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FULL PAPER

Polysubstituted Pyrimidines as Potent Inhibitors of Prostaglandin E_2 Production: Increasing Their Aqueous Solubility

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Abstract: Water-solubility is one of the key features of potential therapeutic agents. In order to enhance poor water-solubility of parent 5-butyl-4-(4-methoxyphenyl)-6-phenylpyrimidin-2-amine, a potent inhibitor of prostaglandin E_2 (PGE_2) production, we synthesized and evaluated a new series of derivatives where the butyl group in the C5 position of pyrimidine ring was replaced with less lipophilic substituent, preferably with a hydrophilic aliphatic moiety. Except for the 5-cyanopyrimidine derivative, all target compounds exhibited increased (2.7 – 87-fold) water-solubility compared to the parent compound. Although non-toxic in mouse peritoneal cells, the prepared compounds were either equipotent or weaker inhibitors of PGE_2 production than the parent compound. The most promising compound from the series was 5-(2,5,8,11-tetraoxadodecyl)pyrimidine derivative (with three polyethylene glycol units in the C5 position), which exhibited 32-fold higher water-solubility and only slightly weaker inhibitory activity (22% of remaining PGE_2 production) compared to the parent compound (15% of remaining PGE_2 production).

Introduction

High *in vitro* biological potency of new drug candidates is not the only key prerequisite for a successful transformation into a drug. Other important physicochemical and pharmacokinetic properties need to be seriously taken into account as well. Nowadays, the poor water-solubility of small molecules represents a common problem as approximately 40% of marketed drugs and up to 75% of molecules in development stages are poorly water-soluble.[1] Drug solubility is considered to be a fundamental property that has to be evaluated already in the early stages of drug discovery. Fine-tuning of the solubility is crucial since both adequate lipophilicity and hydrophilicity are important factors for the pharmacokinetics of the active compound. Hydrophilicity is pivotal for the solubility in an aqueous environment, which is essential for appropriate absorption in the case of highly desirable oral bioavailability of the drug candidate. Lipophilicity, on the other hand, is crucial for the penetration of the molecule through biomembranes of the target cells. Lipophilicity is usually quantified by log *P* which can be predicted *in silico*, giving medicinal chemists some initial hints for the design of novel molecules.[2]

There are various strategies, exploited by pharmaceutical industry, how to increase compound's solubility. These include salt formation, determination of the proper polymorph, introduction of a prodrug moiety, utilization of co-solvents, co-crystals, or surfactants.[1,3–6] A useful way to enhance water-solubility is to form suitable bioisosters via an introduction of a heteroatom into a molecule. For instance, the decrease of log *P* from 3.36 to 0.77 can be achieved by replacing the CH_2 (X) group for oxygen atom in the RCH_2-X-CH_2R segment.[7]

We have recently discovered substituted pyrimidines with potent anti-inflammatory properties. The compounds were shown to be inhibitors of immune-activated nitric oxide (NO) production[8,9] or dual inhibitors of NO and prostaglandin E_2 (PGE_2) production.[10,11] Later, series of 2-amino-4,6-diarylpyrimidines were synthesized and studied as potent inhibitors of PGE_2 production.[12,13] Moreover, the compounds exhibited strong anti-inflammatory *in vivo* effects in the rat model of acute inflammation.[13]

Among the polysubstituted pyrimidines studied, 5-butyl-4-(4-methoxyphenyl)-6-phenylpyrimidin-2-amine (**1**, Figure 1) was identified as a potential lead structure with anti-inflammatory properties,[12,14] but a significant drawback of this compound was its low water-solubility. Thus, boosting the solubility was the next logical step in further development of polysubstituted pyrimidines as anti-inflammatory agents.

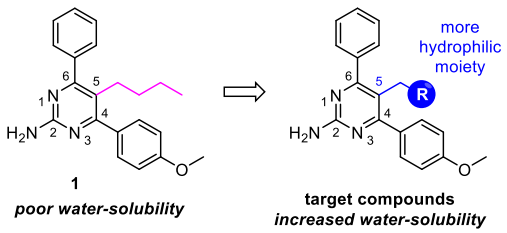


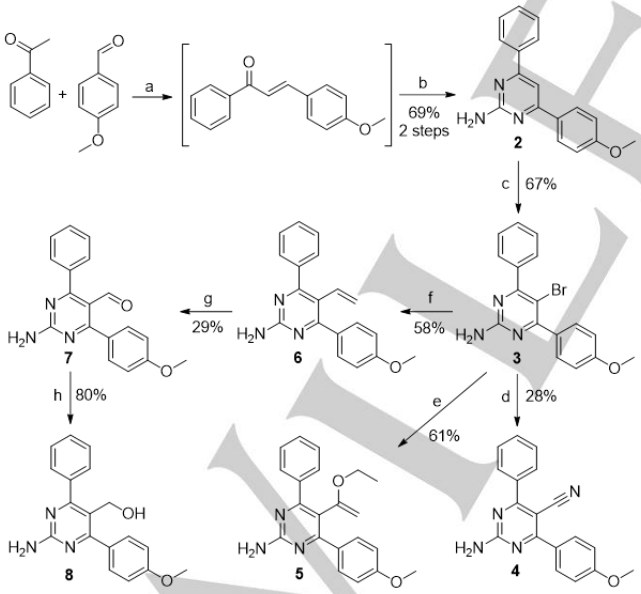
Figure 1. The goal of the current study: a replacement of the 5-butyl group with more hydrophilic aliphatic chain R.

Herein we present structural modifications of compound **1** (Figure 1) with the aim to increase its water-solubility. Previous data[15] suggested that modification of the substituent in the C5 position of pyrimidine moiety should have minimal impact on anti-inflammatory properties. Thus, we decided to replace the 5-butyl group with various aliphatic chains containing heteroatom(s) in order to increase water-solubility of such derivatives. An efficient synthetic approach towards analogues of compound **1** modified at the C5 position with polar substituents was developed and their biological activity as well as solubility were determined.

Results and Discussion

Synthesis

Analogues of compound **1** (Figure 1) bearing a hydrophilic aliphatic chain (instead of lipophilic 5-butyl group) were designed and 10 target molecules were selected for the synthesis, based on calculated log *P* values (see SI) using Virtual Computational Chemistry Laboratory (VCCLAB).[16] The synthetic route started from compound **2** (Scheme 1), which was prepared in a 69% yield from acetophenone and *p*-anisaldehyde using the one-pot assembly reported by Nimkar and co-workers.[17] Compound **2** was brominated using bromine in chloroform to give 5-bromopyrimidine derivative **3** (67%), which was subsequently converted into 5-cyanopyrimidine derivative **4** (by reaction with zinc cyanide in a 28% yield) and into 5-(1-ethoxyvinyl)pyrimidine derivative **5** (via the Heck reaction with ethyl vinyl ether in a 61% yield). 5-Vinylpyrimidine **6**, the key intermediate for further modifications, was obtained in a 58% yield by the Suzuki-Miyaura coupling reaction of 5-bromopyrimidine **3** with pinacol vinylboronate.

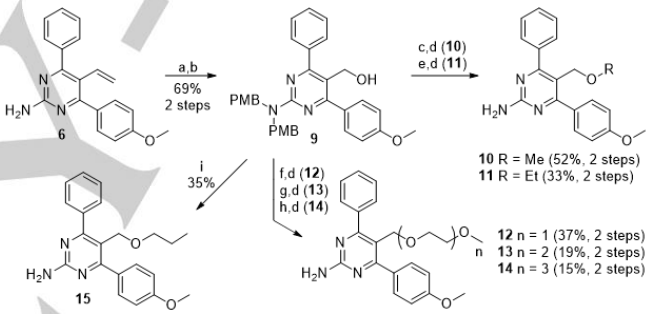


Scheme 1. Synthesis of compounds **4**–**8**. Reagents and conditions: (a) NaOH, EtOH, 30 min, 25 °C; (b) guanidine hydrochloride, 24 h, 85 °C; (c) Br_2, $CaCO_3$, $CHCl_3$, 5 h, 25 °C; (d) $Zn(CN)_2$, $Pd(t\text{-}Bu_3P)_2$, DMF, 16 h, 110 °C; (e) ethyl vinyl ether, Et_3N, $Pd(t\text{-}Bu_3P)_2$, DMF, 16 h, 110 °C; (f) pinacol vinylboronate, Cs_2CO_3, $Pd(t\text{-}Bu_3P)_2$, 1,4-dioxane/H_2O, 15 h, 85 °C; (g) O_3, DCM, -20 °C, Me_2S, 3 h, 25 °C; (h) $NaBH_4$, DMF, 1 h, 0 °C.

Ozonolysis of 5-vinylpyrimidine **6** afforded 5-formylpyrimidine **7** in a 29% yield and its subsequent reduction with $NaBH_4$ in DMF gave 5-(hydroxymethyl)pyrimidine derivative **8** in an 80% yield (Scheme 1).

In view of the fact, that the next synthetic steps were based on alkylation of the 5-(hydroxymethyl)pyrimidine group, a protection of the 2-aminopyrimidine group was necessary. At first, benzyl was selected as a suitable protecting group with regard to its stability under the reaction conditions, however, its standard removal using catalytic hydrogenation with Pd/C failed. Afterwards, *p*-methoxybenzyl (PMB) group was employed in order to achieve easier deprotection.[18] Nevertheless, even the PMB group proved to be relatively stable as it was resistant to catalytic (Pd/C) hydrogenation in glacial acetic acid as well as to treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or cerium(IV) ammonium nitrate (CAN).[18–20] The efficient PMB group removal was ultimately achieved by treatment with trifluoroacetic acid (TFA).

The protection of the 2-aminopyrimidine group with PMB was performed at the stage of 5-vinylpyrimidine **6** (Scheme 2). Thus, treatment of compound **6** with *p*-methoxybenzyl chloride, followed by ozonolysis/reduction afforded the desired protected intermediate **9** in a 69% yield (over 2 steps).



Scheme 2. Synthesis of target compounds **10**–**15**. Reagents and conditions: (a) NaH, 0 °C, DMF, then *p*-methoxybenzyl chloride, 16 h, 65 °C; (b) O_3, DCM, -20 °C, then $NaBH_4$, 168 h, 25 °C; (c) NaH, 0 °C, DMF, MeI, 19 h, 80 °C; (d) TFA, 16 h, 50 °C; (e) NaH, 0 °C, DMF, EtBr, 19 h, 80 °C; (f) NaH, 0 °C, DMF, 2-chloroethyl methyl ether, 19 h, 80 °C; (g) NaH, 0 °C, DMF, 1-bromo-2-(2-methoxyethoxy)ethane, 19 h, 80 °C; (h) NaH, 0 °C, DMF, 1-(2-bromoethoxy)-2-(2-methoxyethoxy)ethane, 19 h, 80 °C; (i) TFA, *n*-PrOH, 5 h, 50 °C.

Key 5-(hydroxymethyl)pyrimidine intermediate **9** (Scheme 2) was treated with NaH in DMF and then with the corresponding alkylating agent, followed by the PMB groups removal with TFA, to afford target derivatives **10**–**14** in 15-52% yields (over two steps). Interestingly, partial decomposition of the 5-alkoxymethyl moiety was observed during the removal of PMB groups under acidic conditions (TFA), leading to a partial formation of 5-(hydroxymethyl) pyrimidine **8** in the reaction mixture (observed in UPLC-MS). In order to corroborate this phenomenon, compound **13** was treated with TFA and in the UPLC-MS, a mixture of compounds **13** and **8** (as the product of decomposition) was observed in MeCN while a mixture of **13** and **10** (as the product of re-etherification) was observed in MeOH (Figure S39 in SI).

This observation was subsequently exploited for the synthesis of 5-(propoxymethyl)pyrimidine derivative **15** (Scheme 2). Treatment of compound **9** with TFA, in *n*-PrOH afforded target compound **15** in a 35% yield.

Since the prepared derivatives were more soluble compared to compound **1** (see water-solubility determination below), we decided to expand this approach and we designed novel compound **17** (Figure 2) as a combination of soluble derivative **14** and previously reported[13] potent inhibitor of PGE_2 production, compound **16** (IC_{50} = 31 nM, 99.7% inhibition of PGE_2 production), which was approx. 150-fold more potent compared to compound **1** (IC_{50} = 4.83 μM, 87.7% inhibition of PGE_2 production).

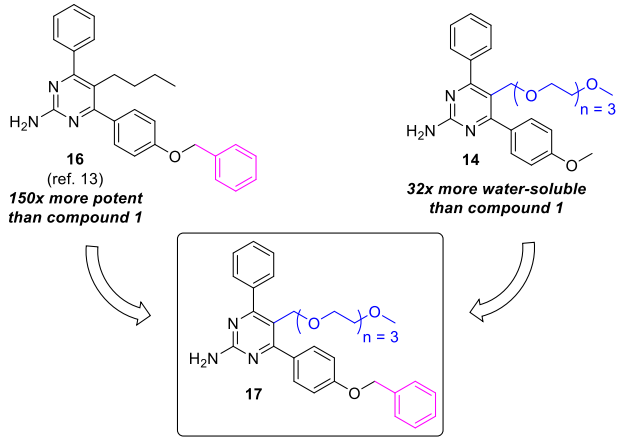


Figure 2. Design of compound **17** based on previously published derivative **16**.

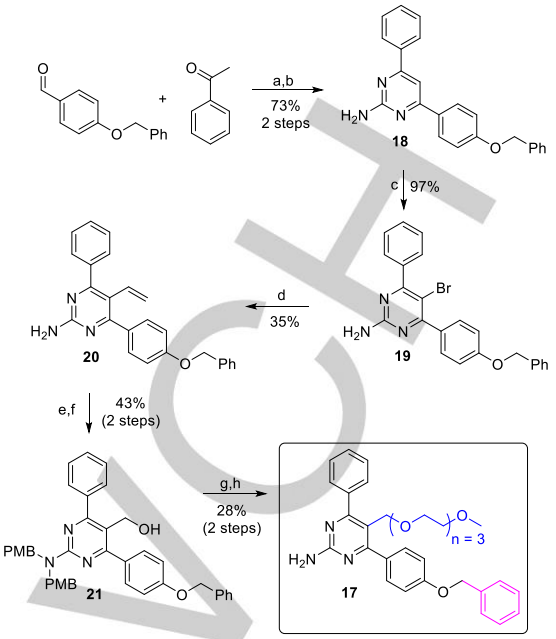
Compound **17** was prepared in analogous way to compound **14**. Pyrimidine **18** (Scheme 3) was prepared in a 73% yield from acetophenone and 4-(benzyloxy)benzaldehyde by the one-pot assembly.[17] Bromination of compound **18** with bromine in chloroform gave 5-bromopyrimidine derivative **19** quantitatively. 5-Vinylpyrimidine **20** was obtained in a 35% yield by the Suzuki-Miyaura coupling of compound **19** with pinacol vinylboronate. Introduction of PMB groups followed by ozonolysis/reduction afforded derivative **21** in a 43% yield (2 steps). Finally, alkylation of compound **21** with 1-(2-bromoethoxy)-2-(2-methoxyethoxy)ethane and with subsequent PMB groups removal gave the target compound **17** in a 28% yield (2 steps).

Water-solubility

The water-solubility of studied compounds was determined by a method exploiting the compounds absorption at 254 nm using HPLC (for details, see SI, p. S17). The area under the curve (AUC) was correlated to the corresponding calibration curve in order to calculate the concentration of dissolved compound.

All target compounds proved to be more soluble compared to the parent compound **1** (Figure 3), with the exception of 5-cyanopyrimidine **4** which had similar solubility as compound **1**. The insertion of one extra oxygen atom into the aliphatic moiety of 5-(propoxymethyl)pyrimidine **15** (Scheme 2) to form 5-(methoxyethoxymethyl)pyrimidine **12** led 5-fold increase in the solubility. Interestingly, despite the calculated log *P* values (see SI, p. S16), the most soluble derivative from the series was compound **13** (with two PEG units in the C5 position), and not **14** (with three PEG units in the C5 position). This result suggests that C5 substituents consisting of 9 or more atoms might start to coil and form micelle-like structures, thus, reducing the polar surface of the molecule available for interactions with water molecules.

The water-solubility of benzyloxyphenyl analogue **17** (Scheme 3) was one order of magnitude higher compared to its parent compound **16**, but compound **17** exhibited significantly lower potency to inhibit PGE_2 production (Figure 4).



Scheme 3. Design and synthesis of compound **17**, as a more soluble PGE_2 production inhibitor of **16**. Reagents and conditions: (a) NaOH, EtOH, 30 min, 25 °C; (b) guanidine hydrochloride, 24 h, 85 °C; (c) Br_2, $CaCO_3$, $CHCl_3$, 5 h, 25 °C; (d) pinacol vinylboronate, Cs_2CO_3, Pd(*t*-$Bu_3P)_2$, 1,4-dioxane/H_2O, 15 h, 85 °C; (e) NaH, 0 °C, DMF, 4-methoxybenzyl chloride, 16 h, 65 °C; (f) O_3, DCM, -70 °C, then $NaBH_4$, 168 h, 25 °C; (g) NaH, 0 °C, DMF, 1-(2-bromoethoxy)-2-(2-methoxyethoxy)ethane, 19 h, 80 °C; (h) TFA, 16 h, 50 °C.

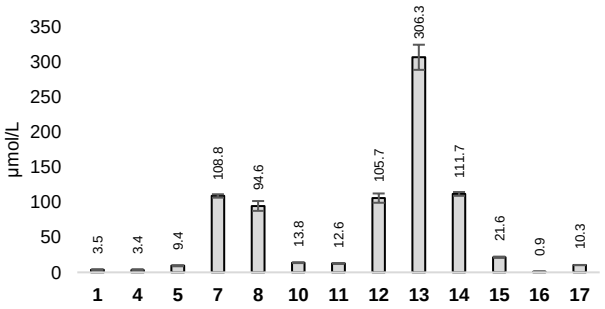


Figure 3. Experimentally determined water-solubility of target compounds. Bars are means ± SEM obtained by averaging results of three experiments for each compound.

Biological data

The target molecules were evaluated *in vitro* for inhibition of PGE_2 production and viability using C57BL6 mouse peritoneal cells (Figure 4). The effects of target compounds on the PGE_2 production were expressed as a percentage change (remaining production of PGE_2) relative to the response of LPS stimulated cells (positive control, 100 %) or unstimulated cells (negative control, 2.50 %). The newly prepared derivatives exhibited comparable (compounds **4**, **5**, **10**, and **14**) or worse activity (compounds **7**, **8**, **11**, **12**, **13**, and **15**) than compound **1**. Hence, replacement of the 5-butyl group for more hydrophilic and longer aliphatic moiety did not lead to an increased potency compared to parent compounds **1** or **16**. The cell viability was expressed as % of control untreated cells and the studied compounds did not exhibit cytotoxic effects within the exposure time of 6 h.

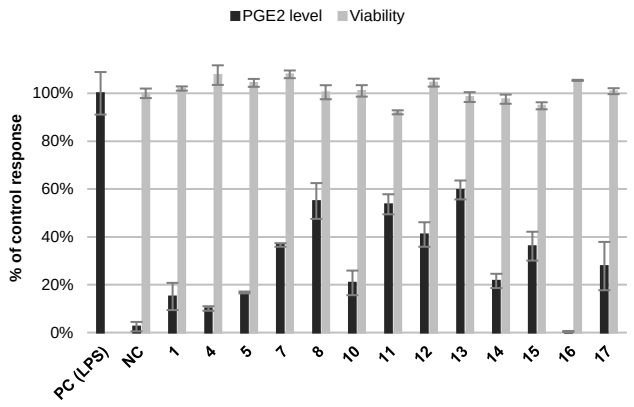


Figure 4. Effect of prepared compounds on *in vitro* production of PGE_2 and on viability of mouse peritoneal cells (interval of 6 h). Effects are expressed as a percentage change relative to the response of LPS stimulated (100%, positive control) or unstimulated (2.5%, negative control) cells. Bars are means ± SEM obtained by averaging results of two to four experiments for each compound. PC – positive control, NC – negative control.

Conclusion

The structure of 5-butyl-4-(4-methoxyphenyl)-6-phenylpyrimidin-2-amine (**1**), a potent inhibitor of PGE_2 production with anti-inflammatory properties, was modified in order to increase its poor water-solubility. A series of compounds was designed and synthesized, where the lipophilic *n*-butyl substituent in the C5 position of pyrimidine moiety of compound **1** was replaced with more hydrophilic substituent, preferably an aliphatic chain. Except for 5-cyanopyrimidine **4** (which had a similar solubility as **1**), all target compound proved to be more water-soluble. Compounds **13** and **14**, bearing two and three PEG units in the C5 position, respectively, were identified as the most promising compounds within the series: compound **13** was the most water-soluble derivative (almost 2 orders of magnitude more water-soluble than **1**), but it was considerably (4-fold) less potent inhibitor of PGE_2 production than compound **1**; compound **14**, on the other hand, was the second most water-soluble derivative (32-fold more soluble compared to **1**), with only slightly weaker (1.5-fold) potency compared to **1**, thus exhibiting relatively favorable potency/solubility ratio. An analogous transformation of 5-butylpyrimidine **16** into compound **17** (with three PEG units in the C5 position of pyrimidine) led to a 1 order of magnitude higher water-solubility compared to parent compound **16**, however, it also led to considerable decrease of potency to inhibit PGE_2 production. Currently, other methods how to increase water-solubility and to keep or increase the potency of parent compound **1** or its derivatives are being investigated.

Experimental Section

See Supporting Information for general experimental information, standard procedures, spectral data, characterization, calculated log *P* values, solubility measurements details, calibration curves, PGE_2 assay, viability assay, and copies of spectra of the prepared compounds.

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Notes

The authors declare no competing financial interest.

Keywords: solubility • anti-inflammatory • pyrimidines • prostaglandin E_2 • synthesis

[1] H. D. Williams, N. L. Trevaskis, S. A. Charman, R. M. Shanker, W. N. Charman, C. W. Pouton, C. J. H. Porter, *Pharmacol. Rev. Pharmacol Rev* **2013**, *65*, 315–499.
[2] J. Kujawski, M. K. Bernard, A. Janusz, W. Kuźma, *J. Chem. Educ.* **2012**, *89*, 64–67.
[3] V. J. Stella, K. W. Nti-Addae, *Adv. Drug Deliv. Rev.* **2007**, *59*, 677–694.
[4] D. Fleisher, R. Bong, B. H. Stewart, *Adv. Drug Deliv. Rev.* **1996**, *19*, 115–130.
[5] T. Heimbach, D. M. Oh, L. Y. Li, M. Forsberg, J. Savolainen, J. Leppänen, Y. Matsunaga, G. Flynn, D. Fleisher, *Pharm. Res.* **2003**, *20*, 848–856.
[6] A. T. M. Serajuddin, *Adv. Drug Deliv. Rev.* **2007**, *59*, 603–616.
[7] N. A. Meanwell, *J. Med. Chem.* **2011**, *54*, 2529–2591.
[8] P. Jansa, A. Holý, M. Dračínský, V. Kolman, Z. Janeba, P. Kostecká, E. Kmoníčková, Z. Zídek, *Med. Chem. Res.* **2014**, *23*, 4482–4490.
[9] P. Jansa, A. Holý, M. Dračínský, V. Kolman, Z. Janeba, E. Kmoníčková, Z. Zídek, *Med. Chem. Res.* **2015**, *24*, 2154–2166.
[10] Z. Zídek, M. Kverka, A. Dusilová, E. Kmoníčková, P. Jansa, *Nitric Oxide* **2016**, *57*, 48–56.
[11] V. Kolman, P. Jansa, F. Kalčic, Z. Janeba, Z. Zídek, *Nitric Oxide* **2017**, *67*, 53–57.
[12] V. Kolman, F. Kalčic, P. Jansa, Z. Zídek, Z. Janeba, *Eur. J. Med. Chem.* **2018**, *156*, 295–301.
[13] F. Kalčic, V. Kolman, H. Ajani, Z. Zídek, Z. Janeba, *ChemMedChem* **2020**, *15*, 1398–1407.
[14] P. Dosoudil, V. Kotek, V. Kolman, O. Baszczyński, M. M. Kaiser, Z. Janeba, M. Havránek, *Org. Process Res. Dev.* **2020**, *24*, 1718–1724.
[15] V. Kolman, F. Kalčic, Z. Janeba, Z. Zídek, *Polysubstituted Pyrimidines as Inhibitors of Prostaglandin E2 Production, Methods of Preparation and Application*, **2019**, 309052.
[16] VCCLAB, Virtual Computational Chemistry Laboratory. **2005**.
[17] M. M. V. Ramana, Amey Nimkar, R. Betkar, P. Ranade, B. Mundhe, *New J. Chem.* **2016**, *40*, 2541–2546.
[18] P. G. M. Wuts, *Greene´s Protective Groups in Organic Synthesis*, Wiley, Kalamazoo, Michigan, USA, **2014**.
[19] B. Bourrie, P. Casellas, P. Ciapetti, J.-M. Deroco, S. Jegham, Y. Muneaux, C.-G. Wermuth, *6-Substituted Pyridoindolone Derivatives, Production and Therapeutic Use Thereof*, **2007**, US2007/129365 A1.
[20] T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, G. D. Anderson, E. G. Burton, J. N. Cogburn, S. A. Gregory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. W. Veenhuizen, Y. Y. Zhang, P. C. Isakson, *J. Med. Chem.* **1997**, *40*, 1347–1365.

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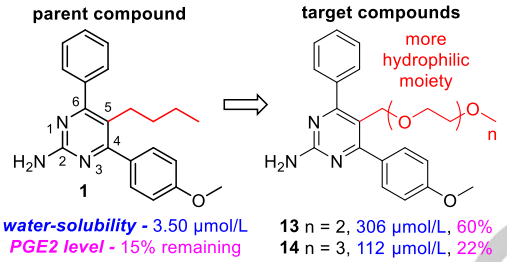

In this study, the goal was to increase water solubility of previously reported inhibitors of prostaglandin E_2 production. The most soluble derivative exhibited 2 orders of magnitude higher solubility although much lower potency compared to the parent compound. The most promising compound (**14**) reached 32-fold higher solubility with only slight decrease in potency.