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Gallic acid-based indanone derivatives as anticancer agents ☆

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ABSTRACT

Gallic acid-based indanone derivatives have been synthesised. Some of the indanones showed very good anticancer activity in MTT assay. Compounds **10**, **11**, **12** and **14** possessed potent anticancer activity against various human cancer cell lines. The most potent indanone (**10**, IC_{50} = 2.2 μM), against MCF-7, that is, hormone-dependent breast cancer cell line, showed no toxicity to human erythrocytes even at higher concentrations (100 μg/ml, 258 μM). While, indanones **11**, **12** and **14** showed toxicities to erythrocytes at higher concentrations.

Indanones and related compounds are important bioactive molecules. These compounds have been studied for various biological activities including cancer and Alzheimer's type of diseases. Indanones are also used as drug intermediates, ligands of olefinic polymerisation catalysts[2a,b] and discotic liquid crystals.[3] Indanocine (**1**, Fig. 1) and its analogues are being developed to combat drug-resistant malignancies.[4] Another indanone analogue Donepezil hydrochloride (**2**, Fig. 1) has been approved by US-FDA for the treatment of mild to moderate Alzheimer's disease. This drug acts as an AChE (Acetylcholinesterase) inhibitor.[5] Dilemmaone A[6] (**3**, Fig. 1) and some other indanones have been isolated from natural products. Being such a useful moiety, several synthetic strategies have also been developed for their synthesis.[7a–j]

In continuation of our studies on modification of plant phenolics,[8a–e] we modified gallic acid to an indanone moiety (**4**, Fig. 1). Gallic acid (**5**), a plant phenolic acid is present as hydrolysable tannins in almost all woody perennials. The modified gallic acid moiety i.e., a 3,4,5-trimethoxy phenyl unit has been established as an essential structural requirement for several anticancer leads[9] like Combretastatin A4, Podophyllotoxin, Colchicine, etc. (Fig. 2). In the present letter, gallic acid-based indanone derivatives have been synthesised and evaluated for their anticancer activity. One of the potent indanone (**10**) has further been modified to establish its structure and activity relationship (SAR). All the compounds showing potent anticancer activity were further evaluated for toxicity to human erythrocytes by performing erythrocyte fragility test.

The synthetic strategy was as depicted in Scheme 1, gallic acid (**5**) was taken as the starting material. It was fully methylated at phenolic as well as carboxylic acid positions by refluxing it with dimethyl sulphate in 20% aqueous alkali to get 3,4,5-trimethyl gallic acid methyl ester (**6**) in 60% yield. The ester **6** underwent Grignard reaction with methylmagnesium iodide to yield the desired substrate 3,4,5-trimethoxyacetophenone (**7**). The acetophenone **7** and aldehyde **8** were condensed together in 3% aqueous methanolic sodium hydroxide to get a corresponding chalcone[10] (**9**). Similarly, other aldehydes were condensed with **7** to get respective chalcones first and then modified to corresponding indanones (**11**–**14**).[11,12] All these chalcones were further modified to corresponding indanones by heating with trifluoroacetic acid in a sealed glass tube (Borosil).[13a] However, indanone **15** was obtained on condensation of 3,4-dimethoxyacetophenone with **8** to get the respective chalcone and further modified to corresponding indanone (**15**) as described for other indanones (**11**–**14**). Chalcones lacking an electron releasing groups in the phenyl ring of benzoyl group did not undergo Nazarov's cyclisation reaction, due to deactivation by the carbonyl group. Therefore, chalcones synthesised from simple acetophenones could not be transformed into indanones. All the compounds were characterised by spectroscopic means.[17]

All these indanones were evaluated for in vitro anticancer activity by MTT assay[14] (Table 1) against various human cancer

☆ See Ref. 1.

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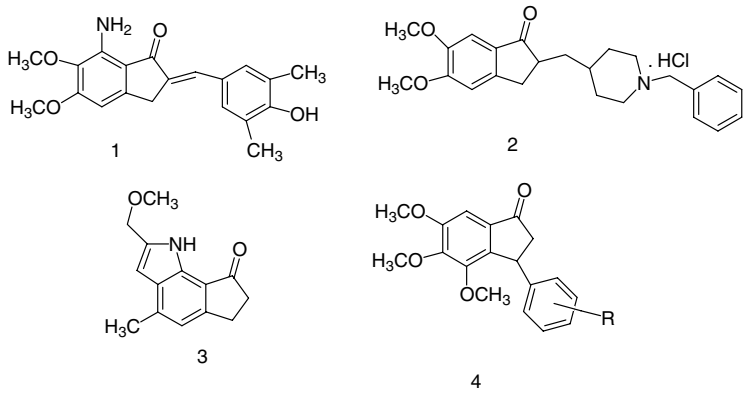


Figure 1. Structures of Indanocine (**1**), Donepezil hydrochloride (**2**), Dilemmaone A (**3**) and gallic acid-based indanone (**4**).

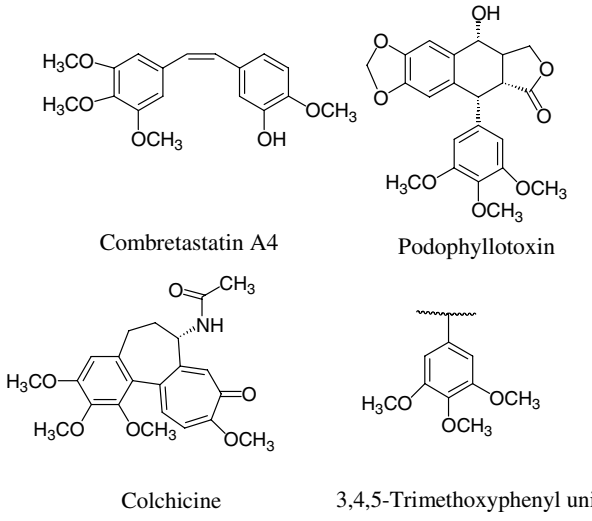


Figure 2. Structures of some lead molecules possessing 3,4,5-trimethoxyphenyl moiety as a common unit.

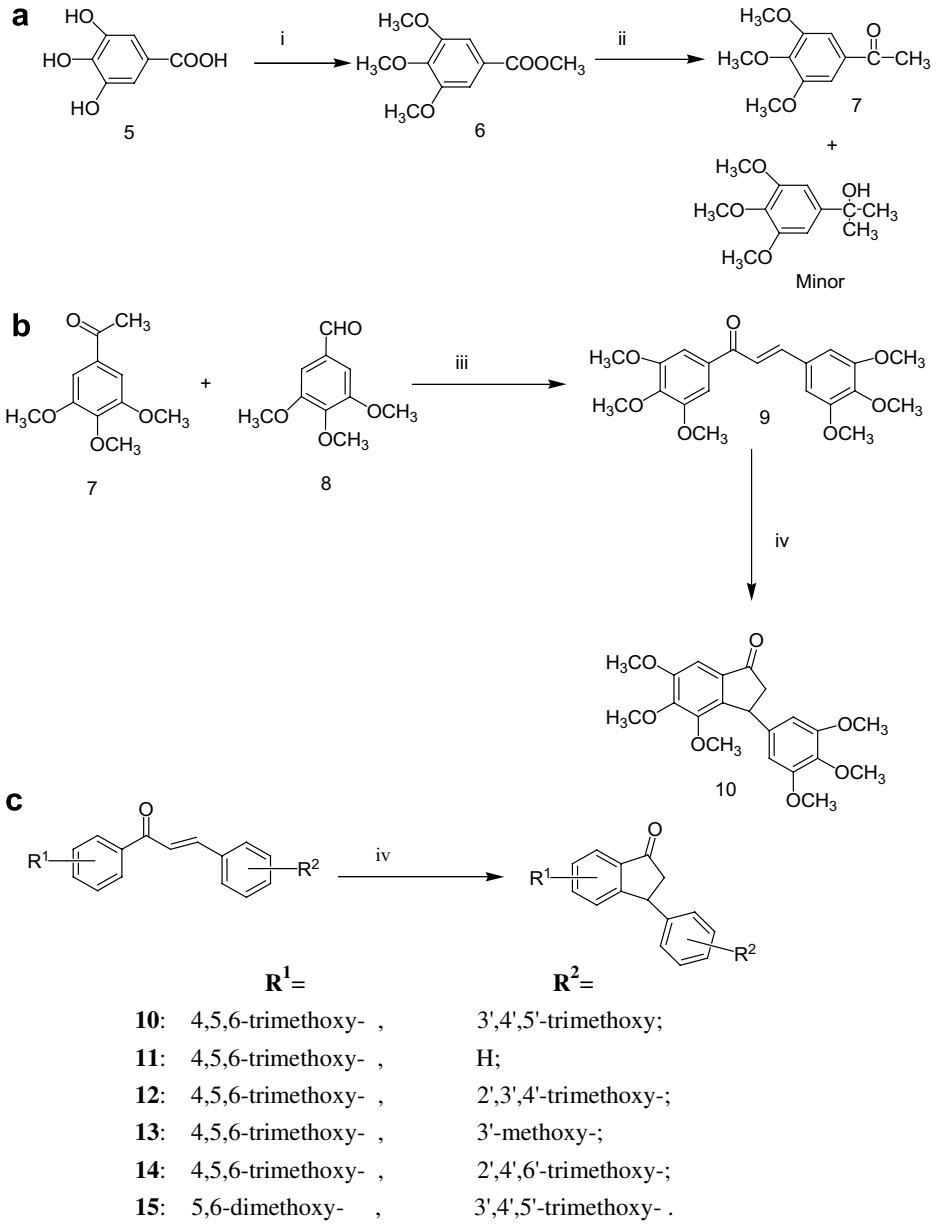
cell lines i.e., KB403 (oral and mouth cancer cells), WRL68 (liver cancer cells), CaCO2 (colon cancer cells), HepG2 (liver cells) and MCF7 (hormone-dependent breast cancer cells). Taxol (Paclitaxel) and Podophyllotoxin were used as reference compounds. Among all these indanone derivatives compound **14** (IC_{50} = 0.022 μM) showed the highest level of activity followed by **12** (IC_{50} = 0.023 μM) against WRL liver cancer cell lines, while indanone **10** (IC_{50} = 2.2 μM) was found to be most active against MCF-7 hormone-dependent breast cancer cell lines. Indanones **11** and **12** possessed highest anticancer activity against KB-403 oral and mouth cancer cell lines. Indanone **13** was found to be inactive against almost all the cell lines. Rest of the compounds showed moderate to low level of activity against these human cancer cell lines.

Compound **10** having trimethoxyphenyl units identical to gallic acid in both the rings possessed potent anticancer activity against MCF-7, HEPG2 and WRL68 human cancer cell lines. To establish structure and activity relationship (SAR) of compound **10**, it was further modified by simple derivatisations (Scheme 2).[13b,c] Compound **10** was refluxed with selenium dioxide in 1,4-dioxane to introduce another keto group at 2-position. On oxidising compound **16** was obtained as 1,2-keto-enol derivative rather than a 1,2-diketo derivative. An unexpected polycyclic derivative **17** (Fig. 3) was also obtained, which was characterised by various spectroscopic means. Compound **10**, on refluxing with hydroxylamine hydrochloride in ethanol and pyridine transformed to its oxime in excellent yields (94%). On sodium borohydride reduction in methanol, a corresponding secondary alcohol **19** was formed in quantitative yield, while sodium borohydride reduction in trifluoroacetic acid yielded a 1-deoxy derivative **20** in good yield. But all these derivatives possessed either lower cytotoxicities or were found to be inactive as compared to the parent molecule **10**. From this, it was concluded that in indanone **10**, all the above modifications are not favourable. Hence, a keto group at 1-position along with no such substitutions at 2-position is desirable for its better activity.

The structure proposed for compound **17** has been confirmed by spectroscopic means using IR, NMR experiments like ^{1}H NMR, ^{13}C NMR, DEPT 135 and HMBC correlation experiments and finally by mass spectrometry. The proton spectra taken on 300 MHz FT NMR in $CDCl_3$ showed six distinct singlets at 3.90, 3.96, 3.98, 4.00, 4.03 and 4.07 ppm for six methoxy groups. Two singlet protons were also observed in the aromatic region at 7.04 and 8.07 ppm. The ^{13}C NMR spectra showed presence of total 21 carbons in **17**. ^{13}C coupled with DEPT 135 experiments clearly indicated the presence of six methyls (all oxygenated) and two methines (aromatic) and 13 quaternary (nine oxygenated) carbons. Absence of one of the aromatic proton of ring C (which is otherwise a singlet for two enantiotopic protons) and enolic hydroxyl suggested the possibility of cyclisation. It was further indicated by the downfield shifts of both the aromatic protons and presence of one more quaternary carbon at the loss of one aromatic methine as compared to the ^{13}C spectrum of **16**. It was further ascertained by HMBC correlations of compound **17** (Fig. 3).

Indanone derivatives **10**, **11**, **12** and **14** showing potent anticancer activity were also evaluated for erythrocyte osmotic fragility (Fig. 4) to determine their toxicity.[15] Among these indanone **10** showing most potent activity against MCF-7 was found to be non-toxic to human erythrocytes even at higher concentrations (100 μg/ml, 258 μM). Indanones **11**, **12** and **14** increased the haemolysis of erythrocytes, hence these may be considered toxic at higher concentrations.

In conclusion, gallic acid-based indanone derivatives showed potent anticancer activity against hormone-dependent breast cancer, oral and liver cancer cell lines. As one of the potent molecules was found non-toxic to human erythrocytes, this compound may further be optimised to better anticancer leads with no or low toxicities to normal cells.

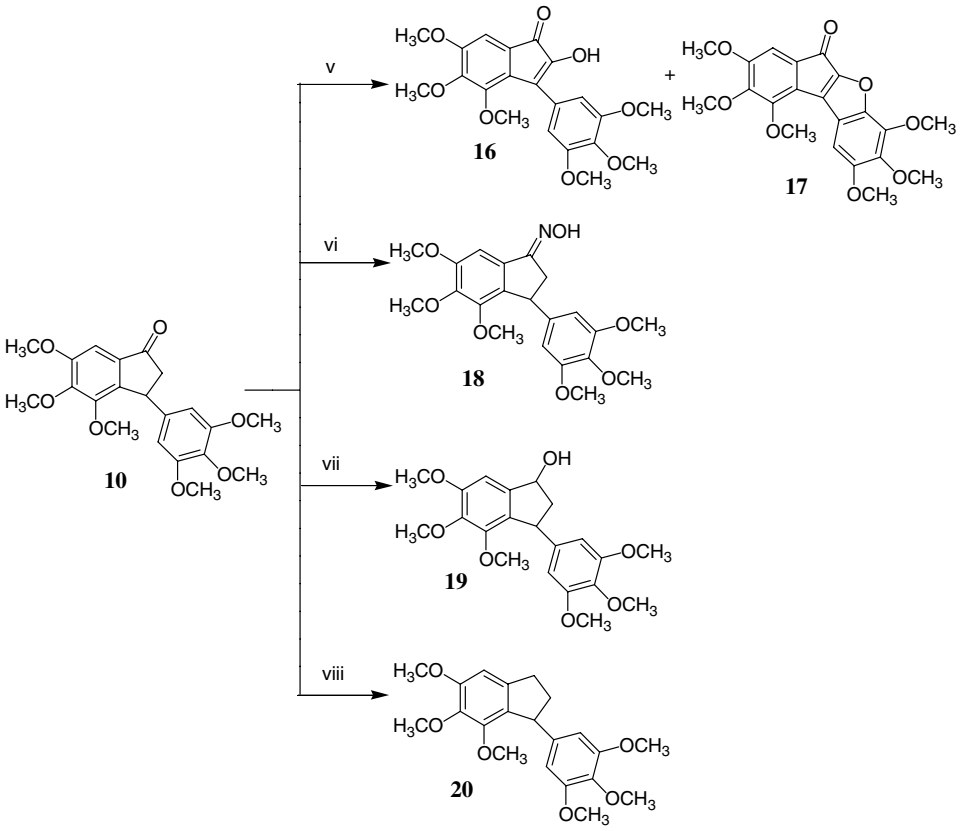


Scheme 1. Reagents and conditions: (i) 20% aq alkali, dimethyl sulphate, refluxed for 3 h, 60%; (ii) CH_3I, Mg turnings, THF, 20 min at RT then reflux for 1 h, 42%; (iii) 3% aq methanolic NaOH, RT, overnight 16–18 h, 62–84% respective acetophenones and aldehydes used; (iv) TFA, refluxed in a sealed tube, 3–4 h, 28–52%.

Table 1
Cytotoxicities[a] of indanones and their analogues against various human cancer cell lines by MTT assay

Compound	Human cancer cell lines				
	KB403 IC_{50} (μM)	WRL68 IC_{50} (μM)	CaCO2 IC_{50} (μM)	HEPG2 IC_{50} (μM)	MCF7 IC_{50} (μM)
10	10.3	12.8	Inactive	9.0	2.20
11	0.84	Inactive	Inactive	188	Inactive
12	1.54	0.023	206	Inactive	Inactive
13	Inactive	Inactive	Inactive	Inactive	Inactive
14	12.90	0.022	226	1.49	211
15	139.6	195.5	139.6	111.7	Inactive
16	Inactive	124.3	Inactive	74.6	Inactive
17	Inactive	62.5	112.5	Inactive	50
18	136.4	223.3	49.6	124	Inactive
19	192.3	192.3	6.4	102.5	243.5
20	Inactive	153.8	115.3	89.7	Inactive
Taxol	0.001	0.004	0.008	0.009	0.006
Podophyllotoxin	20.5	4.8	0.002	4.8	8.5

[a] $IC_{50} \geqslant 250$ μM was considered as inactive.



Scheme 2. Reagents and conditions: (v) SeO_2, 1,4-Dioxane, refluxed for 3 h, **16**: 67%, **17**: 14%; (vi) $NH_2OH{\cdot}HCl$, Ethanol, Pyridine, refluxed for 2 h, 94%; (vii) $NaBH_4$–MeOH, 58%; (viii) $NaBH_4$–TFA, 0 °C–RT, 6 h, 72%.

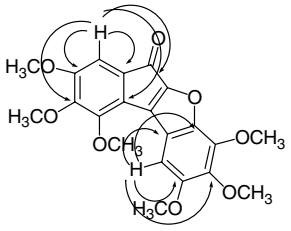


Figure 3. HMBC correlations of compound **17**.

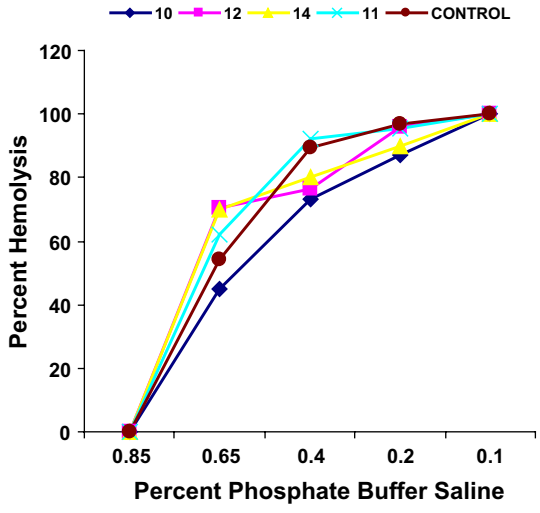


Figure 4. Osmotic haemolysis curve of erythrocytes.

Acknowledgment

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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.2008.06.039.

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13. (a) *Syntheses: General procedure for preparing indanone derivatives from chalcones:* Synthesis of 3-(3′,4′,5′-Trimethoxyphenyl)-4,5,6-trimethoxy-indan-1-one (**10**). In a Borosil test tube, chalcone **9** (150 mg, 0.39 mmol) was taken in trifluoroacetic acid (0.5 ml) and the tube was sealed carefully with flame. The reaction mixture was heated at 120 °C for 4 h. The reaction mixture was poured into crushed ice and extracted with ethyl acetate, organic layer was washed with water, dried over anhydrous sodium sulphate and evaporated in vacuo. The crude residue thus obtained was purified through column chromatography on silica gel using ethyl acetate–hexane as eluent. The desired indanone **10** was obtained as a solid. It was recrystallised with chloroform–hexane (1:3) to get **10** as a light brown solid.
(b) *Synthesis of 3-(3′,4′,5′-Trimethoxyphenyl)-4,5,6-trimethoxy-indan (**20**).* Indanone **10** (100 mg, 0.26 mmol) was taken in trifluoroacetic acid (2.5 ml) and the reaction flask was stirred in an ice-bath. After stirring for 5 min sodium borohydride (100 mg, 2.6 mmol) was added in portions with maintaining the bath temperature 0–15 °C for an hour. After that the reaction mixture was stirred at room temperature for 6 h. On completion, 10 ml water was added to reaction mixture and it was extracted with ethyl acetate, organic layer was washed with water, dried over anhydrous sodium sulphate and evaporated in vacuo. The crude residue thus obtained was purified through column chromatography on silica gel using ethyl acetate–hexane as eluent. The desired indan **20** was obtained as oil.
(c) *2-Hydroxy, 3-(3′,4′,5′-trimethoxyphenyl)-4,5,6-trimethoxy-ind-2-en-1-one (**16**).* Indanone **10** (100 mg, 0.26 mmol) was taken in a round-bottomed flask with 1,4-dioxane (10 ml) and selenium dioxide (290 mg, 2.6 mmol). The reaction mixture was refluxed for 6 h. On completion, reaction mixture was filtered and filtrate was evaporated in vacuo. The crude mass thus obtained was purified through column chromatography on silica gel using ethyl acetate–hexane as eluent. The cyclised product **17** was first obtained followed by the desired derivative **16**.
14. In-vitro anticancer activity using MTT assay. In-vitro cytotoxicity testing was performed as per reported method.[16] 2×10^3 cells/well were incubated in the 5% CO_2 incubator for 24 h to enable them to adhere properly to the 96-well polystyrene microplates (Grenier, Germany). Test compound dissolved in dimethyl sulphoxide (DMSO, Merck, Germany), in at least five concentrations, were added into the wells and left for 4 h. After the incubation, the compound plus media was replaced with fresh media and the cells were incubated for another 48 h in the CO_2 incubator at 37 °C. The concentration of DMSO were always kept below 1.25%, which was found to be non-toxic to cells. Then, 10 μL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well and plates were incubated at 37 °C for 4 h. DMSO (100 μL) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. The plates were read on SpectraMax 190 Microplate reader (Molecular Devices Inc. USA) at 570 nm within 1 h of DMSO addition.
15. *Determination of osmotic haemolysis of erythrocytes:* Blood from healthy human male volunteers ($n = 3$) with informed consent was collected for experiments using heparin (10 U/ml) as the anti-coagulant. The collected blood was stored at 4 °C and was used for experiments within 4 h of collection.[18] Experiments were carried in-vitro by adding heparinised blood to hypotonic solutions of varying concentrations of phosphate buffered saline (0.85% to 0.10%). Phosphate-buffered saline stock (10%) was prepared by dissolving 5 g of sodium chloride, 1.3655 g of disodium hydrogen orthophosphate and 0.243 g of sodium dihydrogen orthophosphate in 100 ml of autoclaved double distilled water. From this stock, working standards of 0.85% to 0.10% were prepared. The tubes were incubated at 37 °C for 60 min with mild shaking and the extent of haemolysis was measured colorimetrically at 540 nm.[19] Results are expressed in terms of mean erythrocyte fragility (MEF_{50}), which is the level of haemolysis of the erythrocytes at 50% saline concentrations. Similarly, prior to the experiment, heparinised blood was incubated with effective concentration of indanone derivatives (5–100 μg/ml) at 37 °C for 60 min. The concentrations of indanones were chosen higher than the concentrations at which the compounds showed anticancer activity.
Aliquots of saline solutions of decreasing concentration (from 10 to 1 g/L) were prepared as described earlier.[19,20] The test compound treated erythrocytes were then transferred to tubes containing decreasing concentrations of saline solutions. After careful mixing, the cell suspensions were left to equilibrate for 30 min and then centrifuged at 3000 rpm for 5 min. The absorbance of supernatants was read at 540 nm, with the standardised against an assay blank (the 10 g/L saline supernatant corresponds to 0% haemolysis). The recorded optical density (OD) of the supernatant reflects the degree of haemolysis of the erythrocytes. The percentage lysis was calculated by dividing the OD of the supernatant obtained from a particular saline concentration by the OD of the standard (1 g/L) representing 100% haemolysis.[21] Osmotic fragility curves were constructed by plotting the percentage lysis against the concentration of saline solutions. The MEF_{50} (mean erythrocyte fragility) value, which is the saline concentration at which 50% of the cells haemolyse at standard pH and temperature, was then obtained from the curve.
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17. *Selected physical data:Compound **10**: Yield = 64%; mp = 107–110 °C;* IR (KBr, cm^{-1}): 2938, 1705, 1591, 1509, 1500, 1129. ^1H NMR($CDCl_3$, 300 MHz) δ *2.58–2.65* (dd, 1H, 2-CH, $J = 2.58$, 19.29 Hz), 3.13–3.22(dd, 1H, 2-CH, $J = 7.98$, 19.26 Hz), 3.42 (s, 3H, OCH_3), 3.78 (s, 6H, 2× OCH_3), 3.81 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 4.50–4.53 (dd, 1H, 3-CH, $J = 7.95$, 2.49 Hz), 6.31 (s, 2H, aromatic protons), 7.09 (s, 1H, aromatic proton); ^{13}C NMR($CDCl_3$, 75.46 MHz) δ 42.30, 47.48, 56.58, 56.68, 56.68, 60.44, 61.14, 61.14, 100.81, 105.03, 132.65, 137.75, 140.42, 140.42, 144.38, 149.21, 150.89, 153.84, 153.84, 155.45, 205.08. EI Mass GC–MS (CH_3CN): 388 [M^+], 373, 357, 181.
*Compound **12**: Yield = 71%; mp = 119–123* °C; IR (KBr, cm^{-1}): 2936, 2838, 1704, 1599, 1498, 1470, 1101. ^1H NMR($CDCl_3$, 300 MHz) δ *2.54–2.61* (dd, 1H, 2-CH, $J = 1.99$, 19.07 Hz), 3.07–3.16 (dd, 1H, 2-CH, $J = 8.03$, 19.09 Hz), 3.38 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.75–4.78 (br d, 1H, -CH, $J = 7.95$, 2.49 Hz), 6.53–6.58 (br s, 2H, aromatic protons), 7.08 (s, 1H, aromatic proton); EI mass GC–MS (CH_3CN): 388 [M^+], 357, 373, 358, 342.
*Compound **13**:* Yield: 48%; mp = 112–115 °C; ^1H NMR($CDCl_3$, 300 MHz) δ *2.58–2.65* (dd, 1H, 2-CH, $J = 2.42$, 19.26 Hz), 3.14–3.23(dd, 1H, 2-CH, $J = 7.97$, 19.27 Hz), 3.39 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 4.54–4.58 (dd, 1H, –CH, $J = 2.36$ and 7.90 Hz), 6.66–7.23 (m, 8H, aromatic protons); EI mass GC–MS (CH_3CN): 328 [M^+], 313, 207.
*Compound **16**: Yield = 67%; mp = oil;* IR (KBr, cm^{-1}): 3444, 2939, 1727, 1594, 1499, 1466, 1126. ^1H NMR($CDCl_3$, 300 MHz) δ 3.33 (s, 3H, OCH_3), 3.78 (s, 6H, 2× OCH_3), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.65 (s, 2H, aromatic protons), 6.97 (s, 1H, aromatic proton); ^{13}C NMR($CDCl_3$, 75 MHz) δ 42.30, 47.48, 56.58, 56.68, 56.68, 60.44, 61.14, 61.14, 100.81, 105.03, 132.65, 137.75, 140.42, 140.42,144.38, 149.21, 150.89, 153.84, 153.84, 155.45, 205.08. EI Mass GC–MS (CH_3CN): 402 [M^+], 387, 195.
*Compound **17**: Yield = 16%; mp = 145–148* °C; IR (KBr, cm^{-1}): 2933, 1697, 1474, 1384, 1096. ^1H NMR($CDCl_3$, 300 MHz) δ 3.90 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 4.07 (s, 3H, OCH_3), 7.04 (s, 1H, aromatic proton), 8.07 (s, 1H, aromatic proton); ^{13}C NMR($CDCl_3$, 75 MHz) δ 56.55, 56.93, 61.10, 61.37, 61.51, 62.09, 105.96, 106.96, 127.09, 130.38, 133.06, 136.87, 137.10, 141.73, 147.17, 147.52, 149.42, 153.86, 154.26, 156.33, 187.84. EI Mass GC–MS (CH_3CN): 400 [M]$^+$.
*Compound **19**: Yield = 58%; mp = 132–136* °C ; IR (KBr, cm^{-1}): 3516, 2941, 1593, 1503, 1463, 1419, 1335, 1120. ^1H NMR($CDCl_3$, 300 MHz) δ *1.92–2.01* (m, 1H, 2-CH), 2.94–2.99 (m, 1H, 2-CH), 3.45 (s, 3H, OCH_3), 3.79 (s, 12H, 4× OCH_3), 3.90 (s, 3H, OCH_3), 4.22–4.27 (m, 1H, 3-CH), 5.16–5.20 (m, 1H, 1-CH), 6.48 (s, 2H, aromatic protons), 6.81 (s, 1H, aromatic proton); ^{13}C NMR($CDCl_3$, 75.46 MHz) δ 46.35, 47.58, 56.45, 56.53, 60.21, 60.42, 60.94, 60.98, 75.35, 103.54, 105.24, 105.88, 130.49, 137.01, 141.54, 142.30, 142.82, 150.36, 153.31, 154.46. EI Mass GC–MS (CH_3CN): 390 [M^+], 372, 357. $[\alpha]_{589}^{28} + 6.99^\circ$ (1.04, MeOH).
*Compound **20**: Yield = 72%; mp = 71–74* °C ; ^1H NMR($CDCl_3$, 300 MHz) δ *1.99–2.06* (m, 1H, 2-CH), 2.52–2.59 (m, 1H, 2-CH), 2.85–2.89 (m, 1H, 1-CH), 2.99–3.06 (m, 1H, 1-CH), 3.51 (s, 3H, OCH_3), 3.77 (s, 6H, 2× OCH_3), 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.37–4.42 (m, 1H, 3-CH), 6.33 (s, 2H, aromatic proton), 6.63 (s, 1H, aromatic proton); ^{13}C NMR($CDCl_3$, 75.46 MHz) δ 22.66, 29.67, 31.93, 49.43, 56.35, 56.35, 60.15, 60.73, 60.81, 104.01, 105.19, 130.84, 139.95, 142.38, 150.29, 153.93, 153.76. EI Mass GC–MS (CH_3CN): 390 [M^+], 372, 357.

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