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# New indane and naphthalene derivatives from the rhizomes of *Kniphofia reflexa* Hutchinson ex Codd

Denis Kehdinga Sema[a,b], Alain Meli Lannang[c,*], Simplice J.N. Tatsimo[c], Mujeeb-ur-Rehman[b], Sammer Yousuf[b], Denis Zoufou[d], Urooj Iqbal[b], Jean Duplex Wansi[e], Norbert Sewald[f], M. Iqbal Choudhary[b,g]

[a] Department of Chemistry, Faculty of Science, The University of Maroua, P. O. Box 46, Maroua, Cameroon
[b] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[c] Department of Chemistry, Higher Teachers' Training College, University of Maroua, P. O. Box 55, Maroua, Cameroon
[d] Medical Research and Applied Biochemistry Laboratory, University of Buea, P. O. Box 63, Buea, Cameroon
[e] Faculty of Sciences, Department of Chemistry, University of Douala, 24157, Douala, Cameroon
[f] Department of Chemistry, Organic and Bioorganic Chemistry, Bielefeld University, P.O. Box 100131, 33501 Bielefeld, Germany
[g] Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21412, Saudi Arabia

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ABSTRACT

Two new (**1-2**) and eight known (**3-10**) secondary metabolites were isolated from the rhizomes of *Kniphofia reflexa* Hutchinson ex Codd. Among them, an indane derivative kniphofiarindane (**1**), and naphthalene derivative kniphofiarexine (**2**), were identified as new natural products. 1D- and 2D-NMR spectroscopic studies together with single-crystal X-ray diffraction techniques were employed in the structural elucidation of the new compounds, **1** and **2**. These compounds were evaluated for their cytotoxic effect against kidney epithelial cell line (LLC-MK2). Compound **3** was found to be highly cytotoxic, while compounds **1**, and **10** showed moderate cytotoxicity with $CC_{50}$ values of 4.44 ± 0.79, 16.35 ± 1.54, and 11.24 ± 1.22 μg/mL, respectively against the tested standard Gleevec (Imatinib), ($CC_{50}$ = 18.50 ± 1.21 μg/mL). Compounds **8**, and **9** also showed moderate anti-inflammatory activity ($CC_{50}$ = 38.7 ± 4.90, and 20.00 ± 4.40 μg/mL, respectively) against ROS production.

## 1. Introduction

*Kniphofia reflexa* Hutchinson ex Codd. (Asphodelaceae) is a perennial rhizomatous herb, with a single sterile, inflorescence about 2 feet high having small campanulate and yellow flowers (Smith and Van Wyk, 1998). *K. reflexa* grows with its base (rhizomes and roots) in water in high altitude swamps, and are found along streams, and bog edges that do not dry up entirely even in the late dry season (Cheek et al., 2000; Maisels et al., 2000). In Kejom Ketingoh (Cameroon), the whole plant, especially the rhizomes are employed for the treatment of high relapsing fever. Several ethnomedicinal uses have been reported in the genus *Kniphofia* ranging from snake deterrent and chest ailment (Ramdhani et al., 2006; Bringmann et al., 2008); enemas, charm against lightening, painful menstruation and infertility (Codd, 1968; Ramdhani et al., 2006); wound healing and abdominal cramps (Abate, 1989; Wube et al., 2005; Bringmann et al., 2008) to gonorrhea and hepatitis B (Wube et al., 2005). The genus *Kniphofia* has been shown to be a rich source of anthraquinones, flavonoids, alkaloids, as well as and benzene, naphthalene and phloroglucinol derivatives. These constituents are well known for their broad range of biological properties including cytotoxic and antimalarial activities (Yumin et al., 2014). To the best of our knowledge, there is no study reported on the chemical constituents of *k. reflexa*.

## 2. Results and discussion

The methanol extract from the rhizome of *K. reflexa* was subjected to several chromatographic techniques for purification amongst which were flash and column chromatography using silica gel, Sephadex LH-20, recycling preparative HPLC and Preparative TLC to afford one new indanone (Kniphofiarindane, **1**) and one new naphthalene derivative (Kniphofiarexine, **2**), along with eight known compounds: 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene (**3**) (Wube et al., 2005), 2-acetyl-1,8-dimethoxy-3-methylnaphthalene (**4**) (Ghoneim et al., 2013) 2-(4-hydroxyphenyl)acetic acid (**5**) (Todorova et al., 2010; Ghoneim et al., 2013), 3,4-dihydroxybenzoic acid (**6**) (Tagashira and Ohtake,

* Corresponding author.
*E-mail address:* alainmeli@yahoo.com (A. Meli Lannang).

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**Table 1**
$^{1}H$ and $^{13}C$ NMR chemical shift data of compounds **1**, and **2**.

| Position | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| | $\delta_c$, type | $\delta_H$, m (*J* in Hz) | HMBC | $\delta_c$, type | $\delta_H$, m (*J* in Hz) | HMBC |
| 1 | 206.4 | | | 204.6 | | |
| 1a | 120.3 | | | 113.3 | | |
| 2 | 82.7 | | | 31.3 | | |
| $H_{2a}$ | | | | | 2.10, ddd (4.5, 13.5, 18.0) | 3, 4, 1a |
| $H_{2b}$ | | | | | 2.43, ddd (4.0,6.5, 18.0) | 3, 1 |
| 3 | 78.3 | 4.93, s | 8, 2, 3a, 4, 1a | 32.8 | | |
| 3a | 154.1 | | | | | |
| $H_{3a}$ | | | | | 3.04,m | 2, 4, 1 |
| $H_{3b}$ | | | | | 2.20, m | 3, 1, 4a, 4 |
| 4 | 116.9 | 7.09, d (7.5) | 6, 1a | 62.9 | 5.19, t (3.0) | 3, 7, 4a, 5 |
| 4a | | | | 134.8 | | |
| 5 | 138.9 | 7.56, t (8.0) | 3a, 7 | 139.5 | | |
| 6 | 116.8 | 6.81, d (8.0) | 4, 1a, 7 | 157.9 | | |
| 7 | 158.4 | | | 109.0 | | |
| 8 | | | | 161.0 | | |
| 2-$CH_3$ | 20.1 | 1.24, s | 3, 2, 1 | | | |
| 7-$CH_3$ | | | | 7.8 | 2.02, s | 5, 6, 8 |
| 5-$OCH_3$ | | | | 61.7 | 3.79, s | 5 |

NMR Chemical shifts were recorded in MeOD for compounds **1** and **2.**

1998), 2′,4′,6′-trimethoxyacetophenone (**7**) (Gonzalez et al., 1973; Chiaradia et al., 2008), knipholone (**8**) (Dagne and Steglich, 1984), chrysophanol (**9**) (Danielsen et al., 1992), microcarpin (**10**) (Fujitake et al., 1998).

Compound **1** was isolated as whitish rectangular crystals whose molecular formula was determined as $C_{10}H_{10}O_4$; HRESI-MS (positive) *m/z* 217.0471 $[M+Na]^+$, calcd 217.0477; data, indicating 6 double bond equivalents. The $^{1}H$ NMR spectrum (Table 1) showed signals for three aromatic protons of a 1,2,3-trisubstituted benzene ring at $\delta_H$ 6.81 (1H, d, $J$ = 8.0 Hz), $\delta_H$ 7.09 (1H, d, $J$ = 7.5 Hz) and 7.56 (1H, t, $J$ = 8.0 Hz); one methyl group proton signal at $\delta_H$ 1.24 (3H, s); one methine proton at $\delta_H$ 4.93 (1H, s). The $^{13}C$ NMR spectrum (Table 1) displayed one ketone carbonyl signal ($\delta_C$ 206.4), four quaternary carbons ($\delta_C$ 158.4, 154.1, 120.3 and 82.7), four methine ($\delta_C$ 138.9, 116.9, 116.8, and 78.3) and one methyl ($\delta_C$ 20.1) carbons. A comparison of the $^{1}H$ and $^{13}C$ NMR spectra of compound **1** with those of 3-hydroxy-2,6-dimethyl-1-indanone (Ruan et al., 2011) shows some similarities but for the number of methyl and hydroxyl groups and protons on the benzene ring. On the basis of their multiplicities and coupling constants, the aromatic protons at $\delta_H$ 6.81, 7.09, and 7.56 were assigned as H-6, H-4 and H-5 respectively, further supported by HMBC correlations of H-4 with C-1a ($\delta_C$ 120.3) and C-6 ($\delta_C$ 116.8) and H-5 with C-7 ($\delta_C$ 158.4) and C-3a ($\delta_C$ 154.1). The methyl $CH_3$-2 ($\delta_H$ 1.24) displayed HMBC correlations with C-1 ($\delta_C$ 206.4), C-2 ($\delta_C$ 82.7), and C-3 ($\delta_C$ 78.3), thus connecting the methyl and hydroxyl groups at C-3, the carbonyl at C-1, and the methine group at C-3. The methine proton (H-3, $\delta_H$ 4.93) showed HMBC correlations with C-1a ($\delta_C$ 120.3), C-4 ($\delta_C$ 116.9), C-8 ($\delta_C$ 20.1), C-2 ($\delta_C$ 82.7), and C-3a ($\delta_C$ 154.1) attesting the attachment of C-1a ($\delta_C$ 120.3) of the benzene ring to the ketone carbonyl. These data along with single-crystal X-ray (Fig. 2) diffraction studies, established the relative configuration structure of compound **1** (Fig. 1) to be 2,3,7-trihydroxy-2-methyl-2,3-dihydro-*1H*-indan-1-one given a trivial name kniphofiarindane.

Compound **2** was isolated as colorless cubic crystals. HRESI-MS (positive) *m/z* 239.0914 $[M+H]^+$; calcd 239.0919; having as molecular formula $C_{12}H_{14}O_5$, indicating 5 double bond equivalents. The $^{1}H$ NMR spectrum (Table 1) revealed one methoxy group at $\delta_H$ 3.79 (3H, s), one methyl group at $\delta_H$ 2.02 (3H, s), two methylene protons $H_2$-2 [$\delta_H$ 2.10 (ddd, $J$ = 18.0, 13.5, 4.5); 2.43 (ddd, $J$ = 18.0, 6.5, 4.0)] and H-3 [$\delta_H$ 3.04 (m); 2.20 (m)]. In the $^{13}C$ NMR spectrum (Table 1), twelve signals were detected which were classified into a ketonic carbonyl carbon ($\delta_C$ 204.6), six olefinic/aromatic quaternary carbons ($\delta_C$ 109.0, 113.3, 134.8, 139.5, 157.9, and 160.9), two aliphatic methylenes ($\delta_C$ 31.3 and 32.8), an oxygenated methine ($\delta_C$ 62.9) and two methyl carbons ($\delta_C$ 7.8, including one oxygenated methyl $\delta_C$ 61.7) with the help of DEPT. The HMBC correlations from H-2a [2.10] to 62.9 (C-4), 32.8 (C-3), 113.3 (C-1a) and 204.6 (C-1), and H-2b [2.43] to C-3 and C-1; while H-3a [3.04] and H-3b [2.20] correlated with C-4, C-1 and 134.8 (C-4a) showing that the aliphatic methylenes are vicinal in the same ring carrying ketonic carbonyl and the oxygenated methine. Also, the HMBC correlations of the methyl group at $\delta_H$ [2.02] showed the correlations with 109.0 (C-7), 157.9 (C-6) and 160.9 (C-8), while the methoxyl group proton at $\delta_H$ [3.79] correlated with 139.5 (C-5) showing that the other ring of compound **2** is fully substituted. The assigned structure was further supported by a single-crystal X-ray crystallography data (Fig. 2), though it was not possible to determine the configuration of C-4 and named as 4,6,8-trihydroxy-5-methoxy-7-methyl-3,4-dihydronaphthalen-1(2*H*)-one attributed a trivial name kniphofiarexine (Fig 1).

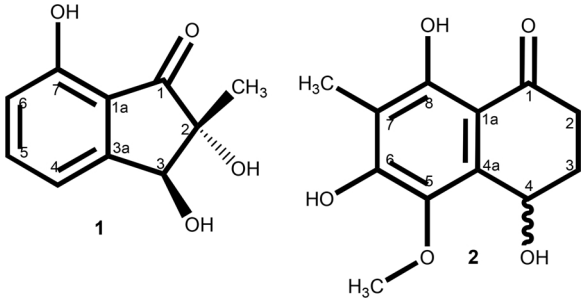


**Fig. 1.** Compounds **1** and **2** isolated from rhizome of *Kniphofiareflexa.*

Compound **3** is isolated here from the genus *Kniphofia* for the second time as it was previously reported from *K. filiosa* (Wube et al., 2005), *Eremurus chinensis* and *Asphodelus tennifolius* (Asphodelaceae) (Li et al., 2000; Abdel-Mogib and Basaif, 2002) where it was found to have good antiplasmodial activities together with compounds **8** and **9** that had previously been reported from *K. filiosa* (Dagne and Steglich, 1984, Dagne et al., 1987). To the best of our knowledge, this is the first report of compounds **4**, **5**, **6**, **7**, and **10** from the genus *Kniphofia*. Compounds **5**, **6**, and **7** were previously reported in *Melilotus officinalis* and *Taraxacum officinale; Dodenaea viscose, Fagopyrum* and *Alnus spp.*; and *Lycoris radiata* respectively reported, are reported here in the Asphodelaceae family for the first time. In the present study, compounds **1**-**4**, **7**, **9**, **10** and methanolic crude extract of *K. reflexa* were evaluated for their cytotoxic activity on the LLC-MK2 Monkey Kidney Epithelial Cell Line against Gleeve (Imatinib) as the positive control using the MTT method. Out of the seven evaluated compounds, one compound (**3**) was highly cytotoxic with a $CC_{50}$ value of 4.437 ± 0.79 µg/mL. Compounds **1** and **10** were also moderately cytotoxic with $CC_{50}$ values of 16.35 ± 1.54 µg/mL, and 11.24 ± 1.22 µg/mL respectively. The crude extract and the rest of the compounds were non-cytotoxic (Table 2). The crude extract, compounds **2**, **8**, and **9** were also evaluated for their effect on phagocyte oxidative burst. Upon activation, phagocytic cells induced the release of free reactive oxygen species (ROS) radicals (oxidative burst) which was then quantified by a luminal-enhanced chemiluminescence assay. Results show that the zymosan-induced oxidative burst in polymorpho-neutrophils (PMNs) was inhibited moderately by compounds **8** and **9** (concentration 100.10 µg/mL) with $CC_{50}$ 38.7 ± 4.90 µg/mL and 20.00 ± 4.40 µg/mL respectively. The crude extract and compound **2** were however less active in suppressing the activity of the monocytes as only 42.4% and 42.2%