SUPPLEMENTARY MATERIAL

Thiotagetin A, a new cytotoxic thiophene from *Tagetes minuta*

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Phytochemical investigation of the *n*-hexane fraction of the methanolic extract of *Tagetes minuta* L. (Asteraceae) aerial parts afforded a new thiophene derivative: thiotagetin A (**3**), together with β -sitosterol (**1**) and stigmasterol (**2**). The structure of the new thiophene was identified by UV, IR, 1D (^1H and ^{13}C), 2D (^1H - ^1H COSY, HSQC, and HMBC) NMR, and HRESIMS spectral data. Compound **3** displayed cytotoxic activity against KB and MCF7 cancer cell lines with ED_{50} values of 2.03 and 3.88 $\mu\text{g/mL}$, respectively compared to adriamycin (0.26 and 0.07 $\mu\text{g/mL}$, respectively).

Keywords: *Tagetes minuta*; Thiotagetin A; Thiophene; Cytotoxic activity.

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Experimental

General experimental procedures

Ultraviolet (UV) spectrum was measured on a Hitachi 300 spectrophotometer. The IR spectrum was recorded on a Shimadzu Infrared-400 spectrophotometer (Kyoto, Japan). HRESIMS was obtained by using LTQ Orbitrap mass spectrometer (ThermoFinnigan, Bremen, Germany). 1D and 2D NMR spectra were recorded on Bruker Avance DRX 500 MHz spectrometer. For column chromatography, silica gel (0.063-0.200 mm, Merck, Darmstadt, Germany) and RP₁₈ (0.04-0.063 mm Merck, Darmstadt, Germany) were used. Pre-coated silica gel 60 F₂₅₄ plates (0.2 mm, Merck, Darmstadt, Germany) were used for thin-layer chromatography. Six mL standard extraction tube (RP₁₈, 40-63 μ m, Merck, Darmstadt, Germany) was used for compounds purification. Spots were detected by spraying with the following reagents: *p*-anisaldehyde:H₂SO₄ for sterols and isatin:H₂SO₄ for thiophenes.

Extraction and isolation

The air-dried powdered aerial parts (0.9 kg) were macerated with MeOH (2 $\times$ 3 L). The total methanolic extract was concentrated under vacuum (22.6 g). The later was mixed with 200 mL distilled water and fractionated successively between *n*-hexane (3 $\times$ 500 mL), EtOAc (3 $\times$ 500 mL), and *n*-BuOH (3 $\times$ 500 mL). Each fraction was concentrated to give *n*-hexane (3.7 g), EtOAc (2.3 g), *n*-BuOH (2.1 g), and aqueous (11.2 g) fractions. The *n*-hexane fraction (3.7 g) was chromatographed on silica gel column (250 g, 100 $\times$ 3 cm) using *n*-hexane:EtOAc gradient elution to obtain ten sub-fractions: TMH-1 to TMH-10. Sub-fraction TMH-3 (240 mg) was subjected to silica gel column (70 g, 50 $\times$ 3 cm), eluting with *n*-hexane:EtOAc gradient to afford **1** (32 mg, white needles). Silica gel column (80 g, 50 $\times$ 3 cm) of sub-fraction TMH-4 (127 mg) using *n*-hexane:EtOAc gradient elution gave impure **2**, which was purified on reversed phase silica gel extraction tube using H₂O:MeOH gradient to yield **2** (18 mg, white needles). Sub-fraction TMH-5 (110 mg) was subjected to silica gel column (50 g, 50 $\times$ 3 cm) using *n*-hexane:EtOAc gradient elution to get impure **3**. Its purification was achieved using

RP₁₈ column eluting with H₂O:MeOH gradient, then on LiChrolut EN/RP-18 solid phase extraction tube, using H₂O with gradual increase of acetonitrile to yield **3** (7.8 mg, yellow amorphous powder). The other sub-fractions were retained for further investigation.

Cytotoxicity assay

The cytotoxic activity of compound **3** was examined towards KB (epidermoid carcinoma) and MCF7 (hormone-dependent breast carcinoma) cancer cell lines using MTT assay. All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were seeded at a density of 25,000 cells/well and incubated for 24 h. Tested sample was added at different concentrations and cells were again incubated for 48 h. At the end of incubation, the cell viability was determined using Neutral Red dye according to a modification of Borenfreund's procedure (Elkhayat et al. 2016; Borenfreund et al. 1990). Compound concentrations that produce 50 % cell growth inhibition (ED₅₀) were calculated from curves constructed by plotting cell survival (%) versus drug concentration (µg/mL). All experiments were carried out in triplicates and repeated three times. As negative controls, media with 0.1% (v/v) EtOH were included in all experiments. The adriamycin was used as a positive control.

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Dr.Gamal
Sample;    CA-1      CDCL3
@C13_NS256_KAAU CDCL13 {D:\outside} jaber 3
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C13DEPT135 CDC13

Figure S3. DEPT ^{13}C NMR spectrum of compound 3.

Dr.Gamal Hussain
 Sample: CA-1 CDCL3
 @COSY_NS2_KAAU CDC13 {D:\outside} jaber 25

Figure S4. ^1H - ^1H COSY spectrum of compound 3.

Dr.Gamal Hussain
Sample: CA-1 CDCL3
@HMBcqp1p2nd_NS4_KAAU CDC13 {D:\outside} jaber 25

Figure S7. ^1H - ^1H COSY and HMBC correlations of compound **3**.

References

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