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The synthesis and spectral properties of new DNA binding ligands

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ABSTRACT

Two new ligands based on anthracene or carbazole planar skeletons, and a phenyloxazoline moiety linked by a vinyl bridge are synthesized as potential DNA-interacting drugs. Their spectral characteristics and DNA binding affinity are assessed.

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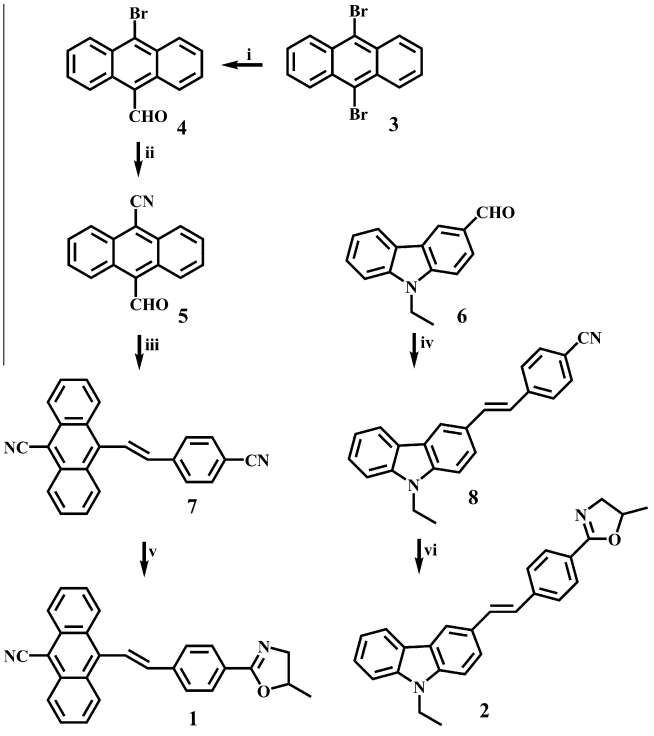
Small organic molecules (ligands) can interact selectively with DNA via the minor groove, or by intercalation, but the interaction preferences depend on the ligand structure and on the nature of DNA. Small differences in the structure of a DNA-interacting ligand may affect the binding preferences and stability of the ligand/DNA complex.[1,2]

G-Quadruplexes are structural forms of nucleic acids and contain G quartets (each consists of four guanines held together by eight hydrogen bonds). These tetraplex structures are stabilized in the presence of specific metal cations (Na^+, K^+). With hypotheses that G-quadruplex structures play an important role in biological processes, the level of interest in these structures has increased. The ligands which selectively interact with the G-quadruplex are called 'G-quadruplex ligands', and are known to exhibit anticancer activities and their number has grown rapidly over the past few years.[2–5]

Our research group is interested in the synthesis of ligands containing anthracene, and carbazole skeletons, which can bind to the secondary structure of guanine-rich DNA. The designed new ligands **1,** and **2**, are expected to interact with the G-quadruplex via external stacking due to the presence of a planar anthracene or carbazole unit and a vinyloxazoline arm coupled with delocalized π-electrons. The polyaromatic moieties may exhibit steric effects, and photochemical activity, and are reported to promote selectively the formation and/or stabilization of higher-order DNA structures.[6] The structures of the ligands can be easily altered by *cis–trans* photoisomerization of a double bond.[7] Finally, the oxazoline moiety may serve as a potential donor of electrons, and can be installed as a chiral non-racemic group.[8] The ligands **1,** and **2** were synthesized via the Arbuzov–Horner modification of the Wittig reaction (Scheme 1).

The starting diethyl[(4-cyanophenyl)methyl]phosphonate was prepared from *p*-toluonitrile by known synthetic procedures in 79% overall yield.[9,10] The 9-cyano-10-anthracenocarboxaldehyde (**5**) was prepared from 9,10-dibromoanthracene (**3**) in two steps.[11–13] Addition of *n*-buthyllithium to **3** was carried out following the general reaction conditions specific for this type of reaction. The best results in terms of chemical yield were achieved using *N*-formylmorpholine as a formylating agent.[14] 9-Bromoanthracene-10-carboxaldehyde (**4**) was reacted with copper(I) cyanide in DMSO to afford **5** in a good yield of 81%,[15] while in DMF the



Scheme 1. Reagents and conditions: (i) (1) *n*-BuLi, THF, −72 °C, 1 h; (2) 4-formylmorpholine, −35 °C, 12 h; (ii) CuCN (10 equiv), DMSO, 190 °C, 6 h; (iii) $CNC_6H_4CH_2PO(OC_2H_5)_2$, MeONa, DMF, 0 °C, 3 h; (iv) $CNC_6H_4CH_2PO(OC_2H_5)_2$, MeONa, DMF, reflux, 22 h; (v) (±)-1-amino-2-propanol (40 equiv), ethylene glycol/glycerol, K_2CO_3, 115 °C, 72 h; (vi) (±)-1-amino-2-propanol (10 equiv), ethylene glycol/glycerol, K_2CO_3, 115 °C, 22 h.

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reaction product was not observed.[16] Application of the Arbuzov–Horner modification of the Wittig reaction using MeONa as base in DMF afforded the expected product in moderate yields.[17] With *t*-BuOK no product was observed.[18] Nitrile **8** was obtained from carbazole **6** in a similar manner.

Nitriles **7,** and **8**,[19] possessing almost exclusively the *E,E*-configuration, as indicated by ^{1}H NMR spectroscopic analysis, were converted into oxazolines **1,** and **2**,[20] by known synthetic procedures in moderate yields.[21] In the case of compound **7** with two nitrile groups (IR: 2224 cm^{-1} (C_6H_4–C≡N) and 2211 cm^{-1} ($C_{14}H_8$–C≡N), only the benzylic nitrile underwent conversion to an oxazoline moiety (disappearance of the band at 2224 cm^{-1}).

The new ligands **1,** and **2** are soluble in several organic solvents and the UV/Vis absorption spectra did not depend on the nature of the solvent (some changes in intensity of the absorption bands occurred without remarkable band shifts) as shown in Table 1.

Small solvent-dependent shifts (1–8 nm) and variations in molar absorptivities were observed, but they did not seem to have any correlation with the polarity of the solvents. In the case of ligand **1** a broad band at ca. 413 nm suggests conjugation between the anthryl chromophore and the phenyl-oxazoline system.

In contrast to organic solvents, ligands **1,** and **2** were only partially soluble in water, and aqueous buffer solution. The absorption spectra of aqueous solutions of the ligands, prepared by injection of a small volume of ligand stock solution in EtOH, showed a gradual decrease in absorbance with time. The same effects were observed in the fluorescence spectra of aqueous solutions, which indicated aggregation phenomena, and slow precipitation processes. Indeed, after 30 min, the absorbance decreased by ca. 30%, showing a noticeable turbidity Fig. 1).

Since ligands **1,** and **2** were designed as potential DNA binding agents, their tendency for aggregation in aqueous solutions made further studies difficult. On the other hand, DNA preserves its higher-order structure in the presence of moderate amounts of organic solvents such as EtOH or DMSO (up to 10%). Therefore, we investigated the spectral properties of the ligands in mixed H_2O/EtOH systems containing 5–80% EtOH. Satisfactory spectral stability was observed in all H_2O/EtOH mixtures, even solutions containing only 5% EtOH exhibited good stability, and reproducibility of spectral parameters.

The absorbance of ligands **1** and **2** in 5% EtOH was noticeably lower than those in pure EtOH, but they did not change with time. It should be noted that for both ligands, molar absorptivities in 5% EtOH solution were remarkably lower than those in EtOH or other organic solvents (Table 1).

The fluorescence spectra of the ligands were recorded in EtOH and in H_2O/EtOH-mixed solvent systems. The most interesting results are shown in Figure 2. The ligand **2** exhibits a broad weak emission band at 477 nm, and at 487 nm in 96% EtOH, and 5% EtOH, respectively, but the latter is slightly red-shifted and broadened. These effects and the much lower molar absorptivity may indicate the formation of less fluorescent dimers in aqueous solution containing only 5% EtOH.

Interestingly, the fluorescence spectra of ligand **1** exhibited more complex behavior depending on the mixed solvent composition. Bimodal fluorescence was observed in 96% EtOH with one emission band at ca. 450 nm, and a second band at ca. 550 nm. The first band shows vibronic structure characteristic of the anthracene emission and may represent the monomer fluorescence whereas the broad long-wavelength fluorescence originates, probably from the excited dimer of ligand **1**. These assignments

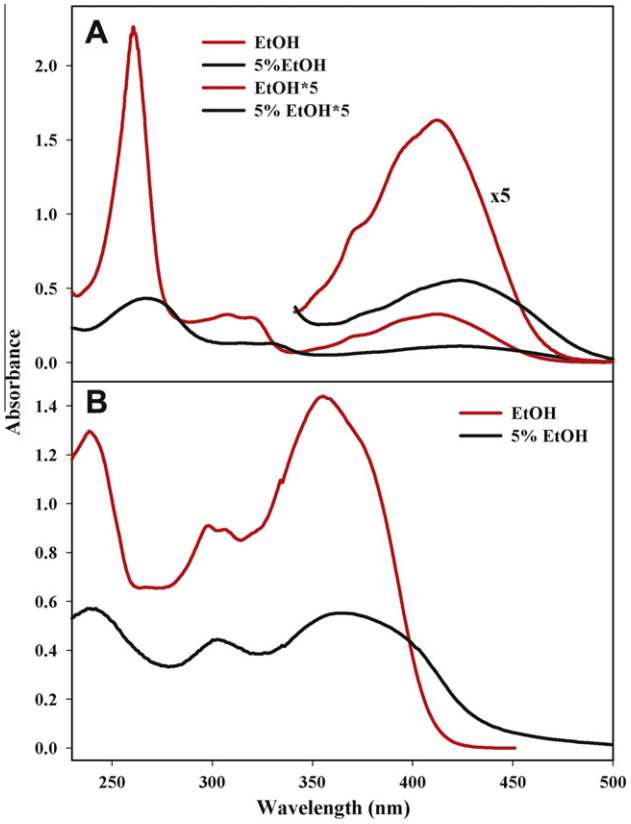

Figure 1. Absorption spectra of ligand **1** (**A**): (1.9×10^{-5} M) and ligand **2** (**B**): (4.3×10^{-5} M) in EtOH and 5% EtOH.

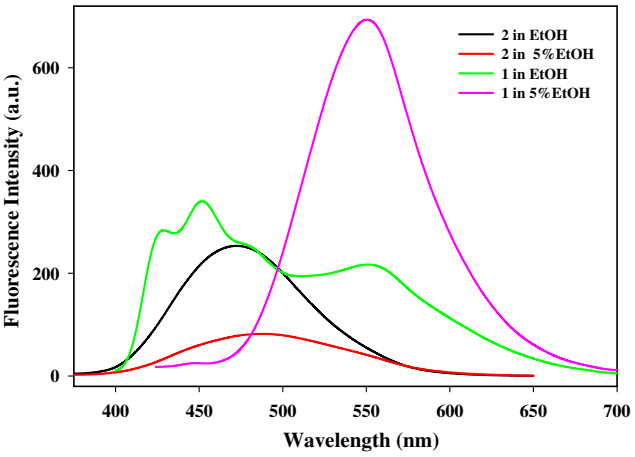

Figure 2. Fluorescence emission spectra of ligand **1** (1.19×10^{-5} M in 96% EtOH (green) and 5% EtOH (pink), λ_{ex} = 388 nm), and ligand **2**(1.5×10^{-5} M in 96% EtOH (black) and 5% EtOH (red), λ_{ex} = 350 nm).

Table 1
Effect of solvent on the spectral characteristics of the absorption spectra of ligands **1** and **2**

Solvent	λ_{max}/nm ($\varepsilon \times 10^{-4}$/$M^{-1}$ cm^{-1})			
	Ligand **1**		Ligand **2**	
MeOH	411 (1.61)	260 (12.7)	356 (3.3)	239 (3.0)
EtOH	412 (1.72)	260 (12.9)	358 (3.3)	237 (2.95)
5% EtOH	424 (0.58)	266 (2.47)	363 (1.27)	241 (1.29)
CH_3CN	412 (1.66)	260 (10.5)	355 (3.4)	239 (3.0)
$CHCl_3$	413 (1.66)	263 (10.2)	357 (3.2)	nd[a]
DMSO	419 (1.54)	nd[a]	359 (2.8)	nd[a]
Toluene	415 (1.61)	nd[a]	358 (3.4)	nd[a]

[a] nd—not determined—insufficient solvent transmittance.

correlate well with the emission characteristics of ligand **1** in 5% EtOH which exhibit only a single broad emission band centered around 550 nm. The position and shape of the long-wavelength emission band resemble those reported for anthrylvinylpyridinium derivatives.[22] The relative intensities of both fluorescence bands change with the content of ethanol in solution and can be ascribed to the extent of dimerization (association) of the ligand. The reversibility of this association process was confirmed by changing the solvent mixing order, which gave the same bimodal-emission spectra at 50% content of EtOH, starting from both 5% EtOH and pure EtOH solutions of **1**.

Preliminary studies on the DNA binding affinity of the ligands were carried out with calf thymus DNA as a double-stranded DNA (dsDNA), and 22-mer oligonucleotide (5′-AGGGTTAGGGTTA GGGTTAGG G-3′), with a sequence related to human telomere DNA (G4 DNA), which can form intramolecular G-quadruplex structures by the folding of a single oligonucleotide molecule. Small spectral changes were observed in the absorption and emission spectra of both ligands in the presence of DNA as shown in Figure 3.

These indicate that the ligands interact with both dsDNA, and G4 DNA, but spectral effects are rather modest. In the case of ligand **1** small quenching and a blue-shift of fluorescence was observed (Fig. 3A). The fluorescence spectrum of ligand **2** undergoes opposite changes in the presence of DNA, that is, a red-shift and a small enhancement effect.

It can be speculated, that the poor solubility of the ligands in aqueous solution, and the tendency to form associates may hamper efficient intercalation of the ligands to the DNA samples. The introduction of a positive charge into the ligand structure (quaternary nitrogen) should improve the solubility and DNA binding affinities of the ligands. Further investigations including new syntheses and DNA binding studies are in progress.

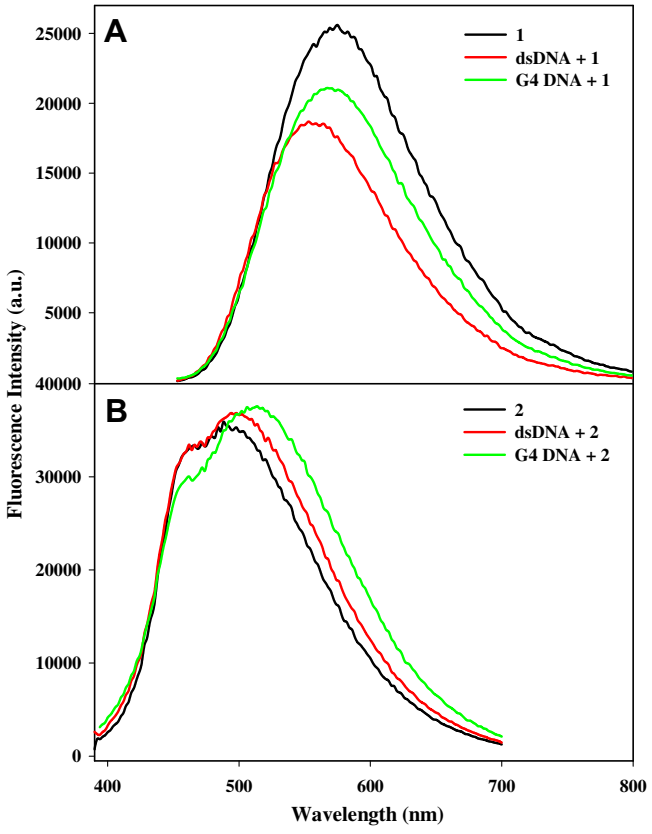

Figure 3. Fluorescence emission spectra of ligand **1** (**A**): (1.62×10^{-5} M, 5% EtOH, 10 mM Tris–HCl pH 7.2, λ_{ex} = 388 nm) and ligand **2** (**B**): (1.2×10^{-5} M, 5% EtOH, 10 mM Tris–HCl pH 7.2, λ_{ex} = 350 nm) in the absence (black) and the presence of dsDNA (1.25 equiv, red) and G4 DNA (1.25 equiv, green).

Acknowledgements

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11. *Compound* **4**: To a vigorously stirred suspension of 9,10-dibromoanthracene (1.00 g, 3 mmol) in THF (60 mL) under an argon atmosphere at −72 °C *n*-BuLi (1.6 M solution in hexane, 6.25 mL, 8.35 mmol, 1.4 equiv) was added dropwise. After 1 h, 4-formylmorpholine (0.72 mL, 7.2 mmol, 1.2 equiv) was added at the same temperature. The yellow clear mixture was kept at −35 °C for 12 h and then quenched with 20% NH_4Cl (20 mL) at the same temperature. After warming to rt, phases were separated and the aqueous one was extracted with Et_2O (3 × 20 mL). The combined organic extracts were dried, and the solvent evaporated under reduced pressure to afford a crude product which was crystallized from CH_2Cl_2/hexane to give 485 mg (70%) of 9-bromoanthracene-10-carboxaldehyde (**4**) as yellow crystals. Mp: 210–213 °C [lit.[12] 207–208 °C (C_6H_6)]. Spectral data were identical with literature data.[12]
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13. *Compound* **5**: To a yellow suspension of 9-bromoanthracene-10-carboxaldehyde (**4**) (300 mg, 1 mmol) in DMSO (30 mL) CuCN (895 mg, 10 mmol) was added. The mixture was heated at reflux with stirring until no more substrate was present (6 h, TLC). After cooling to rt the copper complex of the product was destroyed with 25% NH_4OH (30 mL). The solid was filtered off, washed with H_2O (40 mL), dried, and was crystallized from CH_2Cl_2/hexane to give 194 mg (81%) of 9-cyano-10-anthracenocarboxaldehyde (**5**) a yellow crystals. Mp: 260–263 °C (lit.[12] 263–264 °C). Spectral data were identical with literature data.[12]
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19. Synthesis of nitriles **7** and **8**: To a solution of diethyl[(4-cyanophenyl)-methyl]phosphonate[9,10] (278 mg, 1.1 mmol) in DMF (1 mL), freshly prepared MeONa (0.7 mL, 1.62 mmol, 2.3 M/dm^3) was added. The ylide was generated at room temperature in 10 min under an argon atmosphere. After cooling to 0 °C aldehyde (1 mmol) in DMF (4 mL) was added. The mixture was stirred at this temperature or at rt until completion of the reaction (TLC). The resulting precipitate was filtered and the filtrate poured onto ice. The solid was filtered, the combined solids washed with H_2O (20 mL) and hexane (20 mL), and dried to give the crude product. *Compound* **7**: 3 h, 0 °C. The crude product was crystallized from CH_2Cl_2/hexane; yellow crystals (65%), mp: 268–270 °C (hexane–CH_2Cl_2). IR (KBr) cm^{-1}: 2224 (C≡N), 2211 (C≡N), 964 (E CH=CH). ^{1}H NMR ($CDCl_3$) δ: 6.96 (d, J = 16.7 Hz, 1H, CH=CH), 7.52–7.62 (m, 2H, ArH), 7.72–7.86 (m, 4H, ArH), 8.00 (d, J = 16.5 Hz, 1H, CH=CH), 8.01–8.04 (m, 2H, ArH), 8.32–8.37 (m, 2H, ArH), 8.46–8.50 (m, 2H, ArH). EIMS: m/z (%): 227 (26), 301 (16), 328 (M−2, 14), 329 (M−1, 43), 330 (M+, 100), 331 (M+1, 33), 332 (M+2, 11). HRMS calcd for $C_{24}H_{14}N_2$ 330.11569. Found 330.11614.*Compound* **8**: 22 h, rt; the TLC-pure product **8** was used in the next step without further purification; yellow crystals (yield: 55%), mp: 186–188 °C. IR (KBr) cm^{-1}: 2220 (C≡N), 966 (E CH=CH). ^{1}H NMR ($CDCl_3$) δ: 1.45 (t, J = 7.1 Hz, 3H, CH_2–CH_3), 4.38 (q, J = 7.1 Hz, 2H, CH_2–CH_3), 7.11 (d, J = 16.5 Hz, 1H, CH=CH), 7.24–7.29 (m, 1H, ArH), 7.39–7.52 (m, 3H, ArH), 7.55 (d, J = 16.5 Hz, 1H, CH=CH), 7.58–7.69 (m, 5H, ArH), 8.11–8.14 (m, 1H, ArH). EIMS: m/z (%): 204 (10), 292 (16), 307 (63), 322 (100). HRMS calcd for $C_{23}H_{18}N_2$ 322.14700. Found 322.14591.
20. Synthesis of oxazolines **1** and **2**. *Compound* **1**: To (±)-1-amino-2-propanol (1.1 mL, 14 mmol) and anhydrous K_2CO_3 (81.5 mg) in a mixture of ethylene

glycol (10.8 mL), and glycerol (5.9 mL) *trans*-1-[(9-(10-cyanoanthryl)]-2-(4-cyanophenyl)ethylene (**7**) (82 mg, 0.35 mmol) was added, and the resulting mixture was heated at 115 °C with stirring under an argon atmosphere until no more substrate reacted by TLC (ca. 72 h). After cooling to ambient temperature, the mixture was poured onto ice and after rt was reached the solid was filtered. The solid was washed with H_2O (20 mL) and hexane (20 mL), and was dried to give 41 mg (43%) of crude *trans*-1-[(9-(10-cyanoanthryl)]-2-[4-(5-methyl-2-oxazoline)phenyl]ethylene (**1**), which was purified by HPLC using a preparative column (Inertsil PRER-ODS 100% MeOH, flow 9.9 ml/min, 258 nm. Retention time = 2.9 min), Mp: 210–215 °C. IR (KBr) cm^{-1}: 2211 (C≡N), 1704 (C=N), 975 (E CH=CH). ^1H NMR ($CDCl_3$) δ: 1.31 (d, 3H, J = 6,3 Hz, CH_3), 3.65 (dd, 1H, J = 7.4, 14.6 Hz, C=N–CHH), 4.18 (dd, 1H, J = 9.3, 14.6 Hz, C=N–CHH), 4.89–4.93 (m, 1H, OCH–CH_3), 6.96 (d, 1H, J = 16.5 Hz, CH=CH), 7.58–7.62 (m, 4H, ArH), 7.71–7.74 (m, 2H, ArH), 7.95 (d, 1H, J = 16.5 Hz, CH=CH), 8.03–8.23 (m, 2H, ArH), 8.39–8.45 (m, 2H, ArH), 8.46–8.49 (m, 2H, ArH). EIMS: m/z (%): 388 (M^+, 100), 389 (M+1, 37), 390 (M+2, 12). HRMS calcd for $C_{27}H_{20}N_2O$ 388.15756. Found 388.15911. Compound **2**: To (±)-1-amino-2-propanol (0.175 ml, 2.5 mmol), and anhydrous K_2CO_3 (53 mg) in a mixture of ethylene glycol (0.48 ml), and glycerol (0.27 ml) *trans*-1-[(9-*n*-ethyl)-3-carbazole]-2-(4-cyanophenyl)-ethylene (**8**) (80.5 mg, 0.25 mmol) was added, and the resulting mixture was heated at 115 °C with stirring under an argon atmosphere until no more substrate reacted (ca. 22 h, TLC). After cooling to rt, H_2O (10 mL) was added, and the solid was filtered. The filtrate was extracted with $CHCl_3$ until the Dragendorff test was negative. The combined organic extracts were dried and the solvent was evaporated under reduced pressure to afford crude product **2** (64 mg, 67%). This was washed with Et_2O (15 mL). The ethereal solution was concentrated in vacuo to give TLC-pure *trans*-1-[(9-*n*-ethyl)-3-carbazole]-2-[4-(5-methyl-2-oxazoline)phenyl]-ethylene (**2**) (13 mg, 14%). Mp: 120–122 °C. IR (KBr) cm^{-1}: 1644 (C=N), 961 (E CH=CH). ^1H NMR ($CDCl_3$) δ: 1.44 (d, J = 6.3 Hz, 3H, O–CH–CH_3), 1.45 (t, J = 7.1 Hz, 3H, CH_2–CH_3), 3.63 (dd, J = 14.4, 7.4 Hz, 1H, C=N–CHH), 4.16 (dd, J = 14.4, 9.3 Hz, 1H, C=N–CHH), 4.5 (q, J = 7.1 Hz, 2H, CH_2–CH_3), 4.85–4.88 (m, 1H, O–CH–CH_3), 7.16 (d, J = 16.2 Hz, 1H, CH=CH), 7.41–7.48 (m, 4H, ArH, 7.46 (d, J = 16.2 Hz, 1H, CH=CH), 7.59 (d, J = 8.2, 3H, ArH), 7.93 (d, J = 8.5 Hz, 2H, ArH), 8.1 (d, J = 7.41 Hz, 1H, ArH), 8.25 (d, J = 1.6 Hz, 1H, ArH). ES-MS: (positive mode) m/z 381 $[M+H]^+$, 403 $[M+Na]^+$.

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