

Surface functionalization of chitosan isolated from shrimp shells, using salicylaldehyde ionic liquids in exploration for novel economic and ecofriendly antibiofoulants.

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1. Synthesis and characterization of the key starting materials

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1.1 Materials

Chemicals were obtained from the following suppliers and used without further purification: salicylaldehyde (Sal) and 2-Methoxypyridine (2-MeO-Py) (Sigma–Aldrich), paraformaldehyde ((CH₂O)_n) (Roth), 1-methylimidazole (1-Me-Im) (Alfa Aesar), triethyl amine (Et₃N) and anhydrous zinc chloride (ZnCl₂) (GRÜSSING GmbH).

1.2 Instrumentation

Elemental analyses for C, H, N and S were performed with a Perkin–Elmer 263 elemental analyzer. FT-IR spectra were recorded on a BRUKER Tensor-37 FT-IR spectrophotometer in the range 400–4000 cm⁻¹ as KBr discs or in the 4000–550 cm⁻¹ region with 2 cm⁻¹ resolution with an ATR (attenuated total reflection) unit (Platinum ATR-QL, Diamond). For signal intensities the following abbreviations were used: br (broad), sh (sharp), w (weak), m (medium), s (strong), vs (very strong). NMR-spectra were obtained with a Bruker Avance DRX200 (200 MHz for ¹H) or Bruker Avance DRX500 (500 MHz for ¹³C) spectrometer with calibration to the residual proton solvent signal in DMSO-d₆ (¹H NMR: 2.52 ppm, ¹³C NMR: 39.5 ppm), CDCl₃ (¹H NMR: 7.26 ppm, ¹³C NMR: 77.16 ppm) against TMS with $\delta = 0.00$ ppm. Multiplicities of the signals were specified s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). The mass spectra of the synthesized sal-imidazolium, saldach-imidazolium chlorides and their complexes were acquired in the linear mode for positive ions on a BRUKER Ultraflex MALDI-TOF instrument equipped with a 337 nm nitrogen laser pulsing at a repetition rate of 10 Hz. The MALDI matrix material (1,8-dihydroxy-9(10H)-anthracenone (dithranol, DIT) (C₁₄H₁₀O₃, M= 226.23)) was dissolved in chloroform at a concentration of 10 mg/mL. MALDI probes were prepared by mixing compound solutions (1 mg/mL in CH₂Cl₂) with the matrix solution (1:10, v/v) in a 0.5 mL Eppendorf® micro tube. Finally 0.5 μ L of this mixture was deposited on the sample plate dried at room temperature and then analyzed. The molar conductance 10⁻³ M solution of various salts has been measured at ambient temperature with a digital conductivity meter (S30 SevenEasy™ conductivity, Mettler-Toledo Electronics, LLC, Polaris Parkway, Columbus). The overall accuracy of the conductance measurements was found to be $\pm 0.2\%$.

1.3 5-chloromethyl-2-hydroxybenzaldehyde (**1**)

They were synthesized from the corresponding salicylaldehydes according to the modified

chloromethylation procedure [S1]. In a typical synthesis, (15.2 mmol) of salicylaldehyde was treated with para-formaldehyde (1.0 g, 33.3 mmol) and zinc chloride (0.2 g, 1.46 mmol) in 20 ml of concentrated hydrochloric acid. The mixture was vigorously stirred under HCl_g atmosphere for 24-72 h at 313 K. The reaction mixture was extracted several times with diethyl ether (3x15 mL). Then the collected ether fractions were washed by 2x10 mL 5% aqueous NaHCO_3 solution, 2x10 mL brine, 5x10 mL milli-Q water and dried over anhydrous MgSO_4 . After filtration and removal of the volatiles under reduced pressure, the obtained product was characterized and used in the next step without further purification. 5-Chloromethyl-2-hydroxybenzaldehyde was isolated as white needles (15.2 g, 62.0 % yield). FTIR (ATR, cm^{-1}): 3240 (m, br, ν OH), 3120 (m, br, ν_{asym} CH, Ar), 3050 (m, br, ν_{sym} CH, Ph), 2876 (m, sh, ν CH_2), 1659 (vs, sh, ν C=O), 1578, 1489, 1437 (s, sh, ν C=C_{arom} + ν C-H bend), 1338 (m, sh, ν CH_2), 1252 (s, sh, ν CH_2Cl), 1150 (s, sh, ν HCC, Ar), 772 (s, sh, ν C-Cl). ^1H NMR (200 MHz, CDCl_3) δ (ppm): 11.07 (s, 1H, Ar-OH) 9.90 (s, 1H, Ar-HC=O), 7.57 (m, 2H, 2 x Ar-H), 7.00 (d, 1H, J = 8.34 Hz, Ar-H), 4.60 (s, 2H, CH_2 -Ar).

1.4 Ionic liquid-based salicylaldehydes (Sal-IL, 2a-c)

To a vigorously stirred solution of N-component (21.39 mmol) in dry toluene (25 mL) at room temperature was added the solution of chloromethyl- salicylaldehyde **1** (4.15 g, 19.50 mmol) in dry toluene (25 mL), drop-wise over 30 min, under nitrogen atmosphere. The resulting solution was stirred under nitrogen atmosphere at 60 °C for 24 h. After cooling, the isolated products were washed intensively with 2 x 5 mL dry toluene, several with ether (5x10 mL), to remove the unreacted materials, and dried under vacuum to give the desired products which used for the following preparations without further purification.

5-(1-Methylimidazoliummethyl) salicylaldehyde chloride (2a): Obtained as of white solid, Yield (89 %). FTIR (KBr, cm^{-1}): 3373 (m, br, $\nu_{(\text{O-H})}$), 1669 (vs, sh, $\nu_{(\text{C=O})}$), 1547, 1455, 1399 (s, sh, $\nu_{(\text{C=C}_{\text{Ar}} + \text{C-H}_{\text{bend}})}$), 1274 (s, sh, $\nu_{(\text{Ar-O})}$), 1153 (s, sh, $\nu_{(\text{H-C=C} + \text{H-C=N})_{\text{bend}}}$, Im). ^1H NMR (200 MHz, $\text{DMSO-}d_6$) δ (ppm): 10.80 (s, 1 H, Ar-OH), 10.33 (s, 1 H, Ar-HC=O), 7.84 (d, J = 1.76 Hz, 1 H, N(1)CHCHN(3)), 7.67 (d, J = 2.01 Hz, 2 H, 2 x Ar-H), 7.55 (d, J = 1.69 Hz, 1 H, N(1)CHCHN(3)), 7.41 (m, 3H, 3 x Ar-H), 5.38 (s, 2H, N(3)- CH_2 -Ar), 3.86 (s, 3 H, N(1)- CH_3). ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ (ppm): 197.23, 160.18, 144.71, 137.45, 136.23, 131.03, 127.72, 122.80, 121.91, 120.13, 51.34, and 35.39. ESI-MS: In positive mode peaks at m/z = 217.3 [$\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_2$, M - Cl^-] $^+$ a.m.u.

5-(Triethylammoniummethyl) salicylaldehyde chloride: Obtained as of white solid, Yield (91 %). FTIR (KBr, cm^{-1}): 3429 (m, br, $\nu_{(\text{O-H})}$), 1658 (vs, sh, $\nu_{(\text{C=O})}$), 1567, 1447, 1386 (s, sh, $\nu_{(\text{C=C}_{\text{Ar}} + \text{C-H}_{\text{bend}})}$), 1282 (s, sh, $\nu_{(\text{Ar-O})}$). ^1H NMR (200 MHz, $\text{DMSO-}d_6$) δ (ppm): 10.74 (s, 1 H, Ar-OH), 10.08 (s, 1 H, Ar-HC=O), 7.81 (d, J = 2.5 Hz, 1H, Ar), 7.61 (dd, J_1 = 2.5 Hz, J_2 = 10.0 Hz, 1H, Ar), 7.02 (d, J = 9.5 Hz, 1H, Ar), 4.41 (s, 2H, N- CH_2 -Ar), 3.16 (q, J = 7.0 Hz, 6H, CH_2), 1.35 (t, J = 6.8 Hz, 9H, CH_3). ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ (ppm): 194.56, 162.97, 140.21, 136.32, 122.24, 118.88, 118.45, 59.51, 52.48, and 7.06. ESI-MS: In positive mode peaks at m/z = 236.3 [$\text{C}_{14}\text{H}_{22}\text{NO}_2$, M - Cl^-] $^+$ a.m.u.

3-(2-methoxypyridiniummethyl) salicylaldehyde chloride (2c): Obtained as pale yellow solid (91 %). FTIR (KBr, cm^{-1}): 3385 (m, br, $\nu_{(\text{O-H})}$), 1661 (vs, sh, $\nu_{(\text{C=O})}$), 1573, 1485, 1455 (s, sh, $\nu_{(\text{C}=\text{C}_{\text{Ar}} + \text{C-H}_{\text{bend}})}$), 1270 (s, sh, $\nu_{(\text{Ar-O})}$), 1149 (s, sh, $\nu_{(\text{H-C}=\text{C} + \text{H-C}=\text{N})_{\text{bend}}}$, Py). ^1H NMR (200 MHz, CDCl_3) δ (ppm): 10.83 (s, 1H, Ar-OH) 10.30 (s, 1H, Ar-HC=O), 9.15 (d, $J = 2.10$ Hz, 1H, Py-H), 8.68 (m, 2H, Py-H), 7.84 (d, 1H, $J = 1.39$ Hz, Py-H), 7.75 (d, $J_{\text{HH}} = 1.41$ Hz, 1H, Ar-H), 7.38 (m, 2H, 2 x Ar-H), 5.45 (s, 2H, -CH₂-Ar), 2.73 (s, 3H, CH₃). ESI MS: In positive mode peaks at $m/z = 244.3$ ($[\text{C}_{14}\text{H}_{14}\text{NO}_3]^+$, M - Cl) a.m.u.

1.5 Anion metathesis: synthesis of hexafluorophosphates (3a-c):

General method; To a solution of chlorides 2a-c (11.75 mmol) in milli-Q water (50 mL) was added aqueous solution of HPF₆ (60 w % solution, 2.7 mL, 17.62 mmol) portion-wise with vigorous stirring while cooling in ice bath over 1 h. After the addition was completed, the reaction was stirred at room temperature for 24 h. The solid product was filtered, washed with milli-Q water (to remove HPF₆-solution and any water-soluble impurities) until it was neutral. The final product was dried under vacuum at 40 °C for 24 h. Samples of the isolated products are fully characterized below.

5-(1-Methylimidazoliummethyl) salicylaldehyde hexafluorophosphate (3a): Obtained as of white solid, Yield (93 %). FTIR (KBr, cm^{-1}): 3164 (m, br, $\nu_{(\text{O-H})}$), 1662 (vs, sh, $\nu_{(\text{C=O})}$), 1573, 1458, 1406 (s, sh, $\nu_{(\text{C}=\text{C}_{\text{Ar}} + \text{C-H}_{\text{bend}})}$), 1285 (s, sh, $\nu_{(\text{Ar-O})}$), 825 (vs, sh, $\nu_{(\text{PF}_6^-)_{\text{str}}}$), 558 (s, sh, $\delta_{(\text{P-F})}$). ^1H NMR (200 MHz, DMSO- d_6) δ (ppm): 10.98 (s, 1 H, Ar-OH), 10.30 (s, 1 H, Ar-HC=O), 9.16 (d, $J = 1.78$ Hz, 1 H, N(1)CHCHN(3)), 7.78 (m, 1 H, Ar-H), 7.69 (d, $J = 1.70$ Hz, 1 H, N(1)CHCHN(3)), 7.61 (m, 2H, 2 x Ar-H), 7.06 (d, $J = 8.50$ Hz, 2H, 2 x Ar-H), 5.36 (s, 2H, N(3)-CH₂-Ar), 3.85 (s, 3 H, N(1)-CH₃). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 192.39, 161.09, 136.60, 136.47, 128.93, 125.71, 123.98, 122.47, 122.14, 118.03, 51.09, and 35.84. ^{31}P NMR (202 MHz, DMSO- d_6): -159.43 to -117.33 ppm (septet, $^2J_{\text{PF}} = 711.18$ Hz). ^{19}F NMR (470 MHz, DMSO- d_6): -67.82 to -64.81 ppm (doublet, $^1J_{\text{PF}} = 711.28$ Hz). ESI-MS, m/z : 217.1 $[\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_2, \text{M} - \text{PF}_6^-]^+$ a.m.u.

5-(Triethylammoniummethyl) salicylaldehyde hexafluorophosphate (3b): Obtained as of yellowish white solid, Yield (90 %). FTIR (KBr, cm^{-1}): 3163 (m, br, $\nu_{(\text{O-H})}$), 1654 (vs, sh, $\nu_{(\text{C=O})}$), 1580, 1452, 1393 (s, sh, $\nu_{(\text{C}=\text{C}_{\text{Ar}} + \text{C-H}_{\text{bend}})}$), 1276 (s, sh, $\nu_{(\text{Ar-O})}$), 821 (vs, sh, $\nu_{(\text{PF}_6^-)_{\text{str}}}$), 556 (s, sh, $\delta_{(\text{P-F})}$). ^1H NMR (200 MHz, DMSO- d_6) δ (ppm): 10.68 (s, 1 H, Ar-OH), 10.16 (s, 1 H, Ar-HC=O), 7.71 (s, 2H, 2 x Ar-H), 7.56 (m, 1H, Ar-H), 7.43 (d, $J = 12.9$ Hz, 1H, Ar), 4.37 (s, 2H, N-CH₂-Ar), 3.17 (q, $J = 8.9$ Hz, 6H, CH₂), 1.35 (t, $J = 6.8$ Hz, 9H, CH₃). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 190.23, 166.20, 157.52, 138.91, 132.16, 128.0, 127.33, 121.07, 114.72, 58.83, 51.42 and 7.40. ^{31}P NMR (202 MHz, DMSO- d_6): -159.41 to -117.34 ppm (septet, $^2J_{\text{PF}} = 711.17$ Hz). ^{19}F NMR (470 MHz, DMSO- d_6): -67.83 to -64.80 ppm (doublet, $^1J_{\text{PF}} = 711.26$ Hz). ESI-MS: In positive mode peaks at $m/z = 236.2$ $[\text{C}_{14}\text{H}_{22}\text{NO}_2, \text{M} - \text{PF}_6^-]^+$ a.m.u.

3-(2-methoxypyridiniummethyl) salicylaldehyde hexafluorophosphate (3c): Obtained as pale yellow solid (89 %). FTIR (KBr, cm^{-1}): 3334 (m, br, $\nu_{(\text{O-H})}$), 1676 (vs, sh, $\nu_{(\text{C=O})}$), 1572, 1482, 1439 (s, sh,

$\nu_{(\text{C}=\text{C}_{\text{Ar}} + \text{C-H}_{\text{bend}})}$), 1273 (s, sh, $\nu_{(\text{Ar-O})}$), 1150 (s, sh, $\nu_{(\text{H-C}=\text{C} + \text{H-C}=\text{N})_{\text{bend}}}$, Py), 838 (vs, sh, $\nu_{(\text{PF}_6^-)_{\text{str}}}$), 558 (s, sh, $\delta_{(\text{P-F})}$). ^1H NMR (200 MHz, CDCl_3) δ (ppm): 10.81 (s, 1H, Ar-OH) 10.31 (s, 1H, Ar-HC=O), 9.08 (d, $J = 2.00$ Hz, 1H, Py-H), 8.66 (m, 2H, Py-H), 7.80 (d, 1H, $J = 1.39$ Hz, Py-H), 7.69 (d, $J_{\text{HH}} = 1.51$ Hz, 1H, Ar-H), 7.38 (m, 2H, 2 x Ar-H), 5.47 (s, 2H, -CH₂-Ar), 2.77 (s, 3H, CH₃). ^{31}P NMR (202 MHz, DMSO- d_6): -159.38 to -117.33 ppm (septet, $^2J_{\text{PF}} = 711.19$ Hz). ^{19}F NMR (470 MHz, DMSO- d_6): -67.85 to -64.82 ppm (doublet, $^1J_{\text{PF}} = 711.26$ Hz). ESI MS: In positive mode peaks at $m/z = 244.3$ ($[\text{C}_{14}\text{H}_{14}\text{NO}_3]^+$, $\text{M} - \text{PF}_6^-$) a.m.u.

2. Tables Captions

Table S1: MIC/ MBC (mg/mL) assay results for promising antibacterial compounds against different strains

Table S2 : Approximate fouling cover percentage (%) on acrylic surfaces coated with chitosan functionalized compounds.

Table S1 MIC/ MBC (mg/mL) assay results for promising antibacterial compounds against different strains

Compound	MIC/ MBC (mg/mL)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>A. hydrophila</i>	<i>Vibrio</i>
CS	2.27±1.86/ 5.50±1.80	1.5±0.25/ 3.83±1.26	10.00±2.50/ 12.50±2.50	1.42±0.05/ 1.71±0.08
ILCSB1	1.50±0.18/ 2.83±1.04	0.25±0.05/ 0.64±0.13	0.62±0.13/ 0.64±0.38	1.50±0.25/ 1.31±0.30
ILCSB2	0.50±0.18/ 0.75±0.75	0.16±0.04/ 0.17±0.14	0.50±0.25/ 1.42±0.95	0.28±0.10/ 0.33±0.08
ILCSB3	1.89±0.21/ 3.99±0.53	0.75±0.10/ 1.88±0.30	2.02±0.25/ 1.86±0.23	2.11±0.18/ 1.55±0.20
ILCSB4	0.92±0.14/ 2.83±1.04	0.62±0.13/ 0.65±0.15	1.66±0.14/ 3.17±0.76	0.55±0.18/ 0.61±0.09
ILCSB5	0.55±0.18/ 0.92±0.50	0.25±0.05/ 0.32±0.08	1.08±0.38/ 1.92±0.52	0.30±0.09/ 0.41±0.22
ILCSB6	1.64±0.17/ 3.03±0.32	0.45±0.11/ 1.01±0.24	1.65±0.21/ 1.93±0.12	1.26±0.15/ 1.07±0.16
Diuron	10.00±2.50/ 15.00±2.50	5.50±1.80/ 7.17±1.04	10.00±2.50/ 17.50±2.50	10.00±2.50/ 14.16±0.14

Table S2 : Approximate fouling cover percentage (%) on acrylic surfaces coated with chitosan functionalized compounds.

Compound	Date (days)	Fouling Organisms								
		Algae			Tube Worms	Barnacles		Bryozoan	Ascidian	Zooids
		Brown	Green	Red		alive	dead			
Blank										
	10/6/2015 (29)	50±3.58	20±1.08	50±2.56	95±2.02	20±1.29	0±0	30±2.16	0±0	0±0
	24/6/2015 (43)	50±3.75	35±2.27	80±1.53	93±3.07	40±3.26	0±0	50±2.66	0±0	0±0
	14/7/2015 (63)	60±2.46	20±1.35	30±1.16	90±3.10	50±2.22	0±0	60±3.35	2±0.75	0±0
	11/8/2015 (91)	60±3.28	25±1.46	0±0	95±2.08	65±4.36	1±0.02	60±5.93	2±0.80	20±0.18
	6/10/2015 (147)	0±0	25±1.18	0±0	100±0	65±5.53	1±0.08	60±2.38	5±0.13	20±0.22
Control										
	10/6/2015 (29)	5±0.06	2±0.08	10±0.92	50±1.89	1±0.03	0±0	6±0.09	0±0	0±0
	24/6/2015 (43)	25±1.35	2±0.05	25±0.45	15±2.46	20±1.06	0±0	12±0.24	0±0	0±0
	14/7/2015 (63)	25±1.44	25±2.17	15±0.13	50±2.90	40±2.53	0.5±0.15	50±0.75	0±0	0±0
	11/8/2015 (91)	0±0	0±0	0±0	50±2.67	80±2.70	2.5±0.24	40±3.89	1±0.02	0±0
	6/10/2015 (147)	0±0	0±0	0±0	50±3.92	90±2.95	2.5±0.18	50±2.90	5±0.14	20±1.25
ILCSB2										
	10/6/2015 (29)	0±0	1.5±0.08	0±0	1±0.1	0.25±0.03	0±0	0±0	0±0	0±0
	24/6/2015 (43)	0±0	1.5±0.5	0±0	3±1	0.125±0.01	0±0	10±0.35	0±0	0±0
	14/7/2015 (63)	0±0	0.5±0.01	0±0	3±0.40	0.5±0.02	0.25±0.08	70±2.30	0±0	0±0
	11/8/2015 (91)	0±0	0±0	0±0	3±0.50	4±0.80	11±0.98	40±3.75	1±0.02	0±0
	6/10/2015 (147)	0±0	0±0	0±0	3±0.3	3±0.90	12±0.90	40±1.88	0±0	20±1.28
Standard (Diuron)										
	10/6/2015 (29)	0±0	0±0	5±0.80	30±2.90	0.25±0.03	0±0	4±0.10	0±0	0±0
	24/6/2015 (43)	0±0	0±0	15±0.30	20±0.98	2±0.04	0±0	10±0.51	0±0	0±0
	14/7/2015 (63)	0±0	0±0	10±0.5	10±.58	18±1.3	2±0.02	75±2.75	0±0	0±0
	11/8/2015 (91)	0±0	0±0	0±0	4±0.03	12.5±1.7	47.5±2.1	20±2.10	0±0	0±0
	6/10/2015 (147)	0±0	0±0	0±0	35±2.5	12±1.1	48±2.70	60±5.1	0±0	0±0

3. Figures Captions

Fig. S1: Field anti-macrofouling assays at Eastern Harbor of Alexandria beach; The coated panels were connected to the testing frames with nylon threads through nails bored in the panels



Fig. S1 Field anti-macrofouling assays at Eastern Harbor of Alexandria beach; The coated panels were connected to the testing frames with nylon threads through nails bored in the panels

References

[S1] R .F. M. Elshaarawy and C. Janiak, *Eur. J. Med. Chem.*, 2014, **75**, 31-42.