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Design, synthesis, and evaluation of novel 6-chloro-/fluorochromone derivatives as potential topoisomerase inhibitor anticancer agents

M. P. S. Ishar,[a,*] Gurpinder Singh,[a] Satyajit Singh,[b] K. K. Sreenivasan[b] and Gurmit Singh[a]

[a]*Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar 143 005, Punjab, India*
[b]*Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal 576 104, Karnataka, India*

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Abstract—6-Chloro-2-pyrrolidino-/morpholino-/piperidino-/*N*-methylpiperazino-3-formyl-chromones (**13**–**16**) and 6-fluoro-2,7-dimorpholino-/piperidino-/*N*-methylpiperazino-3-formylchromones (**17**–**19**) have been synthesized as potential topoisomerase inhibitor anticancer agents, and evaluated, in vitro, against Ehrlich ascites carcinoma (EAC) cells, and also in vivo on EAC bearing mice. The compounds displayed promising anticancer activity under these test systems and shall serve as useful 'leads' for further design.

DNA topoisomerases are a class of enzymes involved in regulation of DNA supercoiling and are crucial for replication and transcription of DNA.[1] Consequently, these enzymes are targets for chemotherapeutic intervention in antibacterial and anticancer therapies.[2,3] Though a number of classes of compounds are known to inhibit topoisomerases, their precise target and mode of action are varied and are still being investigated.[2–9] Fluoroquinolone antibacterials such as ciprofloxacin (**1a**) and norfloxacin (**1b**, Fig. 1) are inhibitors of essential bacterial DNA-type-II topoisomerases (DNA gyrase and Topoisomerase IV found in bacteria).[2,3,7] Camptothecin (**2a**) and its synthetic analogs such as topotecin (**2b**, Fig. 1) are DNA topoisomerase-I poisons used as anticancer agents.[6,10] On the other hand, although the natural product podophyllotoxin is an antimitotic agent, its semi-synthetic analogs, etoposides, are topoisomerase-II inhibitors with anticancer applications.[11] In the case of psorospermin (**3**, Fig. 1), a natural antitumor antibiotic,[12] which is apparently an alkylating agent resembling pluramycin A (**4**), it has been shown that it has to intercalate with DNA and its alkylating potential is significantly increased in the presence of topoisomerase-II.[13] Compound A-62176 (**5**, Fig. 1), a fluoroquinolone possessing a quinobenzoxazine moiety, shows good activity against a number of cancer cell lines and competes with psorospermin for topoisomerase II–DNA complex;[5,13,14] binding of A-62176 has been shown[5,14] to involve its two molecules and two Mg^{2+} ions.

Based on the structural similarities, i.e., presence of a benzochromone (xanthone) nucleus in psorospermin, a chromone moiety in pluramycin A, a fluoroquinolone moiety in A62176 along with known involvement of fluorquinolones with topoisomerases, the established SARs[3,7,15] in case of fluoroquinolones, and recent observation that the ketone moiety plays an important role in anticancer activity of some 2-fluorinated phenyl-4-quinolone antimitotic anticancer agents,[16] it was reasonable to infer that N-alkylation in fluoroquinolones is meant to ensure the keto-tautomer. Therefore, it was concluded that a chromone moiety can be a replacement for *N*-alkyl-4-quinolone nucleus, leading to the design of novel chloro/fluorochromone analogs (Fig. 2).

2-Anilino-6-chloro-3-formylchromone and 2-anilino-7-chloro-6-fluoro-3-formylchromone (**11a**), (**11b**) were obtained by recently reported thermal rearrangements of the corresponding nitrones (**10a**), (**10b**)[17] by refluxing in dry benzene. Reactions of (**11a**), (**11b**) with methyl iodide in the presence of anhydrous K_2CO_3 afforded the corresponding *N*-methylanilino derivatives (**12a**), (**12b**). Nucleophilic substitution of *N*-methylanilino moiety in **12a** by heterocyclic amines,[18] on refluxing the equimolar

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* Corresponding author. Tel.: +91 183 2258802 09x3321; fax: +91 183 2258820; e-mail: mpsishar@yahoo.com

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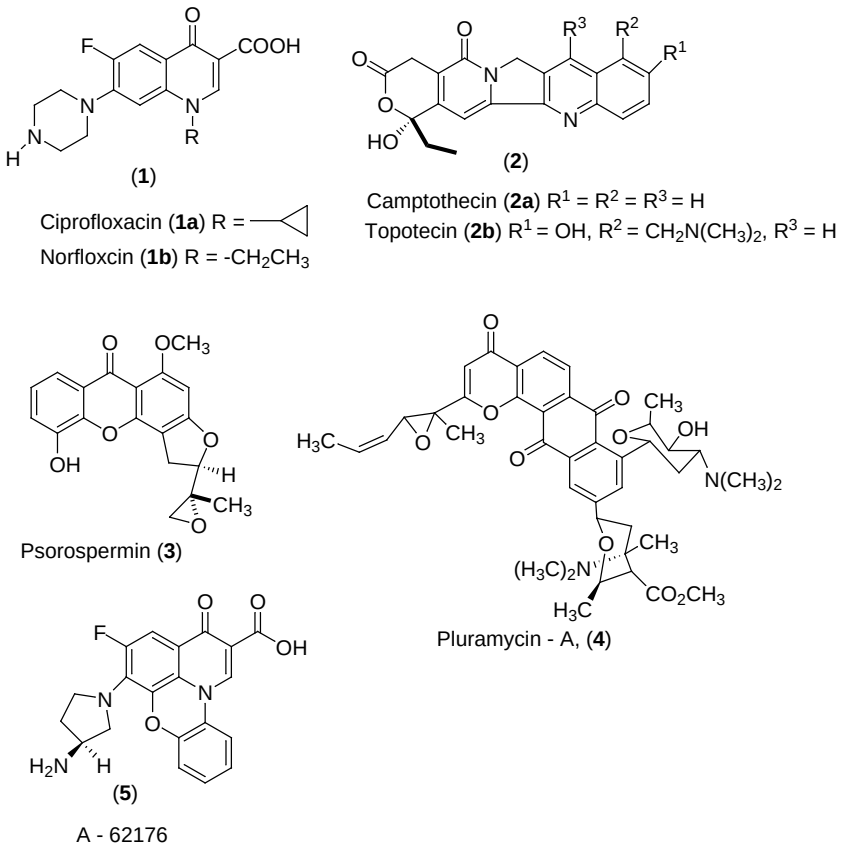


Figure 1.

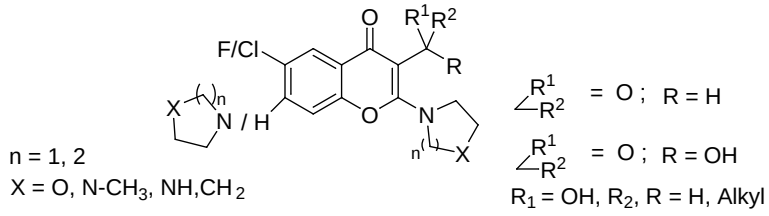
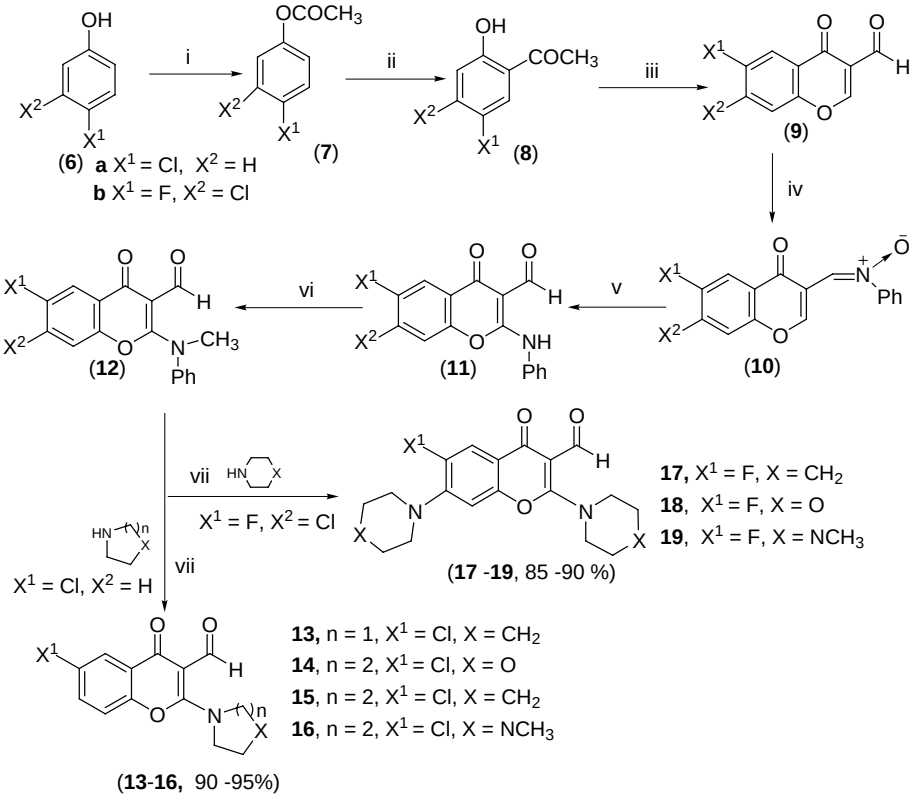


Figure 2.

solutions of reactants in acetonitrile under anhydrous conditions, afforded compounds (**13**–**16**, Scheme 1); progress of the reactions was monitored by TLC and products were isolated by direct crystallization on removal of solvent and re-crystallized from chloroform–hexane (2:1). Similar reaction of **12b** with two molar equivalents of heterocyclic amines, under identical conditions, led to the nucleophilic substitution of both *N*-methylanilino moiety as well as 7-chloro, affording compounds (**17**–**19**, Scheme 1), which were isolated similarly and re-crystallized from chloroform–hexane (2:1). All the intermediates and the final products have been characterized by detailed spectroscopic (UV, IR, ^{1}H and ^{13}C NMR, and EI-MS) and microanalytical data.[19]

The obtained compounds (**13**–**19**) were evaluated for cytotoxicity against Ehrlich ascites carcinoma (EAC) cells and compounds **14**, **17**, and **18** were also evaluated, in vivo, against EAC carcinoma bearing mice.[20] For cytotoxicity evaluation, EAC cells were incubated with different concentrations (125, 250, and 500 μg/ml) of these compounds. Counting of cells was done with hemocytometer and the percentage of dead cells was determined by tryphan blue exclusion method; the results are summarized in Table 1. For in vivo evaluation ascites tumors were induced in Swiss Albino mice by intraperitoneal inoculation of ascitic fluid (containing estimated 106 cells/200 μl) from donor mice and 24 h after inoculation, test compounds were administered intraperitoneally (as 2% suspension with carboxymethyl-cellulose) and continued with a daily dose for nine days.[23] The results of various observations/measurements along with similar measurements on control and standard[22] are summarized in Table 2.

The results in Table 1 clearly indicate that compounds (**13**–**17**) show significant cytotoxicity at a concentration of 250 μg/ml, whereas compound **19** was nontoxic even at 500 μg/ml. The results of in vivo evaluation (Table 2) indicate significant increase in the life span of ascites



Scheme 1. Reagents and conditions: (i) CH_3COCl, Δ; (ii) anhyd $AlCl_3$, Δ; (iii) DMF, $POCl_3$; (iv) PhNHOH, anhyd benzene; (v) anhyd benzene, Δ; (vi) CH_3I, anhyd acetone, Δ; (vii) anhyd acetonitrile, reflux.

Table 1. Cytotoxicity of compounds (**13**–**19**) against Ehrlich ascites carcinoma (EAC) cells

Concentration (μg/ml)	Percentage cytotoxicity (% of dead cells)						
	13	**14**	**15**	**16**	**17**	**18**	**19**
125	90	60	100	50	100	0	0
250	100	100	100	60	100	0	0
500	100	100	100	60	100	70	20

carcinoma bearing mice treated with compounds **14**, **17**, and **18**, relative to control. The treated animals also showed a reduction in body weight as compared to control and also increased glutathione (GSH) level; the latter signifying the decreased utilization of GSH due to suppression of tumors.[21,23] In general, 6-chloro-substituted derivatives gave more consistent results and piperidine bearing analogs (**15**, **17**) were more potent.

In conclusion, the synthesized 2-substituted-6-chloro-3-formylchromones (**13**–**16**) and 2,7-disubstituted-6-fluoro-3-formylchromones (**17**, **18**) have shown promising anticancer activity and shall serve as useful 'leads.' Further developments involving oxidation/reduction of 3-formyl group, preparation of 2,7-disubstituted-6-chloro- and 2-substituted-6-fluoro-analogs, and introduction of an alkylating function on heterocyclic-amine moieties are in progress. A substantial increase in anticancer activity is anticipated after oxidation/reduction of the formyl function.

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Table 2. Median survival time (MST), percent increase in life span (% ILS), percent weight variation, and glutathione level in Ehrlich carcinoma bearing mice treated with compounds **14**, **17**, **18**, and cisplatin (standard) vis-a-vis blank (untreated)

Sr. No.	Compound	MST (in days)	% ILS	Body weight	% weight variation	GSH level (μg/mg protein)
1	Blank (control)	9.8 ± 0.17	0.5 ± 1.77	25.2 ± 0.99	10.40	4.17 ± 1.05
2	Cisplatin (standard)	17.3 ± 0.21	75.4 ± 2.21	23.0 ± 1.16	−17.26	9.63 ± 0.09
3	**14**	12.0 ± 0.13	23.1 ± 1.33	19.3 ± 0.77	−16.81	9.61 ± 0.13
4	**17**	11.6 ± 0.15	17.9 ± 1.59	23.8 ± 1.15	−9.50	8.17 ± 0.18
5	**18**	11.14 ± 0.15	16.9 ± 1.59	21.7 ± 1.27	−15.89	5.96 ± 0.04

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19. Spectroscopic and microanalytical data:
*6-Chloro-2-pyrrolidin-1-yl-3-formylchromone (**13**)*. Pale-yellow solid, mp 188–189 °C (chloroform–hexane, 1:2); ν_{max} (KBr): 1674, 1638, 1624, 1545, 1440 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.19 (s, 1H, CH=O), 8.18 (br s, 1H, C5-H), 7.49 (dd, 1H, J = 8.7 and 2.6 Hz, C7-H), 7.19 (d, 1H, J = 8.7 Hz, C8-H), 3.67 (br, 4H, 2× CH_2), 2.06 (br, 4H, 2× CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.02 (HC=O), 176.88 (C4), 161.19 (C2), 151.48 (C8a), 133.37 (C7), 129.59 (C6), 125.59 (C5), 120.12 (C4a), 118.04 (C8), 101.5 (C3), 51.27 (N-CH_2), 25.16 (CH_2); Mass (EI) *m/z*: 279 (M^+ + 2, 12), 278 (M^+ + 1, 14), 277 (M^+, 21), 249 (65), 241 (41), 221 (100); Anal. Calcd. for $C_{14}H_{12}NO_3Cl$: C, 60.55; H, 4.36; N 5.04. Found: C, 60.39; H, 4.49; N, 4.93.
*6-Chloro-2-morpholin-4-yl-3-formylchromone (**14**)*. Light-yellow solid, mp 177–178 °C (chloroform–hexane, 1:2); ν_{max} (KBr): 1672, 1639, 1619, 1542, 1434 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.11 (s, 1H, HC=O), 8.26 (d, 1H, J = 2.5 Hz, C5-H), 7.55 (dd, 1H, J = 8.8, 2.5 Hz, C7-H), 7.22 (d, 1H, J = 8.8 Hz, C8-H), 3.91 (dist. t, 4H, $J \sim$ 5.1 Hz), 3.69 (dist. t, 4H, $J \sim$ 5.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 186.59 (HC=O), 177.53 (C4), 162.64 (C2), 151.39 (C8a), 133.64 (C7), 131.44 (C6), 124.04 (C5), 120.17 (C4a), 116.16 (C8), 101.69 (C3), 66.6 (CH_2), 49.93 (CH_2); Mass (EI) *m/z*: 295 (M^+ + 2, 9), 294 (M^+ + 1, 5), 293 (M^+, 24), 169 (100), 112 (26); Anal. Calcd for $C_{14}H_{12}NO_4Cl$: C, 57.28; H, 4.12; N, 4.77. Found: C, 57.45; H, 4.21; N, 4.89.
*6-Chloro-2-piperidin-1-yl-3-formylchromone (**15**)*. Cream-colored solid, mp 157–158 °C (chloroform–hexane 1:2); ν_{max} (KBr): 1670, 1620, 1542, 1443, 1416 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.11 (s, 1H, HC=O), 8.13 (d, 1H, J = 2.4 Hz, C5-H), 7.54 (dd, 1H, J = 8.8, 2.4 Hz, C7-H), 7.22 (d, 1H, J = 8.8 Hz, C8-H), 3.66–3.63 (br m, 4H), 1.84–1.77 (br m, 6H, 3× CH_2); ^{13}C NMR (75 MHz, $CDCl_3$):δ 186.28 (CHO), 177.65 (C4), 162.36 (C2), 151.35 (C8a), 133.53 (C7), 130.92 (C6), 126.61 (C5), 125.53 (C4a), 118.15 (C8), 101.49 (C3), 50.86 (CH_2), 26.07 (CH_2), 23.65 (CH_2); Mass (EI) *m/z*: 293 (M^+ + 2, 11) 292 (30), 291 (M^+,30), 167 (25), 149 (79), 71 (58), 70 (47), 58 (100); Anal. Calcd for $C_{15}H_{14}NO_3Cl$: C, 61.76; H, 4.84; N, 4.80. Found: C, 61.63; H, 4.96; N, 4.71.
*6-Chloro-2-(N-methylpiperazin-1-yl)-3-formylchromone (**16**)*. Yellow solid, mp 155–156 °C (chloroform–hexane, 1:2); ν_{max} (KBr): 1673, 1631, 1615, 1544 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.11 (s, 1H, HC=O), 8.10 (d, 1H, J = 2.5 Hz, C5-H), 7.55 (dd, 1H, J = 8.8, 2.5 Hz, C7-H), 7.25 (d, 1H, J = 8.8 Hz, C8-H), 3.72 (dist. t, 4H, $J \sim$ 4.9 Hz, 2× CH_2), 2.63 (dist. t, 4H, $J \sim$ 4.9 Hz, 2× CH_2), 2.37 (s, 3H, *N*-Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ 186.53 (CHO), 177.72 (C4), 162.51 (C2), 151.43 (C8a), 133.79 (C7), 131.33 (C6), 125.81 (C5), 124.07 (C4a), 118.17 (C8), 101.68 (C3), 54.78 (CH_2), 49.57 (CH_2), 45.85 (NCH_3); Mass (EI) *m/z*: 307(M^+, 4), 283 (15), 169 (94), 112 (26), 111 (46), 71 (100); Anal. Calcd for $C_{15}H_{15}N_2O_3Cl$: C, 58.73; H, 4.93; N, 9.13. Found: C, 58.82; H, 5.01; N, 9.36.
*6-Fluoro-2,7-di-piperidin-1-yl-3-formylchromone (**17**)*. Yellow solid, mp 175–176 °C (chloroform–hexane 1:2); ν_{max} (KBr): 1674, 1627, 1558, 1407, 1365, 1131. ^1H NMR ($CDCl_3$, 200 MHz): δ 10.05 (s, 1H, CHO), 7.89 (d, 1H, $^2J_{H,F}$ = 8.26 Hz, C5-H), 7.38 (d, 1H, $^3J_{H,F}$ = 5.50 Hz, C8-H), 3.61 (br, 8H, 4× CH_2), 1.78 (br, 12H, 6× CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz): δ

186.26 (CHO), 177.29 (C4), 162.34 (C2), 156.99 (C6), 148.61 (C8a), 126.46 (C7), 122.84 (C4a), 118.99 (C5), 112.69 (C8), 101.22 (C3), 51.06 (CH_2), 50.94 (CH_2), 26.10 (CH_2), 25.76 (CH_2), 24.46 (CH_2), 23.59 (CH_2). MS (ESI) *m/z*: 359 ($M+H^+$); Anal. Calcd for $C_{20}H_{23}N_2O_3F$: C, 67.02; H, 6.47; N, 7.82. Found: C, 66.89; H, 6.34; N, 7.68.

*6-Fluoro-2,7-di-morpholin-4-yl-3-formylchromone (**18**)*. Pale-yellow solid, mp 178–179 °C (chloroform–hexane 1:2); ν_{max} (KBr): 1676, 1639,1616, 1548, 1440, 1407, 1398. ^1H NMR ($CDCl_3$, 200 MHz): δ 10.09 (s, 1H, CHO), 7.90 (d, 1H, $^2J_{H,F}$ = 8.20 Hz, C5-H), 7.39 (d, 1H, $^3J_{H,F}$ = 5.50 Hz, C8-H), 3.90 (br, 4× CH_2), 3.68 (br, 4× CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 186.44 (CHO), 177.07 (C4), 162.49 (C2), 155.50 (C6), 148.55 (C8a), 127.29 (C7), 122.80 (C4a), 118.93 (C5), 112.60 (C8), 101.28 (C3), 66.50 and 65.89 (O*C*H_2), 51.15 and 49.89 (N-*C*H_2). MS (ESI) *m/z* : 363 ($M+H^+$): Anal. Calcd for $C_{18}H_{19}N_2O_5F$: C, 59.66; H, 5.29; N, 7.73. Found: C, 59.52; H, 5.32; N, 7.59.

*6-Fluoro-2,7-bis-(N-methylpiperazin-1-yl)-3-formylchromone (**19**)*. Yellow solid, mp 196–197 °C (chloroform–hexane 1:2); ν_{max} (KBr): 1668, 1635, 1618, 1434, 1406, 1363, 1315, 1290. ^1H NMR ($CDCl_3$, 200 MHz): δ 10.09 (s, 1H, CHO), 7.88 (d, 1H, J = 8.30 Hz, C5-H), 7.39 (d, 1H, J = 5.38 Hz, C8-H), 3.99 (br, 4H), 3.69 (br, 4H), 3.05 (br, 4H), 2.60 (br, 4H), 2.34 and 2.32 (singlets, 6H, 2× CH_3), ^{13}C NMR ($CDCl_3$, 75 MHz): δ 186.36 (CHO), 117.12 (C4), 162.36 (C2), 155.05 (C6), 148.55 (C8a), 126.98 (C7), 122.78 (C4a), 118.90 (C5), 112.67 (C8), 101.24 (C3), 54.53 (CH_2), 52.93 (CH_2), 49.43 (CH_2), 45.76 (N-CH_3), 45.61 (N-CH_3), 43.65 (CH_2). MS (ESI) *m/z*: 389 ($M+H^+$); Anal. Calcd for $C_{20}H_{25}N_4O_3F$: C, 61.84; H, 6.49; N, 14.42. Found: C, 61.69; H, 6.38; N, 14.27.

20. EAC was obtained from Radiobiology Department, Kasturba Medical College, Manipal, India, and was propagated by serial transplantation in Swiss Albino mice in Central Animal Facility at Manipal Academy of Higher Education, Manipal, India.
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22. (a) Cisplatin was used as standard at 3.5 mg/kg.; (b) Percent increase in life span (% ILS) is calculated[21a] as % ILS = Median Survival Time (MST) ÷ MST of Control group × 100.; (c) Glutathione level has been determined by literature protocol [(a) Lowry, O. H.; Roesebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265. (b) Moron, M. S.; Depierre, J. W.; Mannervik, B. *Biochim. Biophys. Acta* **1979**, *5820*, 62].; (d) One-way analysis of variance (ANOVA) with Dunnet's test was applied to all data.
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