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GREEN SYNTHESIS OF GABAPENTIN DERIVATIVES: THEIR BIOLOGICAL EVALUATION AND *IN SILICO* STUDIES

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Abstract: *N*-Mannich reaction has been carried out using 2-azaspiro[4.5]decan-3-one, formaldehyde, and various primary amines to give compounds 2a-i. All compounds are also formed using green roots and compared with the conventional method to ventilate the importance of the green synthesis method. ^{1}H NMR, ^{13}C NMR, IR, and Mass spectral analysis are used to evaluate the structures of final compounds. All synthesized moieties are subjected to antimicrobial and antitubercular activities. Compounds **2e** and **2c** exhibit significant antitubercular activities compared to reference drugs. *In silico* studies are also carried out such as ADMET studies and dynamic molecular studies. Binding affinities from docking analysis reveal the reason to be an active drug molecule by showing deep interaction details with proteins like *Escherichia coli*, *Alkalihalobacillus halodurans* C-125, *Mycobacterium tuberculosis*, and *Mycobacterium tuberculosis* $H_{37}Rv$. *In vitro* outcomes are supported by these computational probes.

***IndexTerms* - *N*-Mannich reaction, Microwave synthesis, Spiro compounds, Antimicrobial, Antitubercular, Molecular docking, ADMET studies.**

I. INTRODUCTION

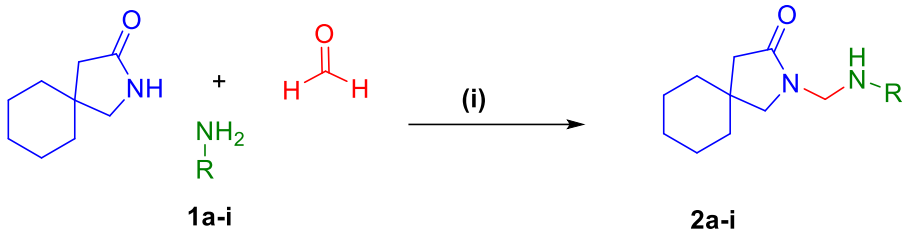
Recently, it is necessary to synthesize a compound with small size and high pharmacological effect. Since, spiro compounds containing hetero atoms came into trends, draw an attention towards researchers. Multicomponent reactions are widely used now days due to their comprehensive applications. It reduces the multiple steps and give important spiro scaffold. [1,2] Mostly spiro compounds were synthesized using multicomponent reaction such as Ugi reaction [3], [3+2]-cycloaddition reaction [4] and Passerini reactions [5]. Ali Darehkordi et. al. (2015) synthesizes gabapentin derivatives using three component Ugi reaction. [6] Jashuva V. P. Katuri et. al. (2016) synthesizes same derivatives via Hoffmann reaction using catalysts trichloroisocyanuric acid (TCCA), Sodium dichloroisocyanurate (NaDCC), 1,3-dichloro-5−5-dimethyl hydantoin (DCDMH) and N-Chlorosuccinimide (NCS). [7] further similar 1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-diones derivatives were designed and prepared by Ze Yang et. al. (2019) and were subjected for antitumor activities which results in promising anticancer drug molecules. [8] So, purpose of this work is to design three dimensional spiro compound with significant biological activities. Spiro moieties were prepared from coumarin are via three component reaction found highly active as antitubercular agents. [9] According to CDC's National Tuberculosis Surveillance System (NTSS) by the 50 U.S. states and the District of Columbia (DC), case of TB has been reported around 7860 during year of 2021 which is more than during year of 2020 (7173 cases) [10]. Recently, Natarajan Arumugam et. al. (2020) investigated spiropyrrolidine derivatives for analysis of antitubercular activity and outcomes are significant as an antimycobacterial agent. [11] Due to their rigid structure, they exhibit anticonvulsant [12], antiepileptic [13], anticancer [14], antitubercular [15], antioxidant [16], antimalarial [17], antiprotozoal [18], antibacterial [19] and antianxiolytic [20] activities.

Not much work has been employed on the gabapentin moiety. Present work comprises of novel gabapentin derivatives using various primary amines via N-Mannich reaction. Reaction is single stepped reaction and carried out using both conventional and microwave method where, microwave method strikes as usual. Molecular docking studies are carried out to check the interaction responsible for their biological activities and to confirm in vitro results. Using the previous docking analysis protein macromolecule has been selected. [21-22] For antimicrobial docking, antimicrobial proteins *Escherichia coli* (PDB: 1ab4) and *Alkalihalobacillus halodurans* C-125 (PDB: 3tg9) proteins are applied as macromolecule. Similarly, for antimycobacterial docking, *Mycobacterium tuberculosis* (PDB: 1oy0) and *Mycobacterium tuberculosis* $H_{37}Rv$ (PDB: 5ugq) are employed. As synthesized drug should be potential and non-toxic, ADMET study of most of drug molecule are carried out to check the drug likeness. [23] Results of ADMET studies are sufficient for to be a good drug molecule. So, in all area these drug molecules carry satisfactory outcomes.

II. RESULTS AND DISCUSSION:

2.1 Chemistry:

N-Mannich base reaction is used in this research article which is a single-step and multicomponent reaction between two amines and formaldehyde. The listed study introduces 2-azaspiro[4.5]decan-3-one, formaldehyde, and various amine. (Scheme 1) Initially, lone pair on the nitrogen of 2-azaspiro[4.5]decan-3-one attacks on formaldehyde molecule to form an alcohol. Further, the -OH group of an alcohol is replaced by the $-NH_2$ group of various primary amines with the removal of H^+.



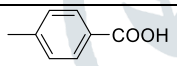
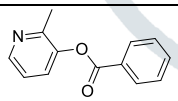
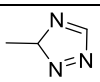
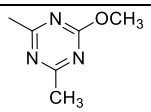
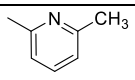
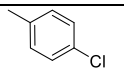
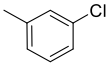
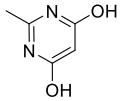
1a-i **2a-i**

(i) MeOH, 65° C, 6-5 hr, $-H_2O$

Scheme 1: General reaction to synthesize compound 2a-i

All synthesized compounds are characterized using various spectral analyses such as IR, ^{1}H NMR, ^{13}C NMR, and Mass spectroscopy. In IR spectroscopy, all compounds possess a γ-lactam band around 1600 cm^{-1}. Further, every compound has N-H stretching band at around 3300 cm^{-1}. From ^{1}H NMR spectroscopy compounds are confirmed by the peak of the proton on a nitrogen atom around 7-9 δ ppm. The active methylene group between two nitrogen shows a peak around 3-4 δ ppm. The peak of spiro carbon is around 70 δ ppm in ^{13}C NMR confirming the formation of a spiro ring. Likewise, in their spectroscopic analyses, all of the compounds provide all of their typical peak or band values. Compounds' molecular weights were resolved using mass spectrometry and the m/z ratio. All of the substances are produced in both traditional and microwave approaches. Due to the obvious difference in yield percentages between the two methods, the microwave approach is demonstrated to be the greener method. Table 1 lists the physiochemical features of all substances.

Table 1: Physical data of synthesized compounds 2a-i

No	Compound	R-	Melting Point (°C)	Conventional		Microwave		Molecular Formula
				Yield %	Time (hr)	Yield %	Time (min)	
1	2a		202	70	6	84	3	$C_{15}H_{21}N_3O$
2	2b		210	75	6	86	3	$C_{17}H_{22}N_2O_3$
3	2c		256	82	5	92	2	$C_{22}H_{25}N_3O_3$
4	2d		200	78	6	88	3	$C_{12}H_{19}N_5O$
5	2e		236	80	6	90	3	$C_{15}H_{23}N_5O_2$
6	2f		230	74	6	83	3	$C_{16}H_{23}N_3O$
7	2g		224	76	5	82	2	$C_{16}H_{21}ClN_2O$
8	2h		204	79	6	85	3	$C_{16}H_{21}ClN_2O$
9	2i		218	66	6	87	3	$C_{14}H_{20}N_4O_3$

2.2 Biology:

2.2.1 *In vitro* antibacterial activity:

The antibacterial activity of the synthesized compounds **2a-i** was examined *in vitro* against Gram-negative bacteria such as *E. Coli* (MTCC 443) and *P. Aeruginosa* (MTCC 1688), as well as Gram-positive bacteria such as *S. Aureus* (MTCC 96) and *S. Pyogenus* (MTCC 442) using Gentamycin, Ciprofloxacin, Ampicillin, Chloramphenicol, and Norfloxacin as standards. Compounds **2c** and **2e** are very active against *E. Coli* compared to Ampicillin and Chloramphenicol. Compound **2a** has some inhibition against *P. Aeruginosa* compared to Ampicillin. Compound **2g** is slightly active against *S. Aureus*. Again, compound **2c** is effective against *S. Pyogenus*. (Table 2)

Table 2: Antibacterial activities of compounds 2a-i

Antibacterial activity table Minimal inhibition concentration [microgram/ml]					
Sr No.	**Code No.**	***E. Coli***	***P. Aeruginosa***	***S. Aureus***	***S. Pyogenus***
		MTCC 443	**MTCC 1688**	**MTCC 96**	**MTCC 442**
1	2a	125	62.5	100	100
2	2b	100	250	125	100
3	2c	50	125	125	62.5
4	2d	100	200	250	500
5	2e	62.5	100	100	125
6	2f	125	100	125	200
7	2g	100	100	62.5	200
8	2h	100	500	125	250
9	2i	250	200	500	200
10	GENTAMYCIN	0.05	1	0.25	0.5
11	AMPICILLIN	100	100	250	100
12	CHLORAMPHENICOL	50	50	50	50
13	CIPROFLOXACIN	25	25	50	50
14	NORFLOXACIN	10	10	10	10

2.2.2 Antifungal activity:

Candida albicans (MTCC 227), *Aspergillus niger* (MTCC 282), and *Aspergillus clavatus* (MTCC 1323) are tested *in vitro* for antifungal activity using widely used medications Nystatin and Griseofulvin. Results of this test found that all compounds are very mildly active against all antifungal strains compared to Griseofulvin. From all these results, compound **2c** shines with a MIC value of 100 mg/mL against Candida albicans. (Table 3)

Table 3: Antifungal activities of compounds 2a-i

Antifungal activity table				
Minimal fungicidal concentration [microgram/ml]				
Sr. No.	**Code No.**	***C. Albicans***	***A. Niger***	***A. Clavatus***
		MTCC 227	**MTCC 282**	**MTCC 1323**
1	2a	250	1000	500
2	2b	1000	1000	500
3	2c	100	250	250
4	2d	250	500	1000
5	2e	1000	200	200
6	2f	200	200	200
7	2g	500	250	250
8	2h	500	500	1000
9	2i	500	1000	1000
10	Nystatin	100	100	100
11	Greseofulvin	500	100	100

2.2.3 Antimycobacterial activity:

Table 4 declares the results of antimycobacterial activities of **2a-i** compounds. These γ-lactam portions of all compounds are responsible for antitubercular activities. Furthermore, various amine with heterocyclic ring enhances inhibition action. All synthesized compounds exhibit extreme activity against antitubercular strains. These results are compared with Rifampicin and Isoniazid. All compounds are strongly active compared to Rifampicin. (Table 4) Substances **2c** and **2e** are highly active having MIC values of 0.73 µg/ml, 0.8, and µg/ml, consecutively.

Table 4: Antimycobacterial activities of compounds 2a-i

Antimycobacterial activity		
SR.NO	**CODE NO**	**MIC µg/ml**
1	2a	0.98
2	2b	1.59
3	2c	0.73
4	2d	1.25
5	2e	0.83
6	2f	1.85
7	2g	0.90

8	2h	1.91
9	2i	0.92
10	**Isoniazid**	0.2
11	**Rifampicin**	40

2.3 Computational Study:

2.3.1 Molecular Docking Study:

Moieties are also subjected to molecular dynamic study against two antimicrobial proteins *Escherichia coli* (PDB: 1ab4) and *Alkalihalobacillus halodurans C-125* (PDB: 3tg9). Compounds **2c** and **2e** have more binding affinity toward these proteins. (Table 5). Responsible interactions for these binding energies are conventional *H*-bond on carbonyl oxygen with LYS A:129, ILE A:130, and ARG A:518 further, the π-alkyl bond also assists the binding affinity in compound **2c** against *Escherichia coli* (PDB: 1ab4). Against *Alkalihalobacillus halodurans C-125* (PDB: 3tg9), conventional *H*-bond on carbonyl oxygen with GLU A:114 and π-π T-shaped interaction with TRP A:117 are accountable for binding affinity.

Table 5: Docking score of compounds 2a-i

No.	Code no.	Binding energy (KJ mol^{-1})			
		***Escherichia coli* (PDB: 1ab4)**	***Alkalihalobacillus halodurans C-125* (PDB: 3tg9)**	***Mycobacterium tuberculosis* (PDB: 1oy0)**	***Mycobacterium tuberculosis $H_{37}Rv$* (PDB: 5ugq)**
1	2a	-6.4	-6.5	-6.5	-7.1
2	2b	-7.4	-7.6	-7.6	-8.4
3	2c	-8.1	-8.3	-8.3	-9.1
4	2d	-6.7	-7.5	-7.8	-7.8
5	2e	-7.8	-7.9	-7.5	-8.7
6	2f	-6.9	-6.4	-6.9	-8.0
7	2g	-6.8	-6.2	-6.9	-7.7
8	2h	-7.3	-5.4	-7.0	-8.0
9	2i	-7.3	-7.8	-7.8	-8.0

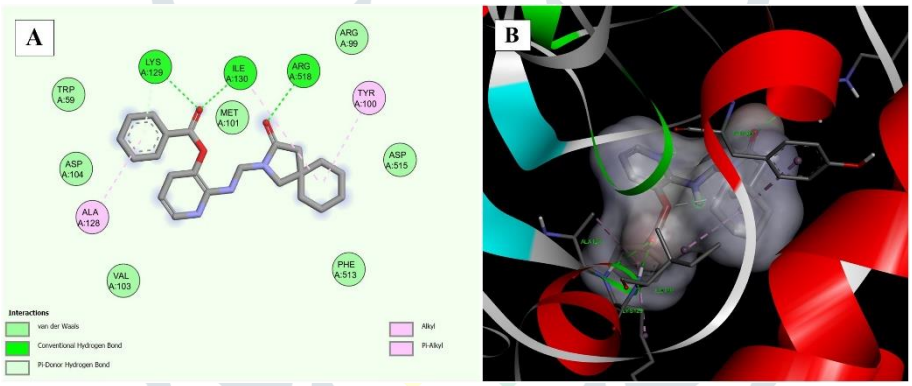


Figure 1: Docking images of compound 2c with antimicrobial protein 1ab4 (A) 2D (B) 3D

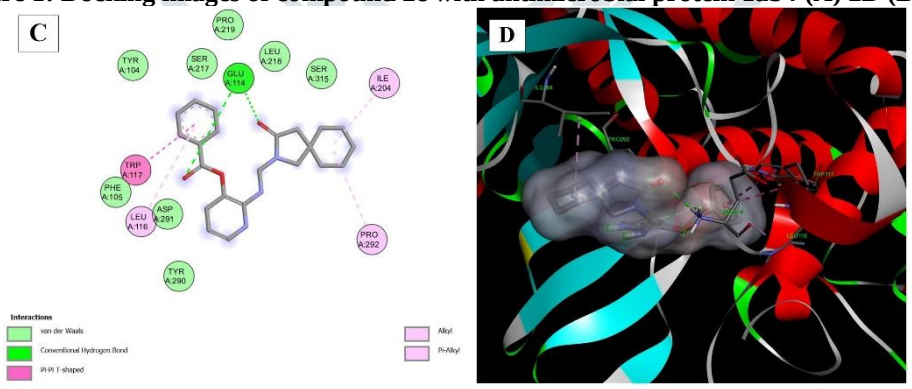


Figure 2: Docking images of compound 2c with antimicrobial protein 3tg9 (C) 2D (D) 3D

For the antitubercular study, *Mycobacterium tuberculosis* (PDB: 1oy0) and *Mycobacterium tuberculosis $H_{37}Rv$* (PDB: 5ugq). It is found that compounds **2c** and **2i** have binding energies of -8.3 KJ mol^{-1} and -7.8 KJ mol^{-1} respectively. Conventional *H*-bond with ASP D:72 and π-alkyl interaction with ARG B:87, Val B:90, ARG B:91, and His B:128 are responsible for high binding affinity for **2c** compound against *Mycobacterium tuberculosis* (PDB: 1oy0). Furthermore, against *Mycobacterium tuberculosis $H_{37}Rv$* (PDB: 5ugq), again conventional *H*-bond with SER A:228, HIS A: 490, π-π staked interaction with TYR A:227 are key interactions for binding affinity.

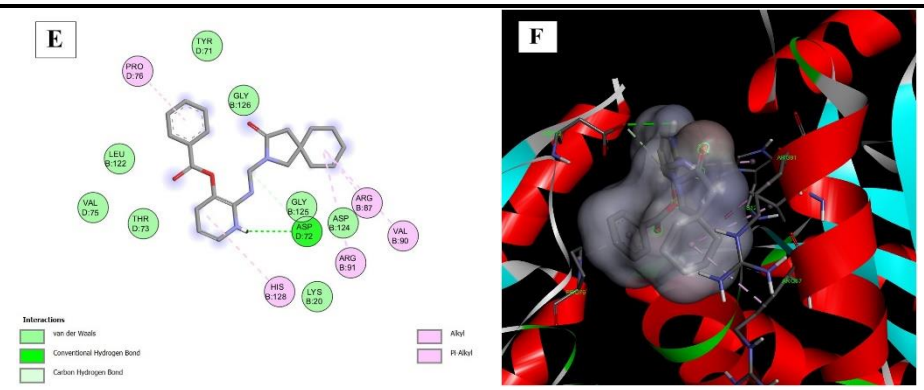


Figure 3: Docking images of compound 2c with antimycobacterial protein 1oy0 (E) 2D (F) 3D

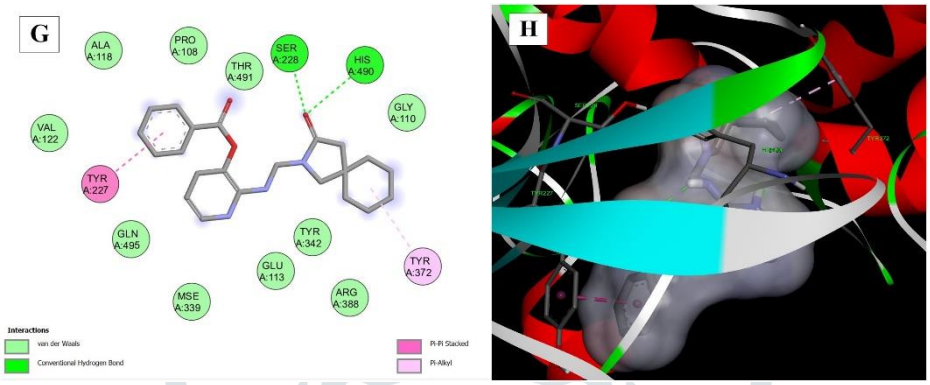


Figure 4: Docking images of compound 2c with antimycobacterial protein 5ugq (G) 2D (H) 3D

2.3.2 *In silico* ADMET evaluation of synthesized compounds:

In silico ADMET (adsorption, distribution, metabolism, excretion, and toxicity) evaluations were used to evaluate the drug-like properties of synthesized molecules [24, 25]. In this study, new compounds with different pharmacokinetic and pharmacodynamic properties such as (nROTB) Number of rotatable bonds, (TPSA) Topological Polar surface area, (LogS) Log of aqueous solubility, (LogP) Log of octanol/water partition, (SA score) Synthetic accessibility score and (PCaco2) Caco2 cell Permeability exhibited the most inhibitory effect. According to this investigation, compounds **2a**, **2c**, **2h**, and **2g** exhibited the best aqueous solubility and the lowest LogS values. Major compounds have a large Topological Polar surface area due to the presence of few nitrogen atoms (TPSA). Furthermore, **2i** possesses the compound's greatest TPSA of about 98.58. The vast majority of the compounds exhibit sufficient Caco2 cell permeability (PCaco2). The data table has further results. (Table 6)

Table 6: *In silico* ADME predictions of targeted compounds (2a-i)

Comp. code.	MW[a]	nHA[b]	nHD[c]	nROTB[d]	TPSA[e]	logS[f]	LogP[g]	SAscore[h]	PCaco2[i]
2a	259.35	2	1	3	45.23	-4.20	2.34	2.67	-4.785
2b	302.37	3	2	4	69.64	-3.93	2.54	2.68	-4.760
2c	379.45	4	1	6	71.53	-6.34	3.46	3.67	-4.659
2d	249.31	5	1	3	69.42	-2.28	2.31	4.17	-4.664
2e	305.38	5	1	4	86.38	-3.95	3.17	3.58	-4.656
2f	273.37	2	1	3	45.23	-4.58	2.73	3.03	-4.651
2g	292.83	1	1	3	32.34	-5.17	3.04	2.69	-4.656
2h	292.83	1	1	3	32.34	-5.17	3.11	2.73	-4.656
2i	292.33	5	3	5	98.58	-2.66	2.40	3.15	-4.714

[a]Molecular weight, [b]Number of hydrogen bond acceptors, [c]Number of hydrogen bond donors, [d]Number of rotatable bonds, [e]Topological Polar surface area, [f]Log of the aqueous solubility, [g]Log of the octanol/water partition, [h]Synthetic accessibility score, (≥ 6, difficult to synthesize & <6 easy to synthesize), [i]Caco2 cell Permeability (Higher than -5.14 Log unit)

III. CONCLUSION

Both conventional and microwave roots offer superior product yield; however, microwave is particularly noteworthy since the product purity is high, time is saved, and the operation is environmentally benign. All compounds' in vitro results against antitubercular strains are very promising, with compound **2c** being the most active antibacterial agent. Similarly, antifungal activities are used, and **2c** is proven to be effective yet again. In vitro antitubercular potency of most of the final compounds is comparable to that of the reference drug Rifampicin; however, compounds **2c** and **2e** are the most potent, with MICs of roughly 0.75 µg/mL. In silico results are excellent since ADMET describes drug similarity and molecular dynamic research backs up in vitro results. Since compound **2c** and **2e** were discovered to be the most suitable drug molecules in the sequence of compounds.

IV. EXPERIMENTAL

4.1 Method:

The majority of the chemicals utilised in this study were obtained from Sigma-Aldrich and Fisher Scientific Ltd. Thin layer chromatography (TLC) plates and column chromatography were used to purify the compounds (silica gel G). The melting points were calculated using the open tube capillary method, and the results have not been altered. FT-IR spectroscopy was used to create IR spectrums from potassium bromide pellets using a Perkin Elmer RZX infrared spectrophotometer and an Agilent resolution Pro FT-IR spectrometer; frequencies are given in cm^{-1}. The ^{1}H-NMR and ^{13}C-NMR spectra were analyzed in dimethyl sulfoxide (DMSO-d6) using a Bruker Advance II 400 spectrometer (500 MHz FT-NMR) with tetramethyl silane as the internal standard (chemical shifts in ppm). The Q-T micro mass spectra of WATERS were obtained (electrospray ionization-MS). On silica gel 60, Merck column chromatography (0.043-0.06 mm) was done. *In vitro* antibacterial tests are performed on *E. Coli*, *P. Aeruginosa*, *S. Aureus*, and *S. Pyogenus*. Antifungal activity of *Candida albicans*, *Candida niger*, and *Candida clavatus* is investigated. *In vitro* antimycobacterial studies against $H_{37}Rv$, isoniazid and Rifampin are employed as standard medicines. The antitubercular activity was determined on Lj medium using the standard procedure. For the *in silico* study, the Autodock Vina software is employed. Further ADMET study has been carried out using online software SwissADME.

4.2 Synthesis of Gabapentin lactam derivatives (2a-i)

Conventional Method:

In 10 mL of methanol, a multicomponent reaction is carried out between 2-azaspiro[4.5]decan-3-one (0.013 mol), formaldehyde (0.013 mol), and various amino compounds (0.013 mol). Initially, 2-azaspiro[4.5]decan-3-one and amino derivatives are dissolved in methanol and agitated for 10 minutes at 0-5° C before adding formaldehyde solution dropwise. After that, the entire batch is heated at 65° C for 5-6 hours. Following the completion of the reaction (on TLC), the entire volume is stored overnight to produce the final product. Further purification is not necessary for this product.

Microwave method:

Similarly, the aforementioned bulk was treated to microwave synthesis, which produced a high yield product in 3 minutes at 340 watts. The finished liquid is chilled and set aside for the night to solidify.

2-((pyridin-4-ylamino)methyl)-2-azaspiro[4.5]decan-3-one (2a)

Light Yellow; M. P.: 202°C; IR (KBr, cm^{-1}): 3410 (N-H, str.), 1650 (C=O, str.); ^{1}H NMR:2.40-2.56 (m, 10H, $5CH_2$), 3.35 (s, 2H, $COCH_2$), 4.90 (s, 2H, -N-CH_2-), 5.03 (s, 2H, -NCH_2-NH-), 5.08 (s, 1H, -NH-), 6.48-6.52 (dd, 2H, aromatic), 6.95-7.10 (dd, 2H, aromatics); ^{13}C NMR: 38.87-39.89 (5-CH_2-), 40.01 (-CH_2-), 49.70 (-N-CH_2-), 58.89 (spiro C), 112.14-116.21 (2 aromatic C), 129.30-130.56 (2 aromatic C), 136.50 (1 aromatic C), 168.43 (C=O);m/z (%): 260.20 (M^+)

4-(((3-oxo-2-azaspiro[4.5]decan-2-yl)methyl)amino)benzoic acid (2b)

Yellow; M. P.: 210°C; IR (KBr, cm^{-1}): 3300 (N-H, str.), 1710 (C=O, str.); ^{1}H NMR:2.43-2.50 (m, 10H, $5CH_2$), 3.33 (s, 2H, $COCH_2$), 5.02 (s, 2H, -N-CH_2-), 5.06 (s, 2H, -NCH_2-NH-), 5.09 (s, 1H, -NH-), 6.40-6.50 (m, 2H, aromatic), 7.03-7.23 (m, 2H, aromatics), 11.12 (s, 1H, -COOH) ; ^{13}C NMR: 32.74-34.98 (5-CH_2-), 39.55 (-CH_2-), 43.45 (-N-CH_2-), 54.23 (spiro C), 111.12-114.21 (2 aromatic C), 129.22-130.56 (2 aromatic C), 135.46-139.79 (2 aromatic C), 163.45 (C=O), 169.33 (C=O);m/z (%): 304.32 (M^+)

4-(((3-oxo-2-azaspiro[4.5]decan-2-yl)methyl)amino)benzoic acid (2c)

Light Yellow; M. P.: 256°C; IR (KBr, cm^{-1}): 3400 (N-H, str.), 1690 (C=O, str.); ^{1}H NMR:2.50-2.52 (m, 10H, $5CH_2$), 3.33 (s, 2H, $COCH_2$), 5.02 (s, 2H, -N-CH_2-), 5.04 (s, 2H, -NCH_2-NH-), 5.08 (s, 1H, -NH-), 6.56-6.59 (m, 3H, aromatic), 7.11-6.66 (m, 5H, aromatics); ^{13}C NMR: 38.90-39.90(5-CH_2-), 39.99 (-CH_2-), 47.81 (-N-CH_2-), 69.00 (spiro C), 112.03-116.17 (3 aromatic C), 127.29-128.25 (2 aromatic C), 136.46-140.89 (2 aromatic C), 149.14 (N-C-N), 162.58 (C=O), 167.54 (C=O);m/z (%): 381.22 (M^+)

2-(((3H-1,2,4-triazol-3-yl)amino)methyl)-2-azaspiro[4.5]decan-3-one (2d)

Brown; M. P.: 200°C; IR (KBr, cm^{-1}): 3460 (N-H, str.), 1700 (C=O, str.); ^{1}H NMR:2.57-2.62 (m, 10H, $5CH_2$), 3.98 (s, 2H, $COCH_2$), 5.10 (s, 2H, -N-CH_2-), 5.16 (s, 2H, -NCH_2-NH-), 5.24 (s, 1H, -NH-), 6.78 (s, 1H, aromatic); ^{13}C NMR: 33.54-36.55 (5-CH_2-), 40.55 (-CH_2-), 49.87 (-N-CH_2-), 57.55 (spiro C), 112.11 (1 aromatic C), 159.34 (1 aromatic C), 159.38 (C=O); m/z (%): 250.27 (M^+)

2-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino)methyl)-2-azaspiro[4.5]decan-3-one (2e)

Light yellow; M. P.: 236°C; IR (KBr, cm^{-1}): 3350 (N-H, str.), 1750 (C=N), 1610 (C=O, str.); ^{1}H NMR:1.28-1.97 (m, 2H, CH_2), 2.50-2.78 (m, 4H, $2CH_2$), 2.98 (s, 2H, $COCH_2$), 3.32 (s, 3H, CH_3), 3.79 (s, 2H, -N-CH_2-), 3.84 (s, 3H, OCH_3), 4.55 (s, 2H, -NCH_2-NH-), 7.44 (s, 1H, -NH-); ^{13}C NMR: 22.32-27.20 ($3CH_2$), 36.08 (-CH_3), 38.25-39.92 (2-CH_2-), 40.01 (Spiro C), 42.90 (OCH_3), 52.53 (-N-CH_2-N), 53.58 (-N-CH_2-), 166.84 (-N_2-C=N-), 170.00 (N(O)-C=N-), 175.79 (C=O), 176.92 (-N(C)-C=N-);m/z (%): 305.68 (M^+)

2-(((6-methylpyridin-2-yl)amino)methyl)-2-azaspiro[4.5]decan-3-one (2f)

Yellow; M. P.: 230°C; IR (KBr, cm^{-1}): 3380 (N-H, str.), 1620 (C=O, str.); ^{1}H NMR:1.56-1.90 (m, 2H, CH_2), 2.52-2.74 (m, 4H, $2CH_2$), 3.05 (s, 2H, $COCH_2$), 3.38 (s, 3H, CH_3), 3.80 (s, 2H, -N-CH_2-), 4.78 (s, 2H, -NCH_2-NH-), 7.23 (s, 1H, -NH-), 8.56-8.98 (m, 4H, aromatic); ^{13}C NMR: 22.28-26.89 ($3CH_2$), 37.34 (-CH_3), 37.56-38.90 (2-CH_2-), 39.67 (Spiro C), 52.46 (-N-CH_2-N), 57.98 (-N-CH_2-), 113.56 (1C aromatic), 126.76 (1C aromatic), 146.76 (1C aromatic), 156.87 (1C aromatic), 178,87 (C=O); m/z (%): 275.56 (M^+)

2-(((4-chlorophenyl)amino)methyl)-2-azaspiro[4.5]decan-3-one (2g)

Brown; M. P.: 224°C; IR (KBr, cm^{-1}): 3400 (N-H, str.), 1600 (C=O, str.); ^{1}H NMR:1.36-2.08 (m, 2H, CH_2), 2.73-2.89 (m, 4H, $2CH_2$), 3.45 (s, 2H, $COCH_2$), 3.56 (s, 3H, CH_3), 3.89 (s, 2H, -N-CH_2-), 4.87 (s, 2H, -NCH_2-NH-), 7.67 (s, 1H, -NH-), 8.78 (dd, 2H, aromatic), 8.98 (dd, 2H, aromatic); ^{13}C NMR: 22.43-27.56 ($3CH_2$), 36.50 (-CH_3), 38.33-39.78 (2-CH_2-), 40.45 (Spiro C), 52.55 (-N-CH_2-N), 53.98 (-N-CH_2-), 114.78 (1C aromatic), 120.76 (1C aromatic), 129.38 (C-Cl), 176.08 (C=O); m/z (%): 294.30 (M^+)

2-(((3-chlorophenyl)amino)methyl)-2-azaspiro[4.5]decan-3-one (2h)

Brown; M. P.: 204°C; IR (KBr, cm^{-1}): 3410 (N-H, str.), 1625 (C=O, str.); ^{1}H NMR: 1.37-2.09 (m, 2H, CH_2), 2.70-2.90 (m, 4H, $2CH_2$), 3.65 (s, 2H, $COCH_2$), 3.92 (s, 2H, -N-CH_2-), 4.79 (s, 2H, -NCH_2-NH-), 7.75 (s, 1H, -NH-), 8.90 (dd, 2H, aromatic), 9.25

(dd, 2H, aromatic); ^{13}C NMR: 22.46-27.59 (3CH_2), 36.59 (-CH_3), 38.29-39.73 (2-CH_2-), 40.53 (Spiro C), 52.65 (-N-CH_2-N), 54.89 (-N-CH_2-), 114.74 (1C aromatic), 120.34 (1C aromatic), 129.92 (C-Cl), 176.29 (C=O); m/z (%): 294.50 (M^+)

2-(((4,6-dihydroxypyrimidin-2-yl)amino)methyl)-2-azaspiro[4.5]decan-3-one (2i)

Brown; M. P.: 218°C; IR (KBr, cm^{-1}): 3350 (N-H, str.), 1789 (C=N), 1590 (C=O, str.); ^{1}H NMR:1.27-1.90 (m, 2H, CH_2), 2.45-2.67 (m, 4H, 2CH_2), 3.08 (s, 2H, $COCH_2$), 3.84 (s, 2H, -N-CH_2-), 4.67 (s, 2H, -NCH_2-NH-), 5.78 (s, 1H, -NH-), 6.76 (s, 1H, aromatic), 10.65 (s, 2H, -OH); ^{13}C NMR: 21.23-27.56 (3CH_2), 38.45-39.90 (2-CH_2-), 38.95 (Spiro C), 52.49 (-N-CH_2-N), 53.79 (-N-CH_2-), 89.15 (1C aromatic), 170. 36 (2C, C-OH), 179.80 (C=O); m/z (%): 293.78 (M^+)

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VII. References

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