Development of 2-Thioxoquinazoline-4-one Derivatives as Dual and Selective Inhibitors of Dynamin-Related Protein 1 (Drp1) and Puromycin-Sensitive Aminopeptidase (PSA)

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An established inhibitor of dynamin-related protein 1 (Drp1), 3-(2,4-dichloro-5-methoxyphenyl)-2-thioxoquinazoline-4-one (mdivi-1), was recently reported also to show potent puromycin-sensitive aminopeptidase (PSA)-inhibitory activity. Herein, we report structural development of mdivi-1 derivatives and structure–activity relationship (SAR) analysis of the synthesized compounds, as well as the structurally related PSA-specific inhibitor 3-(2,6-diethylphenyl)quinazoline-2,4-dione (PAQ-22), with the aim of identifying key structural features for inhibitory activity in order to develop selective inhibitors of Drp1, which is a potential target for treatment of Huntington’s disease. Among the synthesized compounds, 3-(4-chloro-3-methoxyphenyl)-2-thioxoquinazoline-4-one (10g) exhibited more potent Drp1-inhibitory activity than mdivi-1 with high selectivity for Drp1 over PSA.

Key words dynamin-related protein 1; Drp1; puromycin-sensitive aminopeptidase; 2-thioxoquinazoline-4-one

Dynamin related protein 1 (Drp1) is a member of the guanosine triphosphate hydrolase (GTPase) family. It is located mainly in cytosol, but upon activation it is recruited to the mitochondrial surface, where it oligomerizes and bind to mitochondrial adaptors such as Fis1 and Mff to assemble a fission site, at which mitochondrial membrane fission occurs in a GTP hydrolysis-dependent manner. Recently, Drp1 has been identified as a potential target for treatment of Huntington’s disease (HD). HD is an inherited neurodegenerative disorder caused by abnormal polyglutamine (polyQ) expansion in huntingtin exon 1. PolyQ length determines disease onset and severity, with a longer expansion causing earlier onset. Treatment of HD remains a challenge. It is reported that mutant huntingtin interacts with Drp1 and stimulates its enzymatic activity, leading to an increase of mitochondrial fragmentation and enhanced cell death. These defects have been reported to be rescued by reducing Drp1 GTPase activity with dominant-negative mutant Drp1K38A. These defects suggest that Drp1 inhibitors are promising agents for treatment of HD to ameliorate neuronal cell death caused by mutant huntingtin.

To develop Drp1 inhibitors, we selected the 3-phenyl-2-thioxoquinazoline-4-one derivative mdivi-1 (Fig. 1, left), a known Drp1 inhibitor, as a lead compound. On the other hand, we previously developed PAQ-22 (Fig. 1, right), which has 3-phenylquinazoline-2,4-dione structure, as an inhibitor of puromycin-sensitive aminopeptidase (PSA).

Since mdivi-1 is structurally closely related to PAQ-22, we anticipated that mdivi-1 might also inhibit PSA. PSA is a single-chain protein of 99 kDa that hydrolyzes N-terminal amino acids with a preference for basic and hydrophobic residues. This enzyme is an exopeptidase containing Zn²⁺ ion at its catalytic site. It was reported that PSA hydrolyzes polyQ chain and that PSA overexpression reduces polyQ aggregate formation as well as the toxicity of mutant huntingtin exon 1. These facts suggested that the PSA-inhibitory activity of mdivi-1 would be inappropriate in the treatment of HD. Indeed, we found that mdivi-1 strongly inhibits not only Drp1, but also PSA.

On the basis of these considerations, we set out to investigate the structure–activity relationship (SAR) of mdivi-1 derivatives and to conduct structural development studies targeting Drp1-selective inhibitors. In this paper, we describe the design, synthesis, and biological evaluation of a series of 2-thioxoquinazoline-4-one derivatives as Drp1-selective inhibitors.

Structural Development and SAR Studies Mdivi-1 and its o xo-analog (4) were synthesized as shown in Chart 1. 2,4-Dichloro-5-aminoanisole (I) was amidated with 2-nitrobenzoyl chloride to afford 2. The nitro group of 2 was reduced to an amino group by using H₂ in the presence of Pd/C. Annulation of 3 using CS₂ gave 4. Annulation of 5 using triphosgene gave 6. On the other hand, its ring-opened derivatives (1 and 2) all lacked Drp1-inhibitory activity (Table 1). A structurally related PSA inhibitor, PAQ-22, also did not inhibit Drp1. This indicates that the 2-thioxo-quinazoline-
4-one ring is essential for Drp1-inhibitory activity of mdivi-1. Therefore, we next examined the effects of substituents on the 3-phenyl ring of 2-thioxoquinazoline-4-one.

3-Substituted 2-thioxoquinazoline-4-ones were prepared as shown in Chart 2. Briefly, substituted aniline and methyl 2-isothiocyanatobenzoate were condensed in 1,4-dioxane to afford compounds 10a, and 10b (path a in Chart 2). Compounds containing chlorine, i.e., 10c–h, were prepared by annihilation reaction of the corresponding anilines 9c–h with CS₂ (path d in Chart 2).

Table 2 summarized the activities of the synthesized compounds. Among them, 10g and 10f showed Drp1-inhibitory activity, and 10g was about 3 times more potent than mdivi-1. Other compounds (10a–e, 10h) had no Drp1-inhibitory activity. Next, we investigated the PSA-inhibitory activity of these compounds, using the reported method. Briefly, PSA-inhibitory activities were assessed by measuring 7-amino-4-methylcoumaryl-7-amide (Ala-MCA) using intact human acute lymphoblastic leukemia MOLT-4 cells. As we have reported, mdivi-1 exhibited strong PSA-inhibitory activity. On the other hand, 10f showed only moderate PSA-inhibitory activity, and 10g exhibited little activity (inhibitory potency of only 46% at 100 µM). Compounds without an R₁ substituent, i.e., 10a, 10b, 10c, 10g and 10h, did not exhibit PSA-inhibitory activity, and this result is consistent with our previous finding that ortho-substitution of the N-phenyl ring is important for PSA-inhibitory activity.⁵ Among the compounds listed in Table 2, 10g exhibited the most potent Drp1-inhibitory activity and showed high selectivity for Drp1 over PSA.

To optimize the para-substituent of the 3-phenyl ring of compound 10g, various substituents, including halogen, methyl, methoxy, nitrile, and amide were introduced at the para-position; these compounds (19a–d) were synthesized by a similar method to that used for preparation of 10a, b (Chart 3). Compounds 19e–g bearing a halogen atom were synthesized in the same manner as 10e, f, h (Chart 4).

The Drp1- and PSA-inhibitory activities of compounds 19a–g are summarized in Table 3. The Drp1-inhibitory activity of 19f was comparable to that of 10g. All compounds other than 19e and 19f lacked Drp1-inhibitory activity (Table 3). These results indicate that Cl or Br at the para-position in the phenyl group is important for potent Drp1-inhibitory activity. The lack of Drp1-inhibitory activity of 19g might be attributed to the large size of the iodine atom. The PSA-inhibitory activity of 19a–g was weaker than that of mdivi-1 (Table 3).

Next, we optimized the alkoxy group of 10g. Compounds bearing C₂–C₄ alkyl groups (24a–d) were synthesized by a similar method to that used for preparation of 10e, f, h, as shown in Chart 5.

The Drp1- and PSA-inhibitory activities of 24a–d are summarized in Table 4. Ethyl derivative 24a and n-propyl derivative 24b showed Drp1-inhibitory activity comparable to that of mdivi-1, but lower than that of methoxy derivative 10g. On the other hand, 24c and 24d bearing longer alkyl groups lacked Drp1-inhibitory activity. These results suggest that the methoxy group is the most effective for potent Drp1-inhibitory activity with high selectivity over PSA.

Conclusion

Structural development of the known Drp1 inhibitor mdivi-1 afforded 10g, which has a more potent Drp1-inhibitory activity than mdivi-1, together with high selectivity for Drp1 over PSA. Since our previous work showed that a high rotational barrier of the 3-phenyl ring was necessary for PSA-inhibitory activity, the weak PSA-inhibitory activity of 10g may be due to the low rotational barrier of the 3-phenyl group, as evaluated by density functional theory (DFT) calculation. A Cl or Br atom at the p-position of the 3-phenyl group is essential for Drp1-inhibitory activity, and a moderately long alkyl chain in the substituent on the O atom is also important. The structure–activity relationship of mdivi-1 derivatives is summarized in Fig. 2.

Compound 10g should be a promising lead compound for development of highly selective Drp1 inhibitors to treat Huntington’s disease.
Experimental Chemistry

Reagents and conditions: (a) Methyl 2-isothiocyanatobenzoate, Et,N, 1,4-dioxane, r.t., 76–91%; (b) 2-Nitrobenzoyl chloride, K₂CO₃, TBAHS, DCM, H₂O, r.t., 27%-q.y.; (c) 1. SOCl₂, THF, r.t. 2. Substituted aniline, pyridine, THF, r.t., 77%; (d) CS₂, DBU, DMF, 40°C, 34–60%, from 6 to 10d (34% in 2 steps) (e) SnCl₂·2H₂O, solvent, 36–65%; (f) 2-α-Butyloxycarbonylamino-1benzeneacetic acid, HOBT, EDCI, DIEA, DMF, r.t.; (g) TFA, DCM, r.t., 44% from 5g in 2 steps.

Table 2. Drp1-Inhibitory Activity of Mdivi-1 and Compounds 10a–h

| Compound | R¹ | R² | R³ | Drp1 IC₅₀ (µM) | PSA IC₅₀ (µM) |
|----------|----|----|----|----------------|--------------|
| Mdivi-1  | Cl | Cl | OMe| 13             | 0.71         |
| 10a      | H  | H  | H  | N.A.          | >100         |
| 10b      | H  | H  | OMe| 30            |              |
| 10c      | Cl | H  | H  | N.A.          | 44           |
| 10d      | Cl | Cl | H  | N.A.          | 45           |
| 10e      | Cl | H  | OMe| N.A.          | >30          |
| 10f      | Cl | H  | OMe| 9.6          | 31           |
| 10g      | Cl | Cl | OMe| 4.7          | >100         |
| 10h      | H  | OMe| Cl | N.A.          | >100         |

a) N.A. means no activity at 100 µM.
136.29, 136.15, 129.76, 127.48, 124.73, 123.41, 121.93, 115.88, 115.56, 115.22, 56.77. FAB-MS m/z 353 (M\(^+\)), 355, 357. High resolution (HR)-MS (FAB) Calcd for C\(_{15}\)H\(_{10}\)Cl\(_2\)N\(_2\)O\(_2\)S 351.9840, Found 351.9850.

Triphosgene (30.0 mg, 0.101 mmol) in 1,2-dichloroethane (10 mL) was dropped into a solution of 3 (82.6 mg, 0.265 mmol) and Et\(_3\)N (50.0 \(\mu\)L, 0.324 mmol) in 1,2-dichloroethane (5.0 mL) and the mixture was stirred at r.t. overnight. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (n-hexane:AcOEt = 3 : 1 to 1 : 1) to afford the title compound (73.9 mg, 0.219 mmol, 83%) as a white solid. mp 277–278°C. 1H-NMR (500 MHz, CDCl\(_3\)) \(\delta\): 8.84 (1H, s), 8.18 (1H, d, \(J = 7.3\) Hz), 7.68–7.65 (1H, m), 7.59 (1H, s), 7.31–7.28 (1H, m), 7.05 (1H, d, \(J = 8.0\) Hz), 6.92 (1H, s), 3.90 (3H, s). FAB-MS m/z 337 (M\(^+\)), 339.

Anal. Calcd for C\(_{15}\)H\(_{10}\)Cl\(_2\)N\(_2\)O\(_3\)·1/9H\(_2\)O: C, 53.12; H, 3.04; N, 8.26. Found: C, 53.41; H, 3.34; N, 8.14.

3-Phenyl-2-thioxoquinazoline-4-one (10a) To a solution of methyl 2-isothiocyanatobenzoate (46.7 mg, 0.242 mmol) and aniline (22.1 \(\mu\)L, 0.242 mmol) in 1,4-dioxane (2.0 mL) was added Et\(_3\)N (37.4 \(\mu\)L, 0.242 mmol). The mixture was stirred at r.t. for 2 h, and the precipitated white solid was collected by filtration, washed with n-hexane, and recrystallized from CHCl\(_3\) and n-hexane to afford the title compound (47.0 mg, 0.185 mmol, 76%) as a white solid. mp > 300°C. 1H-NMR (500 MHz, DMSO-\(d_6\)) \(\delta\): 7.94 (1H, dd, \(J = 1.4, 7.7\) Hz), 7.79–7.76 (1H, m), 7.48–7.43 (3H, m), 7.41–7.38 (1H, m), 7.36–7.33 (1H, m), 7.28–7.25 (2H, m). 13C-NMR (125 MHz, DMSO-\(d_6\)) \(\delta\): 176.05, 159.81, 139.40, 139.30, 135.61, 128.99, 128.10, 127.41, 124.35, 116.20, 115.69, 115.62. FAB-MS m/z 255 (M\(^+\)). Anal. Calcd for C\(_{14}\)H\(_{10}\)N\(_2\)OS·0.6H\(_2\)O: C, 63.42; H, 4.26, N, 10.57. Found: C, 63.34; H, 4.38; N, 10.36.
3-(3-Methoxyphenyl)-2-thioxoquinazoline-4-one (10b) This compound was prepared from 3-methoxyaniline by a similar method to that described for preparation of 10a. Yellow solid (76.7 mg, 0.270 mmol, 91%) (recrystallized from CHCl3). 1H-NMR (500 MHz, CDCl3) δ: 7.92 (1H, s), 8.17 (1H, d, J = 8.0 Hz), 7.72–7.68 (1H, m), 7.47–7.44 (1H, m), 7.36–7.33 (1H, m), 7.11 (1H, d, J = 7.0 Hz), 7.04–7.02 (1H, m), 6.88–6.87 (1H, m), 6.81 (1H, s), 3.83 (3H, s). FAB-MS m/z 285 (MH+). Anal. Calc’d for C16H13N2O3S: C, 59.86; H, 4.82; N, 9.07. Found: C, 59.86; H, 4.82; N, 9.07.

N-(4-Chlorophenyl)-2-thienobenzamide (7e) To a mixture of 4-chloroaniline (259 mg, 2.03 mmol), K2CO3 (299 mg, 2.17 mmol), and TBAHS (29.1 mg, 0.0857 mmol) in DCM:H2O = 2:1, was added 2-nitrobenzoyl chloride (290 µL, 2.20 mmol) at 0°C. The reaction mixture was stirred for 2 h at r.t., then CHCl3 and MeOH were added. The organic layer was washed with water and brine, dried and concentrated to give the title compound (165 mg, 0.596 mmol, 27%) as a pale yellow solid. 1H-NMR (500 MHz, CDCl3) δ: 8.12 (1H, d, J = 8.5 Hz), 7.74–7.71 (1H, m), 7.64–7.62 (2H, m), 7.53 (2H, d, J = 8.5 Hz), 7.45 (1H, brs), 7.33 (1H, d, J = 8.5 Hz).

2-Amino-N-(4-chlorophenyl)benzamide (9e) To a solution of 7e (165 mg, 0.596 mmol) in DMF (4.0 mL) was added SnCl4·2H2O (417 mg, 1.99 mmol). The mixture was stirred at 90°C for 4 h, then NaHCO3 aq. was added and the whole was filtered through a pad of Celite. The filtrate was diluted with AcOEt and washed with water and brine. The organic layer was dried over MgSO4 and concentrated. The residue was purified by silica gel column chromatography (n-hexane:AcOEt=3:1) to afford the title compound (52.3 mg, 0.212 mmol, 36%) as a yellow solid. 1H-NMR (500 MHz, CDCl3) δ: 7.71 (1H, brs), 7.51 (2H, d, J = 8.5 Hz), 7.43 (1H, dd, J = 8.3, 1.8 Hz), 7.31 (2H, d, J = 8.5 Hz), 7.26–7.23 (2H, m), 6.72–6.69 (2H, m), 5.48 (2H, br s).

3-(4-Chlorophenyl)-2-thioxoquinazoline-4-one (10e) This compound was prepared from 9e by a method similar to that used for preparation of mdivi-1. Yellow solid (24.9 mg, 0.0862 mmol, 34%) (recrystallized from CHCl3/MeOH). mp = 300°C. 1H-NMR (500 MHz, CDCl3) δ: 8.06 (1H, dd, J = 8.0, 1.0 Hz), 7.66–7.62 (1H, m), 7.44–7.42 (2H, m), 7.28–7.25 (1H, m), 7.18 (1H, d, J = 8.0 Hz), 7.15–7.12 (2H, m). FAB-MS m/z 289 (MH+). HR-MS(FAB, MH+) Calc’d for C16H10ClN2O3S: 289.0202, Found: 289.0230.

N-(3-Chloro-4-methoxyphenyl)-2-nitrobenzamide (7h) This compound was prepared from 3-chloro-4-methoxyaniline by a method similar to that used for preparation of 7e. Yellow solid (952 mg, 3.03 mmol, quant.). 1H-NMR (500 MHz, CDCl3) δ: 8.08 (1H, d, J = 8.0 Hz), 7.70–7.67 (1H, m), 7.59–7.57 (3H, m), 7.50 (1H, dd, J = 2.5, 8.5 Hz), 6.88 (1H, d, J = 9.5 Hz), 3.85 (3H, s).

2-Amino-N-(3-chloro-4-methoxyphenyl)benzamide (9h) This compound was prepared from 7h by a method similar to that used for preparation of 9e. Pale yellow solid (61.5 mg, 0.222 mmol, 65%). 1H-NMR (500 MHz, CDCl3) δ: 7.63 (1H, d, J = 2.0 Hz), 7.62 (1H, brs), 7.42 (1H, d, J = 7.5 Hz), 7.40 (1H, dd, J = 2.5, 9.0 Hz), 7.26–7.22 (1H, m), 6.91 (1H, d, J = 8.5 Hz), 6.71–6.68 (1H, m), 5.49 (2H, brs), 3.89 (3H, s).

3-(3-Chloro-4-methoxyphenyl)-2-thioxoquinazoline-4-one (10h) This compound was prepared from 9h by a method similar to that used for preparation of mdivi-1. Yellow solid (74.8 mg, 0.235 mmol, 60%) (recrystallized from CHCl3/MeOH). mp = 300°C. 1H-NMR (500 MHz, DMSO-d6) δ: 7.94 (1H, dd, J = 1.2, 8.0 Hz), 7.79–7.76 (1H, m), 7.44–7.43 (2H, m).
7.35–7.32 (1H, m), 7.24 (1H, dd, J=2.0, 8.9 Hz), 7.22 (1H, d, J=8.6 Hz), 3.91 (3H, s). 13C-NMR (125 MHz, DMSO-d6) δ: 176.23, 159.91, 154.20, 139.54, 135.60, 132.22, 130.40, 129.06, 127.42, 124.36, 126.62, 116.23, 115.68, 112.55, 56.27. FAB-MS m/z 319 (M+H) +. HR-MS (FAB) Calcd for C15H11ClN2O2S: C, 51.39; H, 2.80; N, 8.51. Found: C, 51.39; H, 2.80; N, 8.51.

N-(2-Chloro-5-methoxyphenyl)-2-nitrobenzamide (7f) This compound was prepared from 2-chloro-5-methoxyaniline hydrochloride by a method similar to that used for preparation of 7e. Yellow solid (288 mg, 0.940 mmol, 88%). 1H-NMR (500 MHz, CDCl3) δ: 8.15–8.13 (2H, m), 7.76–7.75 (1H, m), 7.69–7.67 (2H, m), 7.28 (1H, d, J=8.6 Hz), 6.69 (1H, dd, J=3.1, 8.6 Hz), 3.85 (3H, s).

Amino-N-(2-chloro-5-methoxyphenyl)benzamide (9f) This compound was prepared from 9f by a method similar to that used for preparation of 9e. Yellow solid (130 mg, 0.470 mmol, 52%). 1H-NMR (500 MHz, CDCl3) δ: 8.35 (3H, s), 8.18 (1H, d, J=2.7 Hz), 7.53 (1H, d, J=8.0 Hz), 7.29–7.27 (1H, m), 6.75–6.73 (2H, m), 7.16 (1H, dd, J=2.7, 9.0 Hz), 5.59 (brs, 2H), 3.84 (3H, s).

3-(2-Chloro-5-methoxyphenyl)-2-thioxoquinazoline-4-one (10f) This compound was prepared from 9f by a method similar to that used for preparation of mdivi-1. Yellow solid (111 mg, 0.349 mmol, 95%) (recrystallized from 1.4-dioxane/DMF/H2O). mp 232–234°C. 1H-NMR (500 MHz, CDCl3) δ: 9.88 (1H, brs), 8.18 (1H, d, J=7.9 Hz), 7.72–7.69 (1H, m), 7.47 (1H, d, J=9.2 Hz), 7.37–7.34 (1H, m), 7.13 (1H, d, J=7.9 Hz), 6.99 (1H, dd, J=3.1, 9.2 Hz), 6.88 (1H, d, J=2.5 Hz), 3.82 (3H, s). FAB-MS m/z 319 (M+H) +. Anal. Calcd for C9H5ClIN5O2S·0.25H2O: C, 35.73; H, 3.59; N, 8.67. Found: C, 55.84; H, 3.48; N, 8.64.

Amino-N-(2-chlorophenyl)benzamide (9e) To a solution of anthranilic acid (147 mg, 1.07 mmol) in Et2O (5.0 mL) was added SOCl2 (2.0 mL). The mixture was stirred at r.t. for 1 h, and then 2-chloroaniline (255 mg, 2.00 mmol) in pyridine was added SOCl2 (2.0 mL). The mixture was stirred at r.t. for 6.02 mmol. The reaction mixture was stirred for 9 h at r.t. overnight and then diluted with AcOEt. The organic layer was washed with water and brine, dried, and concentrated to afford the title compound as a pale yellow paste, which was used for the next step without further purification.

Amino-N-(4-chloro-3-methoxyphenyl)benzamide (9g) To a solution of 8 (96.0 mg, crude) in DCM (2.0 mL) was slowly added TFA (250 µL) at r.t. The mixture was stirred for 4.5 h, and then NaHCO3aq. and AcOEt were added to it. The organic layer was washed with water and brine, dried, and concentrated to afford the title compound as a yellow paste (61.5 mg, 0.222 mmol, 87%). This compound was used for the next step without further purification.

3-(4-Chloro-3-methoxyphenyl)-2-thioxoquinazoline-4-one (10g) This compound was prepared from 9g by a method similar to that used for preparation of mdivi-1. Yellow solid (41.4 mg, 0.130 mmol, 59%) (recrystallized from DMF/H2O). mp >300°C. 1H-NMR (500 MHz, CDCl3) δ: 7.75 (1H, brs), 7.57 (1H, d, J=1.9 Hz), 7.46 (1H, d, J=7.9 Hz), 7.32–7.31 (2H, m), 6.90 (1H, dd, J=1.9, 8.6 Hz), 6.74–6.71 (1H, m), 5.49 (2H, brs), 3.95 (3H, s).

3-(4-Chloro-3-methoxyphenyl)-2-thioxoquinazoline-4-one (10d) This compound was prepared from 9d by a method similar to that used for preparation of mdivi-1. Yellow solid (115 mg, 0.485 mmol) in DMF (2.0 mL) was added a solution of 4-chloro-3-methoxyaniline (82.2 mg, 0.522 mmol), HOBt (70.0 mg, 0.518 mmol), EDCI–HCl (104 mg, 0.542 mmol) and DIEA (90.0 µL, 0.522 mmol) in DMF (1.0 mL). The resulting mixture was stirred at r.t. overnight. After completion of the reaction, AcOEt was added. The organic layer was washed with water and brine, dried, and concentrated to give the title compound in a pale yellow paste, which was used for the next step without further purification.
and then diluted with AcOEt. The organic layer was washed with water and brine, dried over MgSO₄ and concentrated to give compound 13e as a dark yellow solid (251 mg, 1.50 mmol, 74%), which was used for the next step without further purification. ¹H-NMR (500 MHz, CDCl₃) δ: 7.46 (1H, dd, J=2.0, 8.0Hz), 7.64 (1H, d, J=2.0Hz), 7.23 (1H, d, J=8.0Hz), 3.90 (3H, s), 2.28 (3H, s).

4-Amino-2-methoxy-N-methylbenzamide (14a) Compound 13a (221 mg, 1.05 mmol) was dissolved in MeOH (8.0 mL) and hydrogenated (1 bar H₂) over 10% palladium on charcoal for 5.5 h at r.t. The mixture was filtered through a pad of Celite and the filtrate was concentrated. The residue was recrystallized from DCM and n-hexane to afford the title compound (18.9 mg, 0.0818 mmol, 16%) as a white solid (recrystallized from CHCl₃/MeOH/n-hexane). mp 292–293°C. ¹H-NMR (500 MHz, CDCl₃) δ: 8.02 (1H, d, J=8.6 Hz), 7.63 (1H, brs), 6.32 (1H, dd, J=2.3, 8.6 Hz), 6.18 (1H, d, J=2.3Hz), 4.03 (2H, d, J=4.6Hz), 3.88 (3H, s), 2.95 (3H, d, J=4.6Hz).

4-Amino-2-methoxybenzonitrile (14b) This compound was prepared from 2-methoxy-4-nitrobenzonitrile by a method similar to that used for preparation of 14a. White solid (358 mg, 1.61 mmol, 80%) as a white solid. 1H-NMR (500 MHz, CDCl₃) δ: 7.26 (1H, d, J=8.6Hz), 7.23 (1H, s), 6.19 (1H, dd, J=2.3, 8.7 Hz), 6.12 (1H, d, J=2.3Hz), 4.11 (2H, brs), 3.82 (3H, s).

3-Methoxy-4-methylaniline (14c) This compound was prepared from 13c by a method similar to that used for preparation of 14a. Brown solid (195 mg, 1.42 mmol, 95%). ¹H-NMR (500 MHz, CDCl₃) δ: 8.67 (1H, d, J=7.5), 6.21–6.19 (2H, m), 3.90 (3H, s), 3.53 (2H, brs), 2.08 (3H, s), FAB-MS m/z 138 (MH⁺).

3-(3-Methoxy-4-methylanilino)-2-thioxoquinazoline-4-one (19a) To a solution of methyl 2-isothiocyanato-65.4 mg, 0.338 mmol) and 14a (62.4 mg, 0.346 mmol) in dioxane (2.0 mL) was added Et₂N (80 µL, mmol). The mixture was stirred at r.t. for 10h, and the precipitated white solid was collected by filtration and washed with n-hexane to give the title compound (18.9 mg, 0.0541 mmol, 16%) as a white solid (recrystallized from CHCl₃/MeOH/n-hexane). mp>300°C. ¹H-NMR (500 MHz, CDCl₃) δ: 8.24 (1H, d, J=8.6Hz), 8.05 (1H, dd, J=1.2, 8.0Hz), 7.86 (1H, d, J=4.6Hz), 7.65–7.61 (1H, m), 7.27–7.24 (1H, m), 7.20–7.17 (1H, m), 6.91 (1H, dd, J=1.7, 8.0Hz), 6.82 (1H, d, J=2.3Hz), 3.89 (3H, s), 2.94 (3H, d, J=4.6Hz), FAB-MS m/z 342 (MH⁺). Anal. Calcld for C₂₀H₁₇N₃O₂S·0.3H₂O: C, 58.88; H, 4.53; N, 13.20. Found: C, 58.81; H, 4.64; N, 11.96.

1-Bromo-2-methoxy-4-nitrobenzene (16f) H₂SO₄ (1.5 mL) was added slowly to a cold (0°C) stirred mixture of 2-methoxy-4-nitrobenzene (340 mg, 2.02 mmol) and NaNO₂ (186 mg, 2.69 mmol) in H₂O (4.0 mL). After the addition was completed, stirring was continued for an additional 30 min at 0°C. Then a solution of CuBr (345 mg, 2.40 mmol) in HBr (1.5 mL) was added slowly at 0°C. The whole was stirred at r.t. overnight and diluted with CHCl₃. The organic layer was washed with water and brine, dried and concentrated. Column chromatography (n-hexane:AcOEt=3:1) gave the title compound (358 mg, 1.62 mmol, 80%) as a white solid. ¹H-NMR (500 MHz, CDCl₃) δ: 7.72–7.67 (3H, m), 3.97 (3H, s).

4-Bromo-3-methoxyaniline (16f) To a mixture of 158 mg, 1.61 mmol) and Zn powder (697 mg, 10.66 mmol) in MeOH (16 mL) was added AcOH (4.8 mL). The whole was stirred at r.t. for 4.5 h and then extracted with AcOEt three times. The organic layer was combined, washed with water and brine, dried and concentrated. Column chromatography (n-hexane:AcOEt=3:1 to 2:1) of the residue gave the title compound (205 mg, 1.01 mmol, 63%) as a yellow paste.

1-Iodo-2-methoxy-4-nitrobenzene (16g) HCl (2.0 mL) was added slowly to a cold (0°C) stirred solution of 2-methoxy-4-nitrobenzene (343 mg, 2.04 mmol) and NaNO₂ (170 mg, 2.46 mmol) in H₂O (5.0 mL). After the addition was completed, the solution was stirred for a further 30 min at 0°C. Then a solution of KI (524 mg, 3.16 mmol) in H₂O (2.0 mL) was added and the mixture was stirred for a further 30 min at 0°C. Then a solution of CuBr (345 mg, 2.40 mmol) in HBr (1.5 mL) was added slowly at 0°C. The whole was stirred at r.t. overnight and diluted with CHCl₃. The organic layer was washed with water and brine, dried and concentrated. Column chromatography (n-hexane:AcOEt=5:1 to 3:1) gave the title compound (404 mg, 1.45 mmol, 71%) as a pale yellow solid.

1-H-NMR (500 MHz, CDCl₃) δ: 7.96 (1H, d, J=8.1Hz), 7.62 (1H, d, J=2.8Hz), 7.59 (1H, dd, J=2.8, 8.2Hz), 3.99 (3H, s).

1-Iodo-2-methoxy-4-nitrobenzene (16g) To a mixture of 16g (404 mg, 1.45 mmol) and NH₄Cl (130 mg, 2.43 mmol) in MeOH (7.0 mL), THF (7.0 mL) and H₂O (3.5 mL) was added Fe powder (443 mg, 7.93 mmol). The whole was stirred at 80°C for 4.5 h and then the reaction was quenched with AcOEt and...
To a solution of 1H-NMR (500 MHz, CDCl₃) δ: 7.44 (1H, d, J=8.5 Hz), 6.21 (1H, d, J=2.8 Hz), 6.11 (1H, dd, J=2.8, 8.5 Hz), 3.81 (3H, s), 3.72 (2H, brs). FAB-MS m/z 249 (MH⁺).

N-(4-Fluoro-3-methoxyphenyl)-2-nitrobenzamide (17e) To a mixture of 4-fluoro-3-methoxyaniline (142 mg, 1.01 mmol), K₂CO₃ (157 mg, 1.14 mmol) and TBAHS (21.8 mg, 0.0642 mmol) in DCM : H₂O (2 : 1) was added 2-nitrobenzoyl chloride (297 mg, 1.29 mmol) at 0°C. The reaction mixture was stirred at r.t. overnight and then the reaction was quenched with CHCl₃. The organic layer was washed with water and brine, dried and concentrated. Column chromatography (n-hexane:AcOEt=3 : 1 to 2 : 1) gave the title compound (276 mg, 0.750 mmol, 51% for 2 steps) as a yellow paste. 1H-NMR (500 MHz, CDCl₃) δ: 7.39 (1H, d, J=8.8 Hz), 7.47–7.44 (2H, m), 6.72–6.69 (2H, m), 5.48 (2H, brs), 3.91 (3H, s).

3-(4-Fluoro-3-methoxyphenyl)-2-thioxoquinazoline-4-one (19e) This compound was prepared from 18f by a method similar to that used for preparation of mdivi-1. Yellow solid (18.9 mg, 0.169 mmol, 42%) (recrystallized from DMF/H₂O). mp>300°C. 1H-NMR (500 MHz, DMSO-d₆) δ: 7.95 (1H, d, J=1.4, 7.7 Hz), 7.79–7.76 (1H, m), 7.44 (1H, d, J=8.1 Hz), 7.36–7.28 (1H, m), 7.19 (1H, dd, J=2.6, 7.7 Hz), 6.89–6.86 (1H, m), 3.78 (3H, s). 13C-NMR (125 MHz, DMSO-d₆) δ: 176.08, 159.77, 151.97, 150.03, 147.45, 147.36, 139.55, 135.73, 135.62, 127.39, 124.35, 121.66, 121.61, 116.19, 115.87, 115.71, 115.65, 115.02, 56.16. FAB-MS m/z 303 (MH⁺). Anal. Calcld for C₁₀H₁₁FN₂O₂S·0.5H₂O: C, 58.11; H, 4.03; N, 9.20.

3-(4-Bromo-3-methoxyphenyl)-2-thioxoquinazoline-4-one (19f) This compound was prepared from 18f by a method similar to that used for preparation of mdivi-1. Yellow solid (61.6 mg, 0.169 mmol, 42%) (recrystallized from DMF/H₂O). mp>300°C. 1H-NMR (500 MHz, DMSO-d₆) δ: 7.95 (1H, d, J=8.1 Hz), 7.80–7.77 (1H, m), 6.77 (1H, d, J=8.61 Hz), 7.44 (1H, d, J=8.1 Hz), 7.37–7.33 (1H, m), 7.16 (1H, d, J=1.9 Hz), 6.86 (1H, dd, J=1.9, 8.3 Hz), 3.79 (3H, s). 13C-NMR (125 MHz, DMSO-d₆) δ: 175.78, 159.67, 155.90, 140.01, 139.60, 135.67, 132.84, 127.41, 124.40, 122.80, 116.21, 115.69, 113.89, 110.27, 56.43. Anal. Calcld for C₁₀H₁₁BrN₂O₂S·0.8H₂O: C, 47.71; H, 3.16; N, 7.42. Found: C, 48.05; H, 3.11; N, 7.31.

3)-(4-Iodo-3-methoxyphenyl)-2-thioxoquinazoline-4-one (19g) This compound was prepared from 18f by a method similar to that used for preparation of mdivi-1. Yellow solid (121 mg, 0.295 mmol, 93%) (recrystallized from n-hexane/ AcOEt/MeOH). mp>300°C. 1H-NMR (500 MHz, DMSO-d₆) δ: 7.95 (1H, d, J=8.1 Hz), 7.80–7.77 (1H, m), 6.77 (1H, d, J=8.61 Hz), 7.44 (1H, d, J=8.1 Hz), 7.37–7.33 (1H, m), 7.16 (1H, d, J=1.9 Hz), 6.86 (1H, dd, J=1.9, 8.3 Hz), 3.79 (3H, s). 13C-NMR (125 MHz, DMSO-d₆) δ: 175.78, 159.67, 155.90, 140.01, 139.60, 135.67, 132.84, 127.41, 124.40, 122.80, 116.21, 115.69, 113.89, 110.27, 56.43. Anal. Calcld for C₁₀H₁₁BrN₂O₂S·0.8H₂O: C, 47.71; H, 3.16; N, 7.42. Found: C, 47.62; H, 3.11; N, 7.31.

Chloro-3-ethoxyaniline (21a) To a mixture of 5-amino-2-chlorophenol (148 mg, 1.03 mmol) and K₂CO₃ (296 mg, 2.14 mmol) in DMF (2.0 mL) was added EtI (100 µL, 1.25 mmol). The whole was stirred at 60°C for 2.5 h and then stirred with AcOEt. The organic layer was washed with water and brine, dried and concentrated. Column chromatography (n-hexane:AcOEt=3 : 1) of the residue gave the title compound (88.5 mg, 0.515 mmol, 50%) as a pale yellow paste. 1H-NMR (500 MHz, CDCl₃) δ: 7.07 (1H, d, J=8.61 Hz), 6.25 (1H, d, J=2.9 Hz), 6.19 (1H, dd, J=2.6, 8.3 Hz), 4.02 (2H, q, J=7.0 Hz), 3.63 (2H, brs), 1.43 (3H, t, J=7.0 Hz).

Chloro-3-propoxyaniline (21b) This compound was prepared from 5-amino-2-chlorophenol and n-PrI by a method similar to that used for preparation of 21a. Pale yellow paste (107 mg, 0.572 mmol, 54%). 1H-NMR (500 MHz, CDCl₃) δ:
This compound was prepared from 5-amino-2-chlorophenol and i-PrBr by a method similar to that used for preparation of 21a. Pale yellow paste (141 mg, 0.700 mmol, 68%). 1H-NMR (500 MHz, CDCl3) δ: 8.02–7.99 (1H, m), 7.63–7.60 (1H, m), 7.51–7.49 (2H, m), 6.70–6.67 (2H, m), 5.45 (2H, br s), 4.57 (2H, br s), 4.03 (2H, q, J = 6.8 Hz), 0.74 (3H, t, J = 6.8 Hz). 13C-NMR (125 MHz, DMSO-d6) δ: 175.88, 159.67, 154.09, 153.89, 139.58, 139.16, 135.63, 129.81, 127.40, 124.38, 124.36, 122.10, 121.02, 116.19, 115.66, 115.01, 70.18, 21.78, 10.25. FAB-MS m/z 347 (M+). Anal. Calcd for C19H15ClIN2O3·H2O: C, 59.33; H, 4.03; N, 8.41. Found: C, 59.49; H, 3.57; N, 8.46. 3-(4-Chloro-3-ethoxyphenyl)-2-thioxoquinazoline-4-oxime (23d) This compound was prepared from 21d by a method similar to that used for preparation of 23a. Yellow solid (158 mg, 0.497 mmol, 74%). 1H-NMR (500 MHz, CDCl3) δ: 7.71 (1H, bs), 7.47 (1H, d, J = 2.3 Hz), 7.42–7.41 (1H, m), 7.26 (1H, d, J = 8.6 Hz), 7.25–7.21 (1H, m), 6.85 (1H, dd, J = 2.3, 8.6 Hz), 6.70–6.67 (2H, m), 5.45 (2H, br s), 4.03 (2H, t, J = 6.3 Hz), 1.83–1.78 (2H, m), 1.54–1.47 (2H, m), 0.96 (3H, t, J = 7.4 Hz). 

3-(4-Chloro-3-ethoxyphenyl)-2-thioxoquinazoline-4-one (24a) This compound was prepared from 23a by a method similar to that used for preparation of mdivi-1. Pale yellow solid (141 mg, 0.150 mmol, 53%) (recrystallized from DMF/CH3OH). mp > 300°C. 1H-NMR (500 MHz, DMSO-d6) δ: 7.95 (1H, dd, J = 1.2, 8.0 Hz), 7.78–7.76 (1H, m), 7.50 (1H, d, J = 8.1 Hz), 7.44 (1H, d, J = 8.0 Hz), 7.36–7.33 (1H, m), 7.18 (1H, d, J = 2.3 Hz), 6.89 (1H, dd, J = 2.3, 8.6 Hz), 4.05 (2H, q, J = 6.9 Hz), 0.96 (3H, t, J = 6.9 Hz). 

13C-NMR (125 MHz, DMSO-d6) δ: 175.88, 159.67, 154.09, 153.89, 139.58, 139.16, 135.63, 129.81, 127.40, 124.38, 122.10, 121.02, 116.20, 115.67, 115.02, 64.47, 14.43. FAB-MS m/z 333 (M+). HR-MS (FAB) Caled for C19H15ClIN2O3·H2O: C, 57.38; H, 4.53; N, 7.87. Found: C, 57.49; H, 4.57; N, 7.98.

3-(4-Chloro-3-ethoxyphenyl)-2-thioxoquinazoline-4-one (24b) This compound was prepared from 23b by a method similar to that used for preparation of mdivi-1. Pale yellow solid (141 mg, 0.150 mmol, 53%) (recrystallized from DMF/CH3OH). mp > 300°C. 1H-NMR (500 MHz, DMSO-d6) δ: 7.94 (1H, dd, J = 1.7, 8.1 Hz), 7.78–7.75 (1H, m), 7.49 (1H, d, J = 8.0 Hz), 7.43 (1H, d, J = 8.0 Hz), 7.35–7.32 (1H, m), 7.17 (1H, d, J = 2.3 Hz), 6.88 (1H, dd, J = 2.0, 8.3 Hz), 3.94 (2H, t, J = 6.3 Hz), 1.72 (2H, t, J = 6.3 Hz), 1.07 (3H, t, J = 7.4 Hz). 

13C-NMR (125 MHz, DMSO-d6) δ: 175.86, 159.67, 154.09, 153.89, 139.58, 139.16, 135.63, 129.77, 127.39, 124.36, 122.08, 121.02, 116.19, 115.66, 115.01, 70.18, 21.78, 10.25. FAB-MS m/z 347 (M+). Anal. Caled for C19H15ClIN2O3·H2O: C, 57.38; H, 4.53; N, 7.87. Found: C, 57.49; H, 4.57; N, 7.98.

3-(4-Chloro-3-isoproxyphenyl)-2-thioxoquinazoline-4-one (24c) This compound was prepared from 23c by a method similar to that used for preparation of mdivi-1. Pale yellow solid (60.1 mg, 0.172 mmol, 74%) (recrystallized from CHCl3/n-hexane/MeOH). mp > 300°C. 1H-NMR (500 MHz, DMSO-d6) δ: 7.91 (1H, dd, J = 1.2, 8.0 Hz), 7.75–7.72 (1H, m), 7.44 (1H, d, J = 8.0 Hz), 7.39 (1H, d, J = 8.0 Hz), 7.32–7.30 (1H, m), 7.16 (1H, d, J = 2.3 Hz), 6.83 (1H, dd, J = 2.3, 8.6 Hz), 4.05 (1H, sep, J = 5.7 Hz), 0.96 (6H, t, J = 5.7 Hz). FAB-MS m/z 347 (M+). Anal. Caled for C19H15ClIN2O3·0.4H2O: C, 57.67; H, 4.50; N, 7.91. Found: C, 57.77; H, 4.43; N, 7.73.
3-(3-Butoxy-3-chlorophenyl)-2-thioxoquinazoline-4-one (24d) This compound was prepared from 23d by a method similar to that used for preparation of mdivi-1. Yellow solid (122 mg, 0.338 mmol, 98%) (recrystallized from CHCl3/n-hexane/MeOH). mp 292–293°C. 1H-NMR (500 MHz, CDCl3) δ: 10.20 (1H, brs), 8.13 (1H, dd, J=1.2, 8.0 Hz), 7.68–7.65 (1H, m), 7.47 (1H, d, J=7.5 Hz), 7.33–7.30 (1H, m), 7.10 (1H, d, J=8.0 Hz), 6.78–6.76 (1H, m), 4.02–3.95 (2H, m), 1.81–1.76 (2H, m), 1.52–1.44 (2H, m), 0.94 (3H, t, J=7.5 Hz). FAB-MS m/z 361 (MH+). Anal. Calc'd for C18H17ClN2O2S: C, 59.91; H, 4.75; N, 7.76. Found: C, 59.79; H, 5.05; N, 7.69.

**Biology**

**Cell Culture** MOLT4 cells were cultured in RPMI medium supplemented with 10% FBS and penicillin and streptomycin at 37°C in a humidified incubator (5% CO2 in air).

**Expression and Purification of Drp1 Protein** Histine-directed tagged DRP1 protein (plasmid was purchased from GeneCopoeiaTM) was expressed in transformed Rosetta (DE3) competent cells (Novagen), derived from Escherichia coli BL21, grown in LB by induction with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 25°C for 19 h. Cell suspension (2.0 mL of intact MOLT4 cell suspension (2.0×10^6 cells/mL) at 37°C and the system was pre-incubated for exactly 10 min. Then 50 µL of Ala-AMC (10 µM) was added at 37°C and incubation was continued for exactly 30 min. At this point, 1.5 mL of 1 M AcONa–AcOH (pH 4.0) was added to stop the enzymatic reaction. The amounts of liberated AMC were measured in terms of fluorescence intensity (excitation at 355 nm, emission at 460 nm) with a Wallac 1420 multilabel counter (PerkinElmer, Inc., Life Science) and a Wallac Envision 2104 multilabel reader (PerkinElmer, Inc., Life Science). The assay was performed at least in duplicate, and the mean value was taken.

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**References**

1. Chan D. C., *Annu. Rev. Cell Dev. Biol.*, 22, 79–99 (2006).
2. Chang C. R., Blackstone C., *Ann. N. Y. Acad. Sci.*, 1201, 34–39 (2010).
3. Fannjiang Y., Cheng W. C., Lee S. J., Qi B., Pevsner J., McCaffrey J. M., Hill R. B., Basahez G., Hardwick J. M., *Genes Dev.*, 18, 2785–2797 (2004).
4. James D. I., Parone P. A., Mattenberger Y., Martinou J. C., *J. Biol. Chem.*, 278, 36373–36379 (2003).
5. Palmer C. S., Osselaine L. D., Laine D., Koutoupoulos O. S., Frazier A. E., Ryan M. T., *EMBO Rep.*, 12, 565–573 (2011).
6. Zhao J., Liu T., Jin S., Wang X., Qu M., Uhlen P., Tomilin N., Shupliakov O., Lendahl U., Nistér M., *EMBO J.*, 30, 2762–2778 (2011).
7. Otera H., Wang C., Cleland M. M., Setoguchi K., Yokota S., Youle R. J., Mihara K., *J. Cell Biol.*, 191, 1141–1158 (2010).
8. Bossy-Wetzel E., Petrilli A., Knott A. B., *Trends Neurosci.*, 31, 609–616 (2008).
9. Lin M. T., Beal M. F., *Nature (London)*, 443, 787–795 (2006).
10. Song W., Chen J., Petrilli A., Liu G., Kinglmaey E., Zhou Y., Poquiz P., Tjong J., Pouladi M. A., Hayden M. R., Mitala E., El-Ashman M., Rouiller I., Schwarzenbacher R., Bossy B., Perkins G., Bossy-Wetzel E., *Nat. Med.*, 17, 377–382 (2011).
11. Shirendeb U. P., Calkins M. J., Mactzak M., Anekoza V., Dufour B., McBride J. L., Mao P., Reddy P. H., *Hum. Mol. Genet.*, 21, 406–412 (2012).
12. Cassidy-Stone A., Chipuk J. E., Ingerman E., Song C., Yoo K., Watan K., Kurfeth M. J., Shaw J. T., Hinshaw J. E., Green D. R., Nunnari J., *Dev. Cell.*, 14, 193–204 (2008).
13. Kakuta H., Tanatani A., Nagasawa K., Hashimoto Y., *Chem. Pharm. Bull.*, 51, 1273–1282 (2003).
14. Look A. T., Ashman R. A., Shapiro L. H., Peiper S. C., *J. Clin. Investig.*, 83, 1299–1307 (1989).
15. Hersh L. B., Mckelvy J. F., *J. Neurochem.*, 36, 171–180 (1981).
16. Bauer W. O., Nanda I., Beck G., Schmid M., Jacob F., *EMBO J.*, 19, 377–382 (2011).
17. McBride J. L., Mao P., Reddy P. H., *Hum. Mol. Genet.*, 21, 406–412 (2012).
18. Johnson G. D., Hersh L. B., *Arch. Biochem. Biophys.*, 276, 305–309 (1990).
19. Rawlings N. D., Barrett A. J., *Biochem. J.*, 290, 205–218 (1993).
20. Bhutani N., Venkatraman P., Goldberg A. L., *EMBO J.*, 26, 1385–1396 (2007).
21. Menzies F. M., Houriez R., Imarísio S., Rasper M., Sadiq O., Chandraratna D., O’Kane C., Koczmann P., Reits E., Goldberg A. L., Rubinsztein D. C., *Hum. Mol. Genet.*, 19, 4573–4586 (2010).
22. Matsumoto Y., Noguchi-Yachide T., Nakamura M., Mita Y., Numata A., Hashimoto Y., *Heterocycles*, 86, 1449–1463 (2012).