Bioorganic & Medicinal Chemistry Letters 21 (2011) 794–797

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and bio-evaluation of aryl hydrazono esters for oviposition responses in *Aedes albopictus*

Prabal Bandyopadhyay [a], Lopamudra Guha [a], T. Seenivasagan [a], Manisha Sathe [a], Pratibha Sharma [b], B. D. Parashar [a], M. P. Kaushik [a,*]

[a] Defence R & D Establishment, Jhansi Road, Gwalior 474 002 (M.P.), India
[b] School of Chemical Sciences, Devi Ahilya University, Khandwa Road, Indore 452 001 (M.P.), India

ARTICLE INFO

Article history:
Received 25 September 2010
Revised 12 November 2010
Accepted 20 November 2010
Available online 4 December 2010

Keywords:
Aryl hydrazono ester
Oviposition
Aedes albopictus
Dengue
Chikungunya

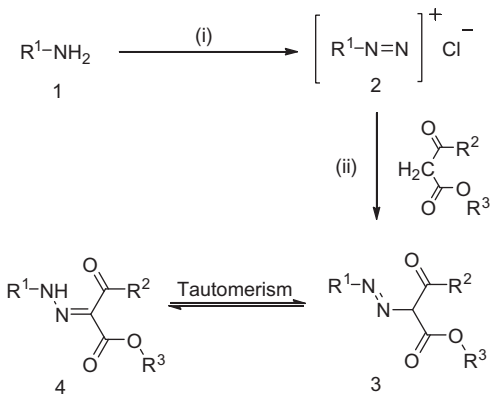
ABSTRACT

A novel series of aryl hydrazono esters (AHE) (1–13) were synthesized (yield 76–98%) to study the oviposition responses in *Aedes albopictus* (Skuse) mosquitoes for the first time. At a concentration of 10 μg ml^{-1} in dual choice experiment, among the screened compounds, AHE-12 showed remarkable oviposition attractant activity with an oviposition activity index (OAI) of +0.299 (greater than 95% confidence limit) comparable to *p*-cresol (OAI +0.320) which is well-reported oviposition attractant for *Aedes aegypti*. Conversely, AHE-10 exhibited highest oviposition deterrent activity with OAI −0.247. The possible utilization of these compounds will be in integrated vector management strategies.

Aedes albopictus (*Ae. albopictus*), is a day biting mosquito, highly anthropophilic and potential vector for dengue and chikungunya. Identification of suitable oviposition sites by mosquitoes is a critical feature of their life history, because it ultimately influences the survivorship of their progeny.[1] 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.[2] 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.[3] 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.[4] 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. Earlier efforts were made to identify a potential synthetic attractant or repellant for mosquitoes using short-chain fatty acid esters against *Aedes aegypti* (*Ae. aegypti*),[5] role of larval water and pre-existing eggs in oviposition by *Ae. aegypti* and *Ae. albopictus*,[6] certain fatty acids and esters identified from egg extracts of *Ae. aegypti* as oviposition attractant[7] and oviposition responses of *Ae. aegypti* and *Ae. albopictus* to certain fatty acid esters[8] laid the foundation to explore synthetic compounds of newer origin. To the best of our knowledge, a finger-count literature is available regarding oviposition responses of *Ae. albopictus* to chemical compounds.[8,9] Pheromone based or chemical mediated oviposition studies in mosquitoes are scanty. Many such reports are available for agriculturally important pests. One of the selected examples is the deterrent ovipositional activity mediated by phenolic acids as compared to non phenolic ones in Spruce Budworm.[10] Floral phenyl esters attract many wide ranges of insects including *Dacus dorsalis*.[11] Methyl benzoate and some of its derivatives mostly nitrogenous allied with host plant aromas were used to study attraction of feral northern and western corn rootworm beetles (*Diabrotica barberi* and *D. virgifera virgifera*).[12] Formic acid 4-(3-oxobutyl)phenylester (raspberry ketone formate [RKF]) was tested successfully against wild populations of male melon flies, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae).[13] *tert*-Butyl ester of methyl cyclohexane carboxylic acids were used to lure male Mediterranean fruit flies *Ceratitis capitata* (Weidman).[14] Certain aromatic compounds (ligands) were found to mediate receptor binding in odorant binding protein in *Ae. aegypti*.[15] Encouraged by these examples, in continuation of our studies on bioactive compounds,[16] herein we report the synthesis of aryl hydrazono esters (AHE)[17] and the oviposition response studies in *Ae. albopictus*. From our preliminary studies we found better attractant properties in case of unsymmetrical aryl hydrazono esters than that of the symmetrical ones. Hence, we focused our

* Corresponding author. Tel.: +91 751 2343972; fax: +91 751 2347542.
E-mail address: mpkaushik@rediffmail.com (M.P. Kaushik).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.11.101



Scheme 1. Reagents and conditions: (i) $NaNO_2$/HCl, 0–5 °C, (ii) CH_3COONa/C_2H_5OH.

Table 1
Synthesized aryl hydrazono ester (AHE) compounds

Entry	Code	R^1	R^2	R^3	Yield* (%)
4a	AHE-1	C_6H_5	CH_3	C_2H_5	90
4b	AHE-2	4-$NO_2C_6H_4$	CH_3	C_2H_5	84
4c	AHE-3	4-MeC_6H_4	CH_3	C_2H_5	77
4d	AHE-4	4-HOC_6H_4	CH_3	C_2H_5	78
4e	AHE-5	2-$MeOCC_6H_4$	CH_3	C_2H_5	98
4f	AHE-6	2-$HOH_2CC_6H_4$	CH_3	C_2H_5	87
4g	AHE-7	4-$MeOCC_6H_4$	CH_3	C_2H_5	76
4h	AHE-8	4-FC_6H_4	CH_3	C_2H_5	98
4i	AHE-9	4-FC_6H_4	CH_3	$CH_2CH(CH_3)_2$	95
4j	AHE-10	3-ClC_6H_4	CH_3	CH_3	97
4k	AHE-11	4-$MeOC_6H_4$	CF_3	C_2H_5	92
4l	AHE-12	Naphthalen-1-yl	CH_3	C_2H_5	84
4m	AHE-13	4-$EtOOCC_6H_4$	CH_3	C_2H_5	81

* Isolated yield.

studies only up to unsymmetrical aryl hydrazono esters. Results from this study will be useful to identify a potential oviposition stimulant or deterrent against dengue and chikungunya vector. The synthetic pathway of the designed AHE was described in Scheme 1. Various aromatic amines were reacted with aqueous sodium nitrite solution in acidic medium under ice-cold condition (0–5 °C) to form the corresponding diazonium chloride salt intermediate, which was further condensed with ethanolic solution of β-keto ester in presence of sodium acetate under ice-cold condition to form the desired aryl hydrazono esters.[18] All the compounds were purified by recrystallisation to get highly pure AHE.

The details of R^1, R^2, and R^3 groups of the various aromatic amines and β-keto esters were illustrated in Table 1.

All the compounds (AHE-1 to AHE-13) were characterized by IR, NMR (^1H and ^{13}C), ESI-MS and elemental analysis. The spectral data and the elemental analysis of all the compounds reported in this study correlate with the proposed structures. The IR spectrum showed absorption at 3159 cm^{-1} and 1193, 1167 cm^{-1} which corresponds to NH stretching of hydrazono group and C–O stretching of ester group. In case of ^1H NMR the sharp singlet at δ 2.6 ppm indicated the presence of ketonic methyl group, while quartet at δ 4.4 and triplet at δ 1.4 ppm confirmed the presence of CH_2 and CH_3 of ethyl group of β-keto ester. The presence of ketonic carbonyl and esteric carbonyl was observed at δ 196 ppm and δ 165 ppm, respectively, in ^{13}C NMR.

Laboratory bioassay[19] on oviposition responses was carried out in fine wire mesh fitted cages (750 × 600 × 600 mm) with a sleeve opening on one side. The oviposition responses of AHE-1 to AHE-13 were evaluated on gravid female mosquitoes at 10 μg ml^{-1} (Fig. 1) using standard procedure.[20] HPLC-grade methanol was used as a control. The basis for measuring the oviposition response was the number of eggs received in both control and treatment bowls. The oviposition activity was expressed as oviposition activity index (OAI) and calculated by using the formula $OAI = (N_t - N_s)/(N_t + N_s)$, where, N_t is the number of eggs laid in test solution and N_s is the number of eggs laid in control. As suggested by Kramer and Mulla,[21] compounds with an OAI of +0.3 and above are considered as attractants, while those with −0.3 and below are considered as repellents. *p*-cresol is well-reported as oviposition attractant for *Ae. aegypti*,[22] we compared the OAI value with it. Out of 13 compounds (AHE-1 to AHE-13), AHE-12 showed significant positive oviposition response with OAI 0.299 (P <0.05) in *Ae. albopictus*. Structurally, AHE-12 consists of naphthalene ring attached to ester via hydrazono group. Interestingly, as such naphthalene was reported as attractant in case of female Tabanidae (Diptera),[23] and also as repellent in *Ae. aegypti*.[24] However, we found AHE-12 was acting as positive stimulant for oviposition of *Ae. albopictus*. Additionally, when phenyl ring of AHE-1 was replaced by naphthalene ring in AHE-12 the oviposition attractant activity was found to be increased significantly with OAI +0.157 to +0.299. It was also evident from the Table 2 that few compounds (AHE-1, AHE-4, AHE-6 and AHE-11) although displayed positive OAI which indicates that more eggs occur in the treated than in the control, though they were not significant (P >0.05). Conversely, AHE-3, AHE-5, AHE-9

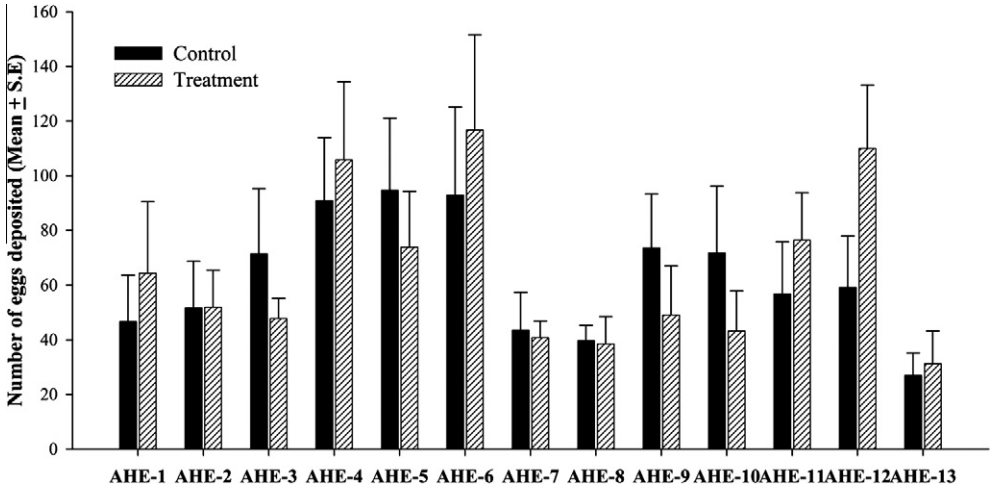


Figure 1. Oviposition response in different aryl hydrazono ester (AHE) compounds.

Table 2
Oviposition response of *Aedes albopictus* to Aryl hydrazono esters

Code	Aryl hydrazono estes	No. of eggs laid in different treatments, (mean + SE)		*P* value* (significance)	OAI value
		Control	10 ppm		
AHE-1	Ethyl 2-(2-phenylhydrazono)-3-oxobutanoate	65.70 ± 14.67	90.20 ± 24.51	>0.05	+0.157
AHE-2	Ethyl 2-(2-(4-nitrophenyl)hydrazono)-3-oxobutanoate	51.71 ± 17.10	52.0 ± 13.50	>0.05	+0.003
AHE-3	Ethyl 2-(2-*p*-tolylhydrazono)-3-oxobutanoate	71.43 ± 23.81	47.86 ± 7.37	>0.05	−0.197
AHE-4	Ethyl 2-(2-(4-hydroxyphenyl)hydrazono)-3-oxobutanoate	90.86 ± 23.02	105.86 ± 28.52	>0.05	+0.076
AHE-5	Ethyl 2-(2-(2-acetylphenyl)hydrazono)-3-oxobutanoate	94.71 ± 26.37	73.86 ± 20.43	>0.05	−0.124
AHE-6	Ethyl 2-(2-(2-(hydroxymethyl)phenyl)hydrazono)-3-oxobutanoate	92.86 ± 32.23	116.71 ± 34.87	>0.05	+0.114
AHE-7	Ethyl 2-(2-(4-acetylphenyl)hydrazono)-3-oxobutanoate	43.57 ± 13.86	40.86 ± 6.08	>0.05	−0.032
AHE-8	Ethyl 2-(2-(4-fluorophenyl)hydrazono)-3-oxobutanoate	39.71 ± 5.60	38.57 ± 9.95	>0.05	−0.015
AHE-9	Isobutyl 2-(2-(4-fluorophenyl)hydrazono)-3-oxobutanoate	73.57 ± 19.67	49.14 ± 17.89	>0.05	−0.199
AHE-10	Methyl 2-(2-(3-chlorophenyl)hydrazono)-3-oxobutanoate	71.71 ± 24.41	43.29 ± 14.62	>0.05	−0.247
AHE-11	Ethyl 4,4,4-trifluoro-2-(2-(4-methoxyphenyl)hydrazono)-3-oxobutanoate	56.86 ± 18.85	76.43 ± 17.37	>0.05	+0.147
AHE-12	Ethyl 2-(2-(naphthalen-1-yl)hydrazono)-3-oxobutanoate	59.29 ± 18.58	110 ± 23.17	<0.05	+0.299
AHE-13	Ethyl 4-(2-(1-ethoxy-1,3-dioxobutan-2-ylidene)hydrazinyl)benzoate	27.0 ± 8.16	31.29 ± 11.98	>0.05	+0.073

* *P* value indicates the level of significance, $P < 0.05$ indicate greater than 95% confidence limit.

and AHE-10 displayed negative OAI which signifies more eggs laid in the control than in the treated one, hence indicating the compounds to be oviposition deterrent. AHE-10 showed highest oviposition deterrent activity with OAI −0.247 against *Ae. albopictus*. The oviposition response was found to be neutral in case of AHE-2, AHE-7, AHE-8 and AHE-13 as in these cases, there was no significant difference ($P > 0.05$) in egg laying between the treatment and control. From this study, it was revealed that compounds which contain hydroxyl group (AHE-4 and AHE-6) or its analog (AHE-11) attached to phenyl ring or its side chain exhibited moderate oviposition attractancy to *Ae. albopictus* females. This was supported by the fact that mosquitoes possess hygroreceptors in their body (mainly tarsi[25]), hence can sense the (active ingredient) vapor content in an oviposition substrate. On the other hand, compounds (AHE-8, AHE-9 and AHE-10) containing halogen groups (Cl and F) displayed moderate to good deterrent properties. In insects, halogenated analogs were reported as inhibitors of chemical communication.[26]

From the present study, it was observed that AHE-11 which had triflouromethyl group in the ester part received more eggs which can be explained by the presence of methoxy group in the phenyl ring and thus methoxy group (analog of OH) predominates over the halogens towards egg laying. Inhibitory activity of the acetyl group present at *ortho* and *para* position of phenyl ring could also be observed in case of AHE-5 and AHE-7 by their negative OAI value (−0.124, −0.032). Further it was interesting to note that the presence of NO_2 and $COOC_2H_5$ in compound AHE-2 and AHE-13 elicited reduced egg deposition by females.

In conclusion, a series of aryl hydrazono esters (AHE) (1–13) were synthesized and evaluated for the oviposition responses against *Ae. albopictus* for the first time. AHE-12 showed significant oviposition attractant activity with an oviposition activity index (OAI) of +0.299 (greater than 95% confidence limit) comparable to well-reported oviposition attractant for *Ae. aegypti*, *p*-cresol (OAI +0.320). Conversely, AHE-10 exhibited highest oviposition deterrent activity with OAI −0.247. The present study shows the potential application of these compounds as oviposition attractants which may have potential application in mosquito trapping for identification, surveillance and control.

Acknowledgements

The authors are thankful to Dr. R. Vijayaraghavan, Director, DRDE, Gwalior for his keen interest and encouragement. We thank Mrs. Ruchi Yadav and all the members of Entomology division for the help in maintenance of the mosquito culture. P.B is thankful to DRDO, New Delhi, for financial support.

Supplementary data

Supplementary data (FT-IR, ^1H, ^{13}C NMR, ESI-MS and elemental analysis) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.101.

References and notes

1. Huang, J.; Walker, E. D.; Giroux, P. Y.; Vulule, J.; Miller, J. R. *Med. Vet. Entomol.* **2005**, *19*, 442.
2. Bentley, M. D.; Day, J. F. *Annu. Rev. Entomol.* **1989**, *34*, 401.
3. (a) Trexler, J. D.; Apperson, C. S.; Schal, C. *J. Med. Entomol.* **1998**, *35*, 967; (b) Takken, W.; Knols, B. G. J. *Annu. Rev. Entomol.* **1999**, *44*, 137; (c) Blackwell, A.; Johnson, S. N. *Ann. Trop. Med. Parasitol.* **2000**, *94*, 389; (d) Knols, B. G. J.; Sumba, L. A.; Guda, T. O.; Deng, A. I.; Hassanali, A.; Beier, J. C. *Int. J. Trop. Insect Sci.* **2004**, *24*, 260.
4. Takken, W. *Invertebr. Reprod. Dev.* **1999**, *36*, 67.
5. Perry, A. S.; Fay, R. W. *Mosq. News* **1967**, *27*, 175.
6. Allan, S. A.; Kline, D. L. *J. Med. Entomol.* **1998**, *35*, 943.
7. Ganesan, K.; Mendki, M. J.; Suryanarayana, M. V. S.; Prakash, S.; Malhotra, R. C. *Aust. J. Entomol.* **2006**, *45*, 75.
8. Sharma, K. R.; Seenivasagan, T.; Rao, A. N.; Ganesan, K.; Agarwal, O. P.; Malhotra, R. C.; Prakash, S. *Parasitol. Res.* **2008**, *103*, 1065.
9. (a) Trexler, J. D.; Apperson, C. S.; Zurek, L.; Gemeno, C.; Perich, M. J.; Carlson, D.; Schal, C. *J. Am. Mosq. Control Assoc.* **2003**, *19*, 228; (b) Trexler, J. D.; Apperson, C. S.; Zurek, L.; Gemeno, C.; Schal, C.; Kaufman, M.; Walker, E.; Watson, D. W.; Wallace, L. *J. Med. Entomol.* **2003**, *40*, 841.
10. Grant, G. G.; Langevin, D. *IOBC/wprs Bulletin* **2002**, *25*, 307.
11. Metcalf, R. L.; Mitchell, W. C.; Fukuto, T. R.; Metcalf, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2501.
12. Hammack, L.; Petroski, R. J. *J. Chem. Ecol.* **2004**, *30*, 1809.
13. Jang, E. B.; Casana-Giner, V.; Oliver, J. E. *J. Econ. Entomol.* **2007**, *100*, 1124.
14. Sonnet, P. E.; McGovern, T. P.; Cunninghum, R. T. *J. Org. Chem.* **1984**, *49*, 4639.
15. Li, S.; Picimbon, J. F.; Ji, S.; Kan, Y.; Chuanling, Q.; Zhou, J. J.; Pelosi, P. *Biochem. Biophys. Res. Commun.* **2008**, *372*, 464.
16. (a) Sathe, M.; Kaushik, M. P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1312; (b) Sathe, M.; Kaushik, M. P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4233; (c) Acharya, B. N.; Saraswat, D.; Tiwari, M.; Shrivastava, A. K.; Ghorpade, R.; Bapna, S.; Kaushik, M. P. *Eur. J. Med. Chem.* **2010**, *45*, 430; (d) Sathe, M.; Ghorpade, R.; Kaushik, M. P. *Chem. Lett.* **2006**, *35*, 86.
17. (a) Farghaly, T. A.; Abdallah, Z. A. *Arkivoc* **2008**, *xvii*, 295; (b) Sharma, P.; Kumar, A.; Sharma, M. *J. Mol. Catal. A: Chem.* **2005**, *237*, 191.
18. The aromatic amine (1–13) (0.01 mol) was dissolved in a mixture of 4.0 mL of concentrated hydrochloric acid (HCl) and 4.0 mL of distilled water. The amine hydrochloride solution was kept at freezing temperature. To this, an aqueous solution of sodium nitrite ($NaNO_2$) 0.69 g (0.01 mol) in 5.0 mL of distilled water was added dropwise with continuous stirring, keeping the temperature of the reaction vessel at 0–5 °C. Meanwhile in another beaker, 6.8 g (0.05 mol) of sodium acetate (CH_3COONa) in a solution of 1.53 mL (0.012 mol) 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 dropwise with thorough stirring. The reaction mixture was kept overnight, filtered under

suction, washed thoroughly with cold water, dried and recrystallised from a mixture of dimethylformamide (DMF) and ethyl alcohol (EtOH) (3:7, v/v) to give the corresponding butanoate.

19. *Ae. albopictus* used for the oviposition experiments were utilized from the laboratory colony maintained since 1973 in our insectaria. The experiments were performed at 27 ± 2 °C, 75 ± 5% RH, L10:D14 regime. The illumination inside the oviposition cages was 80–100 lx. Four to seven-days old adults of *Ae. albopictus* (25 pairs) 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 for two days initially and then every alternative day until the experiment was completed. Laboratory bioassays were performed (duplicates) in two separate cages, enamel bowls with 10 cm diameter filled with 500 mL of dechlorinated water was used as the oviposition substrate. One milliliter of the stock solution in methanol containing the desired compound (to make 10 ppm concentration) was added to the enamel bowls. All stock solution of the test chemicals were dissolved in HPLC-grade methanol (Merck) and stored in deep freeze after every experiment. The bowls were rinsed with tap water and washed with methanol before commencing the experiment. The experiment was completely randomized and in each bioassay females were allowed to choose between the treated and untreated (control) substrates. The position of substrate in dual choice experiment was changed in a clockwise rotation every day. Each compound was evaluated separately in duplicates, and the experiment was repeated for seven days with different batch of mosquitoes. The number of eggs laid in control and treatment were counted manually to assess the oviposition preference of the mosquito species.
20. WHO—World Health Organization, *Technical Report*, **1963**, *Series No. 265*.
21. Kramer, L. W.; Mulla, S. M. *Environ. Entomol.* **1979**, *8*, 1111.
22. Linley, J. R. *Med. Vet. Entomol.* **1989**, *3*, 342.
23. Krčmar, S. *J. Vector Ecol.* **2007**, *32*, 188.
24. Wirtz, R. A.; Turrentine, J. D.; Fox, R. C. *J. Med. Entomol.* **1981**, *18*, 126.
25. Seenivasagan, T.; Sharma, K. R.; Srivastava, A.; Parashar, B. D.; Pant, S. C.; Prakash, S. *J. Vector Borne Dis.* **2009**, *46*, 125.
26. Renou, M.; Guerrero, A. *Annu. Rev. Entomol.* **2000**, *45*, 605.