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"Click" reaction based synthesis of nimbolide derivatives and study of their insect antifeedant activity against *Spodoptera litura* Larvae

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ABSTRACT

A series of Nimbolide-triazole conjugates were synthesized through copper(I)- catalyzed azide–alkyne "click" chemistry approach and these derivatives (**2–4**, **2a–2l**) were characterized using modern spectroscopic techniques. Antifeedant activities of these derivatives were studied on Tobacco Caterpillar, *Spodoptera litura* (F.) using no-choice leaf disk bioassay. Interestingly, the synthesized derivatives were more effective in reducing feedancy by insect species when compared to the parent nimbolide. Among the tested compounds, **2a**, **2c**, and **2d** showed potent antifeedancy with ED_{50} values of **0.49, 0.95** and **0.97** mg/cm^2 against *S. litura*. Several of the analogs were also toxic or caused developmental abnormalities following leaf disc assay.

1. Introduction

Insect pests are well known to cause damage to crops and effect the agricultural productivity. As per the recent estimates, annual post-harvest losses (PHLs) from insect damage are estimated to be 10–30% of the production for major crops [1]. The tobacco caterpillar, *Spodoptera litura*, is one of the most wide spread destructive insect pest of agricultural crops in India, China and Japan. The damage caused by this pest include crops of economic importance like tomato, cotton, chickpea, pigeonpea, sunflower, tobacco, groundnut, sorghum, and soybean [2,3]. The control practices of these pests by most of the farmers primarily dependent upon repeated application of synthetic insecticides [4,5]. Despite their beneficial role in agriculture, repeated use of complex synthetic pesticides led to the development of cross resistance in pest and also severe damage to environment as well as human health. These limitations necessitated a shift to use natural product based agrochemicals, as they are biodegradable, eco-friendly, and safe to the environment [6,7]. Natural products has a long history of usage as insecticidal agents. Numerous plant species have been identified as possessing pesticidal properties and which are alternatives to chemical pesticides. Rotenone (Derris sp), nicotine (Tobacco), and pyrethrum (Chrysanthemum sp) are best examples of botanical pesticides which have been widely used both in small-scale subsistence farming and commercial agriculture [8].

Neem [*Azadiracta indica* A. Juss; family: Meliaceae] is one of the most commonly used natural insecticide since ancient times besides its traditional use as folklore medicine [9]. The oil and organic solvent extracts from the leaves and seeds display several bioactivities against wide range of insect pests [10]. The limonoids are by far the most abundant metabolites and responsible for all the biological effects including antifeedant activity against important insect pests [11]. Azadirachtin (limonoid) is the major active principle extracted from neem seeds and is one of the most promising plant products for integrated pest management (IPM) [12]. As a part of our ongoing search for natural products based pesticides from Indian medicinal plants, we have thus far investigated the potential of species in the meliaeceae family [13–15] and reported several limonoids with potential anti-feedant activities. In this connection, we have isolated large quantities of nimbolide (**1**), as a bioactive marker from A*zadiracta indica*[16]. Despite the prominent cytotoxic potentials of **1**, to the best of our knowledge, very little attention has been devoted to structural modifications of **1** for use as insecticidal agents. This fact, coupled with the ease of acquiring it from neem leaves and functionalities present in this natural product make it well suited for semi-synthetic derivatization for development of more effective natural products-based insecticidal agents.

Recently, development of hybrid molecules between two different types of moieties has emerged as a new approach in the discovery of new therapeutics, as they consist of high potency, improved solubility and different binding sites which are essential to play vital role in

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treatment [17,18]. Thus, present work was undertaken to synthesize triazole derivatives of nimbolide using metal-catalyzed "cycloaddition" reactions and study of their antifeedant properties. The triazole products are more than just passive linkers; they readily associate with biological targets, through hydrogen bonding and dipole interactions [19]. In addition, the chemical reactions involved are concise and straightforward, which make it feasible for the scale-up production of required derivatives of interest. Herein, we report Cu-mediated Huisgen-click reaction of nimbolide (**1**) for the synthesis of triazolyl derivatives and their antifeedant activities against *Spodoptera litura* using no choice bioassay method.

2. Materials and methods

2.1. General

^{1}H and ^{13}C spectra were measured on a Bruker 300 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on Agilent LC/MSD trap SL 1100 series with a 70 eV (ESI probe) and the infrared spectra on a Thermo Nicolet Nexus 670 FTIR spectrometer. Melting points were taken on a Fischer Scientific melting point apparatus and are uncorrected. The synthetic compounds were purified by column chromatography using 60–120 mesh size silica gel (Merck). Thin layer chromatography (TLC) involved the use of precoated silica gel $60F_{254}$ TLC plates of Merck. The optical rotations were measured on Jasco Dip 360 digital Polarimeter.

2.2. Extraction and isolation of nimbolide

The leaves of *Azadiracta indica* (1Kg) were collected during the August–September 2015 from Tirumala forest, Tirupati, Andhra Pradesh, India. A voucher specimen was deposited at the herbarium of Natural Product Laboratory, Indian Institute of Chemical Technology, Tarnaka, hyderabad, India. Air dried leaves were soaked in methanol (3 L) for 72 h and methanol extract of neem (45 g) was partitioned between 1:1 water and hexane (2 × 200 ml) and the water layer was again extracted with ethyl acetate (2 × 200 ml). The combined organic extracts were concentrated under reduced pressure using rotavapor to give a crude extract. This gummy crude extract was then subjected to repeated column chromatography using silica gel (100–200 mesh) eluting with hexane: ethyl acetate (70:30) to afford nimbolide enriched fraction, which was further subjected to repeated washings using hexane-dichloromethane system to yield 1 g of pale yellow amorphous solid of nimbolide (**1**) in pure form in 0.01% yield [20]. Its gross structure was confirmed through ^{1}H, ^{13}C NMR and Mass spectral analysis [16].

2.3. Procedure for the synthesis of **2–4**[20,16]

To a solution of nimbolide (1equivalent) in dry THF under inert atmosphere appropriate amines (3.5 equivalents) were added at room temperature and the reaction was continued till consummation of nimbolide. The progress of reaction was checked by TLC. After completion of reaction, THF was removed under reduced pressure using rotary evovaporator and the crude product was purified using silica gel column chromatography with the elution of hexane:acetone (60:40) to get pure compounds

2.3.1. Methyl 2-((2R,3aR,5R,6R,9aR,10S,10aR)-2-(furan-3-yl)-5-hydroxy-1,6,9a,10a-tetramethyl-9-oxo-6-(prop-2-yn-1-ylcarbamoyl)-3,3a,4a,5,5a,6,9,9a,10,10a-decahydro-2H-cyclopenta[b]naphtho[2,3-d]furan-10-yl)acetate (2)

Yield: 83%; white amorphous powder; IR (KBr) ν_{max} 3526, 3340, 2952, 1731, 1679, 1530, 1495 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (1H, br s), 7.28 (1H, d, J = 9.6) 7.22 (1H, br s), 6.25 (1H, br s), 5.93 (1H, d, J = 9.6), 5.53 (1H, br s), 4.63 (1H, dd, J = 3.8 & 12.8), 4.27 (1H, d, J = 3.6), 3.67 (1H, m), 3.54 (3H, s), 3.25 (1H, d5, J = 5.1 & 16.1), 3.19 (1H, d, J = 12.5), 2.74 (1H, t, J = 5.4 & 11.1), 2.37 (1H, dd, J = 5.1 & 16.1), 2.22 (1H, m), 2.12 (2H, m), 1.70 (3H, s), 1.66 (1H, m), 1.48 (3H, s), 1.37 (3H,s), 1.22 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 202.11, 174.95, 173.52, 148.60, 146.74, 143.01, 138.91, 134.85, 126.79, 126.41, 110.37, 87.50, 86.85, 71.85, 66.31, 51.62, 49.56, 47.95, 47.41, 47.31, 44.03, 41.37, 39.05, 34.41, 31.87, 17.49, 16.62, 16.48, 14.08, 12.82; HRMS (ESI) calcd for $C_{30}H_{35}NNaO_7$ $[M + Na]^+$ calcd. 544.2321, found. 544.2319.

2.3.2. Methyl 2-((2R,3aR,5R,6R,9aR,10S,10aR)-6-((3-fluorophenyl)carbamoyl)-2-(furan-3-yl)-5-hydroxy-1,6,9a,10a-tetramethyl-9-oxo-3,3a,4a,5,5a,6,9,9a,10,10a-decahydro-2H-cyclopenta[b]naphtho[2,3-d]furan-10-yl)acetate (3)

Yield: 89%; white amorphous powder; IR (KBr) ν_{max} 3498, 2961, 2849, 1761, 1705, 1618, 1540, 1352 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (3H, m), 7.32 (1H, s), 7.23 (1H, s), 7.00 (2H, m), 6.49 (1H, d, J = 10.07), 6.31 (1H, s), 5.93 (1H, d, J = 10.07), 5.52 (1H, m), 4.01 (1H, s), 3.97 (1H, m) 3.66 (3H, s), 3.35 (1H, d, J = 11.1), 2.91 (1H, dd, J = 5.7 & 16.4), 2.80 (1H, m), 2.21 (1H, dd, J = 5.7 & 16.4), 2.14 (1H, m), 2.01 (1H, m), 1.71 (3H, s), 1.69 (3H, s), 1.30 (3H, s), 1.27 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.06, 173.55, 173.0, 148.52, 146.64, 143.05, 138.94, 135.05, 126.75, 126.55, 122.21, 122.14, 115.64, 115.47, 110.37, 87.44, 86.93, 66.40, 51.67, 49.59, 48.27, 48.10, 47.33, 44.54, 41.36, 39.10, 34.40, 17.53, 16.91, 16.55, 12.85; HRMS (ESI) calcd for $C_{33}H_{37}NO_7F$ $[M + H]^+$ calcd. 578.2554, found. 578.2539.

2.3.3. Methyl 2-((2R,3aR,5R,6R,9aR,10S,10aR)-6-(4-(2-aminoethyl)piperazine-1-carbonyl)-2-(furan-3-yl)-5-hydroxy-1,6,9a,10a-tetramethyl-9-oxo-3,3a,4a,5,5a,6,9,9a,10,10a-decahydro-2H-cyclopenta[b]naphtho[2,3-d]furan-10-yl)acetate (4)

Yield: 76%; yellow amorphous powder: IR (KBr) ν_{max} 3491, 2958, 2851, 1760, 1701, 1619, 1541, 1351 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (1H, br s), 7.32 (1H, br s), 6.44 (1H, d, J = 10.1), 6.31 (1H, s), 5.84 (1H, d, J = 10.1), 4.00 (1H, d, J = 2.8), 3.92 (1H, J = 2.8 & 11.4), 3.69 (1H, m), 3.67 (1H, m), 3.64 (3H, s), 3.08 (4H, m), 2.50 (4H, m), 2.95 (1H, m), 2.85 (1H, m), 2.71 (1H, m), 2.24 (1H, m), 2.14 (1H, m), 1.98 (1H, m), 1.69 (3H, s), 1.60 (3H, s), 1.29 (3H, s), 1.24 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.78, 175.10, 174.03, 150.22, 146.77, 143.00, 139.00, 134.82, 126.70, 125.59, 110.41, 88.31, 86.54, 65.78, 5 8.32, 52.29, 51.68, 49.50, 48.46, 47.58, 47.47, 45.73, 43.93, 41.33, 39.26, 35.62, 34.32, 17.34, 16.61, 16.41, 12.82; HRMS (ESI) calcd for $C_{33}H_{46}N_3O_7$ $[M + H]^+$ calcd. 596.3336, found 596.3343.

2.4. General procedure for the synthesis of derivatives **2a–2l**[21]

To a solution of alkynes (1.2 eq) and azide (1.2 eq) in dry THF was added catalytic amount of CuI under inert atmosphere and the reaction was allowed to stirr for 6–8 h. After completion of the reaction (Monitored by TLC), reaction mixture was filtered through celite bed and filtrate was quenched with water (10 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were evaporated under reduced pressure using rotavapor to get the crude residue, which was purified by column chromatography using silica gel eluting with hexane/ethyl acetate (50:50) to get the desired products (**2a–2m**) in pure form. All synthesized triazole derivatives were confirmed by the spectral analysis (FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy).

2.4.1. Methyl 2-((2R,3aR,5R,6R,9aR,10S,10aR)-2-(furan-3-yl)-5-hydroxy-6-(((1-(4-hydroxy-3,5-dimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)carbamoyl)-1,6,9a,10a-tetramethyl-9-oxo-3,3a,4a,5,5a,6,9,9a,10,10a-decahydro-2H-cyclopenta[b]naphtho[2,3-d]furan-10-yl)acetate (2a)

Yield: 88%; yellowiest amorphous powder: IR (KBr) ν_{max}3487, 3029, 2951, 2848, 1754, 1705, 1617, 1538, 1351 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (1H, s), 7.31 (1H, s), 7.22 (1H, br s), 6.88