

# 1-Benzyl-1,2,3,4-tetrahydroisoquinoline Derivatives and Optically Pure Isomers as Novel Promising Multidrug Resistance (MDR) Reversing Agents

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**Abstract:** A series of chiral compounds were designed and synthesized as novel multidrug resistance (MDR) modulators on the basis of tetrahydroisoquinolines to reverse cancerous MDR on K562 cells and K562/DOX cells by using the MTT assay. The treatment of K562/DOX cells with **9e**, **10a** and **10e** led to increased intracellular accumulation and decreased efflux of doxorubicin. The pharmacological effects of these tetrahydroisoquinolines on P-glycoprotein (P-gp) mediated MDR were much stronger than that of positive control drug verapamil.

**Keywords:** Tetrahydroisoquinoline, Optical pure isomers, Multidrug resistance, P-glycoprotein, Synthesis, Reversing agents.

## INTRODUCTION

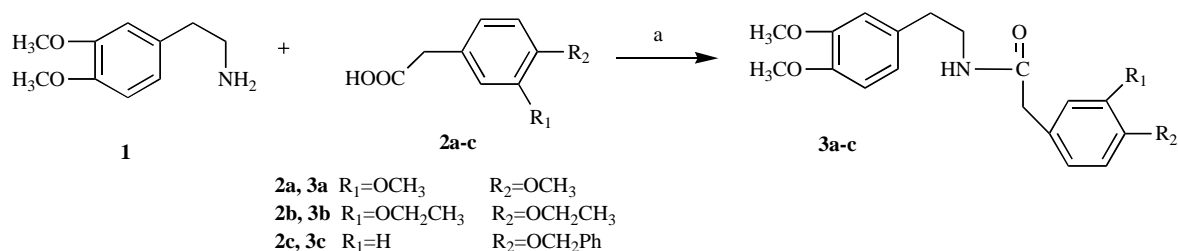
Multidrug Resistance (MDR) has been considered as one of the major obstacles for successful chemotherapy in patients with cancers. It is a type of acquired drug resistance of tumor cells to various kinds of chemotherapeutic agents with different structural and mechanical features. Although the molecular mechanisms leading to MDR include the activation of transport and detoxification systems, enhancement of target repair activities, alterations of drug targets, and dysregulation of cells death pathways, the over-expression of a variety of proteins that act as ATP-dependent extrusion pumps such as P-gp has always been thought to be concerned with this phenomenon [1]. The efflux function of P-gp can be responsible for the decrease of drug concentrations in tumor cells, resulting in chemotherapeutic failure [2]. Much work to overcome MDR by influencing transporter expressions *via* signal transduction pathways or by direct transcriptional control has been done and proven unsuccessful in clinical trials. Verapamil [3] was the first and also the most typical P-gp inhibitor. Tsuruo *et al.* demonstrated that this calcium channel blocker possessed potent activity on reversal of MDR through inhibition of P-gp [4, 5]. Furthermore, a number of other structurally unrelated compounds such as cyclosporin [3], phenothiazines, antimalarials have also been demonstrated to be effective P-gp inhibitors. However, many of these compounds could not enter into clinical trails due to their unacceptable toxicities for antitumor treatment or nonspecific and weak inhibitory effect on P-gp. Therefore, many efforts should be devoted to identifying new kinds of P-gp inhibitors with higher selectivity and stronger potency.

Recently, the natural alkaloid tetrandrine [6] was found to inhibit P-gp, attributing this activity to its bisbenzylisoquinoline scaffold. It has been shown that isoquinoline alkaloid could effectively reverse MDR with low side effects [7, 8]. Based on these observations, many tetrahydroisoquinoline derivatives were designed and synthesized for the search of novel calcium channel blockers by simplifying and optimizing bisbenzylisoquinoline alkaloid tetrandrine. Studies on these novel derivatives have shown that 6,7-dimethoxy-1-(3,4-dimethoxy)benzyl-2-(*N*-n-octyl-*N'*-cyano)guanyl-1,2,3,4-tetrahydroisoquinoline is more active than verapamil and dose not have cardiovascular activity [9]. These derivatives are racemates and the question of the stereoselectivity of transport mediated by P-gp is of relevance [10]. Additionally, according to some fundamental biochemical studies, it could be speculated that P-gp has multiple substrate-binding sites and an allosteric regulatory site, although the number of sites, their chemical selectivities, and their mutual relationships remain uncertain [11]. Based on these facts, we report a new kind of tetrahydroisoquinoline derivatives containing *N*-substituted aminoacetyl groups may further enhance its bioactivity. In an attempt to develop novel and more potent P-gp inhibitors, a number of tetrahydroisoquinoline derivatives were designed and synthesized and their structure-activity relationship against P-gp activity was explored. To investigate the influence of stereoselectivity on pharmacological properties, individual enantiomers of tetrahydropaverine were prepared in comparison with racemate at low or high substrate concentration. The MDR reversal activities of the tetrahydroisoquinoline analogues *in vitro* against K562/DOX cell line [12], a human leukemia cell line, were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay [13].

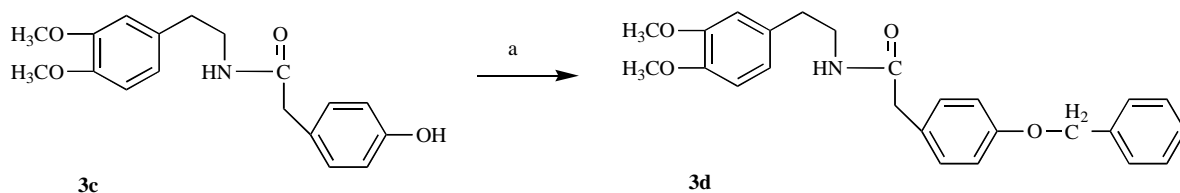
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## SYNTHESIS

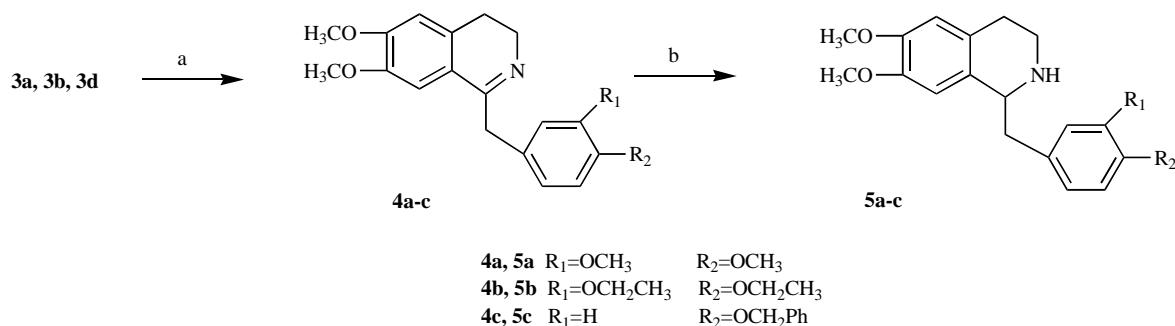
Homoveratrylamine **1** and phenylacetic acid **2a-c** (3,4-dimethoxyphenylacetic acid, 3,4-diethoxyphenylacetic acid,



**Scheme 1.** Reagents: (a) 180 °C, N<sub>2</sub>.



**Scheme 2.** Reagents: (a) TBAB, NaOH, Water, Benzyl chloride, 100 °C.



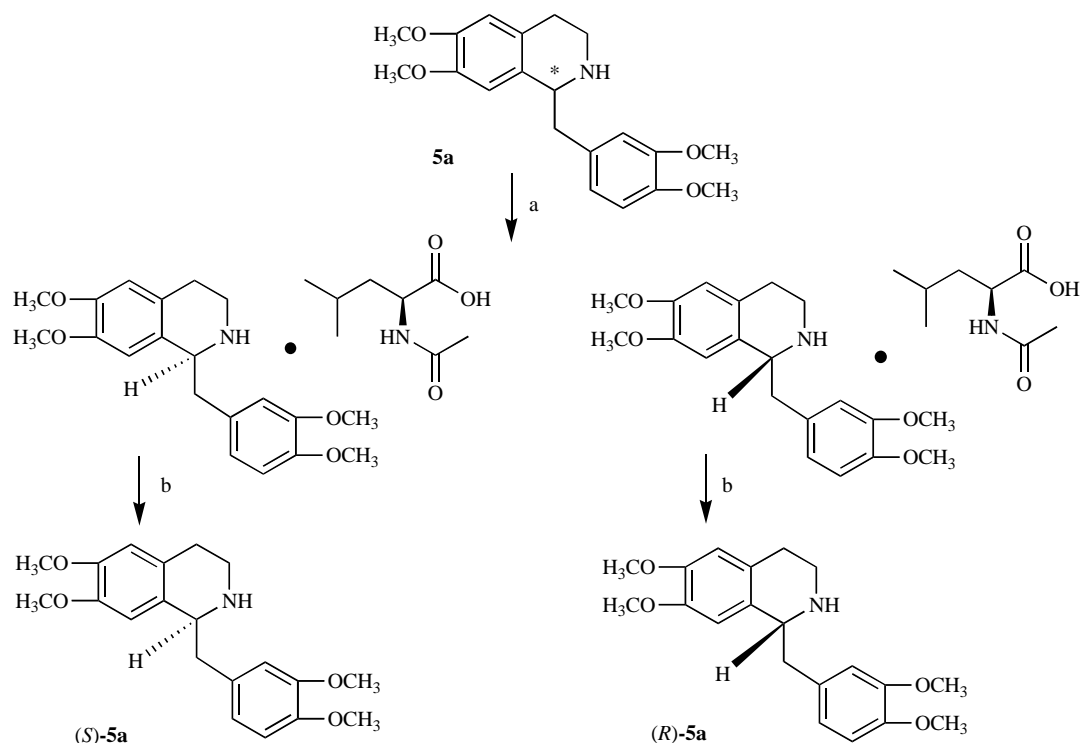
**Scheme 3.** Reagents: (a) POCl<sub>3</sub>, Toluene, 110 °C (b) KBH<sub>4</sub>, MeOH, RT.

4-hydroxyphenylacetic acid) provided phenylacetamides **3a-c** (Scheme (1)). The next conversion from **3c** to **3d** was performed by applying tetrabutylammonium bromide (TBAB) [14], a liquid-liquid phase transfer catalyst (LL-PTC), which catalyzed the reaction between benzyl chloride and phenol **3c** to afford **3d** in an alkaline solution of NaOH and organic solvents (Scheme (2)). The first step of the reaction is the yielding of sodium phenoxide (PhO<sup>-</sup>Na<sup>+</sup>) by reacting phenol with sodium hydroxide in the aqueous phase. Compound **3d** was provided by treatment of phenoxide with **3c** in the organic solvent. The synthesis of 3,4-dihydroisoquinoline analogues **4a-c** was achieved from the corresponding phenylacetamides **3a, 3b** and **3d** in the presence of the dehydrating agent phosphoryl chloride in toluene (Scheme (3)). The imine double bond in compounds **4a-c** provided a prochiral environment for the enantio-selective formation. Imines **4a-c** were subsequently reduced by potassium tetrahydroborate with triethylamine in refluxing methanol for 24 h, affording tetrahydroisoquinolines **5a-c** in over 80% yields.

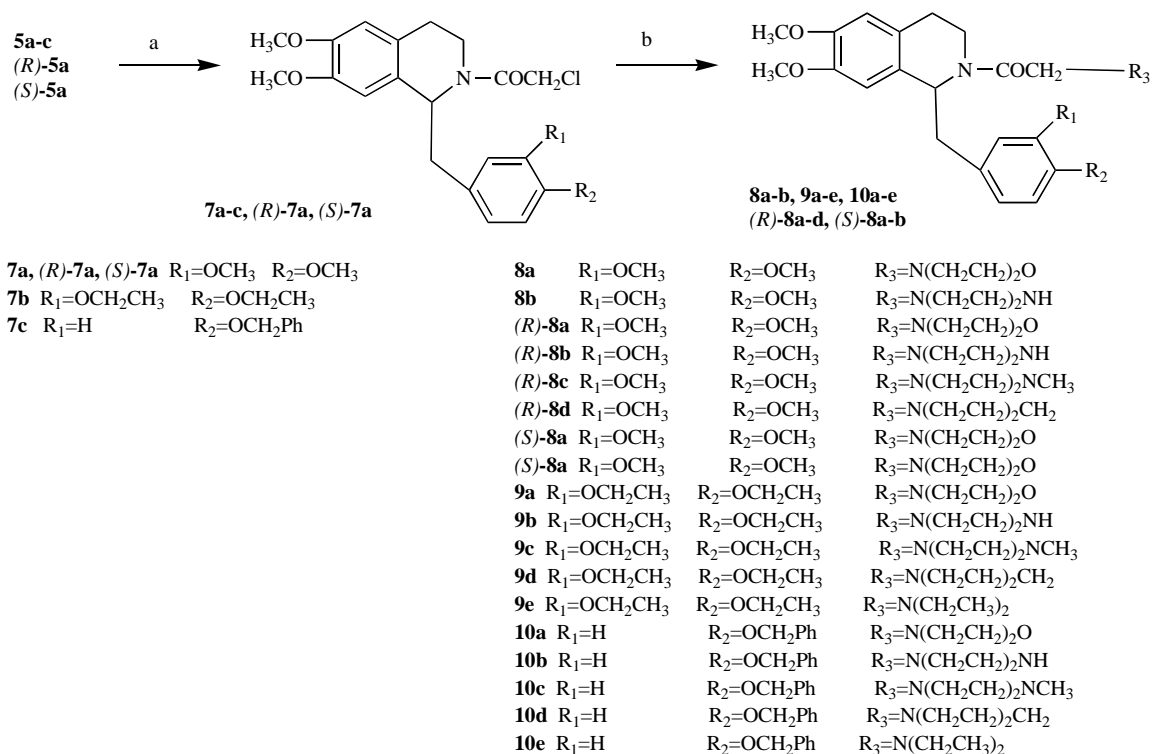
The carbon atom on the tetrahydroisoquinoline scaffold of **5a-c**, as a stereogenic center, has a particular optical isomeric configuration leading to two optically active forms: (*R*) and (*S*). In order to investigate the chiral effects on pharmacological properties, two optical isomers of tetrahydropapaverine **5a** were obtained *via* chemical resolution using *N*-acetyl-*L*-leucine as a chiral resolution reagent [15].

The chiral resolution reagent **6** was prepared by acetylation of commercially available *L*-leucine with acetic anhydride, followed by crystallization of the crude product in water solvent (Scheme (4)). The chemical resolution with racemate **5a** and *N*-acetyl-*L*-leucine can produce their diastereoisomer salts with optical active (*R*)- or (*S*)-tetrahydropapaverine *N*-acetyl-*L*-leucinate, respectively, which were further purified by recrystallization in acetone. The optical active (*R*)- or (*S*)-tetrahydropapaverine *N*-acetyl-*L*-leucinate was alkalinized with aqueous ammonia to obtain the individual enantiomer (*R*)- or (*S*)-**6a** in a good yield and an excellent stereoselectivity (> 99% as indicated by chiral high performance liquid chromatography).

The key intermediates **7a-c**, (*R*)-**7a**, (*S*)-**7a** were prepared by the reaction between the corresponding tetrahydroisoquinolines **5a-c**, (*R*)-**5a**, (*S*)-**5a** and chloroacetyl chloride. Potassium carbonate in the reaction played a role in absorbing the liberating HCl gas and neutralizing the whole reaction system. The final racemates **8a-b**, **9a-e**, **10a-e** were obtained from the alkylation of corresponding amines (diethylamine, morpholine, piperazine, pyrrole, *N*-methylpiperazine). Six chiral derivatives of tetrahydropapaverine (*R*)-**8a-d**, (*S*)-**8a-b** were given by using the same procedure (Scheme (5)). All target compounds were characterized by <sup>1</sup>H NMR, mass spectra and elemental analyses [16].



**Scheme 4.** Reagents: (a) *N*-acetyl-*L*-leucine, MeOH, reflux (b) NaOH, Ether, RT.



**Scheme 5.** Reagents: (a)  $\text{ClCH}_2\text{COCl}$ ,  $\text{K}_2\text{CO}_3$ , Benzene, RT (b) Amine(s),  $\text{K}_2\text{CO}_3$ , EtOH, reflux.

## BIOLOGICAL TESTS

The cytotoxicity [17] and MDR reversal activities [18] of tetrahydroisoquinoline derivatives and their chiral enantiomers were evaluated by the MTT colorimetric assay [13] with the required concentration. The MDR reversal activities

of all the target compounds *in vitro* were listed in Table 1. When tested in combination with doxorubicin against the K562/DOX cell line at 10  $\mu\text{M}$ , compounds **9e**, **10a** and **10e** exhibited a well-defined trend in MDR reversal activities. Compared to the other *N*-substituted acetyl position tetrahy-

**Table 1.** Inhibitory Effects of Tetrahydroisoquinoline Compounds on the Proliferation of K562/DOX Cell Line by MTT Assay ( $\bar{X} \pm s$ , n = 3)

Compd	Conc.	IR	IC <sub>50</sub>	RF
verapamil	10		2.35	11.7
<b>8a</b>	10	-7.57 ± 0.07	8.08	3.41
<b>8b</b>	20	12.50 ± 0.02	24.19	1.14
( <i>R</i> )- <b>8a</b>	20	9.80 ± 0.09	25.12	1.10
( <i>R</i> )- <b>8b</b>	10	3.78 ± 0.06	22.37	1.23
( <i>R</i> )- <b>8c</b>	20	-2.69 ± 0.03	15.81	1.74
( <i>R</i> )- <b>8d</b>	10	-4.77 ± 0.04	34.16	0.81
( <i>S</i> )- <b>8a</b>	40	4.20 ± 0.01	10.70	2.57
( <i>S</i> )- <b>8b</b>	40	5.31 ± 0.01	40.67	0.68
<b>9a</b>	10	2.96 ± 0.06	5.00	5.50
<b>9b</b>	10	-15.83 ± 0.05	14.15	1.95
<b>9c</b>	20	0.43 ± 0.12	18.37	1.50
<b>9d</b>	40	-6.82 ± 0.15	3.61	7.62
<b>9e</b>	40	-6.04 ± 0.17	2.08	13.25
<b>10a</b>	10	2.37 ± 0.13	1.70	16.21
<b>10b</b>	10	10.11 ± 0.12	10.97	2.51
<b>10c</b>	2.5	0.90 ± 0.01	3.45	7.97
<b>10d</b>	2.5	6.62 ± 0.08	2.60	10.60
<b>10e</b>	2.5	-0.64 ± 0.06	2.05	13.45

Conc.: concentration (μM).

IR: inhibitory ratio of compounds against K562 cell lines (%).

IC<sub>50</sub>: half inhibiting concentration (μM).

RF: reversal fold.

droisoquinolines, compounds with morpholine (**10a**) or diethylamine (**10e**) group exhibit were more potent. The compound **9e**, also containing diethylamine group, showed almost the same MDR activity as compound **10e**. Considering their different chemical structures, these results indicated that morpholine and diethylamine groups displayed an essential factor in the interaction of compounds with P-gp by providing an appropriate hydrophilicity which was required by P-gp substrates for better binding with the amine side-chain. Furthermore, in comparison to compounds with 3- and 4-methoxy groups, the substitution of 3- and 4-ethoxy group or O-CH<sub>2</sub>-phenyl on the 1-benzyl position played a significant part to increase the activity of MDR. The optical tetrahydroisoquinolines **8a**, **8b** and their racemates (*R*)-**8a-d**, (*S*)-**8a-b**, at the concentrations tested, displayed minimal modulatory activity.

Hence, the conclusion could be obtained that the phenoxy and amino-acetyl substituent on tetrahydroisoquinoline can enhance its interaction with P-gp and prevent its substrate efflux activity. But the chirality of this series of compounds was independent of MDR modulation activity. The mode of interaction with these modified tetrahydroisoquinolines appears to be quite different, given the broad differences noted in cytotoxicity, inhibition ratio, half inhibiting concentration and reversal fold. In summary, the *N*-substituted tetrahydroiso-

quinolines are considered as very promising modulators of P-gp function and warrant further exploration.

## CONCLUSION

In this work, BNR was applied to the synthesis of several tetrahydroisoquinoline derivatives. These compounds bearing a quaternary carbon atom were evaluated for their MDR reversal activities against K562/DOX cell line *in vitro*. The results showed that compounds **9e**, **10a**, and **10e** exhibited higher MDR reversal activities than verapamil. These compounds all belonged to racemate, and among all the tetrahydro-papaverine derivatives, both enantiomers and racemates had the low MDR reversal activities. Further biological evaluation, structure-activity relationship and mechanistic studies on this new class of compounds are currently in progress.

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## REFERENCES AND NOTES

- [1] Szakács, G.; Paterson, J.K.; Ludwig, J.A.; Booth-Genthe, C.; Gottesman, M.M. Targeting multidrug resistance in cancer. *Nat. Rev. Drug Discov.*, **2006**, 5(3), 219-234.
- [2] Jia, L.; Allen, P.D.; Macey, M.G.; Grahn, M.F.; Newland, A.C.; Kelsey, S.M. Mitochondrial electron transport chain activity, but not ATP synthesis, is required for drug-induced apoptosis in human leukaemic cells: a possible novel mechanism of regulating drug resistance. *Br. J. Haematol.*, **1997**, 98(3), 686-698.
- [3] Saeki, T.; Ueda, K.; Tanigawara, Y.; Hori, R.; Komano, T. Human P-glycoprotein transports cyclosporin A and FK506. *J. Biol. Chem.*, **1993**, 268(9), 6077-6080.
- [4] Tsuruo, T.; Iida, H.; Tsukagoshi, S. Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res.*, **1981**, 41(5), 1967-1972.
- [5] Tsuruo, T.; Iida, H.; Kawataba, H.; Oh-hara, T.; Hamada, H.; Utakoji, T. Characteristics of resistance to adriamycin in human myelogenous leukemia K562 resistant to adriamycin and in isolated clones. *Jpn. J. Cancer Res.*, **1986**, 77(7), 682-692.
- [6] Xu, W. L.; Shen, H. L.; Ao, Z. F.; Chen, B. A.; Xia, W.; Gao, F.; Zhang, Y. N. Combination of tetrandrine as a potential-reversing agent with daunorubicin, etoposide and cytarabine for the treatment of refractory and relapsed acute myelogenous leukemia. *Leuk. Res.*, **2006**, 30(4), 407-413.
- [7] Choi, S. U.; Park, S. H.; Kim, K. H. The bisbenzyl-isoquinoline alkaloids, tetrandine and fangchinoline, enhance the cytotoxicity of multidrug resistance-related drugs via modulation of P-glycoprotein. *Anticancer Drugs*, **1998**, 9(3), 255-261.
- [8] Tian, H.; Pan, Q.C. Modulation of multidrug resistance by three bisbenzyl-isoquinolines in comparison with verapamil. *Acta Pharmacol. Sin.*, **1997**, 18(5), 455-458.
- [9] Li, Y.; Zhang, H. B.; Huang, W. L.; Li, Y. M. Design and synthesis of tetrahydroisoquinoline derivatives as potential multidrug resistance reversal agents in cancer. *Bioorg. Med. Chem. Lett.*, **2008**, 18(12), 3652-3655.
- [10] Yan, F.; Jiang, Y.; Li, Y.M.; Zhen, X.; Cen, J.; Fan, W.R. Reversal of P-glycoprotein and multidrug resistance-associated protein 1 mediated multidrug resistance in cancer cells by HZ08 isomers, tetrahydroisoquinolin derivatives. *Biol. Pharm. Bull.*, **2008**, 31(6), 1258-1264.
- [11] Martin, C.; Berridge, G.; Higgins, C.F.; Mistry, P.; Charlton, P.; Callaghan, R. Communication between multiple drug binding sites on P-glycoprotein. *Mol. Pharmacol.*, **2000**, 58(3), 624-632.
- [12] The drug sensitive human leukemia cell line, K562, and its drug-resistant variant, K562/DOX, were cultured in RPMI-1640 medium supplemented with 10% FBS at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> incubator. The K562/DOX cells were grown in the presence of 1 µg/mL doxorubicin for at least 14 d before used.
- [13] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity. *J. Immunol. Methods*, **1983**, 65(1-2), 55-63.
- [14] Yadav, G.D.; Ceasar, J.L. Novelty of cyanide displacement reaction in ibuprofen amide process by phase transfer catalysis: Solid-liquid versus solid-liquid (omega)-liquid systems. *J. Mol. Catal. A Chem.*, **2006**, 260(1-2), 202-209.
- [15] Hill, D.A.; Turner, G.L. Neuromuscular blocking agents. US Patent 5453510, 1995.
- [16] The tested compounds were purified by silica gel column chromatography, eluting with a mixture of ethyl acetate and petroleum ether. The compounds were characterized by elemental analysis with satisfactory results. Their <sup>1</sup>H NMR and MS spectra were in agreement with the assigned structures. Physical and spectral properties of compounds **8a-b**, **9a-e**, **10a-e**, (*R*)-**8a-d**, (*S*)-**8a-b** are given. <sup>1</sup>H NMR data were obtained at 300MHz, using CDCl<sub>3</sub> as solvent.  
**8a**: mp: 156-160 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.85-6.47 (m, 5H), 5.67, 5.17, 4.75 (m, 1H, CH), 3.84 (s, 6H, 2×OCH<sub>3</sub>), 3.82 (s, 6H, 2×OCH<sub>3</sub>), 3.72 (s, 2H, CH<sub>2</sub>CO), 3.65 (m, 4H, O(CH<sub>2</sub>)<sub>2</sub>), 3.47 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.21-2.51 (m, 4H, PhCH<sub>2</sub>, CHCH<sub>2</sub>), 2.40 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>). MS (ESI, m/z): 471.3 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.56; H, 7.30; N, 5.97.  
**8b**: mp: 64-68 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.86-6.27 (m, 5H), 5.50, 4.75 (m, 1H, CH), 3.83 (s, 6H, 2×OCH<sub>3</sub>), 3.81 (s, 6H, 2×OCH<sub>3</sub>), 3.67 (s, 2H, CH<sub>2</sub>CO), 3.64-3.33 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.12-2.60 (m, 8H, PhCH<sub>2</sub>, CHCH<sub>2</sub>, HN(CH<sub>2</sub>)<sub>2</sub>), 3.09 2.39, 2.29 (d, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 2.09 (brs, 1H, NH). MS (ESI, m/z): 470.3 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>26</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub>: C, 66.50; H, 7.51; N, 8.95. Found: C, 66.70; H, 7.53; N, 8.98.  
(*R*)-**8a**: mp: 140-144 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.85-6.47 (m, 5H), 5.67, 5.17, 4.75 (m, 1H, CH), 3.84 (s, 6H, 2×OCH<sub>3</sub>), 3.82 (s, 6H, 2×OCH<sub>3</sub>), 3.71 (s, 2H, CH<sub>2</sub>CO), 3.65 (m, 4H, O(CH<sub>2</sub>)<sub>2</sub>), 3.47 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.21-2.51 (m, 4H, PhCH<sub>2</sub>, CHCH<sub>2</sub>), 2.40 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>). MS (ESI, m/z): 471.3 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>: C, 66.50; H, 7.28; N, 5.95. Found: C, 66.57; H, 7.30; N, 5.98.  
(*R*)-**8b**: mp: 106-110 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.86-6.27 (m, 5H), 5.50, 4.75 (m, 1H, CH), 3.80 (s, 6H, 2×OCH<sub>3</sub>), 3.81 (s, 6H, 2×OCH<sub>3</sub>), 3.67 (s, 2H, CH<sub>2</sub>CO), 3.64-3.33 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.12-2.60 (m, 8H, PhCH<sub>2</sub>, CHCH<sub>2</sub>, HN(CH<sub>2</sub>)<sub>2</sub>), 3.09 2.39, 2.29 (d, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 2.08 (brs, 1H, NH). MS (ESI, m/z): 470.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>26</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub>: C, 66.50; H, 7.51; N, 8.95. Found: C, 66.72; H, 7.53; N, 8.97.  
(*R*)-**8c**: mp: 135-139 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.78-6.18 (m, 5H), 5.60, 5.24, 4.69 (m, 1H, CH), 3.91(s, 6H, 2×OCH<sub>3</sub>), 3.81 (s, 6H, 2×OCH<sub>3</sub>), 3.60 (s, 2H, CH<sub>2</sub>CO), 3.39-3.15 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.07-2.59 (m, 8H, PhCH<sub>2</sub>, CHCH<sub>2</sub>, N(CH<sub>2</sub>)<sub>2</sub>), 2.35 (d, 4H, CH<sub>3</sub>N(CH<sub>2</sub>)<sub>2</sub>), 2.24 (s, 3H, CH<sub>3</sub>). MS (ESI, m/z): 484.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>27</sub>H<sub>37</sub>N<sub>3</sub>O<sub>5</sub>: C, 67.06; H, 7.71; N, 8.69. Found: C, 67.26; H, 7.73; N, 8.72.  
(*R*)-**8d**: mp: 97-103 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.84-6.21 (m, 5H), 5.62, 5.13, 4.74 (m, 1H, CH), 3.87 (s, 6H, 2×OCH<sub>3</sub>), 3.79 (s, 6H, 2×OCH<sub>3</sub>), 3.63 (s, 2H, CH<sub>2</sub>CO), 3.45-2.59 (m, 6H, PhCH<sub>2</sub>CH<sub>2</sub>, PhCH<sub>2</sub>, CHCH<sub>2</sub>), 2.46 (d, *J* = 9.6 Hz, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 1.73 (d, *J* = 9.6 Hz, 4H, (CH<sub>2</sub>)<sub>2</sub>). MS (ESI, m/z): 455.3 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>5</sub>: C, 68.70; H, 7.54; N, 6.16. Found: C, 68.91; H, 7.56; N, 6.18.  
(*S*)-**8a**: mp: 251-254 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.85-6.47 (m, 5H), 5.67, 5.17, 4.75 (m, 1H, CH), 3.82 (s, 6H, 2×OCH<sub>3</sub>), 3.82 (s, 6H, 2×OCH<sub>3</sub>), 3.73 (s, 2H, CH<sub>2</sub>CO), 3.65 (m, 4H, O(CH<sub>2</sub>)<sub>2</sub>), 3.47 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.21-2.51 (m, 4H, PhCH<sub>2</sub>, CHCH<sub>2</sub>), 2.40 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>). MS (ESI, m/z): 471.3 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>: C, 66.50; H, 7.28; N, 5.95. Found: C, 66.56; H, 7.25; N, 5.97.  
(*S*)-**8b**: mp: 70-73 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.86-6.27 (m, 5H), 5.50, 4.75 (m, 1H, CH), 3.80 (s, 6H, 2×OCH<sub>3</sub>), 3.81 (s, 6H, 2×OCH<sub>3</sub>), 3.67 (s, 2H, CH<sub>2</sub>CO), 3.64-3.33 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.12-2.60 (m, 8H, PhCH<sub>2</sub>, CHCH<sub>2</sub>, HN(CH<sub>2</sub>)<sub>2</sub>), 3.09 2.39, 2.29 (d, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 2.09 (brs, 1H, NH). MS (ESI, m/z): 470.3 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>26</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub>: C, 66.50; H, 7.51; N, 8.95. Found: C, 66.70; H, 7.53; N, 8.98.  
**9a**: mp: 134-137 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.84-6.25 (m, 5H), 5.64, 5.20, 4.73 (m, 1H, CH), 4.03 (m, 4H, 2×OCH<sub>2</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 3.80 (s, 3H, OCH<sub>3</sub>), 3.67 (m, 4H, O(CH<sub>2</sub>)<sub>2</sub>), 3.64-3.39 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.19 (s, 2H, CH<sub>2</sub>CO), 3.06-2.61 (m, 4H, PhCH<sub>2</sub>, CHCH<sub>2</sub>), 2.37 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 1.40 (m, 6H, 2×CH<sub>3</sub>). MS (ESI, m/z): 499.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>28</sub>H<sub>38</sub>N<sub>2</sub>O<sub>6</sub>: C, 67.54; H, 7.68; N, 5.62. Found: C, 67.65; H, 7.71; N, 5.64.  
**9b**: mp: 96-100 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.83-6.24 (m, 5H), 5.64, 5.30, 4.72 (m, 1H, CH), 4.05 (m, 4H, 2×OCH<sub>2</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 3.80 (s, 3H, OCH<sub>3</sub>), 3.64-3.33 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.12-2.60 (m, 8H, PhCH<sub>2</sub>, CHCH<sub>2</sub>, HN(CH<sub>2</sub>)<sub>2</sub>), 3.18 (s, 2H, CH<sub>2</sub>CO), 2.39, 2.29 (d, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 2.12 (brs, 1H, NH), 1.42 (m, 6H, 2×CH<sub>3</sub>). MS (ESI, m/z): 498.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>28</sub>H<sub>39</sub>N<sub>3</sub>O<sub>5</sub>: C, 67.58; H, 7.90; N, 8.44. Found: C, 67.79; H, 7.92; N, 8.47.  
**9c**: mp: 89-92 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.84-6.23 (m, 5H), 5.65, 5.34, 4.74 (m, 1H, CH), 4.05 (m, 4H, 2×OCH<sub>2</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 3.63 (s, 3H, OCH<sub>3</sub>), 3.48 (s, 2H, CH<sub>2</sub>CO), 3.40-2.59 (m, 8H, PhCH<sub>2</sub>CH<sub>2</sub>, CHCH<sub>2</sub>, N(CH<sub>2</sub>)<sub>2</sub>), 2.39, 2.29 (d, 6H, PhCH<sub>2</sub>, CH<sub>3</sub>N(CH<sub>2</sub>)<sub>2</sub>), 2.30 (s, 3H, CH<sub>3</sub>), 1.42 (m, 6H, 2×CH<sub>3</sub>). MS (ESI, m/z): 512.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>29</sub>H<sub>41</sub>N<sub>3</sub>O<sub>5</sub>: C, 68.08; H, 8.08; N, 8.21. Found: C, 68.28; H, 8.10; N, 8.19.  
**9d**: mp: 170-173 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.87-6.25 (m, 5H), 5.60, 5.10, 4.81 (m, 1H, CH), 4.05 (m, 4H, 2×OCH<sub>2</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 3.62 (s, 2H, CH<sub>2</sub>CO), 3.31 (m, 2H,

PhCH<sub>2</sub>CH<sub>2</sub>), 3.13-2.64 (m, 4H, PhCH<sub>2</sub>, CHCH<sub>2</sub>), 2.46 (d, *J* = 9.6 Hz, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 1.73 (d, *J* = 9.6 Hz, 4H, (CH<sub>2</sub>)<sub>2</sub>), 1.46 (m, 6H, 2×CH<sub>3</sub>). MS (ESI, *m/z*): 483.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>28</sub>H<sub>38</sub>N<sub>2</sub>O<sub>5</sub>: C, 69.68; H, 7.94; N, 5.80. Found: C, 69.90; H, 7.97; N, 5.77.

**9e**: mp: 80-84 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.83-6.20 (m, 5H), 5.62, 5.38, 4.76 (m, 1H, CH), 4.03 (m, 4H, 2×OCH<sub>2</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 3.62 (s, 2H, CH<sub>2</sub>CO), 3.12 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>), 3.09-2.68 (m, 4H, PhCH<sub>2</sub>, CHCH<sub>2</sub>), 2.63 (m, *J* = 6.9 Hz, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 1.41 (m, 6H, 2×CH<sub>3</sub>), 1.03 (t, *J* = 6.9 Hz, 6H, 2×CH<sub>3</sub>). MS (ESI, *m/z*): 485.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>28</sub>H<sub>40</sub>N<sub>2</sub>O<sub>5</sub>: C, 69.39; H, 8.32; N, 5.78. Found: C, 69.60; H, 8.35; N, 5.80.

**10a**: mp: 155-158 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.40-7.34 (m, 5H), 7.11-6.84 (m, 2H), 6.60 (d, *J* = 8.4 Hz, 2H), 6.50 (d, *J* = 8.4 Hz, 2H), 4.99 (d, 2H, OCH<sub>2</sub>), 5.65, 5.18, 4.75 (m, 1H, CH), 3.85 (s, 3H, OCH<sub>3</sub>), 3.66 (s, 2H, CH<sub>2</sub>CO), 3.60 (s, 3H, OCH<sub>3</sub>), 3.48 (m, 4H, O(CH<sub>2</sub>)<sub>2</sub>), 3.04-2.80 (m, 4H, PhCH<sub>2</sub>CH<sub>2</sub>, CHCH<sub>2</sub>), 2.60 (m, 2H, PhCH<sub>2</sub>), 2.40 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>). MS (ESI, *m/z*): 517.3 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>31</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>: C, 72.07; H, 7.02; N, 5.42. Found: C, 72.29; H, 6.98; N, 5.39.

**10b**: mp: 118-122 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.39-7.35 (m, 5H), 7.10-6.83 (m, 2H), 6.59 (d, *J* = 8.4 Hz, 2H), 6.26 (d, *J* = 8.4 Hz, 2H), 5.02 (d, 2H, OCH<sub>2</sub>), 5.66, 5.28, 4.72 (m, 1H, CH), 3.83 (s, 3H, OCH<sub>3</sub>), 3.74 (s, 2H, CH<sub>2</sub>CO), 3.60 (s, 3H, OCH<sub>3</sub>), 3.43-2.82 (m, 8H, PhCH<sub>2</sub>CH<sub>2</sub>, CHCH<sub>2</sub>, HN(CH<sub>2</sub>)<sub>2</sub>), 2.65 (m, 2H, PhCH<sub>2</sub>), 2.25 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 2.02 (brs, 1H, NH). MS (ESI, *m/z*): 516.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>31</sub>H<sub>37</sub>N<sub>3</sub>O<sub>4</sub>: C, 72.21; H, 7.23; N, 8.15. Found: C, 72.45; H, 7.19; N, 8.13.

**10c**: mp: 128-131 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.40-7.35 (m, 5H), 7.10-6.83 (m, 2H), 6.60 (d, *J* = 8.4 Hz, 2H), 6.35 (d, *J* = 8.4 Hz, 2H), 5.04 (d, 2H, OCH<sub>2</sub>), 5.85, 5.57, 4.78 (m, 1H, CH), 3.60 (s, 2H, CH<sub>2</sub>CO), 3.48 (s, 3H, OCH<sub>3</sub>), 3.60 (s, 3H, OCH<sub>3</sub>), 3.43-2.82 (m, 8H, PhCH<sub>2</sub>CH<sub>2</sub>, CHCH<sub>2</sub>, N(CH<sub>2</sub>)<sub>2</sub>), 2.65 (m, 2H, PhCH<sub>2</sub>), 2.84 (m, 4H, CH<sub>3</sub>N(CH<sub>2</sub>)<sub>2</sub>), 2.61 (s, 3H, CH<sub>3</sub>). MS (ESI, *m/z*): 530.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>32</sub>H<sub>39</sub>N<sub>3</sub>O<sub>4</sub>: C, 72.56; H, 7.42; N, 7.93. Found: C, 72.79; H, 7.40; N, 7.95.

**10d**: mp: 143-146 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.31-7.26 (m, 5H), 7.07-6.84 (m, 2H), 6.58 (d, *J* = 8.4 Hz, 2H), 6.24 (d, *J* = 8.4 Hz, 2H), 5.03 (s, 2H, OCH<sub>2</sub>), 5.60, 5.10, 4.73 (m, 1H, CH), 3.85 (s, 3H, OCH<sub>3</sub>), 3.72 (s, 2H, CH<sub>2</sub>CO), 3.60 (s, 3H, OCH<sub>3</sub>), 3.45-2.77 (m,

4H, CHCH<sub>2</sub>, PhCH<sub>2</sub>CH<sub>2</sub>), 2.66 (m, 2H, PhCH<sub>2</sub>), 2.46 (d, N(CH<sub>2</sub>)<sub>2</sub>), 1.73 (d, 4H, (CH<sub>2</sub>)<sub>2</sub>). MS (ESI, *m/z*): 501.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>31</sub>H<sub>36</sub>N<sub>2</sub>O<sub>4</sub>: C, 74.37; H, 7.25; N, 5.60. Found: C, 74.59; H, 7.22; N, 5.55.

**10e**: mp: 81-84 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.40-7.31 (m, 5H), 7.10-6.84 (m, 2H), 6.61 (d, *J* = 8.4 Hz, 2H), 6.27 (d, *J* = 8.4 Hz, 2H), 5.03 (s, 2H, OCH<sub>2</sub>), 5.62, 5.35, 4.75 (m, 1H, CH), 3.85 (s, 3H, OCH<sub>3</sub>), 3.77 (s, 2H, CH<sub>2</sub>CO), 3.58 (s, 3H, OCH<sub>3</sub>), 3.21-2.83 (m, 4H, CHCH<sub>2</sub>, PhCH<sub>2</sub>CH<sub>2</sub>), 2.72 (m, 2H, PhCH<sub>2</sub>), 2.52 (m, *J* = 6.9 Hz, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 1.01 (t, *J* = 6.9 Hz, 6H, 2×CH<sub>3</sub>). MS (ESI, *m/z*): 503.4 ([M+H]<sup>+</sup>, base peak). Anal. calcd for C<sub>31</sub>H<sub>38</sub>N<sub>2</sub>O<sub>4</sub>: C, 74.07; H, 7.62; N, 5.57. Found: C, 74.31; H, 7.65; N, 5.55.

[17] Cytotoxicity was assessed by the MTT assay which was performed in K562 and K562/DOX cells. The cells were harvested during logarithmic growth phase and seeded into 96-well culture plates at a density of 5×10<sup>4</sup> cells/mL. Doxorubicin was added alone or with anticancer agents or verapamil in a final volume of 160 μL per well at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> incubator. In the assays of cytotoxic evaluation, cells were incubated in growth medium for 24 h, and were exposed to various anticancer drugs concentrations. After treatment of 40 μL of test compounds solution for 48 h, 20 μL of freshly prepared MTT (5 mg/mL in PBS solution) was added to the wells and incubated for 4 h at 37 °C.

[18] In the assays of drug resistant modulation evaluation, the cells were then cultured for 48 h in the presence of test compounds with or without verapamil. Verapamil, used as the positive control, were added to the wells with the final concentrations of 10 μM. After treatment of 20 μL of test compounds solution for 48 h, 20 μL of MTT solution was added. Then cells were centrifuged for 15 min at 3500 rpm, 160 μL of medium was carefully removed, and 150 μL of DMSO was added until no particulate matter was visible. The cytotoxic effects of drugs were determined according to the OD values using a microplate reader at absorption wavelength of 570 nm. The inhibition ratio (IR) of tumor growth was calculated using the following equation: IR = (1-OD<sub>21</sub>/OD<sub>22</sub>) × 100%. Where OD<sub>21</sub> is the average OD values treated with the test compounds and the OD<sub>22</sub> is the average OD values without the test compounds. The concentrations required to inhibit growth by IC<sub>50</sub> values were calculated from the cytotoxicity curves using Bliss's software.