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Assessing the contribution of reaction components to the overall toxicity of synthesis of 4-methoxy-4′-nitro-1,1′-biphenyl

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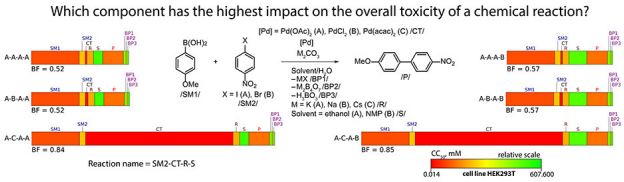
Using bio-Strips and cytotoxicity potentials, the least and most harmful substances involved in the synthesis of 4-methoxy-4′-nitro-1,1′-biphenyl from 4-methoxyphenylboronic acid and aryl halide were identified. A total of 36 synthetic routes were analyzed.



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Fast and reliable assessment of the environmental hazards of chemical processes is one of the most significant and, at the same time, least achievable goals of modern organic chemistry.[1–3] Academia and industry have made considerable efforts to pursue the concept of sustainable development;[4,5] sophisticated approaches have been created to systematize chemical compounds according to their potential toxicity.[6–10] Still, the hazards of widespread chemical reactions have not been significantly reduced.

In recent years, we have been developing a new methodology that allows fast preliminary assessment of the toxicity risks of chemical processes by means of bio-Profiles (bio-Strips) and accompanying metrics (bio-Factors and cytotoxicity potentials).[11,12] We have proposed to evaluate the 'overall cytotoxicity' of a given chemical reaction by calculating the contribution of all substances entering into this reaction or formed during its course. In particular, bio-Strips allow direct comparison of different routes for the synthesis of a particular chemical product in terms of the cytotoxicity of the substances involved.[13,14] It is noteworthy that each new reaction analyzed brings us closer to understanding the ways to reduce the harmful impact of chemical processes on the environment. Here, we address the issue of selecting less harmful starting materials, catalysts, solvents and other reagents by example of 36 synthetic routes of 4-methoxy-4′-nitro-1,1′-biphenyl.

The experimental procedures necessary to obtain the required data were essentially as described previously.† Exact values are given in Table S1 (see Online Supplementary Materials). These data were used for the subsequent construction of bio-Strips of reactions and the calculation of their bio-Factors (BFs), as well as the initial, final and relative final cytotoxicity potentials (CP_i, CP_f and CP_{f_rel}, respectively; see Online Supplementary Materials). The calculated values of BF, CP_i, CP_f and CP_{f_rel} are presented in Table S2. In accordance with our previous works,[16,17] the conversion in reactions is considered to be 100%.

The reactions being analyzed are shown in Figure 1. They differ in their (i) starting material 2 (SM2: 1-iodo-4-nitrobenzene (A) or 1-bromo-4-nitrobenzene (B)), (ii) catalyst (CT: $Pd(OAc)_2$ (A), $PdCl_2$ (B), or $Pd(acac)_2$ (C)), (iii) reagents (R: Na_2CO_3 (A), K_2CO_3 (B), or Cs_2CO_3 (C)), and (iv) solvents (S: ethanol (A) or NMP (B)). Their bio-Strips are built on the basis of half-maximal cytotoxic concentrations upon 24-h incubation (24-h CC_{50}) measured in three cell lines: CaCo-2 (colorectal adenocarcinoma), HEK293T (human embryonic kidney) and FRSN (foreskin mesenchymal stem cells). Figure 1 shows the routes for the synthesis of 4-methoxy-4′-nitro-1,1′-biphenyl with the lowest 'overall cytotoxicity' (and thus the least harmful potential) found for all cell lines used. Bio-Strips of all 36 reactions for CaCo-2, FRSN and HEK293T cells are shown in Figures S1–S3 (see Online Supplementary Materials), respectively, and CPs are presented in Figure 2 and Table S2.

Upon analyzing the bio-Strips and CPs, the following components can be singled out as beneficial in terms of their contribution to the 'overall cytotoxicity' of the reactions under study: 1-iodo-4-nitrobenzene (A) as SM2, $Pd(OAc)_2$ (A) or $PdCl_2$ (B) as CT, Na_2CO_3 (A) or K_2CO_3 (B) or Cs_2CO_3 (C) as R and ethanol (A) as S (see Figures 1 and 2). It is obvious that $Pd(acac)_2$ as a catalyst makes a significant contribution to the 'overall cytotoxicity' of the reaction. The effect is especially pronounced in bio-Strips for FRSN cells, where $Pd(acac)_2$ has the lowest 24-h CC_{50} of 7 μM, compared to 24-h CC_{50} of 37 and 14 μM for CaCo-2 and HEK293T cells, respectively (see Figure 2, Figures S1–S3 and Table S1).

The effects of using 1-bromo-4-nitrobenzene as a starting material and NMP as a solvent are also evident, though to a lesser extent

† All commercial chemicals used in this work were purchased from ABCR, Acros, Alfa Aesar or Sigma-Aldrich and were used as supplied. The synthesis of 4-methoxy-4′-nitro-1,1′-biphenyl[15] and $Cs_2B_4O_7{\cdot}5H_2O$[13] was carried out according to previously published protocols and the purity of the products was confirmed by NMR and GC-MS. Cell cultures CaCo-2 (human colorectal adenocarcinoma), HEK293T (human embryonic kidney) and FRSN (foreskin mesenchymal stem cells) were purchased from the Institute of Cytology RAS, St. Petersburg, Russia, and were cultured and maintained as previously described.[13] The 24-h CC_{50} (half-maximal cytotoxic concentration upon 24-h incubation) values of the test substances were measured using the MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega, USA) as previously described.[13]

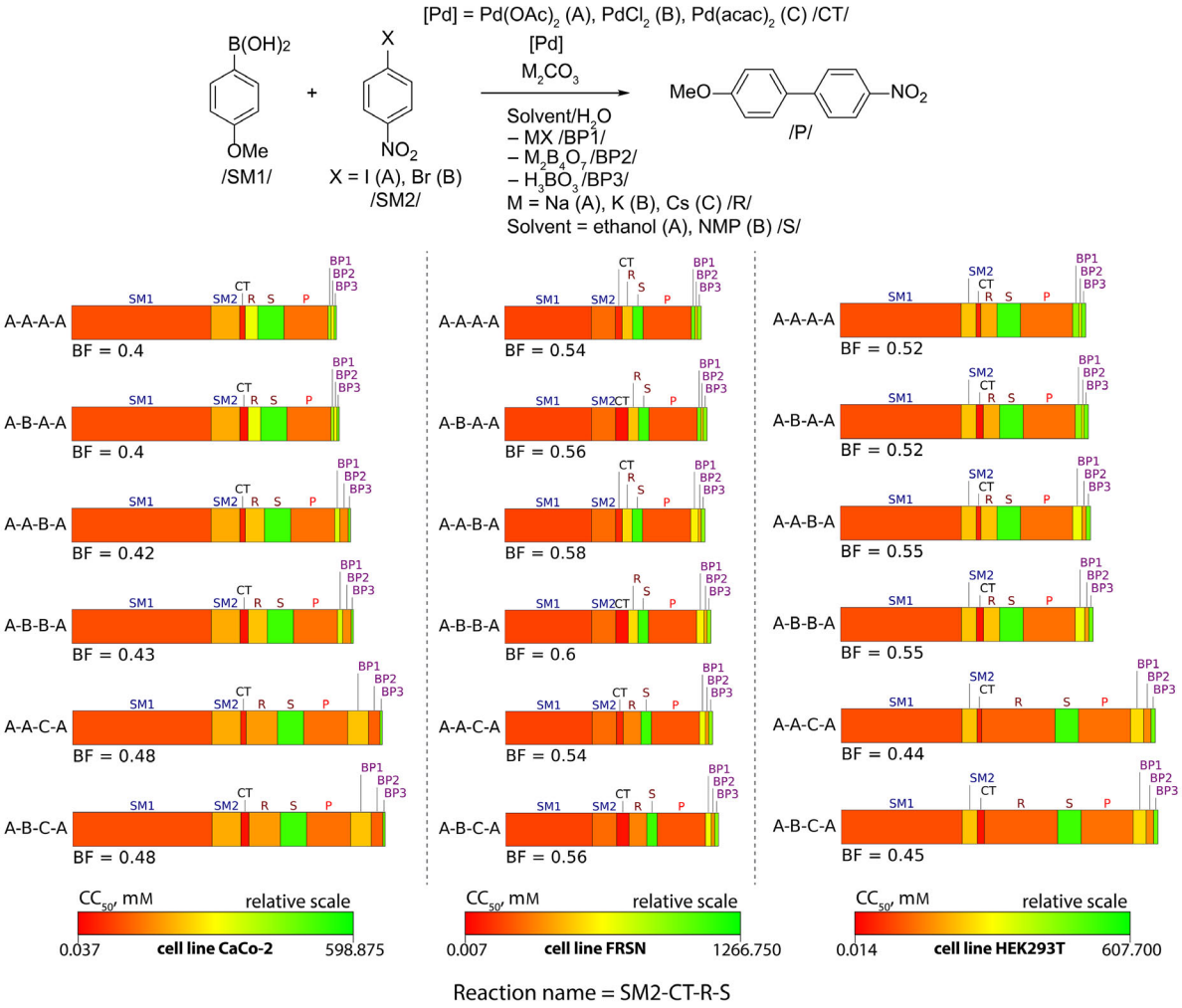

Figure 1 Bio-Strips of the most promising routes for the synthesis of 4-methoxy-4′-nitro-1,1′-biphenyl according to the cytotoxicity of the particular compounds against the CaCo-2, FRSN and HEK293T cell lines. The 1st, 2nd, 3rd and 4th letters in the reaction names correspond to the types of starting material 2 (SM2), catalyst (CT), reagent (R**)** and solvent (S), respectively. The color of the bio-Strip sections reflects the CC_{50} of a particular substance measured against a specific cell line (see cytotoxicity scales under the bio-Strips). Bio-Factors (BFs) are also shown.

(see Figure 2, Figures S1–S3 and Table S2). With regard to the solvent, its relatively low cytotoxicity is to a certain degree counter-balanced by its high amount used in the reaction. As for 1-bromo-4-nitrobenzene, it should be taken into account that KBr, being one of the byproducts produced in the reactions with this starting material, demonstrates significantly higher cytotoxicity against normal (FRSN) and immortalized normal (HEK293T) cells than against cancer cells (CaCo-2). In the case of KI, this effect is less pronounced (see Table S1).

Looking at the 24-h CC_{50} values of the reagents Na_2CO_3, K_2CO_3 and Cs_2CO_3, it can be seen that Cs_2CO_3 and the corresponding byproducts CsI, CsBr and $Cs_2B_4O_7$ generally exhibit higher cytotoxicity. However, due to the low amounts of these compounds used and produced in the reaction, their impacts on the 'overall cytotoxicity' appear small compared to the impacts of catalyst, solvent and starting material 2. This is reflected by the corresponding CPs shown in Figure 2 and Table S2.

It is of note that the BFs of all reactions analyzed in this work are below 1 in all cell lines tested. That is, the 'overall cytotoxicity' decreases during the reaction in all the synthetic routes considered. In accordance with this observation, the conversion also affected the 'overall cytotoxicity' of the reactions studied. For comparison, the CP_{f_rel} values for the reactions at 100, 75, 50 and 25% conversion are shown in Figure 3 (the exact values of CP_{f_rel}s, BFs, CP_is and CP_fs are given in Tables S3–S5). A decrease in the conversion led to an increase in the CP_{f_rel} of the reactions, making them less safe in terms of the remaining substances. It should be noted that for reactions with BFs above 1 the opposite picture would be observed.

Thus, in this work, by means of bio-Strips and cytotoxicity potentials, we identified the least and most harmful substances used in the synthesis of 4-methoxy-4′-nitro-1,1′-biphenyl. Regarding the contribution of the tested compounds to the 'overall cytotoxicity' of the reactions studied, the following observations can be made. Major attention should be paid to the selection of catalysts due to their high cytotoxicity. Starting materials and solvents can also have non-negligible effects on toxicity, the former due to their relatively high CC_{50} values and the latter due to their significant amounts in the reaction.

In this work, we used half-maximal cytotoxic concentrations (CC_{50}) as indicators of toxicity of chemicals because these indices can be measured relatively quickly for a large number of substances, allowing the screening of a large number of synthetic routes. Using different types of cell culture also allows estimating the impact of chemicals on various models of healthy and disease conditions of the human organism. Of course, the introduction of additional toxicity indices measured in different biological objects would provide a more comprehensive picture of the possible harmful impacts of substances on the environment.[18]

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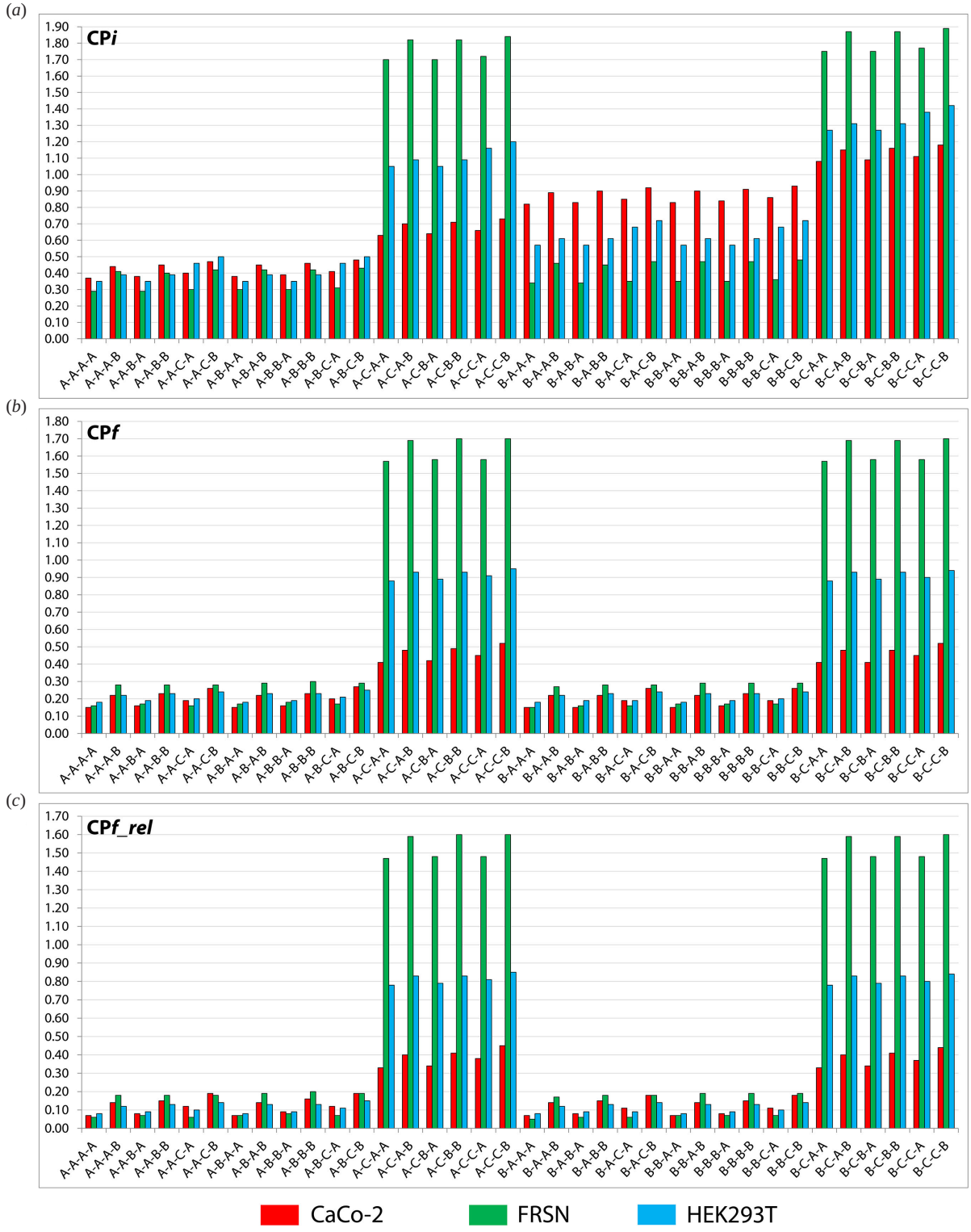

Figure 2 (*a*) Initial, (*b*) final and (*c*) relative final cytotoxicity potentials (CP_i, CP_f and CP_{f_rel}, respectively) of 36 routes for the synthesis of 4-methoxy-4′-nitro-1,1′-biphenyl calculated for CaCo-2, FRSN and HEK293T cells. The 1st, 2nd, 3rd and 4th letters in the reaction names correspond to the types of starting material 2 (SM2), catalyst (CT), reagent (R) and solvent (S), respectively (see Table S2).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.02.007.

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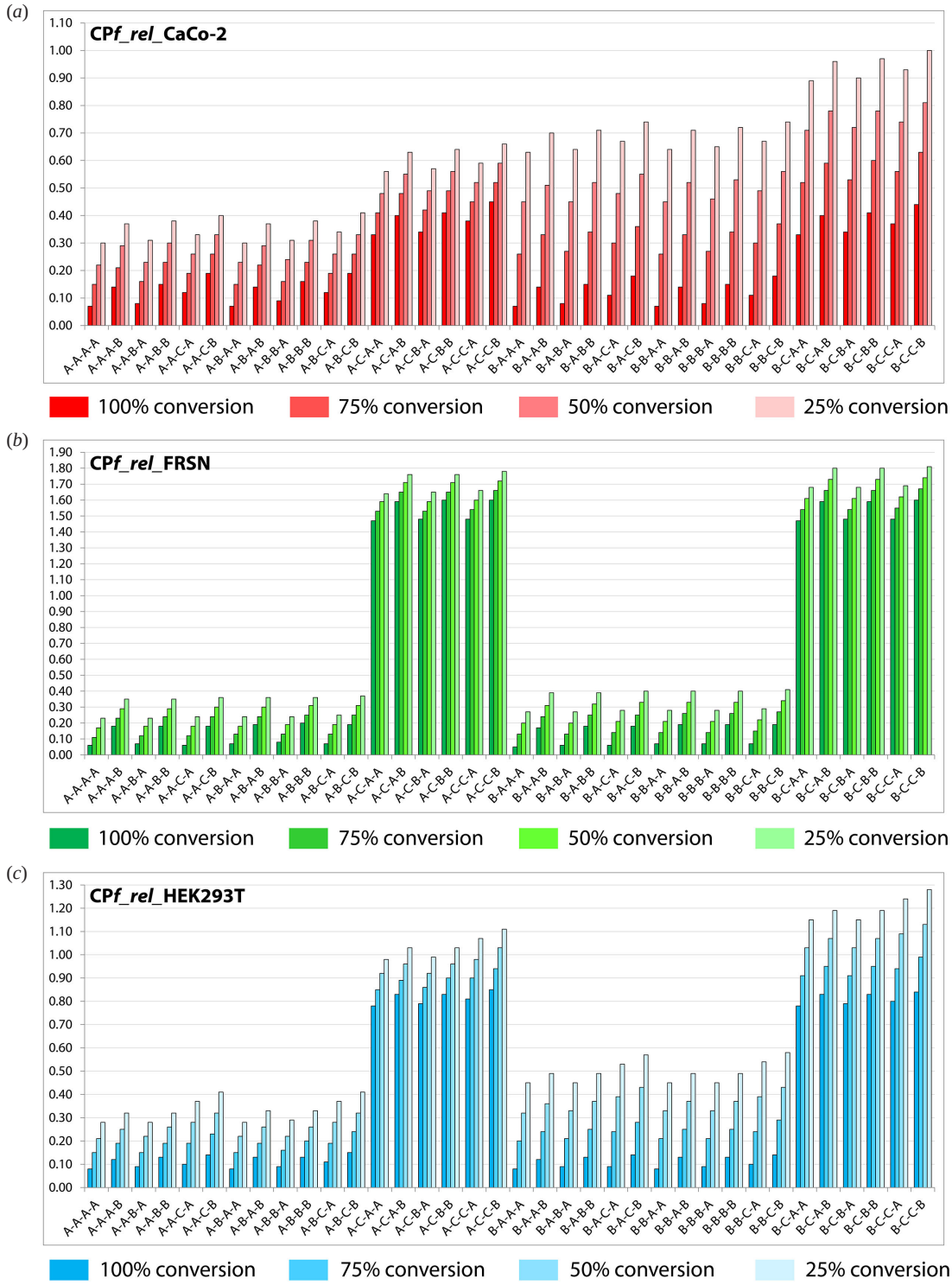

Figure 3 Impact of incomplete conversion on CP_{f_rel} of 36 routes for the synthesis of 4-methoxy-4′-nitro-1,1′-biphenyl, calculated for (*a*) CaCo-2, (*b*) FRSN and (*c*) HEK293T cells. The 1st, 2nd, 3rd and 4th letters in the reaction names correspond to the types of SM2, CT, R and S, respectively (see Tables S2–S5).

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