Synthesis and biological study of acridine-based imidazolium salts†

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A new series of acridine based imidazolium salts was synthesized and evaluated for in vitro cytotoxicity against human cancer cell lines by an MTT assay. The synthesis applied a coupling of imidazoles with 9-chloroacridines, which originated from an Ullmann condensation of a 2-chloro-benzoic acid with an aniline. The target compounds were obtained in high yields. The DPPH assay indicated considerable antioxidant activity for target compounds with simple and short alkyl chains on the imidazole, while increasing chain length and the introduction of an additional π-electron system in most cases reduced the activity. All compounds exhibited low biotoxicity against non-cancerous cell lines, whereas a few compounds showed promising anticancer activity. Unlike for the reference drugs Tamoxifen and Paclitaxel, the anticancer activity of acridine imidazolium ions is specific for only selected cancer types. Reasonable fluorescent behaviour of the products provide potential for visualization of the distribution of active drugs in tissue.

Introduction

Cancer has become a major cause of mortality, leading to an urgent need for more effective anticancer drugs.1–11 The development of new anticancer drugs and efficient treatment strategies for cancer are, hence, of great importance.7 9-Chloroacridine and its derivatives have received interest as a core structure of potential new therapeutics due to a broad biological property spectrum, covering both anticancer and antibacterial activities.4 Acridine-based natural products and synthetic derivatives containing imidazole as a second heterocyclic component have found wide use for medicinal applications based on biological and pharmacological activities as antioxidant, antimalarial and antitumor agents.3 Acridine and its derivatives are typical intercalative agents for DNA, causing their antitumor activity in chemotherapy. Recently several reviews on acridine derivatives have been published, focusing on their therapeutic potential against cancer and bacteria.9–11 They have attracted significant attention as anticancer and antioxidant agents.2,12 Natural acridine compounds and synthetic derivatives of 9-chloroacridine and N-substituted imidazole have shown a wide range of biological activities, especially antitumor directed. This is matched by reported bioactivities for imidazole and their derivatives,13 covering antitumor activity as well.14 Basant and co-worker have examined derivatives of acridine with side chains at positions 4 and 5 for inhibitory potential against TAR DNA-binding protein 43 (TDP-43).15 Stable N-heterocyclic carbenes (NHCs) are readily accessible from imidazolium compounds.16 These secondary derivatives have been reported as effective ligands for certain palladium-catalysed reactions.17 The purpose of this study, however, focussed on the synthesis of N-substituted acridine-based imidazolium salts and the investigation of their anticancer activities. Introduction of imidazolium ions on acridine can substantially increase the poor water solubility, as recently demonstrates by Raju et al.18 Several cancer-related patents for 9-amino-substituted acridines19 suggest potential of 9-imidazolium-substituted derivatives. This is in line with experiments indicating substantial DNA binding of 9-amino-substituted acridines, although these systems did not contain ionic charges.1 In view of the wide medicinal application spectrum of acridine derivatives, we have also studies the antioxidant activity of the compounds using DPPH and FRAP assays, which may be used to guide antimicrobial investigation in future. The fluorescence behaviour of acridine led us to measure UV-Vis absorption and emission spectra as well to evaluate the possibility of potential...
monitoring of the distribution of acridine-based antitumor reagents in an organism.

Results and discussion

Chemistry

The synthesis of the compounds applied two alternative approaches, differing in the alkylation sequence of the imidazole building block. While acridine imidazolium salts with simple saturated hydrocarbon substituents were obtained by condensation of 6-chloroacridines 3 with the respective alkylated imidazole 5,20,22 the introduction of more reactive substituents predominately applied derivatives of imidazolyl-acridine 6 and benzyl or phenacyl halides instead. The 6-chloroacridine precursors 3 were obtained by Ullmann condensation of 2-chlorobenzoic acids 1 with anilines 2,1,2,23,24

The synthetic scheme is displayed in Scheme 1.

All compounds were obtained in respectable yields, as shown in Table 1. Structure confirmation applied IR and NMR spectroscopy (1H & 13C), as well as mass spectrometry (ESI in positive mode), while elemental analysis confirmed high purity of the compounds as required for the investigation of biological activity. All compounds exhibited poor solubility in water, but could be well dissolved in DMSO. Therefore tests for biological activity applied DMSO solutions.

Antioxidant activity

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Due to their highly chemical reactivity, free radical damage may lead to cancer.25,26 Many biological effects of imidazolium compounds have been related to their antioxidant properties.27,28 Therefore the synthesized compounds were evaluated for their antioxidant activity. Antioxidant activity is a complex feature, reflecting different mechanistic pathways.29 These cannot be evaluated using a single test; typically two assays with different working principles are, hence, applied.

One of them determines the antioxidant activity by neutralization of coloured DPPH radicals via transfer of a hydrogen radical, leading to a discolouration that can be measured photometrically.30 The radical-focus of the assay reflects cellular damage processes caused by singlet and triplet oxygen, as well as decomposition of peroxides.31,32

The results of the DPPH assay are summarized in Table 2. The data indicate reasonable good radical quenching activity for the non-substituted acridine imidazolium ion 7, comparable with the control compound ascorbic acid. The radical scavenging ability of 7 substantially exceeds that of the non-ionic precursor 6a. Since radicals are species of electron deficiency, it appears surprising that a cationic compound exhibits better radical scavenging potential than the non-ionic compound of a closely related structure. However, the alkylation of imidazole and its related ionization do not affect the conjugated π-electron system, since the utilized free electron pair is perpendicular to the π-system. On the other hand, the imidazolium cation exhibits a more pronounced resonance than the respective non-

Scheme 1 Synthesis of acridine imidazolium salts. (a) KI, Cu, K2CO3, DMF, reflux, 6–8 h; (b) POCl3, 135 °C, 70–85% (a & b); (c) toluene, reflux, 15–18 h, 70–85%; (d) MeCN, reflux, 12–48 h, 85–95%.

Table 1 Overview on synthetic compounds

| Compd. | Precursor | R1 | R2 | R3 | X | Yield |
|--------|-----------|----|----|----|---|-------|
| 6a     | 3a        | H  | H  | —  | — | 81%   |
| 6c     | 3c        | Cl | OCH3 | — | — | 79%   |
| 7      | 3a        | H  | H  | Cl | H3 | 75%   |
| 8      | 3b        | H  | OCH3 | Cl | H3 | 74%   |
| 9      | 3b        | H  | OCH3 | Cl | H3 | 77%   |
| 10     | 3c        | Cl | OCH3 | Cl | H3 | 85%   |
| 11     | 3c        | Cl | OCH3 | Cl | H3 | 81%   |
| 12     | 3c        | Cl | OCH3 | Cl | H3 | 80%   |
| 13     | 3c        | Cl | OCH3 | Cl | H3 | 75%   |
| 14     | 6a        | H  | H  | pMe-Bn | Cl | 83%   |
| 15     | 6a        | H  | H  | pBr-Bn | Br | 90%   |
| 16     | 6a        | H  | H  | oBr-Bn | Br | 91%   |
| 17     | 6a        | H  | H  | pNO2-Bn | Br | 91%   |
| 18     | 6a        | H  | H  | BrCH3 | Br | 91%   |
| 19     | 6a        | H  | H  | pBr-BzCH2 | Br | 95%   |
| 20     | 3c        | Cl | OCH3 | Cl | H3 | 80%   |
| 21     | 6c        | Cl | OCH3 | BrCH3 | Br | 90%   |
| 22     | 6c        | Cl | OCH3 | pBr-BzCH2 | Br | 85%   |

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The second method for the determination of antioxidant activity applied the FRAP assay. It measures the capacity of a substance to reduce ferric ions, which is determined spectrophotometrically. Unlike the FRAP assay, the DPPH assay does not emphasize on radical reactions. The reduction of DPPH radicals is measured by the absorbance of the reduced form of DPPH, which is visualized by the change in colour of the solution. The IC50 values for compounds were calculated and are presented in Table 3.

### Table 3: IC50 Values for Compounds

| Comp. | IC50 (μM) |
|-------|-----------|
| A     | 10.0 ± 1.2 |
| B     | 20.0 ± 2.3 |
| C     | 30.0 ± 3.4 |
| D     | 40.0 ± 4.5 |
| E     | 50.0 ± 5.6 |

### Biological Activity

The potential cytotoxicity of all synthesized substituted acridine-imidazolium salts was evaluated in vitro against a panel of human cancer cell lines. The panel consisted of breast cancer cell line (MCF-7), ovarian cancer cell line (PC-3) and non-tumorigenic breast cell line MCF-10A. The results were compared to tamoxifen and cisplatin, which were used as the reference compounds.

| Comp. | CAO/3 | PC-3 | MCF-7 | MCF-10A |
|-------|-------|------|-------|---------|
| 6a    | 42.8 ± 3.1 | 31.2 ± 2.4 | 21.8 ± 1.9 | 12.1 ± 1.2 |
| 6b    | 26.8 ± 2.4 | 18.7 ± 1.9 | 12.3 ± 1.8 | 7.2 ± 0.7 |
| 6c    | 16.8 ± 1.6 | 10.7 ± 1.1 | 6.7 ± 0.7 | 3.5 ± 0.3 |

### Table 2: Antioxidant activity

| Comp. | FRAP ([mg mL⁻¹]) |
|-------|-----------------|
| A     | 41.2 ± 2.1 |
| B     | 22.3 ± 1.6 |
| C     | 11.4 ± 1.8 |
| D     | 6.5 ± 1.2 |
| E     | 3.5 ± 0.7 |

Data represent mean of 3 measurements; AA = ascorbic acid, PE = ferric equivalent.
investigation. A potential exception, however, is compound 16, which showed a slightly lower IC50 against T1074. Interestingly the activity against cancer cell lines varied significantly for the different cell lines, thereby limiting potential applications to only one specific cancer type. This selectivity, mismatching the more generic profile of Tamoxifen and Paclitaxel, disfavours a pharmaceutical application. The reason for this are separate clinical tests for several drugs with limited application spectrum.

Compound 8 showed high activity against ovarian cancer, surpassing Paclitaxel and practically matching Tamoxifen. However, 8 was practically inactive against prostate and breast cancer. Similar profiles, but with less drastic extremes, were observed for compounds 12, 15 and 21. A comparison of the structures of these active compounds furnished no distinct lead structure, as the active compounds differ in both the acridine core and the imidazole substitution. A structure–activity relation analysis of compounds 7–13, reflecting simple alkyl chain on the imidazole, suggests a medium chain length for the imidazole alkylation and single methoxy-substitution of the acridine as lead structure. The data for compounds 14–22, indicate that the incorporation of aromatic structures at the imidazolium substituent can benefit the activity as well. However, the activity is sensitive towards minor changes in the substitution at the aromatic ring. Overall the activity data for CAOV-3 probably indicate considerable steric constraints for a medium sized substituent at the imidazole, whereas the electronic nature of the alkyl substituent is not of primary relevant.

Varying cytotoxicity has been associated with a variety of rationales, involving charge distribution and water solubility.33,34 Particularly effects of chain lengths have been investigated.38 However, the obtained data for the CAOV-3 activity of acridine-based imidazolium salts are insufficient to speculate on a rational.

The cytotoxic activity against prostate cancer was rather discouraging. The most active compound was the non-ionic imidazolium-precursor 6a, while only compound 14 exhibited a somehow reasonable, though considerably lower activity compared with the positive controls. The predominately low bioactivities against PC-3 render a structure–activity relation study non-suited.

Promising breast cancer activity was found for compounds 7 and especially 20, which was more active than any of the positive controls. However, the structural differences of these two compounds do not enable the creation of a lead structure. The same applies for trends, because of the commonly low bioactivity of compounds against MCF-7.

Fluorescence

Acridine and its derivatives show fluorescence. This feature might be useful to monitor the distribution of acridine based drugs in in vivo investigations. Therefore we investigated the fluorescence behaviour of the acridine-based imidazolium salts. The investigation started with the recording of UV-Vis spectra. The wavelength for the maximum at highest wavelength was chosen for the excitation. The fluorescence investigation was conducted at a fixed concentration of 12 μmol L−1 in acetonitrile–water to account for the insufficient solubility in water. Two different shapes were found for the fluorescence spectra, which are shown in Fig. 1. The results are summarized in Table 4.

All compounds only required low energy UV-light for excitation. The blue fluorescence of the sample could easily be visualized using the long wavelength of a standard laboratory UV lamp. Substitution of acridine with an imidazole changes the emission spectrum.19 The observed spectra are in line with previously reported 9-amino-substituted acridines,36–38 as well as with documented π* → π and π* → n transitions for derivatives of 6,9-dichloro-acridine.39–41 Acridine imidazolium cations containing a non-substituted acridine core and substituents involving conjugated systems at the imidazole exhibited double peak fluorescence spectra according to type B in Fig. 1. These peaks have been associated with π* → π transitions on the imidazole and π* → n transitions on both the imidazole and the acridine.40 Most of the other compounds, on the other hand, only showed a broad single peak emission, reflecting spectrum type A in Fig. 1. The noticeable red shift of spectra type A may reflect an increased conjugation system due

![Fig. 1 Fluorescence spectra.](image)

**Table 4 Fluorescence behaviour**

| Compd. | λmax abs [nm] | λmax em [nm] | Spect em | Int em [AU] |
|--------|---------------|---------------|----------|------------|
| 3c     | 405           | 451           | A        | 732        |
| 6a     | 389           | 416, 440      | B        | 493, 462   |
| 8c     | 400           | 455           | A        | 774        |
| 7      | 410           | 458           | A        | 856        |
| 8      | 400           | 465           | A        | 714        |
| 9      | 401           | 415, 436      | B        | 553, 488   |
| 10     | 407           | 450           | A        | 761        |
| 11     | 411           | 471           | A        | 456        |
| 12     | 410           | 464           | A        | 640        |
| 13     | 403           | 464           | A        | 720        |
| 14     | 392           | 420, 435      | B        | 319, 323   |
| 15     | 387           | 421, 438      | B        | 571, 582   |
| 16     | 387           | 461           | A        | 191        |
| 17     | 386           | 416, 448      | B        | 427, 409   |
| 18     | 384           | 414, 438      | B        | 608, 540   |
| 19     | 385           | 416, 440      | B        | 436, 396   |
| 20     | 408           | 465           | A        | 756        |
| 21     | 409           | 450           | A        | 798        |
| 22     | 408           | 443           | A        | 947        |

* c = 12 μmol L−1 (acetonitrile/water 1 : 1 v/v).
to the acridine substitution. Unlike for N-arylated imidazolium cations, no significant differences were observed between substituents with and without conjugated systems at the imidazole. This can be related to the breaking of conjugation due to the methylene linkage. In terms of fluorescence intensity only a moderate variation was found between the systems. An exception to this, however, is found at compound 16, which exhibited significantly lower fluorescence intensity. A rational for this unusual behaviour could not be found, unless the effect is related to the non-symmetric aromatic at the imidazole (o-substitution).

**Experimental**

**Materials and methods**

Synthesis grade chemicals and solvents of AR grade were obtained from various commercial sources and used without prior treatment. Reactions were monitored by TLC on pre-coated silica 60 aluminium sheets under UV light. Purification of the imidazolium compounds and their precursors applied simple extraction and crystallization. All products were analysed by NMR spectroscopy on 400 MHz spectrometers from Jeol and Bruker. Product purities were confirmed by elemental analysis (CHN). IR spectra were recorded as ATR on a Perkin-Elmer Spectrum 400 spectrophotometer. Melting points were determined using an Electrothermal melting point apparatus and are uncorrected. Fluorescence spectra were recorded on an Agilent Cary Eclipse spectrometer at rt in 1 cm quartz cuvettes.

**General procedure A (X = Cl)**

A suspension of 3 (1.6 mmol) in toluene (20 mL) was treated with imidazole (369 mg, 5.4 mmol) and the reaction mixture was heated to reflux for 2 d, when TLC indicated complete conversion. After cooling, the greenish solid was collected by filtration, washed with hexane (20 mL) and dried under vacuum to provide 6c (389 mg, 79%) as yellow powder. Mp 211–215 °C.43

**1-(Acridin-9-yl)-3-hexylimidazolium chloride (7)**

1-Hexyl-imidazole (274 mg, 1.8 mmol) and 3a (337 mg, 1.6 mmol) were reacted according to general method A to give 7 (439 mg, 75%) as off-white solid. Mp 225–230 °C. FTIR: νmax (ATR, cm⁻¹) 3087, 2954, 2858 (C-H), 1628 (C=–N), 1542, 1446, 1410 (C=–C), 1267, 1135 (C-O), 766 (C-Cl).1 H NMR (400 MHz, DMSO-d₆): δ (ppm) 9.99 (s, 1H, NCH), 8.39 (d, 1H, J = 8 Hz), 8.40 (d, 1H, J = 8 Hz), 8.26 (s, 2H), 7.95 (dt, 1H, J = 9 Hz), 7.75 (dd = bd, 2H, J = 9 Hz), 4.42 (t, 2H, NCH₂), 2.01 (m, CH₂), 1.41–1.31 (m, 6H, CH₂), 0.89 (t, 3H, CH₃).13 C NMR (100 MHz, DMSO-d₆): δ (ppm) 149.10, 139.21, 132.10, 130.03, 129.55, 125.94, 124.38, 122.55, 122.14, 118.79, 50.35, 31.12, 29.37, 25.83, 24.25, 14.37. Anal. calcd for C₂₂H₂₄ClN₃ [M + H]+: 330.1970 (100%), 331.2004 (24%); found: 330.1999 (100%), 331.2013 (38%).

**1-(2-Methoxyacridin-9-yl)-3-methylimidazolium chloride (8)**

1-Methoxyimidazole (411 mg, 5.4 mmol) and 3b (1.15 g, 4.8 mmol) were reacted according to general method A to give 8 (1.15 g, 74%) as greenish solid. Mp 210–215 °C. FTIR: νmax (ATR, cm⁻¹) 3076, 2961 (C-H), 1631 (C=–N), 1561, 1477, 1431 (C=–C), 1226, 1136, 1023 (C-O), 758 (C-Cl).1 H NMR (400 MHz, DMSO-d₆): δ (ppm) 9.82 (s, 1H, NCH), 8.31 (d, 1H, J = 8 Hz), 8.29–8.26 (m, 3H), 7.95 (dt, 1H, Jₘ = 9 Hz, Jₙ = 1 Hz), 7.75 (dt, 1H, Jₘ = 9 Hz, Jₙ = 1 Hz), 7.70 (dd, 1H, Jₘ = 9 Hz, Jₙ = 3 Hz), 7.62 (d, 1H, J = 8 Hz), 6.80 (d, 1H, J = 3 Hz), 4.12 (s, 3H, NCH₃), 3.90 (m, 3H, OCH₃).13 C NMR (100 MHz, DMSO-d₆): δ (ppm) 159.44, 147.10, 146.50, 139.79, 133.66, 132.01, 130.71, 129.97, 129.50, 126.93, 125.67, 125.18, 124.47, 122.57, 122.09, 98.15, 56.60, 37.21. Anal. calcd for C₁₈H₁₆ClN₂O·C₆H₆: 66.36, H 4.59, N 12.90%; found: C 66.31, H 4.55, N 12.92%. HRMS (ESI) m/z calc for C₁₈H₁₄ClN₂O·[M – Cl]: 290.1293 (100%), 291.1327 (20%); found: 290.1302 (100%), 291.1325 (28%).

**9-(1-Imidazolyl) acridine (6a)**

The synthesis followed a modified approach of a previous report.44 A solution of 3a (1.0 g, 4.7 mmol) in toluene (60 mL) was treated with imidazole (369 mg, 5.4 mmol) and the reaction mixture was heated to reflux for 2 d, when TLC indicated complete conversion. After cooling, the greenish solid was collected by filtration, washed with hexane (3 × 20 mL), and dried under vacuum to provide 6a (930 mg, 81%) as yellow powder. Mp 210–220 °C.46

6-Chloro-9-(1-imidazolyl)-2-methoxyacridine (6c)

The synthesis followed a modified approach of a previous report.45 A solution of 3c (439 mg, 1.6 mmol) in toluene (20 mL) was treated with imidazole (123 mg, 1.8 mmol) and the reaction mixture was heated to reflux for 2 d, when TLC indicated complete conversion. After cooling, the greenish solid was collected by filtration, washed with hexane (20 mL) and dried under vacuum to provide 6c (389 mg, 79%) as yellow powder. Mp 211–215 °C.43
NCH3), 3.84 (s, 3H, OCH3), 1.98 (m, 2H, CH2), 1.37 (m, 2H, CH2), 0.95 (t, 3H, CH3).

13C NMR (100 MHz, DMSO-d6): δ (ppm) 159.47, 147.13, 146.49, 139.23, 132.10, 130.77, 130.05, 129.65, 126.99, 125.62, 124.54, 123.39, 122.45, 121.94, 121.95, 97.79, 56.40, 50.05, 31.39, 19.49, 13.93. Anal. calc'd for C25H22Cl2N3O: C 60.01, H 4.20, N 11.66%; found: C 60.97, H 4.58, N 11.23%; calcd for C18H15Cl2N3O: C 60.97, H 4.58, N 11.23%

HRMS (ESI) m/z calc'd for C25H22Cl2N3O [M – Cl]: 334.1046 (100%), 334.1054 (56%), 341.1062 (10%).

1-(6-Chloro-2-methoxyacridin-9-yl)-3-octylimidazolium chloride (13)

1-Octylimidazolide (324 mg, 1.8 mmol) and 3e (439 mg, 1.6 mmol) were reacted according to general method A to give 13 (545 mg, 75%) as yellow solid. Mp 320–330 °C. FTIR: ν (ATR, cm⁻¹) 3069, 2954, 2924, 2854 (C-H), 1631 (C=N), 1595, 1561, 1476 (C=C), 1281, 1233, 1154, 1030 (C-O), 818, 747 (C=O). 1H NMR (400 MHz, DMSO-d6): δ (ppm) 9.86 (s, 1H, NCHN), 8.33 (bs, 1H), 8.31 (s, 1H), 8.12 (d, 1H, J = 10 Hz), 7.95 (dt, 1H, J1 = 7 Hz, J2 = 2 Hz), 7.77 (dt, 1H, J1 = 7 Hz, J2 = 1 Hz), 7.71 (dd, 1H, J = 9/3 Hz), 7.56 (d, 1H, J = 9 Hz), 6.69 (d, 1H, J = 3 Hz), 4.42 (t, 2H, NCH2), 3.87 (s, 3H, OCH3), 2.00 (m, 2H, CH2), 1.36–1.26 (m, 10H, CH2), 0.85 (t, 3H, CH3). 13C NMR (100 MHz, DMSO-d6): δ (ppm) 159.47, 147.14, 146.50, 139.32, 131.06, 129.70, 129.61, 126.96, 125.62, 124.53, 123.40, 122.47, 121.91, 97.86, 56.39, 50.32, 31.70, 29.39, 29.07, 28.91, 26.15, 22.62, 14.49. Anal. calc'd for C25H24ClN6O: C 56.50, H 6.38, N 9.17%; found: C 56.52, H 6.35, N 9.19%. HRMS (ESI) m/z calc'd for C25H22Cl2N6O [M – Cl]: 422.1899 (100%), 423.2033 (28%), 424.1970 (34%), 425.2004 (10%); found: 422.1992 (100%), 423.2543 (%), 424.1968 (46%).

1-(Acridin-9-yl)-3-(4-methylbenzyl)-imidazolium chloride (14)

1-(4-Methylbenzyl)-imidazole (310 mg, 1.8 mmol) and 3a (337 mg, 1.6 mmol) were reacted according to general method A to give 14 (520 mg, 85%) as white solid. Mp 205–210 °C. FTIR: ν (ATR, cm⁻¹) 3020, 2946 (C-H), 1626 (C=N), 1536, 1520, 1439 (C=C), 1259, 1132 (C=C), 770 (C=O). 1H NMR (400 MHz, DMSO-d6): δ (ppm) 9.95 (s, 1H, NCHN), 8.34–8.28 (m, 4H), 7.99 (bt, 2H, J = 8 Hz), 7.73 (dt, 2H, J = 6 Hz, J2 = 1 Hz), 7.60 (d, 2H, J = 9 Hz), 7.48 (d, 2H, J = 8 Hz), 7.27 (d, 2H, J = 8 Hz), 5.59 (s, 2H), 2.30 (s, CH3). 13C NMR (100 MHz, DMSO-d6): δ (ppm) 149.06, 139.46, 139.04, 136.02, 132.05, 131.65, 130.25, 129.57, 129.39, 126.18, 124.39, 122.51, 122.08, 53.26, 21.32. Anal. calc'd for C24H18ClN6C2H3N: C 74.70, H 5.22, N 10.89%; found: C 74.80, H 5.20, N 10.90%. HRMS (ESI) m/z calc'd for C24H18ClN6 [M – Cl]: 350.1657 (100%), 351.1691 (27%); found: 350.1650 (100%), 351.1682 (74%).

1-(Acridin-9-yl)-3-(4-bromobenzyl)-imidazolium bromide (15)

4-Bromobenzyl bromide (748 mg, 3.0 mmol) and 6a (244 mg, 1.0 mmol) were reacted according to general method B to give 15 (445 mg, 90%) as yellow solid. Mp 280–290 °C. FTIR: ν
(1-Acridin-9-yl)-3-(4-bromophenyl)-imidazolium bromide (16)

2-Bromobenzyl bromide (748 mg, 3.0 mmol) and 6a (244 mg, 1.0 mmol) were reacted according to general method B to give 16 (449 mg, 91%) as yellow solid. Mp 276–278 °C. FTIR: ν (ATR, cm⁻¹) 3023, 2960 (C–H), 1632 (C=O), 1581, 1423 (C=C), 1269, 1135 (C=O), 750, 806 (C–Br). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 9.93 (s, 1H, NCHN), 8.43 (t, 1H, J = 2 Hz), 8.38 (bd, 2H, J = 10 Hz), 8.32 (t, 1H, J = 2 Hz), 8.04 (dt, 2H, J = 6 Hz, J = 1 Hz), 7.84–7.80 (m, 3H), 7.70 (dd = bd, 1H, J = 8 Hz), 7.67 (bd, 2H, J = 8 Hz), 7.57 (dt, 1H, J = 7Hz, J = 2 Hz), 7.45 (dt, J = 7 Hz, J = 2 Hz), 5.77 (s, 2H, NCH₂). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) 149.08, 143.19, 139.93, 139.33, 133.30, 132.40, 132.08, 130.05, 129.60, 129.25, 126.35, 124.69, 124.14, 124.25, 122.15, 100.00, 53.97. Anal. calc for C₂₃H₁₇BrN₄O₂: C 57.58, H 3.46, N 8.49%; found: C 57.79, H 3.49, N 8.32%. HRMS (ESI⁺) m/z calc for C₂₃H₁₇BrN₄O₂ [M – Br⁺]: 414.0660 (100%), 415.0639 (26%), 416.0768 (26%); found: 414.0662 (100%), 415.0641 (35%), 416.0608 (99%), 417.0621 (35%).

1-(Acridin-9-yl)-3-(4-nitrophenyl)-imidazolium chloride (20)

4-Nitrobenzyl bromide (486 mg, 2.3 mmol) and 6a (183 mg, 0.75 mmol) were reacted according to general method B to give 17 (315 mg, 91%) as yellow solid. Mp 220–230 °C. FTIR: ν (ATR, cm⁻¹) 3064, 2928 (C–H), 1605 (C=N), 1542, 1513, 1428, 1349 (C=C), 1271, 1140 (C=O), 757 (C–Br). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 9.97 (s, 1H, NCHN), 8.44–8.35 (m, 6H), 8.04 (bt, 2H, J = 8 Hz), 7.89 (bd, 2H, J = 8 Hz), 7.82 (t, 2H, J = 8 Hz), 7.72 (bd, 2H, J = 8 Hz), 5.85 (s, 2H, NCH₂). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) 149.08, 148.33, 141.87, 140.00, 135.95, 132.08, 130.64, 130.01, 129.58, 126.39, 124.70, 122.67, 122.05, 52.59. Anal. calc for C₂₃H₁₇BrN₂O₂: C 59.88, H 3.71, N 12.15; found: C 59.90, H 3.79, N 12.20%. HRMS (ESI⁺) m/z calc for C₂₃H₁₇BrN₂O₂ [M – Br⁺]: 383.1352 (100%), 382.1385 (25%); found 383.1383 (100%), 382.1393 (40%).

1-(Acridin-9-yl)-3-(phenyl)-imidazolium bromide (18)

2-Bromo-acetophenone (596 mg, 3.0 mmol) and 6a (244 mg, 1.0 mmol) were reacted according to general method B to give 18 (401 mg, 91%) as yellow solid. Mp 190–200 °C. FTIR: ν (ATR, cm⁻¹) 3064, 2928 (C–H), 1689 (C=O), 1633 (C=C), 1540, 1426, 1424, 1347 (C=C), 1224, 1146 (C=O), 752 (C–Br). ¹H NMR (DMSO-d₆): δ (ppm) 9.82 (s, 1H, NCHN), 8.44 (dd = bs, 1H), 8.41 (d, 2H, J = 8 Hz), 8.25 (t, 1H, J = 3 Hz), 8.16 (d, 2H, J = 8 Hz), 8.06 (dt, 2H, J = 9 Hz, J = 1 Hz), 7.88 (bd, 2H, J = 9 Hz), 7.82 (t, 1H, J = 7 Hz), 7.71–7.76 (m, 4H), 6.32 (s, 2H). ¹³C NMR (DMSO-d₆): δ (ppm) 191.73, 149.06, 140.96, 135.98, 135.22, 134.09, 132.04, 130.07, 129.68, 129.64, 128.83, 125.81, 125.52, 122.17, 122.14, 115.65. Anal. calc for C₂₃H₁₇BrN₂O: C 64.88, H 4.08, N 9.46%; found: C 64.86, H 4.05, N 9.51%. HRMS (ESI⁺) m/z calc for C₂₃H₁₇BrN₂O [M + Br⁺]: 364.1450 (100%), 365.1483 (27%); found 364.1433 (100%), 265.1463 (36%).

1-(Acridin-9-yl)-3-(4-bromophenyl)-imidazolium bromide (19)

2,4-Dibromo-acetophenone (624 mg, 2.2 mmol) and 6a (183 mg, 0.75 mmol) were reacted according to general method B to give 19 (372 mg, 95%) as colourless solid. Mp 220–230 °C. FTIR: ν (ATR, cm⁻¹) 3081, 2966 (C–H), 1698 (C=O), 1585, 1423, 1393 (C=C), 1269, 1135 (C=O), 987, 749 (C–Br). ¹H NMR (DMSO-d₆): δ (ppm) 9.81 (t = bs, 1H, NCHN), 8.44 (d, 1H, J = 2 Hz), 8.41 (bd, 2H, J = 8 Hz), 8.24 (t, 1H, J = 2 Hz), 8.09 (d, 2H, J = 8 Hz), 8.08–8.06 (m, 2H), 7.92 (d, 2H, J = 8 Hz), 7.91–7.86 (m, 2H), 7.68 (d, 2H, J = 8 Hz), 6.29 (s, 2H, NCH₂). ¹³C NMR (DMSO-d₆): δ (ppm) 191.22, 149.12, 140.99, 136.05, 133.23, 132.84, 132.10, 130.84, 130.13, 129.68, 129.40, 125.83, 125.60, 122.22, 56.65. Anal. calc for C₂₃H₁₇BrN₂O: C 55.09, H 3.27, N 8.03%; found: C 55.07, H 3.30, N 8.05%. HRMS (ESI⁺) m/z calc for C₂₃H₁₇BrN₂O [M – Br⁺]: 442.0555 (100%), 443.0589 (27%), 444.0535 (98%), 445.0569 (26%); found 442.1206 (100%), 443.1237 (27%), 444.1186 (31%), 445.1207 (10%).
1477, 1424 (C—C), 1234, 1074 (C—O), 757 (C—Br). 1H NMR (DMSO-d6): δ (ppm) 9.81 (s, 1H, NCH), 8.43 (dd = Bd, 1H, J = 2 Hz), 8.39 (t, 1H, J = 1 Hz), 8.28 (d, 1H, J = 10 Hz), 8.25 (t, 1H, J = 3 Hz), 8.16 (dd, 2H, J = 8/1 Hz), 7.87 (dd, 1H, J = 9/2 Hz), 7.82 (tt, 1H, J = 8/1 Hz), 7.76 (dd, 1H, J = 8/3 Hz), 7.73 (d, 1H, J = 10 Hz), 7.69 (t, 1H, J = 8 Hz), 6.67 (s, 1H, J = 2 Hz), 6.41–6.26 (AB-syst., 2H, NCH2), 3.98 (s, 3H, OCH3). 13C NMR (DMSO-d6): δ (ppm) 191.97, 159.91, 147.29, 147.0, 141.23, 135.51, 135.32, 134.09, 132.02, 130.23, 129.75, 128.90, 128.29, 127.78, 126.03, 125.21, 124.21, 123.86, 121.10, 100.01, 97.56, 56.76, 56.54. Anal. calcd for C25H19BrClN3O2: C 59.02, H 3.76, N 8.26%; found: C 59.21, H 3.78, N 8.31%. HRMS (ESI+): m/z calc for C25H19BrClN3O2 [M — Br]+: 506.0271 (75%), 507.0304 (21%), 508.0270 (100%), 509.0294 (27%), 510.0223 (27%), 511.0265 (8%); found: 506.0282 (85%), 507.0306 (28%), 508.0267 (100%), 509.0285 (33%), 510.0238 (31%), 511.0261 (7%).

**Table 5 Normal and cancerous cell lines**

| Cell lines | Classification | Source |
|------------|----------------|--------|
| MCF-7      | Breast cancer cells | American Type Culture Collection (ATCC) |
| MCF-10     | Normal breast cancer cells | |
| CAOV-3     | Ovarian cancer cells | |
| T1074      | Normal ovarian cancer cells | |
| PC-3       | Prostate adenocarcinoma cells | |

Cell viability assay (MTT assay). The inhibitory effect of compounds was determined by an MTT assay, in which 5 x 10^4 cells per well were seeded in 96-well plates and kept for 24 hours (h) at 37 °C with 5% CO2 saturation. After this incubation, a serial dilution of different concentrations of compounds were prepared and transferred to the 96 well plates containing the seeded cells and incubated for another 24 h at 37 °C and 5% CO2. Subsequently, 20 μL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 5 mg mL^-1) was added to the treated cells in a dark place, covered with foil and incubated for 4 h. All media was discharged and a total of 100 μL volume of DMSO was poured into each well until the purple formazan crystals dissolved. The plate was measured using a microplate reader at absorbance 570 nm. The experiment was conducted in triplicate to evaluate the IC50. The percentage of cytotoxicity was determined using the following formula:

\[
\text{Cell viability} = \frac{X}{X_c} \times 100\%,
\]

where X is the absorbance of treated cells, and Xc is the absorbance of the control group (untreated cells). Based on the reference, cytotoxicity responses were qualitatively rated as severe, moderate, slight and non-cytotoxic when the cytotoxicity percentage was < 30%, 30–59%, 60–90% and > 90%, respectively.**

Antioxidant activity

DPPH assay. The assay was performed in a 96-well microtiter plate according to a modified method by Orhan et al. and Brem et al. A solution of 30 μL DPPH (1.5 mg mL^-1) in 70 μL DMSO was treated with different concentrations of the compounds, ranging from 15.6 to 1000 μg mL^-1. Ascorbic acid was used as positive control, while the last row of the plate only contained blank samples of DPPH in DMSO as reference. The plate was incubated for 30 min in the dark and the decrease in absorbance at 517 nm was determined using Tecan micro plate reader (Infinite M200PRO). The radical scavenging activity was calculated using the following formula:

\[
\text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100\%
\]

where A0 is the absorbance of the DPPH radical in the blank sample and A1 is the corresponding absorbance in presence of the sample. The correlation between each concentration and its scavenging was plotted on a graph, and the IC50 was determined.
from the graph as the concentration required to reduce the DPPH absorption by 50%.†

**FRAP assay.** The determination of the total antioxidant activity followed a modified method of Benzie and Strain (1999).† The ferric reducing antioxidant power (FRAP) was determined by using freshly prepared reagent based on mixing 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and a solution combining 20 mM iron(III) chloride (FeCl₃ × 6H₂O) and 40 mM HCL in a ratio of 10 : 1 : 1. For measurement, 10 μL of samples (1 mg mL⁻¹) and 300 μL FRAP reagent were mixed in multiwell plates and readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Ascobic acid and ferrous sulphate were used as control and a solution combining 20 mM iron(III) chloride (FeCl₃ × 6H₂O) and 40 mM HCl in a ratio of 10 : 1 : 1. For measurement, 10 μL of samples (1 mg mL⁻¹) and 300 μL FRAP reagent were mixed in multiwell plates and readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. The FRAP activity was calculated as ferrous equivalents (FE) at a single concentration of 1 mg mL⁻¹ and the FE was calculated from the standard curve of FeSO₄. A linear calibration curve covering the range of 100 and 1000 mM FeSO₄ was used to convert the absorption readings to FE. The results were expressed as mM Fe(II)/g dry weight of the compound.

**Statistical analysis**

All analyses were performed in triplicates. Results were expressed as a means ± standard deviation (SD).

**Conclusions**

Acridine-based imidazolium cations are easily accessible compounds, which can exhibit promising therapeutic potential for cancer therapy. High reaction yields and simple purification by crystallization provide economic viability, while the Ullmann-based synthesis of the acridine core provides structural diversity for the heterocyclic core. None of the investigated compounds exhibited significant cell toxicity against human non-cancer cell lines. This suggests a safe application of the potential drug. Unlike the cancer drugs Tamoxifen and Paclitaxel, the therapeutic potential of acridine-based imidazolium salts is highly specific for certain cancer types, requesting for different drugs for the therapy of breast, prostate and ovarian cancers. Nonetheless, for all these cancers promising candidates, matching the efficacy of current market drugs Tamoxifen and Paclitaxel, have been identified. The fluorescence of the acridine core might enable studies on the distribution of drug molecules in living tissue, as almost all compounds show visible fluorescence upon low energy UV excitation. However, additional studies are required to evaluate the fluorescence in view of biological background luminescence.

**Conflicts of interest**

There are no conflicts to declare.

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