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Design, synthesis and biological evaluation of *N*-phenylsulfonylnicotinamide derivatives as novel antitumor inhibitors

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ABSTRACT

A series of novel *N*-phenylsulfonylnicotinamide derivatives (**1**–**24**) have been synthesized and evaluated as potential EGFR tyrosine kinase (TK) inhibitors. Among all the compounds, compound **10** (5-bromo-*N*-(4-chlorophenylsulfonyl)nicotinamide) showed the most potent growth inhibitory activity against EGFR TK and antiproliferative activity of MCF-7 cancer cell line in vitro, with IC_{50} value of 0.09 and 0.07 μM. Docking simulation was performed to insert compound **10** into the EGFR TK active site to determine the probable binding model. Based on the preliminary results, compound **10** with potent inhibitory activity to tumor growth may be a potential anticancer agent.

1. Introduction

Epidermal growth factor receptor (EGFR) kinase, a member of tyrosine kinases family is a transmembrane protein with an external ligand binding receptor domain and an intracellular tyrosine kinase activity domain. Once a native ligand (e.g., EGF) binds to the extracellular domain, it results in receptor homo- or heterodimerization, activation of intrinsic TK activity, and autophosphorylation of the receptors, which regulates cell growth, differentiation, mitosis, and death.[1–6]

ion, mutation, or coexpression of the ligand and the receptor, these receptors can become hyperactivated; the result of this is uncontrolled cell proliferation.[7,8] The abnormal phosphorylation of EGFR tyrosine kinase (TK) has been studied in nearly all kinds of cancers.[9,10] However breast cancer is most thoroughly studied, which is overexpressed in 25–30% of cases and is correlated with a poor prognosis.[11–14] Compounds that inhibit the kinase activity of EGFR are of potential interest as new therapeutic antitumor agents.

In previous studies, *N*-(phenylsulfonyl)benzamide derivatives (Fig. 1) revealed remarkable growth inhibitory activity against VEGF and antitumor effects against a broad range of human tumor xenografts in SCID mice.[15] Historically, the VEGF and EGFR pathways are closely related, sharing common downstream. Inhibition of VEGF-related pathways is proved to contribute to the mechanism of action of agents targeting the EGFR.[16–18] Furthermore, the target of many drugs for treating breast cancer is not VEGF but EGFR.[19] These results promoted us to hypothesize that *N*-(phenylsulfonyl)benzamide derivatives may also inhibit the overexpression of EGFR. In addition, Kim et al. have reported bioisosteres of terpyridine with considerable protein kinase C (PKC) inhibitory activity and antitumor cytotoxicities against several human cancer cell lines.[20] On the other hand, pyridine which has similar structure with benzene, a small bioactive molecule, is an important pharmacophore that can form hydrogen-bonded structures similar to those encountered with the base-pairing mechanism in DNA and RNA.[21,22] In view of their importance as drugs, biologically active natural products, and in other related applications, extensive studies have been carried out on the synthesis of pyridine compounds in recent years. Therefore, we try to replace the benzene ring with the pyridine ring to improve the biological activity. All of these encouraged us to synthesize and biologically

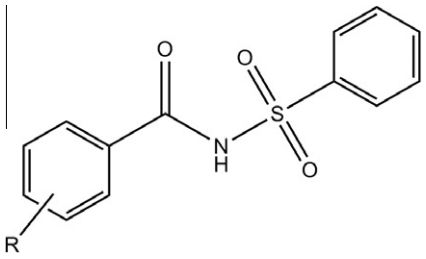

Figure 1. The structure of *N*-(phenylsulfonyl)benzamide derivatives.

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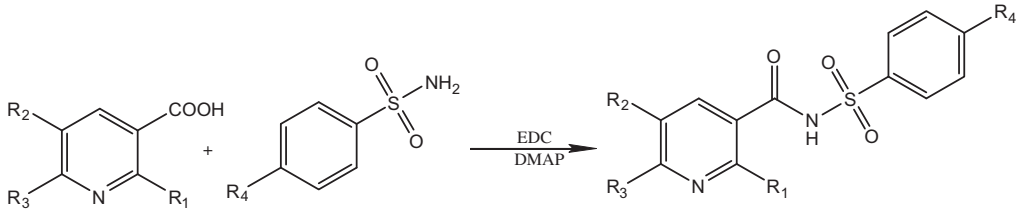

1: R_1= H, R_2= H, R_3=H, R_4=H

2: R_1= H, R_2= H, R_3=H, R_4= Me

3: R_1= H, R_2= H, R_3=H, R_4= F

4: R_1= H, R_2= H, R_3=H, R_4= Br

5: R_1= H, R_2= H, R_3=H, R_4= Cl

6: R_1= H, R_2= Br, R_3=H, R_4=H

7: R_1= H, R_2= Br, R_3=H, R_4= Me

8: R_1= H, R_2= Br, R_3=H, R_4= F

9: R_1= H, R_2= Br, R_3=H, R_4= Br

10: R_1= H, R_2= Br, R_3=H, R_4=Cl

11: R_1= Cl, R_2= H, R_3=H, R_4= H

12: R_1= Cl, R_2= H, R_3=H, R_4= Me

13: R_1= Cl, R_2= H, R_3=H, R_4= F

14: R_1= Cl, R_2= H, R_3=H, R_4= Br

15: R_1= Cl, R_2= H, R_3=H, R_4= Cl

16: R_1= H, R_2= H, R_3=Cl, R_4= H

17: R_1= H, R_2= H, R_3=Cl, R_4= Me

18: R_1= H, R_2= H, R_3=Cl, R_4= F

19: R_1= H, R_2= H, R_3=Cl, R_4= Br

20: R_1= H, R_2= H, R_3=Cl, R_4= Cl

21: R_1= Cl, R_2= H, R_3=Me, R_4=H

22: R_1= Cl, R_2= H, R_3=Me, R_4= Me

23: R_1= Cl, R_2= H, R_3=Me, R_4= Br

24: R_1= Cl, R_2= H, R_3=Me, R_4= Cl

Scheme 1. Synthesis route of *N*-phenylsulfonylnicotinamide derivatives (compounds **1**–**24**). Reagents and conditions: EDC·HCl/DMAP, CH_2Cl_2, Reflux, 6–8 h

Table 1
Inhibition (IC_{50}) of human breast cell lines MCF-7 proliferation and EGFR kinase phosphorylation of compounds **1**–**24**

Compounds	IC_{50} (μM)	
	MCF-7	EGFR inhibition IC_{50}
1	14.42 ± 0.81	>30
2	12.04 ± 0.15	24.83 ± 0.61
3	15.97 ± 0.62	>30
4	14.69 ± 0.57	>30
5	12.97 ± 0.43	25.36 ± 0.29
6	12.89 ± 0.61	20.19 ± 0.32
7	9.37 ± 0.14	12.11 ± 0.14
8	0.98 ± 0.012	3.27 ± 0.23
9	0.34 ± 0.016	2.08 ± 0.06
10	0.07 ± 0.002	0.09 ± 0.004
11	14.02 ± 0.45	>30
12	12.52 ± 0.61	27.05 ± 0.23
13	12.49 ± 0.58	28.66 ± 0.19
14	11.37 ± 0.36	15.68 ± 0.52
15	10.22 ± 0.41	19.83 ± 0.21
16	14.11 ± 0.67	>30
17	17.21 ± 0.51	>30
18	16.83 ± 0.67	>30
19	9.05 ± 0.12	29.07 ± 0.08
20	8.07 ± 0.14	18.15 ± 0.82
21	13.42 ± 0.79	21.56 ± 0.09
22	8.52 ± 0.15	15.02 ± 0.38
23	1.97 ± 0.05	2.64 ± 0.09
24	1.35 ± 0.08	1.19 ± 0.11
Erlotinib	0.02 ± 0.001	0.03 ± 0.001

evaluate of a novel series of *N*-phenylsulfonylnicotinamide derivatives as anti-EGFR TK agents and antiproliferative activity of MCF-7 cancer cell.

2. Results and discussion

2.1. Chemistry

The synthesis of *N*-phenylsulfonylnicotinamide derivatives followed the general reaction pathway outlined in Scheme 1. Compounds **1–24** were synthesized by coupling substituted nicotinic acid with equimolar quantities of substituted benzenesulfonamide amides using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (DMAP) as coupling reagent. Most of the target compounds **1–24** were reported for the first time. All of the synthetic compounds gave satisfactory analytical and spectroscopic data. ^{1}H NMR and ESI-MS spectra were consistent with the assigned structures.

2.2. Biological activity and molecular modeling

The synthesized *N*-phenylsulfonylnicotinamide derivatives **1–24** were evaluated for their ability to inhibit the autophosphorylation of EGFR TK using ELISA kits and western blotting. The half maximal inhibitory concentration (IC_{50}) of the compounds **1–24** are summarized in Table 1. As shown in Table 1, it was observed that some *N*-phenylsulfonylnicotinamide derivatives (such as compounds **8**, **9**, **10**, **23** and **24**) were found to exhibit good inhibitory activity, displaying IC_{50} values between 0.09 and 3.27 μM.

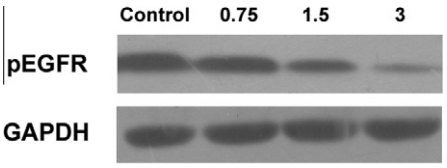

Figure 2. Inhibitory effect of compound **10** on EGFR kinase phosphorylation level. MCF-7 cells were seeded into 6-well plates at a concentration of 1×10^5 cells/ml. After 16 h of growth in serum-containing media, cells were incubated in serum-free media for 24 h. Compound **10** was then added for 1 h at the concentration of 0.75, 1.5 and 3 μM, then stimulated for 15 min with 100 ng/mL EGF. After incubation, cells were lysed, and protein was applied on SDS–polyacrylamide gel. The level of EGFR kinase phosphorylation level was examined by western blotting analysis.

Compound **10** displayed the most potent inhibitory activity with IC_{50} of 0.09 μM (the IC_{50} of positive control Erlotinib = 0.03 μM). The results of western blotting assay further confirmed that compound **10** at the concentration above 1.5 μM could inhibit EGF-induced EGFR autophosphorylation in the MCF-7 cell line (Fig. 2).

Subsequently SAR (structure–activity relationship) studies were performed by modification of the parent compound to determine how the substituents of the subunits affected the EGFR TK inhibitory activities. As shown in Table 1, structure–activity relationships for compounds **1–24** demonstrated that compounds **6–10** with meta-bromide substituents on pyridine ring showed potent EGFR TK inhibitory activities. Compounds **21–24** with both ortho-methyl and chloro groups on the pyridine ring were more active than compounds **11–20** that contain single ortho-chloro group on the pyridine ring. Compounds bearing the same substituents on the pyridine ring exhibited distinct EGFR TK inhibitory activities due to the difference of the substituents on the phenyl ring. The EGFR TK inhibitory activities of compounds **1–24** with different para-substituents on phenyl ring increased in the following order: CH_3 < F < Br < Cl. This result indicated that compounds **1–24** with electronic-withdrawing substituents (halogen) on the phenyl ring showed more potent EGFR inhibitory activities than compounds contains donating methyl substituents.

In order to further understand the SARs observed for EGFR TK and to guide additional SAR studies, molecular docking of the most potent inhibitor (**10**) into ATP binding site of EGFR TK was performed. Molsoft ICM-Pro software was chosen as the virtual docking tool that used the active site of the EGFR/ Erlotinib crystal structure with the inhibitor excluded from the coordinates (PDB accession code 1M17[23]) as a receptor for compound binding. Residues around Erlotinib at a radius of 6 Å were isolated for the construction of the grid for docking screening. As depicted in Figure 3, the oxygen atom of carboxyl group of the compound **10** is potently bound to hydrogen atom of hydroxyl group of Thr 766 in EGFR TK (distance = 2.76 Å). Nitrogen atom of pyridine ring exhibits a hydrogen bond with Met 769 in EGFR TK (distance = 1.80 Å). In addition, the $MeSO_2$–phenyl moiety is oriented towards the EGFR TK secondary pocket (Lys 721 and Thr 830). One of the *O*-atom of *p*-$MeSO_2$ substituent forms a hydrogen binding interaction with amino group of Lys 721 (distance = 2.34 Å) whereas the other *O*-atom is about 2.42 Å away from hydroxy of this amino acid of Thr 830, which can provide a good explanation for the high potency of compound **10** towards EGFR TK. This molecular docking result, along with the enzyme assay data, suggesting that compound **10** is a potential inhibitor of EGFR.

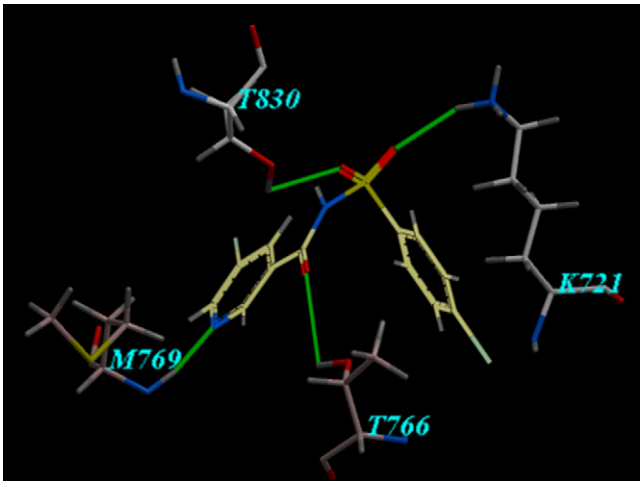

Figure 3. Binding mode of compound **10** with EGFR kinase; Ligand is represented as wire, the interacting residues are depicted as stick and ball. The H-bond (green) is displayed as line.

Besides, in vitro antiproliferative activities of compounds **1–24** were studied in the human breast cancer cell line MCF-7, which overexpresses EGFR TK, by applying the MTT colorimetric assay.[11] As shown in Table 1, most compounds exhibited similar trends with inhibitory activities against EGFR TK. Compound **10** was found to exhibit the most potent activity with IC_{50} values of 0.07 μM (the IC_{50} of positive control Erlotinib = 0.02 μM).

3. Conclusions

In summary, a novel series of *N*-phenylsulfonylnicotinamide derivatives that may function as inhibitors of EGFR TK have been prepared, and some of the synthesized compounds displayed efficient EGFR inhibitory activity. Compound **10** displayed the most potent inhibitory activity (IC_{50} = 0.09 μM). Moreover, antiproliferative assay results indicated that compound **10** showed promising inhibitory activity (IC_{50} = 0.07 μM) against the human breast cancer cell line, MCF-7, which overexpresses EGFR TK. After analysis of the binding model of compound **10** with EGFR, it was found that four hydrogen bonds interaction with the protein residues in the ATP binding site might play a crucial role in its EGFR and antiproliferative activities. Therefore, compound **10** could be a promising lead for the further development of a potential anticancer agent.

4. Experimental

4.1. Chemistry

The synthesis route of compounds **1–24** followed the general pathway outlined in Scheme 1. The substituted nicotinic acid (1 mmol) mixed with benzenesulfonamide (1 mmol) through by using 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) (1.2 mmol) and 4-dimethylaminopyridine (DMAP) (1.2 mmol) in anhydrous CH_2Cl_2 for 6–8 h at 70–80 °C. The reaction was monitored by TLC. The products are extracted with ethyl acetate. The extract is washed successively with 1 N HCl, water, 1 M $NaHCO_3$, and water, dried over $MgSO_4$, filtered and evaporated. The residue is purified by column chromatography using petroleum ether and ethyl acetate (1:1).

4.2. Spectral properties of *N*-phenylsulfonylnicotinamide derivatives

4.2.1. *N*-(Phenylsulfonyl)nicotinamide (1)

White solid, yield 81%, mp: 242–244 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.53–7.56 (m, 1H), 7.63–7.66 (m, 2H), 7.72 (t, J = 7.5 Hz, 1H), 8.00–8.02 (m, 2H), 8.23–8.25 (m, 1H), 8.76–8.78 (m, 1H), 9.00 (m, 1H). MS (ESI): 263.0 ($C_{12}H_{11}N_2O_3S$, $[M+H]^+$). Anal.

Calcd for $C_{12}H_{10}N_2O_3S$: C, 54.95; H, 3.84; N, 10.68. Found: C, 54.74; H, 3.85; N, 10.72.

4.2.2. *N*-Tosylnicotinamide (2)

White solid, yield 83%, mp: 225–227 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 2.40 (s, 3H), 7.44 (d, J = 8.0 Hz, 2H), 7.52–7.57 (m, 1H), 7.90 (d, J = 8.2 Hz, 2H), 8.23 (d, J = 8.0 Hz, 1H), 8.77–8.79 (m, 1H), 8.99 (d, J = 1.8 Hz, 1H). MS (ESI): 277.1 ($C_{13}H_{13}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14. Found: C, 56.38; H, 4.39; N, 10.10.

4.2.3. *N*-(4-Fluorophenylsulfonyl)nicotinamide (3)

White solid, yield 85%, mp: 245–246 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.46–7.49 (m, 2H), 7.56–7.58 (m, 1H), 8.06–8.09 (m, 2H), 8.26–8.28 (m, 1H), 8.77–8.79(m, 1H), 9.01 (d, J = 1.6 Hz, 1H). MS (ESI): 281.0 ($C_{12}H_{10}FN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9FN_2O_3S$: C, 51.42; H, 3.24; N, 9.99. Found: C, 51.24; H, 3.25; N, 9.95.

4.2.4. *N*-(4-Bromophenylsulfonyl)nicotinamide (4)

White solid, yield 79%, mp: 255–256 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.51–7.61 (m, 1H), 7.82–7.85 (m, 2H), 7.90–7.93 (m, 2H), 8.28–8.32 (m, 1H), 8.77–8.79 (m, 1H), 9.01 (d, J = 2.2 Hz, 1H). MS (ESI): 343.0 ($C_{12}H_{10}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9BrN_2O_3S$: C, 42.24; H, 2.66; N, 8.21. Found: C, 42.37; H, 2.67; N, 8.23.

4.2.5. *N*-(4-Chlorophenylsulfonyl)nicotinamide (5)

White solid, yield 82%, mp: 241–243 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.58–7.62 (m, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.93 (d, J = 9.1 Hz, 2H), 8.29–8.33 (m, 1H), 8.78–8.81 (m, 1H), 9.02 (d, J = 2.0 Hz, 1H). MS (ESI): 298.1 ($C_{12}H_{10}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9ClN_2O_3S$: C, 48.57; H, 3.06; N, 9.44. Found: C, 48.31; H, 3.31; N, 9.22.

4.2.6. 5-bromo-*N*-(phenylsulfonyl)nicotinamide (6)

White solid, yield 86%, mp: 206 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.64 (t, J = 7.0 Hz, 2H), 7.73 (d, J = 7.3 Hz, 1H), 8.01 (t, J = 4.4 Hz, 2H), 8.48 (t, J = 2.0 Hz, 1H), 8.91–8.95 (dd, J1=1.8, J2=8.6 Hz, 2H). MS (ESI): 342.9 ($C_{12}H_{10}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9BrN_2O_3S$: C, 42.24; H, 2.66; N, 8.21. Found: C, 42.40; H, 2.65; N, 8.23.

4.2.7. 5-bromo-*N*-tosylnicotinamide (7)

White solid, yield 67%, mp: 250 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 2.20–2.62 (m, 3H), 8.08–8.18 (m, 2H), 8.19–8.23 (m, 1H), 8.56–8.59 (m, 1H), 8.90 (t, J = 2.1 Hz, 1H), 9.33 (d, J = 2.2 Hz, 1H), 9.46 (d, J = 1.8 Hz, 1H), 11.65 (s, 1H). MS (ESI): 357.2 ($C_{13}H_{12}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{11}BrN_2O_3S$: C, 43.96; H, 3.12; N, 7.89. Found: C, 43.80; H, 3.11; N, 7.85.

4.2.8. 5-bromo-N-(4-fluorophenylsulfonyl)nicotinamide (8)

White solid, yield 78%, mp: 165–166 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.48 (t, J = 8.8 Hz, 2H), 8.06–8.10 (m, 2H), 8.48 (s, 1H), 8.91–8.94 (m, 2H). MS (ESI): 361.2 ($C_{12}H_9BrFN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_8BrFN_2O_3S$: C, 40.13; H, 2.25; N, 7.80. Found: C, 40.28; H, 2.26; N, 7.76.

4.2.9. 5-bromo-*N*-(4-bromophenylsulfonyl)nicotinamide (9)

White solid, yield 82%, mp: 249–250 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.44–7.50 (t, J = 8.9 Hz, 2H), 8.05–8.09 (m, 2H), 8.46 (t, J = 2.1 Hz, 1H), 8.89 (d, J = 2.2 Hz, 1H), 8.94 (d, J = 2.0 Hz, 1H). MS (ESI): 422.6 ($C_{12}H_9Br_2N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_8Br_2N_2O_3S$: C, 34.31; H, 1.92; N, 6.67. Found: C, 34.44; H, 1.93; N, 6.65.

4.2.10. 5-bromo-*N*-(4-chlorophenylsulfonyl)nicotinamide (10)

White solid, yield 87%, mp: 230 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.69 (d, J = 8.6 Hz, 2H), 7.99 (d, J = 8.8 Hz, 2H), 8.45 (t, J = 2.0 Hz, 1H), 8.89 (d, J = 2.2 Hz, 1H), 8.94 (d, J = 1.9 Hz, 1H). MS (ESI): 375.9 ($C_{12}H_9BrClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_8BrClN_2O_3S$: C, 38.37; H, 2.15; N, 7.46. Found: C, 38.48; H, 2.16; N, 7.43.

4.2.11. 2-Chloro-*N*-(phenylsulfonyl)nicotinamide (11)

White solid, yield 75%, mp: 170–172 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.48–7.52 (m, 1H), 7.68 (t, J = 7.4 Hz, 2H), 7.77 (t, J = 7.3 Hz, 1H), 7.97–8.02 (m, 3H), 8.50–8.52 (m, 1H), 12.90 (s, 1H). MS (ESI): 297.0 ($C_{12}H_{10}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9ClN_2O_3S$: C, 48.57; H, 3.06; N, 9.44. Found: C, 48.75; H, 3.07; N, 9.47.

4.2.12. 2-Chloro-*N*-tosylnicotinamide (12)

White solid, yield 73%, mp: 182 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 2.92 (s, 3H), 7.92–7.96 (m, 3H), 8.40–8.46 (m, 3H), 8.94–8.96 (m, 1H), 11.59 (s, 1H). MS (ESI): 311.0 ($C_{13}H_{12}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{11}ClN_2O_3S$: C, 50.24; H, 3.57; N, 9.01. Found: C, 50.42; H, 3.58; N, 9.03.

4.2.13. 2-Chloro-*N*-(4-fluorophenylsulfonyl)nicotinamide (13)

White solid, yield 86%, mp: 182 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.92–7.96 (m, 3H), 8.40–8.46 (m, 3H), 8.94–8.96 (m, 1H), 11.59 (s, 1H). MS (ESI): 315.0 ($C_{12}H_9ClFN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_8ClFN_2O_3S$: C, 45.80; H, 2.56; N, 8.90. Found: C, 45.65; H, 2.55; N, 8.85.

4.2.14. *N*-(4-bromophenylsulfonyl)-2-chloronicotinamide (14)

White solid, yield 87%, mp: 202 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.49–7.53 (m, 1H), 7.89–7.95 (m, 4H), 7.99–8.02 (dd, $J1$ = 2.0, $J2$ = 7.5, 1H), 8.51–8.53 (dd, $J1$ = 2.0, $J2$ = 5.0, 1H). MS (ESI): 375.9 ($C_{12}H_9BrClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_8BrClN_2O_3S$: C, 38.37; H, 2.15; N, 7.46. Found: C, 38.28; H, 2.16; N, 7.48.

4.2.15. 2-Chloro-*N*-(4-chlorophenylsulfonyl)nicotinamide (15)

White solid, yield 76%, mp: 196–198 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.49–7.53 (m, 1H), 7.75–7.78 (d, J = 10.3 Hz, 2H), 7.99–8.03 (m, 3H), 8.51–8.53 (dd, $J1$ = 1.8, $J2$ = 4.9 Hz, 1H), 12.94 (s, 1H). MS (ESI): 330.9 ($C_{12}H_9Cl_2N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_8Cl_2N_2O_3S$: C, 43.52; H, 2.43; N, 8.46. Found: C, 43.36; H, 2.42; N, 8.48.

4.2.16. 6-Chloro-*N*-(phenylsulfonyl)nicotinamide (16)

White solid, yield 80%, mp: 191 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 8.04–8.17 (m, 3H), 8.18–8.23 (m, 1H), 8.56–8.59 (m, 2H), 8.73–8.76 (dd, $J1$ = 2.6 Hz, $J2$ = 8.43 Hz, 1H), 9.35–9.36 (d, J = 2.6 Hz, 1H), 11.65 (s, 1H). MS (ESI): 297.0 ($C_{12}H_{10}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_9ClN_2O_3S$: C, 48.57; H, 3.06; N, 9.44. Found: C, 48.68; H, 3.05; N, 9.41.

4.2.17. 6-Chloro-*N*-tosylnicotinamide (17)

White solid, yield 81%, mp: 193–194 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 2.89 (s, 3H), 7.88 (d, J = 8.6 Hz, 2H), 8.03–8.06 (J = 8.4 Hz, 1H), 8.42–8.45 (J = 8.4 Hz, 2H), 8.72–8.75 (dd, $J1$ = 2.6, $J2$ = 8.4 Hz, 1H), 9.34–9.35 (m, 1H), 11.61 (s, 1H). MS (ESI): 312.6 ($C_{13}H_{12}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{11}ClN_2O_3S$: C, 50.24; H, 3.57; N, 9.01. Found: C, 50.48; H, 3.58; N, 9.03.

4.2.18. 6-Chloro-*N*-(4-fluorophenylsulfonyl)nicotinamide (18)

White solid, yield 83%, mp: 199–200 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.83–7.91 (m, 2H), 8.04–8.07 (d, J = 8.4 Hz, 1H), 8.61–8.65 (m, 2H), 8.67–8.77 (dd, $J1$ = 2.6, $J2$ = 8.4 Hz, 1H), 9.35 (d, J = 2.0 Hz, 1H), 11.70 (s, 1H). MS (ESI): 314.9 ($C_{12}H_9ClFN_2O_3S$,

[M+H]$^+$). Anal. Calcd for $C_{12}H_8ClFN_2O_3S$: C, 45.80; H, 2.56; N, 8.90. Found: C, 45.58; H, 2.55; N, 8.88.

4.2.19. *N*-(4-bromophenylsulfonyl)-6-chloronicotinamide (19)

White solid, yield 77%, mp: 235–237 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 8.04–8.07 (d, J = 8.4 Hz, 1H), 8.28–8.33 (m, 2H), 8.47–8.51 (m, 2H), 8.73–8.77 (dd, $J1$ = 2.6, $J2$ = 8.4 Hz, 1H), 9.35–9.36 (d, J = 2.6 Hz, 1H), 11.73 (s, 1H). MS (ESI): 375.9 ($C_{12}H_9BrClN_2O_3S$, [M+H]$^+$). Anal. Calcd for $C_{12}H_8BrClN_2O_3S$: C, 38.37; H, 2.15; N, 7.46. Found: C, 38.48; H, 2.16; N, 7.44.

4.2.20. 6-Chloro-*N*-(4-chlorophenylsulfonyl)nicotinamide (20)

White solid, yield 78%, mp: 221–222 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 7.65 (d, J = 8.2 Hz, 1H), 7.71–7.75 (d, J = 8.67 Hz, 2H), 7.99–8.04 (d, J = 8.76 Hz, 2H), 8.23–8.27 (dd, $J1$ = 2.6 Hz, $J2$ = 8.43 Hz, 1H), 8.84 (d, J = 2.6 Hz, 1H), 11.60 (s, 1H). MS (ESI): 333.8 ($C_{12}H_9Cl_2N_2O_3S$, [M+H]$^+$). Anal. Calcd for $C_{12}H_8Cl_2N_2O_3S$: C, 43.52; H, 2.43; N, 8.46. Found: C, 43.38; H, 2.41; N, 8.43.

4.2.21. 2-Chloro-6-methyl-*N*-(phenylsulfonyl)nicotinamide (21)

Yellow solid, yield 66%, mp: 90–92 °C. ^{1} NMR (500 MHz, DMSO-d_6) δ: 1.99–2.60 (m, 3H), 7.34 (d, J = 7.9 Hz, 1H), 7.66–7.69 (m, 2H), 7.74–7.78 (m, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.99–8.01 (m, 2H), 12.82 (s, 1H). MS (ESI): 311.0 ($C_{13}H_{12}ClN_2O_3S$, [M+H]$^+$). Anal. Calcd for $C_{13}H_{11}ClN_2O_3S$: C, 50.24; H, 3.57; N, 9.01. Found: C, 50.40; H, 3.56; N, 9.04.

4.2.22. 2-Chloro-6-methyl-*N*-tosylnicotinamide (22)

Yellow solid, yield 68%, mp: 96 °C. ^{1} NMR (300 MHz, DMSO-d_6) δ: 1.15–1.19 (s, 3H), 2.40 (s, 3H), 7.74 (d, J = 8.0 Hz, 2H), 7.89 (d, J = 8.2 Hz, 2H), 8.47 (s, 1H), 8.92 (d, J = 5.3 Hz, 2H). MS (ESI): 325.0 ($C_{14}H_{14}ClN_2O_3S$, [M+H]$^+$). Anal. Calcd for $C_{14}H_{13}ClN_2O_3S$: C, 51.77; H, 4.03; N, 8.63. Found: C, 51.68; H, 4.01; N, 8.66.

4.2.23. *N*-(4-bromophenylsulfonyl)-2-chloro-6-methylnicotin amide (23)

Yellow solid, yield 72%, mp: 121 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.04 (s, 3H), 7.35 (d, J = 7.9 Hz, 1H), 7.88 (t, J = 8.6 Hz, 1H), 7.91–7.94 (t, J = 4.8 Hz, 4H). MS (ESI): 391.9 ($C_{13}H_{11}BrClN_2O_3S$, [M+H]$^+$). Anal. Calcd for $C_{13}H_{10}BrClN_2O_3S$: C, 40.07; H, 2.59; N, 7.19. Found: C, 40.33; H, 2.60; N, 7.16.

4.2.24. 2-Chloro-*N*-(4-chlorophenylsulfonyl)-6-methylnicotin amide (24)

Yellow solid, yield 69%, mp: 125 °C. ^{1} NMR (500 MHz, DMSO-d_6) δ: 2.00 (s, 3H), 7.35 (d, J = 7.9 Hz, 1H), 7.70–7.77 (d, J = 8.8 Hz, 2H), 7.88 (d, J = 8.8 Hz, 1H), 8.00 (d, J = 8.8 Hz, 2H), 12.98 (s, 1H). MS (ESI): 344.9 ($C_{13}H_{11}Cl_2N_2O_3S$, [M+H]$^+$). Anal. Calcd for $C_{13}H_{10}Cl_2N_2O_3S$: C, 45.23; H, 2.92; N, 8.12. Found: C, 45.43; H, 2.93; N, 8.15.

4.3. Preparation, and purification of EGFR and inhibitory assay

A 1.6 kb cDNA encoded for the EGFR cytoplasmic domain (EGFR-CD, amino acids 645–1186) were cloned into baculoviral expression vector pFASTBacHTc. A sequence that encodes $(His)_6$ was located at the 50 upstream to the EGFR sequence. Sf-9 cells were infected for 3 days for protein expression. Sf-9 cell pellets were solubilized at 0 °C in a buffer at pH 7.4 containing 50 mM HEPES, 10 mM NaCl, 1% Triton, 10 μM ammonium molybdate, 100 μM sodium vanadate, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL pepstatin, and 16 μg/mL benzamidine HCl for 20 min followed by 20 min centrifugation. Crude extract supernatant was passed through an equilibrated Ni–NTA superflow packed column and washed with 10 mM and then 100 mM imidazole to remove nonspecifically bound material. Histidine tagged proteins were eluted with 250 and 500 mM imidazole and dialyzed against 50 mM NaCl, 20 mM HEPES, 10% glycerol, and 1 μg/mL each of aprotinin, leupeptin, and pepstatin for 2 h. The entire purification procedure was performed at 4 °C or on ice.[24]

The EGFR kinase assay was set up to assess the level of autophosphorylation based on DELFIA/Time-Resolved Fluorometry. Compounds **1**–**24** were dissolved in 100% DMSO and diluted to the appropriate concentrations with 25 mM HEPES at pH 7.4. In each well, 10 μL of compound was incubated with 10 μL (12.5 ng for HER-2 or 5 ng for EGFR) of recombinant enzyme (1:80 dilution in 100 mM HEPES) for 10 min at room temperature. Then, 10 μL of 5 mM buffer (containing 20 mM HEPES, 2 mM $MnCl_2$, 100 μM Na_3VO_4, and 1 mM DTT) and 20 μL of 0.1 mM ATP-50 mM $MgCl_2$ was added for 1 h. Positive and negative controls were included in each plate by incubation of enzyme with or without ATP-$MgCl_2$. At the end of incubation, liquid was aspirated, and plates were washed three times with wash buffer. A 75 μL (400 ng) sample of europium labeled anti-phosphotyrosine antibody was added to each well for another 1 h of incubation. After washing, enhancement solution was added and the signal was detected by Victor (Wallac Inc.) with excitation at 340 nm and emission at 615 nm. The percentage of autophosphorylation inhibition by the compounds was calculated using the following equation: 100%−[(negative control)/(positive control−negative control)]. The IC_{50} was obtained from curves of percentage inhibition with eight concentrations of compound. As the contaminants in the enzyme preparation are fairly low, the majority of the signal detected by the anti-phosphotyrosine antibody is from EGFR.

4.4. Western immunoblot analysis

MCF-7 cells were pretreated with compounds **10** at the concentrations of 0.75, 1.5 and 3 μM for 15 min before treatment with 100 ng/mL EGF and examining the expression of EGFR protein. Cells were lysed with lysis buffer. Western immunoblot analysis was performed using a method described by Cheenpracha, et al.[25]

4.5. Antiproliferative activities assay

The antiproliferative activities of compounds **1–24** were determined using a standard (MTT)-based colorimetric assay (Sigma). Briefly, cell lines were seeded at a density of 7×10^3 cells/well in 96-well microtiter plates (Costar). After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 0.1 to 100 μM. After 48 h, cell survival was determined by the addition of an MTT solution (10 μL of 5 mg/mL MTT in PBS). After 4 h, 100 μL of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37 °C for a further 18 h; optical absorbance was measured at 570 nm on an LX300 Epson Diagnostic microplate reader. Survival ratios are expressed in percentages with respect to untreated cells. IC_{50} values were determined from replicates of six wells from at least two independent experiments.

4.6. Docking simulations

Molecular docking of compounds **10** into the three-dimensional EGFR complex structure (PDB code: 1M17, download from the PDB) was carried out using the Molsoft ICM-Pro software package (version 3.5-0a).[26]

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