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# Naphthofuroquinone derivatives: Inhibition of receptor tyrosine kinases

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**Abstract**—A series of dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione derivatives were synthesized and evaluated for inhibitory activities against receptor tyrosine kinases. The naphthofuroquinone compounds with dialkylaminoethoxy group at C(5)-position (**7**, **8**, **10**, and **11**) manifested strong inhibitory activities against epidermal growth factor receptor and vascular endothelial growth factor receptor. Docking study of **11** with EGFR was also performed.


Tumor growth is dependent on multiple factors and particularly protein kinases are critical for the development of human cancer. Receptor tyrosine kinases (RTKs) play a key role in the physiological process of tumor angiogenesis and now have become the most promising cancer drug targets in recent several years.[1] RTK inhibitors have emerged as important therapeutic agents for cancer intervention.[2] However, selective inhibitors for a particular RTK may not always guarantee better pharmacological antitumor activities than broad-spectrum inhibitors since tumor cell growth is regulated by multiple growth factors and cytokines. Indeed, many RTK inhibitors in clinical studies have certain degree of cross reactivity.[3] Recent pharmacological result suggests that mutiplex RTK inhibitor strategy, which simultaneously targets multiple kinase pathways, may be required to be more effective in cancer treatment.[4]

The recent progress in crystal structures of protein kinases with various ATP-site directed inhibitors has made RTKs even more attractive targets for rational drug design. A number of drug discovery efforts have already yielded several potent and selective small-molecular inhibitors acting at the ATP-binding site, which derived from a number of different structural classes, preferentially including indolin-2-ones, phthalazines, and quinazolines.[5] In our efforts to discover novel small-molecule inhibitors with broad inhibitory activity across RTK families, we found naphthofuroquinone derivatives exhibiting potent inhibitory activities against epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), and platelet-derived growth factor receptor (PDGFR).

The naphthoquinone skeleton is found in many natural products and has been employed as a synthetic intermediate for the preparation of numerous heterocyclic compounds with interesting biological properties such as antitumor, antibacterial, antifungal, and antiinflammatory agents. The quinone core of streptonigrin and lavendamycin has been proposed to be a determining factor in their antitumor activity.[6] In this respect, it is interesting to note that the induced autophosphorylation of the tyrosine residues of kinase domain of EGFR was blocked by addition of naphthofuroquinone

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compounds. Here, we describe synthesis and RTK inhibition of novel naphthofuroquinone derivatives and a protein–ligand docking study.

The parent compound, dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione **3**[7], was easily synthesized by the base-promoted condensation of 2,3-dichloro-1,4-naphthoquinone with methyl 1,4-dihydroxy-2-naphthoate **2**, as shown in Scheme 1. Selective esterification of 1-naphthol **1** with diazomethane provided methyl naphthoate **2** in 86% yield, after recrystallization. The base-promoted condensation of 2,3-dichloro-1,4-naphthoquinone with **2** in the presence of $K_2CO_3$ in refluxing pyridine provided **3** in 64% yield.[7b,8] The known compounds **4**–**6** were prepared according to the literature[7c] and the other naphthofuroquinone derivatives **7**–**18** were readily synthesized by the O-alkylation of **3** with various halides (R–X, $K_2CO_3$, and DMF) in fair to good yield. Thus, starting from **3**, wide ranges of compounds with random alkyl or benzyl group at the C(5)-position, in principle, possessing a hydrophobic or hydrophilic character were prepared and the structures are presented in Table 1.

*In vitro kinase assay*: Kinase activity was determined using AlphaScreen™ phosphotyrosine (P-Tyr 100) assay kit (Perkin Elmer, Shelton, CT, USA). ALPHA (amplified luminescent proximity homogeneous assay) is a bead-based immunoassay monitoring the energy transfer from streptavidin-coated donor bead to anti-phosphotyrosine antibody conjugated acceptor bead, led by the binding of molecules. The kinase assay was performed according to the protocol provided by the manufacturer. The in vitro inhibitory activities of the compounds prepared were obtained as % inhibition at 20 μM against representing RTKs and are shown in Table 1.

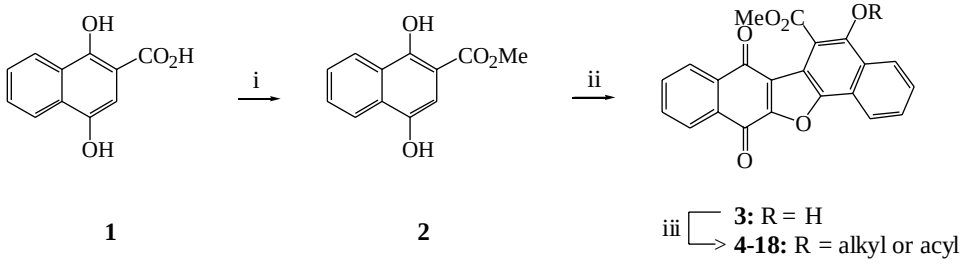
Table 1 lists naphthofuroquinone compounds **3**–**18** with random alkyl or benzyl groups at the C(5)-position, which still commonly possess a characteristic '2-phenylnaphthalene-type' structural framework in which the two rings are coplanar. All prepared compounds are potent inhibitors of protein kinase family, while the benzyl derivatives (compounds **15**–**18**) are slightly less potent than the derivatives with dialkylaminoethoxy or dialkylaminopropoxy group (compounds **7**–**14**). The $IC_{50}$ values for the selected compounds (compounds **7**–**11**) were determined against RTK family and are shown in Table 2. The amino-alcohol derivatives strongly inhibited the kinase activity showing $IC_{50}$ values in the range of 2–30 μM against all receptors. Moreover, the data present that none of these compounds are specific in kinase selectivity profile. In particular, compound**11** was comparable to tyrphostin A51 and the most active from this series, it shows $IC_{50}$ values of 2.8 and 4.6 μM for EGFR and VEGFR2, respectively. While a number of different structural classes are already known, it is noteworthy that a new class of naphthofuroquinone skeleton shows potent inhibitory activity across RTK families.

*Inhibition of EGF-induced autophosphorylation*:[9] The inhibitory effect on EGFR kinase was determined using cultured lung cancer cell line, A431. Cells were pretreated with test compound followed by the addition of 5 nM EGF (epidermal growth factor) for 10 min. Then Western blot experiments were performed to assess the total and phosphorylated forms of EGFR with the whole cell lysates obtained with SDS-sample buffer (6.25 mM Tris–HCl, 2% w/v SDS, 10% glycerol, 50 mM DTT, and 0.1% w/v bromophenol).

EGF-induced autophosphorylation was clearly diminished by addition of compounds **8** or **11** and the subsequent signal transduction of MAPK/ERK cascade was also blocked (Figs. 1A and B). The activity of compound **11** in cell-based assay was superior in blocking autophosphorylation of EGFR kinase domain, which was consistent with the result of in vitro enzyme assay. The inhibition of autophosphorylation was dose-dependently incremented until the concentration became 1 μM and did not further augment at higher concentrations. The phosphorylation of ERK1/2 and MEK1/2 was maximally suppressed at 1 μM treatment and rather intensified at higher concentrations.

*Inhibition of angiogenesis*: The Matrigel (10 mg protein/ml, Clontech, MA, USA) was pipetted into a 96-well culture plate and polymerized. HUVECs were harvested after trypsin–EDTA treatment, re-suspended in M199 and then plated onto Matrigel layer, and followed by the addition of compounds (10, 2, and 0.4 μM). After Matrigel cultures were incubated, the cultures were photographed. Each experiment was performed in duplicate and repeated more than twice.

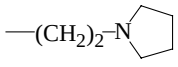
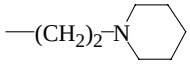
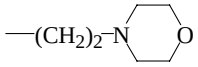
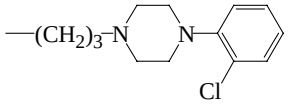
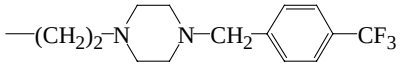
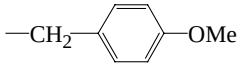
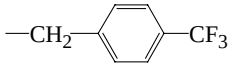
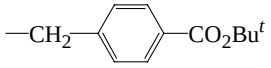
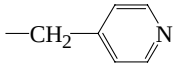
Tube formation assay (Fig. 2) showed moderate suppression of angiogenic activity. Inhibition of tube formation is moderately correlated with the inhibitory




**1** **2**

**Scheme 1.** Reagents and conditions: (i) $CH_2N_2$–$Et_2O$, 86%; (ii) 2,3-dichloro-1,4-naphthoquinone, pyridine, $K_2CO_3$, 90 °C, 64%; (iii) $Ac_2O$, pyridine, DMAP, 94% (for **6**); R–X (X = Cl or Br), $K_2CO_3$, DMF, 58–84% (for **4**–**5** and **7**–**18**).

**Table 1.** Structures and in vitro activity of dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione derivatives

| Compound | R | % inhibition (20 μM) | | | |
|---|---|---|---|---|---|
| | | EGFR | VEGFR2 | FGFR1 | PDGFRβ |
| **3** | –H | 21.4 | 16.0 | 12.6 | 2.8 |
| **4** | –Me | 16.7 | −0.8 | 10.5 | 12.7 |
| **5** | –Pr | 38.7 | −2.1 | 2.4 | 5.7 |
| **6** | –Ac | 21.6 | −25.6 | −8.1 | −0.3 |
| **7** | $-(CH_2)_2-NMe_2$ | 71.0 | 57.1 | 51.0 | 56.5 |
| **8** | $-(CH_2)_2-NEt_2$ | 82.1 | 60.4 | 61.1 | 64.1 |
| **9** | $-(CH_2)_3-NMe_2$ | 72.4 | 57.5 | 51.2 | 57.2 |
| **10** |  | 77.1 | 86.5 | 65.2 | 95.5 |
| **11** |  | 84.3 | 81.7 | 79.1 | 89.8 |
| **12** |  | 67.9 | 40.7 | 41.4 | 46.2 |
| **13** |  | 68.3 | 62.1 | 55.5 | 68.9 |
| **14** |  | 47.5 | 43.3 | 48.2 | 52.0 |
| **15** |  | 72.6 | 48.3 | 46.0 | 69.4 |
| **16** |  | 33.6 | 40.9 | 44.5 | 51.5 |
| **17** |  | 57.1 | 52.2 | 50.8 | 62.2 |
| **18** |  | 19.4 | 3.1 | 2.6 | 2.9 |
| Tyrphostin A51 | — | 90 | 88 | 93 | 82 |

potency against RTK enzyme. Compound **7** suppressed tube formation at the concentration of 2 μM. At 10 μM, compounds **7**, **8**, and **11** completely blocked the tube formation. However, we could not exclude that this result came from cell death rather than anti-angiogenic effect, such compounds exhibiting a dual kinase inhibition with cytotoxic activity.[10] The antiangiogenic activity was also tested by CAM (chorioallantoic membrane) assay. In CAM assay, only **8** (50 μg) inhibited blood vessel formation (data not shown). The compounds, **7** and **11**, did not block the vessel formation even at 50 μg treatment. Even though the compounds have similar

**Table 2.** $IC_{50}$ values for the selected compounds

| Compound | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| | EGFR | VEGFR2 | FGFR1 | PDGFRβ |
| **4** | 12.6 | nd[a] | >50 | >50 |
| **5** | >50 | nd[a] | >50 | >50 |
| **7** | 31 | 13.0 | 23.6 | 7.6 |
| **8** | 5.1 | 12.6 | 8.8 | 15.9 |
| **9** | 13.6 | 15.5 | 38.4 | 10.2 |
| **10** | 9.0 | 5.5 | 23.8 | 8.7 |
| **11** | 2.8 | 4.6 | 7.1 | 5.5 |
| Tyrphostin A51 | 4.1 | 4.3 | 4.9 | 2.8 |

[a] Not determined.

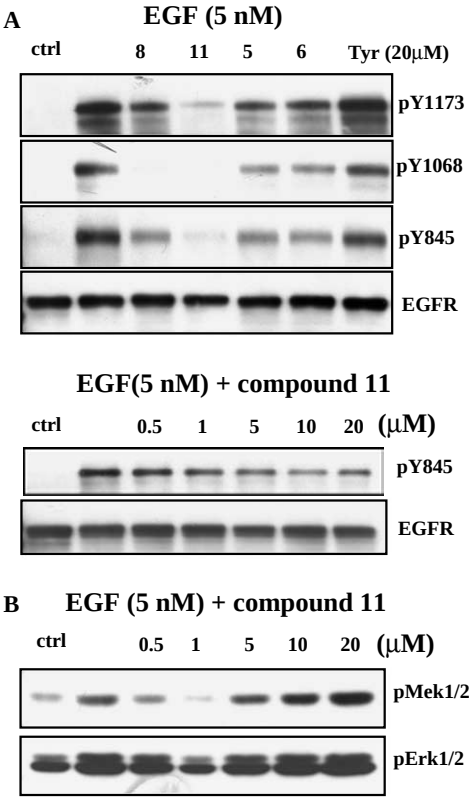



**Figure 1.** (A) Intracellular inhibition of autophosphorylation of EGFR by naphthofuroquinones. (B) Inhibition of phosphorylation of ERK and MEK (Ctrl means control and Tyr means tyrphostin).

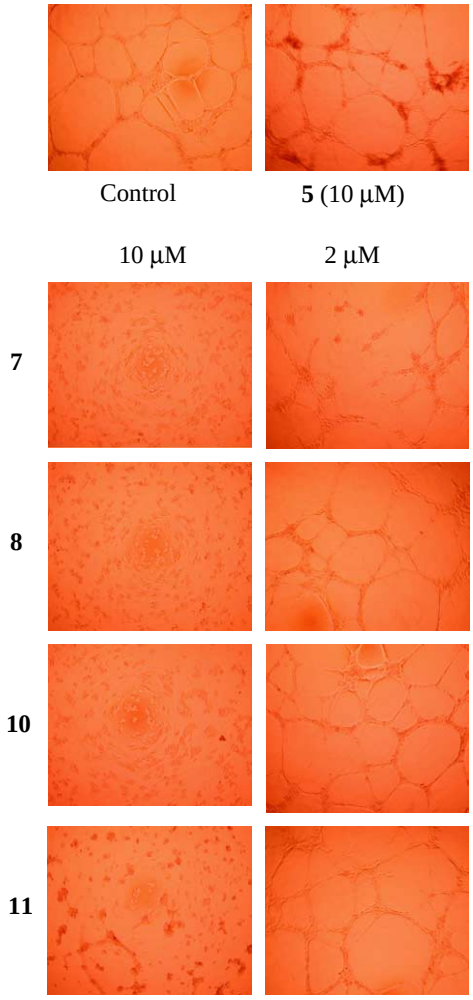



**Figure 2.** Inhibition of in vitro tube formation.

structural features, the reason for the difference in in vivo activity is not clear.

*Computer modeling studies*: The coordinates for the three-dimensional structure of EGFR were obtained from the RCSB Protein Data Bank (PDB entry code 1M17).[11] The 3D structures of naphthofuroquinone compounds were generated using SYBYL 6.9.2 (Tripos Associates). Compound **11** was nicely fitted into the binding pocket of EGFR using 1M17 coordinates (Fig. 3A). The docked complex by *FlexX* suite showed five or six potential hydrogen bonds between compound **11** and the active site (Fig. 3B). It shows that water11 might bridge two H-bonds between the nitrogen at C(5)-position of naphthofuroquinone and OG1 of Thr766/ O of Gln767. There is another H-bond between nitrogen at 5-position of compound **11** and OG1 of Thr830. Therefore, the nitrogen of dialkylaminoethoxy group appears to greatly contribute to the total binding affinity. According to the calculated drug score of virtual docking with *FlexX* module, several conformers of **11** with different torsion angles of side chains showed better fit to the topology of the binding pocket than that of 4-anilinoquinazoline (the original ligand in 1M17).

In summary, a series of dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione derivatives were synthesized and evaluated for inhibitory activities against receptor tyrosine kinases.[12–14] The naphthofuroquinone compounds with dialkylaminoethoxy group at the C(5)-position showed broad spectrum inhibitory activity across RTK family. It is interesting to note that naphthofuroquinone compounds have not been mentioned before in RTK inhibition. The anti-RTK activity was also confirmed by accessing cellular phosphorylation state and virtual docking model. Angiogenic assays showed that naphthoquinone derivatives have moderate antiangiogenic activity. These results clearly suggest that naphthofuroquinone compounds represent a promising new class of RTK inhibitors worthy of further studies.

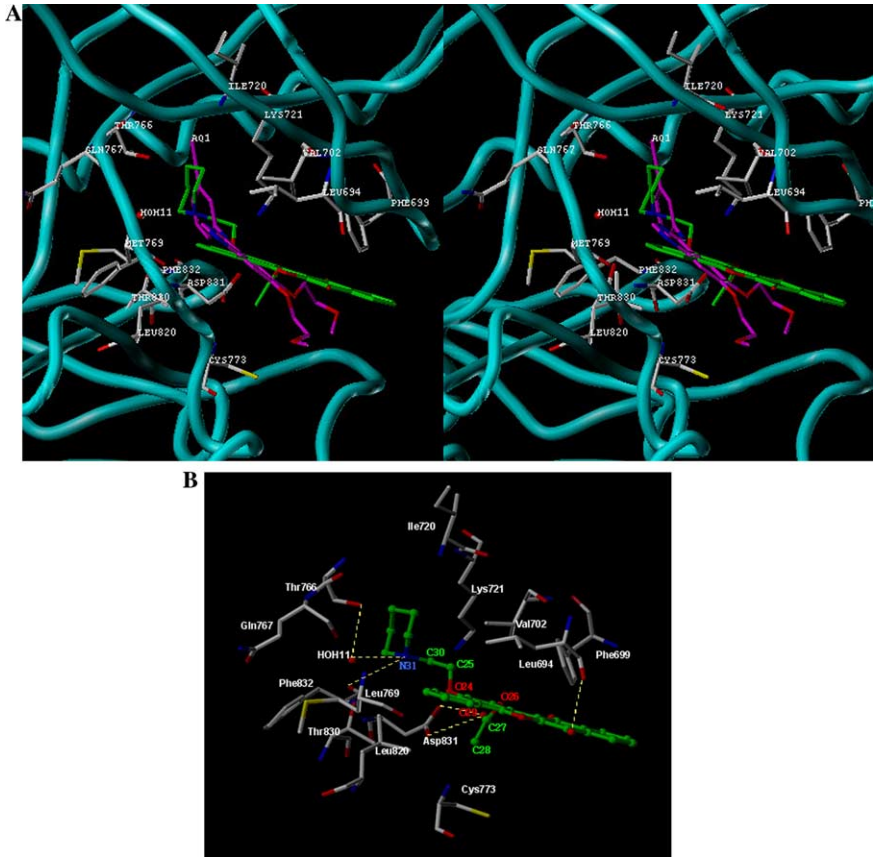



**Figure 3.** Docked model of compound **11** into EGFR. (A) Stereoview of docked model of compound **11** in the binding site and nearby residues from EGFR/4-anilinoquinazoline. The green molecule is **11** and the magenta depicts 4-anilinoquinazoline of crystal structure. (B) Presumable hydrogen bonds of docked model. Dashed line indicates potential hydrogen bonds between **11** and active site residues.


## Acknowledgment

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## References and notes


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10. The cytotoxicity ($IC_{50}$, μM) for the selected compounds against lung cancer cell line A431: **7** (1.1), **8** (1.7), **10** (1.1), and **11** (4.3).
11. Stamos, J.; Sliwkowski, M. X.; Eigenbrot, C. *J. Biol. Chem.* **2002**, *277*, 46265.
12. Synthesis of methyl 1,4-dihydroxy-2-naphthoate **2**. To a prepared ethereal solution of diazomethane (ca. 15–16 mmol, 25 mL $Et_2O$) was added **1** (2.04 g, 10 mmol). The reaction mixture was allowed to stir for 30 min and evaporated under reduced pressure to give the residue. The residue was recrystallized from ethyl acetate to give **2** (1.88 g, 86%) as a solid: mp 192–193.5 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.44 (s, 1H), 9.19 (s, 1H), 8.32 (d, 1H, $J$ = 8.1 Hz), 8.18 (d, 1H, $J$ = 8.1 Hz), 7.63–7.50 (m, 2H), 7.14 (s, 1H), 3.98 (s, 3H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 170.87, 153.04, 145.48, 129.37, 129.06, 126.75, 125.19, 123.54, 122.52, 105.08, 104.13, 52.90; EIMS (70 eV) *m/z* (rel int) 218 (40, $M^+$), 186 (100), 158 (14), 130 (63), 102 (79), 76 (24), 66 (9), 53 (17); Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 65.72; H, 4.78.
13. Synthesis of dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione **3**. A mixture of 2,3-dichloro-1,4-naphthoquinone (1.04 g, 4.6 mmol), **2** (1.5 g, 6.9 mmol), and $K_2CO_3$ (7.1 g,

51 mmol) in pyridine (50 mL) was heated to 90 °C for overnight. The reaction mixture was poured into ice water. The precipitated solid was filtered and washed with $H_2O$, and then recrystallized from chloroform to give **3** (1.11 g, 64%) as a solid: mp 248–250 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.45 (s, 1H), 8.41 (d, 2H, $J$ = 8.1 Hz), 8.17–8.11 (m, 2H), 7.94–7.89 (m, 2H), 7.86–7.78 (m, 2H); EIMS (70 eV) *m*/*z* (rel int) 372 (13, $M^+$), 340 (100), 256 (12), 228 (22), 200 (33), 156 (6), 100 (13), 76 (18), 40 (17); Anal. Calcd for $C_{22}H_{12}O_6$: C, 70.97; H, 3.25. Found: C, 70.09; H, 3.13.

14. General procedure for the synthesis of 5-(2-diethylamino-ethoxy)-7,12-dioxo-7,12-dihydrodinaphtho[1,2-*b*;2′,3′-*d*]-furan-6-carboxylic acid methyl ester (**8**). To a solution of **3** (200 mg, 0.54 mmol) and 2-diethylaminoethyl chloride hydrochloride (275 mg, 1.6 mmol) in DMF (50 mL) was added $K_2CO_3$ (2.0 g, 14.5 mmol) and then stirred for 8 h at room temperature. The reaction mixture was poured into ice water. The precipitated solid was collected by filtration, washed with water, and dried to give **8** (213 mg, 84%) as a yellow soild: mp 151–152 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.53 (d, 1H, $J$ = 8.9 Hz), 8.43 (d, 1H, $J$ = 8.8 Hz), 8.28–8.21 (m, 2H), 7.79–7.75 (m, 4H), 4.27 (t, 2H, $J$ = 6.2 Hz), 4.19 (s, 3H), 3.01 (t, 2H, $J$ = 6.2 Hz), 2.69 (q, 4H, $J$ = 7.1, 14.3 Hz), 1.12 (t, 6H, $J$ = 7.1 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 179.7, 173.9, 166.2, 152.5, 151.2, 149.5, 133.5, 133.2, 132.6, 131.6, 128.2, 128.0, 127.8, 126.6, 126.2, 124.5, 123.8, 121.5, 121.0, 117.8, 115.2, 74.7, 52.3, 52.2, 47.0, 11.3; EIMS (70 eV) *m*/*z* (rel int) 471 (6, $M^+$), 456 (17), 372 (5), 340 (27), 228 (7), 200 (9), 99 (40), 86 (100), 58 (8).