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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of thiopyrano[3,4-*c*]quinoline-9-carboxamide derivatives as inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1)

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Abstract A series of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors, 5-oxo-2,4,5,6-tetrahydro-1*H*-thiopyrano[3,4-*c*]quinoline-9-carboxamide derivatives, were successfully synthesized and their PARP-1 inhibitory activity was evaluated. These compounds were prepared from carboxylic acid **7** and the appropriate amines, and a number of the synthesized compounds were found to have significant PARP-1 activity. Among them, **9m** showed potent activity in a PARP-1 enzymatic assay and cell-based assay ($IC_{50} = 0.045$ μM, $ED_{50} = 0.54$ μM). Molecular modeling studies confirmed the obtained biological results.

Keywords Poly(ADP-ribose)polymerase · PARP-1 inhibitor · Thiopyranoquinoline derivatives

Introduction

PARP-1 is an abundant nuclear enzyme that is responsible for almost 97% of the poly (ADP-ribose) formation in the brain, and its activity increased up to 500 times by DNA damage (D'Amours *et al.*, 1999; Strosznajder *et al.*, 2005). Depending on the cell type and the extent of DNA damage, PARP-1 can be involved in DNA repair and neuronal death through NAD^+ and ATP depletion. The function of PARP-1 under mild to moderate DNA damage is to repair DNA by interacting with other DNA repair effectors, such as DNA polymerase β and DNA ligase III, and adaptor factors, such as XRCC1 (Masson *et al.*, 1998). This pathway occurs in cells that are exposed to certain DNA damaging agents, such as ionizing radiation, topoisomerase inhibitors, and alkylating agents (Tentori and Graziani, 2005; Tentori *et al.*, 2002a, b). Indeed, PARP-1 inhibitors have been demonstrated to enhance cytotoxicity in combination therapy with cytotoxic agents (Calabrese *et al.*, 2003, 2004; Miknyoczki *et al.*, 2003; Tentori *et al.*, 2002a, b, 2003). Therefore, PARP-1 inhibitors could be attractive drug candidates for the treatment of cancer. Under extensive DNA damage, overactivation of PARP-1 is induced, leading to NAD^+ and ATP depletion and necrotic cell death (Alano *et al.*, 2004; Ha and Snyder, 1999; Ying *et al.*, 2005). This pathway has been implicated in the pathogenesis of several diseases, including stroke, myocardial infarction, diabetes, shock, neurodegenerative disorder, and several inflammatory processes (Tentori *et al.*, 2002a, b; Virag and Szabo, 2002). Thus, the development of PARP-1 inhibitor can provide remarkable therapeutic benefits in various diseases including ischemic stroke.

Currently, PARP inhibitors such as AG-014699, AZD2281, ABT-888, and BSI-201 are being developed as mono- and combination therapy for cancer in clinical studies (Fig. 1) (Menear *et al.*, 2008; Penning *et al.*, 2009; Sandhu *et al.*, 2010). Most of the PARP-1 inhibitors compete with NAD^+ and are designed to mimic nicotinamide, which forms three hydrogen bonds to the PARP-1 enzyme. The amide group of inhibitors is essential for binding to the catalytic

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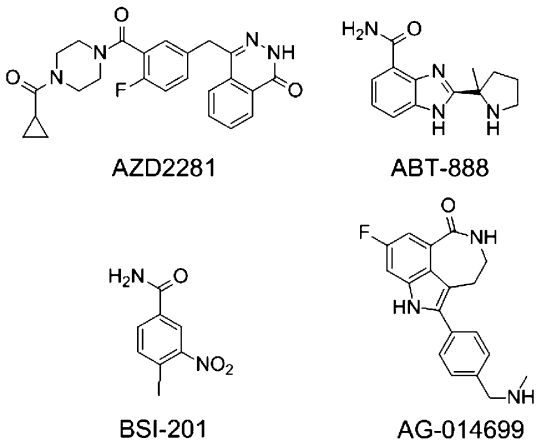


Fig. 1 PARP inhibitors currently in clinical studies

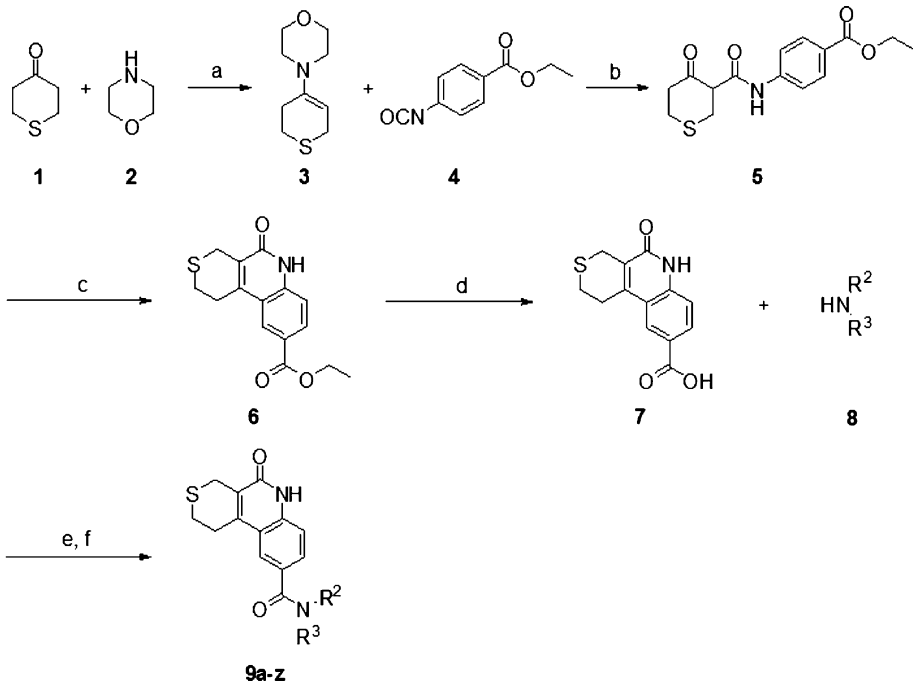
domain of the enzyme. In addition, the A-ring of inhibitors binds effectively to the aryl residues, Tyr907 and Tyr896, through a sandwiched hydrophobic π–π interaction (Ferraris, 2010; Ishida *et al.*, 2006; Kinoshita *et al.*, 2004; Matsumoto *et al.*, 2006).

We have recently reported tricyclic PARP-1 inhibitors, 9-hydroxy-1,2-dihydro-4*H*-thiopyrano[3,4-*c*]quinoline-5(6*H*)-one derivatives (Park *et al.*, 2010). These derivatives include a non-aromatic A-ring and fit well to the active site even though their conformations are not flat. In this study, we describe further modifications of 9-hydroxy-1,2-dihydro-4*H*-thiopyrano[3,4-*c*] quinoline-5(6*H*)-ones. The PARP-1 inhibitors, 5-oxo-2,4,5,6-tetrahydro-1*H*-thiopyrano[3,4-*c*]quinoline-9-carboxamide derivatives, were designed and synthesized. Additionally, docking studies of thiopyranoquinoline carboxylic acid **7** and derivatives of this compound were performed, and their PARP-1 inhibitory activities were evaluated.

Results and discussion

Chemistry

The synthesis of the novel 5-oxo-2,4,5,6-tetrahydro-1*H*-thiopyrano[3,4-*c*]quinoline-9-carboxamides **9a–z** was carried out following the synthetic pathway in Scheme 1. 4-(3,6-dihydro-2*H*-thiopyran-4-yl)morpholine **3** was obtained by refluxing 4-oxothiane **1** with morpholine **2** in the presence of catalytic *p*-TsOH·H_2O and subsequently treated without further purification. The ketamide **5**, prepared by reaction of the enamine **3** with ethyl 4-isocyanobenzoate **4**, was cyclized in 70% sulfuric acid to the thiopyranoquinoline-9-carboxylate **6** (Khuthier *et al.*, 1987; Park *et al.*, 2010; Stork *et al.*, 1963). Hydrolysis of the carboxylate **6** with 1 *N* NaOH solution gave its corresponding carboxylic acid **7**, which was optimized by conversion of acid group to amide. 5-Oxo-2,4,5,6-tetrahydro-1*H*-thiopyrano[3,4-*c*]quinoline-9-carboxamides **9a–z** were synthesized by amide coupling with aliphatic amine or Boc-protected diamine using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI). In particular, the aminoethyl compound **9a** and piperazine compound **9j** were selected and optimized to improve cellular activity. The structure of the synthesized thiopyranoquinoline compounds was established on the basis of ^{1}H NMR and mass spectral analysis.

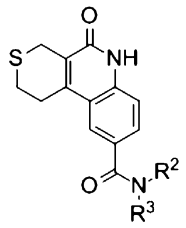


Scheme 1 Reagents and conditions: (a) *p*-TsOH•H_2O, toluene, rt; (b) (i) toluene, rt, (ii) 2 *N* HCl, rt; (c) 70% H_2SO_4, rt; (d) 1 *N* NaOH, MeOH, 50°C; (e) (i) EDC, HOBt, DMF, rt, (ii) 3.7 M HCl in 1,4-dioxane, rt

Biology

The PARP-1 inhibitory activity of all synthesized thiopyranoquinoline compounds **6**, **7**, and **9a–z** was evaluated in vitro PARP-1 enzymatic assay and cell-based assay, which is summarized in Table 1.

Table 1 Enzyme and cellular activity of the synthesized compounds **9a–z**



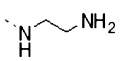
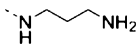
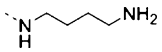
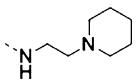
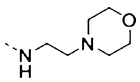
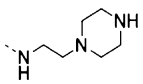
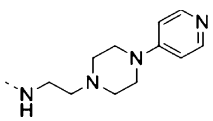
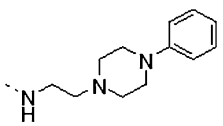
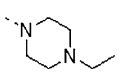
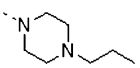
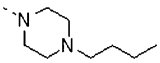
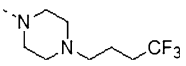
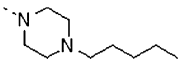
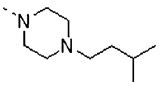
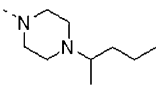
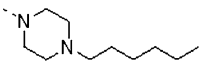
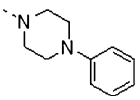
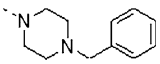
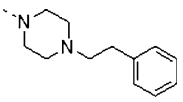
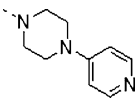
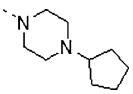
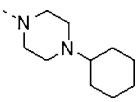
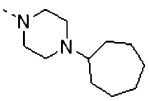
Compounds	NR^2R^3	$IC_{50}(\mu M)$[a]	$ED_{50}(\mu M)$[b]
6	CO_2Et	0.536	–
7	COOH	0.078	–
9a		0.017	>30
9b		0.030	>30
9c		0.027	>30
9d		0.058	2.81
9e		0.060	>30
9f		0.036	>30
9g		0.044	>30
9h		0.166	4.02
9i		0.175	1.26
9j		0.008	>30
9k		0.188	2.53
9l		0.047	2.00

Table 1 continued

Compounds	NR^2R^3	$IC_{50}(\mu M)$[a]	$ED_{50}(\mu M)$[b]
9m		0.045	0.54
9n		0.042	0.77
9o		0.134	2.41
9p		0.079	0.61
9q		0.065	0.84
9r		0.040	0.46
9s		0.094	2.91
9t		0.234	1.29
9u		0.187	2.51
9v		0.019	2.19
9w		0.020	>30
9x		0.047	0.55
9y		0.068	0.32
9z		0.067	0.50

[a] Enzymatic assays followed a commercially available protocol (Trevigen kit, 4671-096-K) in 384 well plates. Values are the mean of triplicate experiments

[b] The CHO-K1 (Chinese hamster ovary) cell line was used for cell-based assay. Values are the mean of quadruplicate experiments

Generally, these compounds showed good enzymatic activity. Some compounds (**9a–c**, **9e–g**, and **9j**), however, displayed poor cellular activity. The polar group at the end

of the derivative chain seems to prevent cell permeability. Among the *N*-alkyl piperazine derivatives (**9k–s**), compounds **9m**, **9n**, and **9p**, which have a linear 3–5 carbon chain, displayed significant activities in enzyme assays and more potent activities in cellular assays. Additionally, *N*-propyl piperazine compound **9m** exhibited good water solubility (>1 mg/ml). Compounds **9q** and **9r**, which have a bulky branched carbon chain, also showed potent activity in a PARP-1 enzymatic assay and cell-based assay. As mentioned above, the compound with the polar group at the end of the chain (**9o**) showed less activity than compound **9n**. *N*-aryl or -arylalkyl piperazine derivatives (**9t**, **9u**, and **9v**) displayed a loss of potency in cellular activity. As expected, the compound with the polar group (**9w**) decreased cellular activity significantly.

A molecular modeling study with automated docking simulation was conducted to explore the interaction between target (PARP-1) and the thiopyranoquinoline-9-carboxylic acid **7**. Ligand included in 1UK0 crystal was very well docked with its protein for validation of docking study. The amide group of the compound **7** formed hydrogen bonds to Ser904 and Gly863 of the enzyme. Furthermore, the hydrogen bond between the acid group of the compound and Asp766 of the enzyme increased PARP-1 activity (Fig. 2) compared to the carboxylate **6** which do not formed hydrogen bond. In an attempt to comprehend the binding conformation of *N*-alkyl piperazine derivatives in the active site of the enzyme, a docking study of highly potent inhibitor (**9m**) was performed, and the result showed that the nitrogen of piperazine analogs is very important for the hydrogen bond to Ala880 of the enzyme backbone (Fig. 3). The docking scores were estimated binding free

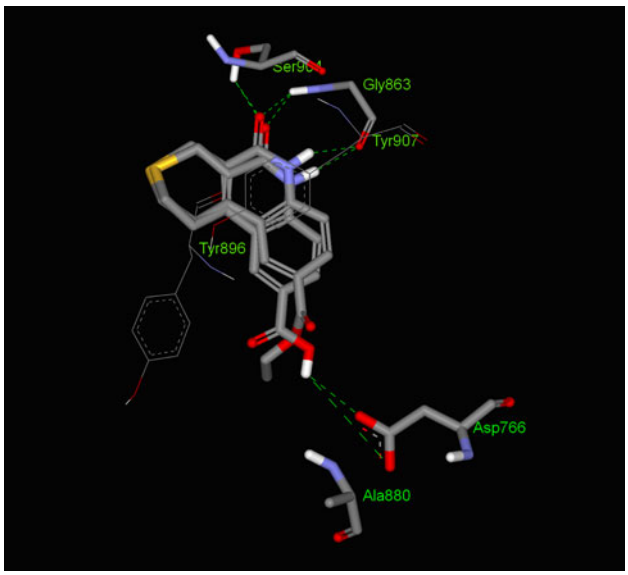


Fig. 2 Docking of compound **7** in the catalytic domain of human PARP-1 (PDB code: 1UK0) (Kinoshita *et al.*, 2004)

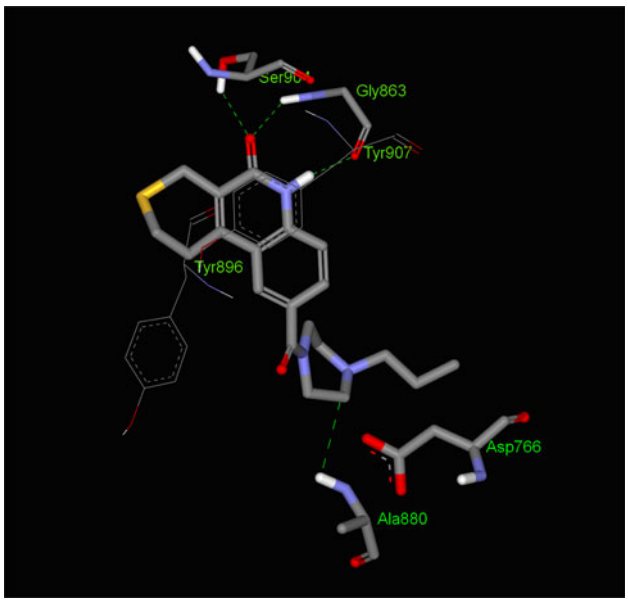


Fig. 3 Docking of compound **9m** in the catalytic domain of human PARP-1 (PDB code: 1UK0) (Kinoshita *et al.*, 2004)

energy using CHARMm implicit salvation models and their results were −65.0, −45.6, and −6.6 kcal/mol for compound **6**, **7**, and **9m**, respectively. Finally, *N*-cycloalkyl piperazine derivatives (**9x–z**) were synthesized by the same method as described above in effort to further optimize *N*-propyl piperazine compound **9m**. From the docking study, the cycloalkyl group of these derivatives was located in a large hydrophobic pocket of the active site; these compounds (**9x–z**) resulted in a significant increase in PARP-1 inhibitory activity. Their activities were, respectively, IC_{50} = 0.047, 0.068, and 0.067 μM in an enzymatic assay, and ED_{50} = 0.55, 0.32, and 0.50 μM in a cellular assay.

Pharmacokinetic properties and microsomal stabilities of selected PARP-1 inhibitors are displayed in Tables 2 and 3, respectively. Compound **9m** displayed high clearance and moderate half-time and showed good metabolic stability, with a 41.7 min half-life in the human liver microsomal stability test compared to those of compounds **9q** and **9x**.

In conclusion, we have described a straightforward synthesis, docking study and biological evaluation of

Table 2 Rat PK profile of **9m** (5 mg/kg), $n = 3$

Compound	**9m**
Dose (mg/kg)	5
$AUC_{0\text{-inf}}$ (h ng/ml)	1303.1
CL (l/h/kg)	3.8
$t_{1/2}$ (h)	1.4

Values were detected by LC/MS/MS after intravenous administration

Table 3 Human liver microsomal stability of selected compounds

Drug	$t_{1/2}$ (min)
Buspirone	4.0
9m	41.7
9q	15.9
9x	14.5

Microsomal activity was detected at 0, 15, 45, and 80 min by LC/MS/MS. The incubation temperature was 37°C. Values are the mean of triplicate experiments. Buspirone was used as a reference

5-oxo-2,4,5,6-tetrahydro-1*H*-thiopyrano[3,4-*c*]quinoline-9-carboxamide derivatives as potent PARP-1 inhibitors. Our studies indicate that compound **9m** was found to be a potent PARP-1 inhibitor, showing $IC_{50} = 0.045$ μM and $ED_{50} = 0.54$ μM in enzyme and cellular activity, respectively. Moreover, compound **9m** showed good metabolic stability and aqueous solubility. These findings suggest that a potent PARP-1 inhibitor **9m** could be a useful therapeutic candidate for cancers and ischemic stroke.

Experimental section

Chemistry

Melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Varian 400 Mercury plus spectrometer; chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (TMS). Mass spectra were performed on an Applied Biosystems API 2000 mass spectrometer and Agilent 1200 series LC system. All compounds were routinely checked by TLC and ^{1}H NMR. TLC was performed on glass-backed silica gel plates (Merck, DC Kieselgel 60 F_{254}). Flash chromatography was carried out on E. Merck Kieselgel 60 silica gel (230–400 mesh). All chemicals were obtained commercially. All solvents were reagent-grade and were used without further purification, unless otherwise stated.

*Ethyl 4-(4-oxotetrahydro-2H-thiopyran-3-carboxamido)benzoate (**5**)*

To a stirred solution of 4-oxothiane **1** (2.0 g, 17.21 mmol) in toluene (20 ml) were added morpholine **2** (2.25 ml, 25.82 mmol) and catalytic p-TsOH·H_2O (160 mg, 0.86 mmol), and the resulting mixture was refluxed for 1 day with a Dean-Stark trap. After completion, the mixture was cooled to room temperature and evaporated under reduced pressure. The residue was concentrated in vacuo to afford the unstable enamine **3**, which was used without further purification.

Ethyl 4-isocyanatobenzoate **4** (6.6 g, 34.43 mmol) in toluene (30 ml) was added dropwise to a stirred solution of unstable enamine **3** in toluene (20 ml) at room temperature, and the stirring was continued at room temperature overnight. Then 2 *N* HCl (8.7 ml, 17.21 mmol) was added, and the resulting mixture was stirred at room temperature for 1 day. The mixture was cautiously neutralized with 2 *N* NaOH, and the precipitate was filtered. The filtrate was taken up with water and extracted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was then purified by flash column chromatography to afford the title compound **5** as a white solid (2.8 g, 53%, 2 step).

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.39 (s, 1H, –C(O)NH–), 8.01 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.63 (d, $J = 8.4$ Hz, 2H, Ar-H), 4.35 (qt, $J = 7.2$ Hz, 2H, –OCH_2CH_3), 3.69 (dd, $J = 10.4$ Hz, $J = 4.8$ Hz, 1H, thiopyran), 3.47–3.40 (m, 1H, thiopyran), 3.22 (dd, $J = 10.4$ Hz, 1H, thiopyran), 3.06–2.98 (m, 2H, thiopyran), 2.91–2.80 (m, 2H, thiopyran), 1.38 (t, $J = 7.2$ Hz, 3H, –OCH_2CH_3); MS (ESI+) m/z: $[M+H]^+$ 308.1.

*Ethyl 5-oxo-2,4,5,6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxylate (**6**)*

Ketamide **5** (1.0 g, 3.25 mmol) was suspended in 15 ml of 70% H_2SO_4 and the resulting mixture was stirred at room temperature for 1 day. The reaction mixture was poured into ice water and stirred for 1 h. The precipitate was filtered, washed with water, and dried in vacuo to afford the title compound **6** as a brown solid (710 mg, 74%).

M.p.: 235°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (s, 1H, –C(O)NH–), 8.29 (s, 1H, Ar-H), 8.02 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.36 (d, $J = 8.4$ Hz, 1H, Ar-H), 4.32 (qt, $J = 7.2$ Hz, 2H, –OCH_2CH_3), 3.59 (s, 2H, thiopyran), 3.15 (d, $J = 4.8$ Hz, 2H, thiopyran), 2.93 (t, $J = 5.6$ Hz, 2H, thiopyran), 1.32 (t, $J = 7.2$ Hz, 3H, –OCH_2CH_3); MS (ESI+) m/z: $[M+H]^+$ 290.1.

*5-Oxo-2,4,5,6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxylic acid (**7**)*

To a stirred solution of thiopyrano[3,4-*c*]quinoline-9-carboxylate **6** (680 mg, 2.35 mmol) in MeOH (12 ml) was added aqueous 1 *N* NaOH solution (11.75 ml). The mixture was then heated to 50°C and stirred for 5 h. After completion of the reaction, the cooled mixture was poured on water and extracted with ethyl acetate. The aqueous layer was then acidified to pH 2 with 2 *N* HCl. The precipitate was filtered and washed with water, yielding the title compound **7** as a light yellow solid (420 mg, 68%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.94 (br, 1H, –C(O)OH), 12.14 (s, 1H, –C(O)NH–), 9.30 (s, 1H, Ar-H),

8.02 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.37 (d, $J = 8.0$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.18 (t, $J = 5.6$ Hz, 2H, thiopyran), 2.95 (t, $J = 6.0$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 262.1.

*General procedure for the synthesis of 5-oxo-2,4,5, 6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide hydrochloride derivatives (**9a–z**)*

To a stirred solution of thiopyrano[3,4-*c*]quinoline-9-carboxylic acid **7** (40 mg, 0.15 mmol) in DMF (3 ml) were added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI, 32 mg, 0.17 mmol), hydroxybenzotriazole (HOBt, 23 mg, 0.17 mmol), and an appropriate amine compound (0.17 mmol). The resulting mixture was stirred at room temperature for 8–12 h and poured into saturated aqueous $NaHCO_3$ solution. The mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was then purified by flash column chromatography to afford thiopyrano[3,4-*c*]quinoline-9-carboxamide as a free base.

3.7 M HCl in 1,4-dioxane (30 equiv.) was added to a stirred solution of thiopyrano[3,4-*c*]quinoline-9-carboxamide in 1,4-dioxane (3–5 ml) at 0°C. After stirring for 1–10 h, the reaction mixture was concentrated in vacuo to give the title compound **9**.

The physical and analytical data of the synthesized title compounds are given, as follows:

*N-(2-Aminoethyl)-5-oxo-2,4,5,6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide (**9a**)*

Yellow solid; yield: 26%; m.p.: 289°C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.07 (s, 1H, –C(O)NH–), 8.97 (br, 1H, Ar-C(O)NH–), 8.32 (s, 1H, Ar-H), 8.01–7.99 (m, 4H, Ar-H, -NH_2·HCl), 7.34 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.56–3.51 (m, 2H, –$(CH_2)_2$N), 3.21 (m, 2H, thiopyran), 3.00–2.95 (m, 4H, thiopyran, –$(CH_2)_2$N); MS (ESI+) m/z: $[M+H]^+$ 304.1.

*N-(3-Aminopropyl)-5-oxo-2,4,5,6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide (**9b**)*

Light yellow solid; yield: 40%; m.p.: 286°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.07 (s, 1H, –C(O)NH–), 8.83 (br, 1H, Ar-C(O)NH–), 8.28 (s, 1H, Ar-H), 7.98 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.87 (br, 2H, –NH_2), 7.34 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.56 (s, 2H, thiopyran), 3.21 (t, $J = 5.6$ Hz, 2H, –$(CH_2)_3$–N), 2.97 (t, $J = 6.0$ Hz, 2H, thiopyran), 2.85 (q, $J = 6.0$ Hz, 2H, –$(CH_2)_3$–N), 1.82 (t, $J = 6.8$ Hz, 2H, –$(CH_2)_3$–N); MS (ESI+) m/z: $[M+H]^+$ 318.1.

*N-(4-Aminobutyl)-5-oxo-2,4,5,6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide (**9c**)*

Light yellow solid; yield: 13%; m.p.: 104°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05(s, 1H, –C(O)NH–), 8.72(t, $J = 6.4$ Hz, 1H, Ar-C(O)NH–), 8.27(s, 1H, Ar-H), 7.97(d, $J = 7.6$ Hz, 1H, Ar-H), 7.90(br, 2H, –NH_2), 7.34(d, $J = 8.0$ Hz, 1H, Ar-H), 3.51(s, 2H, thiopyran), 3.30(d, $J = 5.2$ Hz, 2H, –$(CH_2)_4$–N), 3.20(d, $J = 5.2$ Hz, 2H, thiopyran), 2.97(t, $J = 6.0$ Hz, 2H, thiopyran), 2.80(d, $J = 5.2$ Hz, 2H, –$(CH_2)_4$–N), 1.59(m, 4H, –$(CH_2)_4$–N); MS (ESI+) m/z: $[M+H]^+$ 332.2.

*5-Oxo-N-[2-(piperidin-1-yl)ethyl]-2,4,5,6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide (**9d**)*

White solid; yield: 14%; m.p.: 268°C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, –C(O)NH–), 10.20 (br, 1H, HCl salt), 9.09 (t, $J = 5.4$ Hz, 1H, Ar-C(O)NH–), 8.34 (s, 1H, Ar-H), 8.00 (d, $J = 8.8$ Hz, 1H, Ar-H), 7.33 (d, $J = 8.0$ Hz, 1H, Ar-H), 3.68 (qt, $J = 5.6$ Hz, 2H, –NH–CH_2–), 3.58 (s, 2H, thiopyran), 3.54–3.50 (m, 2H, –CH_2–piperidine), 3.21–3.20 (m, 4H, CH_2, thiopyran and CH_2, piperidine), 2.94 (t, $J = 6.0$ Hz, 2H, thiopyran), 2.91–2.84 (m, 2H, piperidine), 1.77 (m, 4H, piperidine), 1.69–1.66 (m, 1H, piperidine), 1.39–1.34 (m, 1H, piperidine); MS (ESI+) m/z: $[M+H]^+$ 372.2.

*N-(2-Morpholinoethyl)-5-oxo-2,4,5,6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide (**9e**)*

Yellow solid; yield: 31%; m.p.: 253°C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.07 (s, 1H, –C(O)NH–), 10.74 (br, 1H, HCl salt), 9.05 (br, 1H, Ar-C(O)NH–), 8.35 (s, 1H, Ar-H), 8.01 (d, $J = 8.8$ Hz, 1H, Ar-H), 7.35 (d, $J = 8.0$ Hz, 1H, Ar-H), 3.99–3.96 (m, 2H, –NH–CH_2–), 3.82–3.76 (m, 2H, –CH_2–morpholine), 3.71–3.70 (m, 2H, morpholine), 3.60 (s, 2H, thiopyran), 3.45 (m, 2H, morpholine), 3.32–3.31 (m, 2H, morpholine), 3.22 (m, 2H, thiopyran), 3.13–3.10 (m, 2H, morpholine), 2.96 (t, $J = 5.2$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 374.2.

*5-Oxo-N-[2-(piperazin-1-yl)ethyl]-2,4,5,6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide (**9f**)*

Yellow solid; yield: 23%; m.p.: 240°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H, –C(O)NH–), 11.47 (br, 1H, HCl salt), 9.50 (br, 2H, piperazine NH·HCl salt), 8.99 (br, 1H, Ar-C(O)NH–), 8.35 (s, 1H, Ar-H), 8.02 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.34 (d, $J = 8.8$ Hz, 1H, Ar-H), 3.81–3.38 (m, 14H, CH_2, thiopyran and 4CH_2, piperazine and 2CH_2, –$(CH_2)_2$–piperazine), 3.22 (m, 2H, thiopyran),

2.96 (t, $J = 5.6$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 373.2.

*5-Oxo-N-{2-[4-(pyridin-4-yl)piperazin-1-yl]ethyl}-2,4,5, 6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide (**9g**)*

Yellow solid; yield: 20%; m.p.: 252°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H, –C(O)NH–), 11.36 (br, 1H, Ar-C(O)NH–), 9.11 (br, 1H, HCl salt), 8.38–8.36 (m, 3H, CH, Ar-H, 2CH, pyridine), 8.04 (d, $J = 8.8$ Hz, 1H, Ar-H), 7.37–7.30 (m, 3H, CH, Ar-H and 2CH, pyridine), 4.52–4.44 (m, 2H, -NH–CH_2-), 3.84–3.61 (m, 8H, CH_2, thiopyran and $2CH_2$, piperazine and CH_2, –CH_2–piperazine), 3.37–3.35 (m, 2H, thiopyran), 3.27–3.18 (m, 4H, piperazine), 2.96 (t, $J = 5.2$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 450.2.

*5-Oxo-N-[2-(4-phenylpiperazin-1-yl)ethyl]-2,4,5, 6-tetrahydro-1H-thiopyrano[3,4-c]quinoline-9-carboxamide (**9h**)*

Yellow solid; yield: 18%; m.p.: 261°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.06 (s, 1H, –C(O)NH–), 11.04 (s, 1H, HCl salt), 9.16 (t, $J = 4.4$ Hz, 1H, Ar-C(O)NH–), 8.38 (s, 1H, Ar-H), 8.02 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.34 (dd, $J = 8.4$ Hz, $J = 2.0$ Hz, 1H, Ar-H), 7.23 (dd, $J = 14.0$ Hz, $J = 6.8$ Hz, 2H, Ar-H), 6.99 (d, $J = 8.0$ Hz, 2H, Ar-H), 6.84 (t, $J = 8.0$ Hz, 1H, Ar-H), 3.82–3.49 (m, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 3.37 (d, $J = 4.0$ Hz, 2H, –NH–CH_2–), 3.22–3.17 (m, 8H, CH_2, thiopyran and $2CH_2$, piperazine and CH_2, –CH_2–piperazine), 2.93 (t, $J = 4.8$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 449.2.

*9-(Piperidine-1-carbonyl)-4,6-dihydro-1H-thiopyrano [3,4-c]quinolin-5(2H)-one (**9i**)*

Yellow solid; yield: 57%; m.p.: 241°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.88 (s, 1H, –C(O)NH–), 7.67 (s, 1H. Ar-H), 7.37 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.30 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.69(s, 2H, thiopyran), 3.67–3.61 (m, 2H, piperidine), 3.29–3.28 (m, 2H, piperidine), 3.10 (t, $J = 5.8$ Hz, 2H, thiopyran), 2.87 (t, $J = 5.8$ Hz, 2H, thiopyran), 1.59 (m, 4H, piperidine), 1.45 (m, 2H, piperidine); MS (ESI+) m/z: $[M+H]^+$ 329.2.

*9-(Piperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9j**)*

Yellow solid; yield: 63%; m.p.: 230°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.04 (s, 1H, –C(O)NH–), 9.24 (br, 1H, HCl salt), 7.82 (s, 1H, Ar-H), 7.56 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.37 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.70–3.60 (m, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 3.16–3.15 (m, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 2.96–2.93 (m, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 330.2.

*9-(4-Methylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9k**)*

Yellow solid; yield: 36%; m.p.: 327°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, –C(O)NH–), 10.75 (br, 1H, HCl salt), 7.80 (s, 1H, Ar-H), 7.56 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.38 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.40 (m, 4H, piperazine) 3.16–3.06 (m, 6H, CH_2, thiopyran, and $2CH_2$, piperazine), 2.96–2.93 (m, 2H, thiopyran), 2.78 (s, 3H, –N–CH_3); MS (ESI+) m/z: $[M+H]^+$ 344.2.

*9-(4-Ethylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9l**)*

Brown solid; yield: 55%; m.p.: 280°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, –C(O)NH–), 10.61 (br, 1H, HCl salt), 7.81 (s, 1H, Ar-H), 7.56 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.38 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.57–3.22 (m, 4H, piperazine), 3.15–3.11 (m, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 3.05–2.93 (m, 4H, CH_2, thiopyran and CH_2, –N–CH_2CH_3), 1.24 (t, $J = 6.7$ Hz, 3H, –N–CH_2CH_3); MS (ESI+) m/z: $[M+H]^+$ 358.2.

*9-(4-Propylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9m**)*

Yellow solid; yield: 56%; m.p.: 298°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.06 (s, 1H, –C(O)NH–), 11.03 (br, 1H, HCl salt), 7.81 (s, 1H, Ar-H), 7.57 (d, $J = 8.3$ Hz, 1H, Ar-H), 7.38 (d, $J = 8.3$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.60–2.93 (m, 14H, $2CH_2$, thiopyran and $4CH_2$, piperazine and CH_2, –N–$(CH_2)_2CH_3$), 1.70 (m, 2H, –N–$(CH_2)_2CH_3$), 0.91 (t, $J = 7.3$ Hz, 3H, –N–$(CH_2)_2CH_3$); MS (ESI+) m/z: $[M+H]^+$ 372.2.

*9-(4-Butylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9n**)*

Yellow solid; yield: 51%; m.p.: 240°C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, –C(O)NH–), 11.13 (br, 1H, HCl salt), 7.81 (s, 1H, Ar-H), 7.57 (d, $J = 8.3$ Hz, 1H, Ar-H), 7.37 (d, $J = 8.3$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.60–2.93 (m, 14H, $2CH_2$, thiopyran and $4CH_2$, piperazine and CH_2, –N–$(CH_2)_3CH_3$), 1.68 (m, 2H, –N–$(CH_2)_3CH_3$), 1.32 (m, 2H, –N–$(CH_2)_3CH_3$), 0.90 (t, $J = 7.3$ Hz, 3H, –N–$(CH_2)_3CH_3$); MS (ESI+) m/z: $[M+H]^+$ 386.2.

*9-(4-(4,4,4-Trifluorobutyl)piperazine-1-carbonyl)-4, 6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9o**)*

Yellow solid; yield: 72%; m.p.: 285°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, –C(O)NH–), 11.38 (br, 1H, HCl salt), 7.81 (s, 1H, Ar-H), 7.58 (d, $J = 8.3$ Hz, 1H, Ar-H), 7.38 (d, $J = 8.3$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.54–2.93 (m, 14H, $2CH_2$, thiopyran and $4CH_2$, piperazine and CH_2, –N–$(CH_2)_3CF_3$), 2.41 (m, 2H, –N–$(CH_2)_3CF_3$), 1.97 (m, 2H, –N–$(CH_2)_3CF_3$); MS (ESI+) m/z: $[M+H]^+$ 440.2.

*9-(4-Pentylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9p**)*

Yellow solid; yield: 59%; m.p.: 305°C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (s, 1H, –C(O)NH–), 11.10 (br, 1H, HCl salt), 7.79 (s, 1H, Ar-H), 7.57 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.36 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.60 (s, 2H, thiopyran), 3.59–2.91 (m, 14H, $2CH_2$, thiopyran and $4CH_2$, piperazine and CH_2, –N–$(CH_2)_4CH_3$), 1.69 (m, 2H, –N–$(CH_2)_4CH_3$), 1.28 (m, 4H, $2CH_2$, –N–$(CH_2)_4CH_3$), 0.87 (t, $J = 7.0$ Hz, 3H, –N–$(CH_2)_4CH_3$); MS (ESI+) m/z: $[M+H]^+$ 400.2.

*9-(4-Isopentylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9q**)*

Yellow solid; yield: 43%; m.p.: 266°C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, –C(O)NH–), 11.56 (br, 1H, HCl salt), 7.80 (s, 1H, Ar-H), 7.57 (d, $J = 8.3$ Hz, 1H, Ar-H), 7.39 (d, $J = 8.3$ Hz, 1H, Ar-H), 3.60 (s, 2H, thiopyran), 3.60–3.40 (m, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 3.15–2.92 (m, 8H, CH_2, thiopyran, $2CH_2$, piperazine, CH_2, –N–$(CH_2)_2CH(CH_3)_2$), 1.61(m, 3H, –N–$(CH_2)_2CH(CH_3)_2$), 0.88 (d, $J = 5.9$ Hz, 6H, –N–$(CH_2)_2CH(CH_3)_2$); MS (ESI+) m/z: $[M+H]^+$ 400.2.

*9-[4-(Pentan-2-yl)piperazine-1-carbonyl]-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9r**)*

Brown solid; yield: 66%; m.p.: 272°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.04 (s, 1H, –C(O)NH–), 10.29 (br, 1H, HCl salt), 7.83 (s, 1H, Ar-H), 7.60 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.38 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.39–3.34 (m, 6H, piperazine), 3.21–3.13 (m, 4H, CH_2, thiopyran and CH_2, piperazine), 2.97–2.95 (m, 2H, thiopyran), 1.84–1.17 (m, 1H, –N–$CHCH_3(CH_2)_2CH_3$), 1.47–1.31 (m, 2H, –N–$CHCH_3(CH_2)_2CH_3$), 1.28–1.12 (m, 5H, –N–$CHCH_3(CH_2)_2CH_3$), 0.94–0.90 (m, 3H, –N–$CHCH_3(CH_2)_2CH_3$); MS (ESI+) m/z: $[M+H]^+$ 400.2.

*9-(4-Hexylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9s**)*

Yellow solid; yield: 47%; m.p.: 170°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, –C(O)NH–), 10.84 (br, 1H, HCl salt), 7.80 (s, 1H, Ar-H), 7.56 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.38 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.56–3.22 (m, 4H, piperazine), 3.15–3.06 (m, 2H, thiopyran), 3.04–2.96 (m, 4H, piperazine), 2.95–2.93 (m, 2H, thiopyran), 1.68–1.60 (m, 2H, –N–$(CH_2)_5$–CH_3), 1.28–1.21 (m, 8H, –N–$(CH_2)_5$–CH_3), 0.87–0.85 (m, 3H, –N–$(CH_2)_5$–CH_3); MS (ESI+) m/z: $[M+H]^+$ 414.2.

*9-(4-Phenylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9t**)*

Dark brown solid; yield: 92%; m.p.: 258°C; ^{1}H NMR (400 MHz, DMSO-d_6); δ 12.04 (s, 1H, –C(O)NH–), 7.84 (s, 1H, Ar-H), 7.61 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.47–7.37 (m, 5H, piperazine-Ar-H), 7.17 (m, 1H, Ar-H), 3.95–3.82 (m, 4H, piperazine), 3.60 (s, 2H, thiopyran), 3.43–3.42 (m, 4H, piperazine), 3.16 (t, $J = 5.2$ Hz, 2H, thiopyran), 2.94 (t, $J = 5.6$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 406.2.

*9-(4-Benzylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9u**)*

Yellow solid; yield: 55%; m.p.: 222°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, –C(O)NH–), 10.82 (br, 1H, HCl salt), 7.79 (s, 1H, Ar-H), 7.57–7.47 (m, 6H, CH, Ar-H and 5CH, benzl), 7.37 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.61 (s, 2H, CH_2, benzyl), 3.56–3.31 (m, 8H, CH_2, thiopyran and $3CH_2$, piperazine), 3.16–3.13 (m, 4H, CH_2, thiopyran and CH_2, piperazine), 2.95 (t, $J = 5.8$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 420.2.

*9-(4-Phenethylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9v**)*

Yellow solid; yield: 31%; m.p.: 179°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.07 (s, 1H, –C(O)NH–), 11.62(br, 1H, HCl salt), 7.82 (s, 1H, Ar-H), 7.58 (d, J = 8.4 Hz, 1H, Ar-H), 7.40–7.21 (m, 6H, CH, Ar-H and 5CH, piperazine-Ar-H), 3.68–3.42 (m, 8H, CH_2, thiopyran and $2CH_2$, piperazine and CH_2, –N–CH_2CH_2), 3.35–3.28 (m, 2H, –N–CH_2CH_2), 3.20–3.07 (m, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 2.95 (t, $J = 5.6$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 434.2.

*9-[4-(Pyridin-4-yl)piperazine-1-carbonyl]-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9w**)*

White solid; yield: 56%; m.p.: 302°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.71 (br, 1H, HCl salt), 12.06 (s, 1H,

–C(O)NH–), 8.29 (d, $J = 6.4$ Hz, 2H, pyridine), 7.84 (s, 1H, Ar-H), 7.60 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.38 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.18 (m, 2H, pyridine), 3.77 (m, 8H, piperazine), 3.61 (s, 2H, thiopyran), 3.16–3.14 (m, 2H, thiopyran), 2.94 (t, $J = 5.2$ Hz, 2H, thiopyran); MS (ESI+) m/z: $[M+H]^+$ 407.1.

*9-(4-Cyclopentylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9x**)*

White solid; yield: 26%; m.p.: 245°C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.06 (s, 1H, –C(O)NH–), 11.68 (s, 1H, HCl salt), 7.82 (s, 1H, Ar-H), 7.58 (d, $J = 8.8$ Hz, 1H, Ar-H), 7.37 (d, $J = 8.0$ Hz, 1H, Ar-H), 3.60 (s, 2H, thiopyran), 3.45 (m, 5H, $2CH_2$, piperazine and CH, cyclopentane), 3.12–3.05 (m, $J = 8.0$ Hz, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 2.94 (t, $J = 5.6$ Hz, 2H, thiopyran), 1.98 (t, $J = 2.2$ Hz, 2H, cyclopentane), 1.83 (br, 2H, cyclopentane), 1.71 (br, 2H, cyclopentane), 1.53 (t, $J = 5.6$ Hz, 2H, cyclopentane); MS (ESI+) m/z: $[M+H]^+$ 398.2.

*9-(4-Cyclohexylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9y**)*

Yellow solid; yield: 31%; m.p.: 243°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (s, 1H, –C(O)NH–), 10.71 (s, 1H, HCl salt), 7.81 (s, 1H, Ar-H), 7.57 (dd, $J = 2.0$ Hz, $J = 7.4$ Hz, 1H, Ar-H), 7.35 (d, $J = 8.80$ Hz, 1H, Ar-H), 3.60 (s, 2H, thiopyran), 3.42 (br, 5H, $2CH_2$, piperazine and CH, cyclohexane), 3.12 (m, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 2.93 (t, $J = 5.8$ Hz, 2H, thiopyran), 2.08–1.09 (m, 10H, cyclohexane); MS (ESI+) m/z: $[M+H]^+$ 412.2.

*9-(4-Cycloheptylpiperazine-1-carbonyl)-4,6-dihydro-1H-thiopyrano[3,4-c]quinolin-5(2H)-one (**9z**)*

Pale yellow solid; yield: 31%; m.p.: 221°C; ^{1}H NMR(400 MHz, DMSO-d_6): δ 12.00 (s, 1H, –C(O)NH–), 11.00 (s, 1H, HCl salt), 7.81 (s, 1H, Ar-H), 7.58 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.35 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.61 (s, 2H, thiopyran), 3.35 (br, 5H, $2CH_2$, piperazine and CH, cycloheptane), 3.13 (m, 6H, CH_2, thiopyran and $2CH_2$, piperazine), 2.93 (t, $J = 5.6$ Hz, 2H, thiopyran), 2.11 (m, 2H, cycloheptane), 1.69–1.50 (m, 4H, cycloheptane), 1.49–1.40 (m, 6H, cycloheptane); MS (ESI+) m/z: $[M+H]^+$ 426.2.

Docking study

All the molecular docking studies were performed using the docking software LigandFit module of Discovery Studio 2.5 (Accelrys, San Diego, CA). In this study, the docking of PARP-1 inhibitors into the active site of human PARP-1 was performed. The crystal structure of human PARP-1 (pdb code: 1UK0) obtained from the Protein Data Bank was refined to remove water molecules and to add hydrogen atoms to the whole enzyme at a pH of 7.0. The ligands were optimized using a forcefield function, CHARMm. Ten poses were docked for each ligand. Among the docked conformations, the over-fitted conformation was eliminated.

PARP-1 enzyme inhibitory activity

The IC_{50} of PARP inhibitor compounds was determined using Trevigen PARP inhibition kit (Gaithersburg, USA). The assay was performed in 384-well, small volume microplates following a modified previously reported method (Lee *et al.*, 2005), as follows.

The PARP-1 enzyme assay was set up in a volume of 12 µl. Wells were coated with diluted histones and incubated at 25°C for 2 h. After the plates were washed four times with PBS, all the liquid was removed following each wash by tapping the plate onto paper towels. To block the nonspecific signal, the wells were blocked by adding the Strep-diluent and incubated at 25°C for 1 h. The plates were washed four times with PBS, and serial dilutions of compound were added. The diluted PARP-1 enzyme was then added to each well (0.12 unit/well), except for the negative control wells, and combined with a 2× PARP cocktail. The reaction was allowed to proceed for 30 min at 25°C. After washing four times with PBS, streptavidin-linked peroxidase was added to detect the extent of ribosylation, and the plate was incubated at 37°C for 30 min. The plates were then washed four times with PBS, then the TACS-Sapphire substrate was added and the color allowed to develop for 10 min. Finally, the reaction was stopped by adding 0.2 *N* HCl, and the optical densities were read at 450 nm using a Wallac EnVision™ plate reader (Perkin-Elmer Oy, Turku, Finland). All the data points were determined in triplicate and the data were analyzed using SigmaPlot 10 (Systat Software Inc., USA).

Intracellular PARP inhibitory activity

This protocol describes a real-time assay to assess an imbalance of DNA single-strand break repair by indirectly measuring PARP activation through the depletion of intracellular NAD(P)H (Nakamura *et al.*, 2003). Chinese hamster ovary cells (CHO-K1) were cultured in RPMI media supplemented with 10% fetal bovine serum (FBS). The cultured CHO-K1 cells were seeded at a density of 2.9×10^3 cells/well in 96-well plate and cultured at 37°C and 5% CO_2 for 16 h. The cells were then treated with various concentrations of the synthesized compounds and

incubated at 37°C for 2 h. DNA damage was induced using 1.5 mM MMS (methyl methanesulfonate), and the cells were simultaneously treated with a CCK-8 (Cell Count Kit-8) solution (DOJINDO, CK01-13) for colorimetric assay. At 4 h after the treatment with MMS, the amount of NAD(P)H secreted into the culture media was quantified using a Wallac EnVision^{TM} plate reader at 450 nm. The results obtained according to various concentrations of the compounds are the average values obtained from four wells, and the results were calculated by regression analysis.

Human liver microsomal stability

All incubations were conducted in triplicate at 37°C in micro-tubes. Incubation mixtures consisted of human liver microsomes (1 mg/ml), NADPH (1 mM) and PARP-1 inhibitor (**9m**, **9q**, or **9s**; 1 μM) in 0.1 M potassium phosphate buffer (pH 7.4). The micro-tubes containing PARP-1 inhibitor (**9m**, **9q**, or **9s**) and β-NADPH were pre-incubated for 5 min at 37°C. The reaction was terminated at 0, 15, 45, and 80 min by the addition of ice-cold acetonitrile containing carbamazepine as the internal standard. The sample was centrifuged at 3,000 rpm for 10 min and the supernatant was analyzed using an API2000 LC/MS/MS system (Applied Biosystems, Concord, Canada) in positive MRM mode. LC/MS/MS data were analyzed by Analyst 1.4.2 (Applied Biosystems, Concord, Canada).

Rat pharmacokinetic study

Male SD rats (8 week) were acclimated to the testing facility in a temperature and humidity controlled condition for approximately a week prior to the study. A jugular vein catheter purchased from Braintree Scientific, Inc. (Braintree, MMA, USA) was surgically implanted into the right jugular vein under ketamine–xylazine anaesthesia. The PARP-1 inhibitor (**9m**) dissolved in saline was dosed intravenously (5 mg/kg) or orally (10 mg/kg) in SD rats ($n = 3$). About 300 μl of blood samples were collected in heparinized micro-tubes at selected times through the jugular vein cannula more than 24 h post-dosing. Blood samples were centrifuged at 3,000 rpm for 10 min and stored in a freezer until analyzed. Plasma samples were prepared for analysis by the following protein precipitation method. One hundred microliters of the plasma samples were transferred to a micro-tube. Three volumes of acetonitrile containing carbamazepine (IS) were added, and the resulting mixture was vortexed for 5 min on a vortexer. The micro-tube was then centrifuged at 12,000 rpm for 10 min and the supernatant was analyzed to quantify the PARP-1 inhibitor (**9m**). Sample analysis was performed by API2000 LC/MS/MS system in the positive MRM mode (Column: Waters Xterra MS C18 (2.1 × 50 mm, 3.5 μm); Mobile phase: acetonitrile/DI water/0.1% formic acid; Ion source: turbo ion spray). Analytical data were processed using Analyst^{TM} 1.4.2. (Applied Biosystems, Concord, Canada). Pharmacokinetic parameters were obtained by non-compartmental analysis of the plasma concentration–time profiles using PK Solution 2.0 (Summit Research Services, Montrose, CO, USA).

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