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Structure Based Design of Inhibitors of *Trypanosoma cruzi* DHFR as Potential Therapeutic Agents for Chagas' Disease

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Abstract

Dihydrofolate reductase (DHFR) enzyme of the parasite *Trypanosoma cruzi* is a potential target for developing drugs for the treatment of Chagas' disease. We report here the rational design, synthesis, characterization and biological evaluation of four potent inhibitors of this parasitic enzyme. Inhibitory activity of each compound is determined against *T. cruzi* as well as against human DHFR for comparison. Potent nanomolar inhibitors of *T. cruzi* DHFR have been synthesized. Moderate increase in Selectivity Index (SI) has been achieved for some of these inhibitors.

Introduction

Chagas' disease is caused by the protozoan parasite *Trypanosoma cruzi*, which is primarily transmitted by an insect vector. This disease is also transmitted via blood transfusion, organ transplant and genetically from mother to child. The disease affects millions of people in Latin America where the vector and the parasite are endemic.[1] There are two stages for this disease: acute and chronic. There is no effective drug for the treatment of the chronic stage of this disease. Only two drugs are available that to treat the acute stage (Nifurtimox and Benznidazole). However, these two drugs result in toxic side effects. With more than 100 million people in 20 countries at risk and no effective treatments available, there is an immediate need for new drugs to treat this disease. Therefore, validating potential drug targets and identifying novel drug candidates to treat Chagas' disease are needs of considerable global importance. [2-5]

Since the dihydrofolate reductase (*Tc*DHFR) activity of *T. cruzi* is essential for the life of parasite, it represents a potential target for rational drug design. DHFR has a proven track record as a drug target in cancer chemotherapy. More importantly, DHFR inhibitors are successfully used in the treatment of bacterial and parasitic infections.[2-5] Senkovich et al showed that trimetrexate (TMQ, Fig.1) inhibits *Tc*DHFR and kills the growth of *T. cruzi* amastigote and trypomastigote in culture.[6] However, TMQ is a potent inhibitor of human DHFR and is therefore toxic to the cell. It is important to develop inhibitors that are selective to *Tc*DHFR. Even though reports of such selective inhibitors for *Tc*DHFR are limited,[7] recent drug discovery efforts that integrate structural biology with computational and medicinal chemistry reiterate the promise of this enzyme as a novel target for developing drugs against Chagas' disease.[8-10]

We have used structure based drug design strategies to facilitate the rational design of a selective inhibitor of the *Tc*DHFR. As part of this study we previously characterized the structure of the biologically relevant bifunctional form of the parasitic enzyme, dihydrofolate reductase-thymidylate synthase (*Tc*DHFR-TS), and determined the structure of the enzyme with TMQ bound to the DHFR active site.[11] The potent activity of TMQ against the parasite combined with previous studies indicating antiparasitic activity of 2,4-diaminoquinazoline antifolates emphasizes the importance of further structure-activity studies on this class of compounds. So, our initial SAR studies focused on making TMQ analogs as inhibitors of *Tc*DHFR. We have synthesized and evaluated several analogs of TMQ, resulting in the identification of several potent inhibitors of *Tc*DHFR. One such potent inhibitor of *Tc*DHFR is compound 1 (Fig. 1).

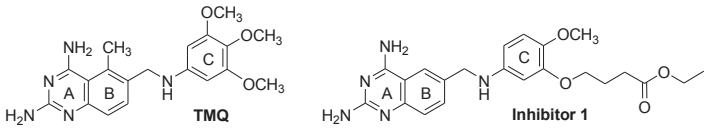


Figure 1: Structures of TMQ and Inhibitor 1

To aid in the SAR studies and further improve the activity and selectivity of inhibitor 1, we have determined the X-ray crystal structure of the enzyme/inhibitor complex using inhibitor 1.[10] The bifunctional *Tc*DHFR-TS enzyme was used in crystallization. The structure of this complex was refined to a resolution of 2.5Å (fig. 2).[10]

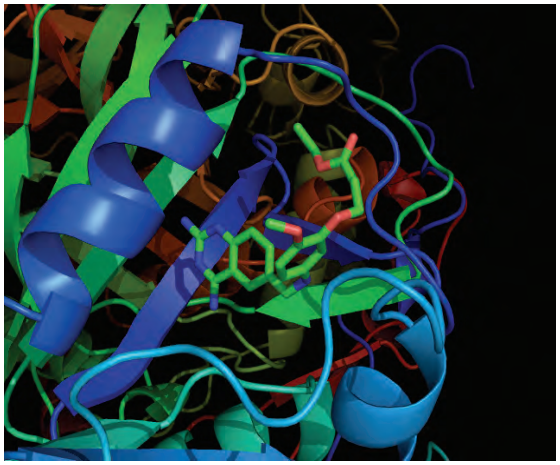


Figure 2: X-ray crystal structure of inhibitor 1 bound to TcDHFR-TS.

Results and Discussion

Analysis of the crystal structure of the enzyme/inhibitor 1 complex clearly shows how inhibitor 1 is bound in the active site. This also has given us insight into the nearby amino acid residues in the active site of *Tc*DHFR around the molecule and the space in to which the structure of the molecule can be expanded to improve binding and selectivity. A comparison of this crystal structure with the crystal structure of *h*DHFR revealed the presence of five amino acid residues (Gly20, Asp21, Phe31, Gln35 and Asn64) around the inhibitor in the active site that are unique to *Tc*DHFR as compared to *h*DHFR. A list of those residues along with corresponding residues in *h*DHFR is given in Table 1 and shown in Fig.3.

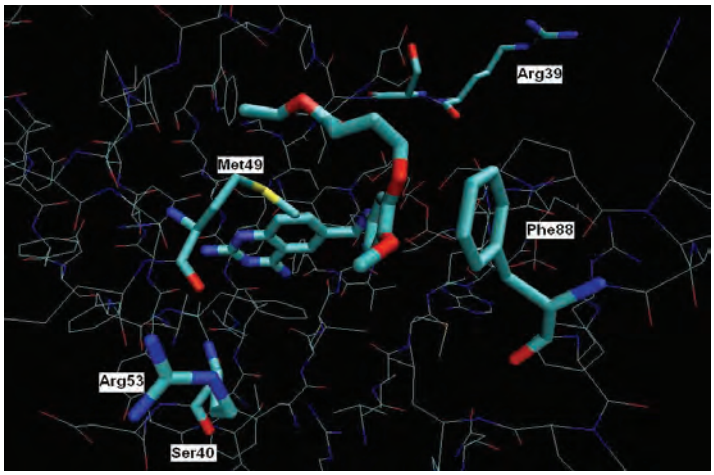


Figure 3: Inhibitor 1 bound in TcDHFR active site. Differences in residues in the DHFR active sites of trypanosomal and human DHFR are labeled.

We hypothesize that the structural modifications of the lead inhibitor 1 targeting these five specific residues in the *Tc*DHFR active site will lead to improvement in selectivity of the inhibitor towards *Tc*DHFR. Specifically, in this manuscript we targeted the residue Phe88, which is near the ethyl butanoate side chain present on the phenyl ring C of inhibitor 1. This side chain is pointed towards the solvent space in the crystal structure and does not have any important interaction with the enzyme in the active site. The side chain substituent is in close proximity to Phe88 on ring C. It is well known that the phenyl ring on phenyl alanine has the ability to stack with the phenyl rings of the inhibitors. Based on this rationale, we propose that the substitution of ethyl butanoate side chain on the ring C oxygen atom with other groups containing phenyl rings might result in stacking interactions with Phe88 leading to better selectivity for *Tc*DHFR. With this objective the following four target compounds (2a-d, Fig. 4) are proposed for synthesis and biological evaluation.

In order to position the phenyl group on the inhibitor at an appropriate distance from Phe88 to maximize the stacking interaction, we proposed to vary the length of attachment of the phenyl group to the oxygen atom on ring C with one carbon (2a), two carbon (2b) and three carbon (2c) linkers. In addition, a target compound with two phenyl groups (2d) has also been proposed. The goal in this case is to maximize the chance of stacking interaction with Phe88. All four target compounds were synthesized and evaluated for their inhibitory activity against *Tc*DHFR and *h*DHFR.

Synthesis of target compounds (2a-d) is outlined in Scheme 1. Treatment of commercially available 2-methoxy-5-nitrophenol (3) with various alkyl halides (R-Br) in the presence of K_2CO_3 in anhydrous DMF afforded the compounds 4a-d in 60-88 % yields. Reduction of nitro groups present in compounds 4a-d using either Pd/C and H_2 (compounds 4b-d) or Sn/HCl (compound 4a) resulted in the formation of amino compounds 5a-d in 70-100 % yield. We had to use Sn/HCl for the reduction of compound 4a as the attempted reduction of this compound using Pd/C and H_2 resulted in the debenzylation of the compound. Reductive amination of 2-fluoro-5-formylbenzonitrile (6) using anilines 5a-d in the presence of $NaCNBH_3$ and $ZnCl_2$ in anhydrous MeOH afforded the compounds 7a-d in 50-76 % yield. Refluxing of compounds 7a-d with guanidine carbonate in N,N-Dimethyl acetamide resulted in the formation of target compounds 2a-d in rather lower yields of 15-62 %.

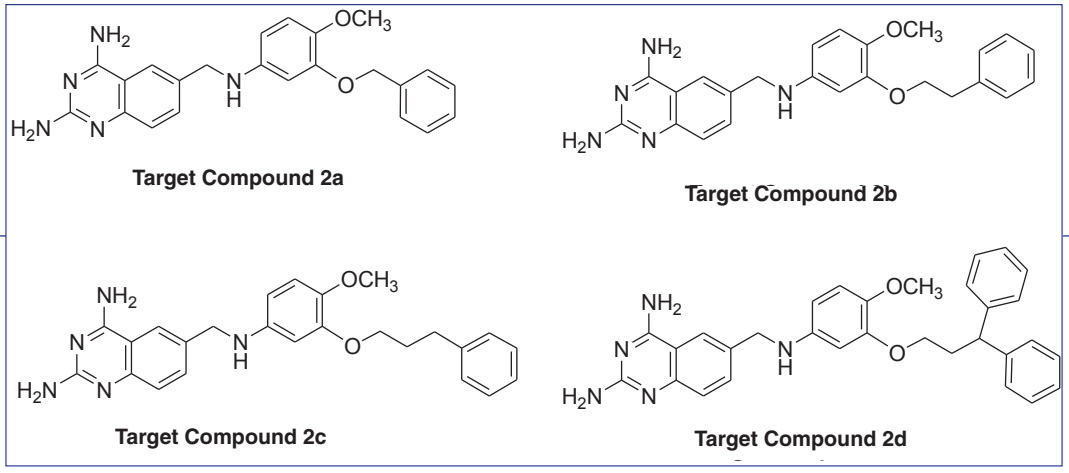
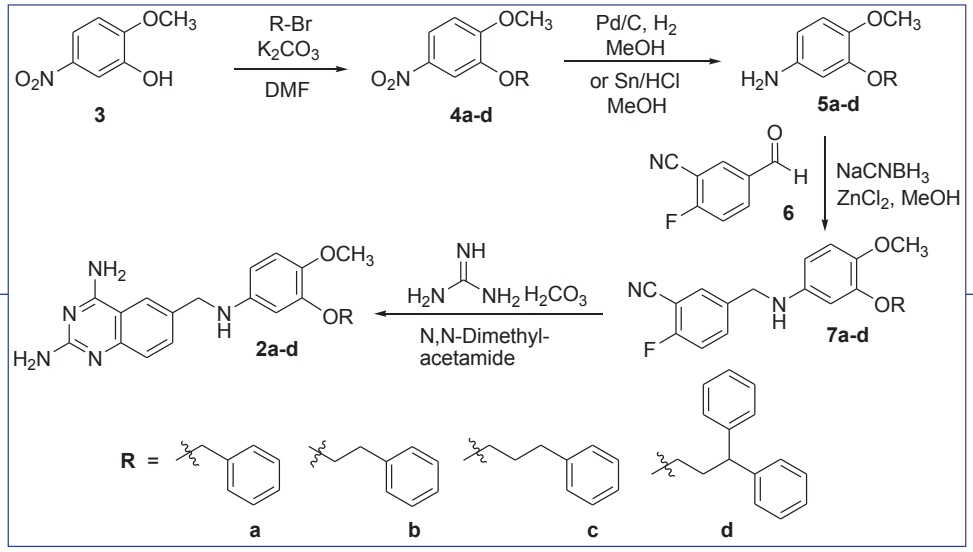


Figure 4: Target compounds 2a-d.



Scheme 1: Synthesis of target compounds 2a–d.

All four final compounds along with TMQ and inhibitor 1 were evaluated for their inhibitory activity against *Tc*DHFR and hDHFR. Recombinant bifunctional enzyme (*Tc*DHFR–TS) was used throughout this study. Inhibitory activity of the reported compounds was measured in a Spectrophotometric assay using the bifunctional enzyme as described previously.[6, 10] Inhibitory activities against *Tc*DHFR and hDHFR are summarized in Table 2. All compounds were found to be potent inhibitors of *Tc*DHFR with IC50 values in nanomolar range. Compounds also showed inhibition of *h*DHFR with the selectivity indices ranging from 2.4 to 17.8. Compound 2a showed a moderate increase in SI (17.8) as compared to TMQ (SI = 3.9) and our earlier lead compound 1 (SI = 2.4). The SI for other derivatives ranged from 2.8 to 4.7.

Conclusions

We have rationally designed and synthesized four inhibitors of *Tc*DHFR. New synthetic methods were developed for making these target compounds. All new compounds and the intermediates were characterized by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The synthesized molecules are potent inhibitors of *Tc*DHFR activity. However, these compounds also showed inhibitory activity against the human enzyme. One of the introduced chemical modifications (benzyl group, 2a) resulted in a moderate increase in SI (17.8) as compared to original lead compound (SI = 2.4). Further work to improve the SI of the current lead compound is in progress.

Experimental

General Methods for Synthesis: Solvent evaporations were carried out *in vacuo* with rotary evaporator. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator (Whatmann, silica gel, UV254, 25 µm plates). Spots were visualized by UV light (254 and 365 nm). Purification by column and flash chromatography was carried out using 'BAKER' silica gel (40 µm) in the solvent systems indicated. The amount (weight) of silica gel for column chromatography was in the range of 50-100 times the amount (weight) of the crude compounds

*h*DHFR	*Tc*DHFR
Gly20	Arg39
Asp21	Ser40
Phe31	Met49
Gln35	Arg53
Asn64	Phe88

Table 1: Differences in residues in the DHFR active sites of trypanosomal and human DHFR

Compound	IC50 (nM)		Selectivity Index
	*Tc*DHFR	*h*DHFR	
TMQ	20.4	80.9	3.9
1	20.5	50.3	2.4
2a	14.7	262.3	17.8
2b	80.7	375.3	4.7
2c	80.8	344.6	4.3
2d	37.5	106.6	2.8

Table 2: Inhibitory activity of TMQ, inhibitor 1 and compounds 2a-d against TcDHFR and hDHFR

being separated. Proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a Bruker Avance DPX-300 spectrometer using TMS as internal standard. The values of chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Mass spectra were recorded on Micromass Platform LCC instrument. Anhydrous solvents used for reactions were purchased in Sure-Seal™ bottles from Aldrich Chemical Company. Other reagents were purchased from Aldrich or Fisher chemical companies and used as received.

General procedure for the preparation of 4a-d:
To a solution of 2-methoxy-5-nitrophenol, 3 (6 mmol) in anhydrous DMF (10 mL), K_2CO_3 (18 mmol) was added and stirred at room temperature for 15 minutes. Alkyl bromide (6 mmol) was added drop-wise to the reaction mixture and stirred at room temperature for 3 hours. After the completion of the reaction (as indicated by TLC, NH_3 saturated $CHCl_3$), the reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL) and brine (1 × 50 mL). The EtOAc layer was then dried over Na_2SO_4. After the removal of the drying agent, the solvent was concentrated to obtain the compounds 4a-d.

1-((2-methoxy-5-nitrophenoxy)methyl)benzene (4a):
(88%); mp. 94°C; ^{1}H NMR ($CDCl_3$) δ 3.98 (s, 3H), 5.20 (s, 2H), 6.93 (d, 1H, J = 9.0 Hz), 7.34-7.49 (m, 5H), 7.81 (d, 1H, J = 2.4 Hz), 7.92 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 56.4, 71.2, 108.6, 110.2, 127.6, 128.4, 128.7, 135.7, 141.3, 141.9, and 155.1; MS (ES+): m/z = 260 [M+H].

1-(2-(2-methoxy-5-nitrophenoxy)ethyl)benzene (4b):
(60%); mp. 97°C; ^{1}H NMR ($CDCl_3$) δ 3.20 (t, 2H, J = 7.3 Hz), 3.97 (s, 3H), 4.28 (t, 2H, J = 7.3 Hz), 6.91 (d, 1H, J = 9.0 Hz), 7.24-7.37 (m, 5H), 7.92 (d, 1H, J = 2.7 Hz), 7.91(dd, 1H, J_1 = 2.7 Hz, J_2 = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 35.5, 56.5, 70.1, 107.9, 110.2, 117.9, 126.8, 128.6, 129.1, 137.4, 141.4, 148.1, 154.9; MS (ES+): m/z = 274 [M+H].

1-(3-(2-methoxy-5-nitrophenoxy)propyl)benzene (4c):
(71%); mp 55°C; ^{1}H NMR ($CDCl_3$) δ 2.18-2.25 (m, 2H), 2.84 (t, 2H, J = 7.5 Hz), 3.97 (s, 3H), 4.08 (t, 2H, J = 6.5 Hz), 6.91 (d, 1H, J = 8.8 Hz), 7.19-7.32 (m, 5H), 7.70 (d, 1H, J = 2.7 Hz), 7.90 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.7 Hz); ^{13}C NMR ($CDCl_3$) δ 30.7, 32.4, 56.8, 68.7, 108.1, 110.4, 118.2, 126.5, 128.9, 128.9, 141.4, 141.8, 148.7, 155.3; MS (ES+): m/z = 288 [M+H].

3-(2-methoxy-5-nitrophenoxy)-1,1-diphenylpropane (4d):
(95%); mp 104°C; ^{1}H NMR ($CDCl_3$) δ 2.60-2.66 (m, 2H), 3.97(s, 3H), 4.01 (t, 2H, J = 6.7 Hz), 4.24 (t, 1H, J = 7.8 Hz), 6.89 (d, 1H, J = 8.9 Hz), 7.18-7.32 (m, 5H), 7.59 (d, 1H, J = 2.5 Hz), 7.88(dd, 1H, J_1 = 2.5 Hz, J_2 = 8.9 Hz); ^{13}C NMR ($CDCl_3$) δ 34.9, 47.6, 56.8, 67.9, 108.1, 110.4, 118.2, 126.9, 128.2, 129.1, 141.7, 144.3, 148.5, 155.3; MS (ES+): m/z = 364 [M+H]

3-(benzyloxy)-4-methoxybenzenamine (5a):
To a stirred solution of compound **4a** (603 mg, 2.33 mmol) in anhydrous MeOH (20 mL), Sn (733 mg, 3.1 mmol) was added, followed by concentrated HCl (8 mL). The reaction mixture was refluxed for 15 minutes. After the completion of the reaction (as indicated by TLC, 50% EtOAc in hexanes), the solvent was removed *in vaccuo*. The residue obtained was then dissolved in $CHCl_3$ (200 mL) and washed with 1M $NaHCO_3$ (2 × 150 mL) and brine (1 × 100 mL). The organic layer was dried over Na_2SO_4.

The drying agent was filtered off and the solvent was removed *in vaccuo* to afford the compound **5a** (480 mg, 70%); ^{1}H-NMR (CDCl3) δ 3.37 (bs, 2H), 3.80 (s, 3H), 5.09 (s, 2H), 6.22 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz), 6.30 (d, 1H, J = 2.7 Hz), 6.72 (d, 1H, J = 8.4 Hz), 7.24-7.43 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 57.1, 71.0, 103.3, 107.3, 114.2, 127.3, 127.8, 128.6, 137.3, 140.7, 142.9, and 149.3; MS (ES+): m/z = 230 [M+H].

General procedure for the preparation of compounds 5b-d:
To a solution of compound **4b-d** (200 mg, 0.83 mmol) in EtOAc (25 mL), 10% Pd/C was added (88 mg, 0.083 mmol). The reaction was stirred at room temperature under H_2 atmosphere (from a balloon) for 12 hours. After the completion of the reaction (as indicated by TLC, 50% EtOAc in hexanes), the reaction mixture was filtered through celite. Celite was washed with EtOAc and the combined EtOAc filtrate was concentrated *in vaccuo* to obtain the compounds **5b-d**.

4-methoxy-3-(phenethyloxy)benzenamine (5b):
(76%); ^{1}H NMR ($CDCl_3$) δ 3.10 (t, 2H, J = 7.5 Hz), 3.35 (bs, 2H), 3.71 (s, 3H), 4.11 (t, 2H, J = 7.5 Hz), 6.15 (d, 1H, J = 8.4 Hz), 6.22 (s, 1H), 6.67 (s, 1H, J = 8.4 Hz), 7.18-7.30 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 36.3, 57.5, 70.1, 102.8, 107.3, 114.8, 127.0, 129.0, 129.6, 138.6, 141.5, 142.8, and 149.7.

3-(3-phenylpropoxy)-4-methoxybenzenamine (5c):
(100%); ^{1}H NMR ($CDCl_3$) δ 2.14-2.24 (m, 2H), 2.86 (t, 2H, J = 7.5 Hz), 3.45 (bs, 2H), 3.84 (s, 3H), 3.99 (t, 2H, J = 6.6 Hz), 6.21-6.28 (m, 2H), 6.76 (d, 1H, J = 8.4 Hz), 7.21-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 30.7, 32.1, 57.1, 67.9, 102.5, 106.8, 114.3, 125.9, 128.4, 128.5, 140.8, 141.6, 142.7, 149.6; MS (ES+): m/z = 257 [M+H].

3-(3,3-diphenylpropoxy)-4-methoxybenzenamine (5d):
(100%); ^{1}H NMR ($CDCl_3$) δ 2.52-2.60 (m, 2H), 3.34 (bs, 2H), 3.75 (s, 3H), 3.85 (t, 2H, J = 6.7 Hz), 4.19 (t, 1H, J = 7.8 Hz), 6.07 (s, 1H), 6.15 (d, 1H, J = 8.4 Hz), 7.12-7.25 (m, 10H), ; ^{13}C NMR (CDCl3) δ 34.8, 47.4, 57.1, 66.9, 102.4, 106.8, 114.2, 126.4, 128.0, 128.6, 140.8, 142.6, 144.4, and 149.4.

General procedure for the preparation of 7a-d:
To a solution of 2-fluoro-5-formylbenzonitrile, **6** (1.31 mmol) in anhydrous MeOH (5 mL), compound **5a-d** (1.31 mmol) was added and stirred at room temperature for 15 minutes. A solution of $NaCNBH_3$ (1.44 mmol) and $ZnCl_2$ (0.65 mmol) in MeOH (5 mL) was added dropwise to the reaction mixture and stirred at room temperature for 12 hours. After the completion of the reaction (as indicated by TLC, 30% EtOAc in hexanes), the solvent was removed *in vaccuo*. The residue obtained was dissolved in EtOAc (60 mL), washed with water (2 × 30 mL) then brine (1 × 30 mL) and dried over Na_2SO_4. The drying agent was filtered off and the solvent was removed *in vaccuo* to obtain the crude product, which was purified by flash chromatography on silica gel (12 × 1 in) using $CHCl_3$ as eluent.

5-((3-(benzyloxy)-4-methoxyphenylamino)methyl)-2-fluorobenzonitrile (7a):
(76%); ^{1}H NMR ($CDCl_3$) δ 3.81 (s, 3H), 4.21 (s, 2H), 5.08 (s, 2H), 6.09 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.5 Hz), 6.75 (d, 1H, J = 8.5 Hz), 7.13 (t, 1H, J = 8.5 Hz), 7.28-7.37 (m, 5H), 7.49-7.55 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 47.5, 57.0, 71.0, 101.4, 101.6 (C–F coupling), 104.8, 114.0, 114.2, 116.4, 116.7, 127.0, 127.8, 128.5, 131.8, 133.6 and 133.7 (C–F coupling), 136.9 and 137.0 (C–F coupling), 137.1, 142.0 and 142.5 (C–F coupling), 149.3, 160.4 and 163.9 (C–F coupling); MS (ES+): m/z = 363 [M+H].

5-((4-methoxy-3-(phenethyloxy)phenylamino)methyl)-2-fluorobenzonitrile (7b):
(71%); ^{1}H NMR ($CDCl_3$) δ 3.12 (t, 2H, J = 7.5 Hz), 3.81 (s, 3H), 4.13 (t, 2H, J = 7.5 Hz), 4.26 (s, 2H), 6.06 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.5 Hz), 6.20 (d, 1H, J = 2.7 Hz), 6.72 (d, 1H, J = 8.5 Hz), 7.15 (t, 1H, J = 8.5 Hz), 7.21-7.32 (m, 5H), 7.55-7.60 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 35.8, 47.6, 57.1, 69.9, 101.1, 101.4 and 101.6 (C–F coupling), 104.4, 114.0, 114.5, 116.4, 116.7, 126.5, 128.5, 129.0,131.9, 133.7 and 133.8 (C–F coupling), 137.0 and 137.1 (C–F coupling), 138.1, 142.2 and 142.6 (C–F coupling), 149.6, 160.5 and 164.0 (C–F coupling).

5-((3-(3-phenylpropoxy)-4-methoxyphenylamino)methyl)-2-fluorobenzonitrile (7c):
(50%); ^{1}H NMR ($CDCl_3$) δ 2.12-2.20 (m, 2H), 2.82 (t, 2H, J = 7.5 Hz), 3.81 (s, 3H), 3.96 (t, 2H, J = 6.4 Hz), 4.29 (s, 2H), 6.07 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz), 6.19 (d, 1H, J = 2.4 Hz), 6.75 (d, 1H, J = 8.4 Hz), 7.15-7.33 (m, 6H), 7.57-7.62 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 30.7, 32.1, 47.7, 57.1, 68.0, 101.1, 101.5 and 101.7 (C–F coupling), 104.1, 114.0, 114.4, 116.5, 116.7, 126.0, 128.5, 128.6, 132.0, 133.8 and 133.9 (C–F coupling), 137.1 (C–F coupling), 141.5, 142.2 and 142.7(C–F coupling), 149.8, 160.6 and 164.0 (C–F coupling).

5-((3-(3,3-diphenylpropoxy)-4-methoxyphenylamino)methyl)-2-fluorobenzonitrile (7d):
(62%); ^{1}H NMR ($CDCl_3$) δ 2.61 (q, 2H, J = 7.0 Hz), 3.83 (s, 3H), 3.92 (t, 2H, J = 7.0 Hz), 4.24 (s, 2H), 4.27 (t, 1H, J = 7.0 Hz), 6.07-6.12 (m, 2H), 6.77 (d, 1H, J = 8.4 Hz), 7.14 (t, 1H, J = 8.5 Hz), 7.20-7.36(m,10H), 7.53-7.60 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 34.6, 47.4, 47.5, 57.1, 67.1, 101.0, 101.3 and 101.5 (C–F coupling), 104.1, 114.1, 114.3, 116.4, 116.7, 126.4, 128.0, 128.6, 131.9, 133.8 and 134.0 (C–F coupling), 137.1 and 137.2 (C–F coupling), 142.2 and 142.5(C–F coupling), 144.4, 149.6, 160.5 and 163.9 (C–F coupling).

General procedure for the preparation of 2a-d:
To a solution of compound **7a-d** (0.67 mmol) in anhydrous N,N-dimethyl acetamide (DMA) (3 mL), guanidine carbonate (0.6 mmol) was added and the reaction mixture was stirred at 140°C for 5 hours. TLC examination (10 % MeOH in NH_3 saturated CH_2C_{l2}) showed that the reaction was complete. Solvent was completely removed and the crude product was purified by flash chromatography on silica gel (12 × 1 in) using 10 % MeOH in

NH_3 saturated CH_2Cl_2 to obtain pure compounds **2a-d**.

6-((3-(benzyloxy)-4-methoxyphenylamino)methyl) quinazoline-2,4-diamine (2a):
(31 %); ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 3H), 4.21 (d, 2H, J = 5.5 Hz), 4.97 (s, 2H), 5.72 (t, 1H, J = 5.5 Hz), 5.91(s, 2H), 6.11 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.5 Hz), 6.70 (d, 1H, J = 8.5 Hz), 7.13-7.49 (m, 9H), 7.98 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 48.6, 57.8, 70.8, 101.7, 104.9, 110.8, 116.0, 123.2, 125.2, 128.4, 128.5, 129.2, 132.6, 133.2, 138.3, 141.6, 144.9, 149.8, 152.6, 161.5, and 163.2; MS (ES+): m/z = 402 [M+H].

6-((4-methoxy-3-(phenethyloxy)phenylamino)methyl) quinazoline-2,4-diamine (2b):
(62 %); mp. 176oC; ^{1}H NMR (DMSO-d_6) δ 2.97 (t, 2H, J = 6.0 Hz), 3.56 (s, 3H), 4.05 (t, 2H, J = 6.0 Hz), 4.15 (d, 2H, J = 6.0 Hz), 5.66 (t, 1H, J = 6.0 Hz), 5.91(s, 2H), 6.09 (dd, 1H, J_1 = 3.0 Hz, J_2 = 9.0 Hz), 6.35 (d, 1H, J = 3.0 Hz), 6.67 (d, 1H, J = 9.0 Hz), 7.13-7.31 (m, 8H), 7.49 (d, 1H, J = 3.0 Hz), 7.96 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 35.5, 48.1, 57.4, 69.2, 100.7, 103.9, 110.4, 115.7, 122.8, 124.6, 126.7, 128.7, 129.4, 132.2, 133.8, 138.9, 140.9, 144.5, 149.5, 151.8, 160.9, and 162.8; MS (ES+): m/z = 416 [M+H].

6-((3-(3-phenylpropoxy)-4-methoxyphenylamino)methyl) quinazoline-2,4-diamine (2c):
(15%); mp. 150oC; ^{1}H NMR ($CDCl_3$) δ 2.11-2.15 (m, 2H), 2.78 (t, 2H, J = 7.5 Hz), 3.80 (s, 3H), 3.95 (t, 2H, J = 7.5 Hz), 4.31(s, 2H), 4.8 (bs, 2H), 5.45 (bs, 2H), 6.17 (d, 1H, J = 9.0 Hz), 6.23 (d, 1H, J = 2.0 Hz), 6.76 (d, 1H, J = 9.0 Hz), 7.18-7.58 (m, 8H); MS (ES+): m/z = 430 [M+H].

6-((3-(3,3-diphenylpropoxy)-4-methoxyphenylamino)methyl) quinazoline-2,4-diamine (2d):
(20 %); mp 216oC; ^{1}H NMR ($CDCl_3$) δ 2.57 (q, 2H, J = 7.2 Hz), 3.80 (s, 3H), 3.89 (t, 2H, J = 7.2 Hz), 4.19 (t, 1H, J = 7.2 Hz), 4.27 (s, 2H), 5.12 (bs, 2H), 5.48 (bs, 2H), 6.11-6.17 (m, 2H), 6.74 (d, 1H, J = 8.5 Hz), 7.14-7.30 (m, 9H), 7.46 (d, 1H, J = 8.5 Hz), 7.54-7.59 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 34.7, 47.3, 49.0, 57.1, 67.0, 101.0, 104.1, 110.1, 114.3, 120.1, 125.9, 126.3, 127.9, 128.5, 133.2, 133.3, 142.4, 142.9, 144.3, 149.5, 151.7, 159.8, and 162.2; MS (ES+): m/z = 506 [M+H].

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