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Synthesis of 6-alkylsulfanyl-1,4-dihydropyridines as potential multidrug resistance modulators

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Abstract: 6-Alkylsulfanyl-1,4-dihydropyridines **5** bearing methoxyphenyl groups at various positions have been prepared by three different approaches. Multidrug resistance modulating (P-glycoprotein and multidrug resistance protein-1 inhibition) activity of 1,4-dihydropyridine derivatives **5** is comparable to that of verapamil.

Keywords: dihydropyridines; efflux pump inhibition; multidrug resistance (MDR); multidrug resistance protein-1 (MRP1); one-pot reaction; P-glycoprotein (P-gp).

Introduction

Multidrug resistance (MDR) against various anticancer drugs remains an important problem in anticancer drug therapies over the last decades. ABCB-1 transporters [1] are the most studied target for reverting MDR. From all the numerous efforts to overcome MDR, like transcription control of ABCB-1 expression, the most promising approach has been the development of MDR modulators, which are able to increase the intracellular drug levels in co-application with MDR substrates by efflux pump inhibition. Substances of different classes have been used as transport protein inhibitors [1, 2]. The calcium channel blocker verapamil is the most investigated and often used as a reference compound but, unfortunately, cardiotoxicity is observed in combination with actual anticancer drugs [3].

Rational approach to drug design – structural analogy with known active agents – has been used in our research to develop effective MDR modulators on the basis of thieno[2,3-*b*]pyridines [4]. A pharmacophore model has been created assuming one part of verapamil as the linker and methoxyphenyl groups as essential features for the pharmacophore (Figure 1).

We have shown previously that substitution of 3-aminothieno[2,3-*b*]pyridine-5-carboxylate or pyridine-5-carboxylate scaffolds with methoxyphenyl groups (hydrophobic aryl groups and methoxy groups as hydrogen bond acceptors) in position 2 and 4 leads to potent P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP-1) and breast cancer resistance protein (BCRP-1) inhibitors. Such derivatives of 3-aminothieno[2,3-*b*]pyridine-5-carboxylates significantly exceed the activity of verapamil, MK-571 and reversan [4], but the activity of pyridine derivatives is comparable to that of verapamil [5].

Results and discussion

In continuation of our research we used the pharmacophore approach mentioned above to modifying the linker from thieno[2,3-*b*]pyridine and pyridine to 1,4-dihydropyridine (DHP). There are many publications in the last years in which DHPs Ca^{2+} channel blockers are investigated as promising MDR reversal agents [6, 7]. It is known that modification of substituents on the DHP ring can lead to the loss of calcium antagonistic properties [6] which in this case would be a positive result. DHPs **5a–d** were prepared in 73–89% yields by one-pot reaction of ethyl 2-arylmethylidenacetoacetate **1** with 2-cyanothioacetamide (**2**) in the presence of equimolar amount of piperidine (**3**) as base in ethanol followed by subsequent alkylation of the resultant thiolate with substituted 2-bromoacetophenone **4** (Scheme 1, pathway A). In turn, DHPs **5e,f** were prepared in 73–89% yields by treatment of the thiolate **6** (prepared according to the procedure described in [8] and used as crude product) with substituted 2-bromoacetophenone **4** (pathway B). Alternative one-pot preparation was the treatment of ethyl acetoacetate **7** with 2-cyano-3-(3,4-dimethoxyphenyl)thioacrylamide **8** in the presence of equimolar amount of piperidine (**3**) in ethanol followed by alkylation of the intermediate thiolate with 2-bromoacetophenone **4** or 2-iodoacetamide **9**, which gave

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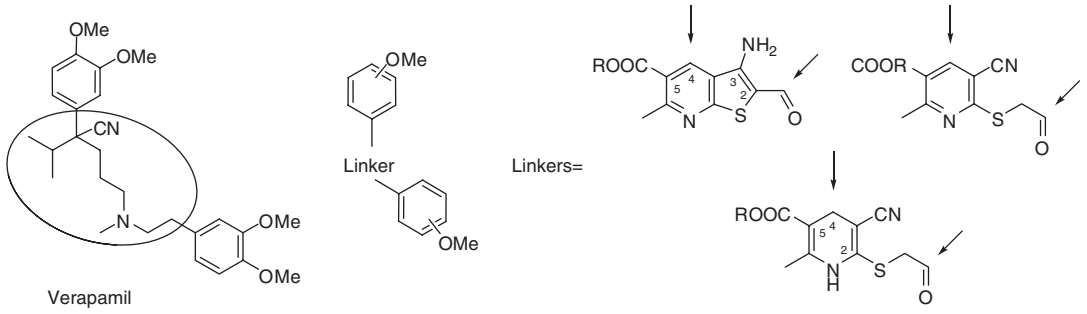
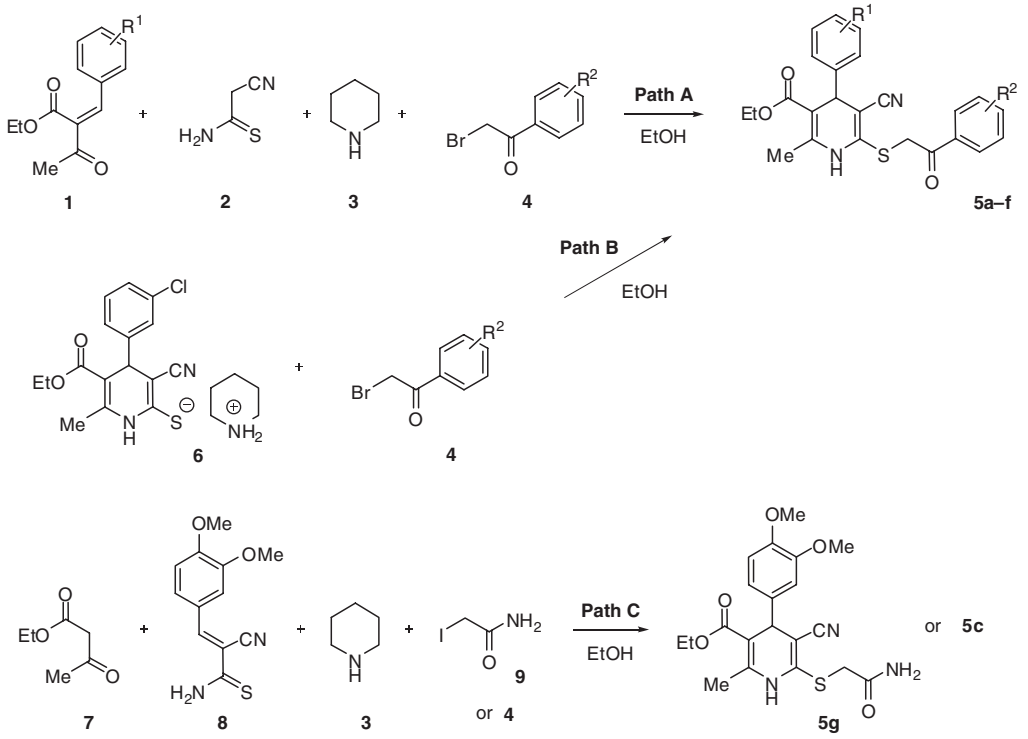


Figure 1 Pharmacophore approach with a modified linker.



a: R^1 = 3,4,5-$(OMe)_3$; R^2 = 4-OMe
b: R^1 = 3,4,5-$(OMe)_3$; R^2 = 2,4-$(OMe)_2$
c: R^1 = 3,4-$(OMe)_2$; R^2 = 2,4-$(OMe)_2$
d: R^1 = 3,4,5-$(OMe)_3$; R^2 = 4-Cl
e: R^1 = 3-Cl; R^2 = 4-Cl
f: R^1 = 3-Cl; R^2 = 4-F

Scheme 1

DHP **5c** and **5g** in 75% and 64% yields (pathway C). As can be seen, the pathway A to **5c** and **5g** is more efficient.

SAR data for the thieno[2,3-*b*]pyridine and pyridine series indicate the necessity of both hydrogen bond donors and hydrogen bond acceptors to reach optimum activity [4]. Pyridines [5] and DHPs **5** are intermediates to thieno[2,3-*b*]pyridines. It was expected that similarly to the thieno[2,3-*b*]pyridine scaffold, the DHP pharmacophore model (Figure 1) the methoxyphenyl groups would play an essential role in activity.

As shown in the Table 1, DHP **5a** at 20 μM concentration displays high P-gp inhibition activity, the activity of DHPs **5c** is comparable, DHPs **5b,d** are slightly less active than verapamil. The aryl group with substituent R^1 in position 4 and benzoylmethylsulfanyl group with substituent R^2 in position 2 of the pyridine ring are essential for MDR modulating activity. The active compound **5a** contains a 3,4,5-$(OMe)_3C_6H_2$ group in position 4 of DHP, while its substitution with 4-$OMeC_6H_4$ [5] or 4-ClC_6H_4 group leads to a reduced activity from 12.0±1.6 (compound **5a**)

Table 1 MDR modulating activity of tested 1,4-dihydropyridine derivatives **5a–g**.

Compound	MDR P-gp, FAR[a] 20 μM	MDR MRP1, FAR[a] 20 μM	Ca^{2+}, A7R5 (IC_{50}, μM)	LD_{50} (mg/kg)
Verapamil	9.4±0.9	1.8±0.4	0.3±0.1	962
5a	12.0±1.6	2.7±0.5	6±0.8	1777
5b	6.7±2.0	3.2±0.5	8±0.7	1820
5c	9.4±1.6	3.7±0.6	6±0.5	2528
5d	6.9±1.6	2.6±0.6	1.9±0.3	2047
5e	1.8	0.8	Not tested	Not tested
5f	2.4	1.3±0.5	Not tested	659
5g	1.2	1.6	Not tested	Not tested

[a]Fluorescence activity ratio (effect is most pronounced when the value is higher).

to 1.8, 2.4 and 1.2 (compounds **5e**, **5f** and **5g**). The highest MDR modulating activity is observed in the case where substituent R^2 in SCH_2COAr group is 4-OMe, but substituents 2,4-$(OMe)_2$, 4-Cl or 4-F lead to a significantly reduced activity.

As shown in Table 1, DHPs **5a–d** at 20 μM concentration are slightly more potent MRP-1 inhibitors than verapamil. Compounds **5e–g** without methoxyphenyl groups appear inactive or display weak potency.

Compounds **5a–d** reveal a weak influence on Ca^{2+} antagonist effect and are less toxic (LD_{50}=1777–2528 mg/kg) in comparison to verapamil (LD_{50}=962 mg/kg). Application of the above mentioned pharmacophore approach with a modified linker to search for new MDR modulators demonstrates that compounds with the 1,4-dihydropyridine core as linker are less active in comparison with the thieno[2,3-*b*]pyridine [4] and pyridine [5] derivatives.

Conclusion

Substituted 6-alkylsulfanyl-1,4-dihydropyridines **5a–g** have been synthesized. These compounds may be considered as analogues of thieno[2,3-*b*]pyridines (highly potent P-gp and MRP1 inhibitors). The MDR modulating (P-gp and MRP1) activity of methoxyphenyl-substituted DHP's **5a–d** is comparable to the reference compound verapamil.

Experimental

Melting points were determined on OptiMelt MPA100 apparatus and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Mercury BB 400 MHz spectrometer. The IR spectra have been recorded on Shimadzu IR Prestige-21 spectrometer in nujol. The progress of the reactions was monitored using silica gel 60 F_{254} plates (Merck) eluting with chloroform/hexane/acetone (2:2:1).

General procedure for synthesis of ethyl 4-aryl-6-(2-aryl-2-oxoethyl)sulfanyl-5-cyano-2-methyl-1,4-dihydropyridine-3-carboxylates 5a–d (pathway A)

A mixture of ethyl 2-[1-(2,3,4-trimethoxyphenyl)-methylidene]-3-oxobutyrate (0.48 g, 1 mmol), 2-cyanothioacetamide (0.1 g, 1 mmol) and piperidine (0.1 mL, 1 mmol) in ethanol (10 mL) was stirred for 10 min at room temperature. Then 2-bromo-4'-methoxyacetophenone (0.23 g, 1 mmol) was added and the resulting mixture was heated under reflux for 5 min. The precipitated crystals were separated by filtration and washed with EtOH and water to give analytically pure product **5a–d**.

Ethyl 5-cyano-6-{[2-(4-methoxyphenyl)]-2-oxoethylsulfanyl}-2-methyl-4-(3,4,5-trimethoxyphenyl)-1,4-dihydropyridine-3-carboxylate (5a) Colorless crystals; yield 88%; mp 141–143°C; IR: 1637, 1696, 2198, 3183 cm^{-1}; ^{1}H NMR: δ 1.11 and 4.00 (t and q, 5H, *J* = 7.0 Hz), 2.37 (s, 3H), 3.75 (s, 9H), 3.84 (s, 3H), 4.00 and 4.34 (d and d, 2H, *J* = 16.6 Hz), 4.58 (s, 1H), 6.38 (s, 2H), 6.92 and 7.88 (d and d, 4H, *J* = 8.6 Hz), 8.51 (s, 1H). Anal. Calcd for $C_{28}H_{30}N_2O_7S$: C, 62.44; H, 5.61; N, 5.20. Found: C, 62.15; H, 5.66; N, 4.95.

Ethyl 5-cyano-6-{[2-(2,4-dimethoxyphenyl)]-2-oxoethylsulfanyl}-2-methyl-4-(3,4,5-trimethoxyphenyl)-1,4-dihydropyridine-3-carboxylate (5b) Colorless crystals; yield 88%; mp 140–142°C; IR: 1692, 2197, 3273 cm^{-1}; ^{1}H NMR: δ 1.17 and 4.07 (t and q, 5H, *J* = 7.0 Hz); 2.44 (s, 3H); 3.81 (s, 9H), 3.89 and 3.92 (2s, 6H), 4.07 and 4.43 (dd, 2H, *J* = 16.00 Hz), 4.63 (s, 1H), 6.44 (s, 2H), 6.59–7.90 (m, 3H), 8.69 (s, 1H). Anal. Calcd for $C_{29}H_{32}N_2O_8S$: C, 61.25; H, 5.67; N, 4.93. Found: C, 61.14; H, 5.66; N, 4.97.

Ethyl 5-cyano-4-(3,4-dimethoxyphenyl)-6-{[2-(2,4-dimethoxyphenyl)]-2-oxoethylsul-fanyl}-2-methyl-1,4-dihydropyridine-3-carboxylate (5c) Colorless crystals; yield 73%; mp 114–116°C; IR: 1641, 1695, 2197, 3280 cm^{-1}; ^{1}H NMR: δ 1.10 and 3.99 (t and q, 5H, *J* = 7.0 Hz), 2.37 (s, 3H), 3.77 and 3.86 (2s, 6H), 3.80 (s, 6H), 4.36 (dd, 2H, *J* = 15.6 Hz), 4.58 (s, 1H), 6.40–7.86 (m, 6H), 8.60 (s, 1H). Anal. Calcd for $C_{28}H_{30}N_2O_7S$: C, 62.44; H, 5.61; N, 5.20. Found: C, 61.94; H, 5.56; N, 5.29.

Ethyl 6-{[2-(4-chlorophenyl)]-2-oxoethylsulfanyl}-5-cyano-2-methyl-4-(3,4,5-trimeth-oxyphenyl)-1,4-dihydropyridine-3-carboxylate (5d) Colorless crystals; yield 89%; mp 67–69°C; IR: 1695, 1699, 2199, 3299 cm^{-1}; ^{1}H NMR: δ 1.16 and 4.06 (t and q, 5H, *J* = 7.0 Hz), 2.42 (s, 3H), 3.80 (s, 9H), 4.05 and 4.40 (dd, 2H, *J* = 16.3 Hz, SCH_2), 4.62 (s, 1H, 4-H), 6.42 (s, 2H), 7.49 and 7.89 (dd, 4H, *J* = 8.4 Hz), 8.02 (s, 1H). Anal. Calcd

for $C_{27}H_{27}ClN_2O_6S$: C, 59.72; H, 5.01; N, 5.16; S, 5.90. Found: C, 59.36; H, 4.94; N, 5.22; S, 6.46.

General procedure for synthesis of ethyl 4-aryl-6-(2-aryl-2-oxoethyl)sulfanyl-3-cyano-2-methyl-1,4-dihydropyridine-3-carboxylates 5 e,f (pathway B)

A mixture of piperidinium 4-(3-chlorophenyl)-3-cyano-5-ethoxycarbonyl-6-ethyl-1,4-dihydropyridine-2-tiolate [8] (0.84 g, 2 mmol) and 2-bromo-1-(4-chlorophenyl)ethanone (0.47g, 2 mmol) in ethanol (10 mL) was was shortly heated under reflux and then stirred at room temperature for 30 min. The resultant crystals were separated by filtration and washed with ethanol and water to give analytically pure product **5e,f**.

Ethyl 4-(3-chlorophenyl)-6-[2-(4-chlorophenyl)-2-oxo-ethyl] sulfanyl-5-cyano-2-methyl-1,4-dihydropyridine-3-carboxylate (5e) Colorless crystals; yield **88**%; mp 142–144°C; IR: 1683, 2200, 2981, 3277 cm^{-1}; ^1H NMR: δ 1.06 and 3.96 (t and q, 5H, J = 7 Hz), 2.36 (s, 3H,), 4.02 and 4.33 (d and d, 2H, J = 16.8 Hz), 4.60 (s, 1H), 7.05–7.83 (m, 8H), 8.03 (s, 1H). Anal. Calcd for $C_{24}H_{20}N_2O_3SCl_2$: C, 59.14; H, 4.14; N, 5.75. Found: C, 58.87; H, 4.09; N, 5.69.

Ethyl 4-(3-chlorophenyl)-5-cyano-6-[2-(4-fluorophenyl)-2-oxo-ethyl]sulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (5f) Colorless crystals; yield **81**%; mp 141–142°C; IR: 1676, 2200, 2981, 3291 cm^{-1}; ^1H NMR: δ 1.06 and 3.96 (t and q, 5H, J = 7 Hz); 2.37 (s, 3H), 4.01 and 4.34 (dd, 2H, J = 16.8 Hz), 4.62 (s, 1H), 7.06–7.17 and 7.94 (m, 8H), 8.12 (s, 1H). Anal. Calcd for $C_{24}H_{20}N_2O_3SClF$: C, 61.21; H, 4.28; N, 5.95. Found: C, 61.07; H, 4.23; N, 5.91.

General procedure for synthesis of ethyl 4-aryl-2-(2-aryl-2-oxoethyl)sulfanyl-3-cyano-6-methyl-1,4-dihydropyridine-3-carboxylates 5c,g (pathway C)

A mixture of 2-cyano-3-(3,4-dimetoxyphenyl)thioacrylamide (0.37 g, 1.5 mmol), ethyl acetoacetate (0.20 g, 1.5 mmol) and piperidine (0.15 mL, 0.15 mmol) in ethanol (5 mL) was stirred at room temperature for 15 min, during which time precipitation of piperidinium thiolate was observed. After addition of 2′,4′-dimethoxy-2-bromoacetophenone or iodoacetamide the mixture was shortly heated under reflux, stirred at room temperature for 20 min and then treated with 0.5 mL of 3N HCl in ethanol. The precipitated crystals were separated by filtration and washed with ethanol and water to give analytically pure product **5c,g**.

Ethyl 6-carbamoylmethylsulfanyl-5-cyano-4-(3,4-dimetoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxylate (5g) Colorless crystals; yield 64%; mp 191–193°C; IR: 1676, 1702, 2198, 3160, 3326 cm^{-1}; ^1H NMR: δ 1.06 and 3.92 (t and q, 5H, J = 7.04 Hz), 2.27 (s, 3H); 3.66 (6H, s), 3.56 and 3.72 (d and d, 2H, J = 15.2 Hz), 4.39 (s, 1H, 4-H), 6.61, 6.67 and 6.83 (4d, 3H, J = 8.2 Hz and 1.6 Hz), 7.51 and 7.83 (2s, 2H), 10.23 (s, 1H, NH). Anal. Calcd for $C_{20}H_{23}N_3O_5S$: C, 57.54; H, 5.55; N, 10.06; Found: C, 57.37; H, 5.35; N, 9.75.

Inhibition activities of compounds 5a–g

Measurement of P-gp and MRP-1 inhibition activities by compounds **5a–g** were carried out according to the procedure described in [4]. As Ca^{2+} channel blocker, verapamil in combination with actual anticancer drugs reveals cardiotoxicity [3]; the influence of some obtained DHP **5** on the cardiovascular system as well as their toxicity were tested according to the procedure described in [4].

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