ll**) proton couple with H_a and H_b proton of benzene ring at both sides.

Table 2. General synthetic scheme and derivatives of AHED by route 'b'



Entry	R^1	R^2	R^3	Yield (%)[a]	Keto to enol ratio (%)[b]
1a	CH_3	CH_2CH_3	$CH(CH_3)CH_2CH_3$	65	67
1b	CH_3	CH_3	$CH(CH_3)CH_2CH_3$	58	59
1c	CH_3	$CH_2CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$	59	65
1d	CF_3	CH_2CH_3	$CH(CH_3)CH_2CH_3$	62	NA[c]
1e	C_6H_5	CH_2CH_3	$CH(CH_3)CH_2CH_3$	64	12[d]
1f	CH_3	CH_2CH_3	CH_2Ph	60	47
1g	CH_3	CH_3	CH_2Ph	58	34
1h	CH_3	$CH_2CH(CH_3)_2$	CH_2Ph	55	47
1i	CF_3	CH_2CH_3	CH_2Ph	65	NA[c]
1j	C_6H_5	CH_2CH_3	CH_2Ph	61	12[d]
1k	CH_3	CH_2CH_3	$CH_2CO_2CH_3$	67	66
1l	CH_3	CH_3	$CH_2CO_2CH_3$	60	57
1m	CH_3	$CH_2CH(CH_3)_2$	$CH_2CO_2CH_3$	59	73
1n	CF_3	CH_2CH_3	$CH_2CO_2CH_3$	58	NA[c]
1o	C_6H_5	CH_2CH_3	$CH_2CO_2CH_3$	62	14[d]

[a]Isolated yield (Only for second step). [b]Quantitatively calculated from methyl proton of R^1. [c]Only enol form exist. [d]Quantitatively calculated from NH and OH protons.

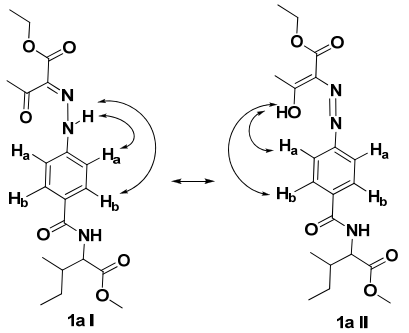


Fig. 2. Exact orientation of compound **1a** in tautomeric form

The plausible mechanism for the formation of AHED is depicted in fig. 3. The treatment of 3 with PS-IIDQ in ACN rapidly generates in situ the corresponding isobutoxycarbonyl mixed anhydride **IV** by nucleophilic substitution and internal rearrangement **II** and **III**. The nucleophilic attack of amine group of amino ester into the **IV** liberates carbon dioxide and isobutanol to form the desired product **1**.

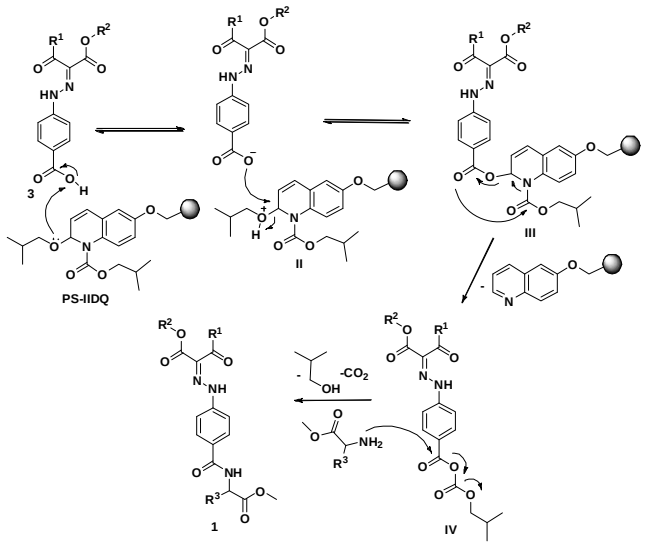


Figure 3. Proposed mechanism for the PS-IIDQ mediated formation of amide

Ae. albopictus used for the oviposition experiments were utilized from the laboratory colony maintained in DRDE insectaries. The experiments were performed at 27±2°C, 75 ± 5 % RH, L10:D14 regime. Three-day-old *Ae. albopictus* after mating (15 females) were kept in separate standard-sized wooden cages (750×600×600 mm) with a sleeve opening on one side. Sucrose (10 %) was provided to adults, and female mosquitoes were fed on rabbit blood on third day after emergence. They were offered with oviposition substrate 48 h post blood meal when they become fully gravid. Laboratory bioassay were performed in separate cages; disposable plastic cup (150 ml capacity) filled with 100 ml of de-chlorinated water were used as the oviposition substrate. HPLC grade methanol was used as solvent to dissolve the compounds and also as control. The glass were rinsed with water and washed with methanol before setting the experiment. The oviposition responses of gravid *Ae. albopictus* were evaluated on simple di-peptide (SDP), aryl hydrazono esters (AHE 1-5) and AHED 1-15 at 1 µg/ml (1 ppm) and 10 µg/ml (10 ppm) for 24 h at optimal room temperature. The dual choice experiment was completely randomized and replicated three times. The basis for measuring the oviposition responses was the number of eggs received in both control and treatment cup. The numbers of eggs laid in control and treatment cups were counted manually to assess the oviposition preference of the mosquito species. The oviposition activity was expressed as oviposition activity index (OAI) and calculated by using the formula OAI = (*Nt* - *Nc*) / (*N*t + *Nc*), where, *Nt* is the number of eggs laid in test cup and *Nc* is the number of eggs laid in control cup. As suggested by Kramer and Mulla, compounds with an OAI of +0.3 and above are considered as attractant, while those with -0.3 and below are considered as repellent.[18]

The oviposition responses of gravid *Ae. albopictus* was evaluated against SDP, AHE-1 to AHE-5 and AHED-1 to AHED-15 (Table 3) at 1 ppm and 10 ppm using standard procedure as mentioned above. Out of 15 compounds from AHED-1-15; AHED-6 showed maximum oviposition attractancy with OAI +0.538 at 10 ppm. Structurally AHED-6 consists of benzyl group at its amino acid side chain along with methyl ketone and ethyl ester on the other side. Additionally at 10 ppm AHED-3 and AHED-5 also elicited increased egg deposition by gravid mosquitoes with OAI +0.362 and +0.413 respectively, both AHED-3 and AHED-5 contains isobutyl group at their amino acid side chains whereas Phenyl ketone group was present in AHED-5 while methyl ketone was present in AHED-3. These results showed that AHED-6, AHED-5 and AHED-3 have potential attractancy towards *Ae. albopictus*. The steric hindrance was minimum in case of AHED-6, which also adds to the attractancy of AHED-6 rather than for AHED-3 and AHED-5.

On the other hand, AHED-13 showed highest negative oviposition response with OAI – 0.774 at 1 ppm; AHED-13 displayed highest repellency at 1 ppm may be due to dose dependence and on increasing the concentration to 10 ppm its deterrence was highly diminished. Structurally AHED-13 consists of additional CO_2Me group at its R^3 position. In addition to this AHED-7 (OAI –0.610) and AHED-14 (- 0.579) also elicited comparable deterrence at 10 ppm *w.r.t.* AHED-13. Structurally AHED-7 consists of benzyl group at its R^3 position while AHED-14 consists of additional CO_2Me group at its R^3 position along with trifloromethane at R^1.

In insects, halogenated analogs were reported as inhibitors of chemical communication.[19] To support this CF_3 group containing AHED-9 and AHED-14 showed negative OAI in both concentrations, except for AHED-4 at 1 ppm which was found to be attractive while its deterrence was observed at 10 ppm.

The OAI value at 1 ppm and 10 ppm was compared (Figure 4), this comparison gave a better idea on the effect of dose dependence towards oviposition responses. While we also conducted experiments on 0.1 ppm concentration for all the compounds but they were not found to be significant.

Furthermore three compounds AHED-1, AHED-4 and AHED-11 showed a sharp dose dependence on concentration in oviposition responses. AHED-1 and AHED-4 exhibited positive response at 1 ppm while the response was inverted at 10 ppm; contrary to these the AHED-11 exhibited the opposite effect. It is very interesting to highlight here that, the mixed oviposition response of *Ae. albopictus* for AHED-1 to AHED-15 was obtained which was due to the structural diversity in AHED than our previously synthesized aryl hydrazono esters.[12]

Table 3. Oviposition response of *Aedes albopictus* to aryl hydrazono ester containing dipeptides

Entry	Code	No. of eggs laid in different treatments (Mean ± Standard Error) Control	1 ppm	OAI Value 1 ppm	No. of eggs laid in different treatments (Mean ± Standard Error) Control	10 ppm	OAI Value 10 ppm
2	SDP	76.00 ± 25.48	88.33 ± 22.05	+0.075	18.00 ± 8.33	20.67 ± 6.36	+0.069
3a	AHE-1	65.67 ± 28.50	45.67 ± 17.57	-0.180	70.33 ± 39.57	77.67 ± 27.81	+0.050
3b	AHE-2	41.33 ± 14.17	59.67 ± 17.70	+0.182	27.00 ± 6.11	33.33 ± 8.88	+0.105
3c	AHE-3	16.66 ± 1.67	21.33 ± 1.86	+0.123	49.67 ± 13.30	82.67 ± 13.93	+0.249
3d	AHE-4	40.00 ± 2.22	23.66 ± 1.20	-0.257	54.67 ± 7.31	29.33 ± 5.46	-0.302
3e	AHE-5	154.33 ± 89.65	150.33 ± 78.19	-0.013	38.67 ± 12.02	30.33 ± 16.86	-0.121
1a	AHED-1	70.00 ± 32.35	78.67 ± 46.71	+0.058	78.33 ± 33.46	67.67 ± 22.28	-0.073
1b	AHED-2	33.66 ± 12.33	19.33 ± 6.98	-0.271	44.33 ± 14.75	23.00 ± 10.15	-0.317
1c	AHED-3	37.33 ± 16.59	34.33 ± 7.75	-0.042	45.00 ± 19.93	96.00 ± 18.50	+0.362
1d	AHED-4	133.33 ± 56.74	188.67 ± 49.82	+0.172	117.67 ± 55.44	70.67 ± 24.61	-0.250
1e	AHED-5	54.00 ± 17.13	57.00 ± 30.01	+0.027	28.00 ± 7.64	67.33 ± 44.85	+0.413
1f	AHED-6	41.33 ± 8.09	40.67 ± 15.56	-0.008	13.33 ± 6.69	44.33 ± 21.46	+0.538
1g	AHED-7	50.67 ± 17.30	23.00 ± 1.33	-0.376	31.67 ± 19.22	7.67 ± 5.36	-0.610
1h	AHED-8	30.33 ± 11.70	21.33 ± 1.33	-0.174	132.00 ± 32.59	63.67 ± 14.45	-0.349
1i	AHED-9	62.67 ± 35.04	31.67 ± 16.76	-0.329	84.67 ± 31.55	66.67 ± 24.58	-0.119
1j	AHED-10	36.33 ± 7.22	21.67 ± 9.21	-0.253	59.33 ± 32.67	58.00 ± 26.85	-0.011
1k	AHED-11	84.33 ± 42.17	61.00 ± 33.15	-0.161	44.33 ± 27.91	64.00 ± 34.22	+0.182
1l	AHED-12	29.00 ± 17.01	35.00 ± 12.01	+0.094	29.00 ± 13.28	42.33 ± 36.38	+0.187
1m	AHED-13	63.00 ± 23.29	9.33 ± 4.91	-0.774	28.33 ± 5.84	23.00 ± 2.08	-0.104
1n	AHED-14	97.00 ± 32.97	70.67 ± 22.17	-0.157	127.67 ± 32.79	34.00 ± 4.93	-0.579
1o	AHED-15	16.33 ± 1.86	13.00 ± 2.08	-0.114	192.67 ± 34.09	114.33 ± 30.80	-0.255

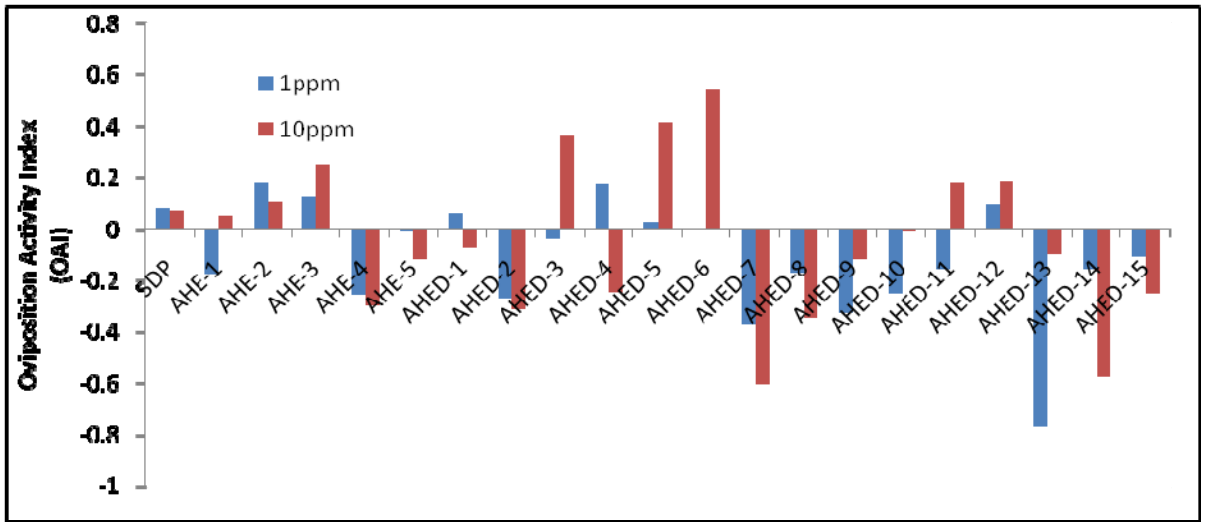


Fig. 4. Comparison of Oviposition Activity Index (OAI) of AHEDs to *Ae. albopictus* at 1 and 10 ppm concentrations

To check the effect of peptide units or amino acid residue on oviposition responses we also evaluated the oviposition responses of mosquitoes on SDP (**2**) and AHE 1-5 (**3a-e**) at 1 and 10 ppm concentration by following the same procedure. The results were illustrated in table 1. It has been clear that as such only peptide unit (SDP) has no effect on oviposition responses; in both the concentrations SDP exhibit neutral responses. While in case of AHE 1-5; AHE-4 showed moderate negative oviposition responses in both the concentrations which is possibly be due to the presence of CF_3 group. Contrary to this AHE-3 showed slightly positive oviposition responses. As such AHE-1, AHE-2 and AHE-5 have neutral effect on oviposition responses in both the concentrations. With these results it has been observed that the peptide units or amino residues were playing significant role on mosquito oviposition behavior. AHED were more functionalized than AHE and simple dipeptides hence showing more diversity in oviposition studies. The diversified oviposition responses were due to functional groups and which can further be examined for other compounds.

Conclusions

In conclusion, we have demonstrated the potential of polymer-supported 2-isobutoxycarbonyl-1,2-dihydroquinolone (PS-IIDQ) as an efficient coupling reagent for the synthesis of a series of hindered and highly functionalized dipeptides. All the fifteen derivatives of novel aryl hydrazono esters containing dipeptides (AHED) have been evaluated for the oviposition responses against *Aedes albopictus*. Among all the compounds; AHED-6 showed maximum oviposition attractancy with an oviposition activity index (OAI) of +0.538 at 10 ppm. Structurally AHED-6 consists of benzyl group at its amino acid side chain along with methyl ketone and ethyl ester on the other side; and therefore having minimum steric hindrance. Whereas AHED-13 exhibited highest oviposition deterrent activity with OAI of –0.774 at 1 ppm which may be due to dose dependence; on increasing the concentration to 10 ppm its deterrence was highly diminished. Structurally AHED-13 consists of additional CO_2Me group at its R^3 position. The present study shows the potential application of these compounds as oviposition attractants and deterrent which may have further applications in mosquito trapping for identification, surveillance and control.

Experimental

General information

All other chemicals were purchased from Sigma-Aldrich, India and used without further purification. Solvents were distilled prior to use. Triple distilled water was used for the reaction. The reactions were performed in air atmosphere without any specific precautions. FT-IR spectra were recorded as KBr pellets on a bruker tensor 27 spectrometer with opus 5.5 software. The ^{1}H NMR spectra (400 MHz) and ^{13}C-NMR (100 MHz) of the synthesized compounds were recorded in bruker avance 400 MHz NMR spectrometer in DMSO-d_6, and $CDCl_3$ solvent and the chemical shifts (d) were expressed in parts per million and coupling constants (J) in hertz. Spin multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass analysis was performed on quadruple-time of flight (Q-Tof) mass spectrometer (Micromass, USA) using electrospray ionization (ESI) in positive mode. TLC is performed using precoated aluminium sheets with silica gel 60 F254. Silica gel column chromatography was performed by using 60-120 mesh size silica manufactured by S. D. Fine-Chem Limited, India.

General Synthetic Procedures

(i) General synthetic procedure for the compound 2 (aromatic amine containing dipeptide) (route 'a', step 1):

To a solution of 4-amino-benzoic acid **4** (1.0 g, 7.299 mmol) and 1,1'-carbonyl diimidazole (1.2 g, 7.407 mmol) in pyridine (5 mL) was added 1,8-diazabicyclo [5,4,0]undec-7-ene (1.1 mL, 7.299 mmol). The mixture was stirred at room temperature for 2 hours followed by the addition of HCl.NH_2-Ile-OCH_3 (1.3 g, 7.3 mmol). The reaction mixture was stirred at room temperature for overnight and diluted with ethyl acetate (100 mL), the solution was washed with water (50 mL x 3). The organic layer was dried over anhydrous sodium sulfate, and concentrated in rotavapour. The resulting residue was purified by silica gel column chromatography (60-120 mesh size) with 20-30% ethyl acetate in hexane as the eluent to afford aromatic amine containing dipeptide methyl ester *i.e.* **2** (1.1 g, 60%).

(ii) General synthetic procedure for the diazotization reaction (aryl hydrazono esters containing dipeptides *i.e.*1a-o (AHED)) (route 'a', step 2 and route 'b', step 1):

The aromatic amine containing dipeptide methyl ester (**2**) or 4-amino-benzoic acid (**4**) (1.0 g, 1 equiv) was dissolved in a mixture of 4.0 mL of concentrated hydrochloric acid (HCl), 4.0 mL of distilled water and 4.0 mL of ethyl alcohol. The amine hydrochloride solution was kept at freezing temperature. To this, an aqueous solution of sodium nitrite ($NaNO_2$) 1.0 equiv in 5.0 mL of distilled water was added drop wise with continuous stirring, keeping the temperature of the reaction vessel at 0–5 ^oC. Meanwhile in another beaker, 5.0 equiv of sodium acetate (CH_3COONa) in a solution of 1.2 equiv ethyl acetoacetate in 25.0 mL of ethyl alcohol was taken and cooled in an ice-bath. Now the diazotized solution was added to this solution drop wise with thorough stirring. The reaction mixture was stirred at the same temperature for 30 minutes.

No solid precipitate formed in case of **2** while solid precipitate formed in case of **4.** Solid was filtered under suction, washed thoroughly with cold water, dried and recrystalized from DMF and ethanol to give the analytically pure diazotized compound that is **3a-e** as yellow solid in 90% yield.

While in case of **2**, the resulting reaction mixture was extracted with ethyl acetate (50 mL x 2). The combined organic layer was washed with water (25 mL x 2) and brine (20 mL), dried over anhydrous sodium sulfate and evaporated in rota vapour. The brown-yellow crude was purified by silica gel column chromatography (60-120 mesh) with 10-15% ethyl acetate in hexane as eluent to afford **1a** as yellow semi solid in 20% yield (0.3 g).

(iii) General synthetic procedure for the preparation of 1a-o using PS-IIDQ (route 'b', step 2):

In a round bottom flask, **3a-e** (acid) (0.2 g, 1 equiv) and amine ester (1 equiv) were stirred in CH_3CN (10 mL) for 10 min. To this, PS-IIDQ (2 equiv) (loading of the resin 1.6 mmol/g) was added. The reaction mixture was then stirred at room temperature for overnight. The un-reacted reagents and by-products (polymer-supported quinolines) remain adsorbed on the resin surface and thereby removed through filtration at the end of the reaction work-up. The mother liquor was then concentrated under reduced pressure. The residue was taken in ethyl acetate (100 mL) and organic layer was washed with 1N HCl solution (10 mL x 2), $NaHCO_3$ solution (10 mL x 2), water and brine (10 mL). The yellow organic layer was dried over anhydrous sodium sulfate and evaporated in rota vapour to afford **1a-o** as yellow semi solid pure product in 55-67%. In some cases column chromatography and washing with n-pentane was done to get the clear NMR spectra.

Acknowledgements

We thank Director, DRDE, Gwalior for his keen interest and encouragement. We also thank Mr. Ajay Pratap and Dr. Avik Mazumder for recording NMR spectra and ROESY spectra respectively. S. K. A. is thankful to DRDO, New Delhi, for financial support.

Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [Experimental Details, NMR Spectra]. See DOI: 10.1039/c000000x/

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