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Synthesis of 1,6,7,8-tetrahydro-naphtho[2,3-*d*]-azepino[4,5-*b*]indole-9,14-diones and their inhibitory effects on pro-inflammatory cytokines

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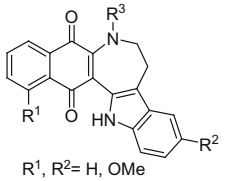
ABSTRACT

A rapid route to a series of naphthoquinone-fused indole derivatives via irradiation in a modified commercial domestic microwave is reported. The desired products were produced in high yields and short reaction times. The naphthoquinone-fused indole derivatives were evaluated for their pro-inflammatory cytokines responses using lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages. The results showed that most of the tested compounds inhibit the production of nitric oxide (NO), prostaglandin (PG)E_2, tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β in RAW264.7 cells treated with LPS.

Quinones are common in a variety of natural products and have been reported to possess a broad range of biological activities including anticancer,[1–5] antibacterial,[1,6–9] antimalarial,[10–12] antifungal[9,13] and anti-inflammatory[14,15] activities. Also aminonaphthoquinones were claimed to show promising antimalarial, antiplatelet, anti-inflammatory and antiallergenic activities.[16,17] A number of naphthoquinones fused with heterocyclic rings have been synthesised and it was found that the presence of a heterocycle fused to the quinone moiety also played a crucial role in the biological activity of the compounds.[18,19] As part of our research program to synthesise novel bioactive compounds, we became interested in naphtho[2,3-*d*]-azepino[4,5-*b*]indole-9,14-dione, in which seven-membered ring heterocyclic naphthoquinones are fused to the indole nucleus, since indoles also possess a wide variety of biological properties. We anticipated that the combined chemical motif of a naphthoquinone, an indole group and an azepine moiety in the same molecule would markedly enhance the anti-inflammatory activity as each compound is predominantly seen in a wide range of anti-inflammatory agents. To the authors' best knowledge, there is no report concerning the synthesis and biological activities of 1,4-naphthoquinones fused through the quinone nucleus to the azepino-indole moiety. With the increased role of microwave chemistry, especially when conventional methods require forcing conditions or prolonged reaction times, a wide variety of organic reactions can be conducted very rapidly and in high yields. On the basis of the above consideration, we now wish to report the synthesis of naphtho[2,3-*d*]-azepino[4,5-*b*]indole-9,14-dione derivatives (Fig. 1) via microwave assisted intramolecular cyclisation and the evaluation of pro-inflammatory activities on lipopolysaccharide (LPS)-induced RAW264.7 cells.

The substituted aminonaphthoquinones (**3**) required for cyclisation were prepared under irradiation using a modified commercial domestic microwave oven.[20] Nucleophilic substitution of naphthoquinones (**1a–c**) with tryptamine derivatives (**2a–b**) afforded the corresponding substituted aminonaphthoquinones (**3a–e**) in moderate to high yields[21] by heating in a microwave reactor at 850 W for 4 min.[20] The palladium catalysed intermolecular reaction of **3a** and **3b** under microwave irradiation at 850 W for 6 min failed to yield the cyclised products. However, under the same



R^1, R^2= H, OMe
R^3= H, CH_3, C_3H_7, Allyl, Bn

Figure 1. General structure of naphtho[2,3-*d*]-azepino[4,5-*b*]indole-9,14-dione.

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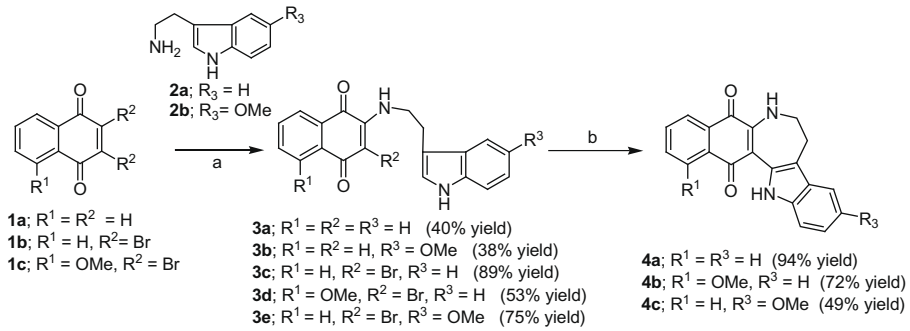
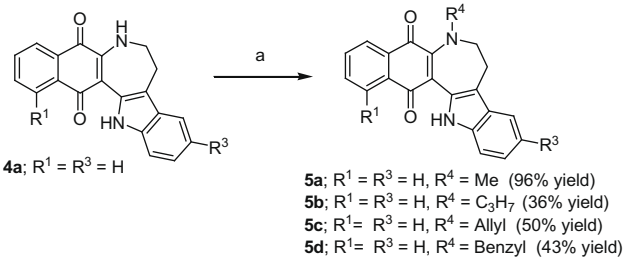


Scheme 1. Reagents and conditions: (a) C_2H_5OH, microwave, 850 W, 4 min (b) 0.1 equiv $Pd(OAc)_2$, 0.2 equiv Ph_3P, 3 equiv K_2CO_3, microwave, 850 W, DMF, 6 min.



Scheme 2. Reagents and conditions: (a) R^4X, K_2CO_3, microwave, 850 W, DMF, 8 min.

reaction conditions, brominated aminonaphthoquinones (**3c–e**) were cyclised to afford the indole fused seven-membered ring heterocyclic naphthoquinones (**4a–c**) (Scheme 1) in high yields unaccompanied by any reduced products.[22]

The effect on activity of eliminating the NH group of the azepines was evaluated by selective N-alkylation of azepine moiety **4a** using various alkyl halides and potassium carbonate under microwave irradiation at 850 W for 8 min (Scheme 2). Compounds **5a–d** were obtained in a short reaction time and in moderate to high yields.[23]

Compounds **4a–c** and **5a–d** were tested in LPS (1 μg/mL)-stimulated RAW264.7 macrophages. The cytotoxic effects of these compounds were evaluated in the presence or absence of LPS using the MTT assay, and cell viabilities were observed to be 58–87% for all tested compounds with the exception of **5c** for which 24% cell viability was observed. In the measurement of NO production stimulated by LPS (1 μg/mL), treatment of the tested compounds (1–5 μg/mL) inhibits the production of NO in a dose-dependent manner (Fig. 2).

The effect of the tested compounds on LPS (1 μg/mL) induced production of PEG_2 in RAW 264.7 was shown in Figure 3. The tested compounds could dramatically inhibit the production of PGE_2 at a concentration of 5 μg/mL, however only compound **4b–c** and **5d** slightly inhibited the production of PGE_2 at a concentration of 1 μg/mL.

Further studies on the pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6[24–26] that are thought to be interlinked in a cascade, being produced serially by macrophages during inflammatory response demonstrated that the tested compounds have inhibitory effects on the production of TNF-α, IL-1β and IL-6 in LPS-stimulated RAW 264.7 macrophages. As shown in Figures 4–6, LPS-induced production of TNF-α was significantly inhibited by the tested compounds in a concentration-dependent manner. However, all of the tested compounds mildly affected the production of IL-1β and IL-6.

In summary, syntheses of the indole-fused azepinonaphthoquinones (**4a–c**) were achieved with an intramolecular palladium-catalysed cyclisation of aminonaphthoquinones bearing an indole nucleus as the key step. In addition, N-alkylation of the NH group on the severn-membered rings afforded the corresponding derivatives **5a–d**. The indole-fused azepinonaphthoquinones (**4a–c** and **5a–d**) were tested for their pro-imflammatory activities. Most of the tested compounds inhibited the production of NO, PGE_2, TNF-α, IL-1β and IL-6 in macrophages stimulated with LPS

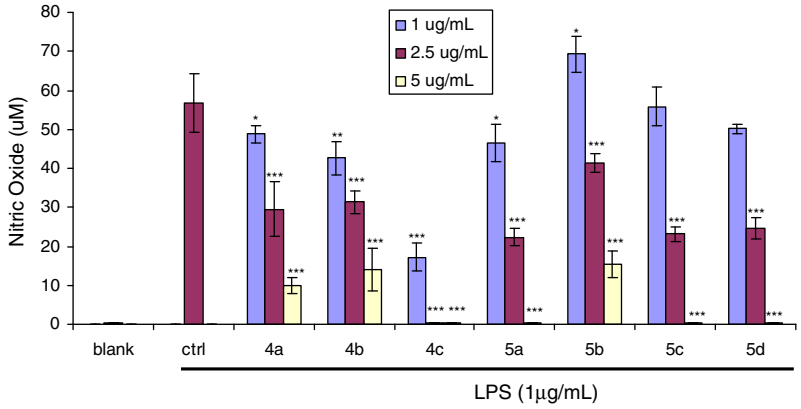


Figure 2. Evaluation of nitric oxide production by RAW 264.7 cells stimulated for 24 h with LPS alone or in combination with increasing concentrations (1.0–5.0 μg/ml) of heterocyclic naphthoquinones derivatives. The values are the means of at least three determinations ± SD. Probability level (student's *t*-test): $^{*}p$ <0.05, $^{**}p$ <0.01,$^{***}p$ <0.001 versus LPS-treated group.

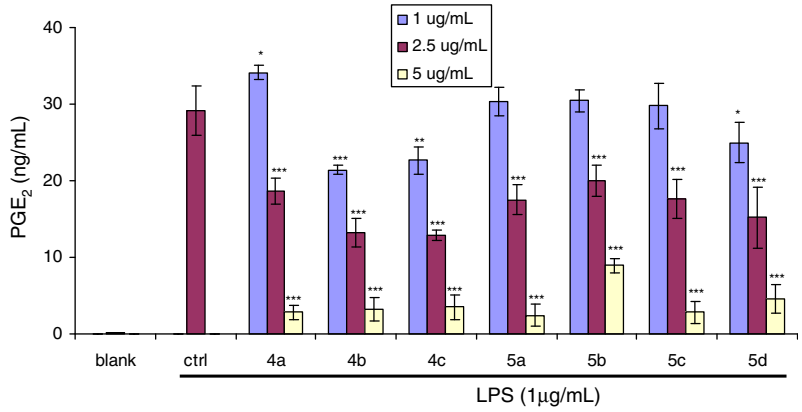


Figure 3. Effect of heterocyclic naphthoquinones derivatives on PGE_2 production in LPS-induced RAW 264.7 macrophage for 24 h. The values are the means of at least three determinations ± SD. Probability level (student's *t*-test): $^{*}p$ <0.05, $^{**}p$ <0.01,$^{***}p$ <0.001 versus LPS-treated group.

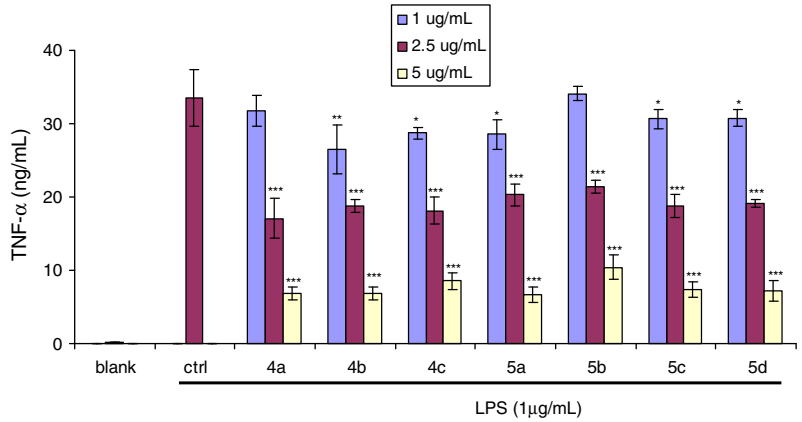


Figure 4. Effect of heterocyclic naphthoquinones derivatives on LPS-induced TNF-α production by RAW 264.7 cells. The values are the means of at least three determinations ± SD. Probability level (student's *t*-test): $^{*}p$ <0.05, $^{**}p$ <0.01,$^{***}p$ <0.001 versus LPS-treated group.

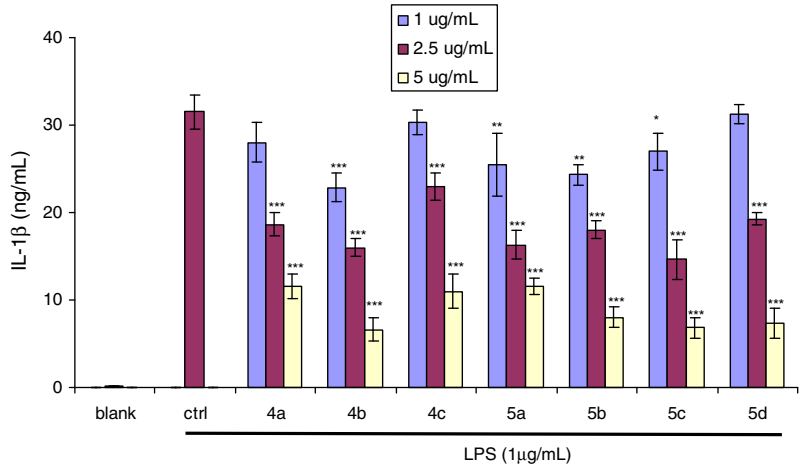


Figure 5. Effect of heterocyclic naphthoquinones derivatives on LPS-induced IL-1β production by RAW 264.7 cells. The values are the means of at least three determinations ± SD. Probability level (student's *t*-test): $^{*}p$ <0.05, $^{**}p$ <0.01,$^{***}p$ <0.001 versus LPS-treated group.

in vitro. We conclude from our investigation that the series of 1,6,7,8-tetra hexahydro-naphtho[2,3-*d*]-azepino[4,5-*b*]indole-9,14-diones could be potential useful for the development of anti-inflammatory agents.

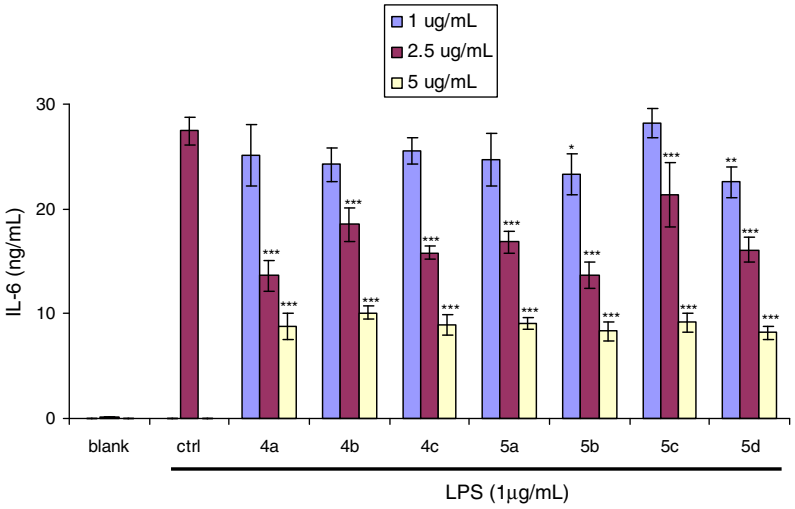


Figure 6. Effect of heterocyclic naphthoquinones derivatives on LPS-induced IL-6 production by RAW 264.7 cells. .The values are the means of at least three determinations ± SD. Probability level (student's *t*-test): $^{*}p$ <0.05, $^{**}p$ <0.01,$^{***}p$ <0.001 versus LPS-treated group.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.154.

References and notes

1. Tandon, V. K.; Singh, R. V.; Yadav, D. B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2901–2904.
2. Yamashita, M.; Kaneko, M.; Iida, A.; Tokuda, H.; Nishimura, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6417–6420.
3. Miguel del Corral, J. M.; Castro, M. A.; Oliveira, A. B.; Gualberto, S. A.; Cuevas, C.; Feliciano, A. S. *Bioorg. Med. Chem.* **2006**, *14*, 7231–7240.
4. Miguel del Corral, J. M.; Castro, M. A.; Gordaliza, M.; Martin, M. L.; Gamito, A. M.; Cuevas, C.; Feliciano, A. S. *Bioorg. Med. Chem.* **2006**, *14*, 2816–2827.
5. Miguel del Corral, J. M.; Castro, M. A.; Gordaliza, M.; Martn, M. L.; Gualberto, S. A.; Gamito, A. M.; Cuevas, C.; Feliciano, A. S. *Bioorg. Med. Chem.* **2005**, *13*, 631–644.
6. Mahapatra, A.; Mativandlela, S. P. N.; Binneman, B.; Fourie, P. B.; Hamilton, C. J.; Meyer, J. J. M.; van der Kooy, F.; Houghton, P.; Lall, N. *Bioorg. Med. Chem.* **2007**, *15*, 7638–7646.
7. Tandon, V. K.; Yadav, D. B.; Singh, R. V.; Chaturvedi, A. K.; Shukla, P. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5324–5328.
8. Machado, T. B.; Pinto, A. V.; Pinto, M. C. F. R.; Leal, I. C. R.; Silva, M. G.; Amaral, A. C. F.; Kuster, R. M.; Netto-dosSantos, K. R. *Int. J. Antimicrob. Agents* **2003**, *21*, 279–284.
9. Gafner, S.; Wolfender, J.-L.; Nianga, M.; Stoeckli-Evans, H.; Hostettmann, K. *Phytochemistry* **1996**, *42*, 1315–1320.
10. Likhitwitayawuid, K.; Kaewamatawong, R.; Ruangrungsi, N.; Krungkrai, J. *Planta Med.* **1998**, *64*, 237–241.
11. dos Santos, E. V. M.; Carneiro, J. W. d. M.; Ferreira, V. F. *Bioorg. Med. Chem.* **2004**, *12*, 87–93.
12. Kittakoop, P.; Punya, J.; Kongsaeree, P.; Lertwerawat, Y.; Jintasirikul, A.; Tanticharoen, M.; Thebtaranonth, Y. *Phytochemistry* **1999**, *52*, 453–457.
13. Tandon, V. K.; Maurya, H. K.; Tripathi, A.; ShivaKeshava, G. B.; Shukla, P. K.; Srivastava, P.; Panda, D. *Eur. J. Med. Chem.* **2009**, *44*, 1086–1092.
14. Checker, R.; Sharma, D.; Sandur, S. K.; Khanam, S.; Poduval, T. B. *Int. Immunopharmacol.* **2009**, *9*, 949–958.
15. Ferreira, M. A. D.; Nunes, O. D. R. H.; Fontenele, J. B.; Pessoa, O. D. L.; Lemos, T. L. G.; Viana, G. S. B. *Phytomedicine* **2004**, *11*, 315–322.
16. Kapadia, G.; Azuine, M. A.; Balasuramanian, V.; Sridhar, R. *Pharmaco. Res.* **2001**, *43*, 363–367.
17. Lien, J.-C.; Huang, L.-J.; Wang, J.-P.; Teng, C.-M.; Lee, K.-H.; Kuo, S.-C. *Bioorg. Med. Chem.* **1997**, *5*, 2111–2120.
18. Wang, X. L.; Zheng, X. F.; Liu, R. H.; Reiner, J.; Chang, J. B. *Tetrahedron* **2007**, *63*, 3389–3394.
19. SungKim, J.; Lee, H.-J.; Suh, M.-E.; Choo, H.-Y. P.; Lee, S. K.; Park, H. J.; Kim, C.; Park, S. W.; Lee, C.-O. *Bioorg. Med. Chem.* **2004**, *12*, 3683–3686.
20. Phutdhawong, W.; Buddhasukh, D. *Molecules* **2005**, *10*, 1409–1412.
21. *Typical procedure: the preparation of 2-(1H-indol-3-yl)ethylamino-1,4-naphthoquinone* (**3a**). To a solution of 1,4 naphthoquinones (**1a**) (50 mg, 0.32 mmol) in DCM (0.5 mL) was added a solution of tryptamine (**2a**) (61 mg, 0.38 mmol) in EtOH (3 mL) at room temperature under argon. The mixture was irradiated in a microwave reactor at 850 W for 4 min and then cooled. The mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (silica gel, hexane/EtOAc (1:1) to give **3a** as a red solid with an R_f value of 0.57 (40 mg, 40%), mp 184–185 °C; IR (CH_2Cl_2) ν_{max}: 1509, 1575, 1609, 1678, 3382, 3469 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.16 (t, J = 6.8 Hz, 2H), 3.52 (q, J = 6.4 Hz, 2H), 5.80 (s, 1H), 6.03 (br s, 1H), 7.09 (s, 1H), 7.15 (td, J = 0.8, 7.3 Hz, 1H), 7.23 (td, J = 1.1, 7.7 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.60 (td, J = 1.0, 7.6 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.72 (td, J = 1.2, 7.5 Hz, 1H), 8.01 (dd, J = 1.0, 7.6 Hz, 1H), 8.10 (dd, J = 0.8, 7.6 Hz, 1H), 8.15 (br s, 1H); ^{13}C-NMR ($CDCl_3$) δ 24.2, 42.5, 100.9, 111.5, 112.7, 118.5, 119.7, 122.2, 122.5, 126.2, 126.3, 127.0, 130.5, 132.0, 133.7, 134.8, 136.5, 147.9, 181.0, 183.0; HRES-MS *m/z* calcd for $[M+H]^+$ $C_{20}H_{17}N_2O_2$: 317.1285, found: 317.1251.
22. *Typical procedure: the preparation of 1,5,6,7,8,14b-hexahydro-naphtho[2,3-d]-azepino[4,5-b]indole-9,14-dione* (**4a**). A mixture of aminonaphthoquinone (**3a**) (46 mg, 0.12 mmol), $Pd(OAc)_2$ (3 mg, 0.012 mmol), $P(Ph)_3$ (7 mg, 0.024 mmol) and K_2CO_3 (48 mg, 0.35 mmol) in DMF (4.0 ml) was irradiate in the modified domestic microwave reactor at 850 W for 6 minutes under an Ar atmosphere. After cooling, the DMF was evaporated, and then EtOAc (15 mL) was added and washed several times with water. The EtOAc layer was washed with brine, dried, evaporated and subjected to flash column chromatography (silica gel, 2:1 hexane/EtOAc) to give **4a** with an R_f value of 0.73 (35 mg, 94%) as a blue solid, mp 218–219 °C; IR (CH_2Cl_2) ν_{max}: 1519, 1566, 1599, 1630, 1668, 3347 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.16 (t, J = 4.5 Hz, 2H), 3.65 (q, J = 4.5 Hz, 2H), 7.00 (td, J = 1.0, 7.5 Hz, 1H), 7.10 (td, J = 1.0, 7.5 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.50 (td, J = 1.2, 7.5 Hz, 1H), 7.62 (td, J = 1.2, 7.5 Hz, 1H), 7.91 (dd, J = 1.0, 7.5 Hz, 1H), 8.05 (dd, J = 1.0, 7.5 Hz, 1H), 11.46 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.4, 44.1, 105.0, 110.1, 114.4, 116.6, 118.2, 121.5, 125.0, 125.7, 126.2, 128.7, 128.8, 131.4, 133.1, 133.9, 134.5, 143.6, 180.1, 183.6; HRES-MS *m/z* cacld for $[M+H]^+$ $C_{20}H_{15}N_2O_3$: 315.1128, found: 315.1032.
23. *Typical procedure*: the preparation of 1,5,6,7,8,14b-hexahydro-naphtho[2,3-*d*]-*N*-methyl-azepino[4,5-*b*]indole-9,14-dione (**5a**). To a solution of heterocyclic naphthoquinones **4a** (45 mg, 0.14 mmol) and K_2CO_3 (48 mg, 0.35 mmol) in dry DMF (4 mL) was stirred at 0–5 °C for 30 minutes under Argon atmosphere was added methyl iodide (0.03 mL, 0.43 mmol) and the mixture irradiated in the microwave reactor at 850 W for 8 minutes. Water (15 ml) was then added to the reaction mixture and the mixture was extracted with EtOAc (2 × 10 ml). The EtOAc layer was dried, evaporated and subjected to flash column chromatography (silica gel, 4:1 hexane/EtOAc) to give **5a** with an R_f value of 0.60 (45 mg, 96%) as a blue solid, mp 218–219 °C; IR (CH_2Cl_2) ν_{max}: 1542, 1595, 1634, 1664, 3379 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.16 (t, J = 4.9 Hz, 2H), 3.27 (t, J = 4.9 Hz, 2H), 3.29 (s, 3H), 7.03 (td, J = 1.0, 7.4 Hz, 1H), 7.15 (td, J = 1.0, 7.4 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.56 (td, J = 1.5, 7.5 Hz, 1H), 7.62 (td, J = 1.5, 7.5 Hz, 1H), 7.92 (dd, J = 1.3, 7.5 Hz, 1H), 8.07 (dd, J = 1.3, 7.5 Hz, 1H), 11.32 (br s, 1H).; ^{13}C NMR ($CDCl_3$) δ 24.3, 42.3, 53.2, 110.0, 112.7, 116.2, 117.6, 118.3, 122.2, 124.9, 125.4, 126.6, 128.9, 130.5, 131.8, 132.3, 132.8, 134.6, 149.7, 181.8, 185.5; HRES-MS *m/z* calcd for $[M+H]^+$ $C_{21}H_{16}N_2O_2$: 329.1290, found: 329.1247.
24. Chang, H. P.; Huang, S. Y. *J. Agric. Food Chem.* **1994**, *53*, 2530–2534.
25. Feldmann, M.; Brennan, F. M.; Maina, R. N. *Annu. Rev. Immunol.* **1996**, *14*, 397–440.
26. Hasko, G.; Nemeth, Z. H.; Szabo, C. *Brain Res. Bull.* **1998**, *45*, 183–187.