Synthesis and Cytotoxic Evaluation of Functionalized Aryl-halo Amide Derivatives

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Abstract

Research in the field of chemistry remains inevitable for the synthesis and manipulation of different drugs and their derivatives. A particular NSAID diclofenac is one of the most widely used drug worldwide. It contains aryl-halo core group in its structure. Altering that core structure with the amide bond formation and thereby assessing its cytotoxic characteristics is the main objective of this study. In order to carry out this task 2-[2-(2,6-dichloroanilino)phenyl]acetate was taken as our starting material which was converted into 2-[2-(2,6-dichloroanilino)phenyl]acetic acid in the first step. After the production of respective carboxylic acid, it was then subjected to amide bond formation by treating it with various substituted anilines using DCC as coupling agent and HOBt as additive in the presence of THF which led to the synthesis of ten different aryl-halo amide derivatives labelled as 4a-j with percentage yields of 82, 84, 81, 82, 80, 83, 81, 79, 83 and 82 respectively. These aryl-halo amide analogues were evaluated for their cytotoxic properties using L929 fibroblast cell line via MTT Assay. The synthesized compounds (4a-j) were shown to have inhibitory concentrations (IC_{50}) of 5.2, 5.3, 5.1, 5.7, 5.0, 5.4, 5.3, 5.9, 5.5, and 5.6 in mM/ml respectively. Among the compounds assessed 4h was found to be least toxic with an inhibitory concentration (IC_{50}) of 5.9 mM/ml

I Introduction

Search for the potential bioactive molecules has been the prime objective of the chemical research. 25% of the available drugsas well as the molecules having therapeutic significance contain amide as a functional group(Ghose et al., 1999). Of all the chemical reactions carried out in the labs associated with drug synthesis 16% pertain to amide bond formation(Roughley & Jordan, 2011). Drugs containing aryl-halo scaffold has long been used to treat conditions such as fever, ache and inflammation(Brandt et al., 2016). The aryl-halo based medications e.g. diclofenac are employed to cure various inflammatory conditions which include spondyloarthritis, rheumatoid arthritis,

gout episodes, polymyositis, and arthritis(Meinicke & Danneskiold-Samsøe, 1980). These medications have been proved to show several biological activities which encompass antibacterialproperties(Dastidar et al., 2000), antimycobacterial effects(Sriram et

al., 2006), effects on ulcerdevelopment and lipid peroxidation, in addition to their anti-inflammatory and analgesicqualities (Amir & Shikha, 2004)(Bhandari et al., 2008)as well as antitumor activity(Barbarić et al., 2007).

A direct reaction between an amine and a carboxylic acid result in the formation of ammonium carboxylate salts. Moreover, extremely harsh conditions are required to carry out this direct condensation which involves no use of a coupling agent. Due to the aforementionedfactors,it has been impractical to perform direct amidation reaction to synthesize compounds of therapeutic significance(Jursic & Zdravkovski, 1993). Therefore,it is necessary to activate carboxylic acid before it can couple with an amine to form amide bond.Amides can be synthesized by utilizing various reactants through different reaction pathways but the condensation reaction between a carboxylic acid and an amine is by far the most popular method currently being used. And this condensation is usually carried out in the presence of a coupling against which facilitates the dehydration reaction by activating carboxylic acid(Lanigan & Sheppard, 2013).

There are many coupling agents which are commonly employed to accomplish amide bond formation, some of them are mentioned here. Phosphorus oxychloride ($POCl_3$), oxalyl chloride($COCl_2$) and thionyl chloride ($SOCl_2$) involving the formation of acid chlorides as intermediates, chloride (PivCl), also referred to as trimethylacetyl chloride which results in the production of acid anhydrides as intermediates are among some of commonly employed coupling reagents(Dunetz et al., 2016). Carbodiimides such as DCC, EDC and DIC(Dunetz et al., 2016), boron species such as boric acid(Anderson et al., 2006) and 3-nitrophenylboronic acid(Bannister et al., 2000), esters involving methyl esters(Fray et al., 2010), ethyl esters(Fleitz et al., 2000), tert-butyl esters(Bellingham et al., 2004), isobutyl esters(Fleck et al., 2003), benzyl esters(Fleck et al., 2003),lactones(Fleck et al., 2003), thioesters(Brands et al., 2002), pentafluorophenyl esters(Zhang et al., 2008)and N-hydroxysuccinimido esters(Westermann et al., 2007), bronsted acids for example acetic acid (AcOH)(van der Linden et al., 2008)and sulfuric acid (H_2SO_4) (Marzoni & Varney, 1997)have also been frequently utilized reagents. Research is also being keenly conducted to find out potent catalysts for direct amidation. Prominent examples of such catalysts include metals likepalladium and copper, organo-catalysts such as proline derivatives and sulfonamides(Lanigan & Sheppard, 2013). The goal of this study is to synthesize aryl-halo based amide derivatives and then subjecting them to cytotoxic evaluation. This will be achieved by altering the aryl-halo scaffold of 2-[2-(2,6-dichloroanilino)phenyl]acetic acid by coupling it with various substituted anilines.

II Experimental

All the chemicals, reagents and solvents were purchased from the Merk, a German company.

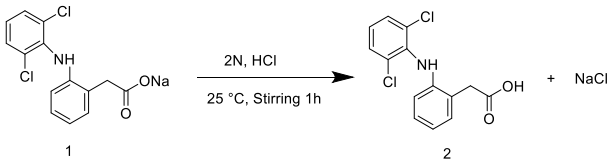
a. Synthesis of Free Diclofenac from Diclofenac Sodium

2g of Sodium;2-[2-(2,6-dichloroanilino)phenyl]acetate (Diclofenac Sodium) was dissolved first in 20 ml of absolute ethanol and was then subjected to stirring for 10 minutes at room temperature. 3 ml of 2N HCl was added to this solution and stirred for 1h afterwards (scheme 2.1). At the end of this reaction cold water was added to the reaction mixture and was then filtered to get precipitates of 2-[2-(2,6-dichloroanilino)phenyl]acetic acid (Free Diclofenac). These precipitates were oven dried at 60 ºC.

b. Synthesis of Aryl-halo Amide Derivatives

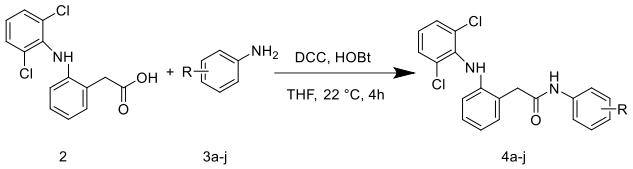
2-[2-(2,6-dichloroanilino)phenyl]acetic acid (0.315mmol) was made to dissolve in 3ml of THF. Solutions of various substituted anilines (0.3mmol) were prepared in 3ml of THF. Each of these solutions were separately mixed with former solution of free diclofenac at 22 ºC. 5 ml of THF was again added to this reaction mixture, afterwards 0.3 mmol of HOBt was incorporated in this solution which then underwent stirring for 10 minutes at 22 ºC. The mixture was cooled down to 0 ºC. A solution of DCC prepared in 5ml of THF was then introduced into the reaction mixture while maintaining the internal temperature of the system at 0 ºC. The temperature of this mixture was gradually raised to 22 ºC over half an hour period and was then stirred for 4 hours at this very temperature (scheme 2.2). After the stirring was complete the solution was again cooled down to 0 ºC.

For the workup procedure 20 mL of 5% $NaHCO_3$ was added to the above reaction mixture at 22 ºC with the subsequent addition of 40 mL of ethyl acetate and then stirred it for 10 minutes. The organic layer was purified and dehydrated by adding brine and 3g of anhydrous Na_2SO_4 respectively and then subjected it to 5 minutes of stirring. The organic layer was then separated and filtered. The filtrate was subjected to evaporation at lower pressure to get the desired product.



1 2

Scheme 2.1:*Conversion of Sodium;2-[2-(2,6-dichloroanilino)phenyl]acetate to 2-[2-(2,6-dichloroanilino)phenyl]acetic acid*



2 3a-j 4a-j

Compound	R
4a	-
4b	2-Cl
4c	2-CH_3
4d	2-OCH_3
4e	2-Br
4f	2,4-Cl
4g	2,4-CH_3
4h	2,5-OCH_3
4i	2-NO_2
4j	2,3-NO_2

Scheme 2.2: *Synthesis of aryl-halo amide analogues from various substituted anilines*

c. Maintenance of Cell Lines

DMEM media was utilized to culture the L929 fibroblast cell lines with the addition of 10% fetal bovine serum, L-glutamine, 1% penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37 ºC. The atmospheric composition was maintained at 5% CO2 and 95% air with the help of controlled CO2 chamber. Trypsin having 0.25% EDTA in it was used to isolate cells and then these were neutralized by DMEM containing PSGF and 10% FBS. Cells were separated mechanically by pipetting. 96 wells plastic culture plates were employed and 200 µl of media was introduced it each well. These plates were then kept in an atmosphere having 5% CO2 and 95% air at 37 ºC for 24 hours.

d. Cell Viability via MTT Assay

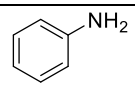
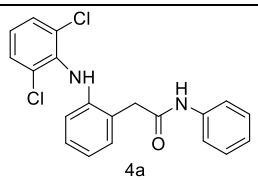
100 µl of cells having a density of 1×10^5 were addedinto the microplates. It served as a negative control. After 24 hours the growth media and the cell monolayer were cleansed with non-FBS MEM twice. Different dilutions (1.0, 2.5, 5.0, 7.5, and 10.0 mM/ml) of aryl-halo amide derivatives in 1 mL of non-FBS media were added to the wells. 20 µl of MTT (5 mg/ml in PBS) was introduced in each well and then the cells were incubated for 6-7 hours in CO_2 incubator. The medium was removed and 1 mL of DMSO was added to each well. The liquid portion was then removed and 50 µl of propanol was added. The plates were subjected to gentle stirring for 15 minutes to dissolve the produced formazan. The absorbance was measured at570nm with the help of MINDRAY90 ELISA reader. Cell survival percentage led to the determination of IC_{50} values of the test samples.

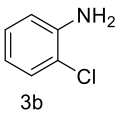
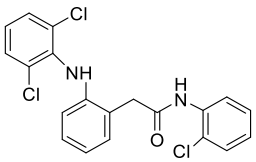
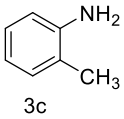
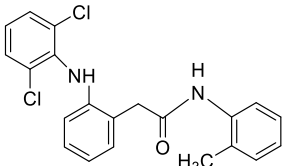
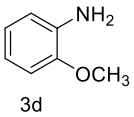
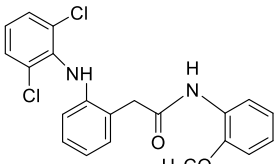
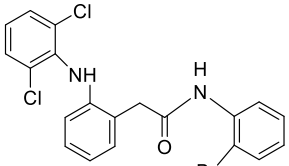
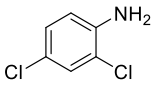
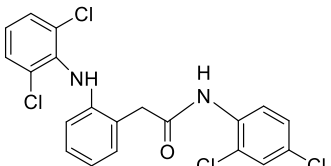
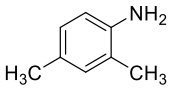
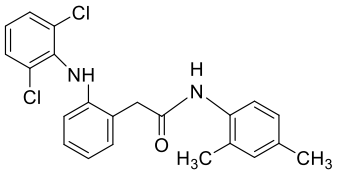
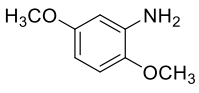
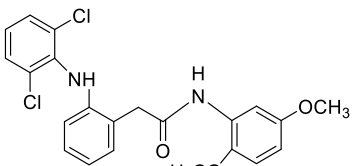
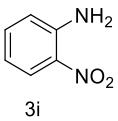
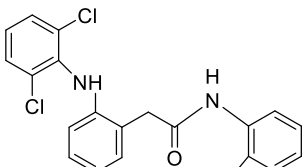
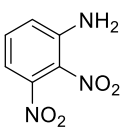
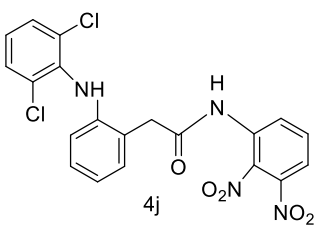
III Results and Discussion

a. Amide Analogues

Table 3.1 depicts how various substituted anilines (3a-j) when subjected to specific reaction conditions yielded different aryl halo amide derivatives (4a-j). The products gave maximum yield when stirring time was increased to 4 hours at 22 ºC using THF as solvent. The maximum yield recorded was 84% given by the compound 4b and lowest one was recorded at 79% produced by the compound 4h.

Table 3.1: *Synthesis of aryl-halo amides derivatives*

Sr. no.	Reactants	Condition	Solvent	Time (h)	Temp. (ºC)	Product	Yield (%)
1	 3a	Stirring	THF	4	22	 4a	82

2	 3b	Stirring	THF	4	22	 4b	84
3	 3c	Stirring	THF	4	22	 4c	81
4	 3d	Stirring	THF	4	22	 4d	82
5	 3e	Stirring	THF	4	22	 4e	80
6	 3f	Stirring	THF	4	22	 4f	83
7	 3g	Stirring	THF	4	22	 4g	81
8	 3h	Stirring	THF	4	22	 4h	79
9	 3i	Stirring	THF	4	22	 4i	83
10	 3j	Stirring	THF	4	22	 4j	82

b. Cytotoxic Evaluation

The cytotoxic analysis of the synthesized compounds was performed using MTT Assay. There was no considerable difference between the cell viability percentage of negative control group and that of the group treated with aryl-halo amide analogues (4a-j) at mM/ml concentration. However, as the concentration of the dose was boosted this difference became conspicuous (table 3.2). The cytotoxic assessment also revealed the inhibitory concentration (IC_{50}) values of the compounds tested. Table 3.3 shows that the least cytotoxic compound among the synthesized ones was 4h with an inhibitory concentration (IC_{50}) of 5.9 mM/mL.

Table 3.2:*Cell viability at different concentrations of aryl-halo amide derivatives*

Test Group	Concentration (mM/mL)				
	1	2.5	5	7.5	10
	Cell Viability (%)				
L929 untreated cells	100 (negative control)				
4a	82.66	64.37	52.74	36.13	23.07
4b	82.07	64.63	52.07	36.01	22.99
4c	83.01	65.14	52.80	37.11	23.91
4d	83.82	65.28	52.98	37.72	24.01
4e	81.21	62.13	51.42	35.01	22.14
4f	80.51	62.12	50.21	34.87	21.98
4g	81.98	63.92	51.74	35.83	22.99
4h	84.68	66.47	55.79	38.31	25.06
4i	80.33	61.15	49.72	33.13	20.70
4j	82.60	64.73	52.12	36.55	23.70

Table 3.3: *Inhibitory concentrations (IC_{50}) of synthesized compounds*

Compounds	IC_{50} (mM/mL)
4a	5.2
4b	5.3
4c	5.1
4d	5.7
4e	5.0
4f	5.4
4g	5.3
4h	5.9
4i	5.5
4j	5.6

Conclusion

There were 10 amide derivatives prepared from the aryl-halo scaffold of diclofenac, namely 4a, 4b, 4c, 4d, 4e, 4f, 4g, 4h, 4i, 4j. At the concentration of 1 mM/mL these compounds did not have a considerable impact on the cell viability but as their concentration was made to increase their effect became pronounced. The cytotoxic evaluation of

demonstrated that the least cytotoxic compound among the synthesized ones was found to be 4h with an inhibitory concentration (IC_{50}) of 5.9 mM/mL as compared to diclofenac sodium whose IC_{50} was reported to be 5.2 mM/mL by (Sowmya et al., 2020).

The use of NSAIDs does not come along without their adverse effects and in the case of Diclofenac the side effects include gastrointestinal harm, absorption, distribution, toxicity, instability and formulation(Kumar et al., 2010). In this study various amide derivatives of diclofenac were synthesized by alternating its aryl-halo scaffold which lowered its cytotoxicity. Further study can be conducted to formulate and assess amide analogues of diclofenac for lowered toxicity.

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